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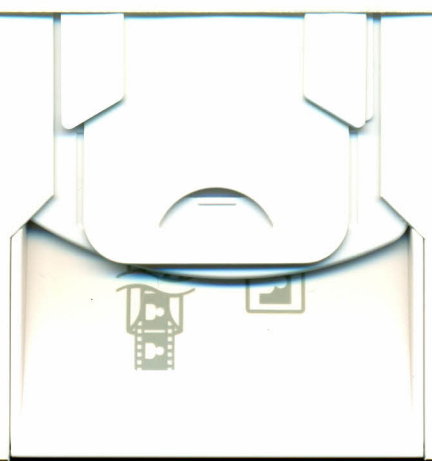

SEED DRESSING WITH CONTROLLED RELEASE FORMULATIONS

Evaluation using a radioisotope technique and yield estimations for the control of aphids and stem nematodes in field beans (Vicia faba L.)

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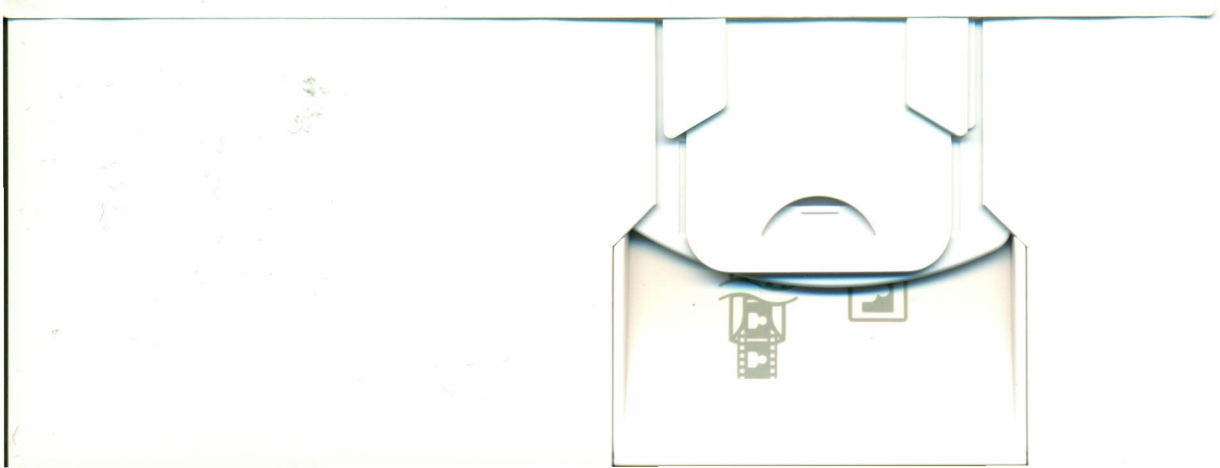
Abstract

SEED DRESSING WITH CONTROLLED RELEASE FORMULATIONS: EVALUATION USING A RADIOISOTOPE TECHNIQUE AND YIELD ESTIMATIONS FOR THE CONTROL OF APHIDS AND STEM NEMATODES IN FIELD BEANS (*Vicia faba* L.).

Previous studies have shown that the incorporation of systemic insecticides in seed coatings, designed as controlled release formulations, is a combined operation (sowing and treatment) which uses much less pesticide for the same period of activity. The carbofuran incorporated into coated field bean seeds (*Vicia faba* L.) at a rate of 3 mg active ingredient per seed resulted in a reduction of up to 96% in the stem nematode populations (*Ditylenchus dipsaci* (Fil.)(Kühn)) found in plants 4 months after sowing; 3 mg of carbofuran per seed correspond to 0.9 to 1.2 kg a.i./ha, depending on the sowing density. The long persistence of nematicide activity for such a quantity of active ingredient is obtained by the slow release of carbofuran from the seed coatings. As might be expected for a systemic pesticide incorporated into the soil, carbofuran has no effect against pollinators and pests, predators or parasites. The calibrated seeds of field beans were coated using the rolling technique. Tritiated carbofuran can be homogeneously incorporated into the matrix, or can be incorporated into a resin or encapsulated in a wide range of matrices. The controlled release effect of all these formulations has been characterized using a radioisotope technique. In a laboratory test, carbofuran was released three times more slowly from coatings than when formulated as commercial microgranules. Infestation in field beans after the growing season was determined by comparing the number of stem nematodes found in treated and untreated plants at different dates. It was found that incorporation of carbofuran into a urea–formaldehyde resin formulation provided the best protection against stem nematodes (95.8% of the control, 4 months after sowing) and aphids, and also increased the yield (+58%). Carbofuran markedly improved the



yields of treated plots. The size of grains harvested on the treated plots and the amount of proteins were significantly higher than those of the untreated plots. The carbofuran residues in the flour of harvested grains, determined by gas–liquid chromatography, were always below the threshold level.

1. INTRODUCTION

Incorporation of systemic pesticides into the soil, as done with granule formulations or seed dressing with pesticides, to prevent drift and inadequate dosage problems results in a marked decrease in pollution risks in the environment [1].

Systemic insecticide granules applied over seed furrows were more effective when placed close to the seeds [2–4]. As pesticides in the soil have half-lives of only a few weeks [5], the results obtained were often better when granules were applied several times. However, this practice greatly increased the residues in harvested products, often beyond the threshold level [3, 4].

Seed dressing is prepared by combining a biologically active agent with excipients (polymer, resin, etc.) which regulate the delivery of the agent to the target. The benefits of seed dressing with controlled release formulations are potentially considerable compared with conventional formulations and application technologies. They facilitate a more precise delivery of pesticide to the appropriate target site; they preserve the biological activity at an effective level during critical periods by controlling the release of active ingredients and protecting them from premature decomposition [5]; they use much less pesticide for the same period of activity, within the limitations of the available pesticide; and they offer the advantage of combining two operations (sowing and treatment) [6, 7].

2. SEED DRESSING WITH CONTROLLED RELEASE FORMULATIONS

The calibrated seeds of field beans (*Vicia faba* L.) (cv. Exelle), with a diameter of 8.5 to 9.5 mm, were coated using the rolling technique [8–11]. Seed dressing was done in a rotating sphere. To the moistened seeds were added alternatively an aqueous solution of an adhesive and a dry mixture (clays, silicates, sawdust, etc.) with one or more adhesive materials. Further careful drying will reduce the excess water, so obtaining a hard matrix around the seeds.

To the seed coating process was added a concentrated suspension of carbofuran (Curater SC 330 flowable) diluted in a solution of adhesive. Water soluble adhesives, such as polyvinyl alcohols, combine on drying and create a network of film around the active agent. This is called a 'one step' or 'classical' (CL) formulation [12].

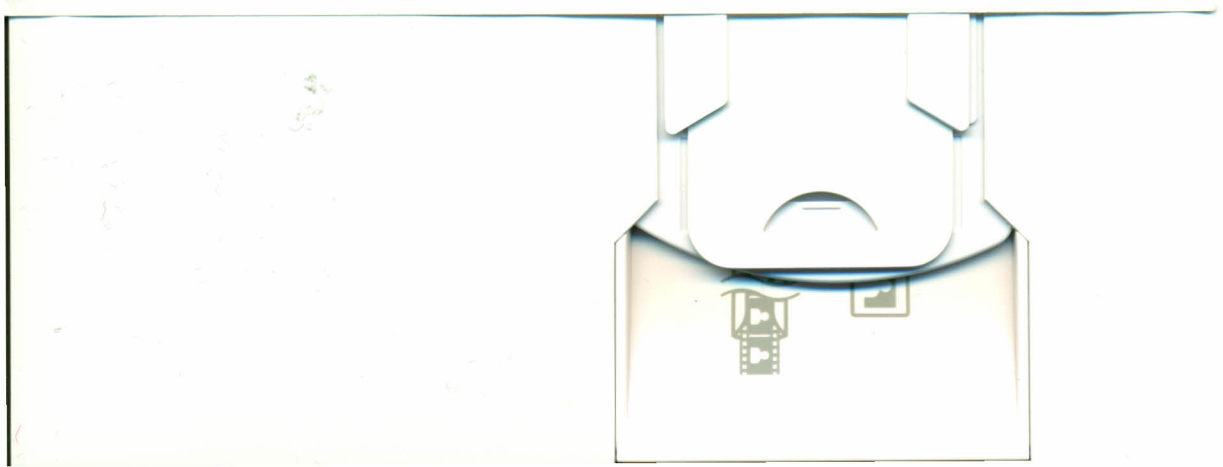


TABLE I. COMPOSITION OF SEED DRESSING WITH CONTROLLED RELEASE FORMULATIONS (3 mg CARBOFURAN/SEED) *(The total amount of coating material added to the field bean seeds corresponds to 30% of their own weight)*

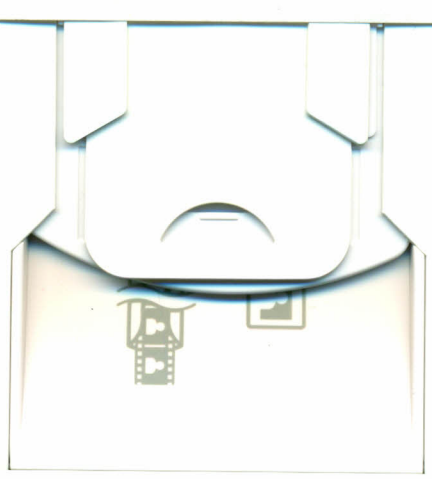
Formulations	Clays		Absorbent	Stickers			Resin
	Bentonite (%)	Vermiculite (%)	Perlite (%)	Versicol S19 (%)	Vinarol ST (%)	Mowilith DL45 (%)	(%)
Classical (CL)	49	10	29	10	2	–	–
Urea–formaldehyde (UF) (resin titrating 4% of carbofuran)	27	14	–	4	–	9	46
Starch xanthide (SX) (resin titrating 12% of carbofuran)	50	10	17	5	–	–	18

Carbofuran can also be incorporated into a resin or encapsulated in a wide range of matrices which are ground to a suitable dimension, mixed with other pelleting adjuvants and added to the seeds. This is called a 'two step' formulation, e.g. a urea–formaldehyde resin (UF) or a starch xanthide matrix (SX).

For the laboratory tests, solutions of tritiated carbofuran (specific activity: 15.5 μCi/mg or 574 kBq/mg) in acetone with concentrations of 3.22, 5.3 and 20.7 mg/mL for CL, UF and SX formulations, respectively, were prepared for incorporation into the seeds. Tagging was achieved by adding 1 mL of the radioactive solution to 3 and 9 g of concentrated suspension (at 33% active ingredient) for CL and SX coatings, respectively, and to 3 g of crystallized carbofuran in acetone for the UF formulation, resulting in about 2 mg of carbofuran per seed.

It was found that seed coating material amounting to 30 wt% of the field bean seeds was enough to incorporate directly or indirectly 3 mg of carbofuran per seed. The details of each formulation are given in Table I.

Previous studies [13] have shown that the rolling technique is able to distribute, on average, the desired dose of carbofuran on to the seeds, with a variation from seed to seed that depends on the amount of coating materials on each seed (linear correlation coefficients: +0.85 and +0.94 for CL and UF formulations, respectively). Carbofuran degradation in the coating material does not occur until at least 12 months.



3. STANDARD WASHING TEST

To evaluate carbofuran release from the coated seeds, a standard washing test was used [13]. This involved placing five seeds on a sand bed (100 g) in a Büchner funnel with a diameter of 7.5 cm and a height of 7 cm, retaining 25 or 30 mL of distillated water in the funnel for 24 hours and then draining it off (Fig. 1). The eluted volume was noted; 1 mL of the eluate was then mixed with 10 mL of a scintillation mixture (Lumagel–Lumac) to measure the radioactivity. The results are expressed in the total weight per cent of carbofuran released from the seed coatings after washing (Table II and Fig. 2). At the end of the test, the radioactivity remaining in the seeds and the sand was measured.

To compare the controlled release of seed coatings with a commercial granular formulation of carbofuran (Curater 5G), the standard washing test was also used; the microgranules contained the same amount of carbofuran as the five coated seeds. The carbofuran released from the microgranules was also extracted from the eluted water and measured by gas chromatography. The results are expressed in the total weight per cent of carbofuran released at the end of the washing test (Table II and Fig. 2).

Some eluate samples were analysed by gas chromatography to identify the radioactivity (GC column: WCOT 20 m × 0.32 mm; phase: Sil 5 CB; temperature: programmed from 70 to 200°C; the on-column injection mode; NPSD detector; azobenzene as the internal standard).

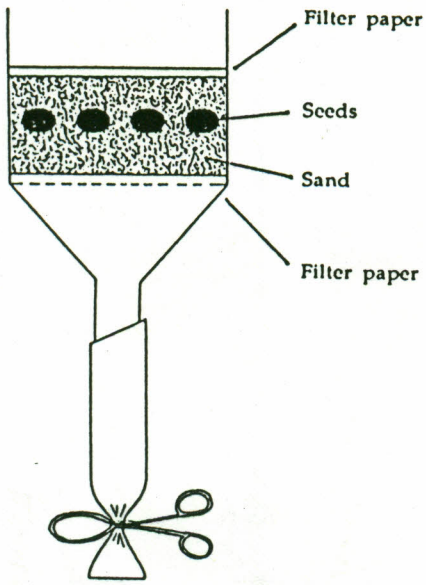
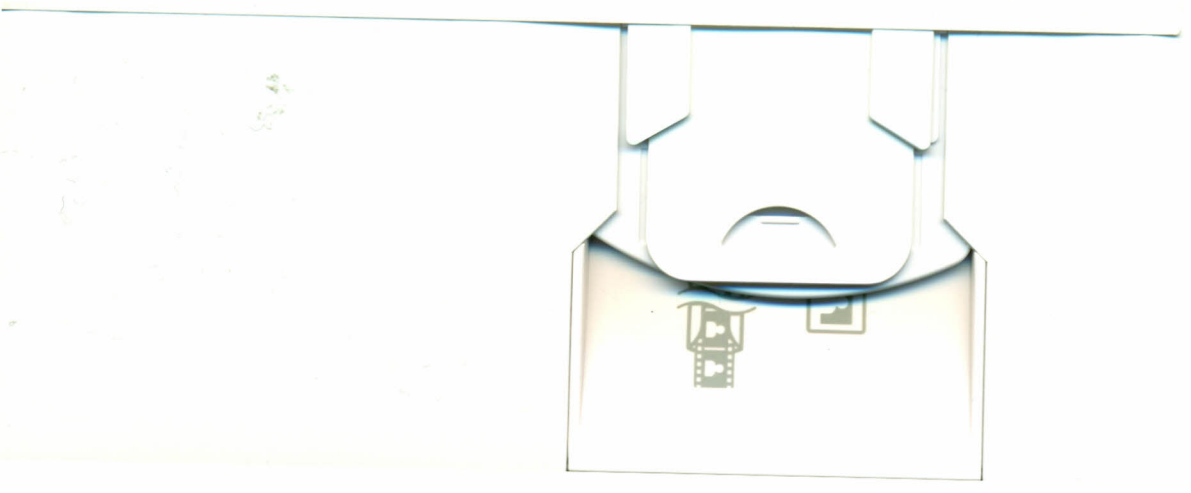


FIG. 1. Büchner funnel used for the standard washing test.

TABLE II. RESULTS OF A STANDARD WASHING TEST EXPRESSED IN PER CENT OF TOTAL WEIGHT OF CARBOFURAN RELEASED AT THE END OF THE TEST FOR EACH FORMULATION (CL: CLASSICAL COATING; SX: STARCH XANTHIDE; UF: UREA–FORMALDEHYDE)
(Average of six replicates)

Cumulated eluate volumes (mL)	CL coating (%)	SX coating (%)	Cumulated eluate volume (mL)	UF coating (%)	Microgranules (Curater 5G) (%)
24	6.4 ± 1.7	6.3 ± 1.0	30	9.0 ± 0.7	26.3 ± 2.8
50	10.3 ± 1.4	13.4 ± 1.9	60	24.1 ± 1.0	20.7 ± 1.5
75	11.1 ± 2.3	13.0 ± 1.9	90	18.9 ± 1.7	17.5 ± 1.2
100	10.7 ± 1.7	13.0 ± 1.9	120	10.4 ± 0.7	16.5 ± 1.0
125	14.9 ± 1.4	13.0 ± 1.9	150	7.5 ± 1.0	9.7 ± 0.9
150	9.9 ± 0.7	9.4 ± 1.5	180	4.8 ± 0.2	5.7 ± 0.8
175	10.4 ± 1.2	8.6 ± 1.5	210	3.4 ± 0.2	2.2 ± 0.3
200	8.4 ± 1.1	6.9 ± 1.6	240	2.5 ± 0.2	0.9 ± 0.3
225	6.5 ± 1.5	4.9 ± 1.8	270	2.0 ± 0.2	0.5 ± 0.3
250	4.8 ± 1.3	3.3 ± 1.0	300	2.0 ± 0.1	
275	3.4 ± 1.4	2.2 ± 0.7	330	1.7 ± 0.1	
300	2.3 ± 1.0	1.5 ± 0.6	360	1.3 ± 0.1	
325	0.8 ± 0.4	1.1 ± 0.4	390	1.6 ± 0.1	
350		0.7 ± 0.2	420	1.5 ± 0.3	
375		0.6 ± 0.2	450	1.4 ± 0.2	
400		0.3 ± 0.1	480	1.3 ± 0.1	
425		0.3 ± 0.1	510	1.3 ± 0.1	
450		0.3 ± 0.1	540	1.1 ± 0.1	
475		0.2 ± 0.0	570	1.0 ± 0.1	
500		0.2 ± 0.1	600	0.9 ± 0.1	
525		0.2 ± 0.1	630	2.1 ± 0.1	
550		0.1 ± 0.1			
575		0.2 ± 0.1			
600		0.2 ± 0.1			
625		0.1 ± 0.1			
650		0.1 ± 0.1			
675		0.1 ± 0.0			



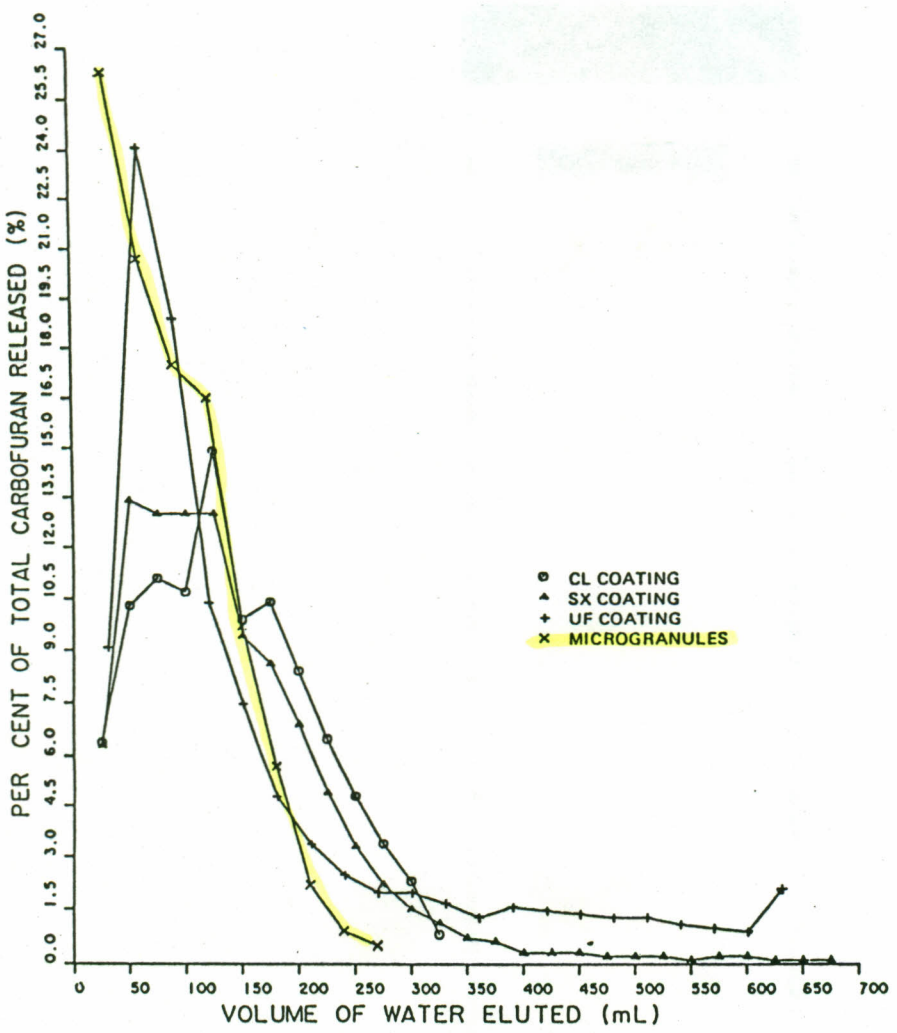


FIG. 2. Per cent of total carbofuran released from microgranules (Curator SG) and seed coatings depending on the volume of water eluted in a standard washing test.

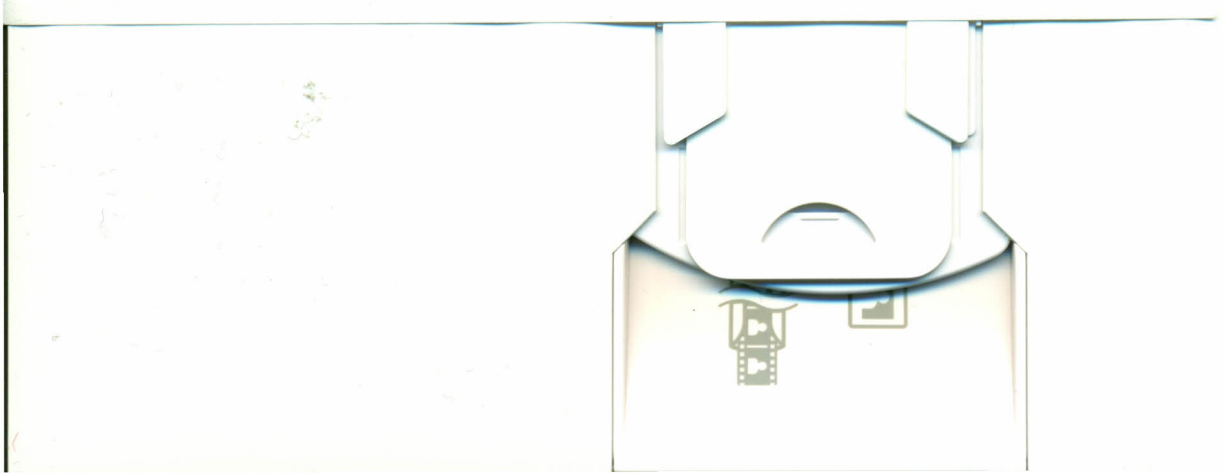


TABLE III. PER CENT OF TOTAL AMOUNT OF CARBOFURAN REMAINING IN COATINGS WHEN COMMERCIAL MICROGRANULES ARE USED UP AND RELEASE RATIO VALUES

Formulations	Microgranules used up (%)	Volume of water needed to release % of the total amount of carbofuran			Release ratio
		50	75	95	
		(mL)			
Microgranules (Curater 5G)	0	90	120	180	1.0
CL coating	26	125	200	275	1.5
UF coating	22	120	210	540	3.0
SX coating	23	125	175	300	1.7

The release of coatings and microgranules can be compared by measuring the total amount of carbofuran remaining in the formulations when the microgranules are used up (A%), and the volume of water needed to release 50, 75 and 95% of the carbofuran. The release ratio (Rr) can be established as Rr = coatings V_{95}/microgranules V_{95} (Table III).

The results of this evaluation indicate that seed dressing formulations appear to offer a higher retention of active agent than commercial microgranules of carbofuran, the best being the UF coating, which has an A% equal to 22% and an Rr three time greater than that of the microgranules. No significant level of radioactivity was found in the sand or the seeds.

4. CARBOFURAN DISTRIBUTION AND METABOLIZATION IN FIELD BEANS

Ten millilitres of a tritiated carbofuran solution (77 μg a.i./mL) were applied to the soil of flower pots (17.2 kBq/pot) in which a single 1 week old field bean was grown. The distribution of carbofuran in field beans was investigated by stem and leaf calcination, in sequence with a BMO–ICN calcinator. Sampling and calcination were carried out 2, 4 and 6 weeks after application. The results (Table IV) showed a very heterogeneous distribution of radioactivity in the plants: the oldest leaves, especially the edges, accumulated much more radioactivity than the youngest leaves.

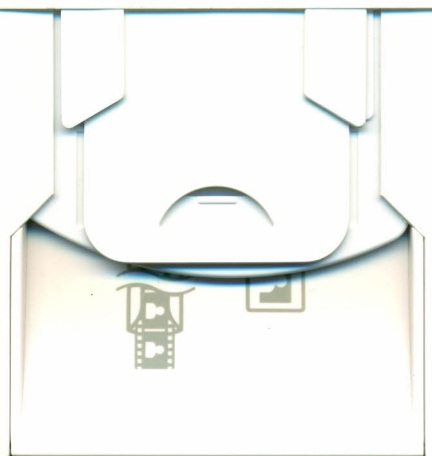
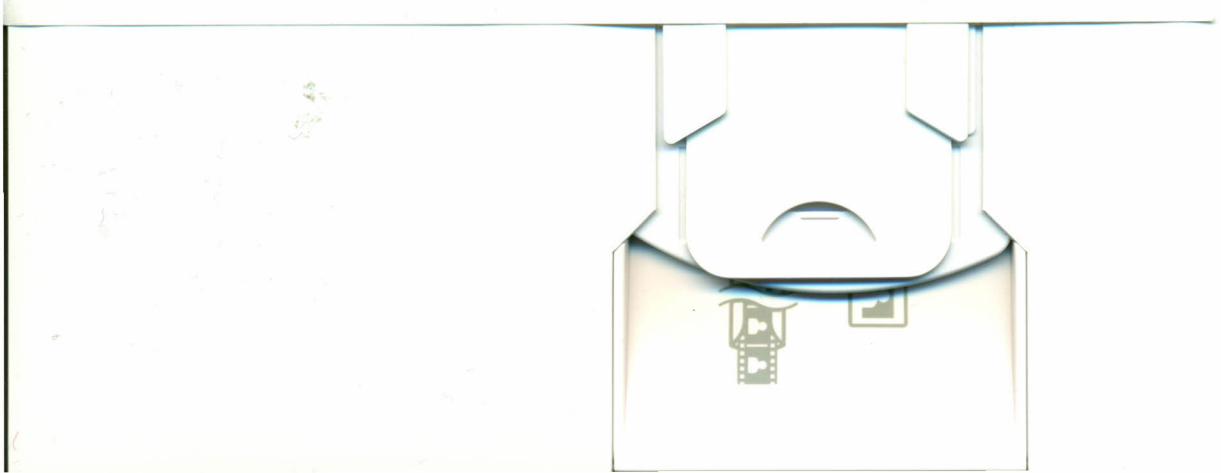


TABLE IV. DISTRIBUTION OF ^{3}H RADIOACTIVITY IN FIELD BEANS 2, 4 AND 6 WEEKS AFTER APPLICATION TO THE SOIL (AVERAGE OF TWO PLANTS) *(The leaves were numbered from 1 to 10 according to their order of formation; sometimes the edges of the leaves were cut and measured separately from the rest of the leaves)*

Sample	Distribution of ^{3}H radioactivity		
	After 2 weeks	After 4 weeks	After 6 weeks
		(Bq/g dry weight)	
Stem	167	224	63
Leaf 1	175	2108	1334
Edge 1	190	-	-
Leaf 2	68	1368	1498
Edge 2	227	4332	-
Leaf 3	139	1211	1675
Edge 3	189	4066	-
Leaf 4	67	1997	-
Leaf 5	50	1536	1014
Leaf 6		1059	543
Leaf 7		775	282
Leaf 8		199	165
Leaf 9			53
Leaf 10			35

The same results were found with ^{14}C-carbofuran. These results are in agreement with those of Ashworth and Sheets in tobacco plants [14].

In another experiment, the metabolization of carbofuran in field beans was investigated. Sixty flower pots each received one field bean seed coated with ^{14}C-carbofuran (coating application with 5.3 kBq and 1.14 mg of carbofuran/pot; specificity activity: 4.6 Bq/μg of carbofuran). Sixty other pots each received one seed and were watered with 10 mL of a ^{14}C-carbofuran solution in water (drench application with 16.8 kBq and 3 mg of carbofuran/pot; specific activity: 5.6 Bq/μg of carbofuran). The control pots received only 10 mL of water. The pots were placed

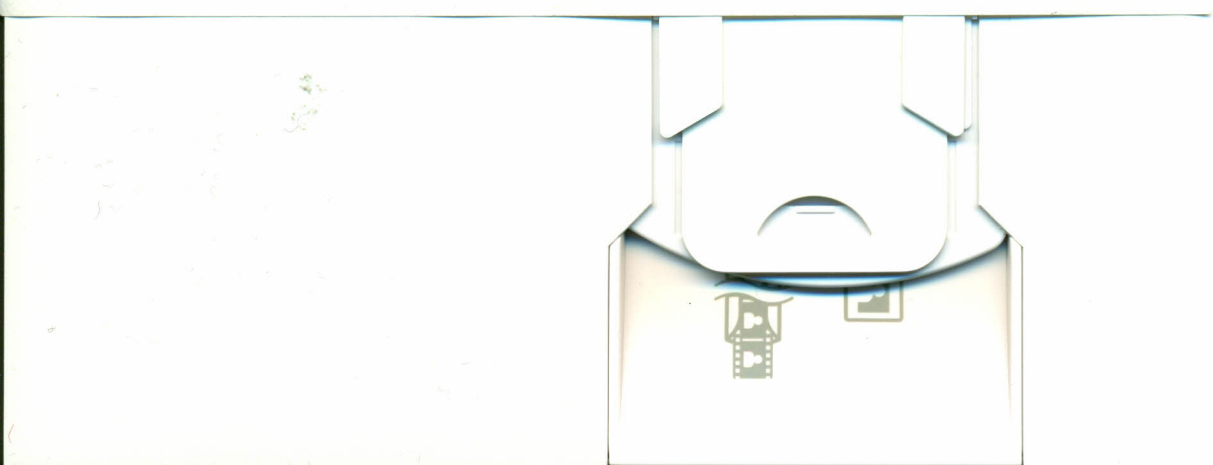


in a greenhouse. All the plants were watered daily with 50 mL of water. On days 17 and 35 after sowing the plants were sampled for carbofuran extraction and three plants from each type of treatment (untreated, coating application and drench application) were placed in a cage and received 20 aphids (*Acyrtosiphum pisum* Harris) per plant to determine the persistence of carbofuran activity.

Two samples taken from a 50 g plant were blended twice with 200 mL of acetone at high speed for 2 minutes and then filtered on glass filter G4. The filtrate was concentrated, extracted four times with 50 mL of chloroform in a separatory

TABLE V. PER CENT CARBOFURAN AND ITS METABOLITES IN FIELD BEANS 17 AND 35 DAYS AFTER SOWING WHEN AN ACTIVE AGENT IS APPLIED BY SEED COATING OR BY SOIL WATERING WITH A RADIOACTIVE SOLUTION *(Means are expressed in becquerels for the total sample extracted)*

Samples	Coating application			Drench application		
	Means (average of six TLC counts)	ppm (dry weight)	% of total activity	Means (average of six TLC counts)	ppm (dry weight)	% of total activity
			After 17 days			
Carbofuran	1151 ± 178	65.0	38.5	177 ± 5	8.3	34.2
3-OH carbofuran	1451 ± 210	81.9	48.6	242 ± 71	11.3	46.8
3-OH glycoside	248 ± 48		8.3	58 ± 23		11.3
Unidentified	137 ± 10		4.6	40 ± 10		7.7
Total	2987		100.0	517		100.0
			After 35 days			
Carbofuran	158 ± 38	5.9	12.3	2 ± 1	0.08	1.2
3-OH carbofuran	564 ± 134	21.1	43.8	47 ± 17	1.6	23.9
3-OH glycoside	488 ± 4		37.9	129 ± 36		65.3
Unidentified	77 ± 31		6.0	19 ± 3		9.6
Total	1287		100.0	197		100.0



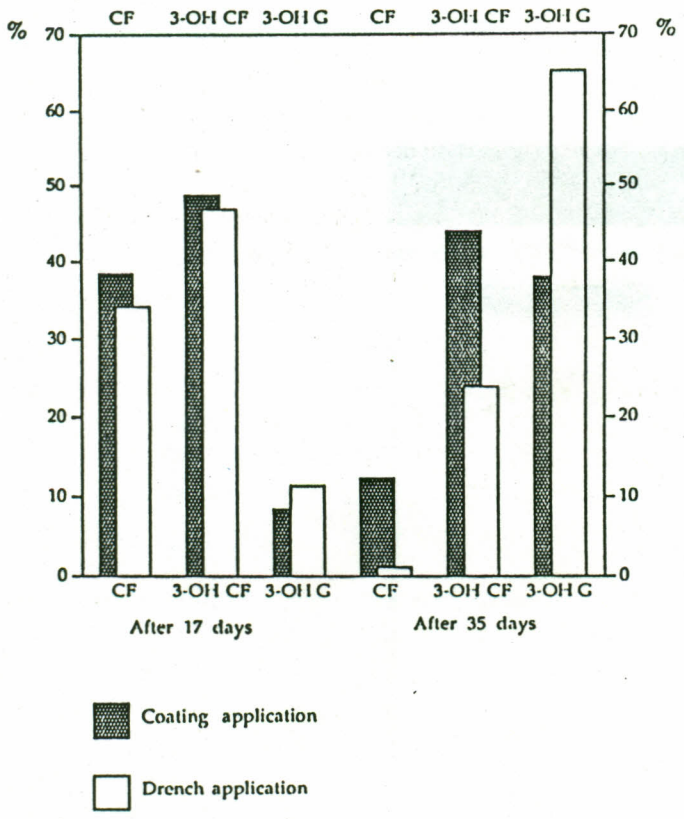


FIG. 3. Comparative evolution of carbofuran and its metabolites in field beans 17 and 35 days after sowing when an active agent is applied by seed coating or soil watering with a radioactive solution (CF = carbofuran; 3-OH CF = 3-hydroxycarbofuran; 3-OH G = 3-hydroxycarbofuran glycoside).

funnel and recovered in 5 mL of chloroform [15, 16]. The water containing 3-hydroxycarbofuran glycoside was refluxed for 30 minutes with 150 mL of HCl 0.25N, extracted four times with 50 mL of dichloromethane and recovered in 5 mL of dichloromethane. Carbofuran and its main metabolites (3-hydroxycarbofuran and 3-hydroxycarbofuran glycoside) were separated by thin layer chromatography (three replicates for each sample) using a mixture of chloroform–acetone–acetonitrile (4:1:1, vol./vol.). After control with a radio TLC scanner, the bands containing CF, 3-OH CF and 3-OH glycoside were scraped and directly measured by liquid scintillation chromatography. The results are given in Table V and illustrated in Fig. 3.

Seventeen days after sowing, no difference was found between the amounts of carbofuran and metabolites in the plants, regardless of the type of treatment. Also,

aphids failed to establish on the plants, except for the control. Thirty-five days after sowing, higher amounts and concentrations of carbofuran and 3-hydroxycarbofuran were determined in coating application plants than in drench application plants (Table V). This explains the failure of aphids to establish on seed coating plants; the evolution of aphid populations was similar for drench application plants and the control.

5. FIELD EXPERIMENT

The experiment was of a randomized block design, consisting of four treatments (untreated and three formulations: CL, UF, SX) and four blocks. Each plot had fifty 1.5 m rows that were 0.25 m apart. Field bean seeds were sown on 3 May 1985 in each row at a sowing density of 40 seeds/m^2. Each plot was then subdivided: 25 rows were set aside for estimation of yield and in the other 25 rows field beans were sampled for nematode extractions. After 117 days withering of the field beans prevented multiplication of the stem nematodes.

For nematode extraction, the stem bases of 10 field beans collected at random were cleaned, chopped up, weighed and extracted by the mixer centrifugal flotation technique described in Ref. [17] and adapted to our laboratory conditions [18, 19].

Infestation of the field beans after different periods of growth was measured; the effects of CL, UF and SX formulations (with 3 mg carbofuran/seed) on the number of stem nematodes per gram of plant tissue at 52, 75, 97 and 117 days after

TABLE VI. EFFECTS OF VARIOUS SEED COATING FORMULATIONS (3 mg CARBOFURAN/SEED) ON THE NUMBER OF STEM NEMATODES RECOVERED FROM YIELD BEANS AT DIFFERENT TIMES AFTER SOWING

Formulations	Average number of stem nematodes/g of stem tissue (days after sowing)				Control (%)
	52	75	97	117	
Untreated	3.6 ± 1.5	11.1 ± 1.8	59.6 ± 38.4	266.9 ± 59.3	0
CL coating	0.4 ± 0.3	1.6 ± 0.7	4.6 ± 2.0	19.9 ± 8.7	92.5
UF coating	0.1 ± 0.1	0.4 ± 0.3	2.4 ± 1.6	11.2 ± 6.0	95.8
SX coating	0.5 ± 0.5	1.1 ± 0.6	22.5 ± 3.4	42.1 ± 16.4	84.3

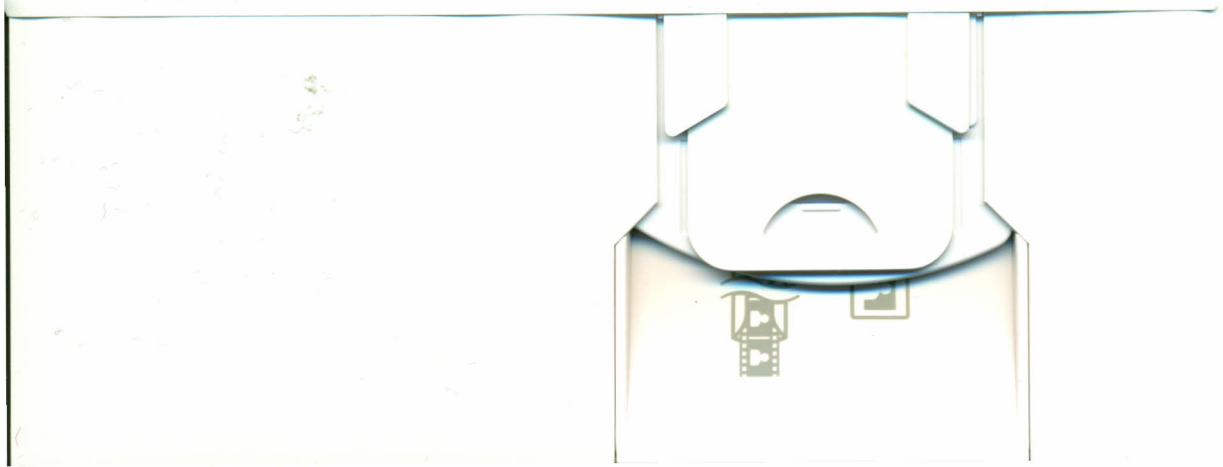


TABLE VII. INFLUENCE OF DIFFERENT FORMULATIONS OF CARBOFURAN ON THE YIELD, THOUSAND GRAIN WEIGHT (TGW) AND PROTEIN CONTENT OF A FIELD BEAN CROP *(Average of four replicates)*

Formulations	Yield (g/m^2)	TGW (g)	Protein content (%)
Untreated	402.8[a]	320.7[a]	24.2[a]
CL coating	604.7[b]	397.5[b]	25.7[b]
UF coating	636.4[b]	412.9[b]	26.1[b]
SX coating	563.9[b]	387.0[b]	25.7[b]

[a, b] Distribution of means following the Newman and Keuls method for alpha = 0.05%.

sowing are shown in Table VI. The efficacy of each nematicide formulation was determined by comparing the percentage of infestation control (C%) at the last extraction date. This is expressed by $C\% = 100 - (X/Y) \times 100$, where X is the number of stem nematodes found in the treated plants at the last extraction date and Y is the number of stem nematodes found in the untreated plants at this date (Table VI).

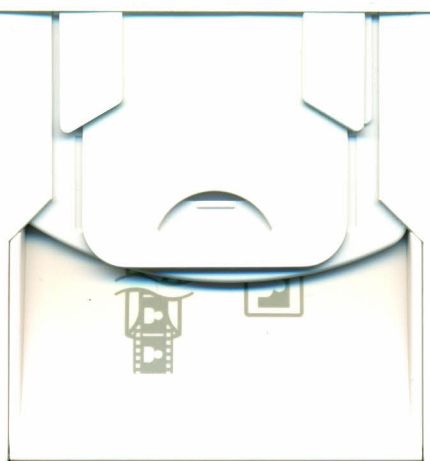
The highest number of stem nematodes was found in the first observation of untreated plants and it increased rapidly. A good control was achieved during the first 3 months with all formulations of carbofuran. At the last observation (117 days after sowing) the SX formulation permitted some nematode penetration.

Half plots were harvested on 10 September 1985 (130 days after sowing). Field bean grains were cleaned and air dried before being weighed. Carbofuran markedly improved the yield ($P < 0.001$), the thousand grain weight and the protein content; all were significantly higher than those in the untreated plots (Table VII). All the values given in the table are significantly different ($P < 0.05$).

After extraction, the carbofuran residues in the flour of the harvested grains were determined by gas–liquid chromatography [19]. The residues attained 0.11 ppm or 0.38 ppm for plants treated with the CL or the UF formulation, respectively.

6. CONCLUSIONS

The controlled release performance of coating and microgranule formulations has been characterized using a radioisotope technique. In a laboratory test, carbo-



furan was released three times more slowly from coatings than when formulated as commercial microgranules.

Accumulation of carbofuran and its metabolites occurs in the oldest parts of the plants. Carbofuran release from seed coatings provides a higher amount of chemical in the plant tissues, which increases the efficacy time of the active agent.

Schiffers et al. [18, 19] demonstrated that carbofuran incorporated in seed coating formulations is an effective nematicide. In the field experiment described here, the chemical markedly reduced the number of stem nematodes found in plants 4 months after sowing (Table VI). Three milligrams of carbofuran per seed correspond to 0.9 to 1.2 kg a.i./ha, depending on the sowing density. The long persistence of nematicide activity for such a quantity of active ingredients is obtained by the slow release of carbofuran from seed coatings, as characterized in a standard washing test.

Seed dressing with the UF formulation provides the best protection against stem nematodes (95.8% of the control, 4 months after sowing) and the best yield (+58%). The SX formulation has a shorter activity than the others, but infestation is nevertheless prevented during the sensitive blossoming period (until 15 July). Therefore, yields of the SX formulation are not significantly smaller than those of the other treatments. The residues of carbofuran at harvest were always below the threshold level (0.50 ppm), but the difference in residue levels between the CL and the UF formulations illustrates the risk of prolonging the release period for too long.

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REFERENCES

[1] HEDIN, P., J. Agric. Food Chem. **30** 2 (1982) 201–215.
[2] WHITEHEAD, A.G., TITE, D.J., FRASER, J.E., FRENCH, E.M., Ann. Appl. Biol. **93** (1979) 213–220.
[3] WHITEHEAD, A.G., TITE, D.J., FRASER, J.E., Ann. Appl. Biol. **103** (1983) 291–299.
[4] WINDRICH, W.A., Crop Prot. **4** 4 (1985) 458–463.
[5] SUETT, D.L., Crop Prot. **5** (1986) 165–169.
[6] McFARLANE, N.R., PEDLEY, J.B., Pestic. Sci. **9** (1978) 411–424.

[7] SCHIFFERS, B.C., Ann. Gembloux **91** (1985) 31–37.
[8] JEFFS, K.A., CIPAC Monograph 2, Heffers Printers Ltd, Cambridge, UK (1978) 99 pp.
[9] LONGDEN, P.C., ADAS Quart. Rev. **18** (1975) 73–80.
[10] FRASELLE, J., SCHIFFERS, B., Med. Fac., Landbouww. Rijksuniv., Gent **47** 2 (1982) 665–673.
[11] SCHIFFERS, B., CORNET, D., FRASELLE, J., BALANDI, Mboka-Unda, Parasitica **38** 2 (1982) 55–63.
[12] SCHIFFERS, B.C., FRASELLE, J., Ann. Gembloux **88** (1982) 165–175.
[13] SCHIFFERS, B.C., FRASELLE, J., GASIA, M., DREZE, P., "Seed dressing with controlled release formulations: Preparation and evaluation using radioisotope techniques", Research and Development of Controlled Release Technology for Agrochemicals Using Isotopes (Proc. Sem. Vienna, 1985), IAEA-TECDOC-404, IAEA, Vienna (1087) 40–41.
[14] ASHWORTH, R.J., SHEETS, T.J., J. Econ. Entomol. **63** 4 (1970) 1301–1304.
[15] BAYOUMI, O.C., SCHIFFERS, B., FRASELLE, J., Med. Fac., Landbouww. Rijksuniv., Gent **48** 4 (1983) 1007–1014.
[16] BAYOUMI, O.C., DREZE, P., Bull. Rech. Agron., Gembloux **19** (1984) 155–163.
[17] COOLEN, W.A., HENDRICKX, J., GOORIS, J., D'HERDE, C.J., Min. Agr. St., Agric. Res. Cent., Gent (1984) 9 pp.
[18] SCHIFFERS, B.C., FRASELLE, J., HUBRECHT, F., JAUMIN, L., Med. Fac., Landbouww. Rijksuniv., Gent **49** 2b (1984) 635–641.
[19] SCHIFFERS, B.C., FRASELLE, J., JAUMIN, L., Med. Fac., Landbouww. Rijksuniv., Gent **50** 3a (1985) 797–807.