

***CONSERVATION OF AN ENDEMIC METALLOPHYTE SPECIES: EFFECT OF  
POPULATION HISTORY AND VEGETATIVE DENSITY ON THE REPRODUCTIVE  
SUCCESS OF VIOLA CALAMINARIA***

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## Abstract

Demographic studies that monitor population dynamics are an essential component in establishing conservation strategies. The conventional view that human disturbance results in negative effects to species and habitats is countered by the fact that some anthropogenic activities result in the origin of new habitat opportunities for species. Faced with an increase in European restoration programs, studies that assess the variability in traits conferring reproductive success among populations is particularly relevant to rare species conservation and further improves our knowledge to achieve restoration success. In the present study, we evaluated reproductive success variation (flower density, percent fructification and seed set) in *Viola calaminaria*, a rare endemic metallophyte, in relationship to population origins (ancestral or recent habitat), plant density and habitat structure. Results indicated that seed set varied significantly among ancestral and recently established populations, with recent populations exhibiting increased seed set ( $P < 0.05$ ). Habitat structure did not influence species reproductive success. A positive significant correlation was detected between vegetative and flower density ( $P < 0.001$ ). Results suggested that population origin (ancestral or recent) and local vegetative density was more important than habitat structure on reproductive success in *Viola calaminaria*. In addition, we demonstrated that *Viola calaminaria* populations distributed in habitats recently created by anthropogenic activity exhibited similar or higher reproductive success than populations from ancestral sites. These results are noteworthy as they show that anthropogenic activities can create new favourable habitats for some rare species.

Keywords: *Viola calaminaria*; Reproductive success; Plant density; Population history.

## Introduction

Reproductive fitness and demography are essential components of the population dynamics of all species. Characterisation of these attributes is particularly important in rare and endemic species conservation because they elucidate critical life cycle stages and facilitate the prediction of future population growth (Lande, 1988; Schemske et al., 1994; Oostermeijer et al., 2003). Consequently, direct conservation strategies can be developed and implemented. However, long-term studies assessing spatio-temporal variation of reproductive success do not adequately address the urgency of conservation measures for species facing short-term threats, particularly relevant to rare endemic species (Oostermeijer et al., 1996; Menges, 2000; Colas et al., 2001; Freville et al., 2004). Therefore, it is vital studies delimit the variability in traits conferring reproductive success among populations or habitats as a first step prior to modelling long-term species dynamics.

The conventional view is that human disturbance results in negative effects to species and habitats, resulting in habitat fragmentation and a reduction in reproductive success (Reed & Frankham, 2003; Reed, 2005; Leimu et al., 2006). However, some anthropogenic activities can result in the origin of new habitat opportunities for populations. Reproductive success may be expected to vary in relation to population history and habitat patch colonisation which affect reproductive success differently. In recently founded population, reproductive success can be reduced as a result of founder effect (Nei et al., 1975; Leimu & Mutikainen, 2005), but rapid growth of populations associated with high gene flow between populations can reduce the impacts of these effects (Leimu & Mutikainen, 2005). Furthermore, habitat quality in a recently established population may differ from an ancestral habitat and subsequently affect the reproductive success of rare endemic species (Brock et al., 2007; Quintana-Ascencio et al., 2007). Colonisation of new habitats by maladapted genotypes can lead to a reduction in the number of individuals until the population reaches extinction (Pulliam, 1988). Alternatively, dispersal from a source population by genotypes well adapted to the novel habitat could increase species selective advantage and persistence in the landscape (Blais & Lechowicz, 1989; Kingsolver et al., 2002; Sultan & Spencer, 2002) and rescue ancestral populations. In this dynamic landscape, long-term species survival may be dependent on a species ability to establish viable populations in newly created habitats (Cristofoli et al., in press). One ecological restoration aim is to recreate viable populations of special status species relative to reference populations (Lake, 2001; Robert et al., 2007; Menges, 2008). Restoration programs throughout the world (Young et al., 2005) and notably in Europe (Cristofoli & Mahy, in press) assess how reproductive success traits vary among population origins could help to determine restoration success, which is particularly relevant in rare species conservation. Any attempts to better understand population dynamics and improve our knowledge for restoration efforts (Esfeld et al., 2008) should include analysis of traits related to individual fitness.

Soils with elevated concentrations of heavy metals (metalliferous soils) represent harsh and restrictive phytotoxic habitats for plants (Antonovics et al., 1971; Ernst, 1990; Brown, 2001). As a result, and also because metalliferous sites are generally of small size and geographically isolated from each other, these sites often host rare, endemic taxa ecologically adapted to high heavy metal soil concentrations (Ernst, 1990; Brown, 2001; Wolf, 2001). Metalliferous sites are extremely important to biodiversity (Whiting et al., 2004) but are directly destroyed by mining activities and/or site remediation. On the other hand, industrial pollution (areal fallout or residual ore) has created new habitat patches for metallophyte species (Faucon et al., in press). In eastern Belgium and western Germany, metalliferous sites occur in calamine soils exhibiting high concentrations of zinc, cadmium and lead. One metallophyte hosted on calamine soil is *Viola calaminaria* (Gingins) Lej. (Violaceae) (Lambinon & Auquier, 1964; Duvigneaud, 1982), a species protected in Belgium and supported by a habitat targeted for biodiversity maintenance at the European scale of Annex 1 in Habitats directive 92/43/EEC of the European Community. From the end of 19th century to date, the *V. calaminaria* habitat network has exhibited dynamics resulting from industrial pollution and site destruction (Bizoux, 2006; Bizoux et al., 2004). Metallophyte species therefore provide suitable models to study the influence of species population history (recent versus historical populations) on the variability in spatial reproductive success. In a previous study (Bizoux et al., 2008), we found that *V. calaminaria* fitness (seed set and percent germination) was higher in recent populations than in ancestral populations, exclusive of population size. Nevertheless, studies of other reproductive success traits appeared essential for viability, particularly for perennial species where adult stages are more important for population survival and growth than recruitment (Silvertown et al., 1993; Colling & Matthies, 2006). In addition to the effects of population origins on fitness, plant density has a substantial influence on reproductive success (Harper, 1977; Lande, 1988; Begon et al., 1990; Roll et al., 1997). Individual reproductive success might decrease with increasing local density due to intraspecific competition for resources (e.g. Harper, 1977). The aim of the current study was to examine the influence of population history on reproductive success variation in *V. calaminaria*. More specifically, this study focused on the following questions: (1) can population origin lead to variation in reproductive success; (2) can the potential variation be explained by differences in *Viola calaminaria* vegetative density or habitat structure in newly established populations; and (3) what are the implications for species management and conservation?

## Materials and methods

### *Plant species and study sites*

*Viola calaminaria* (Gingins) Lej. (Violaceae) is a perennial pansy that reproduces primarily through seeds. Vegetative propagation by means of rhizomes is low and does not extend beyond 40cm from the parent plant (Bizoux & Mahy, 2007). Predominantly allogamous, the species flowers from April to November and is primarily visited by solitary bees, bumblebees and flies (Syrphidae) (personal observation). The species is distributed in eastern Belgium (northeast of the Liege Province), southern Netherlands (southern part of the Dutch Limburg) and western Germany (vicinity of Aachen) (Fig. 1). In Belgium, *V. calaminaria* is considered a rare calamine-endemic (Lambinon et al., 2004) with 23 documented populations (range of population size: 0.4-32,242m<sup>2</sup>) (Bizoux et al., 2004). Of these, two origins of the populations have been ascertained, one ancestral and one recent. Ancestral populations occur on natural calamine sites or on historic mines (dating back as far as the Roman Age; Lefebvre, pers. comm.), while recent populations occur on industrial polluted sites. We performed a study of reproductive success in *V. calaminaria* from five Belgium populations: Theux (T); Schmalgraf (S); Plombières (PI); Prayon (P); and Streupas (St) representing three ancestral (T, S and P1) and two recent (P and St) populations (Table 1).

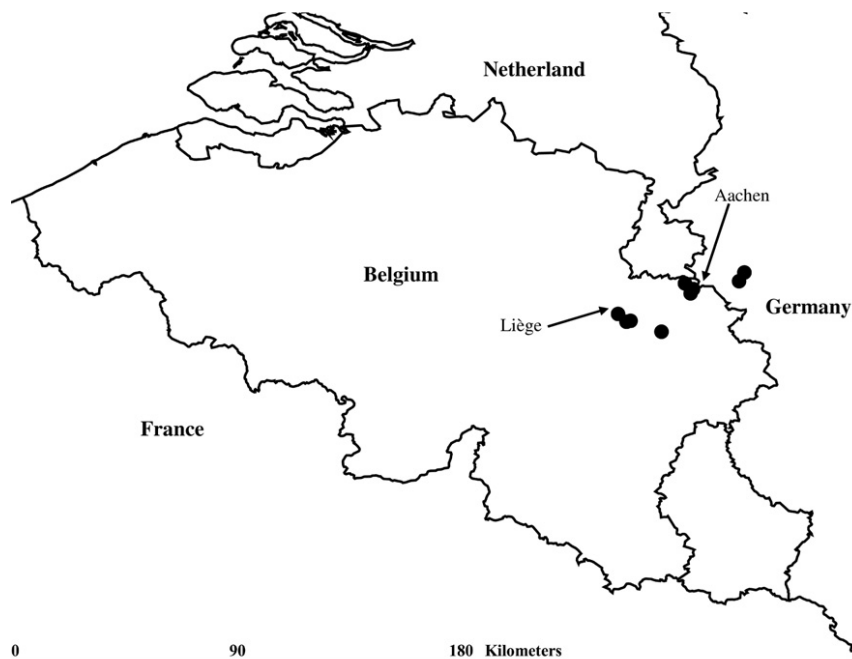


Fig. 1. Distribution of *Viola calaminaria*. Each point represents the area where *Viola calaminaria* populations are located.

Table 1. *Viola calaminaria* samples with localities, and origin. Longitude Latitude Origin

|                 | Longitude | Latitude   | Origin    |
|-----------------|-----------|------------|-----------|
| Theux (T)       | 5°49.79 E | 50°31.41 N | Ancestral |
| Schmalgraf (S)  | 5°59.6 E  | 50°41.64 N | Ancestral |
| Plombières (Pl) | 5°58.07 E | 50°44.04 N | Ancestral |
| Prayon (P)      | 5°40.28 E | 50°35.12 N | Recent    |
| Streupas (St)   | 5°35.95 E | 50°36.37 N | Recent    |

### *Habitat structure characterization and variation in reproductive Success*

In June 2004, habitat types for each of the five populations were a priori identified on the field based on vegetative physiognomy and dominant species. Three different habitat types were chosen in Theux and two in each of the other study sites and were delimited in the field. In each of the different habitat types (T1, T2, T3; S1, S2; Pl1, Pl2; St1, St2; and P1, P2) two transects (transect length were proportional to the habitat types area within populations) were established. The vegetation structure was characterised by estimating linear cover of seven vegetation layers along each transect: trees (% tree); shrubs (% shrub); herbaceous plants higher than 30cm (% herb > 30); herbaceous plants lower than 30cm (% herb < 30); lichens (% lichen); mosses (% moss); and bare ground (% bare ground). In each transect, *V. calaminaria* cover was estimated every metre in 50cm×50cmquadrats by the point contact method (Kent & Coker, 1992) in June (peak of vegetative growth). *V. calaminaria* cover was used as a vegetative density measure. Four quadrats were then permanently marked: two quadrats with high density of *V. calaminaria* (corresponding to quadrats with the highest density in transect); and two quadrats with low density (corresponding to quadrats with intermediate density in transect). Each month, from June to October 2004, flower density (the number of flowers in each quadrat) and percent fructification (number of fruits on a maximum of ten marked flowers per quadrat) was recorded. Fruit density (number of fruits per quadrat) was also estimated by multiplying the density of flowers by the percent fructification. In June and September, up to three mature fruits (from the ten marked flowers) were sampled in each quadrat and well-developed seeds were counted to determine mean seed set (mean number of seed/fruit within a quadrat).

## *Statistical analysis*

Principal Component Analysis (PCA) was performed to characterise transect-vegetation structure. Vegetation structure (linear cover of seven vegetation layers along transects) variation was first analysed by a three-way MANOVA (fixed effect model) (habitat types nested within populations nested within origins) based on all vegetation layers and subsequent three-way ANOVA (habitat types nested within populations nested within origins) for each of the seven vegetation layers.

Pearson correlation coefficients between mean *V. calaminaria* transect density and transect coordinates along the first three PCA axes were used to test the possible relationship between vegetation structure and density of *V. calaminaria*.

Variation in vegetative and flower density, percent fructification and seed set in relation to dates (months), population origin, population locality, habitat types and transects were tested using mixed-crossed hierarchical ANOVA models. Subsequent Tukey's tests were used for pairwise mean comparisons. Vegetative and flower density were log-transformed in order to improve normality and homocedasticity.

Resource competition effects were tested on *V. calaminaria* density and flower density using a linear regression in June (peak of vegetative growth and flowering). The best linear regression model was determined by transformation of the dependant variable. Different dependant variable transformations were tested to improve residual normality and homocedasticity using Minitab macro (Palm, 2002). The form of relationship between vegetative and reproductive density enables the detection of eventual resource limitations on reproductive success. Similarly, the effect of flower density on percent fructification was also tested using Spearman correlations. Finally, the effect of vegetative density on fruit density and on the number of seed set per fruit was evaluated by Spearman correlations to test for competition effects on total seed production (only in June). All ANOVAs and correlation and regression analyses were performed with MINITAB ver. 15 and PCA with XLSTAT ver. 6.0.

## Results

### *Habitat structure characterisation and variation in vegetative density*

All habitat types were dominated by herbaceous plants less than 30cm in height (>50% cover), with the exception of T3 and S2 habitat types, which were dominated by % bare ground and % herb > 30 cover. The first three PCA axes explained 29%, 25% and 19% of the variation in vegetation structure (Fig. 2). The first axis was mainly positively correlated with % herb > 30 cover and negatively correlated with % bare ground and % moss cover. It expressed a gradient from open habitat in P2 and T3 (exhibiting lower scores on axis 1) to more closed habitat in S1, S2, St1, St2 and T2 (exhibiting higher scores on axis 1). The second axis was mainly positively correlated with % herb < 30, lichen and shrub cover and the third axis was positively correlated with % moss and negatively with % bare ground cover. Ancestral population locales represented more diversified habitat types than recent sites that appeared more homogeneous, but group structure that corresponded to population origin was not resolved in the PCA graph.

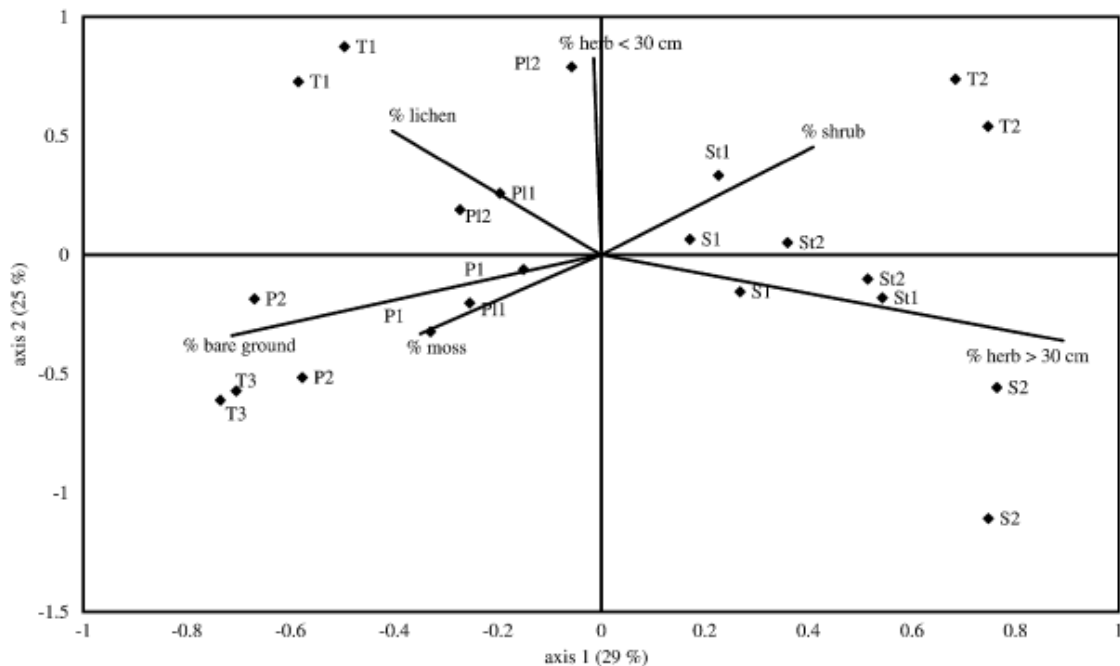


Fig. 2. Transect-vegetation structure biplot along PCA Axes 1 and 2 based on six vegetation layers and 11 habitat types identified in five populations of *Viola calaminaria* (T1, T2, T3, S1, S2, P1, P2, St1, St2, PI1, PI2). The six vegetation layers cover: shrub (% shrub); herbaceous over 30cm in height (% herb > 30); herbaceous less than 30cm in height (% herb < 30); lichen (% lichen); moss (% moss); and bare ground (% bare ground). Transects were identified by the first letter of the population name and the habitat type number.



In general, the three-way MANOVA based on all vegetation layers showed no significant difference in vegetation structure between different origins (Wilks' lambda = 0.243,  $P = 0.097$ ), but significant differences between all populations (Wilks' lambda = 0.00012,  $P < 0.001$ ) and between habitat types (Wilks' lambda = 0.00002,  $P < 0.001$ ) were detected. A three-way ANOVA found significant differences in vegetation structure between populations ( $P < 0.01$ ) and habitat types ( $P < 0.01$ ) for all vegetation layers with the exception of differences between habitat types for %moss cover. In contrast, no effect of population origin for vegetation structure was detected ( $P > 0.05$ ), with the exception of % shrub cover ( $P < 0.001$ ).

A correlation between mean *V. calaminaria* vegetative density and PCA transect scores (along the first three axes) based on vegetation structure was not detected.

**Table 2.** Comparison of spatial and temporal variation in *Viola calaminaria* vegetative density, flower density, percent fructification and seed set in five populations (hierarchical ANOVA). DF, F and P represent respectively the ANOVA degrees of freedom, F-Snedecor value and P-value.

| Source of variation               | Vegetative density |      |              | Flower density |       |                  | Percent fructification |      |                  | Seed set |      |              |
|-----------------------------------|--------------------|------|--------------|----------------|-------|------------------|------------------------|------|------------------|----------|------|--------------|
|                                   | DF                 | F    | P            | DF             | F     | P                | DF                     | F    | P                | DF       | F    | P            |
| Dates                             | -                  | -    | -            | 4              | 50.51 | <b>&lt;0.001</b> | 4                      | 7.92 | <b>&lt;0.001</b> | 1        | 0.7  | 0.402        |
| Origin                            | 1                  | 0.11 | 0.76         | 1              | 3.34  | 0.165            | 1                      | 2.62 | 0.204            | 1        | 13.6 | <b>0.034</b> |
| Dates×origin                      | -                  | -    | -            | 4              | 0.4   | 0.807            | 4                      | 1.68 | 0.186            | 1        | 0.44 | 0.379        |
| Populations (origin)              | 3                  | 0.3  | 0.826        | 3              | 2.41  | 0.165            | 3                      | 1.75 | 0.256            | 3        | 1.85 | 0.238        |
| Dates×populations                 | -                  | -    | -            | 12             | 1.9   | <b>0.032</b>     | 12                     | 0.89 | 0.563            | 3        | 1.16 | 0.327        |
| Habitat types (populations)       | 6                  | 4.96 | <b>0.011</b> | 6              | 2.7   | 0.073            | 6                      | 1.61 | 0.233            | 6        | 0.67 | 0.678        |
| Dates×habitat types (populations) | -                  | -    | -            | -              | -     | -                | 24                     | 1.7  | <b>0.023</b>     | -        | -    | -            |
| Transect (habitat types)          | 11                 | 2.05 | <b>0.025</b> | 11             | 2.71  | <b>0.002</b>     | 11                     | 1.73 | 0.065            | 11       | 2.44 | <b>0.014</b> |
| Quadrat                           | -                  | -    | -            | -              | -     | -                | -                      | -    | -                | 58       | 1.33 | 0.072        |
| Error                             | 370                |      |              | 398            |       |                  | 267                    |      |                  | 261      |      |              |

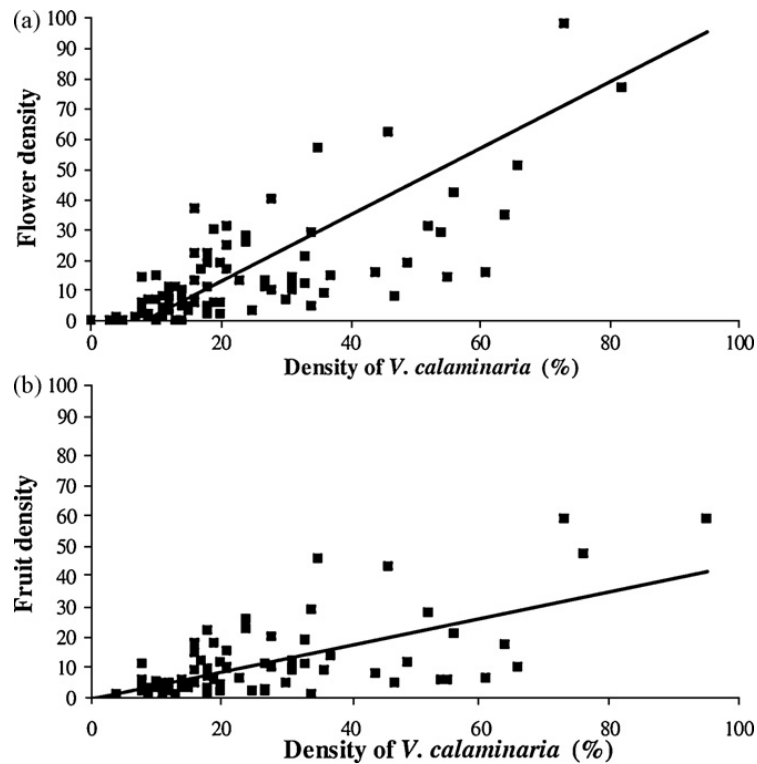
ANOVA (Table 2) indicated no significant differences in *V. calaminaria* vegetative density between origins ( $P = 0.760$ ; mean range: 14.05%-15.5%) or between populations ( $P = 0.896$ ; mean range: 9.9-18.3%). However, significant differences were evident between different habitat types ( $P = 0.011$ ; mean range: 7.5-29.3%) and transects ( $P = 0.025$ ; mean range: 4.7-30.2%). Following mean pairwise comparisons (Tukey's test) significant differences between habitat types were due to differences between habitat type 1 of P1 and habitat type 2 of S2.

### *Variation in reproductive success*

Notable variation between different dates was detected for flower density (Table 2) ( $P < 0.001$ ; mean range: 7.2-73.1 flowers/m<sup>2</sup>). In June and July, the species displayed significantly higher flower density than in other months and in October significantly lower flower density was found compared to all other months (Tukey's test). No significant differences in flower density were found between origins ( $P = 0.165$ ; mean range: 28.04-50.12 flowers/m<sup>2</sup>), between populations ( $P = 0.165$ ; mean range: 15.6-73.6 flowers/m<sup>2</sup>) and between habitat types ( $P = 0.073$ ; mean range: 7.7-102.4 flowers/m<sup>2</sup>). Flower density between transects was significant ( $P = 0.002$ ; mean range: 7.4-95.2 flowers/m<sup>2</sup>). The dates  $\times$  populations interaction was significant ( $P = 0.032$ ), which suggests the evolution of flower density between populations differs according to dates. Consequently, ANOVA was performed on a month-by-month basis. The hierarchical four-way ANOVA showed a significant difference in flower density between populations only in June ( $P = 0.037$ ).

Percent fructification (Table 2) showed significant variation between dates ( $P < 0.001$ ; mean range: 30.9-69%). However, this difference was due to a significant lower percent fructification in October (Tukey's test). No significant difference in percent fructification was found between origin locality ( $P = 0.204$ ; mean range: 57.97-66.34%), populations ( $P = 0.256$ ; mean range: 42.4-74.4%) or habitat types ( $P = 0.233$ ; mean range: 28.2-79%).

ANOVA (Table 2) showed no significant difference in seed set between dates ( $P = 0.402$ ; mean range: 16.7-18.2 seeds/fruit), populations ( $P = 0.238$ ; mean range: 10.9-25.2 seeds/fruit) or habitat types ( $P = 0.678$ ; mean range: 9.9-25.8 seeds/fruit). However, significant differences were indicated between site origins ( $P = 0.034$ ; mean range: 13.2-22.1 seeds/fruit) and transects ( $P = 0.014$ ; mean range: 4.9-26.2 seeds/fruit). Streupas (St) and Prayon (P), the two recent populations, exhibited significantly higher seed set than the ancestral populations (Tukey's test).



**Fig. 3.** Correlation between (a) *Viola calaminaria* density and flower density; (b) *Viola calaminaria* density and fruit density.

A significant positive correlation between vegetative and flower density was detected for *V. calaminaria* in June (Fig. 3a). The best linear model regression for flower density was generated with a square root transformation ( $R^2 = 40.3\%$ ;  $P < 0.001$ ). The correlation between flower density and percent fructification was negative for all months except October and only significant in June ( $r_s = -0.332$ ;  $P = 0.003$ ). Finally, a significant positive correlation was detected between vegetative and fruit density in June ( $r_s = 0.678$ ;  $P < 0.001$ , Fig. 3b), however significant correlations between vegetative density and seed number per fruit were not indicated.

## Discussion

### *Summary of results*

Our study provides the first data to assess the reproductive success of the endemic metallophyte *Viola calaminaria*. The populations and habitat types of *V. calaminaria* sampled did not exhibit variability in reproductive success based on the traits measured in this study. We found a contrasting pattern of temporal (monthly) variation in reproductive traits, with marked differences in flower density, but no variation in percent fructification (with the exception of October) or seed set. However, the temporal variation in flower density influenced the reproductive success of the species as indicated by the total number of seed produced per square metre in each population.

## *Impact of population origins*

It has been well demonstrated that the reproductive success of a species can be influenced by population origins (Leimu & Mutikainen, 2005; Brock et al., 2007; Quintana-Ascencio et al., 2007; Colas et al., 2008). *V. calaminaria* demonstrates similar reproductive success regardless of population origins, suggesting recent populations have not suffered a decrease in fitness. Furthermore, seed set was higher in newly established populations relative to ancestral populations. Bizoux et al. (2008) found an effect of population history on seed set and germination rates, where a recent population exhibited increased fitness relative to the ancestral populations. Colas et al. (2008) observed similar patterns, i.e. diverse demographic parameters could respond differentially between natural and introduced populations of *Centaurea corymbosa* (Asteraceae). This feature is important for management of newly established populations, whatever the origin, i.e. anthropogenic habitat, natural following restoration or artificial by reintroduction. This implies that several reproductive traits and stages should be evaluated to assure the success of population establishment (Colas et al., 2008). Furthermore, the analysis of demographic data in recent populations has great potential to elucidate any evolutionary processes operating in newly established populations and improve knowledge for future management (Menges, 2008).

Genetic effects, i.e. founder effects cannot explain the patterns of variability in reproductive success between recent and ancestral populations revealed in our study (Nei et al., 1975; Leimu & Mutikainen, 2005). Recent populations were likely created from multiple colonisation events from several population sources (Bizoux et al., 2008) and would therefore not experience the effects of genetic drift. This associated with rapid demographic extension and higher gene flow between recent populations (Bizoux, 2006) may have promoted reproductive success in recent populations. Leimu and Mutikainen (2005) proposed a similar scenario in *Vincetoxicum hirundinaria*. Several genetic studies (Brock et al., 2007; Reisch, 2007; Esfeld et al., 2008) demonstrated the maintenance of genetic diversity in recently established populations on anthropogenic habitat, mainly explained by high rates of migration and subsequent population growth. Similar patterns were observed in *V. calaminaria* (Bizoux et al., 2008). In addition, geographic and genetic isolation of ancestral *V. calaminaria* populations (Bizoux et al., 2008) attenuated by reduction and extinction of some populations (Bizoux et al., 2004) could negatively influence seed set.

Furthermore, variation in habitat quality could also lead to variation in reproductive success (Pulliam, 1988), as often observed in habitat fragmentation (Saunders, Hobbs, & Margules, 1991; Lopez-Gallego, 2008). The origin of new *V. calaminaria* habitat is the result of human activities and habitat quality could thus differ between recent and ancestral populations (Quintana-Ascencio et al., 2007). Nevertheless, differences in vegetation structure that could lead to difference of reproductive success between recent and ancestral populations were not

observed. In addition, soil conditions may also explain the pattern. Heavy metal concentration differences between populations were not evident (Bizoux et al., 2004), recent populations do present significantly higher Ca/Mg and significantly lower Zn/Ca, indicating lower toxicity in soils of recent populations (Bizoux, 2006). Calamine sites are generally poor in nitrogen and phosphorus (Simon, 1979; Bizoux, 2006), elements that might be at higher levels in recently established sites (forests, meadows or urban habitats). Therefore, lower toxicity and higher soil nutrients could lead to increased reproductive success (seed set) in more recently established populations.

### *Effect of V. calaminaria vegetative density*

Plant density has been shown to influence reproductive success in various species (Harper, 1977; Lande, 1988; Begon et al., 1990; Roll et al., 1997). In *V. calaminaria*, significant differences in vegetative density and reproductive success were found between transects, suggesting that variation in local density may influence reproductive success of the species. First, individual reproductive success might decrease with increasing local density due to intraspecific competition for resources (e.g. Harper, 1977). However, we found a positive relationship between plant and flower density. The best-fit linear models were obtained with a square root transformation of Y-variables, i.e. increase in flower density was more important than vegetative density. These relationships indicated the absence of intraspecific competition for flower production and can be explained by a high-density patch occurring when resources are ample, which inhibits competition (Kennedy & Gray, 1993; Roll et al., 1997). However, we found a negative effect of flower density on percent fructification in June and July (when flowers were abundant). *Viola* species are known to attract pollinators by pollen or nectar resources (Beattie, 1971; Freitas & Sazima, 2003). This pattern suggested that high flower density leads to a lower chance for a single flower to be pollinated due to increased competition between flowers for pollen transport. Also, in dense flower patches, pollinators could promote, by short distance flights, pollen transfer between flowers of closely related individuals (30 cm; Bizoux & Mahy, 2007) and thus decrease percent fructification and number of seed per fruit due to geitonogamy for example (Roll et al., 1997; Hendrix & Kyhl, 2000). Solitary bees, bumblebees and flies (Syrphidae) (personal observation) are mainly observed visiting flowers of *V. calaminaria* but we advise that additional studies be conducted to explore the relationship between reproductive success, pollinator visitation rate and pollen limitations in *V. calaminaria*.

## Conclusions and implications for conservation

Our results indicated that local vegetative density plays the major role in reproductive success of *V. calaminaria* and lower density leads to lower reproductive success (decreased total seed set) of the species.

The present study also confirmed that recently established populations might play an important role in *V. calaminaria* conservation and should be considered in management strategies due to their similar or higher reproductive success compared to ancestral populations. New populations can have a favourable effect on the metapopulation dynamics of the species. Individuals representing these recent populations could be integrated into rescue areas, especially in the case of a species with a naturally scattered habitat that lacks suitable areas for species durability. Kruger et al. (2002), Brock et al. (2007), Quintana-Ascencio et al. (2007) and Faucon et al. (in press) suggested that anthropogenic activities may play a new favourable role in the conservation of some rare or endemic species.

In addition, reestablishment of viable populations of *V. calaminaria* on recent sites can help to maintain diversity associated with calamine sites, a target habitat of the European Natura 2000 initiative. First, *V. calaminaria* is generally associated with other metallophyte species e.g. *Armeria maritima* subsp. *halleri* (Wallr.) Rothm. and *Thlaspi caerulescens* subsp. *calaminare* (Lej.) Dvorakova also protected by the Natura 2000 initiative (Bizoux et al., 2004). In addition, populations of *V. calaminaria* provide habitat for other species such as the threatened butterfly *Issoria lathonia*, in which one of the most important populations in Belgium is distributed in the recent *V. calaminaria* Prayon site (Ertz & Graitson, 2001). A specific ecosystem restoration or rehabilitation approach has not yet been established for this site, but given the fact that more than one species of concern is supported here (Franklin, 1993), further research should be given priority.

From a restoration perspective, as found by Colas et al. (2008), we showed that reproductive success traits may respond differentially in recent populations. Thus, recently created populations notably in restored habitat should be monitored for several reproductive success traits to evaluate restoration success for special status species. Finally, we know that recent *V. calaminaria* populations appeared at least 40 years ago (C. Lefebvre, Free University of Brussel, personal communication). This return time period is longer than the majority of restoration programs in Europe (max 15-20 years, Young et al., 2005). Our results suggested that viable plant population for several reproductive success traits could be established in newly created habitat after 40 years, especially in the context of former mining areas. This is an encouraging perspective for a number of restoration projects that are currently underway, or in their infancy and too preliminary to evaluate their efficacy (Cristofoli & Mahy, in press; Lake, 2001).

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