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Etude des interactions entre l'entomofaune et un cadavre: approches biologique, comportementale et chémo-écologique du coléoptère nécrophage, *Thanatophilus sinuatus* Fabricius (Col., Silphidae)

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Résumé – La décomposition d'un corps entraîne des changements physiques et biochimiques importants, le cadavre va émettre des odeurs attractives pour certaines espèces et d'autres moins attractives. Au sein des écosystèmes terrestres tempérés, les insectes sont généralement les principaux organismes qui colonisent un corps selon une séquence plus ou moins prédictive. Ces insectes nécrophages et/ou nécrophiles, principalement des Diptères et des Coléoptères, utilisent le micro-habitat créé par le cadavre comme un substrat nourricier, un site de reproduction, un refuge ou encore comme un territoire de chasse. L'objectif principal de cette thèse était de mieux connaitre l'écosystème cadavre et plus précisément les interactions cadavre-entomofaune sous l'angle novateur de l'écologie chimique. Cette approche pluridisciplinaire combine des études biologiques, électrophysiologiques (EAG), comportementales (olfactométrie) ainsi que des analyses de composés volatils par diverses méthodes analytiques ((TDS)-GC-MS, GCxGC-TOF-MS). Le cochon domestique, substitut couramment utilisé pour modéliser la décomposition humaine, a servi de modèle animal pour les études faunistiques de suivis postmortem et les études visant à caractériser la signature olfactive d'un cadavre au cours du processus de décomposition. L'«odeur de la mort» est constituée par un mélange de centaines de composés organiques volatils cadavériques (COVs) dont le profil qualitatif et quantitatif évoluent au cours du processus de décomposition. Les Coléoptères, acteurs importants de l'écosystème-cadavre, étaient jusqu'à présent délaissés par les entomologistes forensiques au profit des Diptères. Ce travail de recherche s'est intéressé à deux familles de Staphylinoidea: les Staphylinidae et les Silphidae. 62 espèces de Staphylinidae ont été recensées au sein de l'écosystème-cadavre. Une espèce de staphylin ressort nettement de ces études *in situ*: *Creophilus maxillosus*. Concernant les Silphidae, 9 espèces de Nicrophorinae et de Silphinae ont été recensées sur les carcasses de porc avec une nette prépondérance des Silphinae. Néanmoins, seul le taxon des Silphinae semble avoir un intérêt potentiel en entomologie forensique et plus particulièrement les *Thanatophilu*s spp. et *Necrodes littoralis*. *T. sinuatus* a été choisi comme insecte modèle, son cycle de développement ainsi que celui de *N. littoralis* ont été étudiés en conditions contrôlées à deux températures constantes. Une approche chémo-écologique, combinant des études EAG et olfactométriques, a été réalisée sur *T. sinuatus* avec une sélection de molécules cadavériques. Ces essais ont mis en avant le rôle attractif du diméthyldisulfure (DMDS) sur *T. sinuatus*. Ce composé semble être un marqueur clé dans les processus de décomposition animale. Le *p*-crésol était quant à lui uniquement attractif pour les mâles de *T. sinuatus*. Ces découvertes améliorent notre compréhension de l'écosystème cadavre et plus particulièrement la communication chimique entre une espèce de Silphinae et un corps en décomposition. Cette recherche a également permis de mieux connaitre les populations de staphylins et de silphes que l'on retrouve au sein de l'écosystème cadavre.

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Abstract – The decay process of vertebrate mammals conducts to physical and biochemical changes. The corpse releases attractive odors for some species and other less attractive. In temperate terrestrial ecosystems, insects are the most specialized organisms in terms of exploitation of a "cadaver-ecosystems". Carrion insects, comprising many Dipterans and Coleopterans, are attracted to the cadaver in a relative predictable sequence. These necrophagous and/ or necrophilous insects use the cadaver as a food source, a reproduction site, a refuge or a hunting site. The main goal of this thesis was to investigate the relationships that may exist between a decaying corpse and insects, using chemical ecology. This multidisciplinary approach was used which mixed biological, electrophysiological (EAG), behavioral (olfactometry) studies and chemical analyses with different analytical methods ((TDS)-GC-MS, GCxGC-TOF-MS). Domestic pig was used as animal model to conduct faunistic studies on pig carcasses and cadaveric volatile samples during the decay process. The "smell of death" is constituted by a blend of several hundred volatile organic compounds (VOCs) which change over time. Until now, Coleoptera were neglected by forensic entomologists. Indeed, many published reports are focused on Diptera pattern colonization and very few looked at beetle succession. This research work was focused on a part of the superfamily of Staphylinoidea: Staphylinidae and Silphidae. 62 species of Staphylinidae were identified in cadaver ecosystem. *Creophilus maxillosus* was the most abundant staphylinid species. Concerning Silphidae, 9 species of Nicrophorinae and Silphinae were identified on decaying pig carcasses. Silphinae specimens were more abundant than *Nicrophorus* spp. All carrion beetles do not have the same forensic interest; species of Silphinae seem to have a more important value as forensic bioindicators and more exactly *Thanatophilus* spp. and *Necrodes littoralis*. *T. sinuatus* was chosen as insect model; its life cycle and the developmental cycle of *N. littoralis* were studied under laboratory conditions at two constant temperatures. A chemo-ecological approach, combining EAG and behavioral studies, was used on *T. sinuatus* with selected cadaveric compounds. These bioassays highlighted the attractive role of dimethyldisulfide (DMDS) on *T. sinuatus*. DMDS seems to be a key component in vertebrate decaying process. *P*-cresol was only attractive for males of *T. sinuatus*. These findings enhance our understanding of the cadaver ecosystem and chemical communication between silphine toward a decaying corpse. It also improves our knowledge of the forensic community of rove beetles and carrion beetles.

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Partie I: Introduction générale, contexte et objectifs

«L'homme est né lorsque pour la première fois, devant un cadavre, il a chuchoté: pourquoi?»

André Malraux

«Mais qu'est-ce donc l'entomologie forensique? Il s'agit d'une science qui a pour but la datation des cadavres grâce aux insectes nécrophages. Passionnant, ce sujet!»

Claude Wyss, 2006, Traité d'entomologie forensique

Chapitre I.1: Introduction générale

L'entomologie forensique est une discipline des sciences forensiques qui étudie les insectes et d'autres arthropodes dans un contexte médico-légal (Hall 2001). Contrairement à ce que l'on pourrait croire, l'entomologie forensique ou médico-légale n'est pas une discipline récente. En effet, l'utilisation des insectes en criminalistique n'est pas neuve…déjà en 1894, paraissait le célèbre ouvrage de Mégnin nous parlant de la «Faune des cadavres: application de l'entomologie à la médecine légale» (Mégnin 1894). Cependant, le premier cas d'entomologie criminelle recensé dans la littérature daterait du 13ème siècle (Benecke 2001). Il est l'œuvre d'un enquêteur chinois (Sung Tz'u) qui a utilisé les insectes afin de démasquer le coupable d'un meurtre à la faucille commis au sein d'une rizière. Ayant réuni les fermiers de la communauté, Sung Tz'u leur a demandé de déposer leurs outils devant eux. Alléchées par l'odeur du sang, les mouches ont mené notre enquêteur vers une seule des faucilles…Ainsi démasqué, le meurtrier a avoué (Benecke 2001, Wyss et Cherix, 2006).

Il faudra cependant attendre la fin du XXème siècle pour que cette discipline se développe véritablement, et soit enfin reconnue comme une science criminalistique à part entière (Charabidzé et Bourel 2007). La création en 2002 de l'Association Européenne pour l'Entomologie Forensique (*European Assiociation for Forensic Entomology*, EAFE) a largement contribué à faire connaitre les avancées de cette discipline un peu partout en Europe. Dans les faits, l'EAFE n'est réellement née qu'en 2003, lors de la première réunion des scientifiques du domaine à Francfort (Wyss et Cherix 2006); celle-ci a désormais un rayonnement international qui dépasse les frontières de l'Europe. On ne peut cependant ignorer l'influence, bien avant les années 2000, d'un pionnier belge en la matière, à savoir le Docteur Marcel Leclercq. Ce célèbre médecin liégeois, décédé en 2008, a largement contribué à la renommée mondiale de l'entomologie médico-légale.
Si l'entomologie forensique n'est pas une nouvelle science au sens strict, la terminologie «forensique» a quant à elle supplanté les autres dénominations telles que l'entomologie (médico-)légale, criminelle ou encore judiciaire. Le terme forensique est dérivé du latin «forum» qui signifie rendre public (Ricciuti 2007). L'entomologie forensique fait partie des sciences forensiques, appelées outre Atlantique *forensics*, terme bien souvent aperçu dans les séries américaines en vogue.

Cependant, bien qu'étant à la mode, il faut admettre que cette science n'en est encore qu'à ses prémisses et que de nombreuses synergies sont encore inconnues. Le micro-habitat temporaire

créé par la décomposition d'un organisme animal attire un faune variée dont les plus spécialisés, dans nos écosystèmes terrestres, sont les insectes nécrophages et nécrophiles. Il s'agit principalement de Diptères et de Coléoptères. Si la biologie de nombreuses espèces de Diptères, appelées communément mouches, fait l'objet d'un grand nombre d'études entomoforensiques; une large partie de cette entomofaune est encore trop peu étudiée. La majorité des travaux de recherche se portent sur les Diptères et plus particulièrement sur la famille des Calliphoridae (*Blowflies*). En effet, leur arrivée précoce sur le cadavre, dans les minutes qui suivent la mort en font de redoutables bioindicateurs quant au moment du décès. Les Coléoptères, acteurs importants de cet écosystème, sont quant à eux, encore trop souvent négligés. Preuve en est le faible nombre de publications scientifiques ayant trait aux Coléoptères d'intérêt forensique par rapport à celles existant sur les Diptères nécrophages.

Très peu étudiée à l'heure actuelle, l'écologie chimique axée sur un organisme animal en décomposition permettrait de mieux comprendre les interactions existant au sein de cet écosystème particulier qu'est le cadavre. En effet, la communication chimique est le principal mode d'interaction des grands groupes d'animaux, y compris chez les insectes. Ceux-ci perçoivent les odeurs principalement grâce à leurs antennes, véritables bio-détecteurs de molécules volatiles. Le corps, en se décomposant, va émettre des odeurs attractives pour une entomofaune particulière: la «faune des cadavres». Encore faut-il pouvoir identifier ces odeurs afin de décrypter le «langage des insectes». Véritable trait d'union entre la chimie analytique et l'écologie, l'approche chémo-écologique de l'écosystème cadavre se révèle être très prometteuse. Ce n'est pas notre Sherlock Holmes chinois qui le démentirait.

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Chapitre I.2: L'entomologie forensique

I.2.1. Prélude

Discipline à part entière des sciences forensiques, l'entomologie forensique est un terme général qui se subdivise en trois sous-disciplines. On y inclut l'entomologie dite «urbaine», l'entomologie des denrées stockées et enfin, celle qui nous intéresse plus particulièrement, l'entomologie criminelle. Seule cette dernière discipline sera abordée dans la suite de ce travail.

Si la datation de la mort est l'une des premières applications qui vient à l'esprit quand on parle d'insectes et d'expertises entomologiques médico-légales; elle n'est pas la seule utilisation des insectes en criminalistique. En effet, les insectes peuvent aussi donner des indices à l'entomologiste forensique en ce qui concerne le lieu du décès, s'il y a eu ou non dissimulation du corps ou encore sur d'éventuels déplacements *postmortem* du cadavre. Les insectes peuvent aussi fournir des informations sur les causes du décès et même en l'absence de cadavre, révéler l'identité de la personne décédée en analysant l'ADN présent dans le système digestif des larves. L'utilisation des insectes en tant que bio-indicateurs et auxiliaires miniaturisés de police se révèlent être précieuse et non négligeable. Outre les phénomènes biochimiques *postmortem* qui se déroulent après le décès d'une personne, le sous-chapitre suivant, intitulé «entomologie médico-légale» développe les champs d'applications de l'expertise entomologique médico-légale ainsi que les méthodes entomologiques d'estimation du délai *postmortem*.

Grâce au legs de sa collection entomologique et de ses écrits à l'unité d'Entomologie fonctionnelle et évolutive (Ulg, Gembloux Agro-Bio Tech), une synthèse des expertises médico-légales du docteur Marcel Leclercq a été réalisée sur bases de ses rapports d'expertises. Pionnier belge à la reconnaissance mondiale, le Docteur M. Leclercq a travaillé pendant près de 36 ans en tant qu'expert médico-légale auprès de la justice belge, mais également auprès des tribunaux français. Il a traité 132 expertises judiciaires et a travaillé sur quelques-uns des cas les plus célèbres de l'histoire judiciaire belge.

I.2.2. L'entomologie médico-légale

Jessica Dekeirsschieter[1], Eric Haubruge[1], Marcel Leclercq (1924 -2008†), Philippe Boxho[2]

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Référence - Dekeirsschieter J., Haubruge E., Leclercq M. (*A paraitre*). Section 3: entomologie médico-légale. Médecine Légale à l'usage des juristes, Anthémis.

1. Préambule

Lorsque la mort remonte à plus de 72h ou que des signes de putréfaction avancée sont visibles, les techniques thanatologiques usuelles (méthodes thermométriques, rigidités, hypostases ou les méthodes biochimiques) ne sont plus efficaces pour évaluer le moment du décès. La présence et l'identification des insectes sur le corps et de façon plus large sur la scène du décès sont de meilleurs bio-indicateurs pour dater la mort. L'étude des insectes ainsi que celles d'autres Arthropodes (Acariens) dans un contexte judiciaire ou médico-légale définit l'entomologie médico-légale (Catts et Goff 1992, Hall 2001). On parle également d'entomologie judiciaire, légale ou criminelle. Cependant, la dénomination «entomologie forensique» tend à supplanter toutes ces dénominations bien qu'elle inclut d'autres secteurs tels que l'entomologie des denrées stockées (sécurité alimentaire) et l'entomologie « urbaine » (infestations d'insectes nuisibles pour l'homme: cafards, termites, *etc.*).

Après la mort, le corps subit une décomposition biologique par des microorganismes tels que des bactéries et des champignons saprophytes (putréfaction), mais également par des Arthropodes dont les plus destructeurs sont les insectes, et plus particulièrement leurs larves. En effet, au sein de nos écosystèmes terrestres tempérés, les insectes associés aux décomposeurs participent à la minéralisation du cadavre. Leur rôle est donc primordial au sein de nos écosystèmes où ils remplissent la fonction « d'éboueurs entomologiques » (Leclercq et Verstraeten 1992). Ils participent activement au recyclage des matières organiques mortes en intervenant dans les cycles biogéochimiques du carbone et de l'azote (Hastir et Gaspar 2001). Il faut cependant noter que toutes les dégradations imposées au cadavre ne sont pas dues à la

seule activité entomologique et microbienne, certains cadavres mal ou non inhumés peuvent subir des déprédations causées par des mammifères (rongeurs, renards, etc.) et des oiseaux (corbeaux, etc.), y compris les animaux domestiques (chiens, chats). On parle de *scavenging* (Devault *et al.* 2003); les animaux consommeraient de 35 à 75% du corps au sein d'un écosystème terrestre et jusqu'à l'entièreté de celui-ci lorsque l'entomofaune et les bactéries sont moins actives (Devault *et al.* 2003).

2. L'entomofaune des cadavres

La dégradation d'un corps et sa colonisation par les insectes sont deux phénomènes intimement liés et sont influencés par de nombreux facteurs intrinsèques et extrinsèques au cadavre (Wells et Lamotte 1995, Campobasso *et al.* 2001). Les facteurs intrinsèques, directement liés à la personne décédée, sont l'âge, la masse corporelle, la cause du décès (drogues, infection, etc.), l'hygiène corporelle, l'intégrité du corps (blessures, plaies) et la présence de vêtements (Campobasso *et al.* 2001). Parmi les facteurs externes, le facteur le plus important est la zone biogéoclimatique incluant l'habitat, la végétation, le type de sol et les conditions météorologiques (température, vent, humidité atmosphérique) du lieu où se situe la dépouille (Anderson 2001, Campobasso *et al.* 2001).

D'autres paramètres ont une influence significative sur la vitesse de décomposition d'un corps; on peut citer la saison, l'emplacement du corps (ombragé *vs* ensoleillé) et enfin l'accessibilité du corps aux organismes vivants qu'ils soient mammifères (animaux domestiques ou sauvages) ou insectes (Anderson 2001, Campobasso *et al.* 2001).

De plus, les processus de décomposition et la faune des cadavres varient fortement en fonction du lieu où se trouve le cadavre. Les corps enterrés ou submergés subiront des évolutions différentes des corps laissés à l'air libre (Anderson 2001).

L'ensemble de ces facteurs variables a été classifié (Mann *et al.* 1990) (Tableau 1), on attribue à chaque item un certain nombre de points sur 5 en fonction de son influence sur le processus de décomposition, la cote de 5 indiquant le maximum d'influence.

Tableau 1. Facteurs affectant le processus de décomposition (d'après Mann *et al.* 1990).

Variables	Effet sur la vitesse de la décomposition
Température	5
Accessibilité aux insectes	5
Enfouissement et profondeur	5
Carnivores & Rongeurs	4
Traumatismes	4
Humidité & Aridité	4
Pluviosité	3
Taille et Poids du corps	3
Embaumement	3
Habillement	2
Surface sous le cadavre	1
pH du sol	inconnu

.

Parmi tous ces facteurs, deux sont prépondérants dans la décomposition d'un corps, il s'agit de la température ambiante et de l'accessibilité du corps aux insectes (Mann *et al.* 1990, Campobasso *et al.* 2001).

La colonisation du corps par les arthropodes

Les insectes sont généralement les premiers organismes à arriver sur le corps peu après la mort et le colonisent selon une séquence plus ou moins prédictible (Smith 1986, Anderson, 2001). En effet, ces derniers sont équipés d'un système sensoriel très réceptif aux molécules odorantes de leur environnement. Grâce à leurs organes olfactifs (antennes et sensilles olfactives), les insectes sarcosaprophages sont parfaitement adaptés à la détection de cadavres humains ou de carcasses animales. La décomposition du corps va entraîner des changements physiques et biochimiques importants, celui- ci va émettre des odeurs plus attractives pour certaines espèces et d'autres moins attractives (Leclercq 1978, Anderson 2001, Dekeirsschieter *et al.* 2009). Les insectes utilisent le micro-habitat créé par le cadavre comme un substrat nourricier, un site de pontes (reproduction), un refuge ou encore comme un territoire de chasse. En fonction de leurs caractéristiques écologiques, on rencontre quatre groupes écologiques autour d'un cadavre (Leclercq 1978, Smith 1986, Wyss et Cherix 2006), une cinquième catégorie est parfois citée, il s'agit des espèces dites accidentelles dont la présence sur le corps est le fait du hasard (Arnaldos *et al.* 2005).

- **les espèces nécrophages**: se nourrissent des tissus cadavériques et plus spécifiquement des liquides. On peut citer parmi cette catégorie, les Diptères

appartenant aux familles des Calliphoridae et des Sarcophagidae, mais également des Coléoptères des familles des Silphidae et des Dermestidae.

- **les espèces nécrophiles**: sont prédateurs ou parasites des espèces nécrophages, principalement des larves et des pupes de Diptères. On rencontre régulièrement des Coléoptères (Silphidae, Histeridae, Staphylinidae), des Diptères (Calliphoridae et Stratiomyidae) ainsi que des Hyménoptères (Campobasso *et al.* 2001, Wyss et Cherix 2006). Les larves de certains Diptères peuvent devenir prédatrices à partir d'un certain stade de développement. C'est le cas, par exemple, des larves de stade III appartenant au genre Muscina (Diptère, Muscidae) (Gaudry 2002) et de certaines Chrysomya (Diptère, Calliphoridae) (Leclercq 1978).

- **Les espèces omnivores**: se nourrissent tant du cadavre que des espèces dites nécrophages et nécrophiles présentent sur la dépouille. Les principales espèces omnivores sont généralement des Hyménoptères (fourmis et guêpes) ainsi que des Coléoptères.

- **Les espèces opportunistes**: perçoivent la présence du cadavre comme une extension de leur habitat. Elles utilisent le cadavre comme une annexe de leur biotope afin de s'abriter, se réchauffer, hiberner et parfois même pour se nourrir (Leclercq et Verstraeten 1992). Elles sont originaires de la végétation environnante ou de la pédofaune et peuvent exceptionnellement être prédateur des espèces nécrophages (Campobasso *et al.* 2001). On y dénombre des collemboles, des araignées, des mille-pattes, des Lépidoptères mais aussi des acariens qui se nourrissent des moisissures et champignons qui peuvent se développer sur le corps en décomposition (Campobasso *et al.* 2001 ; Wyss et Cherix2006).

Seul les deux premiers groupes sont utiles en entomologie forensique, ils regroupent un grand nombre de Diptères et de Coléoptères (Amendt *et al.* 2004).

3. Applications de l'entomologie forensique

La première application qui nous vient à l'esprit quand on parle d'entomologie forensique est l'utilisation des insectes pour estimer la date du décès. On parle plus précisément d'intervalle post-mortem (IPM), celui-ci se définit comme étant le laps de temps écoulé entre la date du décès et la date de découverte du corps (Nuorteva 1977, Benecke 2004, Wyss et Cherix

2006). Différentes méthodes entomologiques existent et permettent d'estimer ou de calculer cet IPM avec plus ou moins de précision, nous les détaillerons dans le paragraphe 4.

Cependant les insectes récoltés sur le lieu du décès et/ou sur le corps peuvent également servir à déterminer les causes de la mort notamment dans les cas de décès par substances toxiques (drogues, poisons, toxines, alcool, etc.), on parle d'entomotoxicologie (Beyer *et al.* 1990, Bourel *et al.* 2001, Introna *et al.* 2001, Amendt *et al.* 2004).

Entomotoxicologie : Lorsque le corps est trop décomposé et que l'on ne dispose plus des tissus ou des fluides (urine, sang) couramment utilisés pour effectuer des analyses toxicologiques, les insectes peuvent s'avérer très utiles (Beyer *et al.* 1990, Introna *et al.* 2001).

En effet, en s'alimentant des tissus cadavériques, ceux- ci vont également ingérer les éventuels toxiques présents (bio-accumulation). On peut détecter et éventuellement effectuer des dosages sur les insectes vivants (larves en croissance active), mais également sur les restes d'insectes imputrescibles qui peuvent persister des années après le décès (enveloppes pupales, exuvies, fragments de cuticule, etc.) (Bourel 2001).

On peut également déterminer s'il y a eu des déplacements, des dissimulations ou des manipulations du cadavre en analysant l'entomofaune présente sur le corps (Haskell *et al.* 1997, Wyss et Cherix 2006). Une autre application récente de l'entomologie forensique est l'utilisation de l'ADN contenu dans le tractus digestif des larves en vue de déterminer le profil génétique de la victime (Benecke et Wells 2001, Campobasso 2005). Cette application trouve son utilité lorsque l'on est en présence d'insectes nécrophages en grande quantité mais en l'absence d'un cadavre (déplacement du corps par exemple) (Gaudry *et al.* 2007).

Les insectes peuvent aussi révéler certains cas de maltraitances et de négligence sur les personnes dépendantes de notre société telles que les personnes âgées ou les enfants en bas âge (Benecke 2004, Gennard 2007). En effet, les larves de Diptères nécrophages peuvent se développer dans les escarres ou les couches et peuvent causer des myiases des plaies ou traumatiques (Wyss et Cherix 2006). Cependant, la médecine a su également tirer parti des mouches. En effet, on emploie leurs larves à des fins thérapeutiques en asticothérapie pour débrider les plaies (Ghilhou *et al.* 2004). On parle également de *maggot therapy*, de larvothérapie ou de luciliathérapie en référence aux espèces de Calliphoridae employées *(Lucilia* sp.).

4. Estimation du délai post-mortem par les méthodes entomologiques

Dans la littérature, on parle souvent de deux méthodes pour déterminer un intervalle post-mortem en utilisant les insectes comme bioindicateurs en fonction du laps de temps écoulé depuis le décès (Swift 2006, Wyss et Cherix 2006, Amendt *et al.* 2007).

La première méthode de datation entomologique se base sur l'âge des premiers insectes colonisant le cadavre (Diptères, Calliphoridae et Sarcophagidae). On utilise cette méthode pour établir un intervalle post-mortem «court», de quelques jours à quelques semaines pour autant qu'une seule génération d'insectes ait colonisé le corps. Pour être correcte, cette méthode doit tenir compte de nombreux facteurs tels que l'accessibilité du corps aux insectes dès le décès et des conditions climatiques favorables à l'activité des mouches. En effet, on part du postulat que si les mouches ont accès au substrat (le cadavre), le jour des premières pontes correspondra au jour du décès.

La deuxième méthode est une méthode de datation à plus long terme, elle se base sur la reconstitution des successions entomologiques pour déterminer l'IPM lorsque plusieurs générations d'insectes ont colonisé le corps. Cette méthode, basée sur la théorie des escouades, est peu précise et est à utiliser avec précaution (Wyss et Cherix 2006). En effet, la succession chronologique des espèces sur un cadavre n'est pas immuable. Le taux de décomposition du corps est variable de même que le cycle biologique de l'insecte, tous deux fortement influencés par les conditions climatiques locales.

4.1. Intervalle post-mortem « court »

4.1.1. Cycles biologiques des Diptères Calliphoridae

Les Diptères nécrophages sont très rapidement attirés par le corps, parfois quelques minutes seulement après le décès (ou même avant celui- ci). Les Calliphoridae (*blow flies*) sont les premières à arriver sur le corps, suivies de près par les Sarcophagidae (*flesh flies*) (Smith 1986, Anderson 2001). Les Calliphoridae sont des insectes holométaboles (métamorphose complète). On distingue les stades de croissance suivants : l'œuf, les trois stades larvaires (L1, L2, L3), les larves migrantes (stade prépupal), la pupe (ou nymphe) et enfin l'adulte appelé imago ou insecte parfait.

Les Calliphoridae, insectes ovipares, pondent à proximité des orifices naturels (nez, bouche, anus, organes génitaux), dans les plis cutanés, au niveau des plaies (le sang étant un élément

très attractif même coagulé) (Amendt *et al.* 2004, Wyss et Cherix 2006, Gennard 2007). En effet, les larves du premier stade (L1) ont besoin d'un substrat protéique liquide pour se nourrir et sont incapables de percer la peau. Les larves de deuxième stade (L2) sont munies de crochets buccaux et peuvent sécréter des enzymes protéolytiques leur permettant de perforer la peau (trous circulaires) et de progresser dans la colonisation du corps. Le troisième stade est le plus actif et se nourrit abondamment des tissus en décomposition. Après le stade III, les larves vont cesser de s'alimenter et se disperser en vue de leur métamorphose (Gomes *et al.* 2005). Les larves peuvent migrer à plusieurs mètres du corps et vont soit s'enfoncer dans le sol (milieu naturel) entre 5 et 20 cm de profondeur, soit se dissimuler dans les vêtements, tapis, literies, sous les meubles, etc. (habitation) (Turner 2005, Gomes *et al.* 2005, Wyss et Cherix 2006).

Figure 1. Calliphoridae à différents stades de développement, (A) femelles en train de pondre (*Calliphora vomitoria* et *Calliphora vicina*), (B) Pontes et présence d'une *Lucilia* sp. adulte, (C) pupes et larves migrantes à différents stades de pupaison de Calliphoridae, (D) larves de deuxième et troisième stades de Calliphoridae, (E) pupes de Calliphoridae, (F) œufs de Calliphoridae.

4.1.2. Calcul de l'intervalle post-mortem

De nombreuses méthodes existent pour calculer l'âge des larves (Diptères, Calliphoridae) (Greenberg 1991, Day et Wallman 2006), mais toutes sont basées sur le même principe : le taux de développement de l'insecte est fonction de la température ambiante (Amendt *et al.* 2004). Nous nous en tiendrons à deux approches : les mesures biométriques sur les stades immatures (larves) et le calcul de l'IPM sur le cycle de développement complet des Diptères Calliphoridae (de l'œuf à l'adulte émergent).

a. Mesures biométriques des larves

Ces différentes méthodes utilisent un modèle de croissance linéaire des larves en fonction de la température afin d'en déterminer leur âge. On peut mesurer différents paramètres sur les larves tels que leur taille (longueur ou largeur) ou encore leur poids.

- **Longueur**: la longueur des larves est le paramètre biométrique le plus souvent utilisé (Levot et Brown 1979, Williams 1984, Davies et Ratcliffe 1994, Wells et Lamotte1995, Von Zuben *et al.* 1998). La longueur est ensuite reportée dans un diagramme à 3 entrées appelé *isomegalen diagram* (courbes de croissance), celui-ci nous permet de déterminer rapidement l'âge pour autant que l'insecte ait été élevé à températures constantes (ce qui peut être le cas de corps trouvés à l'intérieur), mais ce qui est rarement le cas en conditions naturels (corps découvert à l'extérieur). Toutefois, lorsque la température ambiante varie, il est encore possible de déterminer l'âge de la larve en établissant une estimation entre l'intersection de la mesure et les courbes de croissance des températures minimum et maximum enregistrées (Reiter 1984, Grassberger et Reiter 2001, Gaudry *et al.* 2007, Amendt *et al.* 2007). Cette méthode peut être utilisée pour autant que les larves ne soient pas aux stades prépupal (larves migrantes, *postfeeding*) et/ou pupal pour lesquels la longueur n'est plus un critère adapté pour estimer l'âge (Grassberger et Reiter, 2001). Dans ce cas, on utilisera plutôt un diagramme appelé *isomorphen diagram* dans lequel sont reportés tous les stades de développement.

- **Largeur**: selon le même principe, on peut aussi mesurer la largeur des larves (Myskowiak et Doums 2002, Day et Wallman 2006), ce paramètre est cependant moins souvent utilisé que la longueur.

- **Poids**: Il y a également une bonne corrélation entre le poids des larves et leur âge (Nishida 1984, Wells et Lamotte 1995, Myskowiak et Doums 2002). On compare ensuite les données obtenues à des données de référence.

Il y a de nombreuses limitations et précautions à prendre pour utiliser ces méthodes biométriques sous peine d'introduire des biais dans l'estimation de l'âge des larves (Amendt *et al.* 2007). La nature des solutions de préservation et leur titrage peuvent influer sur les tissus larvaires et par conséquent modifier la biométrie des larves (Adams et Hall 2003, Amendt *et al.* 2007, Day et Wallman 2008). Elles offrent toutefois l'avantage de s'affranchir d'un élevage en laboratoire car elles travaillent généralement sur des échantillons morts (stades larvaires fixés dans une solution conservatrice et préalablement ébouillantés afin de fixer au mieux leurs caractéristiques biométriques) ce qui n'est pas le cas des méthodes travaillant sur le cycle biologique complet (Gaudry *et al.* 2007). Un autre inconvénient des mesures biométriques est la nature même de l'échantillon. En travaillant sur des stades larvaires, on augmente les risques d'erreurs d'identification taxonomique pour certains stades immatures (Gaudry *et al.* 2007). Cependant, l'utilisation de cette méthode (longueur des larves) se justifie dans les cas où les échantillons entomologiques sont reçus par le laboratoire dans une solution conservatrice (Adams et Hall 2003).

b. Etudes des cycles de développement complet

La deuxième approche se base sur la durée des cycles de développement des espèces de Calliphoridae et de Sarcophagidae. Chaque espèce de mouche a besoin d'une certaine somme de températures pour boucler son cycle de développement complet, de l'œuf à l'adulte émergent, cette somme ou constante de chaleur est spécifique et propre à chaque espèce. Cette méthode est connue sous le nom « d'accumulation degrés-jours » (ADJ) ou de façon plus fine, si on travaille sur une portion du cycle biologique « accumulation degrés-heures » (ADH) (Greenberg et Kunich 2002, Amendt *et al.* 2004, 2007). La relation qui lie le taux de développement de l'insecte à la température ambiante peut être représentée par une courbe sigmoïde (Figure 2).

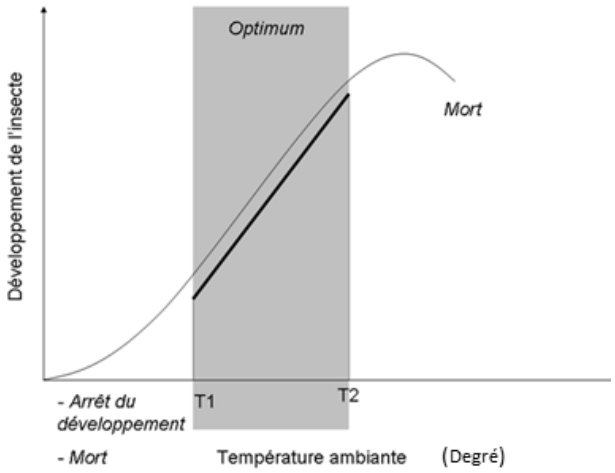

Figure 2. Relation curvilinéaire liant la température ambiante au taux de développement de l'insecte (adapté de Higley et Haskell 2001, Wyss et Cherix 2006).

Cependant, dans une gamme de températures optimales (comprises entre T1 et T2), cette relation est directement proportionnelle à la température ambiante (relation linéaire). Cette relation linaire ne se vérifie plus pour des températures extrêmes, celles-ci peuvent inhiber (léthargie) ou stopper la croissance des insectes. La limite inférieure de température est appelée température seuil minimum, cette valeur est également propre à chaque espèce (Greenberg et Kunich 2002, Amendt *et al.* 2004, 2007). Le principe est donc pour l'entomologiste de calculer précisément le moment des premières pontes de Diptères en «remontant dans le temps» afin d'obtenir la somme total de températures nécessaire au développement de l'espèce. Pour ce faire, le matériel entomologique vivant (œufs, larves, pupes) doit être placé en élevage contrôlé (incubateur thermostatisé) jusqu'à l'émergence des adultes (identification taxonomique certaine). Connaissant la date exacte d'émergence, la ou les espèces mises en élevage (constante de chaleur et température seuil inférieure), les températures moyennes journalières de l'incubateur (températures constantes) et celles ayant régnés sur la scène du décès (moyenne journalière sur 24h), l'entomologiste possède toutes les données nécessaires pour calculer le jour de la ponte et donc la date supposée du décès. Un exemple de calcul est repris à la figure 3.

- **Cas des températures constantes**

La température moyenne journalière est de 18°C et les deux espèces identifiées sont *Calliphora vicina*, cette espèce à une constance de chaleur équivalente 388° et une température seuil inférieure de 2°C. La deuxième espèce est *Lucilia sericata* qui a une constante de chaleur établie à 207° et une température seuil inférieure de 9°C.

Combien de jours durent le cycle de développement à 18°C de ces deux espèces ?

Température effective*

C. vicina : 388 = (18°-2°) * X
X = 388/ 16
X = 24 jours

L. sericata : 207 = (18°-9°) * X
X = 207/9
X = 23 jours

La durée du cycle de développement à 18°C est de 24 jours pour *Calliphora vicina* tandis qu'elle est de 23 jours pour *Lucilia sericata*.

* *Température effective* : différence entre la T° ambiante moyenne (sur 24h) subie par les stades de développement et la température seuil inférieur de développement

- **Cas des températures variables**

Connaissant les différents paramètres repris ci-dessous, l'entomologiste va calculer le jour des pontes.

→ (a) la constante de chaleur (propre à chaque espèce)
→ (b) le seuil inférieur de croissance (propre à chaque espèce)
→ (c) les T° ayant régné les jours précédents la découverte du cadavre
→ (d) les T° effectives avant la découverte du corps sont calculées (c)-(b)
→ (e) les T° effectives subies par les larves et pupes durant leur élevage en laboratoire jusqu'à leur émergence, T° constantes.

En pratique, on utilise un tableur dans lequel on encode tous ces paramètres [(a), (b), (c), (d), (e)] (tableau complet du calcul en annexe).

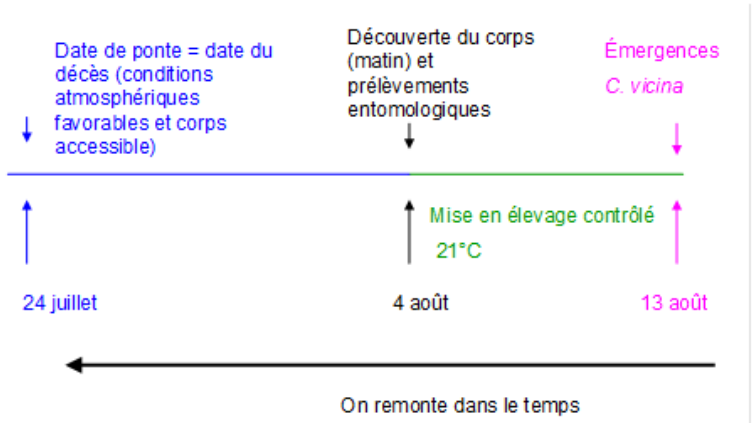

Figure 3. Exemple chiffré de calcul d'un IPM avec la méthode des ADJ (d'après Wyss et Cherix 2006).

Ces méthodes sont recommandées par les experts pour fournir une estimation aussi précise que possible de l'IPM en se basant sur des échantillons vivants (Haskell *et al.* 1997, Wyss et Cherix 2006). De nombreuses publications, basées sur des expérimentations en laboratoire, indiquent les constantes de chaleur propres aux principales espèces d'intérêt forensique sous forme de tables ou index (Kamal 1958, Marchenko 1988, Greenberg 1991, Anderson 2001,

Marchenko 2001, Amendt *et al.* 2007). Mais compte tenu des variations qui peuvent exister entre les populations d'une même espèce, il peut être opportun d'établir ses propres tables en fonction des températures régionales les plus représentatives (Wyss et Cherix 2006, Amendt *et al.* 2007).

Le jour des pontes correspond en principe au jour du décès auquel on ajoute une marge d'erreur de plus ou moins 24h pour autant que les conditions climatiques leur soient favorables (absence de pluie, luminosité adéquate, température suffisante) et que le cadavre leur soit accessible (Wyss et Cherix 2006). Les mouches ne pondent pas durant la nuit, elles ont une activité diurne (Nuorteva 1977, Haskell *et al.* 1997, Amendt *et al.* 2008). Cependant des études récentes ont montré que sous certaines conditions, on pouvait observer un phénomène d'oviposition nocturne (Baldridge *et al.* 2006, Amendt *et al.* 2008). Il s'agira toujours d'un intervalle post-mortem minimum, la victime étant déjà décédée au moment des pontes, sauf dans certains cas exceptionnels de myiases antemortem.

Kit de prélèvements entomologiques pour la Belgique

L'institut de criminalistique et de criminologie (INCC-NICC, Bruxelles) a développé un kit de prélèvements entomologiques. Celui-ci contient tout le matériel nécessaire (pinces, fioles, etc.) ainsi que les protocoles nécessaires à l'échantillonnage optimal des insectes sur la scène du décès et à l'autopsie, il est opérationnel depuis 2004. Les prélèvements peuvent être effectués par le Laboratoire Microtraces de l'INCC ou les techniciens de scène de crime des Laboratoires de la Police Scientifique et Technique (LPTS).

4.2. Intervalle post-mortem « moyen» et « long »

4.2.1. Les escouades successives d'insectes

C'est aux travaux de Mégnin et à son ouvrage intitulé « La faune des cadavres » (Mégnin 1894) que l'on doit la schématisation de la colonisation du cadavre en huit vagues successives d'arthropodes nécrophages sur les corps en plein air et de quatre escouades pour le corps inhumés. Cette théorie associe à chaque stade de décomposition du corps une espèce ou un groupe d'espèces d'arthropodes (Campobasso *et al.* 2001).

a. Corps à l'air libre

Les principaux insectes et acariens se succédant sur un cadavre sont repris dans le Tableau 2, basés sur les escouades de Mégnin (Mégnin 1894, Smith 1986) et revues par le docteur M. Leclercq (Leclercq 1978, Leclercq et Verstraeten 1992, Benecke et Leclercq 1999, Wyss et Cherix 2006):

- **Première escouade**: elle apparaît immédiatement après la mort sur le cadavre frais alors qu'aucune odeur n'est encore perceptible pour l'homme. Il s'agit surtout de Calliphoridae avec les genres *Calliphora* (*Calliphora vicina* Robineau-Desvoidy, *Calliphora vomitoria* L.), *Protophormia* (*Protophormia terraenovae* Robineau-Desvoidy), *Lucilia*, *Phormia* et de Muscidae représentées par les genres *Musca* (*Musca domestica* L.) et *Muscina* (*Muscina stabulans* Fall.).

Les mouches du genre *Calliphora* sont de grosses mouches (4 à 16 mm) avec des couleurs métalliques bleues (mouche bleue de la viande: *Calliphora vomitoria* et *Calliphora vicina*) ou vertes (*Lucilia* spp.). Elles recherchent les cadavres frais pour y pondre leurs œufs, une dizaine à plusieurs centaines sont déposées par femelle (Anderson 2001, Amendt *et al.* 2004, Gennard 2007).

Dans nos régions, nous avons trouvé au cours des expertises entomologiques du Docteur Marcel Leclercq les espèces suivantes: *Calliphora vicina*, *C. vomitaria* (les plus fréquentes), puis *Phormia regina*, *Protophormia terraenovae*, *Lucilia caesar*, *L. illustris*, *L. richardsi*, *L. sericata*.

Les mouches du genre *Musca* sont de couleur terne (grisâtre) avec une taille comprise entre 2 et 18 mm.

- **Deuxième escouade**: elle fait son apparition dès que l'odeur cadavérique ammoniacale se fait sentir (entre 48 et 72 heures post-mortem). Ce sont principalement des mouches Sarcophagidae et des Calliphoridae (genres *Lucilia*, *Cynomya*, *Chrysomia*). *Cynomya mortuorum* se rencontre plutôt sur les petits cadavres animaux et est assez rares sur les cadavres humains (Wyss et Cherix 2006).

Les sarcophagidae sont des mouches trapues qui ont une taille comprise entre 2 et 22 mm de longueur, elles se reconnaissent aisément par la présence d'un motif à damiers (bandes ou des tâches grisées) sur le thorax et par l'absence de coloration métallique (Wyss et Cherix 2006).

- **Troisième escouade**: elle arrive sur le cadavre au moment du rancissement des graisses (fermentation butyrique) qui dégage des acides gras volatils dont l'acide butyrique. Cette escouade est composée de Coléoptères, surtout des Dermestes, et des Lépidoptères du genre *Aglossa*.

Les Dermestidae sont des Coléoptères de petite taille (2 à 12 mm); les adultes ont un corps arrondi, recouvert de poils ou d'écailles de couleur variées et formant des dessins caractéristiques (Wyss et Cherix 2006). Leurs larves sont cylindro-coniques et couvertes de poils.

- **Quatrième escouade**: elle colonise le substrat au moment de la fermentation des matières protéiques (fermentation caséique), elle attire donc les mêmes insectes que le fromage fermenté. On rencontre des Diptères (Piophilidae, Fanniidae) et des Coléoptères appartenant à la famille des Cleridae (genres *Necrobia* et *Corynetes*). Cette escouade correspond également à l'écoulement des liquides putrides qui attirent d'autres Diptères tels que des Drosophilidae et des Sepsidae.

Les Piophilidae sont des Diptères de petite taille (2,5 à 6 mm) de couleur sombre mate ou brillante (Wyss et Cherix 2006). Les larves de piophilides ont un comportement caractéristique, on parle de larves sauteuses.

- **Cinquième escouade**: elle apparaît lorsque le cadavre est au stade de la fermentation ammoniacale qui succède aux deux précédentes fermentations. Les liquides putrides s'évaporent et d'autres Diptères font leur apparition (Phoridae, Muscidae avec le genre *Ophyra*). De nombreux Coléoptères sont également présents, des Silphidae, des Histeridae (*Hister* et *Saprinus*) et des Nitidulidae.

Les Phoridae sont des Diptères de petite taille (1,5 à 6 mm) de couleur brune, noire ou jaune (Wyss et Cherix 2006). Contrairement à beaucoup d'autres espèces de Diptères, les Phoridae sont capables de coloniser les corps inhumés (Leclercq et Verstraeten 1993, Bourel *et al.* 2004).

- **Sixième escouade**: elle va achever d'absorber tous les liquides dont le cadavre reste imprégné et contribuer à sa dessiccation. Cette escouade est constituée de nombreux acariens et est présente de 5 à 10 mois après le décès sur la dépouille.

- **Septième escouade**: elle fait son apparition sur le cadavre lorsque celui-ci est complètement desséché (à partir de 8 mois approximativement). On rencontre de nombreux Dermestes (*Attagenus pellio, Dermestes maculatus, Dermestes lardarius*) et des Lépidoptères (*Aglossa* et *Tineola*) qui se nourrissent habituellement de matières animales sèches (fourrure, laine, etc.).

- **Huitième escouade** : lorsque la mort est ancienne (de 1 à 3 ans), cette escouade vient nettoyer les débris organiques laissés par les escouades précédentes. Il s'agit essentiellement de petits Coléoptères appartenant aux familles des Tenebrionidae (genre *Tenebrio*) et des Ptinidae (genre *Ptinus*).

D'autres scientifiques ont modélisé l'arrivée des insectes sur le cadavre et ont dénombré moins d'escouades que Mégnin. Par exemple, en Italie Porta (1929) dénombre cinq escouades tandis que Fuller (1934), un australien, en dénombre trois (Leclercq 1978).

Tableau 2. Les travailleurs de la mort se succédant sur un corps à l'air libre (Leclercq 1978).

Escouades	Insectes et Acariens	Etat du corps	Intervalle postmortem approximatif
1ère escouade			
Diptères	Calliphoridae *Calliphora vicina* *Calliphora vomitoria* *Lucilia* spp. Muscidae *Musca domestica* *Musca autumnalis* *Muscina stabulans*	Frais et récent (variable selon la saison, les températures	
2ème escouade			3 premiers mois
Diptères	Calliphoridae *Calliphora vicina* *Calliphora vomitoria* *Lucilia* spp. *Cynomyia mortuorum* Sarcophagidae	Odeur développée	
3ème escouade			
Coléoptères Lépidoptères	Dermestidae Aglossa sp.	Graisse rance	
4ème escouade			
Diptères	Piophilidae Sepsidae Drosophilidae Fanniidae Sphaeroceridae Syrphidae	Après la fermentation butyrique et caséique	3 à 6 mois
Coléoptères	Cleridae *Necrobia* sp.		
5ème escouade			
Diptères	Muscidae *Hydrotaea* sp. Phoridae	Fermentation ammoniacale Évaporation des liquides sanieux	4 à 8 mois
Coléoptères	Silphidae Histeridae		
6ème escouade			6 à 12 mois
	Acariens		
7ème escouade		Complètement sec	
Coléoptères	Dermestidae *Dermestes maculatus* *Attagenus pellio*		1 à 3 ans
Lépidoptères	Tineidae		
8ème escouade			
Coléoptères	Ptinidae Tenebrionidae		3 ans et plus

b. Corps inhumés

Les cadavres inhumés de façon légale (cimetières) ou directement enfouis dans le sol (dissimulation) peuvent également subir des dégradations liées à l'activité des insectes, majoritairement des Diptères Phoridae (Turner et Wilshire 1999, Dewaele *et al.* 2000). Mais on retrouve également des Coléoptères de la famille des Rhizophagidae (*Rhizophagus parallelicollis*), fréquemment rencontrés avec les phorides dans les herbes des cimetières. Quatre espèces de Phoridae sont fréquemment observées sur des cadavres humains en Europe:

la mouche des cercueils *Conicera tibialis* SCHMITZ («*coffin fly*»), *Triphleba hyalinata* MEIGEN, *Megaselia rufipes* MEIGEN et *M. scalaris* LOEW (Benecke et Leclercq 1999, Dewaele *et al.* 2002).

La profondeur d'enfouissement et/ou la protection du corps par un cercueil sont des facteurs limitants mais n'empêchent en rien la colonisation post-mortem du corps. Des expériences réalisées sur des carcasses de porc inhumées ou lors d'exhumations de cercueils sur requêtes judiciaires ont montré la présence de Leptoceridae (Trichoptères), de Diptères Sphaeroceridae, Psychodidae, Muscidae et Calliphoridae. On peut également observer des Hyménoptères (fourmis), des Coléoptères appartenant aux familles des Staphylinidae (*Aleochera* sp.), des Histeridae, des Leiodidae et des Silphidae (Payne *et al.* 1968, Vanlaerhoven et Anderson 1999, Bourel *et al.* 2004). Historiquement, Mégnin avait décrit quatre vagues d'Arthropodes se succédant sur les corps inhumés qu'il appela la faune des tombeaux (Mégnin 1894, Leclercq 1978).

Tableau 3. Les travailleurs de la mort se succédant sur un corps inhumés (Mégnin 1894, Leclercq 1978).

Escouades	Insectes et Acariens	Etat du corps	Intervalle postmortem approximatif
1ère escouade			
	Calliphoridae *Calliphora sp.*		
Diptères	Muscidae *Muscina stabulans*		
2ème escouade			
	Muscidae *Hydrotaea (Ophyra)*		
Diptères			
3ème escouade			
Diptères	Phoridae *Conicera* sp.		1 an
4ème escouade			
	Rhizophagidae Staphylinidae		
Coléoptères			2 ans

4.2.2. Limitations de la datation à long terme

L'extrême variabilité des processus biochimiques qui régissent la décomposition d'un corps empêche d'observer les escouades décrites par Mégnin comme une hypothèse strictement reproductible en toutes circonstances (Leclercq 1978, Wyss et Cherix 2006). En effet, le

nombre d'escouades, leur succession et leur composition spécifique (espèces sarcosaprophages) sont fortement remis en question par de nombreuses études entomologiques de terrain concernant la colonisation des cadavres par les insectes. Certaines escouades peuvent coexister, se chevaucher, d'autres peuvent être absentes, mais le plus souvent, elles ne suivent pas l'ordre chronologique établi dans la théorie classique des escouades. Des espèces réputées appartenir aux 4ème ou 5ème escouades peuvent se retrouver très tôt sur le cadavre (Wyss et Cherix 2006).

Paramètres influençant la décomposition d'un corps

La décomposition d'un corps et sa colonisation par les insectes sont deux phénomènes intimement liés et sont influencés par de nombreux facteurs intrinsèques et extrinsèques au cadavre (Campobasso *et al.* 2001).

Les facteurs intrinsèques, directement liés à la personne décédée, sont l'âge, la masse corporelle, la cause du décès (drogues, infection), l'hygiène corporelle, l'intégrité du corps (blessures, plaies) et la présence de vêtements (Campobasso *et al.* 2001). Parmi les facteurs externes, le facteur le plus important est la zone biogéoclimatique incluant l'habitat, la végétation, le type de sol et les conditions météorologiques (température, vent, humidité atmosphérique) du lieu où se situe la dépouille (Anderson, 2001, Campobasso *et al.* 2001).

D'autres paramètres ont une influence significative sur la vitesse de décomposition d'un corps ; on peut citer la saison, l'emplacement du corps (ombragé vs. ensoleillé) et enfin l'accessibilité du corps aux organismes vivants qu'ils soient mammifères (animaux domestiques ou sauvages) ou insectes (Anderson 2001, Campobasso *et al.* 2001).

De plus, les processus de décomposition et la faune des cadavres varient fortement en fonction du lieu où se trouve le cadavre. Les corps enterrés ou submergés subiront des évolutions différentes des corps laissés à l'air libre (Anderson, 2001).

Parmi tous ces facteurs, deux sont prépondérants dans la décomposition d'un corps, il s'agit de la température ambiante et de l'accessibilité du corps aux insectes (Campobasso *et al.* 2001).

5. Les corps immergés ou partiellement immergés

La faune des cadavres immergés est différente de celle des cadavres retrouvés à l'air libre (Anderson 2001). Contrairement aux écosystèmes terrestres, il n'y a pas d'espèces aquatiques exclusivement nécrophages. Cependant, les restes humains (et/ ou animaux) attirent une faune

aquatique diversifiée: des larves d'insectes (Trichoptères, Coléoptères, Diptères etc.), des crustacés, des mollusques et des poissons (Merritt et Wallace 2001). On ne parle plus d'intervalle post-mortem mais d'intervalle post-mortem de submersion (IPMS) (Merritt et Wallace 2001). Le cadavre constitue une source de nourriture pour une large variété d'invertébrés et de poissons, c'est aussi un abri pour de nombreuses autres espèces.

Les algues peuvent également coloniser et croître sur le cadavre (Merritt et Wallace 2001). Par la suite, ces végétaux aquatiques vont attirer des herbivores.

Néanmoins, les corps partiellement immergés peuvent être colonisés par des arthropodes terrestres, notamment les Calliphoridae (Anderson 2001). L'eau limite le nombre et les espèces d'arthropodes nécrophages présents sur le corps. En effet, le tiers de la faune présente sur un cadavre à l'air libre peut être retrouvé sur les corps immergés (Leclercq 1978). Peu d'études étudiant les relations entre insectes et corps immergés existent. Pour dix études réalisées en entomologie forensique, seulement deux sont consacrées aux arthropodes aquatiques (Merritt et Wallace 2001). Une étude récente (Wallace *et al.* 2008) souligne l'importance des Trichoptères (*caddisflies*) en tant que bio-indicateur dans l'estimation de l'IPMS.

6. Les corps calcinés ou partiellement calcinés

Très peu d'études ont été publiées concernant l'influence du feu sur la colonisation post-mortem par les insectes (Anderson 2009). Cependant, ces études mettent en évidence une colonisation différentielle des corps brûlés par rapport aux corps non incendiés (Avila et Goff 1998). Les corps brûlés attirent plus précocement les insectes (craquements de l'épiderme fournissant des zones propices à l'oviposition) que ceux n'ayant pas été brûlés (Avila et Goff 1998, Anderson 2009). Toutefois, on retrouve les mêmes insectes sur les corps brûlés que sur les corps non brûlés, l'importance de la colonisation entomologique est fonction du taux d'incinération du corps (Anderson 2009). Pour que celui-ci reste attractif pour les insectes, la carbonisation doit être partielle; il doit rester sur le corps des masses musculaires humides sans coagulation totale des protéines par la chaleur. De plus, le carburant utilisé doit être volatil et rapidement éliminé sous peine de jouer le rôle de substance répulsive pour les insectes. Dans une autre optique, les traces laissées par les insectes (pupes, exuvies, etc.) sur une scène de décès incendiée peuvent encore être analysées et servir d'indicateurs post-mortem (Anderson 2005). En effet, ces éléments résistent très bien au feu (Anderson 2005).

Exemple d'expertise entomologique réalisée par le Docteur Marcel Leclercq (Leclercq 1993)

Le 6 septembre à 8 h 45, découverte dans un chemin forestier du cadavre partiellement carbonisé d'une petite fille. Un foyer éteint se situe sous le corps, la terre est neutre sans odeur de carburant. Les enquêteurs prennent des clichés photographiques très précis des lieux et du corps et ils pensent à une petite fille âgée de 10 ans, disparue le 26 août tard dans la soirée.

Les documents photographiques révèlent de nombreuses larves qui correspondent au genre *Calliphora*.

Dans le secteur concerné, il existe deux espèces de ces mouches bleues: *Calliphora vicina* et *C. vomitaria* dont la biologie est comparable. Habituellement ces mouches ont une activité diurne à partir de 12-13° centigrade. La station météorologique la plus proche de l'endroit de la découverte du cadavre nous a fourni le relevé des températures maximales, minimales et moyennes du 26 août au 7 septembre, l'intervalle entre la disparition de la victime et la découverte du cadavre est de 10 jours plus environ 10 heures.

Le calcul des températures moyennes en degrés Celsius révèle:

Périodes	Minimales	Maximales	Moyennes
26 au 31 août	12,91	19,38	16,15
1 au 6 sept.	12,73	19,10	15,91
1 au 7 sept.	13,05	20,08	16,57

Retenons qu'à partir du 6 septembre dans l'avant-midi, le cadavre a été évacué, puis placé en chambre froide (ralentissement de la croissance des larves).

L'autopsie a eu lieu le 7 septembre. La durée de vie des larves ne peut pas logiquement être estimée à moins de 9 jours et celle de l'incubation des œufs correspond à environ 24 heures et l'arrivée des premières mouches sur le cadavre paraît bien être limitée au 27 août, après le lever du jour.

La datation de la mort de la victime peut logiquement être estimée durant la dizaine d'heures qui ont suivi son enlèvement le 26 août vers 23 heures.

Il faut nécessairement en déduire la durée de la succession des événements: enlèvement de la victime, manipulation, transport à une vingtaine de kilomètres de distance, déroulement de l'affaire criminelle, carbonisation partielle (preuve de l'utilisation d'un carburant très volatil et

de courte durée, comme par exemple l'essence), puis le feu éteint qui a permis d'attirer des mouches (*Calliphora* sp.).

La datation de la mort de la victime peut ainsi être fixée très tard dans la soirée du 26 août ou éventuellement dès le 27 août dans un délai très court.

L'enquête judiciaire a suivi son cours et la conclusion de cette expertise entomologique a été confirmée par les aveux de l'accusé reconnu coupable d'homicide volontaire avec préméditation et condamné à la plus lourde peine en cours d'assises (Leclercq 1993).

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I.2.3. Forensic entomology investigations from Doctor Marcel Leclercq (1924 -2008†): a review of cases from 1969 to 2005

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Référence - Dekeirsschieter J.R.,Verheggen F.J., Frederickx C., Boxho P., Haubruge E. Forensic entomology investigations from Doctor Marcel Leclercq (1924 -2008†): a review of cases from 1969 to 2005. *Accepted for publication in Journal of Medical Entomology*.

Abstract - Doctor Marcel Leclercq was a pioneer in the field of forensic entomology. He has provided his knowledge of insect biology to many forensic cases, and most of them have found the way to publication. Most of the papers he has written were focused on individual cases, and despite the abundance of entomoforensic investigations he conducted, no synthesis has been published. This paper summarizes 36 years of forensic entomological investigations in temperate Europe, mainly in Belgium. Leclercq's work includes 132 entomological cases involving 141 human corpses found in various death scenes. Under certain conditions, insect specimens found on death scene can provide information on when (postmortem interval estimation), where and how a person died. More or less one hundred insect species associated to a dead body have been identified by Marcel Leclercq.

Key words - Forensic entomology, Forensic science, Doctor Marcel Leclercq, cadavers, human remains, case studies, Europe

1. Introduction

Forensic entomology is a branch of the forensic sciences which studies insects and other arthropods (*e.g.* mites) in a medico-legal context (Nuorteva 1977, Hall 1990, 2001, Greenberg 1991, Amendt *et al.* 2004, 2007, Wallman 2004, Gennard 2007). The main goal of forensic entomology is to establish the postmortem interval (PMI), *i.e* the elapsed time since death (Nuorteva 1977, Greenberg 1991, Amendt *et al.* 2004, 2007, Lefebvre and Gaudry 2009). Many forensic papers (Catts 1992, Byrd and Castner 2001, Amendt *et al.* 2004, Sharanowski *et al.* 2008, Vanin and Gherardi 2009) highlight the scarcity of information about the phenology, distribution, biology and ecology of forensic species. Nevertheless, there is not a lack of real forensic cases but a poverty of synthesis (Vanin and Gherardi 2009). Some European case reports are described for Italy (Turchetto *et al.* 2001, Vanin *et al.* 2008, 2009), Spain (Arnaldos *et al.* 2005), Germany (Benecke 1998, Amendt *et al.* 2000, Shroeder *et al.* 2003), France (Bourel *et al.* 2001), Great Britain (Disney and Manlove 2005), and Finland (Pohjoismaki *et al.* 2010). However, these reports summarize individual forensic cases or a few numbers of cases. A wider review of such cases from one biogeoclimatic country can introduce more understanding concerning the type of entomological evidence that occurs (Sukontason *et al.* 2007). In Belgium, some case reports of Doctor Marcel Leclercq have been already published (Leclercq and Leclercq 1948, Leclercq and Quinet 1949, Leclercq and Watrin 1973, Leclercq and Tinant-Dubois 1973, Leclercq 1983, 1988a,b, 1999, Leclercq and Brahy 1985, Leclercq and Verstraeten 1988, 1993, Leclercq and Vaillant 1988, Dewaele *et al.* 1999, Leclercq 2009), but were never summarized in a review document. This paper reviews 132 forensic entomological investigations of the medical Doctor Marcel Leclercq in Belgium and France over a period of 36 years. Marcel Leclercq was among the first to use forensic entomology for the determination of the postmortem interval in Europe and in French-speaking regions (Leclercq 1978, Benecke 2001, Amendt *et al.* 2004, Leclercq 2009). Smith (Smith 1986) dedicated his well-known book of forensic entomology to Doctor Marcel Leclercq and Pekka Nuorteva (Finland); both were considered as pioneer in forensic entomology (Smith 1986, Benecke 2001, Amendt *et al.* 2004). The bibliographical directory of Marcel Leclercq is constituted by 353 publications (Leclercq 2009) as author or co-author, he was a renowned dipterist. His bibliography concerns various branch of entomology (in bracket, the number of published reports) such as the dipterology (266), acarology (8), human and veterinary parasitology (36), myiasis (22), forensic entomology (39) and insect bites (bees, wasps) (37) (Leclercq 2009). His first forensic entomology case dates from 1947 and

concerned the murder of a child hidden behind a fireplace (Leclercq and Leclercq 1948; Leclercq 2009). However, Dr Marcel Leclercq has written his first case reports in 1969. The Doctor Marcel Leclercq worked for the courts until 2005.

2. General description of the cases

A total of 132 forensic entomological cases were realized by the Doctor Marcel Leclercq for the Belgian and French courts from 1969 to 2005 (Table 4). These cases concern 141 human corpses generally infested with insect specimens and one mammalian carcass for the case 79. There were no insect specimens for the cases 1, 2, 6, 79, 83 and 94. The cases concern sixty-five males (45.8%), forty-eight females (33.8%), two babies (1.4%) without any mention of the sex and twenty six corpses (18.3%) for which the sex was not specified in the forensic entomological report (17.9%). Three cases (cases 5, 48 and 72) concern pair of male and female and two cases include more than one corpse; it is about the cases 55 and 89 with respectively three distinct human remains of women and six distinct human remains without any mentions of the sex. Most corpses were at active decay stage (59 cases) or at advanced decay (51 cases); the decay stage (including active and advanced decay) represents 77.5% of the studied cadavers. Nineteen corpses were skeletonized and four were at the "fresh" decomposition stage. For nine corpses (representing 6.1% of the total of corpses), the decomposition stage was not specified in the forensic entomological report. In most forensic entomological case reports, the causes of death were not mentioned.

Table 4. Characteristics of forensic cases realized by the medical doctor Marcel Leclercq in Belgium and France. According to the case, the season when the corpse was discovered (the month is indicated by roman numerals), the sex of the cadaver(s), number of cadaver(s) concerned by the forensic case and when it is mentioned in the report, the cause of death, the stage of decomposition and its humidity (dry or wet corpse), the complete location of the cadaver(s): municipality, country, area, location of the body (indoor or outdoor). For the case 55, the letters a, b and c indicate three different human remains packed in plastic bag (postmortem dismembered). Estimated postmortem interval and the method used to establish the PMI are resumed in the last two columns.

Case	Season & month	Sexe	Number of cadavers	Area	Location (in/out)	Cause of death	Stage of decomposition	Humidity of cadavers (dry/wet)	PMI estimation	Method of PMI estimation
Case 1	Fall - X	female	1	forest	outdoor	not specified	skeletonized	dry	No insect	-
Case 2	Winter - I	male	1	forest	outdoor	not specified	not specified	not specified	No insect	-
Case 3	Fall - XI	male	1	forest	outdoor	not specified	active decay	wet	8 weeks	Pioneer species
Case 4	Fall - X	male	1	urban	outdoor	not specified	active decay	wet	5 – 6 days	Pioneer species
Case 5	Summer - VIII	male + female	2	urban	indoor	crime	active decay	wet	< 3 days	Pioneer species
Case 6	Fall - X	female	1	suburban	outdoor	not specified	not specified	not specified	No insect (linked to case 60)	
Case 7	Fall - IX	male	1	field	outdoor	not specified	active decay	wet	3 days	Pioneer species
Case 8	Spring - V	male	1	forest	outdoor	not specified	advanced decay	dry	13 -17 weeks	Chronological succession
Case 9	Fall - XII	male	1	forest	outdoor	not specified	advanced decay	wet	No PMI estimation – faunistic inventory	
Case 10	Fall - X	female	1	urban	outdoor	not specified	active decay	wet	10 days	Pioneer species
Case 11	Fall - X	male	1	meadow	outdoor	gunshot wound	active decay	wet	3 weeks	Pioneer species
Case 12	Summer - VIII	male	1	urban	indoor	crime	advanced decay	wet	7 days	Pioneer species
Case 13	Summer - VIII	male	1	forest	outdoor	not specified	advanced decay	wet	No PMI estimation – faunistic inventory	
Case 14	Summer -VIII	male	1	not specified	not specified	not specified	active decay	wet	2 weeks	Pioneer species
Case 15	Summer - VII	male	1	not specified	not specified	not specified	active decay	wet	2 weeks	Pioneer species
Case16	Summer - VI	male	1	urban	indoor	not specified	active decay	wet	3 weeks	Pioneer species
Case 17	Spring - V	female	1	urban	indoor	strangulation	active decay	wet	No PMI estimation – faunistic inventory	
Case18	Winter - I	male	1	forest	outdoor	not specified	active decay	wet	13-14 weeks	Pioneer species
Case 19	Winter - II	male	1	suburban	outdoor	not specified	not specified	not specified	No necrophagous/necrophilous insects	
Case 20	Fall - XII	not specified	1	urban	indoor	not specified	advanced decay	not specified	28-30 weeks	Chronological succession
Case 21	Summer - VIII	not specified	1	not specified	outdoor	not specified	active decay	wet	1.5-2 days	Pioneer species
Case 22	Summer - VII	male	1	urban	not specified	stabbing	active decay	wet	6 days	Pioneer species
Case 23	Summer - VI	not specified	1	suburban	outdoor	not specified	skeletonized	dry	1 year	Chronological succession
Case 24	Spring - V	male	1	forest	outdoor	hanging	active decay	wet	13 -14 days	Pioneer species
Case 25	Spring - III	male	1	suburban	indoor	not specified	advanced decay	not specified	> 1 year	Chronological succession
Case 26	Winter - I	male	1	forest	outdoor	not specified	advanced decay	wet	12-16 weeks	Chronological succession
Case 27	Fall - XII	male	1	urban	indoor	not specified	advanced decay	dry	28-32 weeks	Chronological succession
Case 28	Fall - XI	male	1	urban	indoor	not specified	active decay	wet	2 weeks	Pioneer species
Case 29	Fall - X	not specified	1	meadow	outdoor	not specified	active decay	wet	3 weeks	Pioneer species
Case 30	Summer - IX	baby	1	not specified	outdoor	not specified	active decay	wet	2 weeks	Pioneer species
Case 31	Summer - IX	not specified	1	suburban	indoor	not specified	skeletonized	dry	> 1 year	Chronological succession
Case 32	Summer - VII	male	1	urban	indoor	not specified	active decay	wet	4 days	Pioneer species
Case 33	Summer - VII	female	1	forest	outdoor	not specified	advanced decay	wet	9 weeks	Chronological succession
Case 34	Summer - VII	male	1	not specified	outdoor	stabbing	active decay	wet	1 day	Pioneer species
Case 35	Summer - VII	male	1	not specified	not specified	not specified	skeletonized	dry	44 weeks	Chronological succession
Case 36	Spring - VI	female	1	suburban	outdoor	not specified	fresh	wet	30 hours	Pioneer species
Case 37	Spring - VI	male	1	urban	indoor	not specified	active decay	wet	5-7 days	Pioneer species
Case 38	Spring - IV	female	1	urban	indoor	not specified	active decay	wet	6 days	Pioneer species

Case 39	Summer	male	1	not specified	indoor	not specified	advanced decay	dry	No PMI estimation – faunistic inventory	
Case 40	Fall - XII	female	1	urban	indoor	strangulation	skeletonized	dry	40 weeks	Chronological succession
Case 41	Fall - XI	male	1	urban	indoor	not specified	not specified	not specified	< 1 week	Pioneer species
Case 42	Fall - X	female	1	urban	indoor	not specified	active decay	wet	No estimation of PMI	
Case 43	Fall - X	male	1	urban	indoor	not specified	active decay	wet	15 days	Pioneer species
Case 44	Summer - IX	male	1	urban	indoor	not specified	advanced decay	wet	3 weeks	Pioneer species
Case 45	Summer - VII	male	1	urban	indoor	not specified	active decay	wet	3 days	Pioneer species
Case 46	Spring - V	female	1	suburban	outdoor	not specified	advanced decay	wet	No estimation of PMI	
Case 47	Summer - VIII	female	1	field	outdoor	not specified	active decay	wet	17-18 days	Pioneer species
Case 48	Spring - V	Male + female	2	urban	indoor	not specified	active decay	wet	10-12 days	Pioneer species
Case 49	Fall - X	female	1	urban	indoor	stabbing	advanced decay	dry	24-28 weeks	Chronological succession
Case 50	Winter - XII	male	1	suburban	indoor	natural cause	advanced decay	dry	> 1 year	Chronological succession
Case 51	Summer - VIII	male	1	forest	outdoor	not specified	advanced decay	wet	12 weeks	Chronological succession
Case 52	Summer - VIII	female	1	field	outdoor	not specified	active decay	wet	4-5 days	Pioneer species
Case 53	Spring - VI	female	1	urban	not specified	not specified	active decay	wet	5 days	Pioneer species
Case 54	Winter - III	female	1	urban	indoor	not specified	advanced decay	dry	No PMI estimation – faunistic inventory	
Case 55a	Spring - IV	female	3	urban	outdoor	not specified	active decay	wet	3 weeks	Pioneer species
55b							active decay	wet	5-6 days	Pioneer species
55c							advanced decay	wet	> 6 months	Pioneer species
Case 56	Fall - X	female	1	forest	outdoor	not specified	active decay	wet	4 weeks	Pioneer species
Case 57	Spring - V	not specified	1	urban	indoor	not specified	advanced decay	wet	35-40 days	Pioneer species
Case 58	Fall - XII	male	1	forest	outdoor	not specified	skeletonized	dry	5-6 months	Chronological succession
Case 59	Fall - XI	male	1	not specified	outdoor	not specified	advanced decay	dry	> 1 year	Chronological succession
Case 60	Fall - X	female	1	suburban	outdoor	not specified	active decay	wet	5 days	Pioneer species
Case 61	Fall - X	male	1	forest	outdoor	not specified	advanced decay	wet	7 months	Chronological succession
Case 62	Summer - VII	not specified	1	forest	outdoor	not specified	skeletonized	dry	3-4 weeks	Chronological succession
Case 63	Spring - VI	male	1	suburban	indoor	not specified	skeletonized	dry	11 weeks	Chronological succession
Case 64	Spring - IV	not specified	1	not specified	not specified	not specified	advanced decay	dry	1 year	Chronological succession
Case 65	Spring - IV	not specified	1	suburban	outdoor	not specified	skeletonized	dry	36-40 weeks	Chronological succession
Case 66	Spring - IV	female	1	field	outdoor	not specified	skeletonized	dry	> 1 year	Chronological succession
Case 67	Spring - III	female	1	suburban	outdoor	natural cause	skeletonized	dry	33 weeks	Chronological succession
Case 68	Fall - XI	female	1	forest	outdoor	not specified	active decay	wet	5-6 days	Pioneer species
Case 69	Spring - IV	female	1	urban	indoor	not specified	skeletonized	dry	1 year	Chronological succession
Case 70	Fall - X	male	1	forest	outdoor	not specified	active decay	wet	18-23 days	Pioneer species
Case 71	Summer - IX	female	1	suburban	outdoor	not specified	active decay	wet	11 days	Pioneer species
Case 72	Summer - VII	Male + female	2	forest	outdoor	not specified	active decay	wet	6-7 days	Pioneer species
Case 73	Winter - II	not specified	1	urban	indoor	strangulation	skeletonized	dry	> 1 year	Chronological succession
Case 74	Winter - II	female	1	forest	outdoor	stabbing	skeletonized	dry	> 1 year	Chronological succession
Case 75	Winter - II	male	1	suburban	indoor	not specified	advanced decay	wet	101-113 days	Pioneer species
Case 76	Fall - X	female	1	forest	outdoor	not specified	advanced decay	dry	75 days	Chronological succession
Case 77	Fall - IX	male	1	urban	indoor	not specified	advanced decay	dry	7 months	Chronological succession

Case 78	Fall - IX	female	1	urban	indoor	not specified	advanced decay	not specified	7 weeks	Chronological succession
Case 79	Summer	mammal	1	not specified	outdoor	not specified	not specified	not specified		No insect
Case 80	Spring - VI	not specified	1	suburban	outdoor	not specified	advanced decay	wet	3 weeks	Chronological succession
Case 81	Spring - VI	male	1	suburban	outdoor	stabbing	fresh	wet	< 1 day	Pioneer species
Case 82	Spring - V	not specified	1	not specified	not specified	not specified	advanced decay	not specified	≤ 2 days	Pioneer species
Case 83	Spring - V	not specified	1	suburban	indoor	not specified	not specified	not specified		No insect
Case 84	Winter - I	female	1	suburban	outdoor	not specified	advanced decay	wet	74 days	Chronological succession
Case 85	Fall - XI	male	1	forest	outdoor	not specified	active decay	wet	11 weeks	Chronological succession
Case 86	Fall - X	not specified	1	urban	indoor	not specified	active decay	wet	3-4 weeks	Pioneer species
Case 87	Summer - IX	female	1	forest	outdoor	not specified	active decay	wet	9 days	Pioneer species
Case 88	Summer - VIII	female	1	suburban	outdoor	crime	active decay	wet	11 days	Pioneer species
Case 89	Summer - VIII	not specified	6	urban	indoor	crime	advanced decay	wet	15 weeks	Chronological succession
Case 90	Spring - IV	male	1	urban	indoor	not specified	advanced decay	wet	7 weeks	Pioneer species
Case 91	Winter - I	not specified	1	forest	outdoor	not specified	advanced decay	wet	11-13 weeks	Pioneer species
Case 92	Fall - XII	not specified	1	urban	indoor	not specified	skeletonized	dry	No PMI estimation	
Case 93	Fall - X	female	1	suburban	outdoor	not specified	not specified	not specified	No PMI estimation	
Case 94	Fall	female	1	not specified	not specified	not specified	active decay	wet		No insect
Case 95	Fall - XI	female	1	not specified	outdoor	not specified	active decay	wet	5-6 weeks	Pioneer species
Case 96	Winter - I	male	1	suburban	indoor	not specified	active decay	wet	4 weeks	Pioneer species
Case 97	Fall - XI	male	1	urban	indoor	not specified	active decay	wet	23-28 days	Pioneer species
Case 98	Spring - IV	female	1	forest	outdoor	not specified	skeletonized	dry	> 6 months	Chronological succession
Case 99	Fall - XI	male	1	not specified	outdoor	not specified	active decay	wet	3 weeks	Pioneer species
Case 100	Fall - XI	male	1	urban	indoor	natural cause	advanced decay	wet	6 weeks	Pioneer species
Case 101	Fall - XI	male	1	urban	indoor	stabbing	active decay	wet	10-11 days	Pioneer species
Case 102	Spring - V	male	1	forest	outdoor	not specified	advanced decay	wet	2-3 weeks	Pioneer species
Case 103	Fall - XI	male	1	urban	indoor	not specified	advanced decay	wet	2 months	Chronological succession
Case 104	Winter - II	male	1	suburban	outdoor	stabbing	fresh	wet	15 days	Chronological succession
Case 105	Winter - I	male	1	forest	outdoor	hanging	advanced decay	wet	2 months	Chronological succession
Case 106	Fall - XII	female	1	suburban	outdoor	not specified	advanced decay	not specified	No PMI estimation – faunistic inventory	
Case 107	Summer - VII	male	1	urban	indoor	not specified	advanced decay	dry	10 weeks	Chronological succession
Case 108	Winter - II	not specified	1	not specified	not specified	drowning	not specified	not specified	No necrophilous/necrophagous insects	
Case 109	Fall - IX	male	1	forest	outdoor	not specified	advanced decay	wet	3 weeks	Pioneer species
Case 110	Fall - X	male	1	forest	outdoor	hanging	skeletonized	dry	11-12 weeks	Chronological succession
Case 111	Summer - VIII	male	1	field	outdoor	not specified	active decay	wet	2-3 days	Pioneer species
Case 112	Spring - IV	male	1	urban	indoor	not specified	advanced decay	dry	No PMI estimation – faunistic inventory	
Case 113	Fall - XII	male	1	forest	outdoor	not specified	advanced decay	not specified	10 weeks	Chronological succession
Case 114	Fall - X	male	1	forest	outdoor	not specified	advanced decay	not specified	10 months	Chronological succession
Case 115	Summer - IX	female	1	urban	indoor	stabbing	active decay	wet	10 days	Pioneer species
Case 116	Summer - VIII	female	1	urban	indoor	not specified	active decay	wet	10-15 days	Pioneer species
Case 117	Summer - VIII	male	1	forest	outdoor	not specified	active decay	wet	4-6 weeks	Pioneer species
Case 118	Spring - VI	male	1	forest	outdoor	not specified	fresh	wet	<1 day (13-19h)	Pioneer species

Case 119	Spring - VI	female	1	suburban	outdoor	not specified	advanced decay	wet	< 3 months	Chronological succession
Case 120	Spring - IV	male	1	urban	indoor	not specified	advanced decay	dry	6-8 weeks	Pioneer species
Case 121	Winter -III	male	1	forest	outdoor	hanging	advanced decay	not specified	5 months	Chronological succession
Case 122	Spring - V	female	1	urban	indoor	not specified	active decay	wet	3 weeks	Pioneer species
Case 123	Fall - X	male	1	suburban	outdoor	not specified	active decay	wet	7 days	Pioneer species
Case 124	Summer - VIII	male	1	forest	outddor	hanging	advanced decay	wet	1 month	Pioneer species
Case 125	Spring - V	baby	1	urban	indoor	not specified	advanced decay	wet	4-5 weeks	Pioneer species
Case 126	Summer -VI	male	1	forest	outdoor	not specified	active decay	wet	15 days	Pioneer species
Case 127	Spring - VI	female	1	urban	indoor	not specified	advanced decay	wet	10-15 days	Pioneer species
Case 128	Winter - III	female	1	forest	outdoor	not specified	skeletonized	dry	6 months	Chronological succession
Case 129	Winter - I	female	1	suburban	outdoor	not specified	advanced decay	dry	4-5 months	Chronological succession
Case 130	Winter - XII	female	1	forest	outdoor	not specified	active decay	wet	≈ 19 days	Pioneer species
Case 131	Fall - XII	male	1	forest	outdoor	not specified	skeletonized	dry	6-7 months	Chronological succession
Case 132	Fall - X	female	1	suburban	outdoor	strangulation	active decay	wet	3 days	Pioneer species

Only few cases have information about the cause of death, in more than 80.0% of the cases, the cause of death was not specified. Figure 4 shows the different causes of death and the important proportion of cases where the cause of death was not mentioned in the entomological report. The causes of death are various: stabbing (eight cases), hanging (five cases), strangulation (four cases), drowning (one case), gunshot wound (one case), natural causes including cancer (three cases) and in four cases the only information about death was "crime".

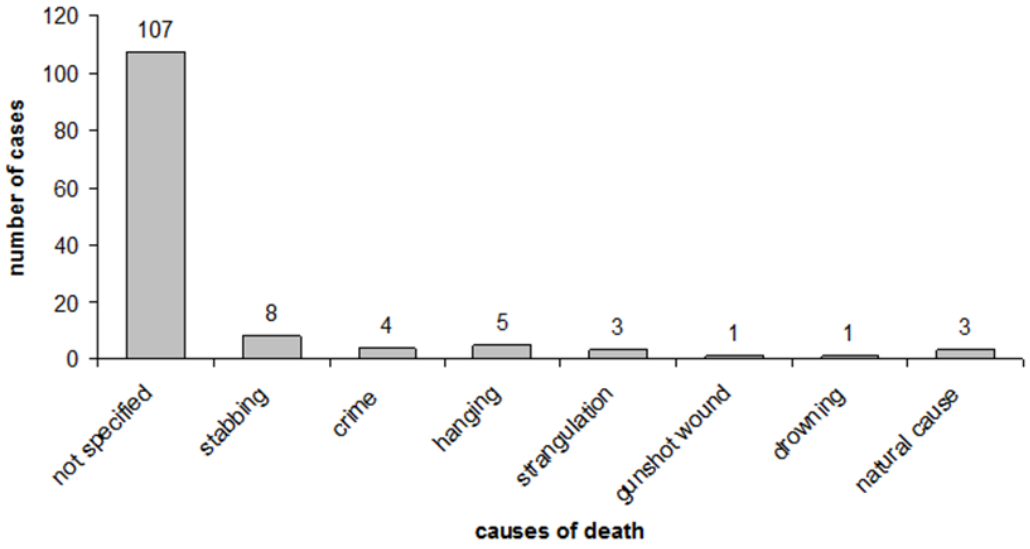

Figure 4. Number of entomoforensic cases of Doctor Marcel Leclercq according to the causes of death referenced in the entomological reports.

The forensic cases of the doctor M. Leclercq concern primarily his native country with 115 cases for Belgium and 16 cases for France. A single case did not mention the concerned country or municipality. For 23 cases the municipality was not specified in the report, 20 cases for Belgium and three cases for France. Figure 5 represents the geographical distribution in Belgium and France of forensic entomological cases, each municipality is represented only once. The Belgian municipality with the most abundant cases realized by Dr. Marcel Leclercq is Liege with 23 cases, the city of Seraing with five cases; the two following cities concern three cases: Mons and Verviers. Two cases were cited for the six following cities of Eupen, Namur, Saint-Nicolas, Saint-Vith, Walcourt and Waterloo. Other Belgian cities report only one forensic case. Concerning the French cities, only one city (Messancy) concerns more than one forensic case (two cases).

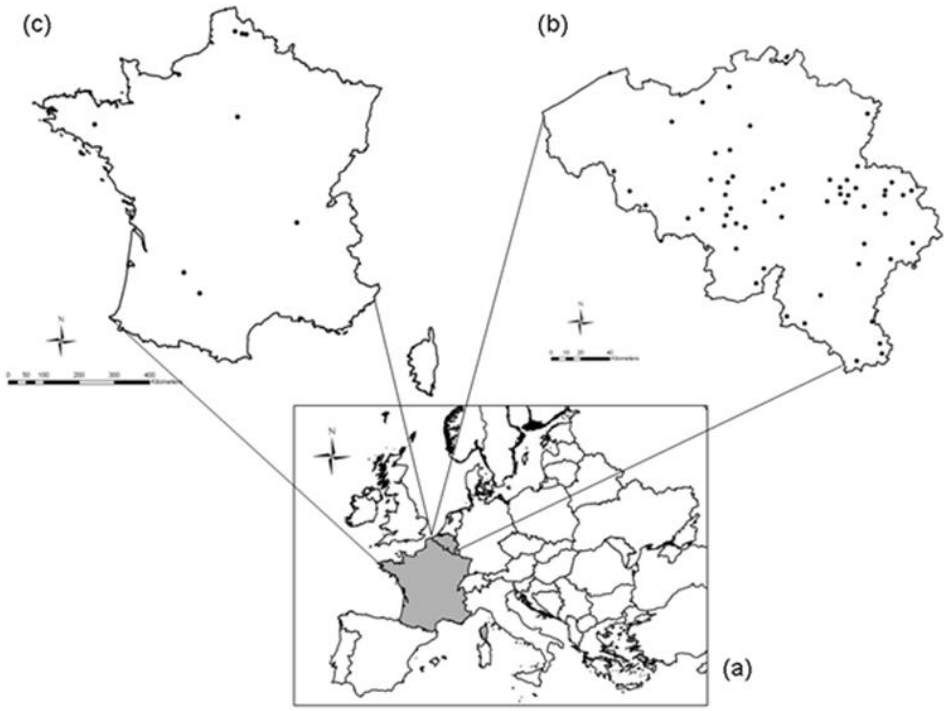

Figure 5. Geographical locations of forensic entomological cases. (a) Map of Europe with Belgium and France, forensic cases are located on enlarged maps of Belgium (b) and France (c). Each municipality concerned by the entomoforensic case(s) is represented by a single point.

Thirty six cases were located in forest (Table 5), seven cases in "open" biotopes such as fields or meadows, 44 cases in urban sites and 26 cases in suburban areas. For 19 cases, the location was not specified. On 132 cases, 63 were found outdoor and 45 inside housing (indoor); the indoor location concern only urban and suburban sites. The location (in- or out-) were not specified for 14 cases. Autumn was the season with the highest number of reported entomological cases (46 cases). Spring and summer had respectively 32 and 35 cases. Nineteen cases were discovered in winter.

Table 5. Repartition by season and biotope of the 132 forensic entomological cases of Marcel Leclercq.

Season	Location									Total
	Forest	Field-Meadow	Urban indoor	Urban outdoor	Urban not specified	Suburban indoor	Suburban outdoor	Not specified outdoor	Not specified	
Spring	5	1	12	1	1	3	7	0	2	**32**
Summer	7	3	9	0	2	1	3	4	6	**35**
Fall	15	3	15	2	0	0	5	4	2	**46**
Winter	9	0	2	0	0	3	4	0	1	**19**
Total	**36**	7	**38**	**3**	**3**	7	**19**	**8**	**11**	**132**

3. Insect evidences

Table 6 lists the insect species identified by Marcel Leclercq. 104 different species are listed: 59 Coleopteran species, 41 Dipteran species, one species of Hymenoptera (Braconidae), one

species of Dermaptera (*Forficula auricularis*), one species of Heteroptera (*Nepa cinerea*) and one species of Lepidoptera (*Tineola sp.*). Figure 6 shows the number of cases by insect order. Diptera is the most abundant insect order, reported in 122 different cases for a total of 257 occurrences. The second most important order is Coleoptera with 54 different cases for a total of 142 occurrences. Lepidopterans are referenced in two cases (27 and 49). While other orders are listed in only one case (Dermaptera: case 15, Heteroptera: case 107, Hemiptera: case 23).

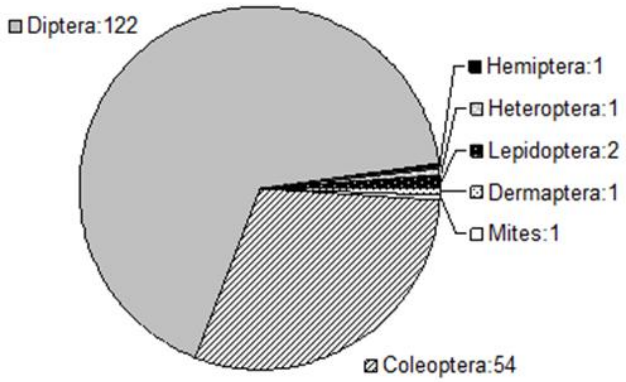

Figure 6. Distribution of the forensic entomology cases by insect group and mites.

3.1. Diptera

Eleven families of Diptera have been encountered (Table 6): Calliphoridae (nine species and two taxonomic identifications at the genus level: *Calliphora* sp. and *Lucilia* sp.), Fanniidae (three species and one at the genus level: *Fannia* sp.), Heleomyzidae (two species), Muscidae (four species and one at the genus level: *Hydrotaea* sp.), Phoridae (four species), Piophilidae (one species and one at the genus level: *Piophila* sp.), Sarcophagidae (one species and one at the genus level: *Sarcophaga* sp.), Sphaeroceridae (two species and one at the genus level: *Sphaerocera* sp.), Syrphidae (one taxonomic identification at the genus level: *Brachyopa* sp.), Trichoceridae (one species: *Trichocera hiemalis*) and Drosophilidae (one identification at the family level). Taxonomic identifications were made at the family level for Calliphoridae within three cases, Drosophilidae (case 33), Heleomyzidae (within ten cases: 31, 51, 54, 55, 58, 59, 61, 63, 74, 82), Muscidae (cases 58, 67 and 108), Phoridae (within eight cases), Piophilidae (cases 23, 62 and 98), Sarcophagidae (cases 33, 67 and 110), and Sphaeroceridae (cases 54, 55 and 58). Figure 7 shows the relative abundance of cases according to Dipteran families. There are 257 occurrences of Diptera in all forensic cases. Among these families, Calliphoridae (blowflies) is the most abundant cited family (47.1%).

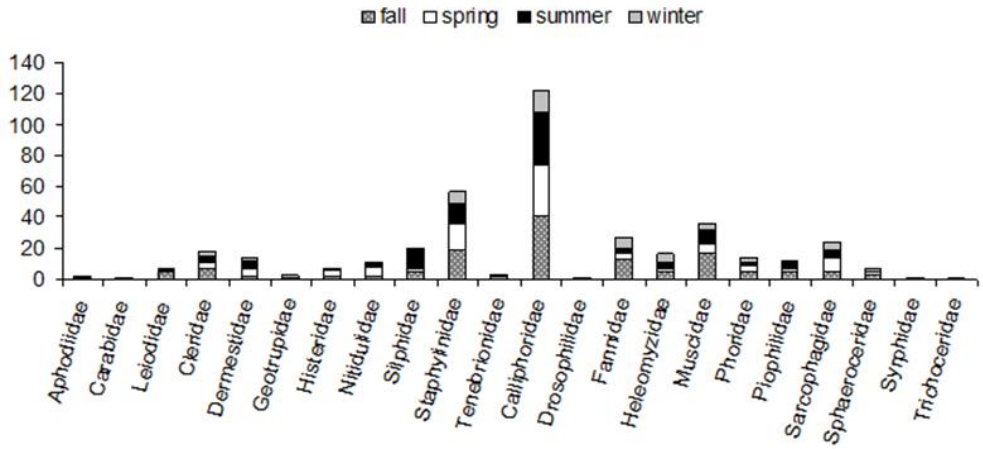

Figure 7. Abundance of forensic cases according to Dipteran and Coleopteran families (and season).

Blowflies were found in 102 different cases of Dr Leclercq (Table 7). In most of these cases (86.3% of blowflies cases), one species of blowflies is listed but in some cases, there are two (11.8%) or three (2.0%) species present simultaneously. Marcel Leclercq identified the following blowflies: *Calliphora vicina*, *Calliphora vomitoria*, *Lucilia caesar*, *Lucilia sericata*, *Lucilia richardsi*, *Lucilia illustris*, *Chrysomya albiceps*, *Phormia regina* and *Protophormia terranovae*. The taxonomic identification at the species level was not made in cases 103, 105 and 110; identifications are at the family level (Calliphoridae). For the cases 58, 69, 73, 82, 87, 115 and 121, identifications were made at the genus level, *Calliphora* sp., except for the case 69 with the genus *Lucilia* sp. The *Calliphora* spp. are the most cited blowflies with respectively 53 and 31 occurrences for *C. vicina* and *C. vomitoria*. *P. regina* (black blowfly) and *P. terraenovae* are reported each in four cases. Luciliinae (green bottle flies) are listed in 19 cases; *L. sericata* and *L. caesar* have the most abundant citations with respectively eleven and five occurrences, while *L. richardsi* and *L. illustris* have been cited only once. *C. albiceps* has also been cited only once. Blowfly developmental milestones identified by Marcel Leclercq are listed in Table 7. Immature stages (including eggs, larval and pupal stages) of Calliphoridae were the predominant developmental stages: 134 occurrences (20 for the eggs, 87 for the larval stage, 27 alive pupae). There are 27 occurrences of empty pupae and sometimes (9 occurrences) both types of pupae (alive and empty pupae) were found. Concerning the adult specimens found in the Leclercq cases, 26 occurrences concern alive specimens and 11 for dead specimens.

Muscidae is the second most important family with 13.6% of the reported individuals. The most abundant muscid fly is *Hydrotaea capensis* which is cited in twelve cases. *Musca domestica* is listed in seven forensic cases and *Muscina stabulans* is referenced within five cases.

Fanniidae is the third family (10.1%); *Fannia scalaris* is the most abundant species within twelve cases. Concerning flesh flies (Sarcophagidae), many specimens were identified at the family level or genus and they contribute to 8.9% of the Diptera occurrences. Phoridae (scuttle flies), Piophilidae (skipper flies) and Heleomyzidae are cited within less than 7.0% of the cases with respectively 5.4%, 4.7% and 6.2% of cases. Three families are cited in only one case which represents 0.4%: Drosophilidae, Syrphidae and Trichoceridae.

Table 6. List of species identified in the entomoforensic cases of Marcel Leclercq classified by order and family. A column "Referenced on carrion" indicates if the species is referenced on human corpses or animal carcasses if not the mention "not referenced" is indicated, that means that there is no relevant information in forensic literature about this species; the sign (-) indicates that the comparison with literature is not relevant due to the lack of specific identification (genus or family taxon). Each entomoforensic case reports of Doctor Marcel Leclercq is listed by species and developmental milestones identified in the case (normal = mature stage, underlined = death mature stage, italic = immature stage, double underlined = empty pupa or nymph, bold = both mature and immature stages). The complete species name was made with the database Fauna Europaea.

Number	Species	Godfather	Referenced on carrion human remains or animal carcasses	Leclercq cases
	Coleoptera			
	Aphodiidae			
1	*Aphodius consputus*	Creutzer, 1799	Not referenced	55
2	*Aphodius sp.*	Illiger, 1798	-	62
	Carabidae			
3	*Limodromus assimilis*	Paykull, 1790	Not referenced	61
	Cleridae			
4	*Necrobia ruficollis*	Fabricius, 1775	[42]	35, 58
5	*Necrobia rufipes*	De Geer, 1775	[14, 18, 42-47,56]	58, 61, 69, **76**, 89, 129, 130
6	*Necrobia violacea*	Linnaeus, 1758	[42, 44-48, 54]	25, 35, 46, **58**, 61, 62, 64, 110
	Dermestidae			
7	*Attagenus pellio*	Linneaus, 1758	[42]	35
8	*Dermestes frischi*	Kugelann, 1792	[17, 42-43, 46-47, 49-50, 54, 56]	89
9	*Dermestes ater*	Degeer, 1774	Not referenced	84, 89, 127
10	*Dermestes lardarius*	Linné, 1758	[44, 48, 50, 54]	**64**, **112**
11	*Dermestes maculatus*	Degeer, 1774	[17, 44, 50-51]	*64*, 69
12	*Dermestes undulatus*	Brahm, 1790	[45-46, 49-50]	**39**, **40**
13	*Dermestes sp.*		-	73, *76*, *107*
	Geotrupidae			
14	*Anoplotrupes stercorosus*	Scriba, 1791	[42, 47-48]	8
15	*Geotrupes stercorarius*	Linné, 1758	[41, 54-55]	33, 62
	Histeridae			33
16	*Margarinotus brunneus*	Fabricius, 1775	[42, 46-47, 56, 70]	110
17	*Margarinotus striola*	Salhberg, 1819	[42, 47-48, 54]	82
18	*Saprinus semistriatus*	Scriba , 1790	[42-43, 47-49, 54]	46, 53, 119
	Leiodidae			

19	*Catops morio*	Fabricius, 1792	[48]	110, 113
20	*Catops picipes*	Fabricius, 1787	[45]	82
21	*Catops tristis*	Panzer, 1794	[41, 48, 54-55]	76, 82
22	*Catops sp.*		-	26, 113
	Monotomidae			
23	*Rhizophagus parallelocollis*	Gyllenhal, 1827	[41]	54
	Nitidulidae			
24	*Amphotis marginata*	Fabricius, 1781	-	**82**
25	*Omosita discoidea*	Fabricius, 1775	[42, 45, 47-49, 54]	23, 46, 55, 62, 63, 64
26	*Soronia punctatissima*	Illiger, 1794	-	46, 58, 61, 62
	Silphidae			
27	*Necrodes littoralis*	Linnaeus, 1758	[41-42, 44, 47, 54]	11, 12, 13, 14, 15, **46**, 51, 53, 70, 117
28	*Nicrophorus humator*	G.A. Olivier, 1790	[42, 45, 47- 49, 52, 54-55]	51, 55, 117
29	*Nicrophorus investigator*	Zetterstedt, 1824	[42, 48, 52, 54]	117
30	*Nicrophorus vespilloides*	Herbst, 1784	[42, 45, 47-48, 52, 54-55]	51
31	*Nicrophorus sp.*		-	*58*, *61*
32	*Thanatophilus sinuatus*	Fabricius, 1775	[47, 54, 56]	15
	Undetermined species		-	*26*, *33*
	Staphylinidae			
33	*Aleochera curtula*	Goeze, 1777	[41-42, 48-50]	46
34	*Aleochera lata*	Gravenhorst, 1802	[46]	46
35	*Aleochera ruficornis*	Gravenhorst, 1802	Not referenced	119
36	*Aleochera sp.*		-	51
37	*Amischa soror*	Kraatz, 1856	Not referenced	98
38	*Anotylus sculpturatus*	Gravenhorst, 1806	[42]	62
39	*Atheta sp.*		[54-55]	98
40	*Coprophilus striatulus*	Fabricius, 1792	-	114
41	*Creophilus maxillosus*	Linné, 1758	[14, 41-42, 44-49, 54,56]	14, 15, 46, 72, 119
42	*Omalium rivulare*	Paykull, 1789	[42, 48-49,54-55]	63, 98, 113, 128, 129, 130
43	*Ontholestes murinus*	Linné, 1758	[42, 46, 48, 54]	46
44	*Oxytelus sp.*		-	51
45	*Philonthus carbonarius*	Gravenhorst, 1802	Not referenced	110
46	*Philonthus cephalotes*	Gravenhorst, 1802	Not referenced	61
47	*Philonthus decorus*	Gravenhorst, 1802	Not referenced	119
48	*Philonthus marginatus*	Strom, 1768	[42, 49, 54]	62

49	*Philonthus sordidus*	Gravenhorst, 1802	[54]	110
50	*Philonthus splendens*	Fabricius, 1792	[42, 54]	62
51	*Philonthus succicola*	Thomson, 1860	[42, 48]	76
52	*Philonthus tenuicornis*	Mulsant & Rey, 1853	[42]	46
53	*Philonthus sp.*		-	51, 58
54	*Proteinus ovalis*	Stephens 1834	Not referenced	130
55	*Tachinus humeralis*	Gravenhorst, 1802	[42, 54]	76
56	*Quedius lateralis*	Gravenhorst, 1802	Not referenced	91
57	*Quedius mesomelimus*	Marsham, 1802	[48]	61, 110
58	*Quedius sp.*		-	58
	Undetermined species		-	11, *12*, *18*, 19, 20, 23, 25, 33, 35, 50, *61*, *64*, 66, 67, *78*, 82, *85*, *95*, **119**
	Tenebrionidae			
59	*Tenebrio molitor*	Linné, 1758	[41]	27, **39**, 40
	Dermaptera			
	Forficulidae			
60	*Forficula auricularis*	Linné, 1758	[43, 44]	15
	Diptera			
	Calliphoridae			
61	*Calliphora vicina*	Robineau-Desvoidy, 1830	[14, 17, 20, 23, 41-45, 47, 49, 54, 56-57]	*3*, *4*, *5*, *16*, *18*, *21*, *22*, *24*, *27*, *28*, *30*, *32*, *33*, *35*, **38**, 39, 40, 41, 48, 49, *53*, *55*, **57**, 63, 64, 69, *71*, *75*, *76*, *77*, *78*, *80*, *81*, *84*, *85*, **86**, *89*, *90*, *91*, *92*, *96*, **97**, *100*, **101**, *107*, *111*, *112*, *113*, 119, *120*, **122**, *125*
62	*Calliphora vomitoria*	Linnaeus, 1758	[14,18, 20, 23, 41-45, 47, 49, 54, 56-57]	*9*, *11*, **14**, **15**, *17*, *29*, **34**, **43**, *46*, *48*, **56**, *60*, 61, **68**, **70**, **72**, *80*, *85*, *93*, *95*, *99*, *102*, *109*, *117*, *118*, *123*, *124*, *126*, *128*, **129**, *132*
63	*Calliphora sp.*		-	58, *73*, *82*, *87*, *115*, 121
64	*Chrysomya albiceps*	Wiedemann, 1819	[17, 40, 43-45, 57-58]	*88*
65	*Lucilia caesar*	Linnaeus, 1758	[18, 41-42, 44-45, 47, 49, 54, 56, 57]	**10**, 15, **36**, *48*, 105
66	*Lucilia illustris*	Meigen, 1826	[41-42, 45, 54, 57]	**30**
67	*Lucilia richardsi*	Collin, 1926	[40, 56]	**86**
68	*Lucilia sericata*	Meigen, 1826	[14, 20, 23, 40, 44-45, 54, 56-57]	**7**, **12**, *16*, **17**, 34, 35, 37, *45*, 112, *116*, *126*
69	*Lucilia sp.*		-	69
70	*Phormia regina*	Meigen, 1826	[20, 40, 42, 44, 47, 54]	*17*, **37**, *44*, *47*
71	*Protophormia terraenovae*	Robineau-Desvoidy, 1830	[18, 23, 40, 44, 57]	*52*, 58, *130*, *131*
	Undetermined species		-	*103*, *105*, *110*
	Drosophilidae			

	Undetermined species		-	
	Fanniidae			
72	*Fannia canicularis*	Linnaeus, 1761	[40, 44-45, 47, 49, 54, 57, 70]	*9*, *12*, 20, 27, 35, *54*
73	*Fannia manicata*	Meigen, 1826	[21, 40, 44, 47, 54, 70]	*46*
74	*Fannia scalaris*	Fabricius, 1794	[21, 40, 44, 49, 54, 57, 70]	*9*, *20*, 27, 35, 54, 55, 67, 76, 107, 128, 129, 131
75	*Fannia sp.*		-	25,49,50,58,59,74,85
	Heleomyzidae			
76	*Neoleria inscripta*	Meigen, 1830	[41, 45, 54]	51
77	*Tephrochlamys flavipes*	Zetterstedt, 1838		20,26,91,121,128
	Undetermined species			31,51,54,55,58,59,61,63,74,82
	Muscidae			
78	*Hydrotaea capensis*	Wiedemann, 1818	[21, 41-42, 45, 56]	3,15,20,27,35,39,40,61,64,77,78,107
79	*Hydrotaea dentipes*	Fabricius, 1805	[41-42, 45, 47, 49, 54]	15,124
80	*Hydrotaea sp.*	Robineau-Desvoidy, 1830	-	25,49,50,74,89,131
81	*Musca domestica*	Linnaeus, 1758	[17, 40, 43, 45, 54, 56-57]	34,69,73,77,78,89,92
82	*Muscina stabulans*	Fallén, 1817	[14, 17-18, 20, 42-44, 54]	13,20,76,112,119
	Undetermined species		-	58,67,110
	Phoridae			
83	*Conicera tibialis*	schmitz, 1925	[21, 41]	50,64
84	*Megaselia rufipes*	Meigen, 1804	[21, 45]	63
85	*Megaselia scalaris*	Loew, 1866	[44]	42
86	*Triphleba hyalinata*	Meigen, 1830	[21]	65,130
	Undetermined species		-	12,50,77,78,89,90,103,119
	Piophilidae			
87	*Piophila casei*	Linnaeus, 1758	[18, 40, 45]	106
88	*Piophila sp.*		-	51,55,58,59,64,128,131
	Undetermined species		-	23,62,98
	Sarcophagidae			
89	*Sarcophaga argyrostoma*	Robineau-Desvoidy, 1830	[18, 41, 43, 45, 57]	115,117
90	*Sarcophaga sp.*		-	18,25,27,35,37,55,58,64,69,73,78,80,89,112,129
	Undetermined species		-	33,67,110
	Sphaeroceridae			
91	*Copromyza pallifrons*	Fallen, 1820	Not referenced	104
92	*Leptocera nigra*	Olivier, 1813	[71]	115
100	*Sphaerocera sp.*	Latreille,1804	[54]	26,85

	Undetermined species		-	54,55,58
	Syrphidae			
101	*Brachyopa sp.*		-	114
	Trichoceridae			
102	*Trichocera hiemalis*	De Geer, 1776	Not referenced	26
	Heteroptera			
	Nepidae			
103	*Nepa cinerea*	Linné, 1758	-	108
	Hymenoptera			
	Braconidae			
	Undetermined species		-	
	Lepidoptera			
	Tineidae			
104	*Tineola sp.*		-	27
	Undetermined species		-	49

Table 7. List of blowflies (Diptera, Calliphoridae) identified by Marcel Leclercq in his entomoforensic investigations. Developmental milestones are divided into four stages: eggs, larva, pupa and adults. Sign (-) indicates that the developmental stage was not found in the forensic cases, (E) indicates that eggs were found in the case; (L) for the larval stage and 1, 2, 3 refer respectively to the first, second, third instar and (PF) for postfeeding larva; pupal stage is indicated by (P) for not hatched pupa and (P) for empty pupa; adults are indicated by (A) for alive adult specimens and (A) for dead specimens. For the case 55, the letters a and b indicate two different locations of the human remains.

Case	Calliphoridae	Developmental stages				Case	Calliphoridae	Developmental stages				Case	Calliphoridae	Developmental stages			
		Eggs	Larva	Pupa	Adults			Eggs	Larva	Pupa	Adults			Eggs	Larva	Pupa	Adults
3	*C. vicina*	-	L3	-	-	46	*C. vomitoria*	-	L3	-	-	90	*C. vicina*	-	L3	-	-
4	*C. vicina*	-	L1, L2	-	-	47	*P.regina*	-	L1, L2, L3	-	-	91	*C. vicina*	-	L	-	-
5	*C. vicina*	-	L2	-	-	48	*C. vicina*	-	L3	P	-	92	*C. vicina*	-	-	P, ♀	-
7	*L. sericata*	E	L2	-	A		*L. caesar*	-	L3	-	-	93	*C. vomitoria*	-	L3	-	-
9	*C. vomitoria*	-	L3	-	-		*C. vomitoria*	-	L3	-	-	95	*C. vomitoria*	-	L2, L3, PF	P	-
10	*L. caesar*	E	L1, L2, L3	-	A	49	*C. vicina*	-	-	♀	-		*C. vicina*	-	L3	-	-
11	*C. vomitoria*	-	L1, L2, L3	-		52	*P. terranovae*	-	L2	-	-	96	*C. vicina*	E	L1, L2, L3, PF	P, ♀	A
12	*L. sericata*	E	L1, L2, L3	-	A	53	*C. vicina*	E	L1, L2, L3	-	-	98	*C. vomitoria*	-	L3	-	-
14	*C. vomitoria*	-	L2, L3	-	A	55a	*C. vicina*	E	L1, L2, L3	-	-	99	*C. vicina*	-	L	-	♂
15	*L. caesar*	-	-	-	A	55b	*C. vicina*	E	L1, L2	-	-	100	*C. vicina*	-	L	-	A
	C. vomitoria	-	L1, L2, L3	-	A	56	*C. vomitoria*	-	L1, L2, L3	-	A	101	*C. vomitoria*	-	L3, PF	P	-
16	*C. vicina*	-	L1, L2, L3	p	-	57	*C. vicina*	-	L3	P, ♀	A	102	Calliphoridae	-	-	P, ♀	A
	L. sericata	-	L2, L3	-	-	58	*Calliphora* sp.	-	-	♀	-	104	Calliphoridae	-	L	P	-
17	*L. sericata*	-	-	P , ♀	A		*P. terranovae*	-	-	♀	-	105	*L. caesar*	-	-	-	♂
	C. vomitoria	E	-	-	♂	60	*C. vomitoria*	E	L1, L2	-	-	106	*C. vicina*	-	L	P	-
	P. regina	-	L2, L3	P	-	61	*C. vomitoria*	-	-	♀	-	108	*C. vomitoria*	-	L3	-	-
18	*C. vicina*	-	L3	-	-	63	*C. vicina*	-	-	♀	-	110	Calliphoridae	-	-	♀	-
21	*C. vicina*	E	L1, L2	-	-	64	*C. vicina*	-	-	♀	-	111	*C. vicina*	-	L	-	-
22	*C. vicina*	-	L1, L2, L3	-	-	68	*C. vomitoria*	-	L	P	♂	112	*C. vicina*	-	-	♀	-
24	*C. vicina*	E	L1, L2, L3	-	-	69	*C. vicina*	-	-	-	♂		*L. sericata*	-	-	-	-
27	*C. vicina*	-	-	♀	♂		*Lucilia* sp.	-	-	♀	♂	113	*C. vicina*	-	-	P	-
28	*C. vicina*	-	L3	-	-	70	*C. vomitoria*	E	L1, L2, L3	-	A	115	*Calliphora* sp.	-	L1, L2, L3	-	-
29	*C. vomitoria*	E	L1, L2, L3	-	-	71	*C. vicina*	-	L2, L3	-	-	116	*L. sericata*	-	L	-	-
30	*C. vicina*	-	L1, L2, L3	-	-	72	*C. vomitoria*	E	L1, L2, L3	-	A	117	*C. vomitoria*	-	L	-	-
	L. illustris	-	L2, L3	-	A	73	*Calliphora* sp.	-	-	P, ♀	-	118	*C. vomitoria*	E	-	-	-
32	*C. vicina*	-	L1	-	-	75	*C. vicina*	-	L3, PF	P	-	119	*C. vicina*	-	-	♀	-
33	*C. vicina*	-	-	♀	-	76	*C. vicina*	-	L3		-	120	*C. vicina*	-	L3	P	-
34	*C. vomitoria*	E	L2	-	A	77	*C. vicina*	-	-	♀	-	121	*Calliphora* sp.	-	-	♀	-
	L. sericata	-	-	-	A	78	*C. vicina*	-	L2	♀	-	122	*C. vicina*	-	L3	P	♂
35	*C. vicina*	-	-	♀	-	80	*C. vicina*	-	L	-	-	123	*C. vomitoria*	-	L3	-	-
	L. sericata	-	-	♀	♂		*C. vomitoria*	-	L	-	-	124	*C. vomitoria*	-	L	P	-
36	*L. caesar*	E	L1	-	A	81	*C. vicina*	E	L1	-	-	125	*C. vicina*	-	-	P	-
37	*P. regina*	-	L1, L2, L3	P	A	82	*Calliphora* sp.	-		P	-	126	*L. sericata*	-	L	-	-
	L. sericata	-		-	♂	84	*C. vicina*	-	L	-	-		*C. vomitoria*	-	L	-	-
38	*C. vicina*	-	L1, L2, L3	-	A	85	*C. vicina*	-	L	-	-	128	*C. vomitoria*	-	L	-	-
39	*C. vicina*	-	-	-	A		*C. vomitoria*	-	L	-	-	129	*C. vomitoria*	-	L3	P, ♀	-
40	*C. vicina*	-	-	♀	♂	86	*L. richardsi*	-	L	P	A	130	*P. terranovae*	-	L3	-	-
41	*C. vicina*	-	-	-	A		*C. vicina*	E	L	P	A	131	*P. terranovae*	-	L3	-	-
43	*C. vomitoria*	-	L1, L2, L3	-	A	87	*Calliphora* sp.	-	L	-	-	132	*C. vomitoria*	-	L3	-	-
44	*P. regina*	-	L1, L2, L3, PF	P, ♀	-	88	*C. albiceps*	-	L	-	-						
45	*L. sericata*	E	L1, L2, L3	-	-	89	*C. vicina*	-	-	P, ♀	A						

3.2. Coleoptera

Twelve families of Coleoptera have been reported (Table 6): Aphodiidae (one species and one taxonomic identification at the genus level: *Aphodius* sp.), Carabidae (on species), Cleridae (three species), Dermestidae (six species and one identification at the genus level: *Dermestes* sp.), Geotrupidae (two species), Histeridae (three species), Leiodidae (three species and one at the genus level: *Catops* sp.), Monotomidae (one species), Nitidulidae (three species), Silphidae (five species and one at the genus level: *Nicrophorus* sp.), Staphylinidae (twenty-one species and five identification at the genus level: *Aleochera* sp., *Atheta* sp., *Oxytelus* sp., *Philonthus* sp., *Quedius* sp.), Tenebrionidae (one species).

Taxonomic identifications were made at the family level for Histeridae (case 33), Silphidae (cases 26 and 33) and for Staphylinidae within nineteen cases. Figure 7 shows the relative abundance of cases according to Coleopteran families. There are 142 occurrences of Coleoptera in all forensic cases. Rove beetles (Staphylinidae) are the most abundant Coleopteran family with 39.4% of the total Coleopteran occurrences. The most abundant species of rove beetles are *Omalium rivulare* and *Creophillus maxillosus*. The genus *Philonthus* is also frequently found on corpses, they were identified in ten cases. Nevertheless, many Staphylinidae were identified at the family level and may include many different species. Silphidae is the second family which represents 14.1%. *Necrodes littoralis* is the most abundant species; this species is cited in ten forensic cases. The third family is Cleridae (checkered beetles) (12.7%) and fourthly, larder beetles (Dermestidae) represented 9.9% of the cases, with mainly *Dermestes* spp. Two species of checkered beetles are most frequent: *Necrobia rufipes* and *Necrobia violaceae*; *Necrobia ruficollis* is cited in two cases. Sap beetles (Nitidulidae) are cited in 7.7% of Coleopteran cases with two species more present: *Omosita discoidea* (six cases) and *Soronia punctatissima* (four cases). Clown beetles (Histeridae) intervene in 4.2% of cases, *Saprinus semistriatus* is cited in three cases. Leiodidae are cited in 4.9% with *Catops* spp. The following families represent less than 6.0%: Geotrupidae and Tenebrionidae for 2.1%, Aphodiidae for 1.4%, Carabidae and Monotomidae in one case (0.7% each family).

4. Postmortem interval with entomological methods

In suspicious death enquiries, the main goal of entomoforensic investigations is the estimation of the postmortem interval (Nuorteva 1977, Greenberg 1991, Amendt *et al.* 2004, 2007, Lefebvre and Gaudry 2009). When a cadaver colonized by insects is discovered, two

situations could be considered for PMI estimations according to the insect specimens growing on the body (Amendt *et al.* 2007, Lefebvre and Gaudry 2009). In early postmortem period (first weeks after death), the PMI estimations are based on the age of the oldest necrophagous flies growing on the corpse (Amendt *et al.* 2007, Lefebvre and Gaudry 2009), mainly blowflies (Diptera, Calliphoridae) (Amendt *et al.* 2007) and fleshflies (Diptera, Sarcophagidae) (Leclercq 1978, Smith 1986) referred as pioneer species (Lefebvre and Gaudry 2009). This method of PMI-estimation, also referred to the minimum postmortem interval, is often used in forensic entomology (Amendt *et al.* 2007, Lefebvre and Gaudry 2009). In late postmortem period (corpses in advanced decay), the estimation of PMI is based on the composition of the chronological succession patterns in the arthropod colonization process (Smith 1986, Amendt *et al.* 2007, Lefebvre and Gaudry 2009).

4.1. PMI-estimations

Doctor Leclercq was a pioneer in the field of forensic entomology with the use of insect biology in death enquiries. The PMI-estimations of Dr Marcel Leclercq are listed in Table 4. The entomological method (Table 4) used by M. Leclercq to estimate PMI is also indicated as “pioneer species” or “chronological succession”. "Pioneer species" refers to the age determination of juvenile stages of blowflies or fleshflies whereas "chronological succession refers to the determination of carrion arthropod community. PMI-estimations are missing for several cases and Leclercq's reports do not give any information about the time elapsed since death but a faunistic list is associated with each cases.

4.2. Entomoforensic cases studied

Some forensic entomology cases studied by Doctor Leclercq were chosen to show the interest of insect in forensic investigations.

Case 127 (Leclercq 1978) - the corpse of a Caucasian woman was found in a flat on the fifth floor of an urban building on June 20th 1972. The corpse was in putrefaction and ambient temperature of the flat was 18-20°C.The entomological evidence consisted of larvae (8 and 18mm in length) and recent pupae of flesh flies. The oldest larvae and pupae were reared to adult and identified as *Sarcophaga argyrostoma* on 8 July 1972 (adult emerging date). At 25°C, the larval growth of *S. argyrostoma* lasts 8-9 days (Leclercq 1978). However in this

case (18°C-20°C), the larval growth must have been slower. Dr M. Leclercq concluded that the death of this woman had occurred about ten days but not beyond 15 days.

Case 37 (unpublished data) – the corpse of a Caucasian man was found in housing on June 2nd 2000.The ambient temperature inside housing was sharply higher than 22 °C. The entomological evidence consisted of larvae (larval stages 1, 2) and pupae of *Phormia regina* and larvae (larval stage 3) and recent pupae of flesh flies. The pupae of *P. regina* and flesh flies were separately reared to adults. The adult emerging data of *P. regina* is reported between June 6th and June 8th whereas the adult emerging data of fleshflies, identified as *Sarcophaga* sp., occurred the 16th June. Dead female adults of *Lucilia sericata* were also found on the death scene. To estimate the PMI, Dr. Marcel Leclercq used the data about the life cycle of *P. regina* (Greenberg 1991) and *Sarcophaga* sp (Leclercq 1978, Smith 1986) and climatic data. The complete life cycle of *P. regina* is of 14 days at 22°c and 12 days (+ 15hours) at 29°C [7] whereas the complete life cycle of *Sarcophaga* sp. is around three weeks at 25°C. Dr. M. Leclercq reported that the oldest stage of *P. regina* was aged of a dozen days. The hatching of these flies occurred between June 6th and June 8th, the laying took place five to seven days before the discovery of the corpse (6/2), between May 26th and May 28th. Concerning the *Sarcophaga* sp., he reported that the oldest stage was aged of seven days + 30 hours, the laying of fleshflies took place approximately one week before the discovery of the corpse (6/2), between May 26th and May 28th. Dr. M. Leclercq concluded that the death of the man probably occurred one week before his discovery on June 2nd 2000.

Case 7 (unpublished data) – the corpse of a Caucasian male was found in a corn field on September 22nd 2003 PM. The entomological evidence consisted of blowfly eggs, larval stage 1 (3.5mm body length), larval stage 2 (4.5 to 7.5 mm body length), adults (males and females) of *Lucilia sericata*. To estimate the PMI, Dr. Marcel Leclercq used the data about the life cycle of *L. sericata* (Greenberg 1991) and climatic data provided by the nearest meteorological station (temperatures) (Table 8).

Table 8. Mean temperatures provided by the nearest meteorological data station.

Date	Mean temperature	Maximal temperature	Minimal temperature
September 18	19.8 °C	28.4 °C	11.2 °C
September 19	19.8 °C	29.2 °C	10.4 °C
September 20	20.9 °C	30.2 °C	11.6 °C
September 21	20.2 °C	29.2 °C	11.4 °C
September 22	20.5 °C	26.2 °C	14.8 °C

The oldest blowfly specimens were the larval stage 2 of *L. sericata* and Marcel Leclercq concluded that these larvae were aged of three days at least. The death occurred probably at least three days before the discovery of the corpse on September 22nd, September 19th conceivably in the morning or the beginning of the afternoon.

Case 110 (Leclercq 1978) – the corpse of a Caucasian male was found hanging in a forest on October 2nd 1975. The cadaver was in advanced decay with the skull and bones at feet of the tree. The arthropod community found on the body was important and varied and consisted of empty pupae of *Calliphora* sp., *Sarcophaga* sp. and Muscidae. Numerous larvae of *Piophila* (*Stearibia*) *foveolata* were found and reared until adult emergence (October 21st). Several mature Coleoptera were also found: *Margarinotus brunneus* (syn. *Hister cadaverinus*), *Necrobia violaceae*, *Catops morio* and rove beetles (*Philonthus sordidus*, *Philonthus carbonarius*, *Quedius mesomelimus*). Numerous Acari were also found as well as some Arachnids (*Meta segmentata* Clerck). Doctor Marcel Leclercq used the meteorological data published by the Royal Meteorological Institute of Belgium during the year 1975 to investigate the insect colonization pattern on the corpse. His conclusions were based on the absence of species of the seventh and eighth squadrons that colonize desiccated corpse after one year. Marcel Leclercq excluded that the corpse was present in the forest during the winter 74-75, one year before his discovery. He approximated that the corpse may be probably at the place of his discovery around 90 to 100 days before October 2nd, in June or the beginning of July 1975. Marcel Leclercq wrote in his report that this estimate was not a rigorous limitation.

5. Discussion

In Europe, many forensic entomological studies were conducted on animal carcasses (pig, rabbit, and rat) (Bourel *et al.* 1999, Kocarek 2003, Grassberger and Frank 2004, Arnaldos *et al.* 2005, Wyss and Chérix 2006, Matuszewski *et al.* 2008, 2010, Ozdemir and Sert 2009) as surrogate human models due to ethical restraint of dealing with human cadavers. This paper synthesizes insect evidences that occur on human cadavers in Leclercq case reports from 1969

to 2005. Species that are reported from these case reports might not be only forensic indicators, because insects are the most numerous animals on earth and they live in various habitats (Benecke 1998). For example, *Nepa cinerea*, a species of water scorpion, has been found in a drowning case (case 108) but Marcel Leclercq wrote in his report that this species has no interest for forensic entomology. Nevertheless, there are more incidences of species that are regarded as forensically important in these entomoforensic cases. Species or families are mainly necrophagous, which feed on the corpse, or necrophilous, which feed on inhabitants of the corpse (necrophagous) by predation or parasitism (Smith 1986, Arnaldos *et al.* 2005). Some species are recognized as coprophagous insects such as Geotrupidae and Aphodiidae (Lefebvre and Gaudry 2009) whereas Geotrupidae can feed on decaying flesh (Smith 1986, Wyss and Chérix 2006). The predominant orders of insects were Diptera with 41 species and Coleoptera with 59 species. Among dipterans, the most abundant family was Calliphoridae with common bluebottle flies (*Calliphora* spp.) and greenbottle flies. These flies are considered as forensically important and they are often use in forensic investigations (Smith 1986, Arnaldos *et al.* 2005, Oliveira and Vasconcelos 2010, Velasquez *et al.* 2010). All species of Calliphoridae identified by Marcel Leclercq are referenced in the Belgian entomofauna. There are only two species of the *Calliphora* genus that are known in Belgium: *C. vicina* and *C. vomitoria*. *C. vicina* is the most common species found on corpses whereas *C. vomitoria* is less frequent (Wyss and Chérix 2006, Velasquez *et al.* 2010). *C vicina* is associated to all types of habitat while *C. vomitoria* is found in less anthropized habitats (Velasquez *et al.* 2010). According to Schroeder and colleagues (Shroeder *et al.* 2003), *C. vicina*, *C. vomitoria* and *Lucilia sericata* are common initial colonizers of corpses in Europe. *L. sericata* is also found in all types of habitat independently of the degree of anthropization whereas *L. caesar* is common in less anthropized habitats (Velasquez *et al.* 2010). *L. illustris* and *L. richardsi* are more often associated with open habitats (Gennard 2007). This latter species is rare (Gennard 2007). However, *L. illustris* is referenced as a wood species in Spain (Velasquez *et al.* 2010). *Protophormia terraenovae* and *Phormia regina* are Northern species, usually observed in cool European regions, and are still little found in corpses in central Europe (including Belgium) (Myskowiak and Doums 2002, Shroeder *et al.* 2003). *C. vicina* and *P. terraenovae* overwinter mainly as adults (Pohjoismaki *et al.* 2010, Velasquez *et al.* 2010). *C. albiceps*, usually found in warm European regions (e.g. Mediterranean countries), has been recently added to the Belgian necrophagous entomofauna, it was found on pig carcasses in 2003 but not in human corpses (Gosselin and Braet 2008). Muscid flies are frequently found on dead bodies (Lefebvre and Pasquerault 2004); the four species identified

by M. Leclercq (*H. capensis*, *H. dentipes*, *M. stabulans*, *M. domestica*) are referenced in the Belgian entomofauna. *Hydrotaea capensis* is sometimes found on confined bodies kept indoors for several months (Bourel *et al.* 1999). However, little biological data is available about *H. capensis* which is frequently found in human cadavers, notably in France (Lefebvre and Pasquerault 2004).

M. domestica, *M. stabulans*, *H. capensis* has also been encountered on human cadavers in Spain (Arnaldos *et al.* 2005, Velasquez *et al.* 2010). Among them, *M. domestica* (Garcia-Rojo 2004, Arnaldos *et al.* 2005, Velasquez *et al.* 2010) and *M. stabulans* were the predominant species (Arnaldos *et al.* 2005). Matuszewski and colleagues (Matuszewski *et al.* 2008, 2010) found on pig carcasses many different *Hydrotaea* species among which *H. capensis* and *H. dentipes*. These both species were also found in Switzerland (Wyss and Cherix 2006). The Phoridae, scuttle flies, are usually associated with the final stage of decay process on exposed corpses or associated with buried bodies (Gennard 2007). Moreover, scuttle flies are able to entering into small openings that are too small for other calyptrate flies (e.g. blowflies, flesflies) and some species are able to move through soil (Disney 2008). *Conicera tibialis*, the coffin fly, is commonly found on buried bodies whereas *Triphleba hyalinata* is more often associated with bodies in wooden coffins (Bourel *et al.* 2004). *Megaselia scalaris* and *Megaselia rufipes* are also associated with buried remains (Bourel *et al.* 2004). *M. scalaris* has also been observed several times in indoor death scenes (Leclercq 2000, Campobasso *et al.* 2004, Disney 2008, Reibe and Madea 2010). It is a warm climate species, frequently found in the Mediterranean countries (Leclercq 2000, Campobasso *et al.* 2004, Disney 2008). Dr Marcel Leclercq and colleagues have published their results on scuttle flies found in human cadavers in 2000 (Dewaele *et al.* 1999). The three species of Fanniidae (*F. canicularis*, *F. manicata* and *F. scalaris*) identified by Marcel Leclercq are referenced in the Belgian entomofauna. The most frequent species on cadavers are *F. canicularis* and *F. scalaris* in Europe (Wyss and Chérix 2006, Velasquez *et al.* 2010); particularly when sanitary conditions are poor (Wyss and Chérix 2006). These species are common on corpses during the coldest months of the year and can be considered as important entomological evidence during this period (Velasquez *et al.* 2010). In Leclercq cases, *Fannia* sp. was mostly found between October and March, the coolest months in Belgium. The only species of identified flesh fly (Sarcophagidae) is *S. argyrostoma*. This species is one of the most frequent flesh flies associated to human cadavers in Spain (Velasquez *et al.* 2010). *S. argysrostoma* is also reported as the only species encountered in a German case (Benecke 1998). The body of a man was discovered on a balcony located at the 8th floor of an urban flat (Benecke 1998). In

Switzerland, *S. argyrostoma* is essentially related to human corpses found inside apartments (Wyss and Chérix 2006). However, flesh flies can colonize corpses located indoor or outdoor (Wyss and Chérix 2006). Nine reported cases mention flesh flies, found in death scenes located indoor or outdoor. Several specimens found in the forensic cases of Leclercq were only identified at the genus or family levels. Indeed, species belonging to the tribe Sarcophagini are often difficult to reliably identify even as adults and immature stages using morphological characters (Pohjoismaki *et al.* 2010). However, correct species determination is a necessary requirement in entomoforensic expertise (Zehner *et al.* 2004, Amendt *et al.* 2007). Genetic identifications were non-existent at the beginning of Marcel Leclercq career as forensic entomologist. Currently, more and more papers refer to this family of forensic interest (e.g. (Zehner *et al.* 2004, Pohjoismaki *et al.* 2010, Velasquez *et al.* 2010)). The only species of Piophilidae identified by Leclercq is *Piophila casei*, the cheese skipper. In most of his forensic cases, the identification of Piophilidae was made at the genus level. Different cases report the presence of *P. casei* at imaginal or larval stages on human cadavers (Benecke 1998, Arnaldos *et al.* 2004, 2005, Wyss and Chérix 2006). Concerning other Dipteran families found in the Leclercq cases, little data with forensic relevance is known about Drosophilidae, Heleomyzidae, Trichoceridae and Sphaeroceridae. Winter gnats, Trichoceridae, could be used to determine time since death in winter (Erzinçlioglu 1980, Smith 1986, Gennard 2007). The only case of Leclercq with Trichoceridae occurred in winter (case 26). The heleomizid *Neoleria inscripta* is the most frequent Heleomyzidae associated with carrion in Great Britain (Smith 1986). Concerning Coleoptera, there are still neglected in research in forensic entomology (Midgley *et al.* 2010). However, their use in forensic entomology could be relevant (Kulshrestha and Satpathy 2001), providing a wide spectrum of sources of potential insect evidence (Midgley *et al.* 2010). However, there is a lack of existing data with forensic relevance. Among Coleopteran families cited in Leclercq's cases, Staphylinidae are commonly found on human cadavers. Most of rove beetles are usually found on the soil surface such as *O. rivulare* and *Philonthus* sp. (Bourel *et al.* 2004). However, their use in forensic entomology is still limited whereas some species are very common on corpse in temperate countries (e.g. *C. maxillosus* (Matuszewski *et al.* 2008, 2010)). Few forensic data are available in the literature on Staphylinidae; little is known about biology of "carrion" rove beetles. In several cases treated by Leclercq, the taxonomic identification of rove beetles was made at the family level. Indeed, one of the main difficulties in Staphylinidae is the specific identification (Wyss and Chérix 2006). Among Silphidae, *Necrodes littoralis* is commonly found on corpse or animal carcasses (Matuszewski *et al.*

2008, 2010). This species is the most frequent Silphinae found in Leclercq's cases and sometimes inside housing (e.g. case 12). Concerning Nicrophorinae (*Nicrophorus* spp.), all species cited in Leclercq cases where found in forest sites. However, Chauvet and colleagues (Chauvet *et al.* 2008) recorded some *Nicrophorus* on human corpses discovered inside houses (e.g. *N. humator*) in France. Dermestidae are generally associated to dry decomposition (e.g. mummification, skeletonization) and can be found on drying corpses (Smith 1986, Schroeder *et al.* 2002, Arnaldos *et al.* 2004, 2005, Wyss and Chérix 2006, Pasquerault *et al.* 2008). Under certain environmental conditions, larder beetles can considerably accelerate human decomposition, notably in indoor death scene (Schroeder *et al.* 2002). In France, Dermestidae were found in 64 entomoforensic cases on a total of 730 treated cases by the French gendarmerie [50] (Pasquerault *et al.* 2008). Pasquerault and colleagues (Pasquerault *et al.* 2008) list the following larder beetles on human cadavers: *D. undulatus*, *D. frischii*, *D. maculatus*, *D. lardarius*, *D. peruviannus* and *D. haemorrhoidalis*. These two latter species were not listed in Leclercq cases. However, one additional *Dermestes* species was listed in the Leclercq expertise: *D. ater*. All identifications at the genus level (*Dermestes* sp.) concern only immature stages found on death scenes. Another coleopteran family generally associated with desiccated bodies is Cleridae which are represented by few *Necrobia* (Smith 1986, Midgley *et al.* 2010). However, *Necrobia* species can be also found on corpse at various stage of decomposition feeding on other inhabitants of the corpse such as eggs or maggot (Smith 1986, Wyss and Chérix 2006). The three species of *Necrobia* (*N. violaceae*, *N. rufipes*, *N. ruficollis*) listed in Leclercq cases are commonly found on human bodies (Wyss and Chérix 2006). However, some forensic entomologists indicate that these species are not useful in forensic investigations (Wyss and Chérix 2006) (e.g. adults of *N. violaceae* (Matuszewski *et al.* 2010)). The nitidulid *Omosita discoidea* can be found on human bodies (Grassberger and Frank 2004). Nitidulidae are generally accompanied with Dermestidae on corpse (Smith 1986, Arnaldos *et al.* 2005, Wyss and Chérix 2006). However, nitidulids support a moistener substrate than dermestids (Smith 1986, Wyss and Chérix 2006). The species *Rhizophagus parallelocollis* and in general Monotomidae (=Rhizophagidae (Benecke 2001, Gaudry 2010)) are associated with buried remains (Smith 1986) or with fungi developing on exposed cadavers (Gaudry 2010). *Rhizophagus parallelocollis* was found on the corpse of a young female hidden into a metal box soon after her murder (case 64). The postmortem interval, *i.e* the time elapsed between death and discovery of the corpse, was about five years in this case. The presence of dung beetles is not surprising in Leclercq cases; Matuszewski and colleagues (Matuszewski *et al.* 2008, 2010) highlight the presence of Geotrupidae on pig carcasses

decaying on forest aboveground. The three cases of Leclercq with geotrupids were located in forest habitats. *Anoplotrupes stercorosus* prefers also forest sites (Kocarek 2003). For one case (case 8), the corpse was buried between 60-80 centimeters depth and *Geotrupes stercorarius* were found in the shallow grave. Gaudry (Gaudry 2010) indicates that *Geotrupes* can colonize buried remains at various depths or partially buried (Nuorteva 1977, Smith 1986). Histeridae, clown beetles, were found in few cases of Marcel Leclercq. In certain cases, they can drastically reduce the maggot population present on a corpse (Smith 1986, Wyss and Chérix 2006). Their use in forensic entomology is still limited (Wyss and Chérix 2006, Gennard 2007). *Tenebrio molitor*, a tenebrionid beetle, is usually associated with desiccated bodies (Smith 1986). Concerning other coleopteran families found in the Leclercq cases, little data with forensic relevance is known about Leiodidae and Aphodiidae.

Concerning the PMI estimation of Doctor Marcel Leclercq, the case studies were diverse and concerned early postmortem interval (seventy case studies) as well as late PMI with chronological succession of carrion community (forty-five case studies). At the beginning of his career as forensic entomologist, Doctor M. Leclercq based his PMI estimation on the works of Mégnin (Mégnin 1894), Kamal (Kamal 1958) Nuorteva and colleagues (Nuorteva 1977, Nuorteva *et al.* 1967), Reiter (Reiter 1974) Smith (Smith 1986) and his own publications (Leclercq and Leclercq 1948, Leclercq and Quinet 1949, Leclercq and Watrin 1971, Leclercq and Tinant-Dubois 1973, Leclercq 1978, 1983, Leclercq and Brahy 1985). In the last two decades, Dr Leclercq mainly used the manual of forensic entomology of Smith (Smith 1986) and the works of Greenberg (Greenberg 1990, 1991), Greenberg and Tantawi (Greenberg and Tantawi 1993), Catts and Haskell (Catts and Haskell 1990) and his own publications (Leclercq and Vaillant 1992, Leclercq and Verstraeten 1993, Leclercq 1999).

It is important to note that this review is based on writing reports of Dr. Marcel Leclercq and not on insect specimens used as entomological evidences. Thus, we have not certainty that all taxonomic identifications are completely reliable. However, Dr Marcel Leclercq was a renowned forensic entomologist and a pioneer of this discipline worldwide. Many forensic entomological papers highlight the necessity to generate data on insect succession and insect seasonal activity on carrion in specific geographic regions and various biotopes within these regions (Byrd and Castner 2001, Amendt *et al.* 2004, Sharanowski *et al.* 2008). This review of the entomoforensic cases of Dr Marcel Leclercq identifies the cadaveric entomofauna found on human corpses in central Europe (Belgium and France) over a period of 36 years.

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Chapitre I.3: Objectifs

L'objectif principal de cette thèse vise à mieux connaitre les interactions existant entre un animal vertébré en décomposition et l'entomofaune qui le colonise au sein de biotopes terrestres grâce à une approche pluridisciplinaire. Cette approche multidisplinaire combine des études faunistiques, biologiques, chimiques et chémo-écologiques.

1) L'entomofaune des cadavres: les Coléoptères Staphylinoidea

Il s'agit principalement d'une partie de la nécrofaune qui a fait l'objet de ce travail de recherche: les Coléoptères nécrophages et plus particulièrement une partie de la super-famille des Staphylinoidea avec les Staphylinidae et les Silphidae. En effet, délaissés par la communauté scientifique au profit des Diptères, les Coléoptères de l'écosystème-cadavre sont assez peu étudiés par les entomologistes forensiques. Etant considérés comme un excellent modèle de la décomposition humaine, les études de suivis *postmortem* ont été réalisées sur le cochon domestique. En effet, principalement pour des raisons éthiques et sanitaires, il est interdit de travailler en Belgique sur des cadavres. Néanmoins, les expérimentations menées au cours de cette thèse ont été approuvées par le comité d'éthique animal de la Faculté des Sciences Agronomiques de Gembloux (actuellement et depuis 2009 Gembloux Agro-Bio Tech, Ulg). La deuxième partie de ce travail fait l'inventaire des Coléoptères Staphylinoidea, appartenant aux familles des Silphidae et des Staphylinidae, que l'on retrouve au sein d'un écosystème terrestre localisé en Belgique.

2) Les odeurs émises par un corps en décomposition: les composés organiques volatils cadavériques

Un des principaux objectifs de ce travail de recherche a été d'aborder les relations cadavres-insectes sous une nouvelle approche: l'écologie chimique de l'écosystème-cadavre. En effet, il est couramment admis que le cadavre en se décomposant va émettre des odeurs attractives pour certains insectes, encore faut-il pouvoir identifier ces odeurs. Ces aspects sont développés dans la troisième partie du travail. Différentes techniques analytiques ont été investiguées au cours de ce travail de recherches.

3) Les composés organiques volatils cadavériques possédant une activité biologique

Afin de mettre en évidence certaines interactions cadavre-entomofaune, un Coléoptère Silphidae a été choisi comme modèle: *Thanatophilus sinuatus* Fabricius. Une étude chémo-écologique combinant des essais électrophysiologiques (EAG) et des études olfactométriques a été réalisée.

Finalement, un des objectifs ultimes de ce travail de recherche a été d'envisager le développement de nouveaux bio-indicateurs utiles en entomologie forensique contemporaine, mais également en sciences forensiques grâce à l'étude des composés organiques volatils cadavériques ou COVs. Les potentialités d'utilisation des Silphidae ont donc été investiguées en tant que «nouveaux» bio-indicateurs utiles à l'entomologie forensique. De même qu'en thanatochimie ou chimie forensique, les COVs cadavériques offrent des potentialités en tant que biomarqueurs du processus de décomposition.

Partie II: Les Coléoptères Staphylinoidea au sein de l'écosystème-cadavre

«Que vont devenir ces petits cadavres et tant d'autres lamentables déchets de la vie? Le regard et l'odorat n'en seront pas longtemps offensés. Les préposés à l'hygiène des champs sont légion. […]Bientôt le fumet de la pièce attire le diptère, générateur de l'odieux asticot. En même temps, s'empressent par escouades, venues on ne sait d'où, le Silphe aplati, l'Escarbot luisant trotte-menu, le Dermeste poudré à neige sous le ventre, le Staphylin fluet, qui tous, d'un zèle jamais lassé, sondent, fouillent, tarissent l'infection. […] L'horreur de ce laboratoire est une belle chose pour qui sait voir et méditer. Surmontons notre dégoût ; relevons du pied l'immonde détritus. Quel grouillement là-dessous, quel tumulte de travailleurs affairés ! Les Silphes, à larges et sombres élytres de deuil, fuient éperdus, se blottissent dans les fissures du sol ; les Saprins, ébène polie où miroite le soleil, trottinent à la hâte, désertent le chantier : les Dermestes, dont l'un porte pèlerine fauve mouchetées de noir, essayent de s'envoler, mais, ivres de sanie, culbutent et montrent la blancheur immaculée de leur ventre, contraste violent avec l'obscurité de leur costume. Que faisaient-ils là, tous ces enfiévrés de besogne? Ils défrichaient la mort en faveur de la vie. Alchimistes transcendants, avec la putridité redoutable ils faisaient produit animé, inoffensif. Ils épuisaient le périlleux cadavre au point de le rendre aride et sonnant ainsi qu'un reste de pantoufle tanné à la voirie par les frimas de l'hiver et les ardeurs de l'été. Ils travaillaient au plus pressé, l'innocuité de la dépouille.»

Jean-Henri Fabre, 1899, Souvenirs entomologiques, VIème Série, Chapitre 7.

Chapitre II.1: Prélude

La première partie de ce travail a brièvement développé la thématique de l'entomologie forensique ainsi que l'entomofaune associée aux cadavres. Deux ordres d'insectes sont largement tributaires de cet écosystème particulier: les Diptères et les Coléoptères nécrophages.

Bien qu'il y ait au moins autant d'espèces de Coléoptères nécrophages que de Diptères nécrophages recensés au sein de l'écosystème-cadavre, peu d'études entomoforensiques s'intéressent aux Coléoptères en tant que bioindicateurs potentiels en entomologie médico-légale. Ce manque d'intérêt pour les Coléoptères de l'écosystème cadavre se retrouve déjà dans l'un des premiers livres de référence d'entomologie forensique contemporain (Midgley *et al.* 2010) à savoir, le manuel d'entomologie forensique de Smith (Smith 1986). En effet, Smith traite des familles de Diptères d'intérêt forensique sur une septantaine de pages tandis que la place dévolue aux Coléoptères se résume à une douzaine de pages seulement (Smith 1986, Midgley *et al.* 2010). En 25 ans de recherche en entomologie forensique contemporaine, cette situation a très peu évolué. Néanmoins, depuis 2009, on assiste à une recrudescence de publications concernant les Coléoptères d'intérêt forensiques. Cette prise de conscience de la communauté scientifique pour d'autres familles que celles des Calliphoridae est d'ailleurs clairement énoncée dans la préface de l'ouvrage intitulé *Current Concepts in Forensic Entomology* (Amendt *et al.* 2010).

Parmi les Coléoptères fréquemment retrouvés au sein de l'écosystème-cadavre la super-famille des Staphylinoidea est particulièrement bien représentée. On recense notamment des staphylins (*rove beetles*) appartenant à la famille des Staphylinidae ainsi que des silphes et des nécrophores (*burying beetles*) faisant partie de la famille des Silphidae. Ces deux familles de Coléoptères font l'objet des deux chapitres suivants. Le suivi de la colonisation entomologique *postmortem* de carcasses de cochon domestique a permis de faire un inventaire exhaustif des espèces de Staphylinidae et de Silphidae que l'on peut retrouver au sein de l'écosystème-cadavre. De par certaines de leurs caractéristiques comportementales, les Silphidae et plus particulièrement la sous-famille des Silphinae semblent répondre à plusieurs critères favorables pour leur utilisation en entomologie forensique. Une étude des cycles de développement de deux espèces de Silphinae, fréquemment et abondamment recensées sur les cadavres, a été réalisée en conditions contrôlées.

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Chapitre II.2: Les Staphylinidae au sein de l'écosystème-cadavre

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Référence - Dekeirsschieter J., Frederickx C., Verheggen F.J., Drugmand D., Haubruge E. (2013). Diversity of forensic rove beetles (Coleoptera, Staphylinidae) associated with decaying pig carcass in a forest biotope. ***Accepted for publication in Journal of Forensic Sciences*****.**

Abstract – Most forensic studies are focused on Diptera pattern colonization while neglecting Coleoptera succession. So far, little information is available on the postmortem colonization by beetles and the decomposition process they initiate under temperate biogeoclimatic countries. These beetles have however been referred to as being part of the entomofaunal colonization of a dead body. Forensic entomologists need increased databases detailing the distribution, ecology and phenology of necrophagous insects, including staphylinids (Coleoptera, Staphylinidae). While pig carcasses are commonly used in forensic entomology studies to surrogate human decomposition and to investigate the entomofaunal succession, very few works have been conducted in Europe on large carcasses. Our work reports the monitoring of the presence of adult rove beetles (Coleoptera, Staphylinidae) on decaying pig carcasses in a forest biotope during four seasons (spring, summer, fall and winter). A total of 23 genera comprising 60 species of rove beetles were collected from pig carcasses.

Keywords – Forensic science, Forensic entomology, Staphylinidae, Coleoptera, Beetle, Carrion ecology, temperate area

1. Introduction

Rapidly after the death of an animal (including human), its carcass becomes a scarce and patchy food source for many organisms (1-3). In temperate natural biotopes, insects are the most specialized organisms in terms of exploitation of a "cadaver-ecosystems" (4). Carrion insects, comprising mainly Dipterans and Coleopterans, are attracted to the cadaver in a relative predictable sequence called the entomofaunal succession or insect succession (5-10). Study of these insects in a medico-legal context is part of forensic entomology (4, 11). Many forensic entomological studies have been conducted on pig carcasses as surrogate human models mainly for physiological, ethical, legal and economic reasons (2, 12-16). However, very few works have been conducted in Europe on swine carcasses for forensic entomological research (2, 17-21). Many published reports are focused on Diptera pattern colonization and very few looked at Coleoptera succession (1, 19, 21-23). However, the use of beetles in forensic entomology can be relevant (23-24). Beetles constitute a taxonomically and ecologically diverse part of the carrion insect community, thus providing a wide spectrum of source of potential entomological evidence (23). Families of beetles of forensic importance are Staphylinidae (rove beetles), Silphidae (carrion beetles), Dermestidae (larder, skin or hide beetles), Histeridae (clown or hister beetles), Cleridae (checker beetles), Geotrupidae and Nitidulidae (sap beetles) (18, 25-26). Most of Staphylinidae are predaceous of smaller arthropods or have specialized diets (*e.g.* diatoms, fungi), whereas other are generalists (18, 25, 27). On decaying carcasses, Staphylinidae feed primarily on maggots but they also prey on other juvenile inhabitants of the corpse (*i.e.* necrophilous species) and are frequently considered as the commonest predators or parasitoid found on cadavers (27). Moreover, some staphylinid species such as *Creophilus maxillosus* are specialized on carrion, which repeatedly breed in carrion and frequently appear late in decay process (18-20, 28-29), even so adults may be also found on carcasses only hours after death (25). However, little is known in general about forensic staphylinid communities (30), mainly because of the difficulties of species identification (18). Staphylinidae is the largest family of beetles with between 45000 and 54000 species known worldwide (25, 31-32) with more than 1100 species for the Belgian entomofauna (33). Many forensic entomological papers highlight the necessity to generate data on insect succession and insect seasonal activity on carrion in specific geographic regions and various biotopes within these regions (4, 13, 25, 29). This paper identifies the staphylinids that occur on large carcasses in a temperate forest biotope during four seasons

2. Material and Methods

2.1. Field site and study periods

This study was conducted during four seasons (summer 2008 from June 6 to July 25, autumn 2008 from October 13 to December 19, winter 2009 from January 23 to March 20, spring 2009 from April 10 to June 4). The study site was a forest biotope, located in Belgium (Lambert-coordinates: 172800.00/167150.00). The field site is a facility research area devoted to forensic research managed by the Disaster Victim Identification (DVI) of the Belgian Federal Police. The tree layer of the field site is dominated by oak trees (*Quercus rubra*) and beech trees (*Fagus sylvatica*). The shrub layer is absent. The soil vegetation is scattered and the herb layer is mainly constituted of bracken (*Pteridium aquilinum*), blackberry (Rubus fructicosus), lily-of-the-Valley (*Convallaria maialis*) and May Lily (*Maianthemum bifolium*). Some spare spots of *Polytrichum* sp. constitute the moss layer.

2.2. Environmental parameters

Ambient air temperature was automatically measured once an hour using a datalogger (HOBO RH/TEMP 8K, Onset computer corporation, USA) placed on the field site during each complete sampling period. The daily mean temperature, the minimal temperature and the maximal temperature were calculated on the basis of ambient air temperature recorded on a time interval of 24 hours.

2.3. Animal model

Domestic pig (*Sus scrofa domesticus* L.) was used to surrogate human models mainly for physiological, biochemical, ethical, legal and economic reasons (2, 12-16, 34-37). Unlike other animals, pigs are considered to be acceptable substitute due to their similarity to human in body mass (torso in weight), skin structure, fat to muscle ratio and hair coverage (16, 33-35, 37). The greatest dissimilarity between pigs and humans are the bones which have a different microstructure (35, 37).

At the beginning of each season, four pigs (average weight of 25 Kg) were killed by penetrative captive bolt (fractured skull) by a veterinary. Pigs were provided from the

experimental farm of the veterinary medical Faculty of the University of Liege and animal ethics were approved for this postmortem field experiment. Immediately after the euthanasia, pig carcasses were packed in double plastic bag to avoid any insect colonization before being placed in the experimental site, within the next 2 hours. Each pig carcasse was placed 30 meters from each other, in metal mesh cages (180 cm × 90 cm × 90 cm) to avoid scavenging by vertebrate carnivores. This pattern of subject spacing was consistent with other carrion entomological studies in spite of that experimental total independence requires intersubject spacing of at least several hundred meters (16).

2.4. Insect collection and identification

In order to quantify insect colonization on pig carcasses, six pitfall traps (glass jars of 15 cm in height and 8 cm in diameter) and four yellow traps (plastic container of 9 cm in height and 27 cm in diameter), both filled with 50% ethylene glycol, were placed around each carcass. Two pitfall traps were placed, flush to the surface, near the ventral face, two near the dorsal face, one near the head and one near the anus (Figure 8). The four yellow traps were placed on the ground around each pig carcass, one near the head, one on the dorsal face, one near the anus and the last on the ventral side.

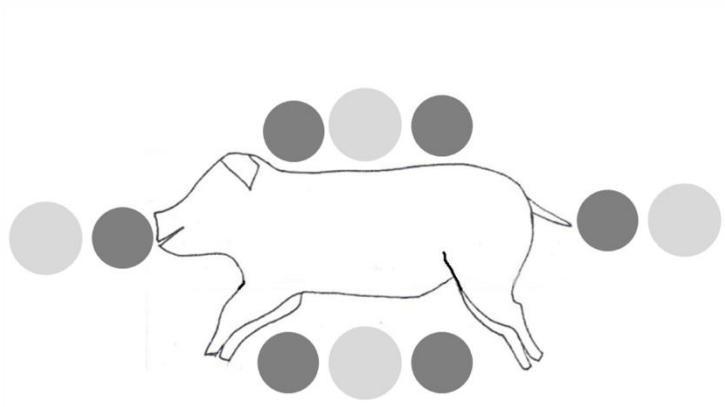

Figure 8. Disposition of the traps around the pig carcass (grey = yellow traps, not coloured = pitfall traps).

The trapped insects were removed weekly and conserved in 80% norvanol D (ethanol denatured with ether). In the laboratory, rove beetles were mounted on insect pins and identified to species. Only adults were included in the counting of collected insects during this field study. The species names follow the taxonomy of Fauna Europaea (38).

3. Results

3.1. Environmental parameters

The mean atmospheric temperature were 13.4°C, 5.5°C, 3.5°C and 14.5°C for spring, fall, winter and summer, respectively. Figure 9 shows the mean, maximal and minimal temperature recorded on the forest site for each season.

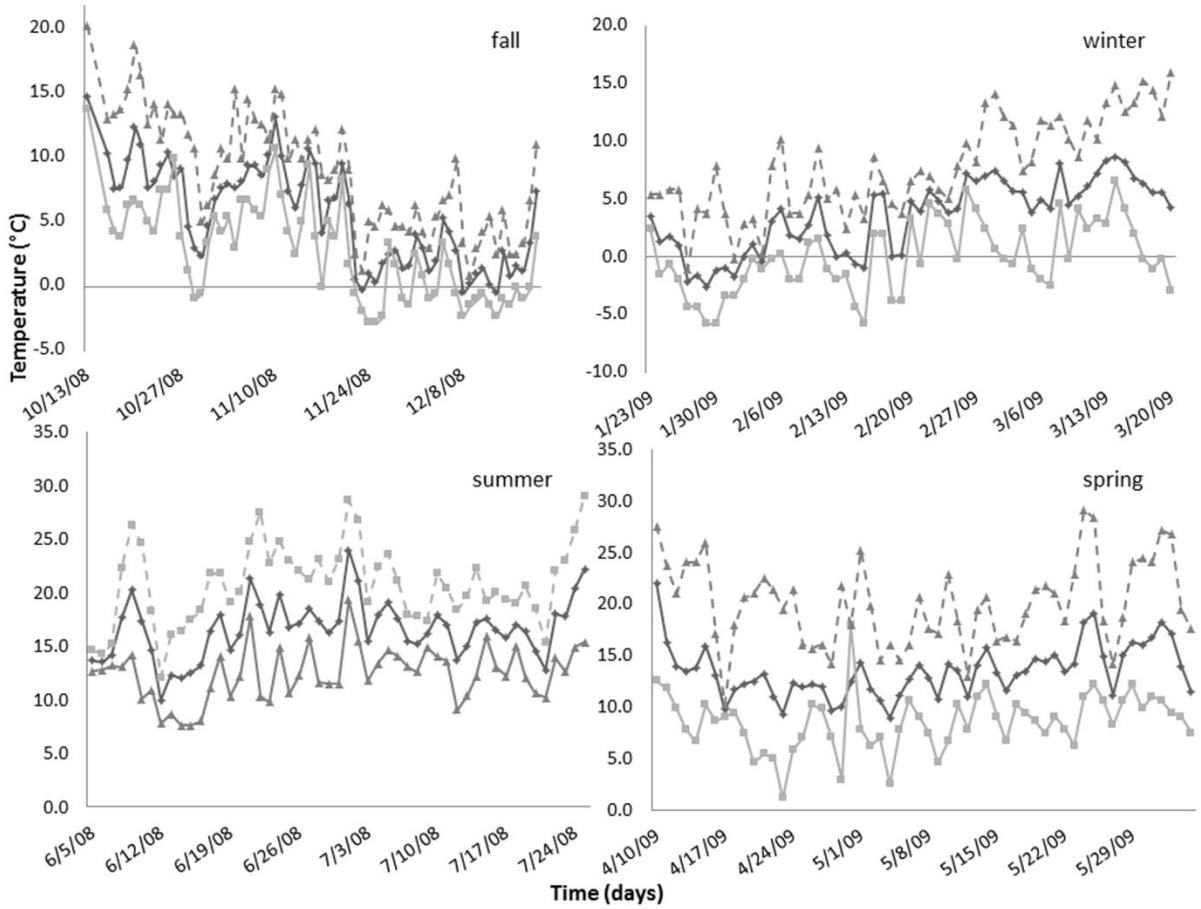

Figure 9. Mean temperature (continuous dark grey stroke), maximal temperature (discontinuous stroke) and minimal temperature (continuous light grey stroke) recorded in the field site during the four inventoried seasons.

3.2. Rove beetles

Sixty-two staphylinid species belonging to six Staphylinidae subfamilies have been identified during the complete sampling period. Table 9 lists the 1980 specimens of rove beetles according to their subfamily and the season.

Table 9. List of rove beetles (Coleoptera, Staphylinidae) collected on pig carcasses and comparison with literature dealing about insect succession on various types of carcass in Europe. ▲ referenced, - not referenced, the species name in bold are staphylinid species referenced in the present study and literature. * indicates necrophilous/ necrophagous species. Ecological data (habitat preference and necrophagous/necrophilous species) result from the monographies of Freude and colleagues (34-35, 48).

	Rove beetles **Present study**	**Ubiquitous species**	**Forest species**	**Body length (mm)**	**Literature (carrion ecology or forensic entomology studies)**												**Abundance (No. of individuals)**			
					Nabaglo (50)	Topp *et al.* (42)	Leclercq *et al.* (43)	Kentner *et al.* (44)	Bourel *et al.* (45)	Kocarek (1)	Garcia-Rojo (17)	Van Wielink (46)	Schlechter (47)	Matuszewski *et al.* (19)	Ozdemir *et al.* (48)	Pardo *et al.* (30)	Summer	Fall	Winter	Spring
	Number of rove beetles species found				21	32	15	37	11	39	11	33	36	50	15	35				
	Thereof in the present study				2	7	4	13	6	12	3	16	13	16	5	5				
Tachyporinae (species richness: 8 sp.)																				
1	***Tachinus laticollis* (Gravenhorst, 1802)**	∨	-	4-5	▲	▲	-	▲	-	▲	-	-	▲	▲	-	-	-	-	1	-
2	****Tachinus signatus* (Gravenhorst, 1802)**	-	∨	5-6.5	-	-	-	-	-	-	-	-	▲	-	-	-	-	1	1	1
3	****Tachinus subterraneus* (L., 1758)**	∨	-	5-6.5	-	▲	-	▲	-	-	-	▲	▲	-	-	-	-	4	24	5
4	**Tachinus elongatus* (Gyllenhal, 1810)	-	∨	7-9	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
5	*Tachinus bipustulatus* (F., 1793)	-	∨	5-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-
6	**Tachinus proximus* (Kraatz, 1855)	-	∨	6-8	-	-	-	-	-	-	-	-	-	-	-	-	-	8	31	2
7	*Tachinus corticinus* (Gravenhorst, 1802)	∨	-	3-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
8	*Bolitobius inclinans*	-	∨	7-8.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-

	(Gravenhorst, 1806)																		
Paederinae (species richness : 1 sp.)																			
9	***Rugilus rufipes* (Germar, 1836)**	V	-	5.5-6	-	-	-	-	-	-	-	▲	▲	-	-	-	-	-	-	1
Omaliinae (species richness : 3 sp.)																			
10	****Omalium rivulare* (Paykull, 1789)**	-	V	3-4	▲	▲	▲	▲	▲	▲	-	▲	▲	▲	-	-	1	14	23	-
11	*Olophrum piceum* (Gyllenhal, 1810)	-	V	5-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
12	***Acidota cruentata* (Mannerheim, 1830)**	V	-	4.5-6	-	-	-	-	-	▲	-	-	-	-	-	-	-	-	1	-
Oxytelinae (species richness : 2 sp.)																			
13	****Anotylus sculpturatus* (Gravenhorst, 1806)**	V	-	3-4	-	▲	-	▲	-	-	-	-	-	▲	-	-	1	7	12	8
14	****Oxytelus (Tanycraerus) laqueatus* (Marsham, 1802)**	V	-	3.8-5	-	-	-	-	-	-	-	▲	-	▲	-	-	-	-	-	1
Staphylininae (species richness : 37 sp.)																			
15	****Creophilus maxillosus* (L., 1758)**	V	-	15-25	-	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	381	-	-	283
16	*Ocypus olens* (O. Muller, 1764)	-	V	22-32	-	-	-	-	-	-	-	-	-	-	-	-	3	116	-	5
17	****Platydrachus chalcocephalus* (F., 1801)**	-	V	12-22	-	-	-	-	-	-	-	-	▲	-	-	-	72	60	-	-
18	****Ontholestes murinus* (L., 1758)**	-	V	10-15	-	-	-	▲	-	▲	-	▲	-	▲	▲	-	-	-	-	12
19	****Ontholestes tesselatus* (Geoffroy, 1785)**	-	V	14-19	-	-	-	▲	-	▲	-	-	-	▲	-	-	1	2	-	1
20	**Xantholinus (s.str.) longiventris* (Heer, 1839)	-	V	7-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
21	*Xantholinus (s.str.) linearis* (Olivier, 1795)	-	V	6-9	-	▲	-	-	-	-	-	-	-	-	-	-	-	-	1	-
22	*Xantholinus (s.str.) gallicus* (Coiffait, 1956)	-	-	6-7.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
23	*Quedius (s.str) levicollis* (Brullé, 1832)	-	V	10-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
24	***Quedius (Distichalius) cinctus* (Paykull, 1790)**	-	V	7.5-8.5	-	-	-	-	-	-	-	-	▲	-	-	-	18	72	21	6
25	****Quedius (Microsaurus) lateralis* (Gravenhorst, 1802)**	-	V	10-14	-	-	-	-	-	-	-	-	▲	-	-	-	-	4	-	-
26	*Quedius (Raphirus) riparius F.* (Kellner, 1863)	-	V	6-7	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
27	***Quedius (Microsaurus) mesomelimus* (Marsham,**	V	-	7-11	-	-	▲	-	-	▲	-	-	-	-	-	-	1	1	1	

	1802)																			
28	*Quedius (Raphirus) semiaenus* (Stephens, 1833)	V	-	6.5-7.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1
29	***Quedius (Raphirus) fumatus* (Stephens, 1833)**	-	V	7-9	-	-	-	-	-	-	-	-	-	-	-	▲	-	-	1	-
30	**Bisnius cephalotes* (Gravenhorst, 1802)	-	V	7-8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
31	****Philonthus (s.str.) succicola* (Thomson, 1860)**	-	V	10.5-13.5	-	-	-	-	-	▲	-	▲	▲	▲	-	-	148	3	1	41
32	****Philonthus (Onychophilonthus) marginatus* (O. Muller, 1764)**	-	V	7-9	-	-	-	▲	▲	-	-	▲	▲	▲	-	-	24	5	-	14
33	****Philonthus (s.str.) splendens* (F., 1793)**	-	V	10-16	-	-	-	▲	-	-	-	▲	-	▲	-	-	11	1	1	12
34	***Philonthus (s.str.) politus* (L., 1758)**	-	V	10.5-13	-	▲	-	▲	-	▲	-	▲	-	▲	▲	-	28	-	-	4
35	****Philonthus (s.str.) laminatus* (Creutzer, 1799)**	-	V	8-10	-	-	-	-	-	-	-	-	-	▲	▲	-	6	-	-	3
36	*Philonthus (s.str.) albipes* (Gravenhorst, 1802)	-	V	5-5.5	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	-
37	***Philonthus (s.str.) varians* (Paykull, 1789)**	V	-	5.5-7.5	-	-		▲	-	▲	-	▲	-	-	-	▲	4	2	-	22
38	****Philonthus (s.str.) tenuicornis* (Mulsant & rey, 1853)**	V	-	11-14	-	-	▲	-	-	-	-	▲	-	▲	-	-	57	3	-	78
39	**Philonthus (s.str.) carbonarius* (Gravenhorst, 1802)	V	-	5-7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
40	*Philonthus (s.str.) corvinus* (Erichson, 1839)	-	-	7-8	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
41	****Philonthus (s.str.) intermedius* (Lacordaire, 1835)**	V	-	8-11	-	-	-	-	-	-	▲	▲	-		-	-	3	-	-	1
42	****Philonthus (s.str.) rectangulus* (Sharp, 1874)**	V	-	6.5-9	-	-	-	-	-	-	-	-	-	▲	-	-	2	1	-	-
43	*Philonthus (s.str.) decorus* (Gravenhorst, 1802)	-	V	11-13	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2
44	****Philonthus (s.str.) cognatus* (Stephens, 1832)**	-	V	8-11.5	-	-	-	-	-	-	▲	-	-	-	-	-	-	2	1	-
45	****Bisnius fimetarius* (Gravenhorst, 1802)**	V	-	5.5-7	-	-		▲	-	▲	-	▲	▲	▲	-	-	8	15	50	19
46	*Philonthus (s.str.) caerulescens* (Lacordaire, 1835)	-	V	9-9.5	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	3
47	*Philonthus (s.str.) quisquiliarius* (Gyllenhal, 1810)	V	-	5.5-7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-

48	***Philonthus (s.str.) cruentatus* (Gmelin, 1790)**	-	V	6-8.5	-	-	-	-	▲	-	-	▲	-	-	-	-	-	1	-	-
49	***Philonthus (s.str.) sanguinolentus* (Gravenhorst, 1802)**	V	-	7-8	-	-	-	-	▲	-	-	-	-	-	-	-	-	-	-	1
50	**Othius subuliformis (Stephens, 1833)*	-	-	4.5-5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
51	*Philonthus sp.*	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Aleocharinae (species richness: 13 sp.)																				
52	**Aleochara lanuginosa* (Gravenhorst, 1802)	V	-	3.5-5	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	14
53	****Aleochara curtula* (Goeze, 1777)**	V	-	4-8	-	-	-	▲	▲	▲	-	▲	▲	▲	-	▲		20	-	-	52
54	*Aleochara bilineata* (Gyllenhal, 1810)	-	V	2.5-5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-
55	****Aleochara intricata* (Mannerheim, 1830)**	-	V	3.5-5	-	-	-	-	-	-	-	-	-	-	▲	▲		-	1	11	-
56	***Oxypoda alternans* (Gravenhorst, 1802)**	V	-	3.2-3.8	-	-	-	-	-	▲	-	-	-	-	-	-		-	1	13	1
57	*Oxypoda umbrata* (Gyllenhal, 1810)	-	-	2.5-3.2	-	-	-	-	-	-	-	-	-	-	-	-		1	-	11	1
58	*Ischnoglossa prolixa* (Gravenhorst, 1802)	-	V	2.6-3.2	-	-	-	-	-	-	-	-	-	-	-	-		-	2	4	-
59	*Aleochara stichai* (Likovsky, 1965)	-	V	3-4.5	-	-	-	-	-	-	-	-	-	-	-	-		-	1	-	-
60	*Haploglossa villosula* (Stephens, 1832)	-	V	2.5-3.5	-	-	-	-	-	-	-	-	-	-	-	-		-	1	8	-
61	*Bolitochara obliqua* (Erichson, 1837)	-	V	3.2-4	-	-	-	-	-	-	-	-	-	-	-	-		-	-	1	1
62	*Bolitochara lunulata* (Paykull, 1789)	-	V	3.5-4.5	-	-	-	-	-	-	-	-	-	-	-	-		1	1	4	2
63	*Silusa rubiginosa* (Erichson, 1837)	-	V	3-3.8	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	2
64	*Aleochara sp.*	-	-		-	-	-	-	-	-	-	-	-	-	-	-		1	-	-	-
																	TOTAL of individuals	**800**	**332**	**238**	**610**

3.3. Species community

The staphylinid subfamilies identified on pig carcasses are the Aleocharinae (13 species), Omaliinae (3 species), Oxytelinae (2 species), Paederinae (1 species), Staphylininae (37 species), and Tachyporinae (8 species). Only two collected specimens have not been identified at the species level, *Philonthus* sp. and *Aleochara* sp. The seasonal species richness is represented by 26 species in summer; 29 species in fall, 32 species in winter and 36 species in spring.

Some rove beetles were collected only during one season (Table9). *Aleochara stichai*, *Philonthus cruentatus*, *Quedius riparius* and *Q. lateralis* were collected only in fall. Twelve species of Staphylinidae were collected only in winter: *Xantholinus gallicus*, *X. lienaris*, *Othius subuliformis*, *Acidota cruentata*, *Olophrum piceum*, *Philonthus quisquiliarius*, *Bisnius cephalotes*, *Quedius fumatus*, *Tachinus corticinus*, *T. bipustulatus*, *T. laticollis* and *Bolitobius inclinans*. Eight species were collected only in spring: *Xantholinus longiventris*, *Philonthus sanguinolentus*, *P. carbonarius*, *Quedius semiaenus*, *Q. levicollis*, *Oxytelus laqueatus*, *Rugilus rufipes* and *Ontholestes murinus*. There is no summer exclusive species of Staphylinidae.

3.4. Abundance

Among all individuals collected, 40.4 % (800 individuals) were collected in summer, 16.8 % (332 individuals) in fall, 12.0 % (238 individuals) in winter and 30.8 % (610 individuals) in spring.

Creophilus maxillosus was the most abundant species overall (33.5 %, 664 individuals), followed by two species of *Philonthus*, *P. succicola* and *P. tenuicornis*, respectively with 9.7 % (193 individuals) and 7.0 % (138 individuals) of the total catching. *Platydrachus chalcocephalus* represented 6.7 % (132 individuals), followed by *Ocypus olens* with 6.3 % (124 individuals), *Quedius cinctus* with 5.9 % (117 individuals), *Philonthus rigidicornis* with 4.6 % (92 individuals) and *Aleochara curtula* with 3.6 % (72 individuals). The remaining species (34 spp.) were infrequently observed, with less than 45 collected. Twenty species were collected once. Most of these single staphylinid specimens were collected in winter (10 species), seven species in spring and three species in fall. Among the twelve species present exclusively in winter, only *T. bipustulatus* (5 specimens) and *O. subuliformis* (2 specimens) were caught more than ones. Among the four species present only in fall, the most often

collected is *Q. lateralis* (4 specimens). For the species collected only in spring, the abundances were very low and most of them were collected only once, except for *S. rubiginosa* with two collected specimens. Figure 10 shows the abundances of Staphylinidae according to the season.

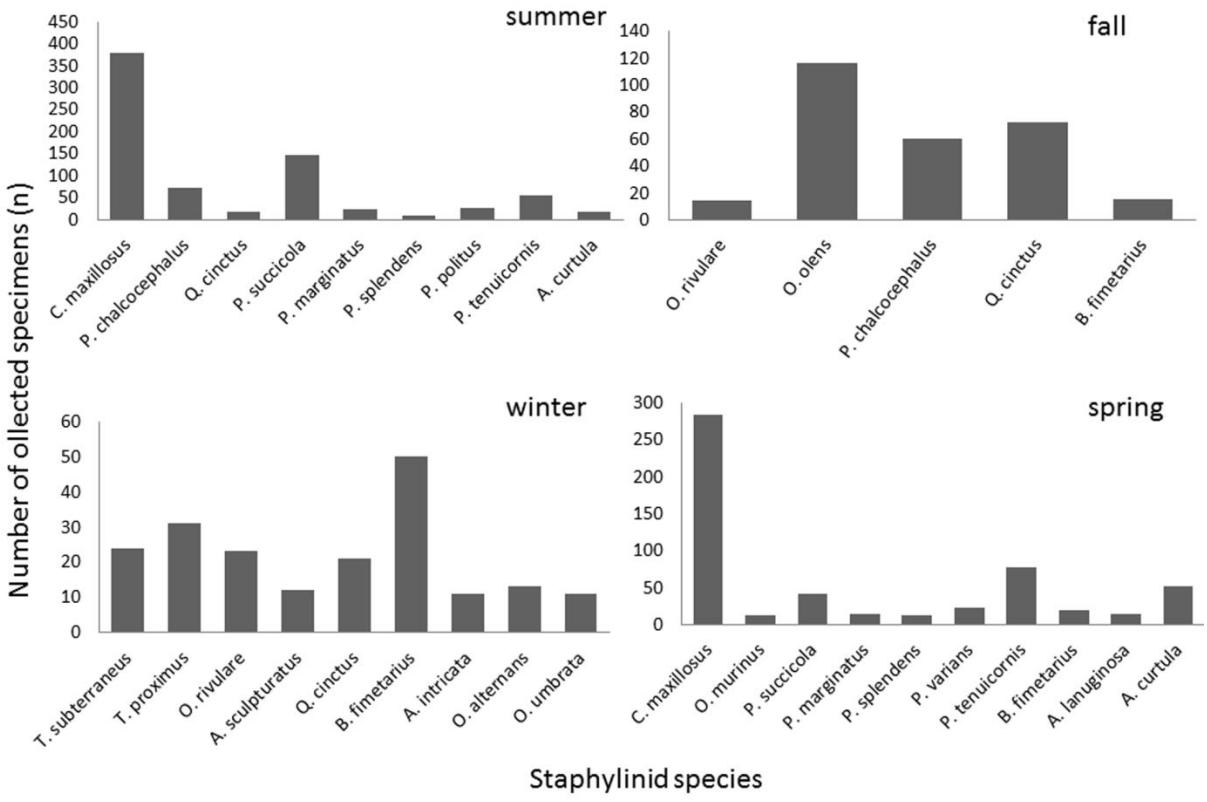

Figure 10. Abundance of rove beetles according to the season. Only the species represented by more than ten individuals are include in the graphs.

During summer, the predominant species were Staphylininae with *C. maxillosus* and different species of *Philonthus*, particularly *P. succicola*. *C. maxillosus* was also the most abundant species observed in spring. Several specimens of *Platydrachus chalcocephalus* were observed in summer, fall and winter whereas *Q. cintus* is principally present in summer and winter. The most predominant species of Aleocharinae was *A. curtula*, particularly in summer and spring. *O. olens* was predominant in fall. *Bisnius fimetarius*, a philonthina species, was present during the four seasons, but was more abundant in winter whereas *Omalium rivulare* was only present during the coldest seasons (fall and winter).The most abundant species in spring were Staphylininae species with many *Philonthus* sp., particularly *P. tenuicornis*.

4. Discussion

In the forest biotope, the carrion rove beetles community is comprising 62 species and was found to change over time. The majority of rove beetle species listed in the present study are forest species (Table 9) (39-40) and could be considered as bio-indicators of forested areas. The assemblage of forest rove beetles community differs among the season. The species diversity was higher during spring, followed by winter, fall and was weaker in summer. However, the lowest abundance of rove beetles occurred in winter with more or less four times fewer trapped specimens than in summer. We have collected the highest number of beetles in summer, followed by spring and fall. The species abundance is not necessarily correlated to the importance of a rove beetle in ecological processes such as predation or carrion scavenging, *e.g.* great amounts of small species may have a similar weight in predation or carrion scavenging than few large insects (41). In this study, the most abundant species, approximately 74% of the total catching, were large rove beetles (body length ≈ 1 cm) such as *Creophilus* sp., *Philonthus* spp., *Platydrachus* sp. and *Ocypus olens*, the latter being the bigger rove beetle in the Belgian entomofauna (body length approximately between 2 and 3 cm) (34-35). Regardless of the season, the predominant species was *C. maxillosus* that represents approximately one third of the total catching. *C. maxillosus* is a necrophilous species frequently found in carrion entomostudies (1, 17, 19, 30, 42-48). *C. maxillosus* was exclusively trapped during spring and summer. Drugmand (49) indicates that the activity of *C. maxillosus* begins in March with a peak activity in May and a decrease until October. The presence of *O. olens* exclusively in fall catching is not surprising. This species has typically a fall phenology (49). Most of rove beetles have a phenology with two peaks activity. The first peak activity is during spring (April-May) and the second, smaller, at the beginning of fall (49).

Table 9 compares the species of Staphylinidae referenced in the literature (in Europe) (1, 17, 19, 30, 42-48, 50) and species caught in this study.

In Central Europe (Poland), Nabaglo (1973) (50) found 21 species of Staphylinidae on dead bank voles in forest habitat. However, only two species are similar to those identified in the present study: *T. laticollis* and *O. rivulare* that were caught only in spring. However, in the present study, *O. rivulare* is more abundant during the coldest months as observed by Torp and colleagues (42) where *O. rivulare* is predominant in fall.

In their study (42), Torp and colleagues found 32 species of rove beetles that inhabited carrion in open air biotopes and seven species of Staphylinidae are similar to those observed in the present study. While studying 49 human corpses in Belgium, Leclercq and Verstraten (43) observed 15 species of rove beetles found in various death scenes. Among the species that were identified in our work, only four species are common with their study. Among them, *O. rivulare* has been found by Leclercq and colleagues in seven medico-legal cases (43). However, the listed staphylinids were the result of manual samplings and not continuous trapping. In their study on decaying rat carcasses, Kentner and Streit report 37 species of rove beetles with respectively 22 species in forest habitat and 28 species in open fields, during spring (44). Contrary to the present study, they did not found *C. maxillosus* in their forest samples; this rove beetle was only found in their open field habitat samples (44). Another study on rat carcasses was conducted by Kocarek (1) in Czech Republic (Central Europe). 39 species of Staphylinidae were found on exposed rat carcasses in meadow and forest habitats of which twelve are the same as those in the present study. *A. curtula* was preferentially encountered in meadow habitat during three seasons (spring, summer and fall) (1). In Northern France (Western Europe), Bourel and colleagues report eleven species of Staphylinidae on rabbit carcasses in sand dunes (45). Among these eleven species, six were also observed here. They collected a great number of *A. curtula* and *O. rivulare* during spring and early summer (40). The species of the genus *Aleochara* are known to have a parasitoid behaviour of cyclorrhapheous dipterans such as Muscidae and Calliphoridae (51-52). While observing rabbit carcasses in woodland biotopes in Luxembourg (Western Europe), 36 species of Staphylinidae were found (47). Among them, 13 species of rove beetles are similar to those we identified. However, there is no information about the coleoptera abundance. In Spanish suburban area (Western Europe), eleven species of rove beetles were found on decaying pig carcasses exposed on sun (17). Among these eleven species, three are the same as those in the present study such as *C. maxillosus* and two *Philonthus* spp. In Poland (Central Europe), Matuszewski and colleagues have been found fifty species of Staphylinidae on pig carcasses (19). However, this study has only 16 species in common with their list of Staphylinidae although their field study was also conducted in forest habitats. In Turkey (Eastern Europe), sixteen species of Staphylinidae have been found on pig carcasses deposited in woodland habitats (pine-forest) (48). Five species of rove beetles are common between this study and the Turkish study (48). *C. maxillosus* were present from early spring to mid-fall (48). Another study conducted on swine carcasses in Portugal (Western Europe) referenced 35 species of Staphylinidae (30). Five species of Staphylinidae were also observed in this work;

the Portuguese study highlights the presence of *C. maxillosus* on corpses. In Netherlands (Western Europe), 28 species of Staphylinidae have been found on a dead fox deposited in the shade of trees and 14 species on a dead roe deer exposed in a sunny fire lane (pine-forest) (46). Sixteen staphylinid species are the same as in this study. The most abundant Staphylinidae was *P. varians* on the fox carcass during early spring whereas *C. maxillosus* was not common on both types of carcass (46).

In comparison with other entomoforensic studies (1, 17, 19, 30, 42-48, 50), this study references a greater number of Staphylinidae species (62 sp.) in a temperate forest biotope. However, we think that the real number of rove beetles species visiting carcasses is greater than 62 species due to the passive sampling procedure with pitfall traps and yellow traps. For instance, the subfamily of Aleocharinae is low represented in our samples and particularly the genus *Atheta*.

Moreover, the various kinds of sampling methods could explain the differences observed between entomological studies. For example, in similar biotopes, the rove beetles composition is quite different (Table 9). Local climate (*e.g.* Western Europe vs Central Europe) is also an hypothesis in the difference observed between field studies. The only species with a strong potential forensic interest is *Creophillus maxillosus*, which is commonly found on carrion (1, 17, 19, 28, 30, 42-48). As recently highlighted by Matuszeswki (28), this coleopteran species seems to be a useful bioindicator in the estimation of late postmortem interval. However, the complete rove beetles community might give information on death scene. Indeed, some species are exclusively found is some biotope (*e.g.* forest species).

To conclude, this is the first entomoforensic study conducted on large vertebrate carcasses used as human body analogues in Western Europe, a temperate biogeoclimatic country, during four seasons. The aim of this study was to identify the carrion rove beetle communities which are under-studied from a forensic point of view.

4.1. Self-critique

Only adult stages were included in this field study and we were unable to provide information about immature stages of rove beetles. However, for forensic purposes, one should consider collecting information about the presence/absence of these immature stages. However, the taxonomic identifications of larval stages are even more difficult than adult stages.

The insect trapping method (pitfall traps and yellow traps) is adapted to sampling carrion visiting insects, except for very small species. One should combine these traps with the use of netting to collect small flying insects.

Moreover, only one temperate biotope has been studied in the present experiment, and it could be interesting to study the entomoforensic communities in various kinds of habitats (*i.e.* meadows, urban, suburban, different kind of forestation areas, *etc*.).

5. Acknowledgements

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The voucher staphylinid specimens are stored at the entomological conservatory of the Department of functional and evolutionary Entomology (Gembloux Agro-Bio Tech, University of Liege, Belgium).

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Chapitre II.3: Les Silphidae au sein de l'écosystème cadavre

II.3.1. Large carrion beetles (Coleoptera, Silphidae) in Western Europe: a review

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Abstract - This review focuses on carrion beetles (Coleoptera, Silphidae) of the Western Palearctic and their potential use in forensic entomology as bioindicators. Few studies have looked at Silphidae in forensic context and investigations. However, some Silphidae present the desirable characteristics of some Diptera used in postmortem estimates and thus may extend the minimum postmortem interval (PMI min). We review here the taxonomy and distribution of Western Palearctic Silphidae. The anatomical and morphological characteristics of both subfamilies are described for adults and larvae. The biology and ecology of silphids are also summarized for Silphinae and Nicrophorinae. A specific chapter gives an overview of the current uses of Silphidae in forensic entomology as postmortem indicator.

Keywords - Silphidae, burying beetles, forensic entomology, taxonomy, identification, Western Europe

1. Introduction

Carrion beetles (Coleoptera, Silphidae) consist of a small group of Coleoptera counting less than 200 species that are worldwide spread (Sikes, 2008).Silphids perform vital ecosystem functions (Wolf *et al.* 2004); they promote the breakdown and recycling of organic matter into terrestrial ecosystems (Peck,1990; Ratcliffe, 1996; Hastir *et al.* 2001; Kalinova *et al.* 2009). Most Silphids are carrion feeders (necrophagous species) but can also prey on other carrion inhabitants such as fly eggs or maggots and other small carrion beetles (necrophilous species) (Racliffe, 1996; Hastir *et al.* 2001; Sikes, 2005; Sikes,2008). The "carrion" terminology is not adapted for all silphid species according to their ecological group, some species (Silphinae) are phytophagous or found in dung or fungi (Anderson *et al.* 1984; Sikes, 2008).Carrion beetles are also referenced as "large carrion beetles" contrary to other smaller carrion beetles such as Agyrtidae, Leiodidae ("small carrion beetles") or Cholevidae (Peck, 1990; Ratcliffe, 1996; Peck, 2001;Sikes, 2008).Their feeding activities on carrion may also destroy some foci of infection of pathogenic bacteria (Peck, 1990). The necrophagous insects, including carrion beetles, have particular relationships with decomposing remains (vertebrate carcass) which constitute a rich ephemeral resource (Anderson *et al.*1996; Grassberger *et al.* 2004; Carter *et al.* 2007).These specialized insects, including mainly Diptera and Coleoptera, are attracted to the cadaver that they colonize in a relative predictable sequence called the entomofaunal succession or insect succession (Megnin, 1894; Putman, 1983; Schoenly *et al.* 1987; Marchenko, 1988; Marchenko, 2001). Their study in a medico-legal context is a part of the forensic entomology (Amendt *et al.* 2004; Amendt *et al.* 2007). Many published reports or reviews are focused on Diptera pattern colonization and neglect Coleoptera succession (Kocarek, 2003; Matuszewski *et al.* 2008; Midgley *et al.* 2009). Carrion beetles have been referenced to as being a part of the entomofaunal colonization of a dead body but very few studies have looked at them in a forensic context. However, the use of beetles in forensic entomology can be relevant (Kulshrestha *et al.* 2001; Watson *et al.* 2005; Midgley *et al.* 2009; Midgley *et al.* 2010). Carrion beetles can provide information on postmortem colonization on remains and time since death (Smith, 1986; Haskell *et al.* 1997; Watson *et al.* 2005). This review focuses on Palearctic carrion beetles (Coleoptera, Silphidae) that are carrion feeder or associated with decomposing remains.

2. Taxonomy and distribution

The family of Silphidae belongs to the superfamily of the Staphylinoidea and is divided into two subfamilies: the Nicrophorinae, called burying beetles or sexton beetles, and the Silphinae (Lawrence *et al.* 1982; Peck *et al.* 1993; Ratcliffe, 1996; Dobler *et al.* 2000; Sikes, 2005). Some taxonomists often include a third subfamily in the silphid beetles: the Agyrtinae (Madge, 1980; Hastir *et al.* 2001; Debreuil, 2003a; Debreuil, 2004a). However, recent phylogenetic analyses (Hansen, 1997; Newton, 1998; Dobler *et al.* 2000; Caterino *et al.* 2005) separate the Agyrtinae of other Silphidae and consider the Agyrtidae as a valid family into itself (Lawrence *et al.* 1982; Peck, 1990; Ratcliffe, 1996; Newton, 1997; Dobler *et al.* 2000; Caterino *et al.* 2005). The world fauna of Silphidae is currently composed of 183 species distributed in 15 genera (Ratcliffe, 1996; Peck, 2001; Sikes, 2005; Sikes, 2008). This family has a worldwide distribution, but is predominant in Holarctic regions (temperate regions) (Peck *et al.* 1985; Peck, 2001; Sikes, 2005). The Palearctic region is considered as the center of their distribution (Peck *et al.* 1985; Dobler *et al.* 2000). There are the most genera and the highest number of species of Silphidae in the Palearctic (Peck *et al.* 1985; Dobler *et al.* 2000). Carrion beetles are rare or absent in tropical regions because they are out-competed by ants, flies and vertebrates (Ratcliffe, 1996). Although, there are some Australian and Latin American endemic species (*Diamesus*, *Ptomophila*, *Nicrophorus mexico*) (Ratcliffe, 1996; Scott, 1998). Nicrophorinae are less widely distributed than Silphinae, being found in the temperate northern climate (Sikes, 2005; Sikes, 2008). Silphines seem to be more tolerant to warmer climate than the nicrophorines (Sikes, 2008). The subfamily of Silphinae has a greatest generic diversity (12 genera) than the Nicrophorinae (3 genera) (Sikes, 2005). In north Western Europe, there are 28 species of Silphidae: 11 species of Nicrophorinae and 17 species of Silphinae. Table 10 lists the species in Western Europe (Heinz, 1971; Hastir *et al.* 2001; Sikes *et al.* 2002; Debreuil, 2003a; Debreuil, 2003b; Debreuil, 2004a; Debreuil, 2004b; Debreuil, 2004c; Ružicka *et al.* 2004; Sikes, 2005). Among them, there are 22 species (11 *Nicrophorus* spp. and 11 Silphinae) that are carrion obligate or predacious species. There is only one genus of nicrophorine in the Western Palearctic: *Nicrophorus*. In the past, the spelling of this genus name varied from *Nicrophorus* to *Necrophorus* and back again to *Nicrophorus*, the valid genus name (Ratcliffe, 1996; Debreuil, 2004b), but it is not rare to see the wrong spelling in some publications.

Table 10. List of the Western European species of "carrion" beetles (including Mediterranean species).

	Subfamily	
	Nicrophorinae KIRBY, 1837	**Silphinae LATREILLE, 1807**
Genus	*Nicrophorus* FABRICIUS, 1775	*Ablattaria* REITTER, 1885 1884
		Aclypea REITTER, 1884
		Oiceoptoma LEACH, 1815
		Phosphuga LEACH, 1817
		Silpha LINNAEUS, 1758
		Thanatophilus LEACH, 1815
		Necrodes LEACH, 1815
		Dendroxena MOTSCHULSKY, 1858
Species	* *Nicrophorus germanicus* LINNAEUS, 1758	**Necrodes littoralis* LINNAEUS, 1758
	* *Nicrophorus humator* GLEDITSCH, 1767	**Thanatophilus dispar* HERBST, 1793
	* *Nicrophorus investigator* ZETTERSTEDT, 1824	**Thanatophilus rugosus* LINNAEUS, 1758
	* *Nicrophorus interruptus* STEPHENS, 1830	**Thanatophilus sinuatus* FABRICIUS, 1775
	* *Nicrophorus sepulchralis* HEER, 1841	**Oiceoptoma thoracicum* LINNAEUS, 1758
	* *Nicrophorus sepultor* CHARPENTIER, 1825	**Silpha carinata* HERBST, 1783
	* *Nicrophorus vespillo* LINNAEUS, 1758	**Silpha obscura obscura* LINNAEUS, 1758
	* *Nicrophorus vespilloides* HERBST, 1783	**Silpha tristis* ILLIGER, 1798
	* *Nicrophorus vestigator* HERSCHEL, 1807	**Silpha olivieri* BEDEL, 1887
	* *Nicrophorus nigricornis* FALDERMANN, 1835	**Silpha puncticollis* LUCAS, 1846
	* *Nicrophorus antennatus* REITER, 1884	**Silpha tyrolensis* LAICHARTING, 1781
		Phosphuga atrata atrata LINNAEUS, 1758
		Dendroxena quadrimaculata SCOPOLI, 1772
		Ablattaria laevigata laevigata FABRICIUS, 1775
		Aclypea opaca LINNAEUS, 1758
		Aclypea undata MULLER, 1776
		Aclypea souverbiei FAIRMAIRE, 1848

* : indicates necrophagous or predaceous species — *indique des espèces nécrophages ou prédatrices* (Heinz, 1971; Hastir et al., 2001; Sikes et al., 2002; Debreuil, 2003a; Debreuil, 2003b; Debreuil, 2004a; Debreuil, 2004b; Debreuil, 2004c; Ružicka et al., 2004; Sikes, 2005)

3. Anatomic and morphological descriptions

Silphid beetles are usually medium to large in size (7 to 45 mm) (Peck, 1990; Ratcliffe, 1996; Hastir *et al.* 2001; Debreuil, 2003a; Sikes, 2008). Although, adults and larvae vary greatly in size and shape (Byrd *et al.* 2009). Adults have an ovate (Silphinae) to moderately elongate shape with protuberant eyes (Sikes, 2005) (Figure 11). They are flattened (Silphinae) or strongly convex (Ratcliffe, 1996). Silphids are often darkened or have distinctive red-orange-yellow markings on the elytra (*Nicrophorus* spp.) that may serve as warning coloration (Ratcliffe, 1996; Hastir *et al.* 2001).

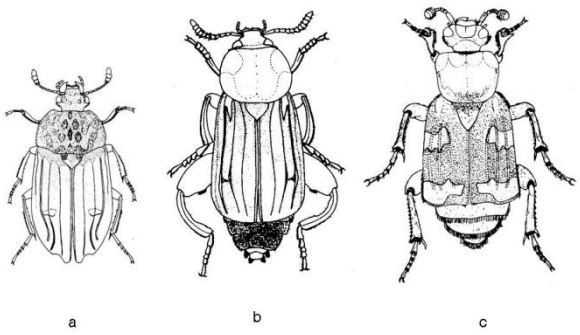

Figure 11. Habitus of Silphinae (a), (b) and Nicrophorinae (c); (a) *Thanatophilus sinuatus* ♀, (b) *Necrodes littoralis*, (c) *Nicrophorus interruptus*. (Source: Sustek, 1981).

The elytra are often short and leave several abdominal segments exposed (1 or 5 abdominal segments among the subfamily). The elytra are punctuate and truncate in *Necrode*s (Silphinae) and Nicrophorinae, not truncate in the remaining Silphinae (Sikes, 2005) (Figure 12).

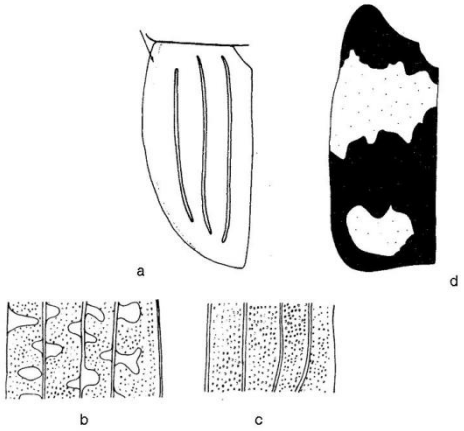

Figure 12. Left elytron of (a) Silphinae with three longitudinal costae and (d) Nicrophorinae with fasciae or maculae. Detail of the elytron of (b) *Thanatophilus rugosus* and (c) *Thanatophilus sinuatus*. (Source: Sustek, 1981).

The scutellum is often very large and the pronotum is enlarged (Peck, 1990; Sikes, 2005; Sikes, 2008). The antennae are constituted by eleven segments and capitate (abruptly clubbed) for Nicrophorinae or clavate (gradually clubbed) for Silphinae (Hastir *et al.* 2001) (Figure 13). The antennae are widely spread and inserted on the lateral side of head. They have often microsetae covering only apical three segments (segments 9 to 11) (Hastir *et al.* 2001). The abdomen with sternite 2 is not visible between hind coxae but visible laterally of metacoxae (Sikes, 2008). The tarsi or terminal portion of each leg has five segments (tarsi 5-5-5) (Peck, 1990; Hastir *et al.* 2001).

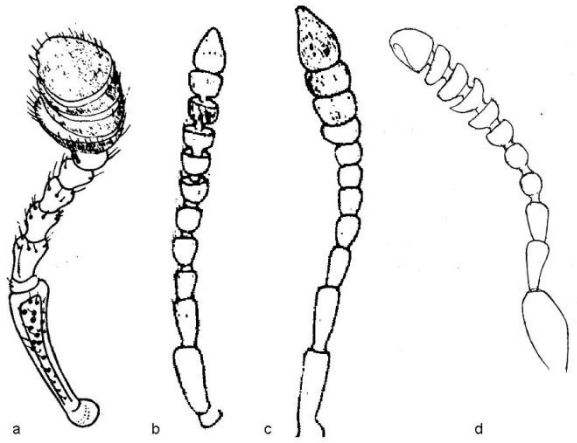

Figure 13. Antennae of (a) *Nicrophorus humator*, (b) *Aclypea undata*, (c) *Aclypea opaca* and (d) *Necrodes littoralis*. (source: a, b, c: Sustek, 1981; d: Portevin, 1926).

Silphid larvae are recognizable by the possession of a combination of mandible without a molar lobe; maxilla with broad, apically cleft mala bearing setae on outer lobe; and usually a two-segmented articulated urogomphi (Newton, 1991). Each subfamily has a very distinctive habitus (Newton, 1991) (Figure 14).

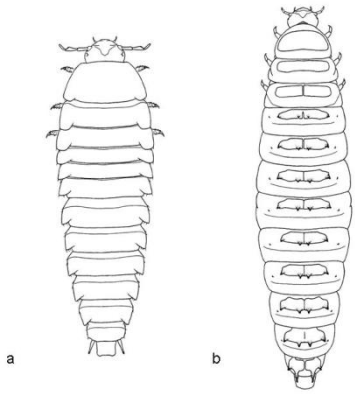

Figure 14. Larval habitus of (a) Silphinae and (b) Nicrophorinae. (source: Ratcliffe, 1996.)

3.1. Silphinae

Adults - Silphine species have often a darkened color and are dorsoventrally flattened (Figure 15). Their size is ranging from 8 to 25 mm (Debreuil, 2003a).The elytra have apices rounded or acute, not truncate or shortened (Peck, 1990; Ratcliffe, 1996). The elytra are usually costate or carinate (0-3 per elytron with 0 for the genus *Ablattaria*) (Peck, 1990; Debreuil, 2003a) but never striate (Figure 12a, 12b, 12c). The frontoclypeal suture is absent (Figure 16b) and the gular sutures are clearly separate, but strongly constricted medially (Peck, 1990; Sikes, 2005).

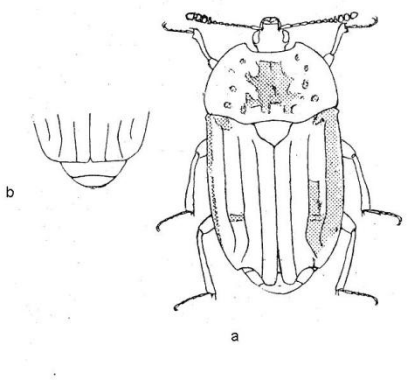

Figure 15. (a) *Thanatophilus sinuatus* ♀, (b) apex of ♂ *Thanatophilus sinuatus*. (source: Portevin, 1926.)

The antennae have eleven well differentiated segments broaden gradually; particularly the last three or four segments (Figure 13b, 13c, 13d).

***Larvae* -** Silphine larvae are campodeiform with body surfaces heavily pigmented and sclerotized (Newton, 1991; Sikes, 2005; Sikes, 2008) (Figure 14a). The body length of mature larvae is ranging from 12 to 40 mm (Newton, 1991). On each side of the head, there are 6 pigmented stemmata (ocelli). The anal lobes bear numerous fine teeth (Sikes, 2005). The tergites are large, laterally produced; each tergite is usually with posterior angles attenuated (Anderson *et al.* 1985; Ratcliffe, 1996).

3.2. Nicrophorinae

Adults - Nicrophorine species are darkened with red-orange markings (bands or spots), called fasciae or maculae, on elytra extending to the epipleura (Figure 11c), except for the species *Nicrophorus humator* and *Nicrophorus germanicus* which are completely darkened (black or brown) (Ratcliffe, 1996; Hastir *et al.* 2001; Sikes, 2008). Elytra have apices truncate (always smooth) and shortened exposing 3 or 4 abdominal segments (Peck, 1990; Ratcliffe, 1996) (Figures 11c and 12d). The frontoclypeal suture is present as a fine line (Peck, 1990; Sikes, 2005) (Figure 16a); the gular sutures are confluent posteriorly and reduce gula to a small triangular piece (Peck, 1990; Sikes, 2005).

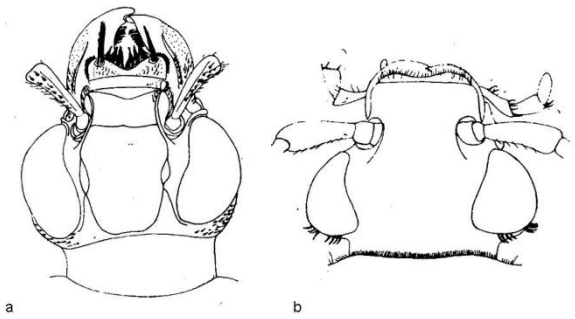

Figure 16. (a) Head of *Nicrophorus* species with clypeal membrane and (b) head of silphine species without frontoclypeal suture (source: Sustek, 1981).

On the fifth dorsal segment, there is a pair of stridulatory files in both sexes which is used for communication (Lane *et al.* 1965; Peck, 1990; Ratcliffe, 1996; Sikes, 2008). The antennae are clubbed, the second antennal segment (pedicel) is often reduced and fewer differentiated than the scape (appearing 10-segmented) (Peck, 1990) (Figure 13a). The compact club is constituted by the last four antennal segments (Peck, 1990; Ratcliffe, 1996; Hastir *et al.* 2001). In most *Nicrophorus* species, there is a sexual dimorphism in tarsomeres. The nicrophorine males possess expanded segments of the protarsus (foretarsus) (Peck, 1990; Ratcliffe, 1996).

Larvae - Nicrophorinae larvae are campodeiform or eruciform and body surfaces are lightly pigmented and unsclerotized with the exception of the head and legs (Newton, 1991; Ružicka, 1992; Sikes, 2005) (Figure 16b). The body length of mature larvae is ranging from 12 to 40 mm (Newton, 1991). The ventral surface is soft and creamy white (Anderson *et al.* 1985; Ružicka, 1992; Ratcliffe, 1996). On each side of the head, there is only one unpigmented stemma (Ratcliffe, 1996; Sikes, 2005). Contrary to Silphinae larvae, the anal lobes are without teeth (Newton, 1991; Sikes, 2005). The tergites are small; those on abdomen have 4 small spines (Anderson *et al.* 1985; Ratcliffe, 1996). For more detailed information about the morphology of larvae of *Nicrophorus*, Ružicka (1992) describes the immature stages of the following European species: *N. vespillo*, *N. vespilloides*, N. humator, *N. investigator*, *N.* (*fossor*) *interruptus*.

4. Ecology and biology

Carrion beetles (Coleoptera, Silphidae) perform vital ecosystem functions (Wolf *et al.* 2004); they promote the breakdown and recycling of organic matter into terrestrial ecosystems (Ratcliffe, 1996; Hastir *et al.* 2001; Kalinova *et al.* 2009). Tables 11 and 12 list the different ecological characteristics of Nicrophorinae and Silphinae Western European species based on the relevant literature (Portevin, 1926; Heinz, 1971; Anderson *et al.* 1984; Ružicka, 1994; Scott, 1998; Kocarek, 2001; Hastir *et al.* 2001; Gueorguiev *et al.* 2002; Kocarek, 2002; Ružicka, 2002; Sikes *et al.* 2002; Kocarek, 2003; Debreuil, 2003a; Debreuil, 2003b; Debreuil, 2004a; Debreuil, 2004b; Debreuil, 2004c; Ružicka *et al.* 2004; Aleksandrowicz *et al.* 2005; Chauvet *et al.* 2008). Silphidae are mainly carrion feeder (necrophagous species) or prey on other carrion inhabitants such as fly eggs or maggots and other carrion beetles (necrophilous species) (Racliffe, 1996; Hastir *et al.* 2001; Sikes, 2005; Sikes, 2008). There are some species of Silphinae that feed on soil invertebrates (snails, caterpillars or slugs predators: *Silpha* spp., *Dendroxena* spp.) or are phytophagous species (e.g. *Aclypea* spp.) (Sikes, 2005; Ikeda *et al.* 2007; Ikeda *et al.* 2008). Some Silphidae may be attracted by decaying fungi (e.g. *Phallus impudicus*), dung or rotten plant (Ratcliffe, 1996; Hastir *et al.* 2001; Sikes, 2005). Silphidae have reduced interspecific competition in partitioning species (niche differentiation) (Anderson, 1982; Peck, 1990; Ohkawara *et al.* 1998; Hocking *et al.* 2007): they have different temporal activities; some species are more active during spring whereas other species are active in summer, few species are active during autumn (Ružicka, 1994; Scott, 1998; Kocarek, 2001). In carrion beetles communities, niche differentiation can occur along dimensions of season, habitat (biotope) and carcass size (Scott, 1998; Hocking *et al.* 2007). The daily activity is also different, Silphidae are primarily nocturnal insect but some species are diurnal (e.g. *Thanatophilus* spp.) or crepuscular (Ohkawara *et al.* 1998; Scott, 1998; Kocarek, 2001). Habitat preferences are also different; some species are subservient to forest biotope (e.g. *O. thoracica*, *N. vespilloides*), whereas other species prefer open habitats (field/meadow species) (Scott, 1998; Kocarek, 2001). The type of carrion (age and carcass size) that they use is an important parameter (Anderson, 1982; Peck, 1990; Scott, 1998). Silphinae tend to use preferentially large vertebrate carcasses whereas Nicrophorinae prefer small carcasses (Anderson, 1982; Peck, 1990; Eggert *et al.* 1992). Both subfamilies have different reproductive strategy. Nicrophorinae have a surprising behavior for insects: the

biparental care of their offspring (Pukowski, 1933; Anderson, 1982; Scott, 1998; Smiseth *et al.* 2006). This is the highest level of sociability attained in the Coleoptera (Milne *et al.* 1976; Ratcliffe, 1996). At contrary, Silphinae show no parental care (Ratcliffe, 1996). Silphidae have particular relationships with nematodes and mites (phoresy) (Springett, 1968; Richter, 1993; Ratcliffe, 1996; Sikes, 2008). These relationships, poorly known, could be mutualism, commensalism or parasitism (Sikes, 2008).

Table 11. Ecological and morphological characteristics of Nicrophorinae of Western Europe (BE: Belgium, FR: France, Ge: Germany, Lu: Luxembourg, NL: Netherlands, DE: Denmark, GB: Great Britain, IT: Italy, PT: Portugal, SP: Spain, SZ: Switzerland).

Species	Distribution	Abundance	Body size (in mm)	Temporal activity	Habitat preference &/or location	Daily activity
Nicrophorus germanicus	BE-FR-GE-LU-NL-DE-GB-IT-SZ	rare	20-35	May-September	field, large cadaver, dung	nocturnal
Nicrophorus humator	BE-FR-GE-LU-NL-DE-GB-IT-PT-SP-SZ	very common	14-33	April-September	forest (hardwood), cadaver, rotten mushrooms	nocturnal
Nicrophorus investigator	BE-FR-GE-LU-NL-DE-GB-IT-SP-SZ	common	11-22	April-September	forest, open areas, cadaver	crepuscular
Nicrophorus interruptus	BE-FR-GE-LU-NL-DE-GB-IT-PT-SP-SZ	rare	10-22	April-October	field, sometimes in forest, cadaver	crepuscular
Nicrophorus sepulchralis	FR-IT-SZ	rare	19-21	N.A.	cadaver, European mountain species (Alps)	N.A.
Nicrophorus sepultor	DE-FR-GE-IT-NL-SP-SZ	very rare	11-22	April-September	forest, open areas, cadaver, rotten mushrooms	N.A.
Nicrophorus vespillo	BE-FR-GE-LU-NL-DE-GB-IT-PT-SP-SZ	common	10-23	May-September	field, open areas, sometimes in forest, cadaver	crepuscular, nocturnal
Nicrophorus vespilloides	BE-FR-GE-LU-NL-DE-GB-IT-SP-SZ	very common	9-19	April-September	forest, cadaver, rotten mushrooms	diurnal
Nicrophorus vestigator	BE-FR-GE-LU-NL-DE-GB-IT-PT-SP-SZ	common	9-23	April-October	field, open areas, cadaver	N.A.
Nicrophorus nigricornis	FR	rare	12-20	N.A.	cadaver, mountain species (Caucasius)	N.A.
Nicrophorus antennatus	FR-IT-NL	rare	N.A.	N.A.	cadaver	N.A.

BE: Belgium — *Belgique*; FR: France — *France*; Ge: Germany — *Allemagne*; Lu: Luxembourg — *Luxembourg*; NL: The Netherlands — *Pays-Bas*; DE: Denmark — *Danemark*; GB: Great Britain — *Grande-Bretagne*; IT: Italy — *Italie*; PT: Portugal — *Portugal*; SP: Spain — *Espagne*; SZ: Switzerland — *Suisse*. N.A.: not available data in the specific literature — *données indisponibles dans la littérature spécialisée* (Portevin, 1926; Heinz, 1971; Anderson et al., 1984; Ružicka, 1994; Scott, 1998; Kocarek, 2001; Hastir et al., 2001; Gueorguiev et al., 2002; Kocarek, 2002; Ružicka, 2002; Sikes et al., 2002; Kocarek, 2003; Debreuil, 2003a; Debreuil, 2003b; Debreuil, 2004a; Debreuil, 2004b; Debreuil, 2004c; Ružicka et al., 2004; Aleksandrowicz et al., 2005; Chauvet et al., 2008).

Table 12. Ecological and morphological characteristics of Silphinae of Western Europe (BE: Belgium, FR: France, Ge: Germany, Lu: Luxembourg, NL: Netherlands, DE: Denmark, GB: Great Britain, IT: Italy, PT: Portugal, SP: Spain, SZ: Switzerland).

Species	Distribution	Abundance	Body size (in mm)	Temporal activity	Habitat preference &/ or location	Daily activity	Trophic group
Necrodes littoralis	BE-FR-LU-GE-NL-SP-DE-GB-IT-PT-SZ	common	15-25	April-September	forest or open areas, cadaver (large carcass), below seaweeds	N.A.	necrophagous predaceous
Thanatophilus dispar	BE-FR-GE-NL-DE-GB-IT-SZ	rare	8-11	May-July	field, cadaver	diurnal	necrophagous
Thanatophilus rugosus	BE-FR-LU-GE-NL-DE-GB-IT-PT-SP-SZ	common	9-12	April-September	field, open areas, cadaver	diurnal	necrophagous predaceous
Thanatophilus sinuatus	BE-FR-LU-GE-NL-DE-GB-IT-PT-SP-SZ	very common	9-12	April-September	field, open areas, cadaver	diurnal	necrophagous predaceous
Oiceoptoma thoracicum	BE-FR-LU-GE-NL-DE-GB-IT-SP-SZ	common	11-16	April-September	forest, cadaver, dung, mushrooms (*e.g.* *Phallus* sp.)	diurnal	necrophagous predaceous
Silpha carinata	BE-FR-LU-GE-NL-DE-GB-IT-SZ	common	13-20	April-September (imago hibernates under barks of trees)	forest, cadaver, crushed slugs and snails, mushrooms	N.A.	necrophagous predaceous
Silpha obscura obscura	BE-FR-LU-GE-NL-DE-GB-IT-SP-SZ	common	11.5-17	May-September (imago hibernates in litter & soil)	field, open areas	N.A.	predaceous necrophagous
Silpha tristis	BE-FR-LU-GE-NL-DE-GB-IT-PT-SP-SZ	rare	13-15	May-September (imago hibernates in litter & soil)	field	N.A.	predaceous necrophagous
Silpha olivieri	FR-IT-PT-SP	rare	15-19	Mid March-Mid September	Mediterranean species	N.A.	predaceous
Silpha puncticollis	FR-IT-PT-SP	rare	14-17	Mid March-Mid September	Mediterranean species	N.A.	predaceous
Silpha tyrolensis	FR-GB-IT-PT-SP	rare	13-14	Mid March-Mid September	mountain species	N.A.	predaceous
Phosphuga atrata atrata	BE-FR-KU-GE-NL-DE-GB-IT-PT-SP-SZ	very common	9-16	May-October (imago hibernates under a bark, moss or litter)	forest	N.A.	predaceous (snails)
Dendroxena quadrimaculata	BE-FR-KU-GE-NL-DE-GB-IT -SP-SZ	common	11-14	May-July	forest (deciduous forest)	N.A.	predaceous (caterpillars)
Ablattaria laevigata laevigata	BE-FR-LU-GE-NL-GB-IT-SP-SZ	rare	11-16	May-October	field, gardens	N.A.	predaceous (snails)
Aclypea opaca	BE-FR-LU-GE-NL-DE-GB-IT-SP-SZ	common	9-12	April-September	field	N.A.	phytophagous (Chenopodiaceae, pest of sugar beets)
Aclypea undata	BE-FR-LU-GE-NL-DE-GB-IT-PT-SP-SZ	rare	11-16	April-September	field	N.A.	phytophagous (Chenopodiaceae)
Aclypea souverbiei	FR-GE-SP	rare	10-12	N.A.	N.A.	N.A.	phytophagous

legend — *légende*: see **table 2** - *voir* ***tableau 2***.

4.1. Silphinae

Contrary to the Nicrophorinae, little is known about the biology and ecology of the Silphinae (Ratcliffe, 1996; Hoback *et al.* 2004; Ikeda *et al.* 2007). Concerning necrophagous silphine species, females are semelparous and lay their eggs in or on the soil around large vertebrate carcasses and provide no care of their offspring (Sikes, 2005; Ikeda *et al.* 2008). Silphine appear usually on large carcasses (> 300 g) (Peck, 1990; Sikes, 2005) because these provide sufficient food resource for the great number of beetles that may be present (Anderson, 1982; Watson *et al.* 2005). Eggs hatch in 4-5 days and silphine larvae feed on carrion remains (Anderson, 1982). They may also compete with nicrophorine species for small vertebrate carcasses that they use for feed but not for reproduction and larval development (Bishop, 2001; Hoback *et al.* 2004). Silphinae colonize a carcass during the early or mid-stage of decay

and thus compete with flies (Diptera) for the food resource (Payne, 1965; Anderson, 1982). Contrary to Nicrophorinae, there are some flightless silphine species or some flight-dimorphic species (Ikeda *et al.* 2007; Ikeda *et al.* 2008).

4.2. Nicrophorinae

Many studies on burying beetles behavior have been published since the pioneer work of Pukowski (Pukowski, 1933; Milne *et al.* 1976; Sikes, 2005). More than 150 behavioral ecology studies on the Nicrophorus spp. were conducted in the past 25 years (Sikes, 2008). The reviews of Milne *et al.* (1976), Ratcliffe (1996), Scott (1998) or Sikes (2005; 2008) provide detailed information about the nicrophorine (or Silphidae) ecology. Burying beetles specialized on carrion (necrophagous and necrophilous species) are subsocial (Pukowski, 1933; Milne *et al.* 1976; Trumbo *et al.* 1993; Ohkawara *et al.* 1998; Scott, 1998). Nicrophorus species use small vertebrate carcasses (< 300 g, usually < 100 g) such as rodents or birds that they bury and prepare for rearing offspring (Pukowski, 1933; Milne *et al.* 1976; Trumbo, 1990b; Scott, 1998; Smith *et al.* 2001; Sikes, 2005; Sikes, 2008). When the carcass is found by a single pair of male and female, they search a suitable spot for burial that is usually completed in 5 to 8 hours during the night (Ratcliffe, 1996; Scott, 1998). Carcasses are often located by several individuals of both sexes (Pukowski, 1933; Müller *et al.* 1998; Steiger *et al.* 2009). In this case, fighting occurs between individuals for the ownership of the carcass by a single male-female pair (Pukowski, 1933; Müller *et al.* 1998; Steiger *et al.* 2009). Searching behavior is guided by olfaction; burying beetles have sensitive chemosensors located on their antennae adapted to detect the smell of a recently dead animal (Shubeck, 1975; Bartlett, 1987; Peck, 1990; Ratcliffe, 1996; Kalinova *et al.* 2009; Steiger *et al.* 2009). If a male discovers a suitable carcass for reproduction, it emits a sexual pheromone to attract the female (Eggert *et al.* 1989; Eggert, 1992; Ohkawara *et al.* 1998). After the burial [10-20 cm depth (Hastir *et al.* 2001)], Nicrophorine removes the skin (fur or feathers) and the remains are fashioned into a compact ball. Then, they inoculate the carrion ball with oral and anal secretions that have antimicrobial properties to delay the decomposition process (Ratcliffe, 1996; Eggert *et al.* 1997; Scott, 1998; Hoback *et al.* 2004; Rozen *et al.* 2008; Cotter *et al.* 2010) and remove also fungi (Scott, 1998). The female makes a chamber above the carrion ball in which it lays 10-50 eggs. Both parents regurgitate food in this crypt for feeding their larvae. Larvae may also feed directly on the surface of the carrion ball (Ratcliffe, 1996; Ohkawara *et al.* 1998; Sikes, 2008).

The larvae receive parental care during their entire development (Ratcliffe, 1996; Scott, 1998). Parents provide extensive care: they feed their offspring, they protect them from predators and intruding burying beetles (inter- and intraspecific competitions) and they maintain a pathogen free nest with preservative secretions (Ratcliffe, 1996; Scott, 1998; Smiseth *et al.* 2006). Female stays on the crypt until complete larval development (1-4 weeks), whereas the male abandons the brood a few days earlier (Trumbo, 1991; Müller *et al.* 1998). If the brood is too large for a successful development, adults may regulate brood size by selective cannibalism. They kill smaller larvae during the first 24 h after hatching (Trumbo, 1990a; Ratcliffe, 1996). After one week, the larvae have consumed the entire carrion ball and pupate in the nearby soil during two weeks (Ratcliffe, 1996; Ohkawara *et al.* 1998). Then, adults emerge and the overwintering occurs in the adult stage or in the pre-pupal stage for some species (Ratcliffe, 1996; Ohkawara *et al.* 1998). Sometimes, nicrophorine may colonize large carcass (too large to bury) and several male-female pairs breed communally their larvae in a subsocial fashion: cooperative breeding (Pukowski, 1933; Eggert *et al.* 1992; Ratcliffle, 1996; Scott, 1998).

5. The utility of carrion beetles in forensic entomology

Most forensic researches have focused on flies while beetles have been neglected (Midgley *et al.* 2009; Midgley *et al.* 2010). When a corpse colonized by insects is found, two situations could be considered (Amendt *et al.* 2007; Lefebvre *et al.* 2009). In the first situation, which is the most frequent case in forensic investigations, insects are pioneer species and the minimum postmortem interval (PMI) is estimated with the age of the oldest specimens found on the death scene, principally blowflies (Amendt *et al.* 2007; Lefebvre *et al.* 2009). In the second situation, later necrophagous species colonize the corpse with a delay, often after the departure of pioneer species. The estimation of the PMI is only possible by analyzing the chronological succession (Amendt *et al.* 2007; Lefebvre *et al.* 2009). A frequent objection against the use of Coleoptera in forensic investigations is the fact that flies (pioneer species) locate corpses faster than beetles (later necrophagous species). Thus, the minimum postmortem interval estimates are more accurate with Diptera, especially with the families of Calliphoridae and Sarcophagidae (Smith, 1986; Midgley *et al.* 2010). However, recent researches have shown that some Silphidae (*e.g. Thanatophilus micans* FABRICIUS) can locate a corpse within 24 h and their larvae have been observed soon after death, during the

early stage of decomposition (Midgley *et al.* 2009; Midgley *et al.* 2010). This implies that some carrion beetles have the same forensic interesting characteristics than carrion flies and can be considered as pioneer species. In this case, some species of Coleoptera can be used as reliable forensic indicators such as blowflies (Midgley *et al.* 2009; Midgley *et al.* 2010). However, there is no available information about early postmortem colonization by European carrion beetles such as in South Africa with *T. micans*. Some recent publications (Matuszewski *et al.* 2008; Matuszewski *et al.* 2010) associate the silphid activity on carcasses during the active decay stage, primarily for Silphinae (*N. littoralis*, *Thanatophilus* spp.). The most important application of insects in forensic investigations is the estimation of the minimum PMI (Greenberg, 1991; Amendt *et al.* 2004; Amendt *et al.* 2007). These minimum PMI estimates are primarily based on the duration of immature stages of Diptera (development models) (Amendt *et al.* 2007). Contrary to flies, there are few studies on the rates of development of Coleoptera with forensic interest (Midgley *et al.* 2009; Midgley *et al.* 2010). For example, Midgley *et al.* (2009) studied the development of *T. micans* at ten constant temperatures. They established a robust statistical model of development for this common African species. Currently, there is no development model ("size-at-age data") for forensically relevant European silphids. However, research on development of Coleoptera with a forensic interest can be a useful tool for medico-legal entomologists (Midgley *et al.* 2010). In addition, carrion beetles have generally a longer life cycle than forensic Diptera (Midgley *et al.* 2009; Midgley *et al.* 2010). They can colonize a corpse during later decay stages when many maggots have already left the corpses (Kocarek, 2003; Matuszewski *et al.* 2008; Midgley *et al.* 2010). The PMI estimates can be established by analyzing the arthropod community present on a corpse including many Coleoptera during the later stage of decomposition (Smith, 1986). However, the biology and ecology of most forensically relevant species of Coleoptera are unknown (Midgley *et al.* 2010). To increase the accuracy and the validity of the PMI estimates based on ecological successions, there is a necessity to generate data on insect succession and insect seasonal activity on carrion in specific geographic regions and various biotopes within these regions (Catts *et al.* 1992; Amendt *et al.* 2004; Sharanowski *et al.* 2008; Lefebvre *et al.* 2009). All carrion beetles do not have the same forensic interest; species of Silphinae seem to have a more important value as forensic bioindicators (Watson *et al.* 2005; Matuszewski *et al.* 2010). Indeed they have ecological preferences for small vertebrate carcasses, while Nicrophorinae present less interest in forensic entomology

(Watson *et al.* 2005). However, *Nicrophorus* spp. could be frequently found on human corpses, including in houses (Chauvet *et al.* 2008). This is an inventory extracted from 700 real forensic cases that occurred during 15 years in France. Midgley *et al.* (2010) suggest to focus on the biology of both Silphinae (*Silpha* and *Thanatophilu*s) while Matuszewski *et al.* (2010) highlight the forensic usefulness of the following silphine species: *Necrodes littoralis* (larvae and adults), *Thanatophilus* spp. (larvae and adults) and *O. thoracica* (larvae). In some cases, necrophagous beetles can also provide information on the presence of drugs or poisons by bioaccumulation (entomotoxicology) (Bourel *et al.* 2001; Introna *et al.* 2001; Carvalho, 2010). Adults, larvae or beetle remain such as exuviae, puparial cases or fecal material of Coleopterans may be used for toxicological analysis when conventional toxicological samples (blood, urine, internal organs) are not available (Miller *et al.* 1994; Bourel *et al.* 2001; Introna *et al.* 2001; Carvalho, 2010).

6. Conclusion

The potential uses of European carrion beetles as bioindicators in forensic entomology are obvious. Silphids and principally burying beetles (*Nicrophorus* spp.) are widely studied in various contexts including biology and ecology (Ratcliffe, 1996; Scott, 1998). Indeed, carrion beetles are poorly studied in a forensic context (Midgley *et al.* 2010). Nevertheless, their use in forensic investigations can be relevant (Watson *et al.* 2005; Midgley *et al.* 2009; Midgley *et al.* 2010). Are there some European carrion beetles with forensically interesting characteristics? Before creating development model for Paleartic Silphidae of forensic value, forensic entomologists need to increase data on carrion beetle's ecology and insect succession.

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II.3.2. Carrion beetles visiting pig carcasses during early spring in urban, forest and agricultural biotopes of Western Europe

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Abstract - Carrion beetles are important in terrestrial ecosystems, consuming dead mammals and promoting the recycling of organic matter into ecosystems. Most forensic studies are focused on succession of Diptera while neglecting Coleoptera. So far, little information is available on carrion beetles postmortem colonization and decomposition process in temperate biogeoclimatic countries. These beetles are however part of the entomofaunal colonization of a dead body. Forensic entomologists need databases concerning the distribution, ecology and phenology of necrophagous insects, including silphids. Forensic entomology uses pig carcasses to surrogate human decomposition and to investigate entomofaunal succession. However, few studies have been conducted in Europe on large carcasses. The work reported here monitored the presence of the carrion beetles (Coleoptera: Silphidae) on decaying pig carcasses in three selected biotopes (forest, crop field, urban site) at the beginning of spring. Seven species of Silphidae were recorded: *Nicrophorus humator* (Gleditsch), *Nicrophorus vespillo* (L.), *Nicrophorus vespilloides* (Herbst), *Necrodes littoralis* L., *Oiceoptoma thoracica* L., *Thanatophilus sinuatus* (Fabricius), *Thanatophilus rugosus* (L.). All of these species were caught in the forest biotope, and all but *O. thoracica* were caught in the agricultural biotope. No silphids were caught in the urban site.

Key Words - Silphidae, carrion ecology, decomposition, forensic entomology, insect succession

1. Introduction

Carrion beetles (Coleoptera: Silphidae) perform vital ecosystem functions (Wolf and Gibbs 2004) by promoting the breakdown and recycling of organic matter into terrestrial ecosystems (Ratcliffe 1996; Hastir and Gaspar 2001; Kalinova *et al.* 2009). Most silphids are carrion feeders (necrophagous species) or prey on other carrion inhabitants such as fly eggs or maggots and other carrion beetles (necrophilous species) (Ratcliffe 1996; Hastir and Gaspar 2001; Sikes 2005, 2008). The necrophagous insects, including flies and carrion beetles, have particular relationships with decomposing remains of vertebrate carcasses that constitute a rich, but ephemeral resource (Anderson and VanLaerhoven 1996; Grassberger and Frank 2004; Carter *et al.* 2007). These specialized insects are attracted to the cadaver that they colonize in a relative predictable sequence called the entomofaunal succession or insect succession (Megnin 1894; Putman 1983; Schoenly and Reid 1987; Marchenko 1988, 2001; Benecke 2004). Study of these insects in a medico-legal context is part of forensic entomology (Hall 1990; Amendt *et al.* 2004). Many forensic entomological studies have been conducted on pig carcasses as surrogate human models for physiological, ethical and economical reasons (Rodriguez and Bass 1983; Catts and Goff 1992; Anderson and VanLaerhoven 1996; Grassberger and Frank 2004; Hart and Whitaker 2005), but few were conducted in Europe with pig carcasses (Grassberger and Frank 2004; Garcia-Rojo 2004; Wyss and Cherix 2006; Matuszewski *et al.* 2008). Many published reports are focused on Diptera pattern colonization and very few looked at Coleoptera succession (Kocarek 2003; Matuszewski *et al.* 2008; Midgley and Villet 2009, Midgley *et al.* 2010). However, the use of beetles in forensic entomology can be relevant (Kulshrestha and Satpathy 2001; Midgley *et al.* 2010). Families of beetles of forensic importance are Silphidae (carrion beetles), Dermestidae (larder, skin or hide beetles), Staphylinidae (rove beetles), Histeridae (clown or hister beetles), Cleridae (checker beetles) and Nitidulidae (sap beetles) (Haskell *et al.* 1997; Byrd and Castner 2001; Wyss and Cherix 2006). Among them, carrion beetles can provide information on postmortem colonization on remains and time since death (Haskell *et al.* 1997; Smith 1986; Watson and Carlton 2005). So far, little information is available on carrion beetles postmortem colonization and the process of decomposition in temperate biogeoclimatic countries.

The world fauna of Silphidae is composed of fewer than 200 species distributed in 15 genera (Portevin 1926; Peck 2001; Sikes 2005). This family has a Holarctic distribution (Peck 2001). In Western Europe, this family is divided in two subfamilies: Nicrophorinae (i.e. burying

beetles) with eleven species and Silphinae including seventeen species (Portevin 1926; Du Chatenet 1986; Hastir and Gaspar 2001; Debreuil 2003a,b, 2004a,b,c). There are seven species of Nicrophorinae and thirteen species of Silphinae reported in Belgium (Hastir and Gaspar 2001; Ružicka and Schneider 2004). Many forensic entomological papers highlight the necessity to generate data on insect succession and insect seasonal activity on carrion in specific geographic regions and various biotopes within these regions (Catts and Goff 1992; Byrd and Castner 2001; Amendt *et al.* 2004; Sharanowski *et al.* 2008). This paper identifies the early activity of silphids that occur on large carcasses in a temperate biogeoclimatic region in three different biotopes (forest, agricultural and urban site).

2. Methods and Materials

2.1. Sites and study period

This study was conducted during spring 2007 (29 March - 11 May) in three distinct biotopes: a forest biotope, an agricultural biotope and an urban site, located in Belgium. The forest habitat consisted of pedonculate oaks, *Quercus robur* L. (Fagales: Fagaceae), European beeches, *Fagus sylvatica* L. and sycamore maples, *Acer pseudoplatanus* L. (Sapindales: Sapindaceae). The agricultural biotope was a transect (5 meters width) of meadow with an alignment of willows (*Salix* sp.) between a barley *Hordeum vulgare* L. (Poales: Poaceae) field and an enclosed grassland. The meadow was not grazed for the duration of the experiment. The urban biotope was an abandoned building of two floors with broken windows and inside vegetation *Clematis vitalba* L. (Ranunculales: Ranunculaceae). The building was located on a secure site belonging to the National Institute of Criminalistic and Criminology (INCC-NICC, Brussels, Belgium).

2.2. Animal model

Six piglets, *Sus domesticus* L. (Artiodactyla: Suidae), (25 Kg) were killed by penetrative captive bolt (fractured skull) and disposed in the experimental sites within the next 4 hours. Immediately after the euthanasia, the pig carcasses were packed in double plastic bag to avoid any insect colonization before being placed in the experimental biotope. In each site, two pig carcasses were placed 50 meters from each other, in metal mesh cages (180 cm x 90 cm x 90 cm) to avoid scavenging by vertebrate carnivores.

2.3. Insect collection and identification

In order to quantify insect colonization on pig carcasses, pitfall traps and yellow traps were used to collect sarcosaprophagous insects. For continuous surveillance, six pitfall traps (glass jars of 15 cm in height and 8 cm in diameter) and two yellow traps (plastic container of 9 cm in height and 27 cm in diameter), both filled with soapy water, were placed around each carcass. The disposition of the pitfall traps flush to the surface, was the following: two near the ventral face, two near the dorsal face, one near the head and one near the anus. One yellow trap was placed near the head and the other was placed near the anus (Figure 17).

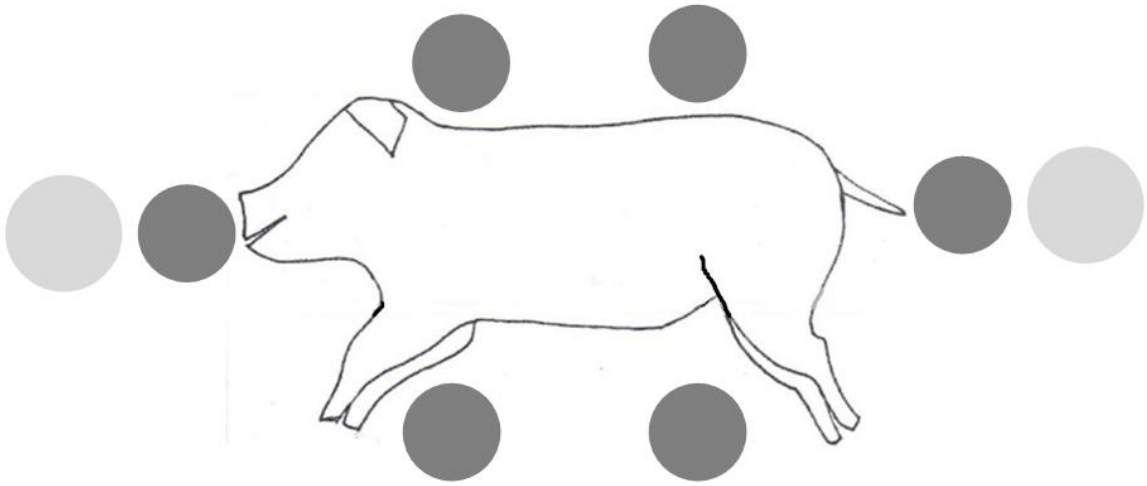

Figure 17. Disposition of the traps around the pig carcass (dark grey,pitfall traps; light grey, yellow traps).

The insect traps were removed every four days and the collected specimens were conserved in 80% norvanol D (ethanol denatured with ether). Only the adult stages were included in the counting of collected insects during this study.

Silphidae specimens were determining using different identification keys (Hastir and Gaspar 2001; Debreuil 2003a,b, 2004a,b,c) and reference collections from the entomological conservatory at Gembloux Agro-Bio Tech, University of Liege (Department of Functional and Evolutionary Entomology).

2.4. Environmental parameters

As temperature is one of the most important parameters influencing the decomposition rate (Vass *et al.* 1992; Gill-King 1997; Anderson 2001; Vass 2001; Campobasso *et al.* 2001; Vass *et al.* 2002), the ambient air temperature was automatically measured once an hour using a datalogger (Testo 175-T1, www.testo.com) placed on the lateral side of each cage. The daily

mean temperature was calculated on the basis of ambient air temperature recorded on a time interval of 24 hours. Other environmental parameters (humidity, wind velocity, wind direction) were recorded thanks to a Vantage Pro Plus™ Stations (Davis instruments, www.davis.com).

2.5. Statistical analyses

Statistical analyses were conducted with R v2.8.0 (www.r-project.org). The number of captured individuals of the two pigs was summed prior to statistical analysis. A generalized linear model with a Poisson error distribution on the absolute count of captured individuals by species and date was first adjusted to confirm the effects of the biotope on the profile of the different species of Silphidae trapped. As the *Thanatophilus* genus was the most abundant genus caught, this model was applied to the *Thanatophilus* genus only. Profiles of the two species of *Thanatophilus* trapped showed similarities within a biotope that were hidden by differences in the total abundance of the species in the sample. That bias was eliminated by fitting a general linear model on the relative abundance of each species, calculated by dividing each insect count by the total abundance of that species for a particular biotope.

3. Results

3.1. Environmental parameters

The mean atmospheric temperature measured during the decompositional process was 13.22° C for the forest site, 13.76° C for the agricultural site and 16.25° C for the urban site (Figure 18).

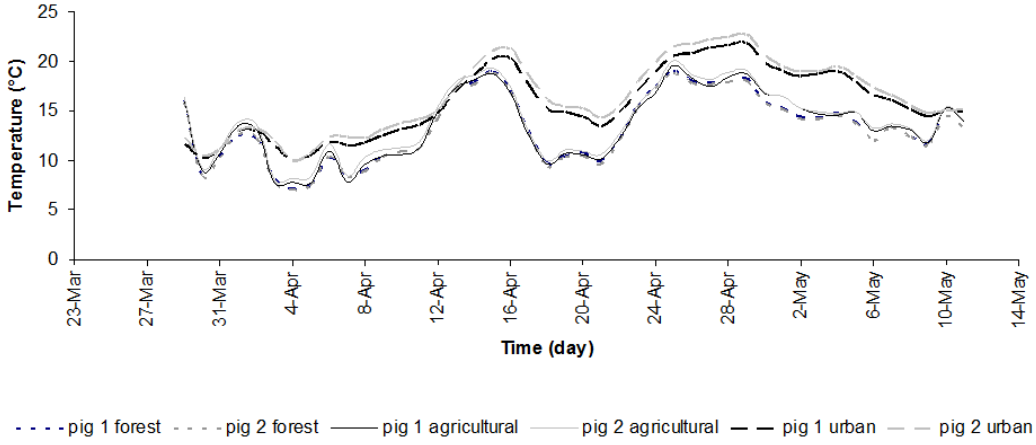

Figure 18. Temperature recordings on the six carcasses ("pig 1 forest" for the first pig carcass on the forest site, "pig 2 forest" for the second carcass on the forest site, "pig 1 agricultural" for the first pig on the agricultural site and "pig 2 agricultural" for the second carcass on the agricultural site, "pig 1 urban" and "pig 2 urban" respectively for the firsta and aad the second carcass in the urban site)..and the second carcass in the urban site)

The temperature curves had a similar pattern over time, but the urban site was warmer than the other sites: there was a difference of 3.03° C compared to the forest biotope and 2.49° C compared to the agricultural site. The mean relative humidity was 68.30 % for the "open-air" biotopes and 62.00 % for the urban site.

3.2. Carrion beetles

Seven species of Silphidae were identified during the sampling period, three Nicrophorinae: *Nicrophorus humator* (Gleditsch), *Nicrophorus vespillo* (L.), *Nicrophorus vespilloides* (Herbst) and four Silphinae: *Necrodes littoralis* L., *Oiceoptoma thoracica* L.; *Thanatophilus sinuatus* (Fabricius), *Thanatophilus rugosus* (L.).

In total, 1579 individuals were collected at the beginning of spring. Regardless of the biotope, the subfamily of Silphinae (1402 specimens) was more represented than the Nicrophorinae with 177 collected specimens. Silphidae were not found in the urban habitat. Six species of Silphidae were trapped in the agricultural biotope: *N. humator*, *N. vespillo*, *N. vespilloides, N. littoralis, T. sinuatus* and *T. rugosus*. In the forest biotope, seven species of Silphidae were collected: *N. humator, N. vespillo, N. vespilloides, N. littoralis, O. thoracica, T. sinuatus* and *T. rugosus*. More individuals were caught in the agricultural biotope (with a total of 960 specimens) than in the forest biotope (619 individuals) (Figure 19).

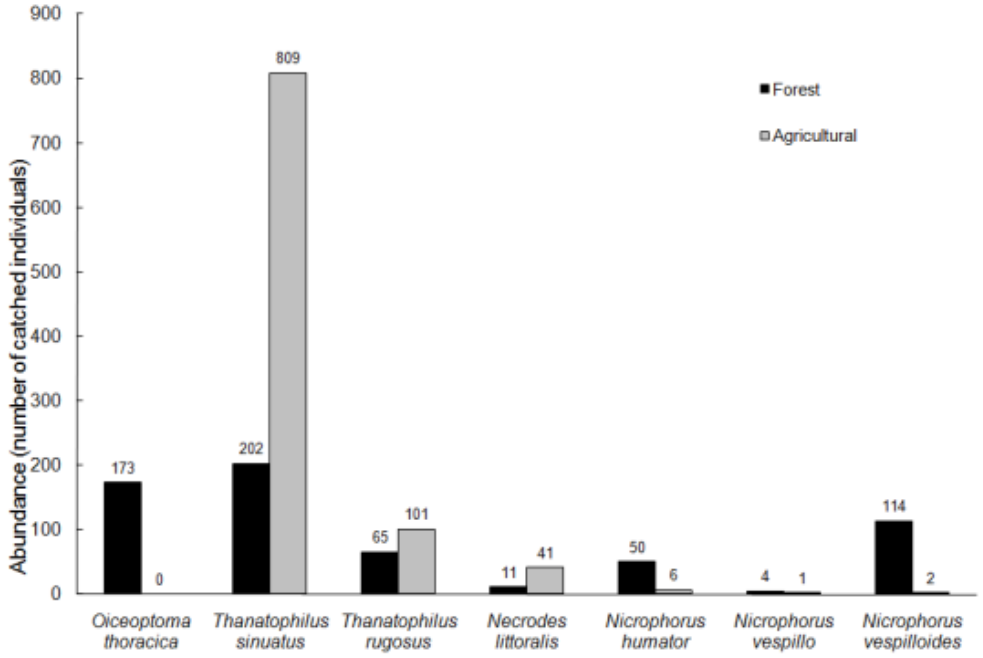

Figure 19. Absolute abundances of Silphidae according to the forest biotope (in dark) and the agricultural biotope (in grey).

Silphinae were more abundant in the agricultural biotope with a total of 951 trapped individuals (60.3% of the total of Silphidae) than in the forest habitat with 451 individuals (28.6%). The Nicrophorinae were more abundant in the forest biotope (168 individuals corresponding to 10.6% of the total of Silphidae) than in the field habitat with only 9 collected individuals (0.6%). The profiles for all species of collected Silphidae are shown in Figure 20.

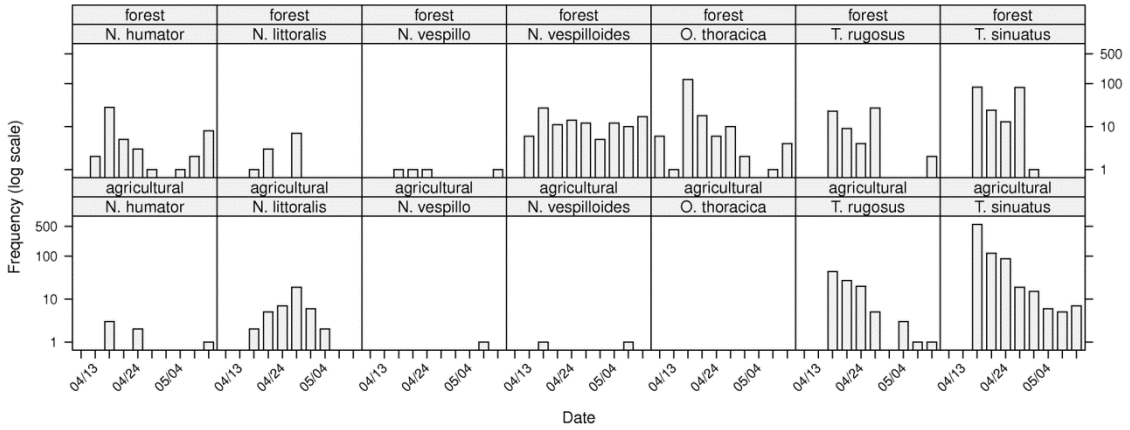

Figure 20. Profiles of Silphidae trapped for the forest and the agricultural biotopes (X-axis represents the time by chronological sampling dates and Y-axis represents the frequency (logarithmic scale).

The generalized linear model for all species of Silphidae confirmed significant differences of the profiles between collected species ($\chi^2 54 = 451.4$, $p < 0.001$) and between habitats ($\chi^2 9 = 200.1$, $p < 0.001$). In both habitats, *T. sinuatus* was the most abundant species with 809 individuals collected in the agricultural biotope and 202 trapped individuals in the forest habitat. The generalized linear model for the members of the genus *Thanatophilus* trapped confirmed significant differences of the profiles between habitats ($\chi^2 9 = 252.5$, $p < 0.001$). Despite the similarities observed in Figure 21, the profiles of collections of the two *Thanatophilus* species showed significant differences ($\chi^2 9 = 28.6$, $p < 0.001$). Since the model is additive and based on absolute counts, this could be a consequence of the significant difference in the total abundance of the two species in the collected sample ($\chi^2 1 = 676.1$, $p < 0.001$). As expected, the analysis of the relative abundance of the two *Thanatophilus* species (general linear model for the genus *Thanatophilus*) did not show any significant differences between the relative profiles of the two species ($F_{9,9} = 2.183$, $p = 0.130$). But the relative profiles (Figure 21) of the *Thanatophilus* species trapped were still significantly different between agricultural and forest habitats ($F_{9,9} = 13.03$, $p < 0.001$).

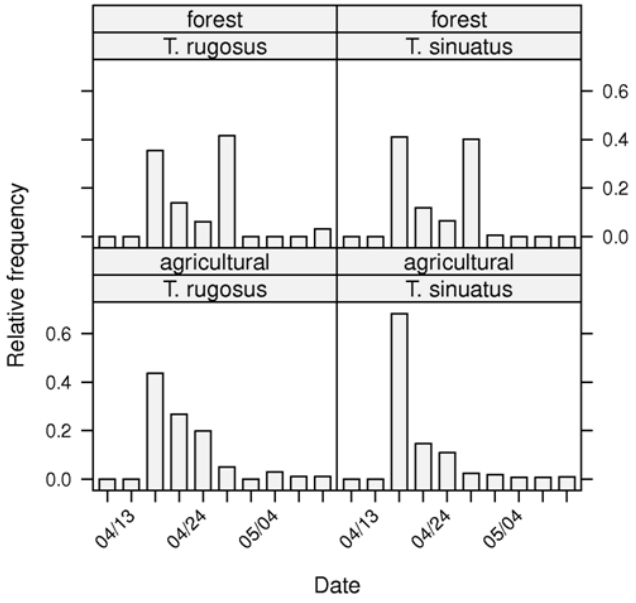

Figure 21. Profiles of members the Thanatophilus genus trapped in the forest and the agricultural biotopes. X-axis represents the time by chronological sampling dates and Y-axis represents the relative frequency.

The species *O. thoracica* was exclusively found in the forest habitat (173 individuals). *N. littoralis* (41 specimens) and *T. rugosus* (101 specimens) were more abundant in the agricultural biotope.

Among the Nicrophorinae, N. *vespilloides* was the most abundant species and represented 65.5% of the total of Nicrophorinae, followed by *N. humator* (31.6%) and *N. vespillo* (3.0%).

4. Discussion

4.1. Carrion beetles

The absence of Silphidae in the urban biotope can be due to the fact that carcasses were deposited in the second floor of a building in an industrial site. Few forest patches are available for native entomofauna within urban ecosystems (Wolf and Gibbs 2004). The forest fragmentation and urbanization alter the diversity of burying beetles communities (Wolf and Gibbs 2004). Nevertheless, Chauvet *et al.* (2008) listed Silphidae (*N. humator*) in houses on human cadavers from in May in France.

Among the seven species of *Nicrophorus* referenced in Belgium (Hastir and Gaspar, 2001; Ružicka and Schneider, 2004), three of them were observed in early spring: *N. humator*, *N. vespillo* and *N. vespilloides*, while *N. germanicus*, *N. investigator*, *N. interruptus* and *N. vestigator* were not collected. However, these four *Nicrophorus* species are less common and

are active later in the season (Hastir and Gaspar 2001; Aleksandrowicz 2005). For example, *N. germanicus* is a rare species and has a very localized distribution (Hastir and Gaspar 2001). Among the Belgian Silphinae, eight species are carrion feeders (Hastir and Gaspar 2001). Among the Belgian Silphinae, eight species are carrion feeders (necrophagous species) or predators (necrophilous species).

Table 13 compares the species of Silphidae referenced in the literature (in Europe) (Leclercq and Verstraeten 1992; Ružicka 1994; Leclercq 1996; Bourel *et al.* 1999; Kocarek 2003; Garcia-Rojo 2004; Grassberger and Frank 2004; Wyss and Cherix 2006; Chauvet *et al.* 2008; Matuszewski *et al.* 2008; Zdemir and Sert 2009) and species caught in this study.

Table 13. List of the 7 species of Silphidae collected in this study and comparison with literature about insect succession on various types of carcass in Europe.. ▲ referenced, - not referenced. * indicates human corpses. N.i. not included in the mentioned study.

			Silphidae						
	European country	Type of carcass	*Nicrophorus humator*	*Nicrophorus vespillo*	*Nicrophorus vespilloides*	*Necrodes littoralis*	*Oiceoptoma thoracica*	*Thanatophilus sinuatus*	*Thanatophilus rugosus*
Present study	Belgium	pig	▲	▲	▲	▲	▲	▲	▲
Nabaglo 1973	Poland	bank vole	▲	–	▲	–	▲	–	–
Kentner and Streit 1990	Germany	rat	▲	▲	▲	▲	▲	▲	▲
Leclercq and Verstraeten 1992	France	lama	–	–	–	▲	–	–	–
Ružicka 1994	Czech Republic	pitfall traps	▲	▲	▲	▲	▲	▲	▲
Leclercq 1996	France	wild boar	–	–	–	–	–	–	–
Bourel et al. 1999	France	rabbit	▲	▲	–	–	▲	▲	▲
Kocarek 2003	Czech Republic	rat	▲	–	▲	–	▲	▲	▲
Grassberger and Frank 2004	Austria	pig		–	–	▲	–	▲	–
Garcia-Rojo 2004	Spain	pig	–	–	–	–	–	▲	▲
Wyss and Cherix 2006	Switzerland	pig/human*	▲	–	▲	–	▲	–	▲
Chauvet et al. 2008	France	human*	▲	▲	▲	n.i.	n.i.	n.i.	n.i.
Matuszewski et al. 2008	Poland	pig	▲	▲	▲	▲	–	▲	▲
Zdemir and Sert 2009	Turkey	pig	–	–	–	–	–	▲	▲
Matuszewski et al. 2010	Poland	pig	▲	▲	▲	▲	▲	▲	▲

In Poland, Matuszewski and colleagues (Matuszewski *et al.* 2008) found seven species of Silphidae in various forest biotopes (*N. littoralis*, *T. sinuatus*, *T. rugosus*, *N. humator*, *N. investigator*, *N. vespilloides* and *N. vespillo*) but they did not collect *O. thoracica* on pig carcasses. However, in further studies of Matuszewski *et al.* (2010), *O. thoracica* was found exclusively during the spring (from mid-April) in forest habitats (pine-oak, hornbeam-oak and alder forests). The absence of *O. thoracica* in their previous field study (Matuszewski *et al.* 2008) could be due to the later study time as it was conducted at the end of the summer and the beginning of fall (September 2nd to October 28th) (Matuszewski *et al.* 2008). Although the literature reports that *O. thoracica* may be found from April to September (Hastir and Gaspar 2001; Debreuil 2004c), field studies suggest that this species has a spring seasonality. Ružicka (1994) lists 14 species of Silphidae caught in two biotopes (forest and field sites) with pitfall traps in Bohemia.

Among the 14 species trapped, seven species are the same as those in the present study but some species that were listed in their study are not carrion feeder such as *Dendroxena quadrimaculata*, *Aclypea opaca* and *Phosphuga atrata atrata*. These differences in trapping could be due to the fact that some of the pitfall traps were baited with ripened cheese and were attractive for more silphid species than the pig carcasses used in our work. *N. investigator*, *N. interruptus* and *N. sepultor* are reported in the Ružicka study (Ružicka 1994); but *N. sepultor* does not occur in Belgium (Ružicka and Schneider, 2004). Silpha tristis was frequently caught in field sites in central Bohemia (Czech Republic) but not in the present study. This species is not active early in the season. Ružicka reports that the captures are more frequent from June to October (Ružicka 1994; Aleksandrowicz and Komosinski 2005) while Hastir and Gaspar (2001) report a better capture period beginning in May.Kocarek (2003), collected eight species of necrophagous carrion beetles on exposed rat carcasses and six are the same as in this study: *N. humator*, *N. vespilloides*, *N. vespillo*, T. *rugosus*, *T. sinuatus* and *O. thoracica*. The two species additionally found in Kocarek study were *N. interruptus* and *N. investigator*, but these species are not found in early spring. *N. interruptus* shows a seasonal preference for summer (July-August), whereas *N. investigator* for summer and fall (Kocarek 2003; Aleksandrowicz and Komosinski 2005). In their study on decaying rodent carcasses, Kentner and Streit (1990) report five Nicrophorinae (*N. interruptus*, *N. humator*, *N. investigator*, *N. vespillo*, *N. vespilloides*) but *N. interruptus* and *N. investigator* were collected in small numbers (Kentner and Streit 1990). *N. vespilloides* was the dominant species in the forest habitat and was not collected in the open field site, while *N. vespillo* and *N. humator* were collected in both open field and forest. However, *N. vespillo* showed a preference for the open field habitat and *N. humator* for the forest habitat. Six Silphinae were reported (Kentner and Streit 1990): *N. littoralis*, *O. thoracica*, *S. obscura*, *S. tristis*, *T. sinuatus*, *T. rugosus*. Any *Thanatophilus* spp. and *Silpha* spp. were collected in a forest biotope, while *N. littoralis* and *O. thoracica* were exclusively found in an open field biotope. The dominant species of Silphinae was *T. sinuatus* (Kentner and Streit 1990). An entomofaunal colonization study on rabbit carcasses in sand dune lists five species of Silphidae in the spring: *N. humator*, *N. vespillo*, *O. thoracica*, *T. sinuatus* and *T. rugosus*. *T. sinuatus* was the most abundant species trapped (Bourel *et al.* 1999).

As *O. thoracica* is a forest inhabiting species and its absence in the agricultural biotope in our study is not surprising. Other studies (Kentner and Streit 1990; Kocarek 2001; Kocarek 2003) have shown the forest preference of *O. thoracica*.

However, *O. thoracica* has been reported in open biotope in Northern France (sand dune) (Bourel *et al.* 1999). *N. vespilloides* and *N. humator* are also considered to be forest species (Kentner and Streit 1990; Scott 1998; Hastir and Gaspar 2001; Kocarek 2001; Kocarek 2003; Aleksandrowicz and Komosinski 2005). This ecological habitat preference explains the great difference we observed between both biotopes with clearly more trappings for the forest biotope. *Thanatophilus* spp. are referenced as field or meadow species (Ružicka 1994; Hastir and Gaspar 2001; Kocarek 2001; Kocarek 2003; Aleksandrowicz and Komosinski 2005). The latter species were more abundant in the agricultural biotope than in the forest habitat in our study. Ružicka (1994) and Kocarek (2001; 2003) show the same habitat preference: *T. sinuatus* (Ružicka 1994; Kocarek 2003) and *T. rugosus* (Kocarek 2003) were collected mostly in the field sites. However, Matuszewski and colleagues (Matuszewski *et al.* 2008; Matuszewski *et al.* 2010) have collected numerous adults of *Thanatophilu*s spp. on pig carcasses located in various forest habitats. As in our work, *T. sinuatus* was found early in the season starting in April) by Ružicka (1994). In a suburban area of Madrid (Spain) (Garcia-Rojo 2004), *T. rugosus* and *T. sinuatus* were also found from mid-April on decaying pigs. *N. vespillo*, a meadow species (Scott 1998; Kentner and Streit 1990), has been less trapped during the sampling period (early spring) and the comparison between the two biotopes is not relevant. The small number of collections-, may be due to the fact that *N. vespillo* becomes reproductively active in summer (May-July) (Scott 1998), while other species of *Nicrophorus* have a reproductive period in spring starting in April (Scott 1998) or later (*N. interruptus*, *N. investigator*) (Kentner and Streit 1990). *N. littoralis* has been collected in both biotopes. However, several studies (Kentner and Streit 1990; Ružicka 1994) report that *N. littoralis* was absent in open field habitats and only collected in forest habitats. It was a dominant species of carrion beetles (larvae and adults) collected in forest studies of Matuszewski and colleagues (Matuszewski *et al.* 2008; Matuszewski *et al.* 2010). Concerning Silphinae, they are active from April to September (Hastir and Gaspar 2001; Peck 2001). However, some species of Silphinae seem to have seasonal preferences (e.g., *O. thoracica* in spring).Food source preferences (niche differentiation) and carcass size could explain the great differences observed between the two subfamilies (Scott 1998; Watson and Carlton 2005; Ikeda *et al.* 2006). Nicrophorinae (*Nicrophorus* spp.) prefer small vertebrate carcasses (rodents, birds) because of their burying behaviour, and their subsocial behaviour for breeding their offspring (biparental care) (Pukowski 1933; Anderson 1982; Kentner and Streit 1990; Trumbo 1992; Scott 1998; Ikeda *et al.* 2006; Sikes 2008).

However, reproductively immature Nicrophorus could be found on larger carcasses (> 300g) where they feed on fly eggs or maggots (necrophilous species) and rarely on decaying meat (Kentner and Streit 1990; Scott 1998; Matuszewski *et al.* 2008; Sikes 2005; Sikes 2008). Contrary to Nicrophorinae, Silphinae show no parental care (Ratcliffe, 1996). Silphine species tend to use larger carcasses for reproduction and larval development (Ratcliffe 1996; Bishop 2001; Hoback *et al.* 2004). Pig carcasses are considered as large carcasses and suitable for Silphinae. Concerning the decompositional stage preference, Silphidae tend to arrive during the mid-stage of decay (Ratcliffe 1972; Ratcliffe 1996; Hoback *et al.* 2004). Other studies (Kocarek 2003; Matuszewski *et al.* 2008; Matuszewski *et al.* 2010) associate silphid activity of N. *littoralis*, *Thanatophilus* spp. and *Nicrophorus* spp. on carcasses during the active decay stage. However, an African species of Silphidae, *Thanatophilus micans*, could be found within 24 hours of death on animal carcasses (Midgley *et al.* 2009).In carrion beetles communities, niche differentiation can occur along dimensions of season, habitat (biotope) and carcass size (Scott 1998; Kentner and Streit 1990; Kocarek 2001; Hocking *et al.* 2007). Further studies on carrion beetles (Coleoptera: Silphidae) and insect postmortem colonization are currently being conducted at the Department of Functional and Evolutionary Entomology (Belgium, Gembloux Agro-Bio Tech, University of Liege).

4.2. Self-critique

In this experiment, an infrequent sampling (every fourth days) was used for collecting sarcosaprophagous insects in a six-week period on decaying pigs. The use of soapy water in traps without any preservative solution is not recommended for infrequent sampling. Indeed, insects themselves could rot in pitfall and yellow traps, although this is less of a problem for adults. For further field studies, it would be better to use traps filled with ethylene glycol (50%) in case of infrequent sampling or use a daily sampling. Only adult stages were included in this study and there is no information about immature stages of carrion beetles. However, for forensic purposes, it is important to have information about the presence/absence of these immature stages.

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II.3.3. Carrion beetles (Coleoptera, Silphidae) visiting pig carcasses exposed on a forest biotope of Western Europe during four seasons.

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Abstract

Carrion beetles are important in terrestrial ecosystems, consuming dead mammals and promoting the recycling of organic matter into ecosystems. Most forensic studies are focused on succession of Diptera while neglecting Coleoptera. So far, little information is available on carrion beetles postmortem colonization and decomposition process in temperate biogeoclimatic countries. These beetles are however part of the entomofaunal colonization of a dead body. Forensic entomologists need databases concerning the distribution, ecology and phenology of necrophagous insects, including silphids. Forensic entomology uses pig carcasses to surrogate human decomposition and to investigate entomofaunal succession. However, few studies have been conducted in Europe on large carcasses. The work reported here monitored the presence of the carrion beetles (Coleoptera: Silphidae) on decaying pig carcasses in a forest biotope during four seasons. Nine species of Silphidae were recorded: *Nicrophorus humator* (Gleditsch), *Nicrophorus vespillo* (L.), *Nicrophorus vespilloides* (Herbst), *Nicrophorus interruptus* (Stephens), *Nicrophorus investigator* (Zetterstedt), *Necrodes littoralis* L., *Oiceoptoma thoracica* L., *Thanatophilus sinuatus* (Fabricius), *Thanatophilus rugosus* (L.). All of these species were caught during summer; seven were caught during spring, and three during fall. No silphids were caught during winter.

Keywords

Silphidae, Silphinae, Nicrophorinae, necrophagous insects, forensic entomology, carrion ecology

1. Introduction

Carrion beetles (Coleoptera: Silphidae) perform vital ecosystem functions (Wolf and Gibbs 2004) by promoting the breakdown and recycling of organic matter into terrestrial ecosystems (Ratcliffe 1996; Hastir and Gaspar 2001; Kalinova et al. 2009). Most silphids are carrion feeders (necrophagous species) or prey on other carrion inhabitants such as fly eggs or maggots and other carrion beetles (necrophilous species) (Ratcliffe 1996; Hastir and Gaspar 2001; Sikes 2005, 2008). The necrophagous insects, including flies andcarrion beetles, have particular relationships with decomposing remains of vertebrate carcasses that constitute a rich, but ephemeral resource (Anderson and VanLaerhoven 1996; Grassberger and Frank 2004; Carter et al.2007). These specialized insects are attracted to the cadaver that they colonize in a relative predictable sequence called the entomofaunal succession or insect succession (Megnin 1894; Putman 1983; Schoenly and Reid 1987; Marchenko 1988, 2001; Benecke 2004). Study of these insects in a medico-legal context is part of forensic entomology (Hall 2001; Amendt et al. 2004). Many forensic entomological studies have been conducted on pig carcasses as surrogate human models for physiological, ethical and economical reasons (Rodriguez and Bass 1983; Catts and Goff 1992; Anderson and VanLaerhoven 1996; Grassberger and Frank 2004; Hart and Whitaker 2005), but few were conducted in Europe with pig carcasses (Grassberger and Frank 2004; Garcia-Rojo 2004; Wyss and Cherix 2006; Matuszewski et al. 2008, 2010). Many published reports are focused on Diptera pattern colonization and very few looked at Coleoptera succession (Kocarek 2003; Matuszewski et al. 2008; Midgley and Villet 2009, 2010). However, the use of beetles in forensic entomology can be relevant (Kulshrestha and Satpathy 2001; Midgley et al. 2010). Families of beetles of forensic importance are Silphidae (carrion beetles), Dermestidae (larder, skin or hide beetles), Staphylinidae (rove beetles), Histeridae (clown or hister beetles), Cleridae (checker beetles) and Nitidulidae (sap beetles) (Haskell et al. 1997; Byrd and Castner 2001; Wyss and Cherix 2006). Among them, carrion beetles can provide information on postmortem colonization on remains and time since death (Haskell et al. 1997; Smith 1986; Watson and Carlton 2005). So far, little information is available on carrion beetles postmortem colonization and the process of decomposition in temperate biogeoclimatic

countries. The world fauna of Silphidae is composed of fewer than 200 species distributed in 15 genera (Portevin 1926; Peck 2001; Sikes 2005). This family has a Holarctic distribution (Peck 2001). In Western Europe, this family is divided in two subfamilies: Nicrophorinae (i.e. burying beetles) with eleven species and Silphinae including seventeen species (Portevin 1926; Hastir and Gaspar 2001; Debreuil 2003a,b, 2004a,b,c; Dekeirsschieter et al. 2011).There are seven species of Nicrophorinae and thirteen species of Silphinae reported in Belgium (Hastir and Gaspar 2001; Ružicka and Schneider 2004). Many forensic entomological papers highlight the necessity to generate data on insect succession and insect seasonal activity on carrion in specific geographic regions and various biotopes within these regions (Catts and Goff 1992; Byrd and Castner 2001; Amendt et al. 2004; Sharanowski et al. 2008). This paper identifies the seasonal activity of silphids that occur on large carcasses in a temperate biogeoclimatic region in a forest biotope.

2. Material and method

2.1. Field site and study periods

This study was conducted during four seasons (summer 2008 from June 6 to July 25, autumn 2008 from October 13 to December 19, winter 2009 from January 23 to March 20, spring 2009 from April 10 to June 4). The study site was a forest biotope, located in Belgium (Lambert-coordinates: 172800.00/167150.00). The field site is a facility research area devoted to forensic research managed by the Disaster Victim Identification (DVI) of the Belgian Federal Police. The tree layer of the field site is dominated by oak trees (*Quercus rubra*) and beech trees (*Fagus sylvatica*). The shrub layer is absent. The soil vegetation is scattered and the herb layer is mainly constituted of bracken (*Pteridium aquilinum*), blackberry (*Rubus fructicosus*), lily-of-the-Valley (*Convallaria maialis*) and May Lily (*Maianthemum bifolium*). Some spare spots of *Polytrichum* sp. constitute the moss layer.

2.2. Environmental parameters

Ambient air temperature was automatically measured once an hour using a datalogger (HOBO RH/TEMP 8K, Onset computer corporation, USA) placed on the field site during each complete sampling period. The daily mean temperature, the minimal temperature and the maximal temperature were calculated on the basis of ambient air temperature recorded on a time interval of 24 hours.

2.3. Animal model

At the beginning of each season, four piglets (*Sus domesticus* L.) (25 Kg) were killed by penetrative captive bolt (fractured skull) by a veterinary. Piglets were provided from the experimental farm of the veterinary medical Faculty of the University of Liege. Immediately after the euthanasia, pig carcasses were packed in double plastic bag to avoid any insect colonization before being placed in the experimental site, within the next 2 hours. Each pig carcasse was placed 30 meters from each other, in metal mesh cages (180 cm × 90 cm × 90 cm) to avoid scavenging by vertebrate carnivores.

2.4. Insect collection and identification

In order to quantify insect colonization on pig carcasses, six pitfall traps (glass jars of 15 cm in height and 8 cm in diameter) and four yellow traps (plastic container of 9 cm in height and 27 cm in diameter), both filled with 50% ethylene glycol, were placed around each carcass. Two pitfall traps were placed, flush to the surface, near the ventral face, two near the dorsal face, one near the head and one near the anus (Figure 22).

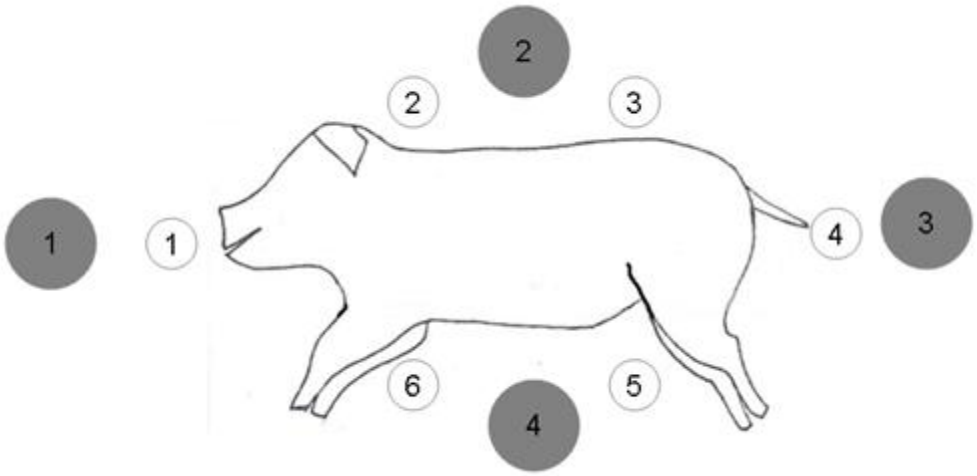

Figure 22. Disposition of the traps around the pig carcass (grey = yellow traps, not coloured = pitfall traps).

The four yellow traps were placed on the ground around each pig carcass, one near the head, one on the dorsal face, one near the anus and the last on the ventral side. The trapped insects were removed weekly and conserved in 80% *norvanol D* (ethanol denatured with ether). In the laboratory, carrion beetles were mounted on insect pins and identified to species. Only adults were included in the counting of collected insects during this field study. The species names follow the taxonomy of Fauna Europaea. Silphidae specimens were determining using different identification keys (Portevein, 1926; Heinz, 1971; Hastir & Gaspar, 2001; Debreuil,

2003a,b; Debreuil, 2004a,b,c) and reference collections of Silphidae from the entomological conservatory of Gembloux Agro-Bio Tech, University of Liege (Department of functional and evolutionary Entomology).

3. Results

3.1. Environmental parameters

The mean atmospheric temperature were 13.4°C, 5.5°C, 3.5°C and 14.5°C for spring, fall, winter and summer, respectively. Figure 23 shows the mean, maximal and minimal temperature recorded on the forest site for each season.

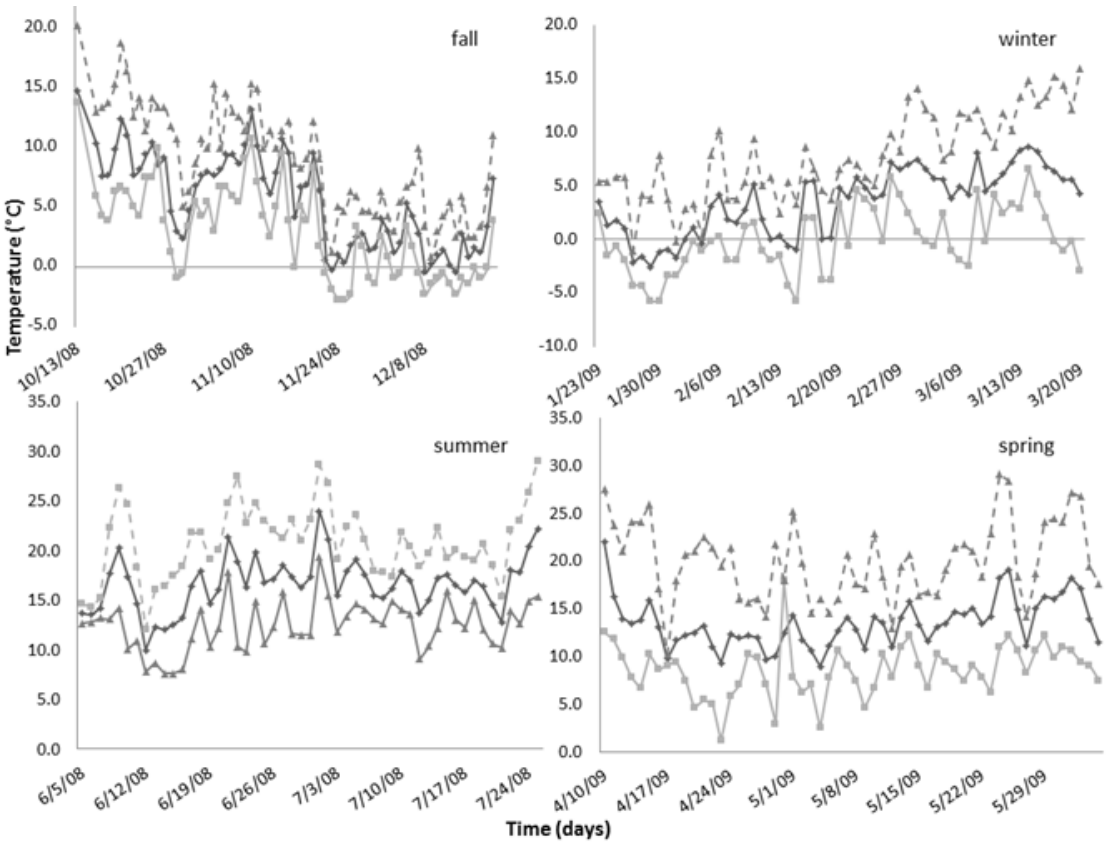

Figure 23. Mean temperature (continuous dark grey stroke), maximal temperature (discontinuous stroke) and minimal temperature (continuous light grey stroke) recorded in the field site during the four inventoried seasons.

3.2. Carrion beetles

Nine species of Silphidae were identified on the pig carcasses, four Silphinae: *Necrodes littoralis* LINNAEUS, 1758; *Oiceoptoma thoracica* LINNAEUS, 1758*; Thanatophilus sinuatus* FABRICIUS, 1775; *Thanatophilus rugosus* LINNAEUS, 1758 and five Nicrophorinae:

Nicrophorus humator OLIVIER, 1758; *Nicrophorus vespillo* LINNAEUS, 1758; *Nicrophorus vespilloides* HERBST, 1783; *Nicrophorus investigator* ZETTERSTEDT, 1824; *Nicrophorus interruptus* STEPHENS, 1830. In total, 3072 individuals were collected during the four seasons with 2245 Silphinae and 827 Nicrophorinae (Table 14).

Table 14. Absolute abundances of Silphidae according to the season.

		Species	Season				
			Summer	Fall	Winter	Spring	Total/species
Silphinae	1	*O. thoracica*	47	0	0	216	263
	2	*N. littoralis*	616	1	0	672	1289
	3	*T. sinuatus*	110	0	0	475	585
	4	*T. rugosus*	18	0	0	90	108
Nicrophorinae	5	*N. humator*	88	4	0	45	137
	6	*N. investigator*	105	0	0	0	105
	7	*N. vespilloides*	408	48	0	103	559
	8	*N. vespillo*	10	0	0	8	18
	9	*N. interruptus*	8	0	0	0	8
Total/season: 3072			1410	53	0	1609	3072
Number of species			9	3	0	7	

Summer was the season with the highest specific diversity (nine species), followed by spring (seven species) and only three species during fall whereas there was no Silphidae during the winter collects. Independently of the season, the most abundant species of Silphidae was *Necrodes littoralis* with 1289 captures which represents more or less 42% of the total (Figure 24).

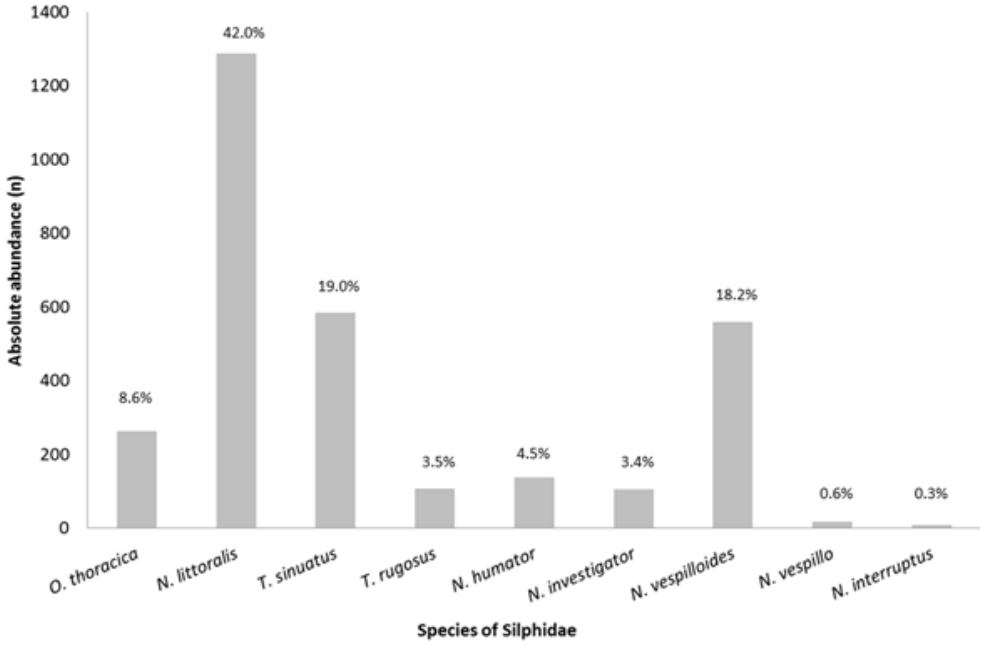

Figure 24. Number of individuals by species of Silphidae.

585 individuals of *Thanatophilus sinuatus* were caught which represent 19% of the Silphidae followed by *Nicrophorus vespilloides* with 559 captures (18.2%). These three species represent 80% of the Silphidae captures on decaying pigs. Other species were much less abundant on pig carcasses; there are 263 individuals of *O. thoracica* (= 8.6%), 137 individuals of *N. humator* (= 4.5%), 108 individuals of *T. rugosus* (= 3.5%) and 105 individuals of *N. investigator* (= 3.4%). The last two species of *Nicrophorus*, *N. vespillo* and *N. interruptus*, represent less than one percent of the collected Silphidae in the forest biotope.

4. Discussion

Among the seven species of Nicrophorus referenced in Belgium (Hastir and Gaspar 2001, Ruzicka and Schneider, 2004; Dekeirsschieter et al. 2011), five of them were observed on pig carcasses: *N. humator*, *N. vespillo*, *N. vespilloides*, *N. investigator* and *N. interruptus* while *N. germanicus* and *N. vestigator* were not collected in the forest biotope. *N. germanicus* is a rare species with a very localized distribution and has a preference for field habitats (Hastir and Gaspar 2001, Dekeirsschieter et al. 2011). *N. vestigator* is a common species but has preference for field and open areas (Hastir and Gaspar 2001, Dekeirsschieter et al. 2011). The absence of these two nicrophorine species is not surprising in our forest samples due to their preference for field habitats. For Belgian silphine species, eight species can be found in

cadaver-ecosystems as carrions feeders (i.e. necrophagous species) and/or predators (i.e. necrophilous species) (Hastir and Gaspar 2001, Dekeirsschieter et al. 2011), four of them were collected: *N. littoralis*, *O. thoracica*, *T. sinuatus* and T. *rugosus*. No *Silpha* species were found in our forest samples.

A niche differentiation exists in carrion beetles communities along dimensions of season (temporal activities), habitat (biotopes) and carcass size (food source preferences) (Anderson 1982; Scott 1998; Kentner and Streit 1990; Kocarek 2001; Hocking et al. 2007). The great differences observed between Silphinae and Nicrophinae trappings could be explained by their different reproductive strategy (Scott 1998; Watson and Carlton 2005; Ikeda et al. 2006). *Nicrophorus* spp. are subsocial, providing bi-parental care of their offspring, and preferentially use small vertebrate carcasses (i.e. rodents or birds) that they bury for rearing offspring (Pukowski 1933; Anderson 1982, Scott 1998; Ikeda et al. 2006; Sikes 2008). Contrary to the Nicrophorinae, Silphinae tend to use large vertebrate carcasses which provide sufficient food resource for the great number of beetles that may be present (Anderson 1982; Watson and Carlton 2005); females lay their eggs in or on the soil around carcasses and shown no parental care (Ratcliffe 1996, Sikes 2005; Ikeda et al. 2008). However, reproductively immature *Nicrophorus* could be found on larger carcasses where they prey on other carrion inhabiting such as fly eggs or maggots and rarely feed on decaying meat (Kentner and Streit 1990; Scott 1998; Matuszewski et al. 2008; Sikes 2005; Sikes 2008). Inversely, Silphinae could be found on smaller carcasses that they use for feed but not for reproduction and larval development (Bishop 2001; Hoback et al. 2004).

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II.3.4. Life cycle of two Palearctic silphine of forensic interest: *Thanatophilus sinuatus* (Fabricius 1775) and *Necrodes littoralis* (L. 1758) (Coleoptera, Silphidae)

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Abstract- *Necrodes littoralis* and *Thanatophilus sinuatus* are common carrion beetles (Coleoptera, Silphidae) found in temperate biogeoclimatic countries. Their use in forensic entomology could be useful for estimating post-mortem intervals. Particularly in advanced or late decay stages of the remains when fly larvae have left the corpse. Carrion beetle larvae were reared under laboratory conditions at 18°C and 23°C. Larval specimens (94 individuals of *N. littoralis* and 90 individuals of *T. sinuatus*) were measured twice a day during their active growth. The life cycles of these two forensic interesting carrion beetles were unknown until now.

Key words

Forensic entomology, Silphidae, carrion beetles, Silphinae, *Thanatophilus sinuatus*, *Necrodes littoralis*, development, temperature

1. Introduction

Carrion beetles have been referenced to as being a part of the entomofaunal colonization of a dead body but very few studies have looked at them in a forensic context. Many published reports or reviews are focused on Diptera pattern colonization and neglect Coleoptera succession (Kocarek, 2003; Matuszewski *et al.* 2008; Matuszewski *et al.* 2010 Midgley *et al.*

2009; Midgley *et al.* 2010). However, the use of beetles in forensic entomology can be relevant (Kulshrestha *et al.* 2001; Watson *et al.* 2005; Midgley *et al.* 2009; Velasquez *et al*, 2009; Midgley *et al.* 2010; Velasquez *et al*, 2010). Carrion beetles can provide information on postmortem colonization on remains and time since death (Haskell *et al.* 1997; Smith, 1986; Watson *et al.* 2005). However, all carrion beetles do not have the same forensic interest; some species of the subfamily of Silphinae seem to have a more important value as forensic bioindicators (Watson *et al.* 2005; Matuszewski *et al.* 2010), whereas the subfamily of Nicrophorinae presents less interest (Watson *et al.* 2005). Indeed, Nicrophorinae have ecological preferences for small vertebrate carcasses (e.g. birds or rodents), while Silphinae tend to use large carcasses (including human corpses) (Anderson, 1982; Peck, 1990; Eggert *et al.* 1992; Ratcliffe, 1996). However, Nicrophorus spp. could be frequently found on human corpses, including in houses (Chauvet *et al.* 2008). Among Silphinae, Necrodes littoralis and T. sinuatus seems to be the most frequent and abundant carrion beetles found in field experiment in Central Europe (Matuszewski *et al.* 2008; Matuszewski *et al.* 2010, Dekeirsschieter *et al.* 2011). Contrary to flies, there are few studies on the rates of development of Coleoptera with forensic interest (Midgley *et al.* 2009; Midgley *et al.* 2010). For example, Midgley and Villet (Midgley *et al.* 2009) studied the development of Thanatophilus micans Fabricius 1794, an Afrotropical species, at ten constant temperatures. They established a robust statistical model of development for this common species in South Africa. In Venezuela, Velasquez and Viloria (Velasquez *et al.* 2009, 2010) studied the development of Oxelytrum discicolle Brullé 1840, a Neotropical beetle, under three constant temperatures and under natural conditions. Currently, there is no development model ("size-at-age data") for forensically relevant European silphids. However, research on development of Coleoptera with a forensic interest can be a useful tool for medico-legal entomologists (Midgley *et al.* 2010). Nevertheless, the biology and ecology of most forensically relevant species of Coleoptera are unknown (Midgley *et al.* 2010). It is particularly true for silphine species (Ratcliffe, 1996; Hoback *et al.* 2004; Ikeda *et al*. 2007). This paper presents the rate of development of two species of Palearctic carrion beetles, *Necrodes littoralis* and *Thanatophilus sinuatus*, at two constant temperatures. This study is the first to present developmental data for these beetles of forensic interest.

2. Materials and methods

2.1. Rearing laboratory colonies of Silphinae

Adults of *Thanatophilus sinuatus* and *Necrodes littoralis* were collected from baited pitfall traps (chicken meat) located in various habitats in Belgium, and decaying pig carcasses. Each species is separately reared in group (30 couples) in several glass container (dimensions: 40x25x20 cm) filled with compost (≈10 cm) to provide laboratory colonies. The daylight regime is 16:8 (L: D) and the temperature in the rearing room is 21°C+-2°C with 70-80% RH. A glass vial containing water with a wick of cotton wool and food are placed in each rearing box. Food source is a mix of dry dog food and decaying chicken and pork meat.

2.2. Experimental design

Pairs (female/male) of unmated *T. sinuatus* and *N. littoralis* were removed from the laboratory colonies and isolated into small plastic boxes (20x15x10 cm) filled with compost (oviposition substrate), food source (chicken liver) and water (moistened cotton wool) to provide fresh eggs. Both species reproduction boxes are placed at two different constant temperatures (18°C and 23°C) in controlled incubator (L:D = 16:8, fixed T°: 18°C or 23°C, RH = 70%). The experimental protocol is adapted from the study of Midgley and Villet on *Thanatophilus* micans (Midgley and Villet, 2009). Each freshly hatched first instar larva was isolated into glass Petri dish (dimensions: 90x15mm) filled half with slightly damp sand and compost. In each Petri dish, there were piece of chicken liver as food source and a small ball of moistened absorbent cotton to provide water. Every Petri dishes were clearly identified with the species name, each isolated individual was identified by a number and the rearing temperature. The Petri dishes were vertically placed in the incubator and closed with elastic bands. Each developmental milestone was identified and monitored twice a day (9 AM and 3 PM) by checking all individuals during their complete development. Mortality was recorded at the same time. During active growth (period which larvae feed on decaying meat and are out the pupation substrate), length of the larvae were measured using a triangle micrometer (Villet, 2007; Midgley and Villet, 2009). Each individual was measured (total body length) four times and twice a day (9 AM and 3 PM) during their active growth (larval stage I, II, III).

The following developmental stages were identified: eggs, first instar (LI), second instar (LII), third instar (LIII), postfeeding larva, nymph and imago (Figure 25).

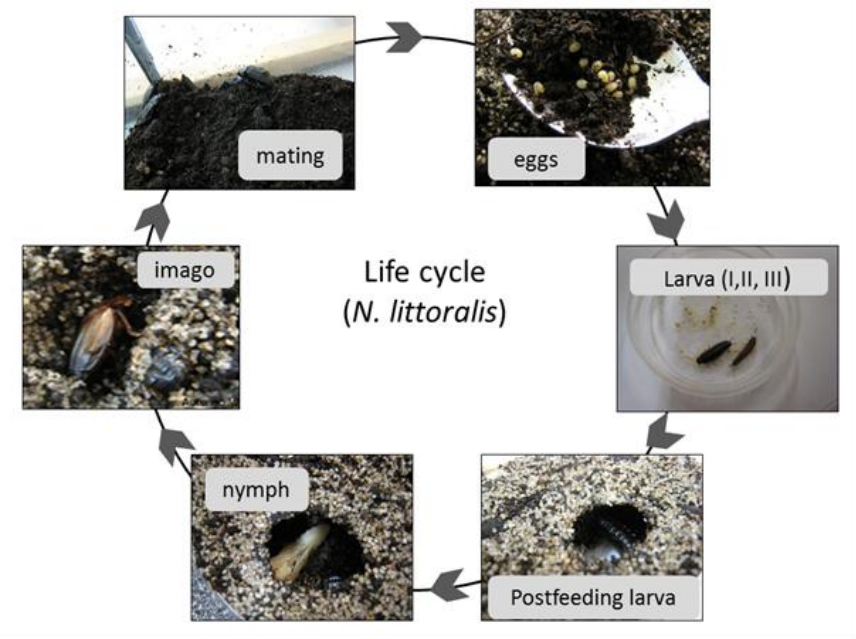

Figure 25. Life cycle of *Necrodes littoralis.*

The egg stage was not included in our measurements due to the difficulties in sampling eggs of Silphidae. In our preliminary trials, their manipulation involved very high mortality rate and it was impossible to isolate a single egg by Petry dish. Indeed, females of *Necrodes littoralis* and *Thanatophilus sinuatus* lay clusters of eggs in the soil. In field conditions, females of Silphinae oviposite in or on the soil around large vertebrate carcasses (>300g) Sikes 2005, Dekeirsschieter et al. 2011, Matuszewski 2011).

2.3. Statistical analyses

After graphical examination of the collected body length measures, the growth curves of larvae were approximated by a linear trend for each species, temperature and development stage. To parameterize those curves, a single linear mixed model was adjusted, with three fixed factors (temperature, species and developmental stages), a covariable (time) and their interactions, with individuals as random effect. A stepwise selection was used to include only the significant ($\alpha = 0.05$) main effects and interactions in the final model.

The effect of temperature on the maximal size at each developmental stage of the larvae was also assessed. The maximal size of each individual at each of the three larval stage was first extracted and then analyzed by a linear mixed model for each species and stage, with temperature as a fixed factor and individuals as random effect.

3. Results

3.1. Duration of life cycle (development time)

Table 15 shows the duration of each developmental stage of *Necrodes littoralis* and *Thanatophilus sinuatus* for both experimental temperatures.

Table 15. Duration (days) of developmental stages of *N. littoralis* and *T. sinuatus* for each constant temperature.

		Stage duration (mean ± S.D.)			
		Necrodes littoralis		*Thanatophilus sinuatus*	
		18°C	23°C	18°C	23°C
		N=44	N=50	N=47	N=43
Active feeding period	1st instar larva	4.68 ± 0.33	2.15 ± 0.31	2.76 ± 0.55	1.29 ± 0.41
	2nd instar larva	4.95 ± 0.64	2.28 ± 0.42	2.98 ± 0.47	1.64 ± 0.49
	3rd instar larva	5.82 ± 1.19	4.71 ± 1.35	5.14 ± 0.62	3.47 ± 0.55
	Postfeeding larva	13.20 ± 0.92	7.59 ± 1.14	9.79 ± 1.06	6.30 ± 0.68
	Nymph	14.14 ± 0.89	6.89 ± 0.50	13.04 ± 1.25	6.14 ± 0.80
	Complete life cycle	42.79 days	23.60 days	33.70 days	18.85 days

At 18°C, the complete life cycle (from first instar larva to adult emergence) of *N. littoralis* took 42.8 days and 23.6 days at 23°C. Concerning *T. sinuatus*, the life cycle took 33.7 days at 18°C and 18.9 days at 23°C.

The egg stage took between 3 and 5 days for both species at 18°C and 23°C.

3.2. Mortality

For both species, larval mortality was higher for 23°C rearing temperature than 18°C. Seven larvae of *Thanatophilus sinuatus* died at 18°C which represent a total larval mortality of 14.9 %. For the 23°C, eight larvae of *T. sinuatus* died which represents a total mortality of 18.6%. Concerning the second species, seven larvae of *Necrodes littoralis* died at 18°C (15.9%) while 15 larvae died at 23°C which represents a total mortality of 30%. Figure 26 presents the occurrences of mortality for each species at two rearing temperatures.

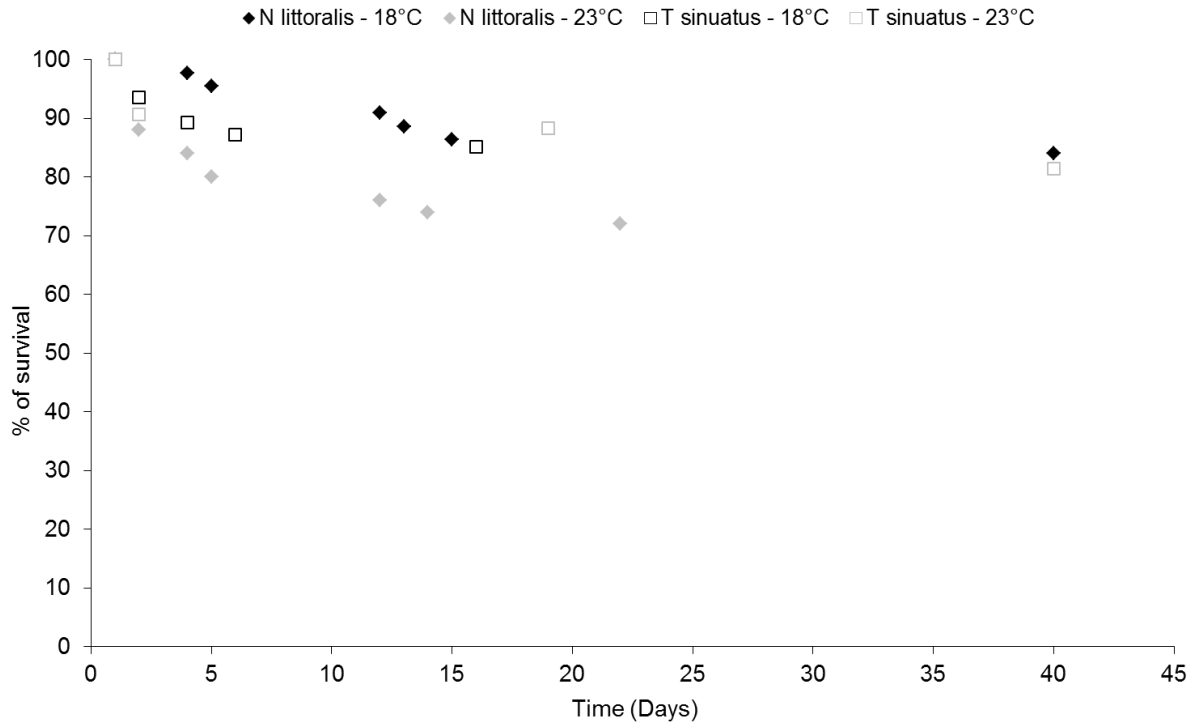

Figure 26. Mortality curves of *Necrodes littoralis* and *Thanatophilus sinuatus* at 23°C and 18°C depending on the development time (in days).

3.3. Growth curves

Independently of the species and the rearing temperature, 12208 length measurements were realized on beetle larvae during the active growth. Figures 27 to 30 show the larval growth curves for *T. sinuatus* and *N. littoralis*. For both species, growth curves were sigmoidal for 18°C and 23°C. Growth rate decreased when larvae reached third instar.

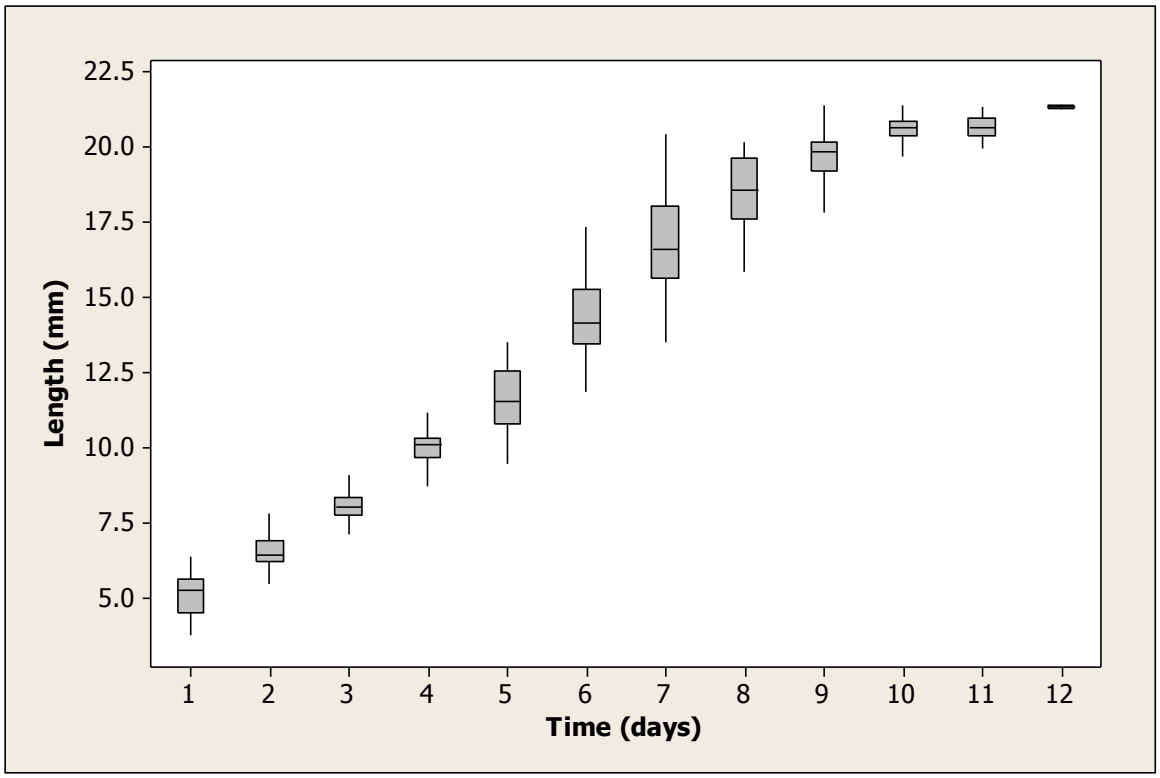

Figure 27. Growth curve of *Necrodes littoralis* at 23°C.

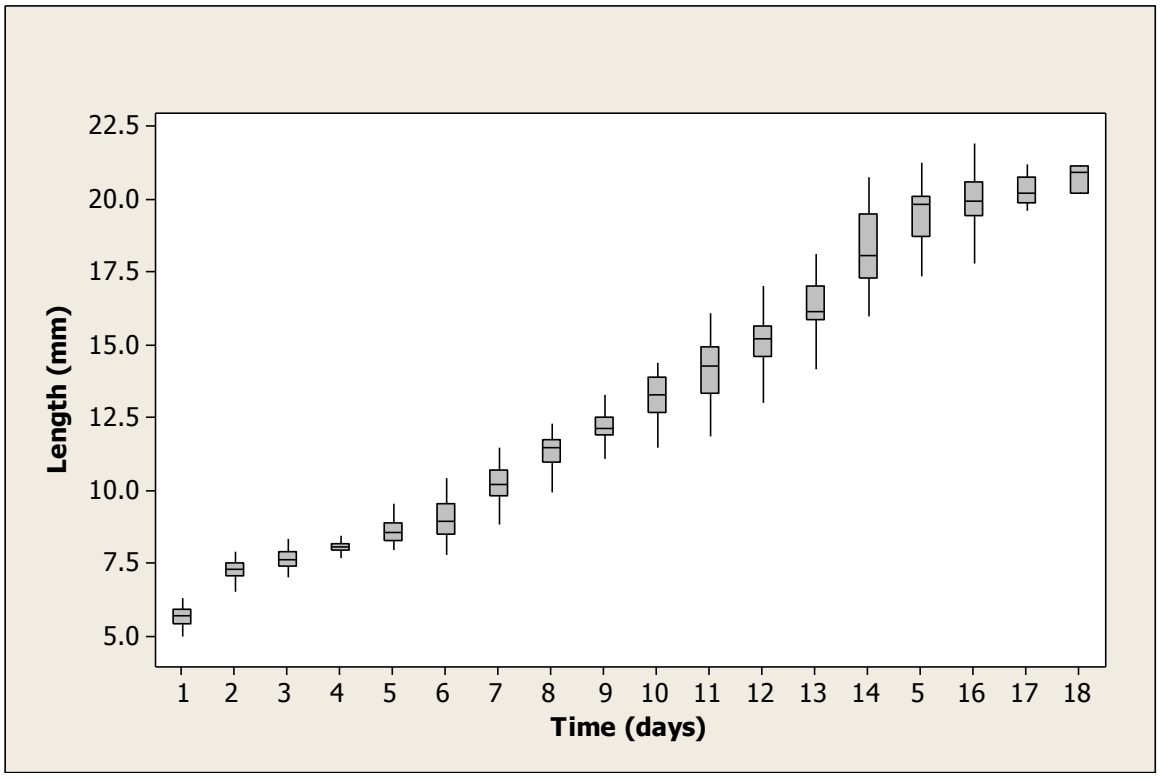

Figure 28. Growth curve of *Necrodes littoralis* a18°C.

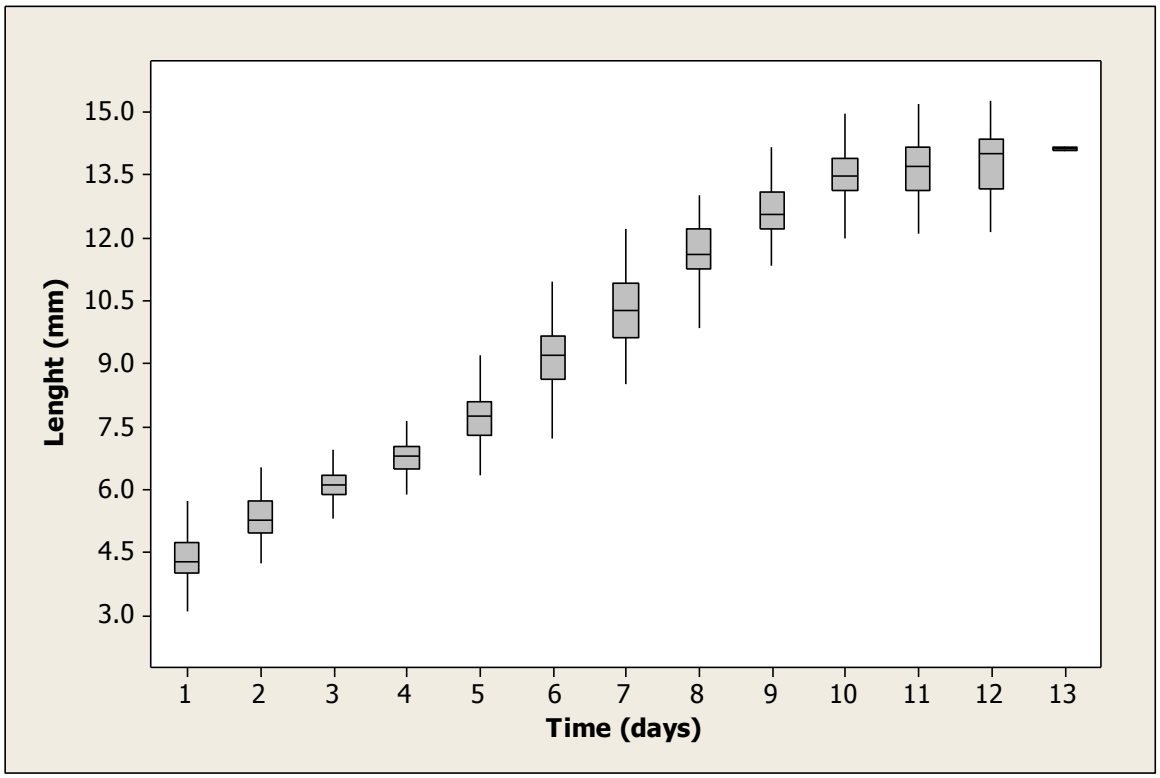

Figure 29. Growth curve of *Thanatophilus sinuatus* at 18°C.

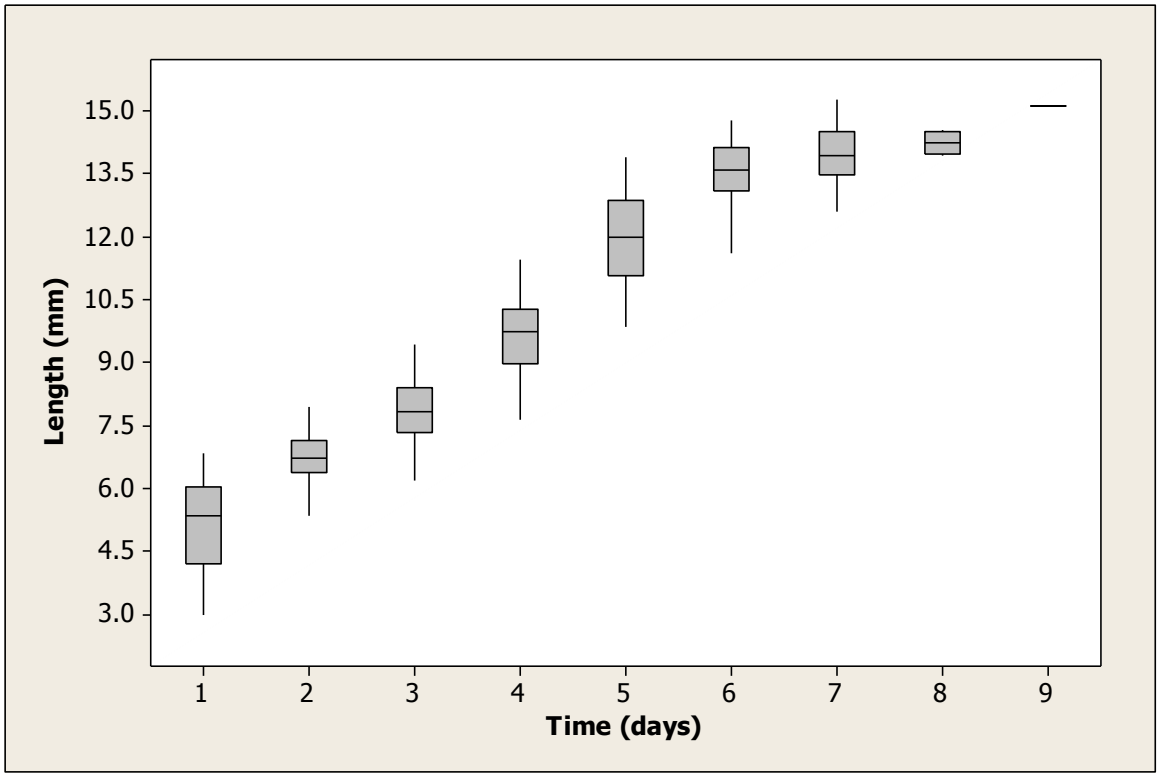

Figure 30. Growth curve of *Thanatophilus sinuatus* at 23°C.

The linear mixed model representing the growth curves shows a significant effect of the species and larval stage on the intercept of the model, and a significant effect of species,

temperature, larval stage, interaction of species and larval stage and interaction of species and temperature on the slope of the growth curves. Fixed coefficients of the global linear mixed model were aggregated to deliver the growth curves' linear equations for each temperature, species and larval stage. Table 16 shows the resulting developmental models for active growth for both species.

Table 16. Linear model of length growth (y = a+b.Time).

	N. littoralis					
	18°C			**23°C**		
Intercept (a)	5.31873	4.11883	5.48226	5.31873	4.11883	5.48226
Slope (b)	0.8442	1.01224	0.96038	1.33853	1.87407	1.72045
	T. sinuatus					
	18°C			**23°C**		
Intercept (a)	4.77245	3.57255	4.93597	4.77245	3.57255	4.93597
Slope (b)	0.73959	1.06059	0.86014	1.23391	1.92242	1.6202

Analysis of maximal stage sizes shows significant effect of temperature for larval stages I and II of *N. littoralis* and larval stage II of *T. sinuatus* only (Table 17).

Table 17. Maximal size of larval stages (in mm).

	Larval stages	**18°C**	**23°C**	***p-value***
N. littoralis	I	8.68 ± 0.14	7.52 ± 0.13	< 0.001 ***
	II	13.43 ± 0.19	11.60 ± 0.19	< 0.001 ***
	III	19.52 ± 0.28	19.98 ± 0.28	0.244
T. sinuatus	I	6.29 ± 0.12	6.40 ± 0.12	0.487
	II	9.71 ± 0.15	9.04 ± 0.16	0.002 **
	III	13.69 ± 0.11	13.94 ± 0.12	0.124

4. Discussion

For both species, the complete life cycle is shorter at 23°c with 42.79 days for *N. littoralis* and 33.70 days for *T. sinuatus*, than at 18°C with 23.60 days for *N. littoralis* and 18.85 days for *T. sinuatus. Thanatophilus sinuatus* has a shorter life cycle than *Necrodes littoralis* for both rearing temperatures. In comparison with other silphine species of forensic interest, the complete development of the African carrion beetle, *Thanatophilus micans*, took 63.16 days at 15°C and 19.33 days at 28.4°C whereas the Neotropical carrion beetle, *Oxyletrum discicolle* required 40.00 (± 2.73) days at 15°C and 20.33 (± 0.89) days at 28°C. Quite as for *Thanatophilus sinuatus* and *Necrodes littoralis*, the duration time of *T. micans* and *O. discicolle* required to complete their development is inversely related to temperature. In higher temperatures, the duration of development becomes shortened. However, too high temperatures avoid the complete development and conduct to more larval mortality (Midgley and Villet 2009) (lethal temperature). The active growth, *i.e* the period of time when larvae breed on carcass, is shorter than the ground development period, *i.e* the postfeeding larval and the nymphal development. Indeed, after breeding on the carcass, the larvae dug into the soil to complete their development. The active growth of *N. littoralis* lasts a little more than fifteen days at 18°C (15.45 days) and a little less than ten days at 23°C (9.14 days). For *T. sinuatus*, the active growth lasts a little more than ten days at 18°C (10.88 days) and moreover six days at 23°C.

Self-critiques

Only two constant temperatures were used to study the development of *Thanatophilus sinuatus* and *Necrodes littoralis*, additional rearing temperatures are required to build isomorphen and isomegalen diagrams.

Some studies indicate that the body length is an ambiguous indicator of the physiological age due to great variation for body length, *e.g.* for *T. micans* (Midgley & Villet, 2009) or *O. discolle* (Velasquez & Viloria, 2010). However, other morphological measurements than the total body length can be used to determine instars larvae such as the pronotal width or the distance between dorsal stemmata (Velasquez & Viloria, 2010; Watson & Carlton, 2005). As suggested by Velasquez and Viloria (Velasquez & Viloria, 2010), additional studies are required to study these morphological variables on other silphid species, including *T. sinuatus* and *N. littoralis*. However, our trials were made on alive larvae and not on killed larvae in

ethanol such as in other studies (Velasquez & Viloria, 2009, Velasquez & Viloria, 2010; Watson & Carlton, 2005).

There is no previous study exploring the effect of temperatures on the development of *Necrodes littoralis* and *Thanatophilus sinuatus*, two carrion beetles of forensic interest. However, further studies under controlled laboratory conditions (additional temperatures, effect of relative humidity, fluctuating temperatures, *etc*) and outdoor natural conditions are necessary to improve developmental models.

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Partie III: Approche chémo-écologique de l'écosystème «cadavre»

« […] Avertis par l'infection, qui au loin se propage, accourent au vol divers insectes charcutiers […]; il trépignent, grisés par la senteur cadavérique, leur délice. […] Nulle part ailleurs ne se verrait telle cohue. C'est une délirante mêlée d'échines et de ventres, d'élytres et de pattes, qui grouille, roule sur elle-même avec des grincements d'articulations accrochées, se soulève et s'affaisse, remonte, et replonge, mise en branle par un continuel remous. C'est une bacchanale, un accès général de delirium tremens. […] Ils étaient venus, convoqués par un fumet de bête crevée, leur suprême délice ; la griserie cadavérique les avais saisis, et ils tournoyaient affolés en un festival de croque-morts. […] Et cette griserie de l'odorat les attire de tous les environs, de bien loin peut-être, on ne sait au juste. De même les Nécrophores, en quête d'un établissement de famille, accourent de la campagne à mes pourrissoirs. Les uns et les autres sont informés par un fumet puissant, qui nous offense nous-mêmes à des cents pas, plonge avant et les délecte à des distances où cesse le pouvoir de notre olfaction».

Jean-Henri Fabre, 1900, Souvenirs entomologiques, VIIème Série, Chapitre 25.

Chapitre III.1: Prélude

La partie précédente de ce travail de recherche a recensé, au travers d'études bibliographiques, *in situ* et en laboratoire, les potentialités d'utilisation des Coléoptères en entomologie forensique. Bien qu'ayant un rôle écologique non négligeable, les staphylins, à l'exception peut-être de *Creophilus maxillosus*, semblent avoir une utilisation potentielle limitée en entomologie forensique. Cependant, les études de terrain nous ont permis de sélectionner plusieurs candidats appartenant à la famille des Silphidae comme bio-indicateurs potentiels. Deux espèces de Silphinae semblent particulièrement intéressantes du point vue forensique. Il s'agit de *Necrodes littoralis* et de *Thanatophilus sinuatus*.

Encore peu étudiée à l'heure actuelle mais en plein développement, l'écologie chimique, axée sur un organisme animal en décomposition, permettrait de mieux comprendre les interactions existant au sein de cet écosystème particulier qu'est le cadavre. En effet, la communication chimique est le principal mode d'interaction des grands groupes d'animaux, y compris chez les insectes. L'entomofaune des cadavres n'y fait pas exception. Ces insectes nécrophages perçoivent les effluves cadavériques principalement grâce à leurs antennes, véritables capteurs des molécules volatiles environnantes; on parle d'olfaction. Le corps en décomposition et ses habitants vont émettre tantôt des odeurs attractives pour certains insectes et tantôt répulsives pour ces mêmes insectes. Encore faut-il pouvoir identifier ces odeurs cadavériques au cours du processus de décomposition afin de décrypter le «langage» de cette entomofaune nécrophage. Grâce aux puissantes techniques de chimie analytique, c'est chose faite... l'odeur de la mort est en passe d'être complètement disséquée.

Le chapitre suivant introduit la chémo-écologie des insectes nécrophages et nécrophiles et replace cette approche novatrice dans un contexte plus généraliste. Alors que les composés organiques volatils (COVs ou *VOCs* en anglais) sont étudiés dans de nombreux domaines tels que l'agroalimentaire, l'écologie chimique, la qualité de l'air, ainsi qu'en médecine pour ne citer que quelques domaines, celui de de la décomposition de la matière organique d'origine animale est délaissé. A ce jour, très peu d'études concernant les COVs cadavériques ont été réalisées dans le domaine des sciences forensiques. Néanmoins, ce domaine de la thanatochimie est actuellement en pleine expansion. Le troisième chapitre développe les techniques d'analyses utilisées pour décrypter l'odeur de la mort. Différentes techniques d'analyses et de prélèvements des COVs cadavériques ont été utilisées au cours de cette recherche telles que la chromatographie gazeuse couplée à la spectrométrie de masse (GC-

MS) et plus récemment la chromatographie bidimensionnelle couplée à la spectrométrie de masse avec détecteur à temps de vol (GCxGC-TOFMS).

Connaitre la composition des odeurs cadavériques est un pré-requis nécessaire à la compréhension des interactions entre le cadavre et l'entomofaune des cadavres. Pourtant, elle ne permet pas de discriminer quels sont les composés odorants réellement perçus par l'insecte et qui vont induire chez lui un comportement spécifique. L'approche électrophysiologique permet d'identifier les composés odorants détectés par les antennes de l'insecte. Déjà explorée chez plusieurs espèces de Diptères d'intérêt forensique, l'électrophysiologie n'a encore jamais été étudiée chez *Thanatophilus sinuatus*. De par son abondance au sein de l'écosystème-cadavre lorsque celui-ci est localisé dans un biotope peu urbanisé, cette espèce de Silphinae a été choisie comme espèce modèle. L'approche électrophysiologique donne des informations sur la perception des odeurs par l'insecte, mais elle ne nous fournit pas d'information sur l'impact des composés odorants sur le comportement de l'insecte étudié. Une approche complémentaire est nécessaire, il s'agit d'une approche comportementale. Le quatrième chapitre détaille les expérimentations électroantennographiques (EAG) et comportementales (tests olfactométriques) réalisées sur des mâles et femelles de l'espèce *T. sinuatus* soumis à différents composés cadavériques.

Chapitre III.2: La communication chimique au sein de l'écosystème cadavre

III.2.1. Comment les insectes communiquent-ils au sein de l'«écosystème-cadavre»? L'écologie chimique des insectes nécrophages et nécrophiles

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Abstract- L'entomologie forensique est une discipline des sciences forensiques qui étudie les insectes et d'autres arthropodes dans un contexte médico-légal. Les insectes nécrophages et nécrophiles, principalement des Diptères et des Coléoptères, sont fréquemment retrouvés au sein de l'écosystème-cadavre. Pour ces insectes, le cadavre est une ressource éphémère très riche qu'ils vont coloniser de manière plus ou moins prévisible. L'entomofaune des cadavres seraient attirées par les odeurs cadavériques émises par le corps en décomposition. A l'heure actuelle, la thanatochimie est encore peu étudiée et l'information disponible concernant les COVs émis après la mort est limitée. Grâce à l'utilisation des méthodes de chimie analytique (TDS)GC-MS, GCxGC-TOF-MS), la signature olfactive d'un cadavre peut être étudiée au cours du processus de décomposition. L' «odeur de la mort» est constituée par un mélange de plus de cent composés organiques volatils qui évoluent au cours de la décomposition. Cependant, les sémiochimiques qui sont réellement attractifs pour les insectes nécrophages et/ou nécrophiles ne sont pas encore clairement identifiés. Les espèces pionnières pourraient être attirées par des COVs cadavériques. Toutefois, les espèces plus tardives pourraient aussi être attirées par d'autres types de sémiochimiques produits par les insectes sarcosaprophages

eux-mêmes (par exemple: des asticots, des insectes nécrophages). Plusieurs techniques d'écologie chimique peuvent être utiles en vue d'investiguer le rôle des sémiochimiques cadavériques dans le comportement des insectes sarcosaprophages. Une meilleure compréhension de l'écologie chimique des insectes nécrophages/nécrophiles et la thanatochimie pourraient avoir de nombreuses applications en science forensique et plus particulièrement en entomologie forensique.

Key words - Entomologie forensique, chémo-écologie, thanatochimie, insects nécrophages, décomposition, COVs cadavériques, odeurs cadavériques.

1. Introduction

Lorsqu'une espèce animale meurt, elle est rapidement visitée et colonisée par de nombreux organismes tels que des bactéries, des champignons, des arthropodes dont les insectes ainsi que des vertébrés (mammifères et oiseaux) (Carter *et al.* 2007). Au sein de nos écosystèmes terrestres tempérés, parmi les animaux consommateurs, les insectes nécrophages sont les plus spécialisés. Associés aux décomposeurs, ils participent à la minéralisation des matières organiques. Leur rôle est donc primordial au sein des écosystèmes terrestres où ils remplissent la fonction « d'éboueurs entomologiques » (Leclercq & Verstraeten, 1992; Marchenko, 2001). Le cadavre constitue pour ces différentes espèces un substrat nourricier, un site de reproduction, un refuge ou encore un territoire idéal bien que fluctuant au rythme des processus de décomposition. Parmi les insectes nécrophages, deux ordres sont largement présents sur les carcasses animales en décomposition: les Diptères et les Coléoptères. L'utilisation des insectes et d'autres arthropodes (acariens) à des fins médico-légales est le centre d'intérêt de l'entomologie forensique. On parle aussi d'entomologie (médico)-légale, judiciaire ou criminelle (Hall, 2001).

2. L' "écosystème - cadavre"

2.1. L'entomofaune des cadavres

Les insectes sont généralement les premiers organismes à arriver sur le corps peu après la mort et le colonisent selon une séquence plus ou moins prédictible (Smith, 1986; Anderson, 2001). Les insectes utilisent le micro-habitat créé par le cadavre comme un substrat nourricier, un site de pontes (reproduction), un refuge ou encore comme un territoire de chasse. En fonction de leurs caractéristiques écologiques, on distingue quatre groupes écologiques autour

d'un cadavre (Leclercq, 1978; Smith, 1986; Wyss & Chérix, 2006), une cinquième catégorie est parfois citée, il s'agit des espèces dites accidentelles dont la présence sur le corps est le fait du hasard (Arnaldos *et al*. 2005).

- **Les espèces nécrophages**: se nourrissent des tissus cadavériques et plus spécifiquement des liquides. On peut citer parmi cette catégorie, les Diptères appartenant aux familles des Calliphoridae et des Sarcophagidae, mais également des Coléoptères des familles des Silphidae et des Dermestidae.

- **Les espèces nécrophiles**: sont prédateurs ou parasites des espèces nécrophages, principalement des larves et des pupes de Diptères. On rencontre régulièrement des Coléoptères (Silphidae, Histeridae, Staphylinidae), des Diptères (Calliphoridae et Stratiomyidae) ainsi que des Hyménoptères (Campobasso *et al.* 2001; Wyss & Cherix, 2006). Les larves de certains Diptères peuvent devenir prédatrices à partir d'un certain stade de développement. C'est le cas, par exemple, des larves de stade III appartenant au genre *Muscina* (Diptère, Muscidae) (Gaudry, 2002) et de certaines *Chrysomya* (Diptère, Calliphoridae) (Leclercq, 1978).

- **Les espèces omnivores**: se nourrissent tant du cadavre que des espèces dites nécrophages et nécrophiles présentent sur la dépouille. Les principales espèces omnivores sont généralement des Hyménoptères (fourmis et guêpes) ainsi que des Coléoptères.

- **Les espèces opportunistes**: perçoivent la présence du cadavre comme une extension de leur habitat. Elles utilisent le cadavre comme une annexe de leur biotope afin de s'abriter, se réchauffer, hiberner et parfois même pour se nourrir (Leclercq & Verstraeten, 1992). Elles sont originaires de la végétation environnante ou de la pédofaune et peuvent exceptionnellement être prédateur des espèces nécrophages (Campobasso *et al.* 2001). On y dénombre des collemboles, des araignées, des mille-pattes, des Lépidoptères mais aussi des acariens qui se nourrissent des moisissures et champignons qui peuvent se développer sur le corps en décomposition (Campobasso *et al.* 2001; Wyss & Cherix, 2006).

Seul les deux premiers groupes sont utiles en entomologie forensique, ils regroupent un grand nombre de Diptères et de Coléoptères (Amendt *et al.* 2004).

2.2. La décomposition d'un cadavre à l'air libre

La décomposition d'un corps comporte une série de processus dynamiques qui vont entraîner des changements physiques, chimiques et biologiques au niveau du cadavre (Anderson, 2001). Hormis la décomposition biologique du corps par des microorganismes (bactéries, champignons saprophytes), des Arthropodes (dont les Insectes) et sa destruction par les Vertébrés (mammifères, oiseaux) (Marchenko, 2001), le corps subit une thanatomorphose. Après la mort, les processus de décomposition s'enclenchent plus ou moins rapidement selon les conditions environnantes (température et humidité principalement) (Anderson, 2001). Les entomologistes forensiques divisent le processus de décomposition en plusieurs stades ou phases. Cependant, ces phases de dégradation du corps doivent être interprétées comme étant une séquence de phénomènes qui se superposent et se combinent et non comme étant des stades clairement identifiables les uns des autres. En effet, il n'y a pas de distinction précise entre la fin d'un stade et le début du suivant (Campobasso *et al.* 2001; Goff, 2009). On distingue classiquement cinq stades de décomposition (Figure 31) (Anderson & VanLaerhoven, 1996; Galloway, 1997; Goff, 2009): le stade initial, le gonflement, la décomposition active, la décomposition avancée et la squelettisation.

Figure 31. Les stades de décomposition d'une carcasse de vertébré (cochon domestique) exposée à l'air libre.

La plupart des cadavres vont se putréfier, cependant, sous certaines conditions environnementales, la putréfaction n'a pas lieu et fait place à d'autres mécanismes tels que la momification du corps ou la formation d'adipocire (Campobasso *et al.* 2001). La putréfaction est la destruction des tissus mous du corps sous l'action des microorganismes (bactéries, protozoaires et champignons) endogènes et exogènes (Leclercq, 1978; Vass, 2001). La décomposition d'un corps à l'air libre provoque l'écoulement de nombreux liquides putrides qui vont progressivement imprégner le sol (*Gravesoil*). Ces infiltrations vont enrichir le sol et former des îlots de décomposition cadavériques (CDI) (Carter *et al.* 2007).

3. La communication chimique chez les insectes

La communication chimique ou chimioréception est le principal mode d'interaction des grands groupes d'animaux incluant les insectes (Brossut, 1996; Picimbon, 2002). Les insectes nécrophages n'y font pas exception (Leblanc & Logan, 2010). On peut définir la chiomoréception comme étant l'aptitude des organismes vivants à identifier des composés chimiques naturels ou synthétiques présents dans leur environnement et à en évaluer les concentrations (Meierhenrich *et al.* 2004). La décomposition du corps va entraîner des changements physiques et biochimiques importants, celui-ci va émettre en se décomposant des odeurs plus attractives pour certaines espèces et d'autres moins attractives (Leclercq, 1978; Anderson, 2001). La principale caractéristique de la communication chimique est la spécificité (Cassier *et al.* 2000). L'insecte est capable d'extraire du bruit de fond odorant qui l'entoure un certain nombre de molécules odorantes qui peuvent déclencher chez lui des comportements spécifiques (Cassier *et al.* 2000). C'est principalement l'olfaction qui permet à l'insecte de détecter et de reconnaitre les signaux chimiques provenant de son environnement (Cassier *et al.* 2000). La décomposition d'un corps à l'air libre provoque l'émission d'une large gamme de molécules chimiques dans l'environnement très facilement perceptibles par les insectes sarcosaprophages grâce à leur système olfactif très sensible aux effluves cadavériques (Statheropoulos *et al.* 2005).

3.1. L'olfaction chez les insectes

Chez les insectes, l'olfaction joue un rôle vital dans la reconnaissance de leur environnement en leur permettant de détecter les signaux chimiques environnants (Cassier *et al.* 2000; Picimbon, 2002; Reinhard, 2004). Le système olfactif des insectes est composé de quatre unités distinctes (Cassier *et al.* 2000). On distingue en partant du niveau périphérique au

niveau centrale: les antennes qui sont le support des organes sensoriels ou sensilles olfactives, les lobes antennaires qui sont le premier relais synaptique et enfin, le second relais synaptique composé des corps pédonculés ou *mushroom bodies* (Nagnan-le Meillour, 1998) où le traitement de l'information olfactive conduit à déclencher ou à modifier un comportement (Cassier *et al.* 2000). L'olfaction s'organise en trois étapes: la réception, la transduction et l'intégration du message olfactif (Brossut, 1996; Picimbon, 2002).

- **La réception**: les molécules odorantes qui pénètrent dans les sensilles olfactives par de nombreux pores cuticulaires sont véhiculées dans la lymphe sensillaire par un système de pores-tubules extracellulaires et de protéines de transport (les *Odorant Binding Proteins*, OBPs, et les *Pheromon Binding Proteins*, PBPs) jusqu'aux dendrites des neurones sensoriels (Cassier *et al.* 2000; Reinhard, 2004; Rutzler & Zwiebel, 2005). Les molécules odorantes, molécules hydrophobes et volatiles, qui pénètrent à l'intérieur du sensille peuvent soit activer le récepteur (complexe OBP/PBP-molécule odorante), soit l'inactiver et être éliminées par une estérase sensillaire (ODEs: *Odor Degrading Enzymes*) (Picimbon, 2002; Rutzler & Zwiebel, 2005). Les sensilles, spécifiques à une molécule ou à une famille de molécules chimiques, fonctionnement comme des microcapteurs périphériques des molécules odorantes de l'environnement (Picimbon, 2002). Il existe une grande diversité morphologique de sensilles ; on retrouve principalement trois types de sensilles sur les antennes : les sensilles trichodéiques, les sensilles basiconiques et des sensilles placodéiques ou plaques olfactives (Picimbon, 2002; Jefferis, 2005). La fixation de la molécule odorante sur la partie extracellulaire du récepteur va induire son activation ainsi qu'une succession d'interactions moléculaires intracellulaires, c'est la transduction (Nagnan- le Meillour, 1998).
- **La transduction ou transmission**: Les neurones olfactifs, encore appelés neurones sensoriels ou récepteurs (= ORNs: *Olfactory Receptor Neurons*) sont le siège de la transduction (Cassier *et al.* 2000; Rutzler & Zwiebel, 2005). Ils permettent la transformation d'un signal chimique, l'odeur, en un signal électrique, un potentiel d'action (Cassier *et al.* 2000). L'activation du récepteur membranaire dendritique induit une succession d'interactions cytoplasmiques qui vont provoquer l'ouverture des canaux ioniques et finalement aboutir à la formation d'un potentiel d'action qui va se propager le long de l'axone (Nagnan-le Meillour, 1998; Cassier *et al.* 2000). Chez les insectes, il n'existe qu'une seule voie pour la transduction olfactive, il s'agit de la

voie de l'inositol triphosphate (IP3) (Brossut, 1996; Cassier *et al.* 2000). Le signal sensoriel est ensuite envoyé jusqu'au centre de traitement primaire du système nerveux. Ensuite, cette information est transmise aux régions centrales du système nerveux où aura lieu l'intégration du message sensoriel (Nagnan-le Meillour, 1998).

- **L'intégration**: La réunion des axones de chaque neurone sensoriel forment les nerfs antennaires qui vont conduire le signal sensoriel jusqu'au premier relais synaptique : les lobes antennaires (un par hémisphère cérébral) qui sont situés dans le deutérocérébron (Brossut, 1996). Le traitement de l'information est ensuite effectué par les régions centrales du système nerveux et va aboutir à l'identification d'une molécule en tant qu'odeur (Nagnan-le Meillour, 1998). Les lobes antennaires, formés d'un ensemble de glomérules interconnectés via des interneurones locaux, constituent les centres de convergence entre les dendrites des récepteurs sensoriels et les centres d'intégration supérieurs (Brossut, 1996; Reinhard, 2004). Des neurones de projection assurent le cheminement du signal vers ces centres d'intégration supérieurs, notamment vers les corps pédonculés localisés dans le protocérébron (Brossut, 1996; Reinhard, 2004). C'est donc au niveau du protocérébron qu'ont lieu que les phénomènes de mémorisation et d'apprentissage des informations olfactives (Brossut, 1996).

3.2. Les sémiochimiques

Les sémiochimiques sont des médiateurs chimiques faisant intervenir des organes sensoriels externes et favorisant les interactions entre organismes (Arnaud *et al.* 2003). Les sémiochimiques impliqués dans l'olfaction appartiennent à deux grandes catégories : les phéromones et les allélochimiques (Cassier *et al.* 2000). Les phéromones interviennent dans les communications intraspécifiques et les allélochimiques interviennent dans les relations interspécifiques (Cassier *et al.* 2000, Arnaud *et al.* 2003). La classification des sémiochimiques est reprise à la Figure 32. On parlera plus particulièrement d'apneumones dans le cas de substances chimiques émises par un matériel non vivant et bénéficiant à l'organisme récepteur (Cassier *et al.* 2000 ; LeBlanc & Logan, 2010), en l'occurrence l'entomofaune des cadavres lors de la décomposition d'un corps. Les odeurs émises au cours de la décomposition sont constituées d'un très grand nombre de molécules chimiques (Dekeirsschieter *et al.* 2009; LeBlanc & Logan, 2010) et peuvent être perçues très rapidement par les insectes nécrophages alors qu'aucune odeur cadavérique n'est perceptible à l'odorat humain (LeBlanc & Logan, 2010).

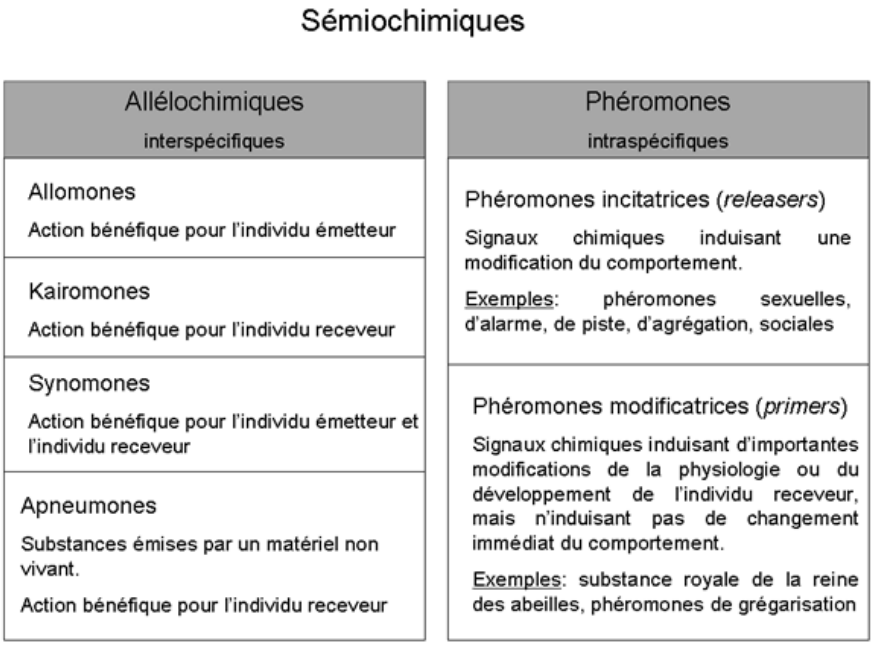

Figure 32. Classification des sémiochimiques impliqués dans l'olfaction chez les insectes (adaptés de Cassier *et al.* 2000; Arnaud *et al.* 2003; Leblanc & Logan, 2010).

4. Les techniques de l'écologie chimique appliquées aux insectes des cadavres

Très peu étudiée à l'heure actuelle, l'écologie chimique axée sur un organisme animal en décomposition permettrait de mieux comprendre les interactions au sein d'un écosystème particulier : le cadavre (Dekeirsschieter *et al.* 2010). Cependant, on n'a pas encore pu identifier ce qui attire vraiment les insectes sur un corps en décomposition et qui déclenche une modification du comportement telle que l'accouplement ou encore l'oviposition sur le cadavre (LeBlanc & Logan, 2010). Il semblerait que les odeurs émises par le cadavre (ou ses hôtes) attirent une grande diversité d'insectes au sein de cet écosystème si particulier (Anderson, 2001; Hart & Whitaker, 2005; Statheropoulos *et al*, 2007; Dekeirsschieter *et al.* 2009, LeBlanc & Logan, 2010). Encore faut-il pouvoir identifier ces odeurs afin de décrypter le «langage des insectes». Les odeurs sont constituées d'un ensemble de molécules chimiques volatiles, appelées Composés Organiques Volatils (COVs), qu'il faut pouvoir prélever, identifier et quantifier. Il faut également pouvoir identifier le rôle de ces molécules odorantes sur la physiologie et le comportement des insectes nécrophages. En combinant des études électrophysiologiques et comportementales, il est possible de mettre en évidence le rôle des molécules cadavériques sur les insectes nécrophages.

4.1. Les odeurs cadavériques

Les odeurs cadavériques forment un mélange complexe de molécules chimiques volatiles qui changent au cours de la décomposition (Dekeirsschieter *et al.* 2009). Le cadavre dégage des odeurs très fortes surtout pendant les phases de décomposition humide lorsque la production de gaz putréfactifs et d'amines est la plus forte (Dix & Graham, 2000). En général, l'odeur de décomposition tend à se dissiper avec la disparition progressive des tissus mous du corps. Les cadavres momifiés et/ ou partiellement squeletisés ont tendance à dégager une odeur de moisi tandis que les restes complètement squeletisés n'émettent plus d'odeur perceptible à l'odorat humain (Dix & Graham, 2000). Le prélèvement de ces molécules odorantes est un pré-requis nécessaire à leur identification et à la caractérisation de la signature olfactive d'un corps en décomposition.

4.1.1. Les méthodes de prélèvements d'odeurs et leurs analyses

Il existe deux grands types de prélèvements d'odeurs: l'échantillonnage dynamique et l'échantillonnage passif ou statique. La distinction entre ces deux types d'échantillonnage est la mise en mouvement de l'air ou non autour de la source d'émissions de molécules volatiles. Contrairement aux méthodes passives, les techniques dynamiques requièrent des dispositifs actifs tels que des pompes pour faire circuler le flux d'air et des débitmètres (Namiesnik *et al.* 2004). Les méthodes dynamiques se basent sur la mise en mouvement de l'air contenu dans l'espace de tête de l'organisme étudié (*headspace collection*). Un système de filtrage de l'air va permettre de piéger les molécules volatiles sur un adsorbant (Charbon actif, Tenax®, PDMS®, Porapak®, *etc*) (Millar & Sims, 1998; Jones & Oldham, 1999). Le principe de l'échantillonneur passif est basé sur la diffusion des molécules volatiles dans l'air ambiant, celles-ci vont se fixer sur un support adsorbant (loi de Fick) (Cocheo *et al.* 1996; Varshney & Singh, 2003). Outre la SPME (Microextraction sur phase solide) qui est très souvent utilisée pour étudier les odeurs, il existe également de nombreux types d'échantillonneurs passifs que l'on peut employer. Dans la plupart des échantillonneurs passifs, le parcours diffusif est axial, il existe cependant des capteurs dont la géométrie est radiale (Cocheo *et al.* 1996). C'est le cas du dispositif Radiello®. Les prélèvements peuvent se faire en enceinte close, semi- ouverte ou ouverte (Tholl *et al.* 2006) ou encore directement *in situ*. Concernant les COVs cadavériques les deux méthodes de prélèvements d'odeurs ont déjà été utilisées avec succès soit sur cadavres ou restes humains (Vass *et al.* 2004; Statheropoulos *et al.* 2005, 2006, 2007; Vass *et al.* 2008; Hoffman *et al.* 2009) soit sur carcasses animales (LeBlanc, 2008; Dekeirsschieter *et al.* 2009; Kalinova *et al.* 2009). La SPME a quant à elle été utilisée par une équipe de

chercheurs américains afin de caractériser les composés organiques volatils présents dans l'espace de tête de différents types de tissus humains en décomposition (Hoffman *et al.* 2009). Cette technique a également été utilisée afin de caractériser la signature olfactive de carcasses de souris en décomposition (Kalinova *et al.* 2009). Les composés organiques volatils piégés seront désorbés soit par solvant (élution avec un solvant pur ou un mélange de solvants organiques) soit par thermodésorption (Millar & Sims, 1998; Tholl *et al.* 2006). Les molécules piégées doivent être séparées avant de pouvoir être identifiées, la chromatographie en phase gazeuse (GC) est une méthode couramment utilisée pour analyser des composés volatils ou semi-volatils (Heath & Dueben, 1998). L'identification des composés odorants est rendue possible grâce l'utilisation d'un détecteur couplé au GC tel que le spectromètre de masse (GC-MS). Le GC-MS permet d'obtenir directement le spectre de masse de chaque constituant d'un mélange au fur et à mesure de leur séparation sur la colonne chromatographique qui fonctionne en amont du spectromètre de masse (Strebler, 1989). Dans les études de thanatochimie, la méthode d'analyse des COVs cadavériques est généralement la chromatographie gazeuse couplée à un spectromètre de masse (Vass *et al.* 2004; Statheropoulos *et al.* 2005, 2006, 2007; Vass *et al.* 2008; Dekeirsschieter *et al.* 2009; Hoffman *et al.* 2009). Cependant, une récente étude utilise la chromatographie bidimensionnelle couplée à un spectromètre de masse à temps de vol (GCxGC-TOFMS) (Kalinova *et al.* 2009).

4.1.2. Les COVs cadavériques

Les composés organiques volatils émis au cours de la décomposition sont en fait des produits de décomposition intermédiaires (Vass *et al.* 2002). Il s'agit de co-produits issus du catabolisme des molécules biologiques: les protéines, les acides nucléiques, les lipides et les glucides (Vass *et al.* 2002 ; Statheropoulos *et al.* 2005). La dégradation complète de ces macromolécules biologiques conduira à la restitution de leurs constituants (carbone, hydrogène, soufre, azote, phosphore, oxygène) dans l'écosystème (Vass *et al.* 2002; Statheropoulos *et al.* 2005). La dégradation des protéines (protéolyse) fournit des gaz (CO_2, H_2S, CH_4, SO_2, NH_3), des diamines (putrescine et cadavérine), des composés soufrés (diméthyldisulfide, diméthyltrisulfide) et des composés phénoliques (indole, scatole) (Clark *et al.* 1997; Vass, 2001; Vass *et al.* 2002; Dent *et al.* 2004; Statheropoulos *et al.* 2005). La dégradation des lipides produit des acides gras (ex: acides oléique et palmitique) et des composés oxygénés, azotés et phosphorés ainsi que des hydrates de carbone (Gill-King, 1997; Statheropoulos *et al.* 2005). Les glucides sont principalement dégradés en composés oxygénés

tels que des acides organiques, des alcools, des cétones, des esters et des aldéhydes (Gill-King, 1997; Dent *et al.* 2004; Statheropoulos *et al.* 2005). Concernant les COVs cadavériques émis au cours du processus de décomposition, toutes les études menées jusqu'à présent mettent en avant un grand nombre de molécules chimiques qui peuvent varier d'une étude à l'autre (Vass *et al.* 2004; Statheropoulos *et al.* 2005, 2006, 2007; LeBlanc, 2008; Vass *et al.* 2008; Dekeirsschieter *et al.* 2009; Hoffman *et al.* 2009; Kalinova *et al.* 2009). Si certains composés sont identiques dans toutes les études (LeBlanc & Logan, 2010) telles que le DMDS ou le DMTS; il n'y a aucune signature chimique unique de la décomposition (LeBlanc & Logan, 2010) que ce soit sur cadavres humains ou carcasses animales.

4.2. L'approche électrophysiologique

Connaitre la composition des odeurs cadavériques est un pré-requis nécessaire à la compréhension des interactions entre le cadavre et la faune des cadavres. Mais, elle ne permet pas de discriminer quels sont les composés odorants réellement perçus par l'insecte et qui vont induire chez lui un comportement spécifique. L'approche électrophysiologique permet d'identifier les composés odorants détectés par les antennes de l'insecte et plus précisément par les neurones récepteurs olfactifs (Bjostad, 1998). L'électroantennographie (EAG) et le Single Cell Recording (SCR) sont deux techniques électrophysiologiques qui permettent de mesurer respectivement la réponse électrique antennaire pour l'EAG et d'une seule sensille olfactive pour le SCR (Bjostad, 1998). Cependant, l'utilisation de l'électroantennographie est plus fréquente et permet de détecter les composés électrophyisologiquement actifs pour un insecte. L'électroantennographe va enregistrer et amplifier des variations de potentiel électrique entre deux électrodes placées sur les antennes de l'insecte lorsque celui-ci est soumis à un stimulus odorant. On observera une différence de potentiel lorsque l'antenne est stimulée par une molécule odorante active. L'EAG peut également être couplé à un chromatographe en phase gazeuse (GC-EAD) (Moorhouse *et al.* 1969; Bjostad, 1998), celui-ci permet de détecter et de positionner sur un chromatogramme les composés électrophysiologiquement actifs présents dans un mélange d'odeurs complexe (LeBlanc & Logan, 2010). Le même principe de couplage existe pour le SCR, on parlera de GC-SCR (Wadhams *et al.* 1982). Lorsque le composé testé (EAG) ou un des composés du mélange testé (GC-EAD) induit une réponse électrique, on observera une dépolarisation des sensilles qui se marquera sur l'électroantennogramme par une variation d'amplitude du signal EAG (Jones & Oldham, 1999). Cette variation de potentiel électrique peut aller de quelques microvolts à plusieurs millivolts et dépend de plusieurs facteurs tels que la nature et la

concentration du stimulus olfactif, mais également de l'espèce, de l'âge et du sexe de l'insecte testé (Verheggen, 2005). Au vu du grand nombre d'insectes que l'on peut retrouver au sein de l'écosystème-cadavre et d'une certaine phénologie dans la colonisation postmortem du cadavre, les études électrophysiologiques sur les insectes appartenant à cet écosystème particulier devraient mettre en avant des différences dans les composés actifs perçus par les insectes « nécrophages ». En effet, certains insectes tels que les Calliphoridae colonisent très rapidement les corps (= espèces pionnières), attirés semble- t'- il par les odeurs cadavériques apparaissant au début du processus de décomposition (Greenberg, 1991; Wall & Warnes, 1994; Anderson, 2001 ; LeBlanc & Logan, 2010). Tandis que d'autres insectes seraient attirés par des stades de décomposition plus avancés voir par l'entomofaune déjà présente sur le cadavre ou encore repoussés par les odeurs de décomposition précoces (LeBlanc & Logan, 2010). A ce jour, très peu d'études électrophysiologiques ont été effectuées avec des insectes appartenant à cet écosystème (Stensmyr, 2004 ; Frederickx, 2008 ; LeBlanc, 2008, Kalinova *et al.* 2009). On peut notamment citer les travaux de GC-EAD d'Hélène LeBlanc (LeBlanc, 2008) sur *Calliphora vomitoria* (Diptera, Calliphoridae), de Frederickx (Frederickx, 2008) qui a réalisé de l'EAG sur *Nasonnia vitripennnis* (Hymenoptera, Braconidae), un parasite de pupes de Diptères. Des composés soufrés ont également été testés sur *Nicrophorus vespilloides* et *Nicrophorus vespillo* (Coleoptera, Silphidae) par EAG (Kalinova *et al.* 2009). L'approche électrophysiologique donne des informations sur la perception des odeurs par l'insecte, mais elle ne nous fournit pas d'information sur l'impact des composés odorants sur le comportement de l'insecte étudié. Une approche complémentaire est nécessaire, il s'agit d'une approche comportementale.

4.3. L'approche comportementale

Identifier les composés électrophysiologiquement actifs ne permet pas de comprendre le rôle biologique de ces composés odorants sur le comportement des insectes étudiés. Une approche comportementale olfactométrique permet de mieux cerner les réactions comportementales de l'insecte lorsque celui-ci est exposé à un stimulus olfactif. Cette approche comportementale se base sur l'utilisation d'olfactomètres. Les olfactomètres sont des dispositifs permettant l'étude du comportement d'un organisme lorsque celui-ci est mis en présence d'une source d'odeurs (Strebler, 1989; Verheggen, 2005). Le choix du type d'olfactomètre sera fonction de l'espèce d'insecte étudiée et du type de comportement induit par les stimuli olfactifs (Strebler, 1989). Il existe deux grandes catégories d'olfactomètres, les appareillages statiques qui ne nécessitent pas la mise en mouvement de l'air au sein du

dispositif et les systèmes dynamiques qui reposent sur la circulation d'un flux d'air au sein de l'enceinte (Hare, 1998). Au sein des dispositifs dynamiques, un système de pompes met en mouvement l'air qui va entrainer avec lui le composé volatil testé. Tandis qu'au sein des dispositifs statiques, un diffuseur dispersa la molécule odorante dans le dispositif (Hare, 1998). L'analyse comportementale permet donc d'identifier l'influence d'une ou plusieurs molécule(s) odorante(s) sur l'insecte étudié, et permet par exemple de démontrer l'effet attractif d'un composé sur un insecte.

5. Conclusion et perspectives

Une meilleure compréhension de l'écologie chimique des insectes nécrophages et/ou nécrophiles permettraient de mieux cerner l'écosystème-cadavre et d'identifier les composés odorants responsables de l'attraction de ces insectes sur un cadavre. Outre une meilleure connaissance de la chemoécologie des insectes nécrophages, la caractérisation de la signature olfactive d'un corps en décomposition trouve des applications dans de nombreux domaines des sciences forensiques. Parmi celles–ci, on peut citer la mise au point d'appareils de détection de cadavre (Vass *et al.* 2004; Statheropoulos *et al.* 2006; Vass *et al.* 2008) un meilleur entrainement des chiens pisteurs (*rescue dog* et/ou *cadaver dog*) (Killam, 1990; Komar, 1999; Oesterhelweg *et al.* 2008; Hoffman *et al.* 2009) ou encore une nouvelle méthode pour déterminer l'intervalle postmortem (IPM) (Vass, 2001; Statheropoulos *et al.* 2007; LeBlanc & Logan, 2010). Les recherches en thanatochimie se poursuivent et les composés organiques volatils qui caractérisent la décomposition de matière organique animale ouvrent de larges perspectives. Cependant, les insectes restent encore une des seules méthodes fiables pour déterminer l'intervalle postmortem (LeBlanc & Logan, 2010), notamment lorsque les méthodes médicales font défaut. Malgré tout, l'étude conjointe des odeurs cadavériques (thanatochimie) et des insectes nécrophages (entomologie forensique) doit aller plus loin afin de déterminer les liens spécifiques qui existent entre la datation de la mort par des méthodes entomologiques et les processus de décomposition (LeBlanc & Logan, 2010). Une approche chemoécologique de l'écosystème-cadavre permettrait de mieux comprendre les interactions spécifiques qui évoluent au gré du processus de décomposition.

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Chapitre III.3: Les odeurs cadavériques

III.3.1. Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes

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Abstract - Forensic entomology uses pig carcasses to surrogate human decomposition and to investigate the entomofaunal colonization. Insects communicate with their environment through the use of chemical mediators, which in the case of necrophagous insects, may consist in the cadaveric volatile organic compounds (VOCs) released by the corpse under decomposition. Previous studies have focused on cadaveric VOCs released from human corpses. Nevertheless, studies on human corpses are restricted for many reasons, including ethics. Forensic entomologists use pig as animal model but very few information are available about the decompositional VOCs released by a decaying pig carcass. We here tested a passive sampling technique, the Radiello® diffusive sampler, to monitor the cadaveric VOCs released by decomposing pig carcasses in three biotopes (crop field, forest, urban site). A total of 104 chemical compounds, exclusively produced by the decompositional process, were identified by thermal desorption interfaced with gas chromatography and mass spectrometry (TDS-GC-MS). Ninety, 85 and 57 cadaveric VOCs were identified on pig carcasses laying on the

agricultural site, the forest biotope and in the urban site, respectively. The main cadaveric VOCs are acids, cyclic hydrocarbons, oxygenated compounds, sulfur and nitrogen compounds. A better knowledge of the smell of death and their volatile constituents may have many applications in forensic sciences.

Key words - Forensic entomology, odour analysis, pig carcasses, decompositional process, volatile passive sampling, Radiello®.

1. Introduction

Forensic entomology is a branch of the forensic sciences which studies insects and other arthropods (e.g. mites) in a medico-legal context (Hall 1990, 2001, Amendt *et al.* 2004, Wallman 2004, Gennard 2007). The necrophagous insects, mainly Diptera and Coleoptera, have particular relationships with decomposing remains which constitute a rich ephemeral resource (Anderson and Van Laerhoven 1996, Grassberger and Frank 2004, Carter *et al.* 2007). These insects are attracted to the cadaver that they colonize in a relative predictable sequence called the entomofaunal succession (Mégnin 1894, Putman 1983, Schoenly and Reid 1987, Marchenko 1988, 2001, Benecke 2004). Undeniably, the first cadaver colonizers are blowflies (Diptera, Calliphoridae) that are attracted by the early decomposition odour (Greenberg 1991, Wall and Warnes 1994). Other insects are attracted by a decaying body during later decompositional stages (e.g. active decay, advanced decay) or by the presence of dry remains (*i.e.* skin,bones) (Anderson 2001). It is speculated that the volatile organic chemicals (VOCs) released during the decompositional process attract a wide range of insects (Anderson 2001, Hart and Whitaker 2005, Statheropoulos *et al.* 2007). Thanatochemistry, also named "chemistry of death", is poorly studied and the available information regarding the VOCs released after death are rather limited (Gill-King 1997, Vass *et al.* 2004, 2008, Statheropoulos *et al.* 2005, 2007, Oesterhelweg *et al.* 2008). Numerous applications would however benefit from a better understanding of the olfactive signature of a human or animal corpse. The cadaveric VOCs find applications in forensic sciences and the etiology of death (Statheropoulos *et al.* 2005), in training of cadaver dogs (killam 1990, Komar 1999, Oesterhelweg *et al.* 2008), in the development of cadaveric material detection device (Vass *et al.* 2004, 2008, Statheropoulos *et al.* 2006) or in the determination of the post-mortem interval (Vass 2001, Statheropoulos *et al.* 2005). Forensic entomologists often raise the hypothesis that the cadaveric VOCs regulate the necrophagous insects behaviour. Many forensic entomological studies were conducted on pig carcasses as surrogate human models for

physiological, ethical and economical reasons (Rodriguez and Bass 1983, Catts and Goff 1992, Anderson and Van Laerhoven 1996, Grassberger and Frank 2004, Hart and Whitaker 2005), but no information is available on the VOCs a pig's carcass releases, and few were focused on human decomposition in natural conditions. As examples, Vass and colleagues (Vass *et al.* 2004) identified 424 specific volatile chemicals released during the burial decompositional process of four human cadavers.

Statheropoulos and colleagues (Statheropoulos *et al.* 2005) detected more than 80 volatile substances on two human putrefied bodies put in "body bags". More recently, the Decompositional Odor Analysis Database (Vass *et al*; 2004, 2008) identified 478 specific volatile compounds associated with buried human remains. All these experiments were conducted in enclosed (i.e. burial, "body bag") environment without access to the corpse for the necrofauna. In opposition to these studies, this paper identifies the VOCs released in the headspace of decaying pigs during the decompositional process in three different biotopes, and using a passive sampling method.

2. Materials and method

2.1. Sites and animals

Six piglets (*Sus domesticus* L.) (25 kg) were killed by penetrative captive bolt and disposed in the experimental sites within the next 4 h. Immediately after the euthanasia, each pig carcass was packed in a double plastic bag to avoid any insect colonization before the laying on the experimental biotope. Three private areas, located in Belgium, were used to monitor the VOCs released by the pig carcasses: a forest, an agricultural and an urban site. In each site, two pig carcasses were placed 50 m from each other, in metal mesh cages (180 cm x 90 cm x 90 cm) to avoid scavenging by carnivores. The forest habitat consisted of pedonculate oaks (*Quercus robur* L.), European beeches (*Fagus sylvatica* L.) and sycamore maples (*Acer pseudoplatanus* L.). The agricultural biotope is a transect (5 m width) of meadow with an alignment of willows (*Salix sp.*) between a barley (*Hordeum vulgare* L.) field and an enclosed grassland. The meadow has not been grazed for the duration of the experiment. The urban biotope was an abandoned building of two floors with broken windows and inside vegetation (*Clematis vitalba* L.). The building was located on a secure site belonging to the National Institute of Criminalistic and Criminology (INCC-NICC, Brussels, Belgium). The experiment was conducted in spring 2007 (March 29-May 11).

As temperature is one of the most important parameters influencing the decomposition rate (Vass *et al.* 1992, 2002, Gill-King 1997, Anderson 2001, Campobasso *et al.* 2001, Vass 2001), the ambient air temperature was automatically measured once an hour using a datalogger (Testo 175-T1® temperature data logger, Germany) placed on the lateral side of each cage, at a height of 75 cm. The daily mean temperature was calculated on the basis of ambient air temperature recorded on a time interval of 24 h. Other environmental parameters (humidity, wind velocity, wind direction) were recorded thanks to a Vantage Pro Plus™ Stations® (Davis instruments, Hayward, CA, USA).

2.2. Volatile collection

A passive sampling technique was used to collect the VOCs released by the decaying carcasses. Radiello® tubes (Foundation Salvatore Maugeri - IRCCS, Padova, Italy) were made of three assembled parts (Cocheo *et al.* 1996): the sorbent cartridge, the diffusive body and the supporting plate. The cylindrical diffusive tube (60 mm x 16 mm diameter) was made of microporous polyethylene (porosity: 10 ± 2 µm) and the triangular supporting plate was made of polycarbonate. The adsorbent cartridge was made of stainless steel with 3 µm x 8 µm mesh, filled with the graphitised carbon (dimensions: 60 mm x 4.8 mm). Carbograph 4 (350 ± 10 mg) was selected as adsorbent material for trapping volatile organic compounds (Foundation Salvatore Maugeri - IRCCS, Padova, Italy). Previous studies on thermally desorbed VOCs have used this sorbent material (Crescenzi *et al.* 1996, Bruno *et al.* 2005, Pennequin-Cardinal *et al.* 2005). Moreover, different sorbents were tried during this experimental study as preliminary step: Tenax Ta and Carbograph 4. These two sorbent materials suitable to Radiello® cartridges were tested in outdoor conditions on wood pigeon carcass (*Columba palumbus* L.). This preliminary study highlighted the best performance of Carbograph 4 to collect the cadaveric volatile compounds. Before their use, the carbograph cartridges were conditioned for 12 h at 300 °C with constant helium flow (200 mL/min). The reconditioning is done in a series of 10 tubes. The conditioned cartridges were sealed in glass tubes, packed in a brown jar and stored at 4 °C.

Two Radiello® samplers were placed above each carcass. To protect them from the rain and prevailing wind, they were enclosed in a specific shelter made of polypropylene. The shelter was placed horizontally on the carcass so that both samplers were disposed close to the abdominal cavity (±3 cm). Volatile organic chemicals were collected passively for 7 days, before being replaced. As controls, two additional samplers were placed in the experimental

areas to collect the atmospheric VOCs, at 50 m from the decomposing carcasses. A total of six samplers were therefore disposed in each experimental site.

2.3. Chromatographic analysis

The adsorbed VOCs were thermally desorbed to a gas chromatograph HP6890 coupled with an automatic thermal desorber (ATD 400, PerkinElmer). Analytical parameters are provided in Table 18. Volatile chemicals were first thermally desorbed from the Radiello tube before cryofocusing (temperature of cryofocusing -30 °C) on an electrical adsorbent trap. The cooled trap was then heated (desorbing temperature: 350 °C during 4 min) and the desorbed chemicals were instantaneously injected in the chromatographic system. Identifications were made by comparing of the retention times with those of known standards and confirmed by mass spectrometry, using an HP5972a mass spectrometer (Agilent Technologies).

Table 18. Analytical parameters of the TDS-GC-MS analysis.

ATD 400	**Autosystem GC-MS**
	GC 6890
Desorption temperature: 350 °C during 4 min	Carrier gas: helium at 1.0 mL/min
Desorption flow: 80 mL/min (helium)	Column: Rtx/MXT-1301/624
Outlet split: 15.1 mL/min	60 m x 0.32 mm x 1.8 mm
Column pressure: 8.7 psi	Oven temperature program:
Trap temperature: -30 to 300 °C	Initial temperature: 35 °C during 5 min
Transfer line temperature: 250 °C	First ramp: 3 °C/min, 50 °C
	Second ramp: 6 °C/min, 110 °C
	Third ramp: 10 °C/min, 245 °C during 5 min
	Detector: MS
	MS 5972 A
	Solvent delay: 5 min
	Mass scan: *m*/*z* 35-300

2.4. Decomposition stages

The decomposition process was observed according to different stages. We decided to discriminate five major decompositional stages based on different visual criteria adapted from the literature (Bornemissza 1957, Reed 1958, Clark *et al.* 1997, Galloway 1997, Gennard 2007, Campobasso *et al.* 2001, Adlam and Simmons 2007): (1) fresh, (2) bloated, (3) active decay, (4) advanced decay and (5) dry remains. The decay stages are presented in Table 19.

Table 19. The five decompositional stages defined in this study and their descriptions.

Decompositional stage	Description	Literature (references)
(1) *Fresh*	From death until the first signsof bloating	(Gennard 2007)
	Autolysis	(Gill-King 1997)
		(Vass 2001)
		(Reed 1958)
(2) *Bloated*	Putrefaction mechanism generates accumulation of breakdown gases causing bloating of the corpse. The first signs of the bloated stage appear in the abdomen. Then the whole body swells	(Gennard 2007)
	Anaerobic fermentations	(Vass 2001)
		(Reed 1958)
		(Galloway 1997)
(3) *Active decay*	Darkening of the skin	(Gennard 2007)
	The skin is breaking up and the body began to deflate. Protein sources are broken down into fatty acids and other decomposition products such as skatole, indole, cadaverine, putrescine	(Vass *et al.* 2004)
		(Vass 2001)
		(Reed 1958)
		(Galloway 1997)
(4) *Advanced decay*	Corpse dries and the remains are skin, cartilage, hair, bones and some fragments of flesh	(Gennard 2007)
		(Reed 1958)
		(Galloway 1997)
(5) *Dry remains or skeletonisation*	The only remains are bones and hair	(Gennard 2007)
	Diagenesis	(Reed 1958)

2.5. Statistical analysis

In order to analyze the spatial distribution of our data set, a statistical analysis was conducted through a multivariate analysis: the principal component analysis (PCA) (Minitab® v15.0, State College, PA, USA). PCA is a descriptive technique which groups the variables (X-variables: volatile chemicals, Y-variables: pigs; more precisely, a combination of the biotope and the decompositional stage) according to their correlation coefficient. The original data set is a matrix of *c* X *n (c* = objects, *n* = variables) corresponding to a matrix of 12 x 104. The area of chromatogram's peak corresponding to VOC was used for PCA.

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3. Results

3.1. Environmental parameters

The mean atmospheric temperature measured during the decompositional process was 13.22 °C for the forest site, 13.76 °C for the agricultural site and 16.25 °C for the urban site (Fig. 33). The temperature curves have a similar pattern over time. Nevertheless, the urban site is warmer than the other sites. There is a difference of 3.03 °C compared to the forest biotope and 2.49 °C compared to the agricultural site. The mean relative humidity was 68.30% for the "open-air" biotopes and 62.00% for the urban site.

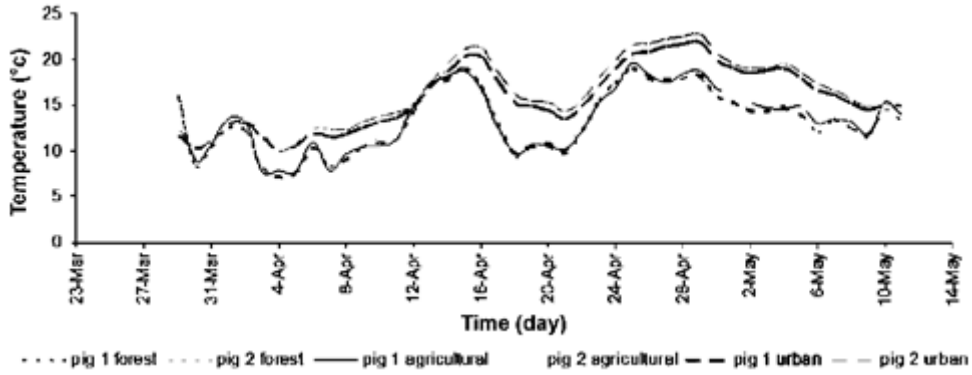

Figure 33. Temperature recordings on the six carcasses (''pig 1 forest'' for the first pig carcass on the forest site, ''pig 2 forest'' for the second carcass on the forest site, ''pig 1 agricultural'' for the first pig on the agricultural site and ''pig 2 agricultural'' for the second carcass on the agricultural site, ''pig 1 urban'' and ''pig 2 urban'', respectively for the first and the second carcass in the urban site).

3.2. Volatile collection

In this study, 104 VOCs specifically released under the pig decomposition process were identified by TDS-GC-MS (Table 20). Many chemical families were represented (in parentheses, the number of chemical compounds identified): acids (14), esters (11), ketones (8), aldehydes (8), alcohols (9), nitrogen compounds (19), sulfur compounds (7), halogen compounds (1), cyclic hydrocarbons (16) and non-cyclic hydrocarbons (11). Table 21 presents the VOCs identified according to their specific biotope. There are some major differences between the three biotopes in terms of sampled chemical molecules. Indeed, while only 57 VOCs were identified in the urban site, 85 and 90 VOCs were identified in the forest and the agricultural site, respectively.

A total of 35 molecules (Table 21) constitute the common core of VOCs associated with pig decaying carcasses, i.e. the chemical compounds sampled in the three experimental biotopes:

7 acids (propanoic acid, butanoic acid, 2-methylpropanoic acid, 2-methylbutanoic acid, 3, methylbutanoic acid, pentanoic acid and hexanoic acid), 4 esters (butyl formate, butyl butanoate, butyl 3-methylbutanoate, 2-methylester, propanoic acid), 1 ketone (pen-tan-2-one), 1 aldehyde (butanal), 5 alcohols (2-methyl-l-propan-l-ol, butan-lol, (2S)-butan-2-ol, 3-methylbutan-l-ol, pentan-1-ol), 6 nitrogen compounds (acetamide, N-methylacetamide, N,N-dimethylformamide, N,N-dimethylacetamide, N,N-dimethylni-trous amide, trimethylamine), 5 sulfur compounds (methanethiol, SO_2, methyldisulfanylmethane (dimethyldisulfide), methylsulfa-nyldisulfanylmethane (dimethyltrisulfide), 1-methylsulfonyloxy-butane), 4 cyclic hydrocarbons (cyclohexylcyclohexane, phenol, 4-methylphenol, piperidin-2-one) and 2 non-cyclic hydrocarbons (eicosane, 2-methylprop-1-ene).

Table 20. The 104 volatile organic compounds specifically released by the pig carcasses, classified by chemical families. The column ''present study'' references the VOCs detected in this study according to their specific biotope (forest biotope, agricultural biotope, urban biotope), (() represented a VOC detected in a specific biotope or (-) for a VOC not detected. The column ''literature'' lists the VOCs referenced in the specific literature (numbers correspond to the different papers concerning the smell of death and are listed in ''References'' section) or not referenced ((-) for not referenced).

Volatile chemicals	Present study			Literature
	Forest biotope	Agricultural biotope	Urban biotope	
Acids				
Formic acid	▲	▲	-	-
Propanoic acid	▲	▲	▲	[20,22,28]
Butanoic acid	▲	▲	▲	[20,22,28,46]
2-Methylpropanoic acid	▲	▲	▲	[46]
2-Methylbutanoic acid	▲	▲	▲	[46]
3-Methylbutanoic acid	▲	▲	▲	[46]
Pentanoic acid	▲	▲	▲	-
4-Methylpentanoic acid	▲	▲	-	-
Hexanoic acid	▲	▲	▲	-
Benzoic acid	▲	▲	-	-
Heptanoic acid	▲	▲	-	-
Octanoic acid	-	▲	-	-
Benzoylformic acid	-	▲	-	-
Nonanoic acid	-	▲	-	-
Esters				
Propan-2-yl-nitrate	▲	▲	-	-
Butyl formate	▲	▲	▲	-
Propyl acetate	-	-	▲	-
Butyl acetate	-	-	▲	-

Butyl 2-methylpropanoate	-	▲	▲	-
Butyl butanoate	▲	▲	▲	-
Butyl 3-methylbutanoate	▲	▲	▲	-
Butyl 2-methylbutanoate	-	▲	▲	-
Butyl pentanoate	-	▲	-	-
Phenyle butanoate	-	▲	-	-
2-Methylester, propanoic acid	▲	▲	▲	-
Ketones				
Acetone	▲	-	-	[20,22,44,50]
Butan-2-one	▲	-	-	[22,28]
2,3 Butanedione	▲	-	▲	[46]
Pentan-2-one	▲	▲	▲	[22]
(E)-Pent-3-en-2-one	-	▲	-	-
Heptan-2-one	▲	▲	-	[22,46]
1 -Phenylethanone	▲	▲	-	[28]
Nonan-2-one	▲	-	-	[24,28]
Aldehydes				
Propen-2-al	▲	-	-	-
But-2-enal	▲	▲	-	-
2-Methylpropanal	-	-	▲	-
Butanal	▲	▲	▲	[24]
2-Methylbutanal	▲	-	-	[46]
Pentanal		▲	▲	[22,24,46]
Benzaldehyde	▲	▲	-	[21]
Heptanal	▲	▲	-	[24,46]
Alcohols				
Ethanol	-	▲	-	[20,22,24,28,44,50]
2-Methyl-l-Propan-l-ol	▲	▲	▲	[22,50]
Butan-l-ol	▲	▲	▲	[20,22,44,50]
(2S)-Butan-2-ol	▲	▲	▲	[50]
3-Methylbutan-1-ol	▲	▲	▲	[46,50]
3-Methylbutan-2-ol	-	-	▲	-
Pentan-1-ol	▲	▲	▲	[22,24,46]
2-PhenylethanoI	▲	-	-	[50]
2-Ethylhexan-1 -ol	▲	-	-	[21,28]
Nitrogen compounds				

Acetamide	▲	▲	▲	-
N-Methylacetamide	▲	▲	▲	-
N,N-Dimethylformamide	▲	▲	▲	-
Propanamide	-	▲	-	-
N-Methylpropanamide	-	▲	-	-
N.N-Dimethylacetamide	▲	▲	▲	[24]
Butanamide	▲	▲	-	-
N.N-Dimethylpropanamide	-	▲	-	-
3-Methylbutanamide	-	▲	-	-
N-Methylbenzamide	▲	▲	-	-
N.N-Dimethylbenzamide	-	▲	-	-
N.N-Dimethylnitrous amide	▲	▲	▲	-
Trimethylamine	▲	▲	▲	-
Acetonitrile	▲	-	▲	-
2-Dimethylaminoacetonitrile	-	▲	-	-
Benzonitrile	▲	▲	-	[21]
2-Phenylacetonitrile	-	-	▲	-
Octanenitrile	▲	-	-	-
Nonanenitrile	▲	-	-	-
Sulfur compounds				
Methadithione	▲	-	▲	[21,22,24]
Methanethiol	▲	▲	▲	-
Sulfur dioxide	▲	▲	▲	[21,22,24.28,33]
Methyldisulfanylmethane	▲	▲	▲	[21,22,24.28,46]
Methylsulfanyldisulfanylmethane	▲	▲	▲	[21,22,24,28]
1 -Methylsulfanylethanone	▲	-	-	-
1 -Methylsulfonyloxybutane	▲	▲	▲	-
Cyclic hydrocarbons				
1 -Methyl-4-(1 -methylethyl) benzene	▲	▲	-	-
Cyclohexylcyclohexane	▲	▲	▲	-
Phenol	▲	▲	▲	[24,28]
4-MethylphenoI	▲	▲	▲	[28]
Benzene-l,4-diol	▲	▲	-	-
Piperidin-2-one	▲	▲	▲	-
Benzooxazole	▲	▲	-	-
1 H-pyrrole	-	▲	-	-
Pyridine	▲	-	-	-
3-Methylpyridine	-	▲	-	-

lH-Indole	-	-	▲	[22,28,33,44]
2.3,5-Trimethylpyrazine	▲	-	-	-
Quinazoline	▲	▲	▲	-
Quinoxaline-	-	▲	-	-
2-Nitrophenol	▲	▲	-	-
3-Methylthiophene	-	▲		-
Halogen compounds				
1-Chloro-butane	▲	-	-	-
Non cyclic hydrocarbons				
4-Methylheptane	-	▲	▲	-
2-Methyldecane	-	-	▲	-
2,6-Dimethylundecane	▲	-	-	-
3-Hexylpentadecane	▲	-	-	-
Eicosane	▲	▲	▲	-
2-Methylprop-1 -ene	▲	▲	▲	[22]
But-1-ene	▲	▲	-	-
(E)-But-2-ene	▲	▲	-	-
Pent-1-ene	-	▲	-	[22]
(Z)-Hex-2-ene	-	-	▲	-
Hept-1-ene	-	▲	▲	-

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[20]:Gill-King 1997, [21]: Vass *et al*. 2004, [22]: Statheropoulos *et al*. 2005, [24]: Vass *et al*. 2008, [28]: Vass 2001, [33]: Vass *et al*. 2002, [44]: Dent *et al*. 2004, [46]: Kondjoyan *et al*. 1997, [50]: O'Neal et al. 1996

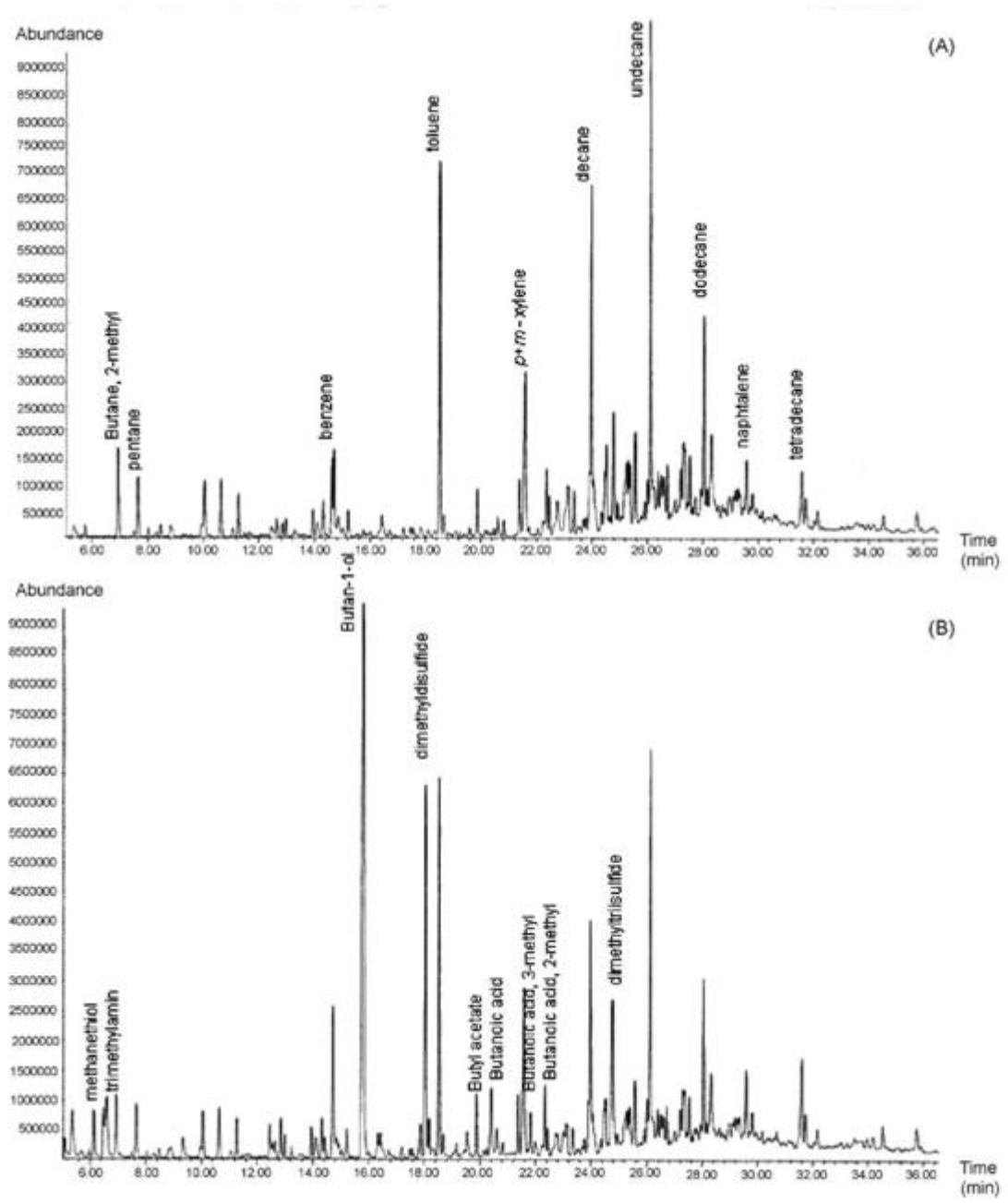

Figure 34. Comparison between a chromatogram resulting from an ambient air sampling (A) and a chromatogram resulting from a pig carcass headspace sampling (B) during the third post-mortem week in the urban site.

3.3. Decompositional stages

No cadaveric VOC was detected during the "fresh decompositional stage". During the bloated stage, many alcohols (butan-1-ol), sulfur compounds such as dimethyldisulfide (IUPAC name: methyldisulfanylmethane), dimethyltrisulfide (IUPAC name: methylsulfanyldisulfanylmethane) and sulfur dioxide were present. The nitrogen containing compound, trimethylamine, was detected during this decompositional stage. The "Active decay" is the decompositional stage with the strongest olfactive signature as many chemicals

were detected. At this decay stage, the main cyclic compounds are indole, phenol and 4-methylphenol. Dimethydi-sulfide and dimethyltrisulfide are also important decompositional products at this decay stage. A great portion of organic acids is present: butanoic acid, 2- and 3-methylbutanoic acid. During the "advanced decay" stage when the soft tissues are removed, the portion of aldehydes is increased (heptanal).

3.4. Principal component analysis

The PCA diagram (Figure 35) shows the distribution of the six carcasses in a score-plot. The first factorial plan (Fl, σ = 23.827) separates the urban pigs from the others (those placed in the agricultural and the forest sites). Urban pig carcasses are located at the bottom of the diagram, while the carcasses deposited outdoors are on the top of the chart. The second factorial plan (F2, σ = 17.741) distinguishes the decompositional stages. For the six carcasses, active decay is on the left side of the diagram, while the bloated and the advanced decay stages are on the right of the chart and are less differentiated from the active decay decompositional stage. Unlike the pig carcasses deposited in the forest and urban biotopes, the pig carcasses placed in the agricultural site have shown different evolution. The two carcasses passed trough the same decompositional stages but not at the same time. In fact, pig-A-1 underwent decomposition faster than pig-A-2. When pig-A-1 was already at advanced decay, pig-A-2 was in active decompositional stage. Moreover, pig-A-2 was slightly bigger than the five other pig carcasses. This weight difference may explain that pig-A-2 at the active decay appears to be an outlier.

The six pig carcasses follow similar spatial evolution with a distinction between the decompositional stages.

Table 21. The 35 common compounds associated with pig decaying carcasses sorted by chemical compounds.

Acids	Esters	Ketones	Aldehydes	Alcohols	Nitrogen compounds	Sulfur compounds	Cyclic hydrocarbons	Non-cyclic hydrocarbons
Propanoic acid	Butyl formate	Pentan-2-one	Butanal	2-Methyl-l-propan-l-ol	Acetamide	Methanethiol	Cyclohexytcyclohexane	Eicosane
Butanoic acid	Butyl butanoate	-	-	Butan-lol	N-Methylacetamide	SO_2	Phenol	2-Methylprop-l -ene
2-Methylpropanoic acid	Butyl 3-methylbutanoate	-	-	(2S)-Butan-2-ol	N,N-Dimethylformamide	Dimethyldisulfide	4-Methylphenol	-
2-Methylbutanoic acid	2-Methyiester, propanoic acid	-	-	3-Methylbutan-1-ol	N.N-Dimethylacetamide	Dimethyltrisulfide	Piperidin-2-one	-
3, Methylbutanoic acid	-	-	-	Pentan-l-ol	N,N-Dimethylnitrous amide	1-Methylsulfonyloxybutane	-	-
Pentanoic acid	-	-	-	-	Trimethylamine	-	-	-
Hexanoic acid	-	-	-	-	-	-	-	-

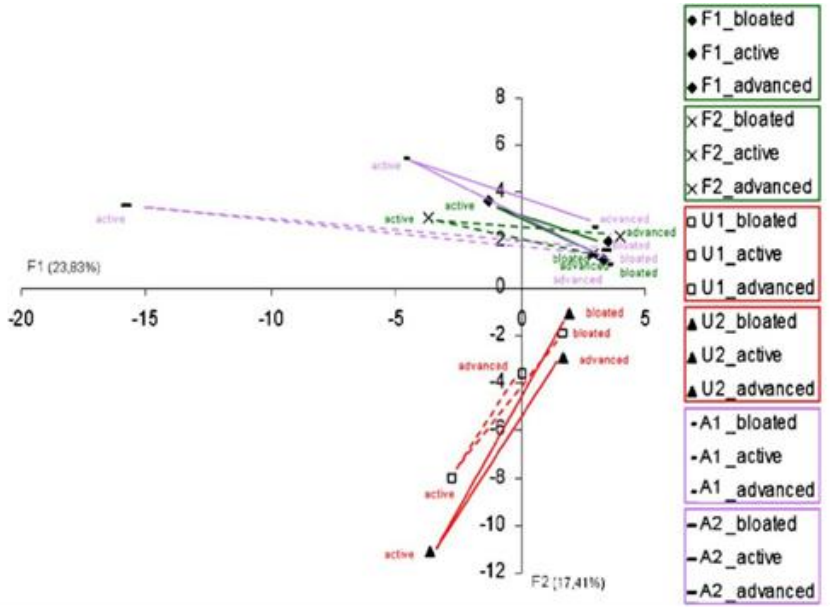

Figure 35. Spatial distribution of Y-variables in a score-plot. Visualization of decompositional process divided in decay stages (bloated, active, advanced) with chemical compounds detected.

4. Discussion

The volatile organic compounds released after death are intermediate products of decomposition (Vass *et al.* 2002). These decompositional by-products come from the catabolism of the four major categories of biological molecules in living organisms: (1) proteins, (2) nucleic acids, (3) lipids and (4) carbohydrates (Statheropoulos *et al.* 2005, Vass *et al.* 2002). Proteins are broken down into amino acids (Vass *et al.* 2002), proteoses, peptones and polypeptides (Evans 1963, Dent *et al.* 2004). A continuing proteolysis produces some gases, diamines (putrescine, cadaverine), sulfur compounds (e.g. dimethyldisulfide) and phenolic molecules (indole, skatole) (Evans 1963,Vass *et al.* 1992, Vass 2001, Dent *et al.* 2004, Statheropoulos *et al.* 2005). The degradation of nucleic acids provides nitrogenous bases, phosphates and sugars (Vass *et al.* 2002). The carbohydrates are mainly decomposed into oxygenated compounds: some organic acids, alcohols (Gill-King 1997, Dent *et al.* 2004, Statheropoulos *et al.* 2005), ketones, aldehydes, esters and ethers (Statheropoulos *et al.* 2005). Lipid degradation produces the palmitic and oleic acids (Cabirol *et al.* 1998, Vass *et al.* 2002) as well as hydrocarbons, nitrogen, phosphorus and other oxygenated compounds (e.g. acetone) (Gill-King 1997, Statheropoulos *et al.* 2005). The ultimate degradation of the large biological macromolecules is the restitution of their building components (C, H, O, N, P and S) in the ecosystem (Vass *et al.* 2002, Statheropoulos *et al.* 2005). The bacterial transformation of lipids, carbohydrates and proteins produce organic acids (propionic and butyric acids) and decompositional gases (carbon dioxide, methane, ammonia, hydrogen, hydrogen sulfide, sulfur dioxide) (Killam 1990, Clark *et al.* 1997, Gill-King 1997, Vass *et al.* 2002, Dent *et al.* 2004). These very volatile compounds (NH3, H2, CO2, and CH4) were not detected with the sampling method used in the present study. Indeed, the triple sorbent traps used in DOA Database do not detected these light volatile compounds (Vass *et al.* 2004, Vass *et al.* 2008). Nevertheless, these compounds could contribute to the cadaveric odour during the early stage of decomposition (Vass *et al.* 2008). The sulfur dioxide was detected in early decompositional stage on each pig carcasses. The same acidic compounds which are referenced in the literature as human decompositional process (Gill-King 1997, Vass *et al.* 2001, Statheropoulos *et al.* 2005) were detected with the radial diffusive sampler: propanoic acid and butanoic acid. Another study concerning the volatile fraction of dry-cured hams from pigs (Kondjoyan *et al.* 1997) lists 42 VOCs including the following acids: butanoic, 2-methylpropanoic, 2-methylbutanoic and 3-methylbutanoic acids. The 2,3-butanedione (or

diacetyl), detected in this study and from dry-curred hams, comes from the breakdown of glycogen (Evans 1963).

Cadaverine and putrescine are other decompositional compounds usually associated with the decaying processes (Gill-King 1997, Vass *et al.* 2002). These two diamines were not detected as volatile compounds in this study. Some hypotheses concerning their absence were formulated by Vass and his colleagues (Vass *et al.* 2004) who used volatile trapping. Statheropoulos and his colleagues (Vass *et al.* 2004, 2008) have not detected cadaverine and putrescine in the headspace of human putrefied bodies. Even so, we have detected the piperidin-2-one in each pig carcass. This compound could be a metabolite of cadaverine (Callery and Geelhaar, 1986). Skatole and indole, two other aromatic phenolic compounds associated with the decompositional process (Vass *et al.* 2002, Dent *et al.* 2004), were detected in previous work, but only indole has been detected once in our study.

With the monitoring of the cadaveric VOCs released by decomposing pig carcasses during 6 weeks, we have shown that the volatile pattern changes over time. The Decompositional Odor Analysis Database (DOA Database) (Vass *et al.* 2002, 2008) has also shown that the smell of death undergoes changes over time. Moreover, the olfactive signature of the cadaver is different depending on our selected biotope, probably because the decomposition process is strongly influenced by the biogeoclimatic zone and by the local micro-climate that includes the entomofauna. Decomposition rates were different in the three biotopes. The most important difference occurs between the urban biotope and the two others "open-air" biotopes. Moreover, many atmospheric VOCs (pollutants) are sampled in the urban biotope creating an important background noise (Fig. 34). These atmospheric compounds could mask the presence of cadaveric VOCs.

In addition to putrefactive bacteria (Gill-King 1997, Vass 2001, Dent *et al.* 2004, Hopkins *et al.* 2000), the different volatile patterns between the three biotopes may be caused by additional environmental microorganisms (e.g. soil and air bacteria). Indeed, the three selected biotopes are very different and create separate microhabitat for the development of environmental microorganisms (Torsvik and Ovreas 2002). These microbes, such as putrefactive bacteria, may contribute to the production of volatile substances. The different biotopes are spatially represented on the ACP diagram (Fig. 35). In this figure, the urban site is clearly separated of the other biotopes.

All chemicals were not seen in all biotopes probably because the environment (soil, vegetation, air, microorganisms and insects) around each carcass were strongly different and interacts with the decompositional process and the production of cadaveric VOCs.

Moreover, some VOCs were perhaps in trace (concentration issue) and were not identified within this study. Nevertheless, a common core of 35 VOCs was identified in the three selected biotopes.

The olfactive signature of decaying pig carcasses shows similarities with the smell of human decomposition in terms of released chemical compounds. Nevertheless, many nitrogen compounds such as amides and cyclic hydrocarbons detected during this study are not referenced as decompositional byproducts.

Further studies on cadaveric volatile organic compounds are currently conducted at the Department of Functional and Evolutionary Entomology (Belgium, Gembloux, FUSAGx). Different volatile sampling techniques are investigated, included the Radiello® sampler, using pig as animal model.

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III.3.2. Enhanced Characterization of the Smell of Death by Comprehensive Two-dimensional Gas Chromatography-time-of-flight Mass Spectrometry (GCxGC-TOFMS)

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Abstract - Soon after death, the decay process of mammalian soft tissues begins and leads to the release of cadaveric volatile compounds in the surrounding environment. The study of postmortem decomposition products is an emerging field of study in forensic science. However, a better knowledge of the smell of death and its volatile constituents may have many applications in forensic sciences. Domestic pigs are the most widely used human body analogues in forensic experiments, mainly due to ethical restrictions. Indeed, decomposition trials on human corpses are restricted in many countries worldwide. This article reports on the use of comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOFMS) for thanatochemistry applications. A total of 832 VOCs released by a decaying pig carcass in terrestrial ecosystem, *i.e*. a forest biotope, were identified by GCxGC-TOFMS. These postmortem compounds belong to many kinds of chemical class, mainly oxygen compounds (alcohols, acids, ketones, aldehydes, esters), sulfur and nitrogen compounds, aromatic compounds such as phenolic molecules and hydrocarbons. The use of GCxGC-TOFMS in study of postmortem volatile compounds instead of conventional GC-MS was successful.

Key Words - Forensic science, forensic chemistry, odor analysis, cadaver decomposition, volatile organic compounds (VOCs), comprehensive two-dimensional gas chromatography (GCxGC), Time-of-flight mass spectrometry (TOFMS), mass spectral deconvolution.

1. Introduction

The decay process of vertebrates begins rapidly after death (*i.e.* four minutes after death) [1] and leads to the release of postmortem compounds in the ecosystem [2-3]. These cadaveric compounds, mainly volatile organic compounds (*i.e.* VOCs), are by- or end-products of the decay process [2,4]. They come from the catabolism of the four major categories of biological macromolecules in living organisms: proteins, nucleic acid, lipids and carbohydrates [2-3]. The principal decay pathways and the metabolic origin of the main vertebrate postmortem volatiles were reviewed by Dent and colleagues [5], Boumba and colleagues [6] and recently by Paczkoski and Schütz [4]. However, the metabolic origin of many cadaveric compounds is still unknown [4,7]. Only a few research groups have studied the postmortem VOCs emanating from human remains [8-13] and animal carcasses (pig [7, 14-15], mouse [16], rabbit [17]). Nevertheless, the majority of these studies is focused on burial decomposition or in closed environments ("body bag") and limits the access to the corpse for the necrofauna, mainly insects [7-11]. The available information concerning postmortem chemistry of above-ground decomposition is rather limited. Numerous applications would however benefit from a better understanding of the postmortem volatiles emitted during the decay process.

The cadaveric VOCs find applications in forensic sciences and the etiology of death [3], in training of cadaver dogs (human remains detection or HRD detection) [12-13, 18-20], in the development of cadaveric material detection devices [8-10, 17] (electrochemical sensors ("electronic nose") [8-9, 17, 21], in exploiting insect olfaction (biosensor or biodetector [4, 22-23]) or in the determination of the post-mortem interval (PMI) [1, 3-4]. The smell of death is constituted by a blend of chemical compounds changing over time and according to animal remains [8-9, 14]. Indeed, a recent study has shown that the odor of human remains is different from that of animals [13].

For the analysis of cadaveric VOCs, gas chromatography (GC) coupled to mass spectrometry (MS) is the technique of choice [3, 7-13, 24]. However, the volatile profile of the decomposition odor is constituted by a large number of VOCs [8-9] and GC easily suffers from peak capacity limitations. The complexity of volatile postmortem samples requires the use of more complex analytical methods. For example, GC is unable to detect quantitatively both short and long chain acids (*e.g.* present in decomposition fluid) due to the polarity difference in the molecules [25-26]. Comprehensive two-dimensional gas chromatography (GCxGC) has been developed to meet an increasing need for complex sample analysis and to address limitations such as peak capacity, dynamic range and restricted specificity of one-

dimensional (conventional) GC systems (1D-GC) (*i.e.* to improve the global efficiency of the separation). GCxGC can be defined as a chromatographic technique during which a sample is subjected to two different separation processes coupled online [27] and as a result every compound is characterized by a retention time in each dimension (^1t_R and ^1t_R) [4]. In practice, the end of the first dimension (^{1}D) column is placed in a temperature controlled interface named 'the modulator' and further serially connected to the second dimension (^{2}D) column. The cryogenic modulator ensures high sampling rates and transfer of the sample to ^{2}D column [28]. The entire ^{1}D chromatogram is thus 'sliced' following a modulation period (P_M) of a few seconds and sent into ^{2}D for a fast GC-type separation, resulting in peak widths of 200-600 ms [29]. By fine-tuning of the GC phase combination, compounds potentially still coeluting at the end of the ^{1}D separation can be separated on the basis of their different behavior as regards of the ^{2}D phase. Globally, the separation power is increased and the sensitivity is also enhanced by cryogenic zone compression [30, 31]. In terms of detector, in addition to flame-ionization and other element selective detectors, various mass-spectrometric (MS) detectors, providing structural information (an additional dimension), can be used since the first coupling was reported in early 2000 [32]. Although double focusing sector, quadrupole, and ion traps are popular MS detectors for GC, they have limited use in GCxGC because of their relatively slow scanning rates, compared to fast acquisition time-of-flight (TOF) MS better suited to characterize very narrow ^{2}D peaks. Additionally, the absence of concentration skewing in the TOFMS instrument ensures spectral continuity and allows mass spectral deconvolution of coeluting chromatographic peaks characterized by different fragmentation patterns [33]. This capability allows the identification of different compounds if the peak apexes of coeluting analytes are at least separated by three scans and differ somewhat in their mass spectra. The use of deconvoluted ion current (DIC) makes the TOFMS almost like a third dimension for the separation system. Consequently, the GC×GC–TOFMS coupling is a powerful instrument combining improved chromatographic resolution of the GC × GC and the analytical resolving power of the TOFMS [34-35]. GCxGC-TOFMS, has thus been used to analyze complex samples in various fields [36], including VOC analyses [37-39].

Here, we report on a first field trial using GCxGC-TOFMS for the study of postmortem VOCs released by above-ground decomposition of pig carcasses, using it as a human body analogue.

2. Material and methods

2.1. Animal model and field site

Domestic pig (*Sus scrofa domesticus* L.) (25 kg) was used to surrogate human models mainly for physiological, biochemical, ethical, legal and economic reasons [25, 40-45]. Unlike other animals, pigs are considered to be an acceptable substitute due to their similarity to humans in body mass (torso in weight), skin structure, fat to muscle ratio and hair coverage [5, 25, 45-46]. The greatest dissimilarity between pigs and humans are the bones, which have a different microstructure [46-47]. The piglet was killed by a penetrative captive bolt and disposed in the experimental site within the next 4 hours. Immediately after the euthanasia, the pig carcass was packed in a double plastic bag to avoid any insect colonization before laying on the experimental biotope. This study was approved by the committee on the Ethics of Animal Experiments of the University Faculty of Agricultural Sciences of Gembloux (since 2009, Gembloux Agro-Bio Tech, University of Liege).

The study site was a forest biotope, located in Belgium (Lambert-coordinates: 141512.00/149844.00) with pedunculate oaks (*Quercus robur* L.), European beeches (*Fagus sylvatica* L.) and sycamore maples (*Acer pseudoplatanus* L.). This field study was conducted with the permission of the forest administrator.

The decaying pig was placed in metal mesh cages (180 cm x 90 cm x 90 cm) to avoid scavenging by carnivores. The experiment was conducted during six weeks in spring 2007 (March 29-May 11). As control samples, volatile collection of the atmospheric VOCs was performed simultaneously to the pig samples, at 50 m from the decomposing swine carcass.

As temperature is one of the most important parameters influencing the decomposition rate [1-2, 48-50], the ambient air temperature was automatically measured once an hour using a data logger (Testo 175-T1® temperature data logger, Germany) placed on the lateral side of the cage, at a height of 75 cm. The daily mean temperature was calculated on the basis of ambient air temperature recorded at a time interval of 24 hours. Other environmental parameters (humidity, wind velocity, wind direction) were recorded thanks to Vantage Pro Plus™ Stations® (Davis, Hayward, CA, USA).

2.2. Volatile collection

A dynamic sampling technique was used to collect volatile organic compounds released by the decaying pig carcass. The VOCs were collected in the headspace of the decaying pig with a pump device for 1 hour at 1 $Lmin^{-1}$ every two days during the field experiment. Simultaneously to the cadaveric VOC collection, air samples were collected as blank references. VOCs were trapped on cartridges, constituted of glass and Teflon®, containing a 40 µg SuperQ® adsorbent filter (80-100 mesh, Alltech Associates, Inc. Deerfield, IL, USA). The sorbent cartridge was connected to the pump device with Teflon® tubing and a glass funnel. The air sampling device (funnel and the sorbent cartridge) was disposed close to the abdominal cavity of the carcass (± 3 cm).In the laboratory, VOCs were solvent eluted from the SuperQ® adsorbent with 150 µl of diethyl ether (HPLC grade, Sigma-Aldrich SA, Bornem, Belgium) and capped in GC-type vials. Before chromatographic analyses, liquid volatile samples were conserved at -80°C.

2.3. Chemicals

All solvents were Pestanal reagents (Riedel-de Haën, Seelze, Germany). Liquid nitrogen was purchased from Air Liquide (Liege, Belgium). Chromatographic pure grade helium gas, 99.9999%, was purchased from Air Products (Vilvoorde, Belgium).

2.4. Measurement by GCxGC-TOFMS

The GCxGC-TOFMS instrument was the Pegasus 4D (LECO Corp., St Joseph, MI, USA). This system is based on a non-moving quad-jet modulator consisting of two permanent cold nitrogen jets and two pulsed hot-air jets, which are responsible for the trapping and refocusing of compounds eluting from the first dimension (^{1}D) column. This modulator was mounted in an Agilent 7890 GC oven and liquid nitrogen was used to create the cold jets. The column set was made of a 30m Rtx®-5 (0.18 mm ID x 0.20 µm df) (Restek Corp., Bellefonte, PA, USA) in the first dimension (^{1}D) and a 1.0 m Rxi®-17 (0.10 mm ID x 0.08 µm df) (SGE, Austin, TX, USA) in the second dimension (^{2}D). The two columns were connected using a universal glass press tight connector (Restek Corp.). The modulation period (P_M) was 4 s. The hot pulse duration was 600 ms. Helium was used as the carrier gas at a constant flow rate of 1 ml/min. 1 µl of the final extract in diethyl ether was injected into a split/splitless injector held at 200 °C in splitless mode and equipped with a Double Gooseneck Restek liner. The primary oven was

programmed as follows: 40 °C for 5min, at 5 °C/min to 220 °C and held for 5 min. The secondary oven temperature offset was 5 °C, with a parallel temperature program. The modulator temperature offset was 10 °C. The MS transfer line temperature was 225 °C. A solvent delay of 300 seconds was used. The ion source temperature was 250 °C with an electron impact (EI) energy of 70 eV. The collected mass range was 35–600 amu. The acquisition rate was 100 scans per second and the detector voltage was 1500 V.

2.5. Data processing

Data processing and display of the GCxGC chromatograms were achieved using the integrated LECO ChromaTOF^{TM} software, version 4.33. Peak apexes of deconvoluted signals were found automatically and were further corrected manually when required. The ^{2}D chromatographic peaks were recombined based on their second dimension retention time ($^{2}t_R$) and mass spectral similarity. Mass spectral data (DIC) were compared to the Wiley (2008) and the NIST (2008) libraries for temptative identification (similarity >700 and reverse match >900). Moreover, the area of each peak was calculated based on the apex mass reconstruction trace to increase the specificity during the comparison. All sample-blank couples were analyzed using the "Reference" option of the software to compare every sample to its blank. This part of the software is based on an algorithmic comparison of the data sets [51]. To analyze one sample-blank couple, the comparison is based on both $^{1}t_R$ and $^{2}t_R$, as well as the respective area and mass spectra data of each peak. The main comparison parameters were fixed at a tolerable shift of 8 seconds on $^{1}t_R$ (twice P_M value) and 0.2 seconds on $^{2}t_R$, and a tolerance of 50% absolute variation on the area. Following this comparison exercise, every single peak was categorized in one of the following groups: match (the compound is found in both injections), not found (the compound is only present in the sample), out of tolerance (the compound is present in both injections but at a different concentration level), and unknown (the compound is only present in the blank). The compounds sorted in the "not found" and "out of tolerance" (with a concentration under 10%) groups were classified as specific to the decomposition process.

2.6. Decomposition stages

The decomposition process was observed according to different stages. We decided to discriminate five major decompositional stages based on different visual criteria adapted from

the literature [50, 52-58]: (1) fresh, (2) bloated, (3) active decay, (4) advanced decay and (5) dry remains. The decay stages are presented in Table 22.

Table 22. The five decompositional stages defined in this study and their descriptions.

Decompositional stage	Description	Literature report
(1) *Fresh*	From death until the first signs of bloating	[1,49, 52, 54]
	Autolysis	
(2) *Bloated*	Putrefaction mechanism generates accumulation of breakdown gases causing bloating of the corpse. The first signs of the bloated stage appear in the abdomen. Then the whole body swells	[1, 52, 54, 56]
	Anaerobic fermentations	
(3) *Active decay*	Darkening of the skin	[1, 52, 54, 56]
	The skin is breaking up and the body began to deflate. Protein sources are broken down into fatty acids and other decomposition products such as skatole, indole, cadaverine, putrescine	
(4) *Advanced decay*	Corpse dries and the remains are skin, cartilage, hair, bones and some fragments of flesh	[52, 54, 56]
(5) *Dry remains or skeletonisation*	The only remains are bones and hair	[52,54]
	Diagenesis	

2.7. Statistical analysis

In order to analyze the spatial distribution of our data set, a statistical analysis was conducted through multivariate principal component analysis (PCA) (Minitab® v15.1, State College, PA, USA). The original data set is a matrix of c X n (c = objects, n = variables) corresponding to a matrix of 12 x 225. Chemical compounds emitted only once were excluded from the multivariate analysis. The relative area of the chromatogram's peak corresponding to VOC was used for the multivariate analysis.

3. Results

3.1. Environmental parameters

The mean atmospheric temperature measured during the decay process was 13.1°C. Figure 36 shows the temperature recordings in the forest site. The mean atmospheric maximal temperature was 20.2°C whereas the mean atmospheric minimal temperature was 6.7°C.

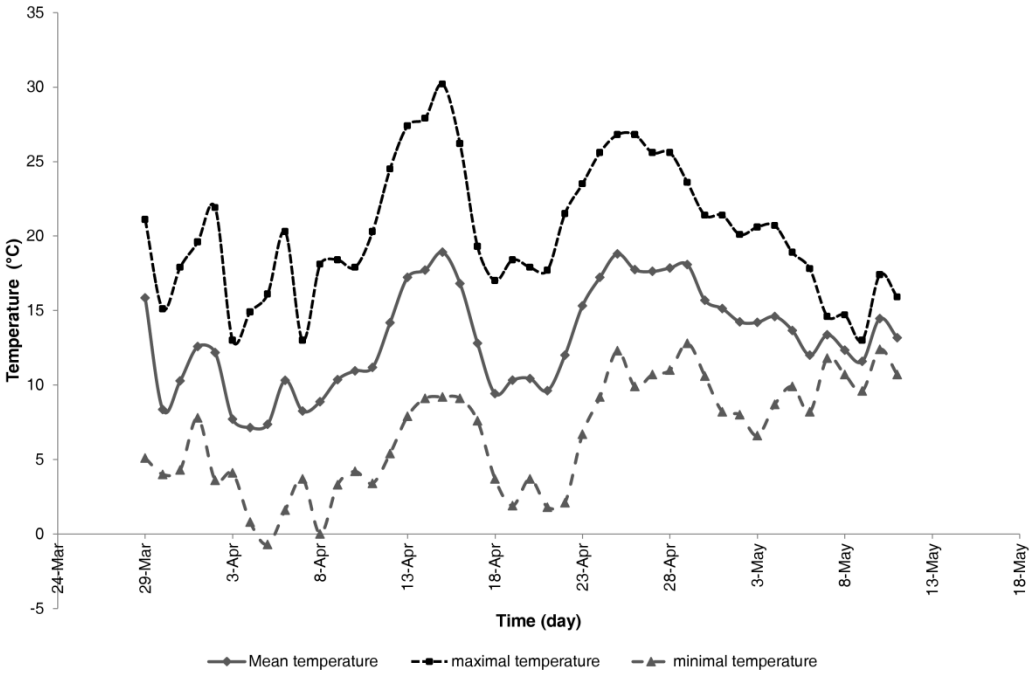

Figure 36. Temperature recordings in the forest biotope on the lateral cage of the pig carcass.

The mean relative humidity was 68.3%. Local atmospheric temperature and relative humidity values were different to seasonal averages reported for the last 20 years by the royal Belgian meteorological institute (KMI-IRM). Mean atmospheric temperature from the Belgian KMI-IRM archives for spring 2007 (March to May) was 12.3°C (14.3°C for April and 14.6 for May 2007) whereas seasonal average was 9.5°C (9°C for April and 12.7°c for May). The maximal mean temperature from the Belgian KMI-IRM archives, for April 2007, was 20.5°C and 19°C for May. The minimal mean temperature from the Belgian KMI-IRM archives was 7.6°C for April 2007 and 10.3°C for May. The seasonal average for maximal temperature was 13.1°C for April and 17.2°C for May. For minimal temperature, the seasonal average was 5°C for April and 8.3°C for May. Spring 2007 was the warmest season since 100 years. Mean relative humidity from the Belgian KMI-IRM archives was 62% for April 2007 and 75% for May

whereas seasonal averages were respectively 76.6% and 75.5%. Moreover, there was no day of precipitations during April 2007.

3.2. Decay stages

Figure 37 illustrates the decay stages followed by the swine carcass in the forest biotope.

Figure 37. Typical decay stages followed by the pig carcass in a forest biotope.

The "fresh" stage began the day of the death (March 29, 2007) until 2nd of April, *i.e.* five postmortem days. The "bloating" stage began on the 3rd of April and finished on the 17th of April, the duration of the bloating stage was fifteen days. The "active decay" stage began on the 18th of April until the 30th of April. The duration of the active decay stage was thirteen days. The "advanced decay" stage began on the 1st of May until the 11th of May; this decomposition stage had duration of eleven days. The decay process was followed during six postmortem weeks.

3.3. Two-dimensional chromatographic screening

Figure 38 shows the GCxGC apex plot of a sample (1st of May) of the advanced decay stage.

Figure 38A clearly illustrates the added value of GCxGC for such analyses for which classical GC would suffer from repeated co-elution issues (same $^{1}t_R$).

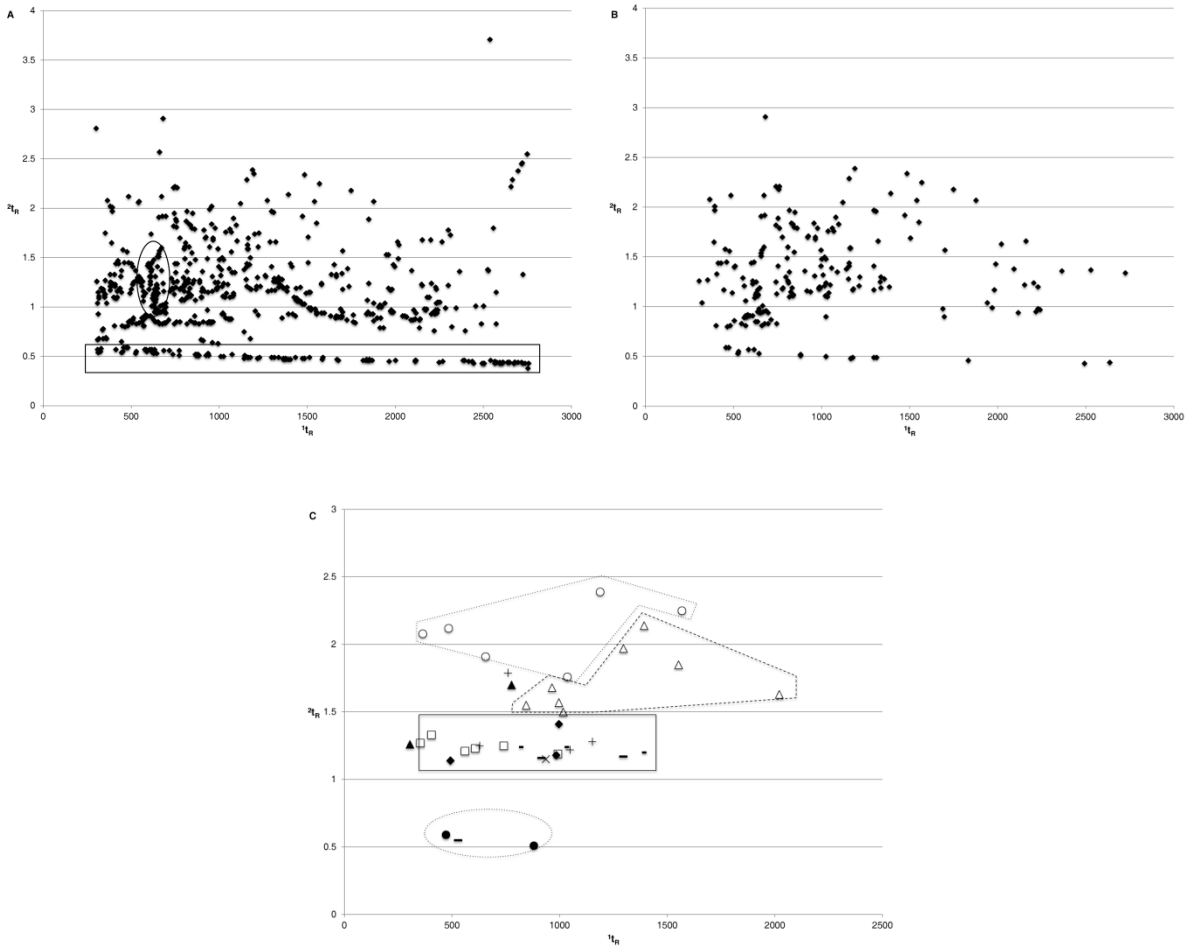

Figure 38. GCxGC-TOFMS apex plot of a sample (1st of May) of the advanced decay stage. All tR are in seconds. (A) 633 hits were identified after raw data processing. (B) 218 peaks were identified after removal of column bleed (rectangle region), solvent signals (circled region), and analytes present in the reference blank samples. (C) chromatographic distribution of the 42 specific VOC compounds present in that particular sample and in at least three other samples (◆Alcohols; + Aldehydes; ○ Amides; • Amines; ▵ Aromatic compounds; □ Carboxylic acids; × Ester; – Ketones ; ▲ Sulfur compounds; − Others compounds).

After processing, the peak table of this chromatogram contained 633 hits. Hits included in the circled region could easily and repeatably be attributed to the extraction solvent. Hits included in the rectangle region were related to column bleed. After further removal of the hits present in the reference samples, 218 peaks were isolated (Figure 38B). Amongst these peaks, 42 were specifically found with at least 4 occurrences in other sample extracts (*e.g.* part of the 60

compounds listed in Table 23, see next paragraph). Figure 38C illustrates their distribution over the chromatographic space. All samples were processed the same way before compilation of the list of specific compounds.

3.4. Cadaveric volatile organic compounds

More than 4,000 hits were reported from extract analyses. After clean-up of the lists from compounds present in reference soils, as well as from GC column bleed related and potential peak artifacts, a list of 830 VOCs specifically released under the pig decomposition process was extracted by GCxGC-TOFMS. Almost all chemical families of VOCs were represented (in parentheses, the number of chemical compounds identified): alkanes (*i.e.* saturated hydrocarbons) (82), alkenes (*i.e*. unsaturated hydrocarbons) (50), alcohols (64), carboxylic acids (45), aromatic compounds (84), esters (87), sulfur compounds (31), nitrogen compounds (141), aldehydes (32), ketones (110), halogen compounds (42), ethers (32) and unclassed compounds (30). Table 24 presents the 225 cadaveric VOCs which have at least two occurrences during the decay process; 605 chemical compounds were detected only once. Among these 225 chemical compounds, 1H-indole was the compound with the highest occurrence (eight occurrences), followed by five compounds with seven occurrences (2-methyl-1-pentene; 1,2,3-propanetriol; ethanol; 4-methylphenol and acetaldehyde). Ten compounds were found 6 times, for example: 1-butanol, butanoic acid, 2- methylpropanoic acid, DMDS (*i.e*. dimethyldisulfide) and DMTS (*i.e.* dimethyltrisulfide), 1-amino-2-propanol, N-butylformamide and N,N-formamide. Sixteen compounds had five occurrences, for instance: 2- and 3-methylbutanoic acid, pentanoic acid, trimethylamine, 2-octanone and 2-undecanone, nonanal. An exhaustive list of compound occurrences (only the chemical volatiles with four to eight occurrences) is compiled in Table 24. Figure 39 shows the number of cadaveric volatile chemicals released by sampling date.

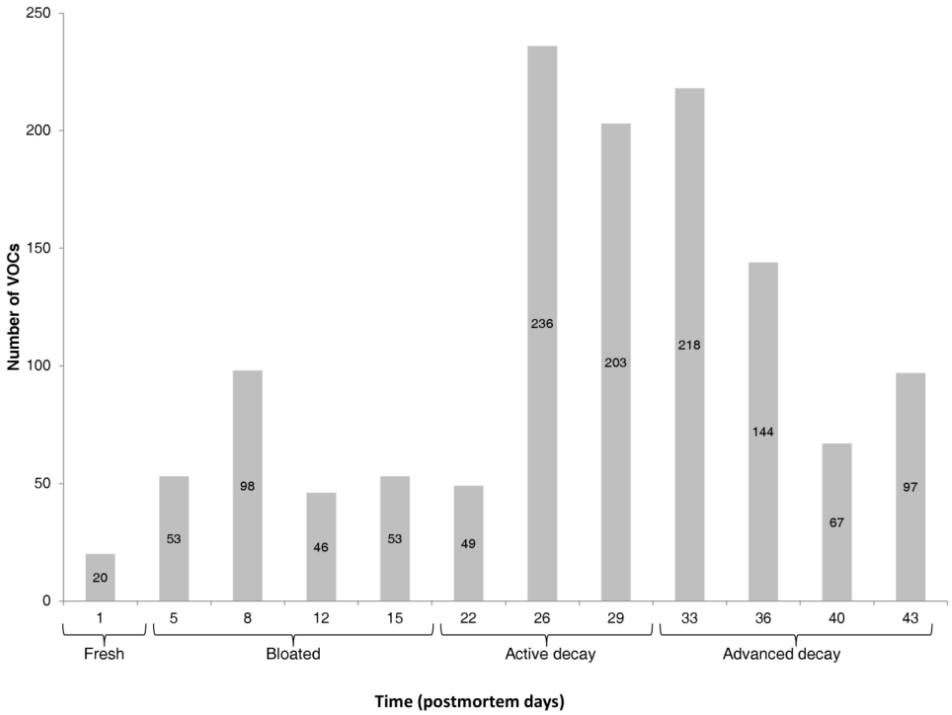

Figure 39. Number of released compounds according to the decay stages and postmortem time.

The highest number of postmortem compounds was monitored during the decay stages, more precisely during the active decay stage and early advanced decay stage. The active decay is the decompositional stage with the strongest olfactive signature as many chemicals were detected. On the contrary, few volatiles were detected during the fresh stage (one day postmortem time). Additionally, no decompositional odor was perceptible to the human sense of smell in the very early decompositional stages. The number of cadaveric VOCs increased with the course of time and decreased with the disappearance of soft tissues (advanced decay). The PCA diagram (Figure 40) shows the distribution of the postmortem time (dates) in a score-plot (F1, σ = 57.501; F2, σ = 26.086). The fresh and bloated stages are less differentiated from the decay stages.

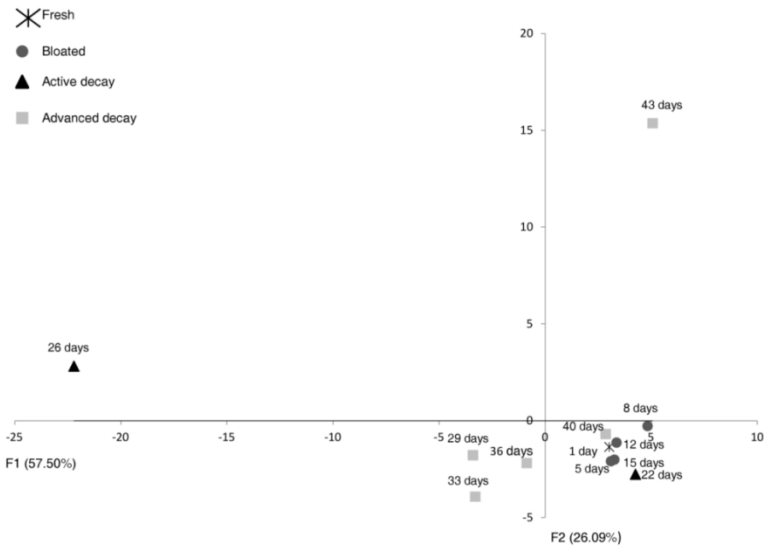

Figure 40. Spatial distribution of Y-variables in a score-plot based on relative area of VOCs.

Figure 41 shows the repartition of chemical families detected in the headspace of decaying swine carcass by postmortem time.

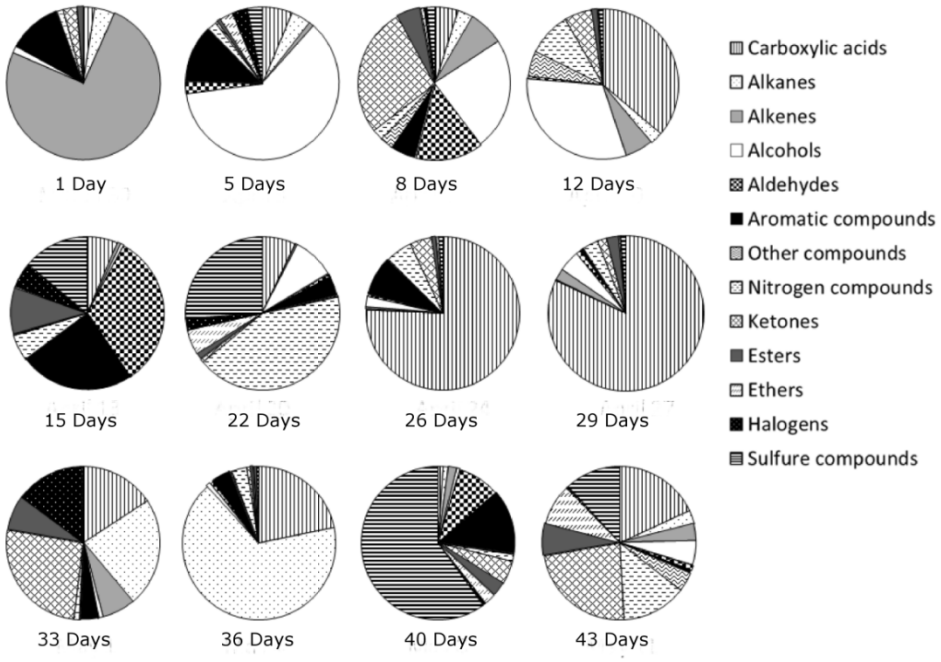

Figure 41. Distribution of chemical classes according to postmortem time (days).

The volatile pattern of decaying pig carcass changes over time. One day post-mortem (30th of March), alkene was the predominant chemical class with γ-terpinene and o-xylene. In the fresh decay stage (5 postmortem days), alcohol was the predominant chemical class with mainly 1-butanol. 8 postmortem days, the presence of alcohols decreased and ketones became the main chemical class with principally cyclohexanone (≈ 20% of the total of emitted volatiles) and 4-methyl-3-pent-2-one. Aldehydes (hexanal, pentadecanal, 2,2-

dimethylpropanal, 3-methylbutanal, acetaldehyde) were also more abundant and represented approximately 15% of the total of emitted volatiles. Furthermore, in bloated stage, alcohols represent more than a third of the volatile emissions whereas carboxylic acids represented 35% with mainly one chemical compound (borinic acid, diethyl-). Nitrogen compounds became more important and represented moreover 10 % of the total of volatile emissions. 15 postmortem days, aldehydes, aromatic and sulfur compounds were the main chemical classes. 2,2 dimethylpropanal was the predominant aldehyde and represented a third of the total of volatile emissions whereas 1H-indole (aromatic compound) represented approximately a quarter of the volatile emissions. With regards to sulfur compounds, dimethyltrisulfide (*i.e.* DMTS) and butyl isopropylsulfone were the predominant compounds detected. During the active decay stage, nitrogen compounds (1-butanol, 4-amino; trimethylamine) and sulfur compounds (DMTS ≈ 23% of the total of volatile emissions of 22 postmortem days) are the main chemical families. Butanoic acid (carboxylic acid) became more important in the volatile emissions. 26 postmortem days, 75% of the volatile emissions were carboxylic acids with 3-methylbutanoic acid (*i.e.* isovaleric acid), butanoic acid, 2-methylbutanoic acid, 2-methylpropanoic acid (*i.e.* isobutyric acid), 4-methylpentanoic acid (*i.e.* isocaproic acid). Many aromatic compounds (37 chemical compounds) were also detected on the 26 postmortem days, but the main compound was 4-methylphenol (*i.e. p*-cresol). 29 postmortem days, the main chemical class was still the carboxylic acids (butanoic acid, 2- and 3-methylbutanoic acid, 2-methylpropanoic acid) with moreover 80 % of the total of volatile emissions. In the early advanced decay stage (33 to 36 postmortem days), the presence of carboxylic acids decreased and the predominant chemical classes were ketones and alkanes. Further in the advanced decay stage, the alkanes increased; the main saturated hydrocarbon was 1,3-diethylcyclopentane (≈50 % of the total of volatile emissions of 36 postmortem days). Carboxylic acids, approximately 20 % of the total of volatile emissions, were still present but to a lesser extent. 40 postmortem days, sulfur compounds were the predominant chemical class with 4-hydroxybenzenesulfonic acid and dimethyldisulfide (DMDS), which represented more than 60% of the volatile emissions. Aldehydes (mainly nonanal) and aromatic compounds (mainly 1H-indole) intervened respectively for 9 % and 14 % of the total of volatile emissions. Later in the advanced decay stage (43 postmortem days)), sulfur compounds (main compound: DMDS) decreased whereas ketones (2-hexanone, 3-hexanone and cyclohexanone), nitrogen compounds and carboxylic acid increased (2,3-dihydroxysuccinic acid).

Table 23. List of occurrences for the detected postmortem chemical compounds by GCxGC-TOFMS.

8 occurrences	7 occurrences	6 occurrences	5 occurrences	4 occurrences
✓ 1H-indole	✓ 1-Pentene, 2-methyl-	✓ 1-Butanol	✓ 1-Octanol	✓ 1-Hexen-3-ol
	✓ 1,2,3-Propanetriol	✓ Butanoic acid	✓ 1-Propanol, 2-methyl-	✓ 1-Pentanol, 4-methyl-
	✓ Ethanol	✓ Propanoic acid, 2-methyl-	✓ Butanoic acid, 2-methyl	✓ 2-Hexanol
	✓ Phenol, 4-methyl-	✓ Disulfide, dimethyl	✓ Butanoic acid, 3-methyl	✓ 2-Hexen-1-ol
	✓ Acetaldehyde	✓ Trisulfide, dimethyl	✓ Pentanoic acid	✓ Hexanoic acid
		✓ 1-Butanamine, 3-methyl-	✓ Benzenemethanol, à-methyl-	✓ Pentanoic acid, 4-methyl-
		✓ 2-Propanol, 1-amino-	✓ Pyrazine, tetramethyl-	✓ Naphthalene, 2,6-diisopropyl
		✓ Formamide, N-butyl-	✓ Pyrazine, trimethyl-	✓ Quinazoline, 2,4-dimethyl-
		✓ Formamide, N,N-dimethyl-	✓ 1-Butanol, 4-Amino-	✓ Quinoline
		✓ 1,3-Dioxolane, 2-acetyl-	✓ Formamide, N-methyl-	✓ Acetic acid, ethyl ester
			✓ Trimethylamine	✓ Butanoic acid, 3-methyl-, butyl ester
			✓ Nonanal	✓ Dothiepin*
			✓ 2-Octanone	✓ 2-Piperidinone
			✓ 2-Undecanone	✓ Acetamide, N-methyl-
			✓ Butyl isocyanatoacetate	✓ Butanamide
			✓ Hydroperoxide, 1-ethylbutyl	✓ Formamide, N-phenyl-
				✓ Hexanamide, N-methyl
				✓ Methanamine, N,N-dimethyl-
				✓ Benzaldehyde
				✓ Heptanal
				✓ Nonenal
				✓ Propanal, 2,2-dimethyl-
				✓ 2-Nonanone
				✓ 3-Octanone
				✓ Cyclohexanone
				✓ Ethanone, 1-phenyl-
				✓ Propane, 1-bromo-2-methyl-
				✓ Hydroperoxide, 1-methylbutyl
1 compound	**5 compounds**	**10 compounds**	**16 compounds**	**28 compounds**

* Dothiepin is not considered as a cadaveric compound

4. Discussion

In previous study on swine decay chemistry (above ground decomposition in a forest biotope) [14], only 85 specific cadaveric volatiles were identified with conventional GC-MS whereas approximately ten times more compounds were detected with two-dimensional GCxGC-TOFMS (832 VOCs). Moreover, no cadaveric compounds were detected with conventional GC-quadrupole(q)MS during the first postmortem week (fresh decay and beginning of the bloated stages) [14] whereas cadaveric volatiles were detected from the first postmortem day with bi-dimensional gas chromatography. This is most probably to be attributed to the enhanced chromatographic resolution and detectability of the zone-compressed peaks. Figure 40A also illustrates that separating column bleed and/or solvent related peaks in the second dimension contributes to better peak identification. Furthermore, the use of the chromatographic space rather than a classical chromatographic temporal line allows classes of compounds to be separated from each other (Figure 40C). Amides, aromatics, carboxylic acids exhibit different retention behaviors towards the ^{2}D GC phase. The availability of two t_R values is an additional piece of information to potentially identify compounds with lower similarity values versus library spectra. The separation could still be improved for alcohols, ketones, carboxylic acids, and aldehydes, but the current situation is clearly improved compared to a situation where all peaks would be present on the top of each other on the x-axis of the chromatogram. For now, the deconvolution software of the TOFMS system can separate them for individual identification.

Other studies on the swine cadaveric decompositional process [7, 14] or human decay [7-12] used GC-qMS [7-13, 15, 17] or GC-TOFMS [7] to analyze the decompositional odor. However, fewer cadaveric compounds were detected with GC-MS or GC-TOFMS than the two-dimensional gas chromatography. For example, in the Decompositional Odor Analysis Database (DOA Database), 478 specific volatile compounds associated with buried human remains were recovered [8-9] whereas Statheropoulos and colleagues [3] detected more than 80 VOCs on putrefied human remains and 150 VOCs were identified on pig carcasses closed in a "body bag" during the early stages of decay [7]. The volatile profiles of different types of human remain (blood, bones, adipose, teeth, skin, body fat, *etc*.), placed in small sealed vials, were constituted of 33 specific VOCs [12].

A recent study [16] used the two dimensional GCxGC-TOFMS analysis to study the chemical composition of volatiles emanating from fresh laboratory mouse carcasses during the early decay stages. A fresh killed mouse (0-30 min old) emits the same volatile pattern as a living

mouse. As the decay progresses, only sulfur compounds (S-VOCs) were detected during the first three days of postmortem time. In older mice carcasses, other decompositional by-products were identified such as aromatic phenolic compounds (indole, scatole), amines and mercaptoacetic acid [16]. However, the olfactive signature of decaying mouse carcasses was not completely described and, except for S-VOCs, few cadaveric compounds were referenced in Kalinova and colleagues' [16] study. Again, their volatile samplings on mouse models were made in a small closed environment with solid-phase microextraction (SPME) during the beginning of the decompositional process, which may explain the differences. Indeed, postmortem volatiles may be concentrated in closed environments and are detectable earlier.

There is a large discrepancy in decomposition odor compounds reported in the literature [13]. This difference could be explained by the various analytical protocols, including volatile extractions (sampling techniques and sorbent materials) and analytical separations, used to study the postmortem volatiles of vertebrate tissues [4, 59]. Moreover, the result of volatile analysis from decomposition of mammalian soft tissues is also influenced by abiotic factors such as temperature and moisture [4]. The use of different mammalian models or remains (tissues samples *vs.* whole corpses) could also lead to differences in cadaveric volatile profiles. There are some recommendations to examine the potential differences between human and animal models [25]. Indeed, the olfactive signature of decaying pig carcasses shows similarities with the smell of human decomposition in terms of released chemical compounds [13-14], but also dissimilarities [13]. Recent research in this field indicated that the odor from human remains is different from that of animals [13]. Two compounds were exclusively found on human remains and not on animal samples: styrene and benzoic acid methyl ester [13]. These two compounds were not detected in our pig decaying samples. However, the principal identified cadaveric VOCs are, in general, in agreement with previous studies conducted on pig decaying carcasses [7, 14-15]. These cadaveric compounds come from the chemical breakdown of the principal body constituents: proteins, lipids, nucleic acid and carbohydrates [2-3, 25]. Indole and other phenolic compounds (*e.g. p*-cresol) might originate from protein and fat decomposition [1, 7]. Indeed, proteolysis (*i.e.* the breakdown of proteins by the action of bacterial enzymes [5, 60]) yields gases, diamines (cadaverine, putrescine), sulfur compounds and phenolic compounds including indole and skatole [1, 3, 5, 60- 61].

Skatole (*i.e.* 3-methylindole) was not detected in the present study, confirming the findings of the previous study [14]. In addition, cadaverine and putrescine were not detected as cadaveric VOCs in this study. The absence of these biogenic amines, usually associated with the decay

process [2, 49], is also confirmed in previous studies on volatile chemistry of human decay [3, 8-9] or swine decay [7, 14]. Indeed, biogenic amines have a low volatility and therefore are not frequently identified with gas chromatography [4]. Liquid chromatography (LC) is more suitable to detect biogenic amines [4]. Even so, 2-piperidone, a cadaverine metabolite [62], was detected in our cadaveric emanation. Sulfur compounds such as DMDS and DMTS are very frequent biomarkers in cadaveric samples of vertebrate tissues [3-5, 7-17]. Volatile sulfur compounds (VSCs or S-VOCs) come from the microbial breakdown of sulfur-containing amino acids (cysteine, methionine) [7]. The oxygenated compounds (organic acids, alcohols [3, 5, 49], ketones, aldehydes, esters and ethers [3]) come from the carbohydrate decomposition. The breakdown of nucleic acids provides nitrogenous bases, phosphates and sugars [2] whereas lipid degradation produces mainly fatty acids, hydrocarbons, oxygenated, phosphorus and nitrogen compounds [3, 49]. Nevertheless, it is important to remember that for some cadaveric compounds the pathways of their formation are not known in detail or are still completely unknown [4, 7].

It is interesting to note that the dothiepin (also called dosulepin [63]: 3-(dibenzo[b,e]thiepin-11(6H)-ylidene)-N,Ndimethylpropylamine [64-65]) was frequently found in our postmortem volatile samples. However, dothiepin is not a specific cadaveric VOC. Dothiepin is a tricyclic antidepressant with tranquilizing properties [63-65]. This compound was probably dosed antemortem to the pig by the veterinarian to improve the animal welfare. Indeed, psychotherapeutic drugs such as tricyclic antidepressants can be used to eliminate the anxiety-related behavior of animals [66]. To the best of our knowledge, this is the first time that drug was detected on a decaying corpse with headspace collection and bi-dimensional gas chromatography. Nevertheless, some basic drugs, including dothiepin, could be detected in postmortem blood samples using gas chromatography coupled to mass spectrometry with ion trap detection [67]. The headspace detection of volatile compounds of forensic interest (*e.g.* drugs, ethanol) on decaying corpse opens up new possibilities in forensic toxicology. Recent research has been conducted into the development of direct headspace sampling methods to analyze volatile compounds of forensic interest in human biological fluids [68-69].

In conclusion, this study provides the first documentation of the use of GCxGC-TOFMS to analyze pig decaying volatile compounds. The use of comprehensive GC could improve the characterization of the smell of death in terms of volatile constitution, rather than conventional GC. Indeed, the complexity of postmortem volatile samples requires more complex analytical methods [25]. Concerning data analysis, it would be interesting to include chemometrics analysis in future work. Nevertheless, the solvent extraction of the volatile

organic compounds from the sorbent cartridges as well as the storage of the liquid fraction prior analyses is not adequate for the most volatile polar compounds, compared to the use of thermal desorption techniques, which is currently under investigation. However, our results demonstrated that bi-dimensional gas chromatography coupled with time-of-flight mass spectrometry is a powerful tool to analyze the volatile cadaveric emissions.

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Table 24. Volatile chemicals released in the headspace of decaying pig carcass according to the decay stages, ordered by chemical families.

VOCs	Decay stages				Literature report
	Fresh	Bloated	Active decay	Advanced decay	
Alkanes					
Butane	▲	-	-	▲	[17]
Cyclopentane, butyl-	-	-	▲	▲	N.R.
Dodecane	-	▲	▲	-	[7]
Eicosane	-	▲	-	▲	[14]
Heneicosane	▲	-	-	-	N.R.
Heptacosane	-	▲	▲	-	N.R.
Heptane, 2,4-dimethyl-	-	-	-	▲	[3]
Hexadecane	-	▲	▲	▲	[59]
Nonane	-	▲	-	▲	[7]
Octane	-	▲	-	▲	[3, 10-11, 17]
Tetradecane	-	▲	▲	▲	[7, 59]
Tricosane	-	-	▲	▲	N.R.
Tridecane, 4-methyl-	-	▲	▲	-	N.R.
Undecane	-	▲	-	▲	[7-9]
Undecane, 2,5-dimethyl-	-	▲	-	-	N.R.
Alkenes					
1-Butene, 3-methyl-	-	-	▲	▲	N.R.
1-Decene	-	-	▲	-	N.R.
1-Pentene, 2-methyl-	-	▲	▲	▲	N.R.
1,3,6-Octatriene, 3,7-dimethyl-	-	-	▲	-	N.R.
α-Phellandrene	-	▲	▲	-	N.R.
α-Pinene	-	-	▲	▲	[3, 7, 15]
γ-Terpinene	▲	-	▲	▲	N.R.
α-Thujene	-	▲	▲	-	N.R.
o-Xylene	▲	▲	-	-	[3, 7, 9, 11, 13]
p-Xylene	-	-	-	▲	[3, 8-13]
Alcohols					
1-Butanol	-	▲	-	▲	[3- 5, 7, 13-14, 17]
1-Heptanol	-	-	▲	▲	[15]
1-Hexanol	-	▲	▲	-	[3-4, 7, 12-13, 15]
1-Hexen-3-ol	-	▲	▲	-	N.R.
1-Octanol	-		▲	▲	[4, 12-13, 15]
1-Pentanol	-	-	▲	▲	[3-4, 6, 9, 12-14, 17]
1-Pentanol, 4-methyl-	-	-	▲	▲	N.R.
1-Propanol, 2-methyl-	-	▲	▲	▲	[3-4, 6-7, 14]
1-Tridecanol	-	▲	-	-	N.R.
1,2,3-Propanetriol	-	▲	▲	▲	N.R.
2-Hexanol	-	▲	▲	▲	N.R.
2-Hexen-1-ol	-	▲	▲	-	N.R.
3-Hexanol	-	▲	-	▲	N.R.
3-Hexene-2,5-diol	-	▲	-	▲	N.R.
7-Octen-4-ol	-	-	-	▲	N.R.
Cyclohexanol	-	▲	▲	-	N.R.
Cyclopentanedecaol	-	▲	▲	-	N.R.
Ethanol	▲	▲	▲	▲	[3-6, 11, 13-15]
carboxylic acids					
2,3-Dihydroxysuccinic acid	-	▲	-	▲	N.R.
3-Pentenoic acid, 4-methyl-	-	-	▲	▲	N.R.
Butanoic acid	-	▲	▲	▲	[1, 3- 5, 12-14, 17, 25, 49]
Butanoic acid, 2-methyl	-	-	▲	▲	[3, 9, 14, 17]

Butanoic acid, 3-methyl- *(iso-valeric)*	-	▲	▲	▲	[14, 25]
Butyric acid, γ-amino-	-	-	▲	▲	N.R.
Heptanoic acid	▲	-	▲	▲	N.R.
Hexadecanoic acid (*palimitic*)	-	-	▲	▲	[2, 4, 25]
Hexanoic acid (*caproic*)	-	▲	▲	▲	[4, 12-14]
Isobutanoic acid, α-Amino	-	-	▲	-	N.R.
Octanoic acid (*caprylic*)	-	-	▲	▲	[2, 13-14]
Pentanoic acid (*valeric*)	-	-	▲	▲	[12-14, 25]
Pentanoic acid, 4-methyl- (*iso-caproic*)	-	-	▲	▲	[14, 25]
Propanoic acid, 2-methyl- *(iso-butyric)*	-	-	▲	▲	[14, 25]
Propanoic acid, 2,2-dimethyl-	-	-	▲	▲	N.R.
succinic acid, 2,3-dihydroxy-	-	-	▲	▲	N.R.
Aromatic coumpounds					
1(3H)-Isobenzofuranone	-	-	▲	▲	N.R.
1H-Indole	-	▲	▲	▲	[3-5, 12-15]
1H-Indole, 3-methyl-	-	-	▲	▲	N.R.
1H-Pyrrole, 2,5-dimethyl-	-	-	▲	▲	N.R.
Benzene, 1-methyl-4-(1-methylethyl)-	-	-	▲	▲	[14]
Benzene, 1-methylethyl-	-	▲	-	▲	N.R.
Benzene, ethyl-	-	▲	-	▲	N.R.
Benzene, methyl-	-	▲	▲	-	N.R.
Benzeneethanol	-	-	▲	▲	N.R.
Benzenemethanol	-	-	▲	-	N.R.
Benzenemethanol, α-methyl-	-	-	▲	▲	N.R.
Furan, 2-pentyl-	-	-	▲	▲	[12-13]
Isoquinoline	-	-	-	▲	N.R.
Naphthalene	-	-	▲	▲	[4, 7-9, 11, 13]
Naphthalene, 2,6-diisopropyl	-	-	▲	▲	N.R.
Phenol, 2-ethyl-	-	-	▲	▲	N.R.
Phenol, 4-ethyl-	-	-	▲	-	[15]
Phenol, 4-methyl-	-	-	▲	▲	[4, 11, 13-15]
2-phenylethanol	-	-	▲	▲	[14]
Pyrazine, 2-butyl-3,5-dimethyl	-	-	▲	▲	N.R.
Pyrazine, 2,3-dimethyl-	-	-	▲	▲	N.R.
Pyrazine, 2,5-dimethyl-	-	-	▲	▲	N.R.
Pyrazine, 2,6-dimethyl-	-	-	▲	▲	N.R.
Pyrazine, 3-ethyl-2,5-dimethyl-	-	-	-	▲	N.R.
Pyrazine, 3,5-diethyl-2-methyl-	-	-	-	▲	N.R.
Pyrazine, methyl-	-	-	▲	▲	N.R.
Pyrazine, tetramethyl-	-	-	▲	▲	N.R.
Pyrazine, trimethyl-	-	-	▲	▲	[14]
Pyridine, 2-methyl-	-	-	▲	▲	N.R.
Pyridine, 2,6-dimethyl-	-	-	▲	▲	N.R.
Quinazoline	-	-	▲	-	[14]
Quinazoline, 2,4-dimethyl-	-	-	▲	▲	N.R.
Quinazoline, 4-methyl-	-	-	▲	▲	N.R.
Quinoline	-	-	▲	▲	N.R.
Esters					
1,2-Benzenedicarboxylic acid, dihexyl ester	-	▲	-	▲	N.R.
2-Propenoic acid, 3-methoxybutyl ester	-	-	▲	-	N.R.
3-Hexen-1-ol, acetate	-	-	▲	▲	N.R.
3-octanyl acetate	-	▲	▲	-	N.R.
Acetic acid, butoxyhydroxy-, butyl ester	-	-	▲	▲	N.R.

Acetic acid, ethyl ester	-	▲	▲	▲	[3, 7, 17]
Allyl tert-Butyl carbonate	-	▲	-	▲	N.R.
Butanoic acid, 1-methylpropyl ester	-	-	▲	▲	N.R.
Butanoic acid, 3-methyl-, butyl ester	-	-	▲	▲	N.R.
Butanoic acid, butyl ester	-	-	▲	▲	[4, 9, 12, 14, 17]
Butyl 2-methylbutanoate	-	-	▲	-	[14]
Ethyl Acetate	-	▲	-	▲	[13]
Formic acid, ethenyl ester	-	▲	-	▲	N.R.
Hexadecanoic acid, ethyl ester	-	▲	-	-	N.R.
Hexanoic acid, butyl ester	-	-	▲	-	N.R.
Oxalic acid, hexyl propyl ester	-	-	▲	▲	N.R.
Propanoate, 2-hexen-1-ol	-	▲	▲	-	N.R.
Propanoic acid, 2-hydroxy-2-methyl-, ethyl ester	-	-	-	▲	N.R.
Propanoic acid, 2-hydroxy-2-methyl-, methyl ester	▲	-	▲	-	N.R.
Propanoic acid, 2-methyl-, butyl ester	-	-	▲	▲	N.R.
Propanoic acid, butyl ester	-	-	▲	▲	N.R.
Vinyl butyrate	-	▲	▲	-	N.R.
Sulfur compounds					
Benzenesulfonic acid, 4-hydroxy-	-	-	▲	▲	N.R.
Dicyclohexyldisulphide	-	▲	-	▲	N.R.
Disulfide, dimethyl	-	-	▲	▲	[3-5, 7-17]
Dothiepin	-	▲	▲	▲	N.R.
Methane, sulfonylbis-	-	-	▲	▲	N.R.
Sulfone, butyl isopropyl	-	▲	-	▲	N.R.
Sulfurous acid, dicyclohexyl ester	-	▲	-	▲	N.R.
Trisulfide, dimethyl	-	▲	▲	▲	[3-5, 7-11, 13-16]
Nitrogen compounds					
1-Butanamine, 3-methyl-	-	▲	▲	▲	N.R.
1-Butanol, 4-Amino-	▲	▲	▲	-	N.R.
1-Decanamine	-	▲	▲	▲	N.R.
1-Heptadecanamine	-	▲	▲	-	N.R.
2-Piperidinone	-	-	▲	▲	[14-15]
2-Propanol, 1-amino-	-	▲	▲	▲	N.R.
2,3-Butanediol, dinitrate	-	▲	-	-	N.R.
2,3-Dihydrooxazole, 2-t-butyl-4-(1-hydroxy-1-methylethyl)-3-methoxycarbonyl-5-methyl-	-	-	▲	▲	N.R.
5,5-Dimethylimidazolidin-2,4-diimine	▲	-	▲	-	N.R.
Acetamide	-	-	▲	-	[14]
Acetamide, N-methyl-	-	-	▲	▲	[14]
Acetamide, N,N-dimethyl-	-	-	▲	▲	[9, 14]
Acetic acid, [(aminocarbonyl)amino]oxo-	-	▲	-	▲	N.R.
Benzaldehyde, 2-amino-	-	-	▲	▲	N.R.
Butanamide	-	-	▲	▲	[14]
Butanamide, 3-methyl-	-	-	▲	▲	[14]
Formamide, (2-acetylphenyl)-	-	-	▲	▲	N.R.
Formamide, N-(2-methylpropyl)-	-	-	▲	▲	N.R.
Formamide, N-butyl-	-	-	▲	▲	N.R.
Formamide, N-methyl-	-	-	▲	▲	N.R.
Formamide, N-phenyl-	-	▲	▲	▲	N.R.
Formamide, N,N-dimethyl-	-	▲	▲	▲	[7, 14]
Heptanonitrile	-	-	▲	▲	N.R.
Hexanamide	-	-	▲	▲	N.R.
Hexanamide, N-methyl	-	-	▲	▲	N.R.

Methanamine, N,N-dimethyl-	-	▲	▲	-	N.R.
Methanediamine, N,N,N',N'-tetramethyl-	-	▲	▲	-	[13]
N-Methylvaleramide	-	-	▲	▲	N.R.
Pentanamide	-	-	▲	▲	N.R.
Propanamide	-	-	▲	-	[14]
Propanamide, 2-methyl-	-	-	▲	-	N.R.
Propanamide, N-methyl-	-	-	▲	-	[14]
Propanamide, N,2-dimethyl-	-	-	▲	▲	N.R.
Propanenitrile, 3-dimethylamino-	-	-	▲	▲	N.R.
Propylamine	-	-	▲	-	N.R.
Propylamine, N,N,2,2-tetramethyl-, N-oxide	-	-	▲	▲	N.R.
Trimethylamine	-	▲	▲	▲	[4, 7, 13-14]
Aldehydes					
2-Butenal, 3-methyl-	-	▲	▲	▲	N.R.
2-Octenal	-	-	▲	-	[12-13]
Acetaldehyde	-	▲	▲	▲	[3, 17]
Benzaldehyde	-	-	▲	▲	[3-4, 7-8, 12-14]
Heptanal	-	▲	▲	▲	[2, 4, 9, 12-14, 59]
Hexanal	-	▲	-	▲	[3-4, 10, 12-13]
Methylglyoxal	-	▲	▲	-	N.R.
Nonanal	-	-	▲	▲	[4, 8-9, 12-13, 17, 59]
Nonenal	-	-	▲	▲	N.R.
Octanal	-	▲	▲	-	[12-13]
Pentanal, 2-methyl-	-	-	▲	▲	N.R.
Propanal	-	▲	▲	-	N.R.
Propanal, 2-hydroxy-	-	▲	-	▲	N.R.
Propanal, 2,2-dimethyl-	-	▲	-	▲	N.R.
Ketones					
1-Octen-3-one	-	-	-	▲	N.R.
2-Cyclohexen-1-one, 3-methyl-	-	-	▲	▲	N.R.
2-Decanone	-	-	▲	▲	[15]
2-Heptanone	-	-	▲	▲	[3-4, 12-14]
2-Hexanone	-	-	▲	▲	[3, 10]
2-Nonanone	-	-	▲	▲	[1, 4, 9, 11, 13-15]
2-Octanone	-	-	▲	▲	[15]
2-Propanone, 1-phenyl-	-	-	▲	▲	N.R.
2-Undecanone	-	-	▲	▲	N.R.
2,3-Octadione	-	-	▲	▲	N.R.
2,4,6-Cycloheptatrien-1-one	-	-		▲	N.R.
2,5-Cyclohexadiene-1,4-dione	-	-	▲	▲	N.R.
2,5-Hexanedione	-	-	▲	▲	N.R.
2(3H)-Furanone, 5-butyldihydro- 2	-	-	▲	▲	N.R.
2(3H)-Furanone, dihydro-5-methyl-	-	-	▲	▲	N.R.
3-Hexanone	-	-	▲	▲	N.R.
3-Hexanone, 2-hydroxy-	-	-	▲	▲	N.R.
3-Octanone	-	-	▲	▲	N.R.
3-Pentanone, 2-hydroxy	-	-	▲	▲	N.R.
3-Penten-2-one, 4-methyl-	-	▲	▲	-	N.R.
4-Penten-2-one, 4-methyl-	-	▲	▲	-	N.R.
5-Hepten-2-one, 6-methyl	-	-	▲	▲	[13]
Ç-Valerolactone	-	-	▲	▲	N.R.
Cyclohept-4-enone	-	-	▲	▲	N.R.

Cyclohexanone	▲	▲	-	▲	[3-4, 7, 12-13]
Cyclopentanone	-	-	-	▲	N.R.
Cyclopentanone, 2-(1-methylpropyl)-	-	▲	▲	-	N.R.
Ethanone, 1-phenyl-	-	-	▲	▲	[1, 11, 13-14]
exo-5-Methyl-2-oxabicyclo[4.1.0]heptan-3-one	-	▲	▲	-	N.R.
Tridecan-2-one, 10-Methyl	-	-	▲	▲	N.R.
Ethers					
1,3-Dioxolane, 2-acetyl-	-	▲	▲	▲	N.R.
2-Propanol, 1-propoxy-	-	-	▲	▲	N.R.
Ethene, methoxy-	-	▲	▲	-	N.R.
Furan, 2-butyltetrahydro-	-	▲	▲	▲	N.R.
Furan, 2,3-dihydro-2,5-dimethyl-	-	-	▲	▲	N.R.
Oxirane, 2,3-dimethyl-	-	-	▲	-	N.R.
Oxiranemethanol	-	-	▲	-	N.R.
halogen compounds					
1-Chloroheptylacetate	-	-	▲	▲	N.R.
1-Iodo-2-methylundecane	-	▲	▲	-	N.R.
Acetamidine, hydrochloride-	-	▲	▲	-	N.R.
Butane, 1-bromo-2-methyl-	-	-	▲	▲	N.R.
Propane, 1-bromo-2-methyl-	-	▲	-	▲	N.R.
Other compounds					
Butyl isocyanatoacetate	-	▲	-	▲	N.R.
Cyanic acid, 2-methylpropyl ester	-	-	▲	▲	N.R.
Cyanic acid, propyl ester	-	▲	-	▲	N.R.
Hydroperoxide, 1-ethylbutyl	-	▲	▲	▲	N.R.
Hydroperoxide, 1-methylbutyl	-	▲	▲	▲	N.R.
Hydroperoxide, 1-methylpentyl	-	-	▲	▲	N.R.

(▲) indicated a VOC detected or (-) for a VOC not detected. The column "literature report" lists the VOCs referenced in peer-reviewed literature (number correspond to the different papers concerning the decompositional chemistry and are listed in "References" section or not referenced (N.R.).

Chapitre III.4: L'écologie chimique d'un Silphidae modèle: le cas de *Thanatophilus sinuatus* F.

III.4.1. Electrophysiological and behavioural responses of *Thanatophilus sinuatus* Fabricius (Coleoptera: Silphidae) to selected cadaveric volatile organic compounds

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Abstract – Soon after death, carcasses release volatile chemicals that attract carrion insects including Silphidae. Nevertheless, it is not known which chemical cues are involved in the attractiveness of the carcass. So far, little information is available on the chemical ecology of carrion beetles, particularly concerning the subfamily of Silphinae. The biological role of selected cadaveric volatile organic compounds including: dimethyldisulfide (DMDS), butan-1-ol, n-butanoic acid, indole, phenol, *p*-cresol, putrescine, and cadaverine on the silphine species, *Thanatophilus sinuatus* Fabricius, was investigated by using both electrophysiological and behavioural techniques. Among the tested cadaveric compounds, butan-1-ol and DMDS elicited the strongest EAG from both *T. sinuatus* male and female antennae. In a two-arm olfactometer, males and females were significantly attracted to dimethyldisulfide (DMDS) for both tested doses, whereas only males were attracted to *p*-cresol at 100 ng. Putrescine was repellent to males at the dose of 1 µg.

Key Words - Forensic science, Forensic entomology, Chemical ecology, Carrion beetles, Silphinae, Carrion ecology, cadaveric VOCs, Insect olfaction, Electroantennography, Olfactometry.

1. Introduction

Forensic entomology is a broad field of forensic science where arthropod science, mainly insects, and the judicial system interact (1). In regards to medico-legal fields, necrophagous insects are predominantly used to determine the time elapsed since death, otherwise known as the postmortem interval or PMI (2). It is well documented that different species of insects, mainly Diptera (*i.e.* flies) and Coleoptera (*i.e.* beetles), are attracted and colonize a vertebrate carcass in a relative predictable sequence called the entomofaunal succession (3-8), each associated with distinct stages of decay (9-10). Most forensic studies have focused on flies while beetles have been neglected (11-12). The beetles are however part of the entomofaunal colonization of a dead body. Among them, carrion beetles (Coleoptera: Silphidae) can provide information on postmortem colonization on remains and time since death (13-15). To an ecological point of view, carrion beetles perform vital ecosystem functions (16) by promoting the breakdown and recycling of organic matter into terrestrial ecosystems (17-19). Searching for carrion, a scarce and patchy resource, is crucial for Silphidae (20-21). Most of them use vertebrate carcasses as food and breeding resources (17, 21-23). Carrion beetles are distributed into two subfamilies: the Nicrophorinae also called burying or sexton beetles and the Silphinae (17, 24-27). There are some ecological and behavioural differences between both subfamilies in the use of vertebrate carcasses (28-30). Nicrophorinae prefer small vertebrate carcasses (*e.g.* rodents, birds) that they bury whereas Silphinae are found in large carcasses (without burying) (22, 28, 31-33). Contrary to humans that use principally vision, touch and sound to recognize their environment, insects perceive the world differently and their ecology relies mainly on chemical cues (34). The sense of smell or olfaction is crucial for them; most insects have a highly developed olfactory system (34-35), primarily located on antennae (34-35). Carrion insects are not exception to this (34), they have adapted their olfactory system over millions of years to specific cadaveric chemicals (9). The odor of carrion contains a wide range of chemicals (34, 36-41) also called cadaveric volatile organic compounds (VOCs) changing over time (36, 40). Forensic entomologists often raise the hypothesis that the cadaveric VOCs regulate the necrophagous insects behavior (40) and are responsible to the attraction of carrion insects to the corpses or animal carcasses (34).

However, it is not known which semiochemicals mediate the attractiveness of carcasses toward insect necrofauna (19, 34). The chemical ecology of a "cadaver-ecosystem", *i.e* the relationship that may exist between cadaveric VOCs and carrion insects, is poorly studied (10, 19, 34). However, advances in forensic entomology are continually being made and incorporate new approaches (34) such as the chemical ecology of necrophagous insects. A better understanding of the chemical ecology of carrion insects would however benefit to the development of cadaveric detection devices based on necrophagous insect olfaction. The use of insects like biosensors or biodetectors begins to be considered in forensic science (34, 42-43). Because of the sensitivity of their olfactory system, it appears that insects also might be used to develop novel methods for detecting and locating chemicals associated with the smell of death (44-45). Moreover, insect olfaction is already used for detecting chemicals associated with drugs and explosives (42, 44-45). Indeed, insects are highly sensitive, flexible, portable and cheap to reproduce, and it is easy to condition them to detect target odorants (42, 44).

Recent studies on the chemoecology of insect of forensic interest are focused on pioneer species such as blowflies (Diptera, Calliphoridae): *Lucilia sericata* (10) and *Calliphora vomitoria* (34) and little is known about latter postmortem colonizers such as carrion beetles. As underlined by Haberer *et al.* (46) and Kalinova *et al.* (19), there have been few studies on the chemoecology of the Silphidae. Currently, only organosulfur compounds (S-VOCs) such as dimethylsulfide (DMS), dimethyldisulfide (DMDS) and dimethyltrisulfide (DMTS) are described as attractants for the burying beetles *Nicrophorus vespillo* Linnaeus and *N. vespilloides* Herbst (Silphidae: Nicrophorinae) (19-20). Podskalska *et al.* (20) speculate that other carrion beetle species may react to different concentrations of S-VOCs that they used or to other infochemicals. However, nothing is known about the semiochemicals involved in the attractiveness of carcasses for other carrion insects, in particular for Silphinae for which there are no published chemoecological studies. All carrion beetles do not have the same forensic interest; species of Silphinae seem to have a more important value as forensic bioindicators (15, 47). Due to their ecological preferences for small vertebrate carcasses, Nicrophorinae present less interest in forensic entomology (15). Silphinae are frequently found on vertebrate carcasses (47-52) and may be present in great numbers (15). *Thanatophilus sinuatus* Fabricius is frequently found in Europe on vertebrate carcasses (47-52). The goal of the study presented herein is to identify the semiochemicals mediating the carcass attractiveness to *Thanatophilus sinuatus*, based upon electrophysiological and laboratory behavioural experiments.

2. Materials and methods

2.1. Rearing colonies of *Thanatophilus sinuatus*

Adults of *Thanatophilus sinuatus* were collected from baited pitfall traps (chicken meat) located in various habitats in Belgium. Males and females of *T. sinuatus* were reared in group (30 couples) in glass containers (dimensions: 40x25x20 cm) filled with compost (≈10 cm) to provide laboratory colonies. The daylight regime was 16:8 (L: D) and the temperature in the rearing room was 21±2°C with 70-80% RH. A glass vial containing water with a wick of cotton wool and food were placed in each rearing box. Food sources consisted in a mix of dry dog food and decaying chicken and pork meat. The experiments were conducted with insects aged 10-20 days. 24h prior the bio-assays, insects were separated from the colonies and placed into aerated plastic boxes without any food source.

2.2. Chemical Compounds

Cadaveric chemical compounds were selected according to their relative abundance during the decay process (36-40, 53). Eight synthetic chemicals belonging to different chemical families were chosen: butan-1-ol, n-butanoic acid, dimethyldisulfide (DMDS), phenol, 4-methylphenol (*p*-cresol), indole, cadaverine and putrescine. Table 25 lists the chemicals and the tested doses for each compound. Among six different solvents (n-hexane, n-pentane, dichloromethane, diethylether, paraffin oil and sunflower oil), paraffin oil was selected latter to dilute the chemical solutions because it was found to elicit the lowest EAG responses from *T. sinuatus* antennae. Because phenol and 4- methylphenol were insoluble in paraffin oil at the dose of 10 mg, these compounds were not tested in bio-assays. Moreover, because indole was not soluble in paraffin oil at the doses of 10 mg, 1 mg, 0.1 mg, it was not tested in bio-assays at these doses.

Table 25. Cadaveric volatile organic compounds tested on *T. sinuatus*. the pictogram (-) means that the chemical is not soluble in paraffin oil at this dose.

Tested chemicals	Chemical family	Tested doses							
		10mg	1 mg	0.1 mg	0.01 mg	1 µg	0.1 µg	0.01 µg	1 ng
n-butanoic acid (Fluka 19210, purity > 99.5%) IUPAC name: n-butanoic acid	acid	▲	▲	▲	▲	▲	▲	▲	▲
butan-1-ol (Sigma 24124, purity > 99%) IUPAC name: butan-1-ol	alcohol	▲	▲	▲	▲	▲	▲	▲	▲
dimethyldisulfide (DMDS) (Fluka 40221, purity > 98%) IUPAC name: methyldisulfanylmethane	organosulfur compound	▲	▲	▲	▲	▲	▲	▲	▲
cadaverine (Fluka 33211, purity > 97%) IUPAC name: pentane-1,5-diamine	diamine	▲	▲	▲	▲	▲	▲	▲	▲
putrescine (Fluka 32790, purity > 99%) IUPAC name: butane-1,4-diamine	diamine	▲	▲	▲	▲	▲	▲	▲	▲
phenol (Fluka 77612, purity > 99%) IUPAC name: hydroxybenzene	aromatic compound	-	▲	▲	▲	▲	▲	▲	▲
***p* cresol** (Fluka 61030, purity > 99%) IUPAC name: 4 methylphenol	aromatic compound	-	▲	▲	▲	▲	▲	▲	▲
indole (Fluka 57190, purity > 98.5%) IUPAC name: indole	aromatic compound	-	-	-	▲	▲	▲	▲	▲

2.3. Electroantennography (EAG)

Electroantennograms allow measurement of the electrophysiological responses of insect antennae on stimulation with an odour (54). EAG recordings were conducted using whole alive insects previously immobilized using odourless modelling clay. The antennae were mounted in saturated AgCl-Ag glass capillary electrodes (Harvard Apparatus, Holliston, MA, USA; 1.5 mm o.d. x 1.17 mm i.d.) filled with saline solution (Ringer solution: NaCl, 7.5 g/l; $CaCl_2$, 0.21 g/l; KCl, 0.35 g/l; $NaHCO_3$, 0.2 g/l) in contact with a silver wire. The half of the last distal antennal segment was immersed into the saline solution of the recording electrode. The second antenna was completely immersed into the saline solution of the reference electrode (ground glass electrode). The DC potential was recorded on a computer with the Autospike® software (Autospike v.3.2, Syntech, Hilversum, Netherlands) by using an amplifier (IDAC-4, Syntech, Hilversum, Netherlands) with 100-fold amplification. A 0.5 cm^2 piece of filter paper, which was impregnated with the tested chemical compound at the selected dose, was placed in a Pasteur pipette and used to puff an air sample in a constant 1.5 l/min air flow during 0.5 s. Charcoal-filtered and humidified air was continuously flowing over the mounted insect (200 ml/min air flow); the air was puffed over the exposed half of the

distal antenna. As negative control, the working antenna was first stimulated with filter paper impregnated with 10 µl of paraffin oil (mechanical and solvent stimulus). All selected chemicals at all tested doses were randomly tested on each insect (10 females and 10 males per chemical per dose). The tested doses were 10 mg, 1 mg, 0.1 mg, 0.01 mg, 1 µg, 0.1 µg, 0.01 µg, and 1 ng. At the end of each cadaveric chemical stimulations, the control stimulation was repeated (blank filter paper with pure paraffin). To allow the repolarization of the antenna, 30 seconds separated each stimulation puff.

2.4. Behavioural Bio-assays

A two-arm olfactometer (Y-olfactometer) was used to investigate the behavioural responses of *T. sinuatus* exposed to olfactive stimuli. The Y-olfactometer was previously described by Fredericks *et al.* (10) when studying the behavioural responses of the necrophagous blowfly *Lucilia sericata* Meigen exposed to selected cadaveric compounds. The main arm of the Y-olfactometer (15 cm long and 1.5 cm I.D.) and the two arms (20 cm long and 1.5 cm I.D.) were made of Teflon® (Fig. 42).

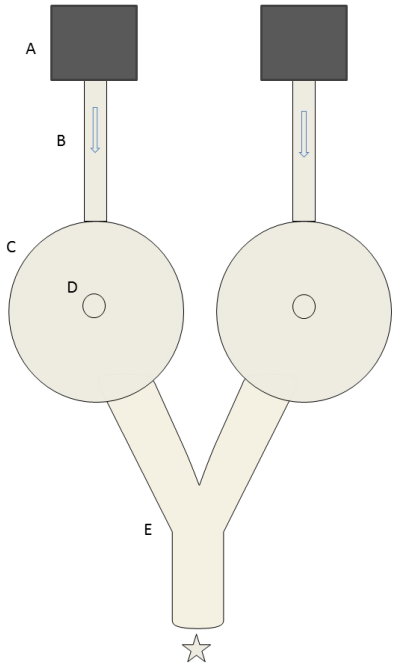

Figure 42. Y-tube olfactometer (A: pushing pump, B: Teflon tubing, C: glass arena containing (D) the impregnated or not impregnated filter paper, E: Y-tube in Teflon), arrow indicates the air direction and star indicates the starting point for the insect.

Each arm of the Y-olfactometer was connected to a glass chamber (16 cm of basal diameter and 16 cm height). A pump (Penn State University, State College, USA) was connected to each arena with Teflon® tubing and allowed to pull air (constant airflow of 500 ml/min)

through the glass chamber and the Y-tube. The olfactometer was placed below incandescent white light (1350 Lux) which provided uniform lighting. Indeed, *T. sinuatus* is a diurnal species (29). Bio-assays were performed at room temperature (21°C). Each chemical compound was tested alone on 50 starved adults of both sexes (50 males and 50 females), at the doses of 100 ng and 1 µg. The two different doses were tested on different carrion beetles. The chemicals were applied on a filter paper (1 cm^2) and randomly placed at the center of one of the two glass chambers. Each individual was introduced into the Y-tube at the entrance of the main branch and had the choice between the tested compound and the control (glass chamber without chemical, non-impregnated filter paper). New impregnated filter papers were used for each insect. The complete Y-tube was washed between each tested insect with hot water and Norvanol D (VWR, 88.5 % v/v ethanol, 2.88% v/v diethylether, water 8.60% v/v). Each insect was allowed to spend a maximum of 15 minutes in the olfactometer. The behavioural test was stopped when an insect made a choice, i.e. when the insect moved into one of the glass chambers. The measured response in each test was calculated as the number of *Thanatophilus sinuatus* attracted by the tested chemical compound.

2.5. Statistical Analyses

Statistical analyses were performed using Minitab® (Minitab v15.0, State College, Pennsylvania, USA). The EAG responses were analyzed by a three-way ANOVA. Three different analyses of variance (with factors being gender (male or female), chemical compounds (8 compounds) and doses (8 doses)) were conducted because phenol, 4-methylphenol and indole were not tested at all doses. When a significant difference of EAG responses based on the cadaveric chemical compounds was observed, a multiple comparison of the means by the method of Newman and Keuls ($\alpha = 0.05$) was undertaken to take into account the rise of the alpha risk with multiple comparisons of the means. When a significant difference of EAG responses based on doses of chemical compounds tested was observed, a multiple comparison of the means by the method of orthogonal polynomials was carried out. For the behavioural experiment, a chi-square Goodness-of-fit test (Minitab v15.0; $n = 50$, $\alpha = 5\%$, 1 *df*) was used to compare theoretical distribution (respectively, 50%–50% for each possibility) and observed distribution. According to the screening process used, non-probability correction was applied to behavioural responses' tests to avoid rejecting potentially active molecules. False positive risk is then set to $\alpha = 5\%$ for each molecule.

3. Results

3.1. Electroantennography

All the tested cadaveric compounds elicited antennal depolarization. The responses were significantly different from the negative controls (Fig. 43). The three-way ANOVA indicated that for each of the doses, the tested chemical compounds were perceived differently, except for the lowest dose, 1 ng ($F_{7,720}$ = 1.01, P > 0.05).

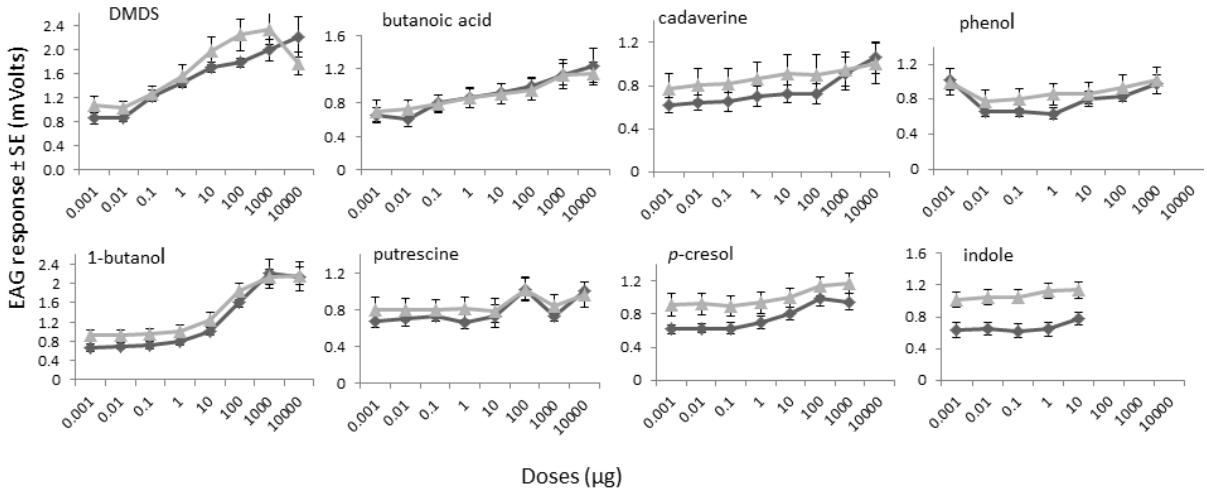

Figure 43. Electroantennography dose-responses obtained from male and female T. sinuatus antennae toward dimethylsulfide (DMDS), butanoic acid, cadaverine, phenol, 1-butanol, putrescine, p-cresol and indole. The light grey line with triangles refers to females (n =10) and the dark grey curve with rhombi refers to males (n=10). Each dot represents a mean of 10 EAG recordings and error bars represent the standard error (± SE).

The multiple mean comparisons by the Newman and Keuls test (α = 0.05) are gathered in Table 26. This comparison showed that DMDS and butan-1-ol induced higher electrical responses than other cadaveric compounds at the higher doses (10 mg, 1 mg, 0.1 mg). At 10 mg and 1 mg doses, electrical responses under stimulations of DMDS and butan-1-ol were similar. However, at lower doses (0.1 mg and 0.01 mg), these two compounds induced different responses. At the dose of 1 µg and 0.1 µg, the tested insects were more sensitive to DMDS than to the other chemicals. Males were also more sensitive to the chemical compounds than females at some tested doses. Significant differences between males and females were observed at 0.1 mg ($F_{1,882}$ 4.79, P = 0.0289), 0.01 mg ($F_{1,720}$ 8.98, P = 0.0028), 1 µg ($F_{1,720}$ 13.46, P = 0.0003), 0.1 µg ($F_{1,720}$ 9.74, P = 0.0019) and 0.01 µg ($F_{1,720}$ 13.76, P = 0.0002). There was no difference between males and females at the highest tested doses of 10 mg ($F_{1,720}$ 1.31, P = 0.2525) and 1 mg ($F_{1,882}$ 2.59, P = 0.1078). The antennal responses were dose dependent for butan-1-ol ($F_{7,720}$ 36.92, P < 0.001), DMDS ($F_{7,720}$ 22.57, P < 0.001),n-butanoic acid ($F_{7,720}$ 3.83, P < 0.001) and 4-methylphenol ($F_{6,882}$ 2.38, P = 0.0278). For other

tested chemical compounds, the EAG responses were not dose dependant, including putrescine ($F_{7,720}$ 1.35, P = 0.2256), cadaverine ($F_{7,720}$ 1.31, P = 0.2417), phenol ($F_{6,882}$ 1.89, P = 0.0802) and indole ($F_{4,720}$ 0.42, P = 0.7945)). The multiple comparisons of the means by the method of orthogonal polynomials showed that antennal responses induced by n-butanoic acid and 4-methylphenol increased linearly with the logarithm of the doses.

Table 26. Multiple comparisons of the average eags responses (±se) (n=20). for each dose, chemical compounds sharing * or ** induce similar eag responses as a result of newman and keuls test (α = 0.05).

10 mg	putrescine * 983 ± 78	cadaverine * 1033 ± 116	butanoic acid * 1193 ± 120	DMDS ** 1927± 184	butan-1-ol ** 2149 ± 169			
1 mg	putrescine * 787 ± 68	cadaverine * 981 ± 105	phenol * 996 ± 94	p cresol * 1064 ± 79	butanoic acid * 1135 ± 102	DMDS ** 2113 ± 152	butan-1-ol ** 2168 ± 167	
0.1 mg	cadaverine * 814 ± 103	phenol * 895 ± 79	butanoic acid * 978 ± 77	putrescine * 1022 ± 86	p cresol * 1064 ± 74	butan-1-ol 1726 ± 98	DMDS 1970 ± 136	
0.01 mg	putrescine * 759 ± 87	cadavérine * 817 ± 95	phenol * 842 ± 76	p cresol * 905 ± 71	butanoic acid * 913 ± 75	indol * 941 ± 74	butan-1-ol 1128 ± 90	DMDS 1781 ± 119
1 µg	putrescine * 739 ± 72	phenol * 749 ± 75	cadaverine * 781 ± 90	p cresol * 817 ± 80	butanoic acid * 855 ± 79	indol * 879 ± 80	butan-1-ol * 903 ± 70	DMDS 1474 ± 88
0.1 µg	cadaverine * 732 ± 83	phenol * 741 ± 70	putrescine * 763 ± 64	p cresol * 765 ± 74	butanoic acid * 791 ± 69	butan-1-ol * 826 ± 74	indol * 833 ± 73	DMDS 1225 ± 74
0.01 µg	butanoic acid * 675 ± 72	cadaverine * 697 ± 78	putrescine * 738 ± 71	p cresol * 765 ± 77	butan-1-ol * 791 ± 73	indol * 815 ± 79	DMDS 956 ± 81	phenol 1036 ± 96
1 ng	butanoic acid 667 ± 70	cadaverine 713 ± 82	phenol 725 ± 76	putrescine 751 ± 72	p cresol 777 ± 76	butan-1-ol 813 ± 70	indol 835 ± 80	DMDS 907 ± 66

3.2. Behavioral bio-assays

Table 27 shows the behavioural data. In the Y-tube bio-assay, dimethyldisulfide (DMDS) attracted both males and females at 100 ng (χ^2males = 6.72, P = 0.010 and χ^2females =5, P = 0.025) as well as at 1 µg (χ^2males = 6.15, P = 0.013 and χ^2females = 5, P = 0.025). *p*-Cresol was attractive for males at the dose of 100 ng (χ^2males = 4.45, P = 0.035), while putrescine was repellent for males at the dose of 1 µg (χ^2males = 4.12, P = 0.042). The other compounds were neither attractive nor repulsive to *T. sinuatus* at the two tested doses.

Table 27. Y-tube bio-assays (* and ** respectively indicate differences between control and voc at $p < 0.05$ and $p \leq 0.01$, respectively, with $\alpha = 0.05$).

Chemical compound	Males			Females		
	Responding beetles	χ^2	*P*	Responding beetles	χ^2	*P*
1 µg						
dimethyldisulfide	94%	6.15	0.013*	90%	5.00	0.025*
butan-1-ol	88%	0.09	0.763	84%	0.38	0.537
butanoic acid	94%	3.60	0.058	92%	0.35	0.555
phenol	92%	0.00	1.00	88%	0.36	0.546
p-cresol	94%	3.60	0.058	84%	0.10	0.758
indole	86%	1.14	0.286	88%	1.45	0.228
putrescine	82%	4.12	0.042*	80%	3.60	0.058
cadaverine	90%	0.56	0.456	88%	0.00	1.00
100 ng						
dimethyldisulfide	86%	6.72	0.010**	90%	5.00	0.025*
butan-1-ol	82%	0.22	0.639	86%	0.21	0.647
butanoic acid	80%	0.00	1.00	84%	1.53	0.217
phenol	92%	0.78	1.00	84%	0.10	0.758
p-cresol	88%	4.45	0.035*	92%	0.00	1.00
indole	84%	0.10	0.758	80%	0.40	0.527
putrescine	88%	0.09	0.763	82%	0.22	0.639
cadaverine	86%	1.14	0.286	84%	0.10	0.758

4. Discussion

To the best of our knowledge, this is the first report of successful electroantennogram recordings from whole alive Silphinae species, *Thanatophilus sinuatus* Fabricius. Other electrophysiological studies (EAG, GC-EAD) on Silphidae concern recordings on excised antennae (19). DMDS and butan-1-ol elicited the largest antennal depolarization at the highest tested doses (10 mg (= 100% v/v) to 1 µg). These two compounds may be considered as key components released from decaying pig carcasses (40) and organosulfur compounds, including DMDS, from mouse carcass (19). At 10 mg and 1 mg, butan-1-ol induced higher responses (10 mg: 2149 ±170 µv, 1 mg: 2168 ±167 µv) than DMDS (10 mg: 1927 ±184 µv, 1 mg: 2113 ± 152 µv). For the other EAG dose-response tests, DMDS induced the highest electrical responses on carrion beetle antennae, except for 0.01 µg where phenol induced

stronger depolarisations (1036 ± 96 µv). However, butan-1-ol showed no significant attractiveness for both sexes in behavioural bioassays whereas DMDS may be considered an attractant for males and females of *T. sinuatus* at both tested doses (100 ng and 1 µg)). In previous studies, it has been demonstrated that organosulfur compounds such as DMDS strongly attract the carrion flies *Lucilia sericata* Meigen (Diptera, Calliphoridae) (10, 55-57), the synanthropic fly *Musca domestica* Linnaeus (Diptera, Muscidae) (58-61) and, *Nicrophorus vespillo* L. (Coleoptera, Silphidae) (19-20). The attractiveness of DMDS was also tested on *N. vespilloides* Herbst (19), but no effect was noted in the Y-tube bioassays for this sulphide. Our results demonstrate that dimethyldisulfide acts as a semiochemical for males and females of *T. sinuatus*. Kalinova and colleagues (19) have shown that the antennal responses to S-VOCs were dose-dependent for males of *N. vespillo* and *N. vespilloides*. They also suggest that burying beetle antennae bear specific receptors for organosulfur compounds (19). Our antennal responses to synthetic dimethyldisulfide were also dose-dependent for both sexes of *T. sinuatus*.

p-Cresol, also called 4-methylphenol, is the second compound that carries a biological activity in our behavioural bioassays. This phenolic compound is significantly attractive for male *T. sinuatus* at the dose of 100 ng. Nevertheless, we observed the largest number of males in the arena which contained *p*-cresol at 1 µg (χ^2males = 3.60, P = 0.058, n = 47). *p*-Cresol is known to act as an attractant to different species of Diptera such as mosquitoes (62-63) and tsetse flies (*Glossina* spp. Wiedemann) (58). It is also referenced as a chemostimulant in the selection of oviposition substrates by the stable fly *Stomoxys calcitrans* Linnaeus (Diptera, Muscidae) (63). *p*-Cresol, along with phenol, was also described as a female sex pheromone component of a Coleoptera species, *Phyllophaga cuyabana* Moser (Coleoptera, Melolonthidae)) (64); in field experiments, only males of *P. cuyabana* were captured in traps baited with *p*-cresol and phenol (ratio 1:1) (64). As for *P. cuyabana*, *p*-cresol could be a component of the sex pheromone of female *T. sinuatus*. This hypothesis could be supported by the difference in terms of biological responses observed between male and female in our behavioural experiments. Indeed, *p*-cresol is not attractive for *T. sinuatus* females whereas it attracted males. Nevertheless, further studies on silphine pheromone are necessary.

In conclusion, of eight tested chemical compounds, two have shown a biological activity on *T. sinuatus*. Statistical analyses revealed that DMDS was attractive for males and females at the doses of 100 ng and 1 µg whereas *p*-cresol is only attractive at 100 ng for males. Despite of the laboratory attractiveness of DMDS on *T. sinuatus* and *p*-cresol on males, future research is necessary to validate this assumption with field experiments. Podskalska *et al.* (20)

successfully used pitfall traps baited with S-VOCs to sample burying beetles in agricultural biotopes. It would be interesting to test these infochemicals under field conditions in various biotopes. Moreover, recent research in the chemoecology of Silphidae has shown that the use of individual chemical compounds was less efficient in attracting carrion beetles than a blend of chemicals (20). Combinations of different S-VOCs increase trapping efficiency for carrion beetles (20). Studies on house fly attractants led to the same conclusions; mixtures were better attractants than single compounds (synergistic effect) (58). However, further research will be needed with mixtures of cadaveric VOCs on Silphidae. In general, there have been few studies on the chemical ecology of the Silphidae (46). There are few research projects carried out examining the influence of odor on the behavior of Nicrophorinae (19) and even fewer on Silphinae. Our results may have potential implications in a better understanding of attractiveness of silphine species toward a decaying corpse. Nevertheless, our behavioural responses based on statistical analysis are exploratory results. The comprehension of the role of odors in the behavior of necrophagous insects would make it possible new developments in forensic sciences and the research of the corpses with the use of insect olfaction that may act as cadaveric "biodetectors or biosensors" (42).

5. Acknowledments

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Partie IV: Discussion générale

«*La vue d'un cadavre était toujours choquante, jusqu'à ce que la télé finisse par nous y habituer.*»

Roland Topor

1) L'entomofaune des cadavres: les Coléoptères Staphylinoidea

La plupart des études en entomologie forensique sont axées sur la biologie et la colonisation postmortem de quelques espèces de Diptères, principalement des Calliphoridae et, dans une moindre mesure, des Sarcophagidae. Les Coléoptères que l'on peut retrouver au sein de l'écosystème cadavre étaient encore, jusqu'à très récemment, peu étudiés par les entomologistes forensiques. Or à l'instar de certaines familles de Diptères d'intérêt forensique, ces derniers peuvent aussi présenter des caractéristiques qui pourraient être exploitées dans les investigations médico-légales et notamment, dans l'amélioration de l'estimation de l'intervalle postmortem par les méthodes entomologiques classiques (Midgley et Villet 2009, Midgley *et al.* 2010). On assiste depuis peu à une recrudescence des études se focalisant sur certaines espèces de Coléoptères nécrophages et/ou nécrophiles et plus particulièrement des espèces appartenant aux taxons des Silphidae (Chauvet *et al.* 2008, Midgley et Villet 2009, Velasquez et Viloria 2009, Midgley *et al.* 2010, Velasquez et Viloria 2010) et des Dermestidae (Schroeder *et al.* 2002, Pasquerault *et al.* 2008). Encore faut-il pouvoir identifier les Coléoptères présents au sein cet écosystème cadavre et, par la suite sélectionner les espèces potentiellement utiles en entomologie forensique. Cela ne peut se faire que grâce à des études de suivis entomologiques postmortem se rapprochant le plus possible des conditions réelles. La caractérisation et la quantification de l'entomofaune associée aux cadavres est donc un prérequis nécessaire à la sélection de bioindicateurs d'intérêt forensique. En outre, certains entomologistes forensiques insistent sur le fait qu'il faut augmenter les bases de données entomologiques. Ceci afin d'affiner nos connaissances concernant l'écologie de l'écosystème cadavre et plus particulièrement les successions d'insectes postmortem dans différentes régions géographiques de même que dans différents biotopes parmi ces régions (Catts et Goff 1992, Byrd et Castner 2001, Amendt *et al.* 2004, Sharanowski *et al.* 2008). L'entomofaune des cadavres peut varier d'une région à l'autre du globe. Si plusieurs espèces de Calliphoridae (par exemples: *Calliphora vicina*, *C. vomitoria*, *Lucilia sericata*, etc.) sont déjà réputées pour être des bioindicateurs hautement fiables pour la datation de la mort, il n'est est rien pour les autres espèces et plus particulièrement en ce qui concerne les Coléoptères nécrophages. Les insectes collectés aux cours des expertises entomologiques judiciaires sont principalement utilisés pour dater la mort d'une personne; on parle aussi d'intervalle postmortem ou d'IPM. En effet, lorsque la mort remonte à plus de 72h ou que des signes de putréfaction avancée sont visibles, les techniques médico-légales classiques ne sont plus assez efficaces pour évaluer le moment du décès. La présence et

l'identification des insectes sur le corps et de façon plus large, sur la scène du décès, deviennent de meilleurs bioindicateurs pour dater la mort. La littérature évoque souvent deux types de méthodes pour déterminer un intervalle postmortem en utilisant les insectes comme bioindicateurs en fonction du temps écoulé depuis le décès (Swift 2006, Wyss et Chérix 2006, Amendt *et al.* 2007). La première méthode de datation entomologique se base sur l'âge des premiers insectes pouvant coloniser le cadavre (Calliphoridae et/ou Sarcophagidae); on peut aussi parler d'espèces pionnières (Lefèbvre et Gaudry, 2009). On utilise cette méthode pour établir un intervalle postmortem «court», de quelques jours à quelques semaines pour autant qu'une seule génération d'insectes ait colonisé le corps. En raison de sa précision, c'est la méthode de datation entomologique la plus couramment utilisée actuellement dans les expertises entomologiques médico-légales. Moins précise, la deuxième méthode permet la datation du décès à plus ou moins long terme, Elle se base sur la reconstitution des successions entomologiques pour déterminer l'IPM. C'est le cas lorsque plusieurs générations d'insectes ont colonisé le corps. Si tous les insectes de l'écosystème-cadavre n'ont pas la même importance du point vue forensique, tous ont néanmoins un rôle écologique non négligeable. Une famille de Coléoptères semble avoir un intérêt forensique plus prononcé que les autres: les Silphidae (*carrion beetles*) et tout particulièrement les Silphinae (Watson et Carlton 2005, Matuszewski *et al.* 2010) avec les genres *Silpha*, *Thanatophilus* et *Necrodes* (Midgley *et al.* 2010, Matuszewski *et al.* 2010, Bonacci *et al.* 2010). En effet, bien qu'abondamment étudiés en raison de leurs particularités éthologiques, les Nicrophorinae présentent un intérêt limité en entomologie forensique (Watson et Carlton 2005). Parmi le genre *Thanatophilus*, Matuszewski et ses collègues (Matuszewski *et al.* 2010) soulignent l'importance des espèces *T. rugosus* et *sinuatus* aux stades juvéniles et adultes en tant que bioindicateurs potentiels utiles en entomologie forensique. Il a d'ailleurs été récemment rapporté que ces deux espèces peuvent, sous certaines conditions particulières, complètement remplacer les asticots de Calliphoridae et participer ainsi activement à la dégradation biologique de la dépouille (Bonacci *et al.* 2010). Une autre espèce, remarquée du point de vue forensique, aussi bien pour les stades immatures et adultes, est *Necrodes littoralis*; seule espèce du genre *Necrodes* en Europe. On peut également relever l'utilité forensique de l'espèce *Oiceoptoma thoracica*, mais uniquement en ce qui concerne les stades immatures (Matuszewski *et al.* 2010). En Belgique, cette espèce est inféodée aux biotopes forestiers. En plus d'être un candidat bioindicateur potentiel de datation de la mort, cette espèce pourrait également être utilisée comme bioindicateur de lieu. Il faut néanmoins relever le fait que nos études de suivis entomologiques postmortem sur carcasses de porc se sont concentrées sur les

stades adultes et n'ont donc pas pris en compte l'apparition et la présence des stades immatures de ces mêmes coléoptères. Du point de vue forensique, il serait opportun d'inclure des données de présence/absence des stades juvéniles dans des études ultérieures. Cependant l'identification taxonomique des stades immatures est plus difficile que celle des imagos. On pourrait néanmoins envisager d'utiliser des techniques d'identifications moléculaires pour confirmer l'identification spécifique des larves récoltées comme c'est déjà le cas pour certains Diptères forensiques (Debry *et al.* 2012, Samarakoon *et al.* 2012). Une des objections fréquentes à l'utilisation des Coléoptères en entomologie forensique provient du fait que les espèces pionnières telles que les Calliphoridae peuvent localiser et coloniser un cadavre beaucoup plus rapidement que les espèces plus tardives dont font partie la majorité des Coléoptères de l'écosystème cadavre (Midgley et Villet 2009). Ce délai de colonisation postmortem, existant pour la plupart des espèces de Coléoptères, implique que l'estimation de l'IPM minimum est beaucoup moins précise que celui calculé grâce aux espèces de Diptères d'intérêt forensique (Midgley et Villet 2009). Cependant, des études récentes (Midgley et Villet 2009, Midgley *et al.* 2010) montrent que certains Coléoptères de la famille des Silphidae, et notamment *Thanatophilus micans*, peuvent très rapidement localiser un corps (endéans les 24h). Leurs larves sont observées peu après sur le cadavre. Cette observation les rapproche de certains Diptères Calliphoridae et ceux-ci pourraient donc être utilisés comme des indicateurs postmortem fiables et précis (Midgley et Villet 2009) pour autant que l'on puisse connaitre leur cycle de développement biologique sur le cadavre. Cependant, contrairement à l'espèce de Silphinae *T. micans* que l'on retrouve en Afrique, aucune espèce de Silphinae européenne, et a fortiori belge, n'est présente aussi rapidement sur un organisme animal en décomposition, si ce n'est par le fruit du hasard. En effet, la majorité des Coléoptères, y compris les Silphidae, apparaissent plus tardivement sur le cadavre. Ce délai de colonisation postmortem est variable et pose problème dans l'estimation du délai postmortem ou IPM. Pour pallier à cette problématique, il faudrait pouvoir modéliser l'arrivée des espèces de Coléoptères d'intérêt forensique sur le cadavre et ainsi estimer la durée de cette «fenêtre temporelle» (Midgley *et al.* 2010, Matuszewski 2011). Matuszewski parle d'intervalle de pré-apparition (PAI ou *pre-appearance interval*) et suggère de diviser l'intervalle postmortem en deux sous-intervalles distincts (Matuszewski 2011). La figure 46 illustre le concept d'IPA et la division de l'IPM en deux sous-intervalles: l'intervalle de pré-apparition et, en fonction du stade de développement des insectes collectés sur le corps, l'intervalle de présence pour les insectes matures ou l'intervalle de développement pour les stades immatures (Matuszewski 2011).

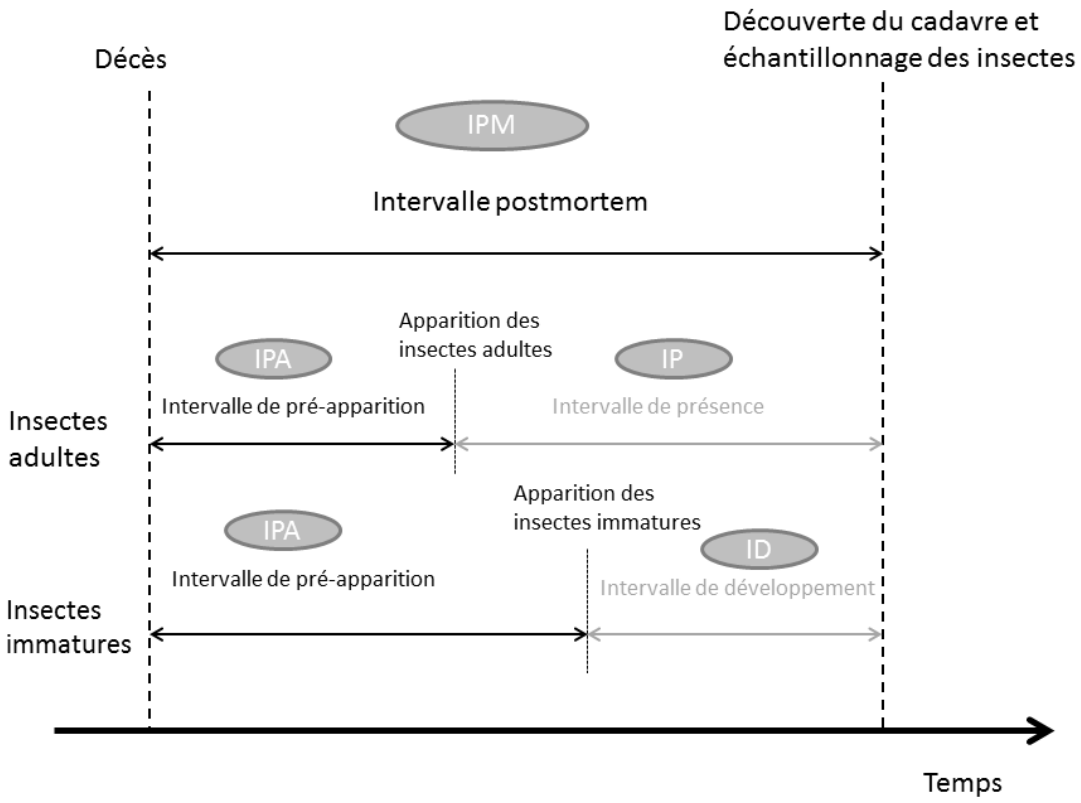

Figure 46. Division de l'intervalle postmortem (IPM) par la méthode entomologique en intervalle de pré-apparition (IPA) et intervalle de présence (IP) pour les spécimens adultes collectés sur le corps et en intervalle de développement pour les spécimens immatures (ID). Adapté de (Matuszewski 2011).

Pour certains taxons, cet intervalle de pré-apparition peut être estimé à partir de la température moyenne journalière environnante (Matuszewski 2011, 2012). Matuszewski a récemment modélisé ces intervalles de pré-apparition pour deux espèces d'intérêt forensique: une espèce de Silphidae (*Necrodes littoralis*) (Matuszewski 2011) et une espèce de Staphylinidae (*Creophilus maxillosus*) (Matuszewski 2012). L'utilisation en tant que bioindicateur de *Necrodes littoralis* en entomologie forensique tend à être corroborée par plusieurs publications récentes (Matuszewski *et al.* 2008, 2010, Matuszewski 2011). *N. littoralis* est très fréquemment et abondamment retrouvé au cours des études entomoforensiques (Matuszewski *et al.* 2008, 2010, Matuszewski 2011) se déroulant en milieux peu anthropisés et peut même dans certains cas remplacer les masses larvaires de Calliphoridae à certains stades de décomposition, notamment au cours de la phase de décomposition active du corps (Matuszewski 2011). En effet, les Silphinae peuvent parfois être en compétition avec les Diptères pour la ressource alimentaire constituée par le cadavre ou la carcasse animale (Payne 1965, Anderson 1982, Bonacci *et al.* 2010). La figure 47 illustre l'activité larvaire de *N. littoralis* sur une carcasse de porc formant, à l'instar des Diptères Calliphoridae, de véritables masses larvaires pouvant réunir des centaines d'individus.

Figure 47. Larves de *Necrodes littoralis* sur une carcasse de porc (photographies J. Dekeirsschieter).

Estimer la durée de l'intervalle de pré-apparition n'est cependant pas suffisant pour en faire des bioindicateurs utiles en entomologie forensique. A l'instar des modèles de développement existant pour les Calliphoridae (par exemple, les modèles d'ADJ), il faudrait également mieux connaitre la biologie des espèces de Silphinae ayant un potentiel d'utilisation en entomologie forensique. Cependant, il faut admettre que les connaissances relatives à la biologie et à l'écologie des Silphinae sont encore très limitées (Ratcliffe 1996, Hoback *et al.* 2004, Ikeda *et al.* 2007). Plusieurs équipes de chercheurs ont récemment travaillé sur la biologie de certaines espèces de Silphinae d'intérêt forensique pour leurs zones biogéoclimatiques. Citons les travaux des sud-africains John Midgley et Martin Villet sur *Thanatophilus micans* (Midgley et Villet 2009). Cette équipe a établi des modèles de développement à températures constantes pour *T. micans*. De même qu'une équipe hispano-vénézuélienne a étudié le cycle de vie du Silphidae néo-tropicale *Oxelytrum discicolle* à trois températures fixées (Velasquez et Viloria 2009, 2010). Aucune information concernant la biologie, et a fortiori les cycles de

développement de Silphinae européens, n'est disponible dans la littérature. En raison de leur importance au sein de micro-habitat constitué par le cadavre dans certaines régions biogéographiques d'Europe occidentale, les cycles de développement de *Necrodes littoralis* et de *Thanatophilus sinuatus* ont été étudiés à deux températures constantes (18 et 23 °C). Ces modèles de croissance doivent être pris comme des résultats préliminaires permettant d'évaluer la durée du cycle biologique de ces deux espèces. En effet, ces modèles de développement ont été établis sur base de données collectées en conditions contrôlées de laboratoire et n'ont pas été validés par des expérimentations *in situ* ou en conditions semi- ou non contrôlées. Si l'utilisation de certaines espèces de Silphinae en entomologie forensique semble être pertinente, l'utilisation des Staphylinidae est quant à elle plus anecdotique. Délaissée par les entomologistes forensiques, principalement pour des raisons de difficultés d'identifications taxonomiques (Wyss et Chérix 2006), cette grande famille de Coléoptères présente peu d'intérêt en tant que bioindicateur du délai postmortem, à l'exception d'une espèce déjà citée précédemment: *Creophilus maxillosus*. Cette espèce est souvent récoltée sur les cadavres (Matuszewski *et al.* 2008, Ozdemir et Sert 2009, Matuszewski *et al.* 2010, Matuszewski 2012); on la retrouve principalement au cours des saisons printanières et estivales. Parmi les 62 espèces de staphylins recensées au cours de nos suivis de colonisation postmortem, cette seule espèce représente plus d'un tiers des captures de staphylins réalisées sur des carcasses de porc. Cependant, si ceux-ci présentent peu d'intérêt au niveau de la datation de la mort, leur présence peut, dans certains cas, fournir des indications quant au biotope. En effet, la majorité des espèces de Staphylinidae recensées au cours de cette étude de suivi saisonnier sont des espèces typiquement forestières. L'ensemble de la communauté de Staphylinidae peut donc donner des indications sur le lieu de la découverte du corps et/ ou sur des manipulations du corps postmortem (par exemple, un déplacement du cadavre). Ce n'est pas telle ou telle espèce de staphylins prise isolément qui permet de tirer des conclusions, mais bien l'ensemble des Staphylinidae récoltés qui permet de dégager des préférences écologiques et de faire converger un faisceau d'indices biologiques.

2) Les odeurs cadavériques émises par un corps en décomposition:

Le cadavre, en se décomposant, va attirer les insectes nécrophages et d'une manière plus générale les insectes sarcosaprophages. C'est plus précisément les odeurs émises par le cadavre (et/ou ses hôtes) au cours de sa décomposition qui seraient responsables de cette colonisation entomologique (Anderson 2001, Hart et Whitaker 2005, Statheropoulos *et al.* 2005, Dekeirsschieter *et al.* 2009, LeBlanc et Logan 2010). En effet, le cadavre, en se

décomposant à l'air libre, va émettre dans l'environnement des odeurs tantôt attractives pour certaines espèces nécrophages et tantôt répulsives pour ces mêmes espèces (Leclercq 1978, Anderson 2001). Ces odeurs cadavériques sont composées d'un grand nombre de molécules chimiques encore appelées composés organiques volatils cadavériques (COVs). Ces sémiochimiques peuvent rapidement être perçus par les insectes nécrophages grâce à leur système olfactif hautement sensible aux effluves cadavériques alors qu'aucune odeur de décomposition n'est perceptible à l'odorat humain (Statheropoulos *et al.* 2005, LeBlanc et Logan 2010). Caractériser «l'odeur de la mort» est une étape cruciale de l'approche chémo-écologique de l'écosystème cadavre, car c'est grâce à cette étape d'analyse des molécules volatiles que l'on peut mieux comprendre les interactions cadavre-entomofaune. Les molécules cadavériques sont à la base des mécanismes d'attraction des insectes nécrophages sur le cadavre. Ces insectes sont capables d'extraire du bruit de fond environnant certaines molécules ou un cocktail de molécules leur indiquant un site propice à leur survie et parfois même à des distances très éloignées de l'émetteur, c'est-à-dire du cadavre ou de la carcasse animale.

Afin d'identifier la signature olfactive de la décomposition animale, il faut pouvoir prélever ces molécules volatiles et les identifier. Plusieurs techniques ont été utilisées au cours de ce travail de recherche. En raison de nombreuses similarités physiologiques avec l'homme, le cochon domestique a servi de substitut à la décomposition humaine pour les études de suivis postmortem entomologiques, de même que pour les études relatives aux prélèvements de composés organiques volatils cadavériques. Pour des raisons éthiques et sanitaires, il est interdit de travailler sur du matériel d'origine humaine en Belgique de même que dans le reste de l'espace européen. Néanmoins, une question se pose à l'heure actuelle, s'il ne fait aucun doute que l'entomofaune colonisant les corps humains est la même que celles pouvant infester les dépouilles animales tel que le cochon (Schoenly *et al.* 2007), l'odeur de la décomposition d'un corps humain est-elle identique à celle d'une carcasse de porc? Il semblerait qu'un petit nombre de composés soient spécifiques aux espèces animales et diffèreraient donc d'une espèce à l'autre (DeGreef et Furton 2011). Selon une récente étude, deux composés sont spécifiques à la décomposition humaine: le styrène et l'acide benzoïque méthylester (DeGreef et Furton 2011). Ces deux composés n'ont jamais été retrouvés au sein de nos échantillons prélevés sur carcasse de porc. Néanmoins, la grande majorité des molécules volatiles cadavériques sont similaires quelle que soit l'espèce animale en décomposition bien qu'il existe aussi des dissimilarités (DeGreef et Furton 2011). Outre le modèle animal, ces

divergences peuvent s'expliquer par différents paramètres dont le plus évident est la grande variabilité des protocoles analytiques employés par les diverses équipes de recherche en thanatochimie. Par protocole analytique, il faut également inclure la méthode de prélèvements des COVs (techniques d'échantillonnage, types de matériaux adsorbants, techniques d'élution) ainsi que la méthode de séparation analytique employée (Prada *et al.* 2010, Packowski et Schütz 2011). Un autre paramètre pouvant influencer l'échantillonnage des COVs postmortem sont les facteurs abiotiques tels que la température et l'humidité (Packowski et Schütz 2011) qui sont, quant à eux, tributaires du biotope, une autre source potentielle de variabilité. Nos études sur les composés volatils cadavériques ont également permis de mettre en évidence l'évolution de la signature olfactive d'un corps en décomposition en fonction de la progression du processus de décomposition (Dekeirsschieter *et al.* 2009, 2012). Jusqu'à très récemment, la technique de choix utilisée pour l'analyse des prélèvements d'odeurs cadavériques était la chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS) (Vass *et al.* 2004, Statheropoulos *et al.* 2005, 2006, 2007, Vass *et al.* 2008, Dekeirsschieter *et al.* 2009, Hoffman *et al.* 2009). Cependant, en raison des profils d'émissions de volatils complexes obtenus, il s'avère que l'utilisation d'une technique d'analyse plus complexe soit plus pertinente à utiliser dans les études futures concernant les composés volatils forensiques. La chromatographie bidimensionnelle peut pallier aux limitations existant en GC classique et plus précisément la chromatographie bidimensionnelle couplée à un détecteur de masse à temps de vol (GCxGC-TOFMS). Si on compare les deux méthodes d'analyses chromatographiques employées au cours de cette recherche, le résultat est sans appel et penche nettement en faveur de la GC bidimensionnelle (Dekeirsschieter *et al.* 2009, 2012). En effet, bien que les techniques d'échantillonnages des COVs soient différentes pour les deux études, une centaine de composés volatils postmortem ont été identifiés par chromatographie conventionnelle (104 COVs (Dekeirsschieter *et al.* 2009) contre huit fois plus avec la chromatographie compréhensive (830 COVs (Dekeirsschieter *et al.* 2012) et environ dix fois plus si on compare les mêmes biotopes entre eux (85 COVs cadavériques spécifiques au biotope forestier avec la GC-qMS). Un autre fait important à souligner est l'absence de détection de COVs cadavériques spécifiques avec la GC-qMS au début du processus de décomposition, alors que des COVs spécifiques à la décomposition sont identifiés avec la GC-TOFMS dès le premier jour de prélèvements de COVs postmortem. La GCxGC a aussi mis en évidence la détection d'un composé non spécifique à la décomposition dans les échantillons de composés volatils: la dothiépine. L'origine de cet antidépresseur

tricyclique provient probablement d'une administration antemortem par le vétérinaire en charge de l'euthanasie des cochons.

3) Les COVs cadavériques possédant une activité biologique

Connaitre la composition des odeurs cadavériques est un prérequis nécessaire à la compréhension des interactions entre le cadavre et l'entomofaune qui y est associée. Néanmoins, la connaissance de la signature olfactive d'un corps en décomposition ne permet pas de discriminer quels sont les composés odorants réellement perçus par l'insecte et qui vont l'attirer sur ou près du cadavre. L'approche électrophysiologique permet d'identifier les composés odorants détectés par les récepteurs olfactifs antennaires en réponse à un stimulus olfactif, en l'occurrence des composés organiques volatils identifiés comme faisant partie de la signature olfactive d'un corps en décomposition. Déjà explorée chez plusieurs espèces de Diptères d'intérêt forensique tels que *Lucilia sericata* (Frederickx *et al.* 2012), l'électroantennographie n'avait encore jamais été réalisée avec des Silphinae vivants appartenant à l'espèce *Thanatophilus sinuatus*. Les huit composés cadavériques, sélectionnés en raison de leur importance au sein de l'écosystème cadavre, ont été testés en EAG sur des mâles et des femelles (Dekeirsschieter *et al. in press*). Ceux-ci ont tous induits des dépolarisations antennaires quelques soit la dose testée (de 1 ng à 10 mg) et sont également perçus différemment, à l'exception de la plus petite dose testée de 1 ng. Il ressort de cette étude que le DMDS et le 1-butanol induisent les plus grandes réponses antennaires aux doses les plus élevées qui s'échelonnent de 1 µg à 10 mg. Néanmoins, il faut signaler que ces fortes doses sont peu probables en situation réelle. Tandis qu'aux doses intermédiaires testées, seule le DMDS induit les plus grandes réponses antennaires, à l'exception de la dose de 0,01 µg où le phénol entraine les plus grandes dépolarisations. Une étude récente suggère que les Silphidae et notamment deux Nicrophorinae, *N. vespillo* et *N. vespilloides*, possèdent des récepteurs spécifiques aux molécules organosoufrées sur les antennes (Kalinova *et al.* 2009) ce qui semble également être le cas pour *Thanatophilus sinuatus*. Si on compare nos résultats électroantennographiques avec ceux obtenus avec les mêmes composés cadavériques testés sur une espèce qui arrive précocement au sein de l'écosystème-cadavre, *Lucilia sericata* (Diptère, Calliphoridae) (Frederickx *et al.* 2012), on remarque que c'est également le 1-butanol et le DMDS qui induisent les plus grandes dépolarisations électriques aux doses testées de 1 mg et 10 mg. Comme pour *T. sinuatus*, *L. sericata* est plus sensible au DMDS aux doses intermédiaires qu'aux autres composés testés (Frederickx *et al.* 2012). Si l'approche électrophysiologique permet d'obtenir des informations sur la perception des

odeurs par l'insecte, elle ne nous permet pas d'identifier les composés odorants qui interagissent sur le comportement de l'insecte étudié. Des essais comportementaux ont donc été réalisés en parallèle aux tests EAGs, le stimulus étudié étant de nature olfactive, ces essais comportementaux ont été réalisés grâce à des olfactomètres adaptés au comportement de *Thanatophilus sinuatus*. Les essais comportementaux sur *T. sinuatus* ont montré que le DMDS était attractif aussi bien pour les mâles que pour les femelles. Cette molécule est aussi réputée attractive pour d'autres espèces d'insecte que l'on peut retrouver au sein de l'écosystème cadavre. Citons notamment *Lucilia sericata* (Cragg et Thurston 1950, Park et Cork 1999, Archer et Elgar 2003, Frederickx *et al.* 2012), *Musca domestica* (Brown *et al.* 1961, Frishman et Matthyse 1966, Mulla *et al.* 1977, Cossé et Baker 1996) et un Silphidae, *N. vespillo* (Kalinova *et al.* 2009). Au cours de nos essais olfactométriques, le *p*-crésol à la dose de 100 ng avaient un rôle attractif pour les mâles *T. sinuatus*. Le *p*-crésol ou 4-methylphénol est connu pour être attractif pour différentes espèces de Diptères telles que les moustiques (Du et Millar 1999, Jeanbourquin et Guerin 2007) ou encore les glossines ou mouches tsé-tsé (Cossé et Baker 1996). Une autre hypothèse avancée est que ce composé, déjà connu comme étant une phéromone sexuelle émise par les femelles du Coléoptère *Phyllophaga cuyabana* (Zarbin *et al.* 2007), pourrait également être une phéromone sexuelle des femelles de *T. sinuatus*. Ce qui expliquerait que ce composé ne joue aucun rôle attractif pour les femelles de *Thanatophilus sinuatus* mais seulement pour les mâles de cette même espèce. Cependant, aucune étude n'a été à ce jour effectuée pour étudier les sémiochimiques potentiellement émis par *T. sinuatus*. Des études sur les phéromones sexuelles de cette espèce sont à envisager. La seule molécule identifiée comme ayant un rôle répulsif est la putrescine à la dose testée de 1 µg, mais uniquement pour les mâles. Les cinq autres composés testés en olfactométrie n'ont induit aucun comportement particulier chez *T. sinuatus*. Alors que le 1-butanol était attractif pour *L. sericata* (Frederickx *et al.* 2012), ce composé n'a induit aucune attractivité sur *T. sinuatus*.

Très peu d'études ont été réalisées en abordant le cadavre et ses hôtes sous l'aspect de l'écologie chimique. Les résultats obtenus au cours de ce travail de recherche permettent de mieux caractériser l'écosystème cadavre et les interactions existant entre celui-ci et une espèce particulière de Silphidae: *Thanatophilus sinuatus*. Cependant, nos résultats comportementaux, basés sur des analyses statistiques, sont des résultats exploratoires. Des études complémentaires sont donc à envisager afin de valider certaines de nos hypothèses. Néanmoins, l'étude de l'écosystème cadavre sous l'angle de l'écologie chimique permet

d'ouvrir de nombreuses perspectives en entomologie forensique et de façon plus globale en sciences forensiques, si on inclut la partie thanatochimie avec le domaine des COVs cadavériques. En effet, la compréhension du rôle des odeurs cadavériques sur le comportement des insectes nécrophages permet d'envisager de nouveaux développements en sciences forensiques et dans la localisation des corps en exploitant l'olfaction des insectes (LeBlanc et Logan 2010, Frederickx *et al.* 2011, Paczkowski *et al.* 2011) comme biosenseurs ou biodétecteurs (Frederickx *et al.* 2011, Paczkowski *et al.* 2011).

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Partie V: Conclusions générales et perspectives

«Comme la lumière, l'odeur a ses rayons x. Que la Science, instruite par la bête, nous dote un jour du radiographe des odeurs, et ce nez articficiel nous ouvrira tout un monde de merveilles.»

Jean-Henri Fabre, 1900, Souvenirs entomologiques, VIIème Série, chapitre 25.

Les études faunistiques de suivis postmortem ont permis de caractériser une portion de la nécrofaune belge peu étudiée du point de vue forensique: les staphylins (Col., Staphylinidae) et les silphes (Col., Silphidae), ces deux taxons font partie des Staphylinoidea. Un grand nombre d'espèces de Staphylinidae (62 espèces) ont été recensées au sein de l'écosystème cadavre. Cette population de staphylins, majoritairement inféodés aux milieux forestiers, fluctue au sein de l'écosystème cadavre en fonction des saisons. Cependant parmi toutes ces espèces, seule *Creophilus maxillosus* semble avoir un intérêt potentiel en entomologie forensique à l'heure actuelle. Concernant les Silphidae, neuf espèces de Silphinae et de Nicrophorinae ont été identifiées au cours des suivis de colonisation postmortem sur carcasses de porc. Bien que fréquemment collectés au sein de l'écosystème cadavre, les Nicrophorinae présentent peu d'intérêt d'un point vue forensique ce qui n'est pas le cas des Silphinae et plus particulièrement, pour les espèces de *Thanatophilus* spp. et *Necrodes littoralis*. Parmi les trois espèces du genre *Thanatophilu*s recensées dans la faune belge, *Thanatophilus sinuatus* semble être un meilleur candidat bioindicateur que les autres en raison de son abondance au sein de l'écosystème cadavre et plus précisément au début de la saison de reproduction (au printemps); cette observation a également été faite par une équipe italienne (Bonacci *et al*. 2010). Néanmoins, *Necrodes littoralis* semble être un bien meilleur bioindicateur en raison de sa plus longue période d'activité qui s'étale du printemps à la fin de l'été. C'est l'espèce majoritaire collectée sur nos carcasses de porc situées dans la forêt de Meerdaal. Il a d'ailleurs été observé sur le terrain que les larves de *Necrodes littoralis* pouvaient coloniser un corps de la même manière que des masses larvaires de Calliphoridae le feraient. Ces études faunistiques nous ont permis de mieux caractériser les populations de staphylins et de Silphidae qui fréquentent l'écosystème cadavre et ainsi de pouvoir sélectionner des candidats bioindicateurs potentiellement utilisables en entomologie forensique. Cependant, d'autres études de terrain restent à envisager. Etudes dans lesquelles il serait souhaitable d'inclure des données sur les stades immatures appartenant à ces insectes, nos études de suivis postmortem n'étant focalisées que sur les imagos. Il faudrait aussi multiplier les biotopes expérimentaux étudiés et différentes situations qui reproduisent des scènes de crime. En effet, que se passerait-il si ces insectes avaient face à eux, non pas un corps directement accessible, mais un corps avec des entraves à leur colonisation? Citons par exemple, le cas de corps emballés (plastique, tapis, couverture, *etc.*), de corps calcinés ou partiellement brulés ou encore tout simplement avec la présence d'habits. *Quid* de la présence de substances répulsives sur le corps? Est-ce que, dans ce type de situations, les Silphidae seraient encore capable de coloniser le cadavre? Très probablement, mais peut-être pas de la même manière. Le corps, en

se décomposant, émettra toujours des odeurs, signalant par la même occasion sa présence aux insectes nécrophages. En effet, les effluves cadavériques attirent une large gamme d'insectes au niveau du cadavre. Cependant, l'étude de l'écosystème-cadavre sous l'angle de l'écologie chimie est une approche très récente. Afin de mieux comprendre les interactions existant entre le cadavre et les insectes le colonisant et plus particulièrement les Silphidae, il faut identifier ces composés odorants cadavériques. Plusieurs techniques de prélèvements d'odeurs (passives et dynamiques) ont été utilisées au cours de ce travail de recherche ainsi que différentes méthodes analytiques. La TD-GC-qMS et le système d'échantillonnage Radiello a permis d'identifier une petite centaine de composés organiques volatils (104 COVs) et de mettre en évidence une évolution temporelle des COVs cadavériques au cours du processus de décomposition. Tandis que la technique plus complexe de GCxGC-TOFMS a permis d'identifier 830 composés cadavériques. C'est la première fois que la chromatographie bidimensionnelle a été utilisée pour caractériser la signature olfactive d'un corps en décomposition à l'air libre. En raison de la complexité des échantillons de molécules volatiles cadavériques obtenus, l'utilisation de la chromatographie compréhensive semble être plus pertinente que la GC conventionnelle qui était jusqu'à présent la technique de choix pour ce type d'analyses. Outre les composés spécifiques à la décomposition animale, la GCxGC a également permis de détecter d'autres composés non spécifiques au cadavre telle que la dothiépine, un antidépresseur. Cette découverte ouvre de nouvelles perspectives en science forensique et plus particulièrement en toxicologie forensique.

Néanmoins une question reste en suspens, l'odeur d'une carcasse animale, et tout particulièrement celle du cochon domestique, est-elle la même que celle d'un corps humain en décomposition? Comme le suggère Swann et al. (Swann *et al*. 2010), il serait intéressant de comparer, dans les mêmes situations et avec les mêmes protocoles analytiques, l'odeur d'un corps humain en décomposition avec celles d'autres modèles animaux tel que le cochon, fréquemment utilisé comme substitut de la décomposition humaine. Une étude récente précise qu'il existe des différences en termes de composés volatils organiques postmortem entre l'homme et d'autres carcasses animales (DeGreef et Furton 2011). Il faudrait dans un avenir proche envisager de réaliser des prélèvements d'odeurs sur du matériel d'origine humaine, à défaut de pouvoir travailler sur des corps entiers *in situ*, afin de comparer rigoureusement la signature olfactive d'un cadavre et d'une carcasse de porc. Les recherches en thanatochimie doivent être poursuivies; les composés organiques volatils qui caractérisent la décomposition de matière organique animale ouvrent de larges perspectives dans le domaine des sciences

forensiques, notamment dans l'utilisation des COVs comme marqueurs de l'intervalle postmortem (Vass 2001, Statheropoulos *et al.* 2007; LeBlanc & Logan 2010, Stefanuto 2011). Il faudrait aussi pouvoir mettre au point et valider des techniques qui permettront la quantification des COVs cadavériques prélevés car à ce jour très peu d'informations strictement quantitatives existent.

Concernant les études électrophysiologiques et comportementales sur les Silphinae, le DMDS (le diméthyldisulfure) a été identifié comme étant une substance attractive pour les deux sexes de l'espèce *Thanatophilus sinuatus*. Tandis que seul le *p*-crésol était attractif pour les mâles de cette même espèce. Cette molécule pourrait jouer un rôle dans les relations intraspécifiques de *T. sinuatus* en intervenant comme phéromone sexuelle femelle. Des études complémentaires relatives aux émissions de substances phéromonales de *T. sinuatus* seraient à envisager. Comme suggéré par l'équipe tchèque de Kalinova *et al.* pour les *Nicrophorus* spp. (Kalinova *et al*. 2009), il serait également opportun de compléter nos études exploratoires avec des études de terrain en vue de valider le rôle biologique du DMDS sur *T. sinuatus* en réalisant des essais de piégeages avec des substances odorantes tels que les COVs soufrés et plus particulièrement avec le diméthyldisulfure. Compte tenu du grand nombre de composés émis au cours du processus de décomposition animale, une démarche plus avancée en électrophysiologie serait également bénéfique pour une meilleure compréhension des relations «cadavre-insecte» en étudiant des mélanges complexes de molécules cadavériques. Citons par exemple la mise en place du couplage EAG avec la chromatographie en phase gazeuse (GC-EAD), déjà réalisée sur une espèce d'intérêt forensique: *Calliphora vomitoria* (LeBlanc 2008) et de nombreux autres insectes. Il serait également très innovant de réaliser le couplage GC-EAD avec un spectromètre de masse (GCMS-EAD) afin de pouvoir caractériser simultanément les réponses biologiques de l'insecte étudié avec la structure chimique des molécules cadavériques perçues par l'insecte. Le développement d'appareillages portatifs utilisables *in situ* permettrait également de faire progresser les connaissances de l'écosystème-cadavre en échantillonnant et en analysant directement sur place les émissions cadavériques.

Bien que l'étude des COVs cadavériques semble prometteuse dans l'estimation du délai postmortem, les insectes restent une des seules méthodes fiables pour déterminer l'intervalle postmortem lorsque les méthodes médicales font défaut (LeBlanc & Logan, 2010). Malgré tout, l'étude conjointe des odeurs cadavériques (thanatochimie) et des insectes nécrophages (entomologie forensique) doit aller plus loin afin de déterminer les liens spécifiques qui

existent entre la datation de la mort par des méthodes entomologiques et les processus de décomposition (LeBlanc & Logan, 2010).

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Partie VI: Liste des publications, communications orales et posters

«*Notre language ne vaut rien pour décrire le monde des odeurs…*»

Patrick Süskind, Le parfum.

«*Qui maitrisait les odeurs, maitrisait le cœur des hommes.*»

Patrick Süskind, Le parfum.

1. Publications

1.1. En tant que 1er auteur

Dekeirsschieter J., Verheggen F.J., Gohy M., Hubrecht F., Bourguignon L., Lognay G., Haubruge E. (2009). Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes. *Forensic Science International* 189: 46-53.

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decaying pig carcass in a forest biotope. *Accepted for publication in Journal of Forensic Sciences*.

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Dekeirsschieter J.R.,Verheggen F.J., Frederickx C., Boxho P., Haubruge E. Forensic entomology investigations from Doctor Marcel Leclercq (1924 -2008†): a review of cases from 1969 to 2005. *Accepted for publication in Journal of Medical Entomology*.

1.2. En tant que co-auteur

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Frederickx C. **Dekeirsschieter J.,** Verheggen F., Haubruge E. (2010). *Utilité des composés organiques volatils (COVs) émis par les Diptères nécrophages dans l'estimation de l'intervalle post mortem*. VIIème Congrès International Francophone d'Entomologie (CIFE 2010), Louvain la neuve, Belgique. 5-10 juillet 2010.

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3. Posters

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Table 26. Multiple comparisons of the average eags responses (±se) (n=20). for each dose, chemical compounds sharing * or ** induce similar eag responses as a result of newman and keuls test ($\alpha = 0.05$).

Table 27. Y-tube bio-assays (* and ** respectively indicate differences between control and voc at $p < 0.05$ and $p \leq 0.01$, respectively, with $\alpha = 0.05$