Determination and Uptake of Carbosulfan and Carbofuran in Young Douglas Firs (*Pseudotsuga menziesii* Mirb.)

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Protection of young Douglas firs and other conifers from attacks by the large pine weevil, *Hylobius abietis* L. (Col. Curculionidae), consisted of repeated insecticide treatments by dipping or spraying with organochlorine or pyrethroid insecticides. The use of a controlled-release formulation as Marshal/SuSCon containing the systemic carbamate insecticide carbosulfan in nurseries or on reforestation sites as a single treatment at planting time ensures a good level of protection. Using gas chromatography as a standard analytical method, carbofuran, a byproduct of carbosulfan, was detected and admitted to be the effective product. Its penetration in young plants is quick, and high levels of this product are observed 24 months after treatment. Comparisons between nursery and field trials show good protection of young Douglas firs over the critical period of establishment of the plantation with this formulation.

Keywords: *Insecticide, carbofuran, carbosulfan, Hylobius weevil, Douglas fir, uptake, formulation*

INTRODUCTION

The large pine weevil, *Hylobius abietis* L. (Coleoptera: Curculionidae), is one of the most serious pests of young conifer plantations throughout Europe, generally after Scotch pine or Norway spruce mature forests have been clear-cut. The insect develops in the roots of fresh stumps, and adult weevils emerge in March–April and feed on the bark of newly planted trees, causing damage and death of the plants by ring-barking. Young conifers are most susceptible during the first 2 years after planting.

To limit this damage, a controlled-release formulation of the carbamate insecticide carbosulfan (100 g/kg) was tested with a single application at planting time. The dry, dust-free granular formulation Marshal/SuSCon provided superior protection from attack by *H. abietis* when compared to the less environmentally benign standard practice of repeated spraying/dipping with synthetic pyrethroids or organochlorine insecticides (Julien *et al.*, 1990). Marshal is the trade name from FMC Corp. for the active ingredient carbosulfan, and SuSCon is the trademark of Incitec Ltd. for the controlled-release granule formulation of carbosulfan. Protection of young Douglas fir, *Pseudotsuga mensiezii* (Mirb.), was demonstrated with the Marshal/SuSCon formulation through a set of 22 field trials (Lemperiere and Julien, 1989). The mode of action of the systemic insecticide and its uptake into the plant have been characterized using analytical methods.

This study was designed to determine the rate and pathways of penetration of both carbosulfan and its main byproduct, carbofuran, in Douglas fir (Figure 1).

MATERIALS AND METHODS

Nursery Plots. The field trials were conducted at Saint-Junien-la-Brégère (Creuse, France), in a forest nursery, and at Perols-sur-Vezere (Correze, France), on a reforestation site, both on brown acid soil, pH 5.5. In the nursery, Marshal/SuSCon granules were applied at the rates of 25, 50, and 100 g of ai/m^2 incorporated into the soil, near the root system of 3-year-old Douglas firs. On the basis of our calculation, an approximate level of 0.2 g of ai per plant could be found with the higher rate.

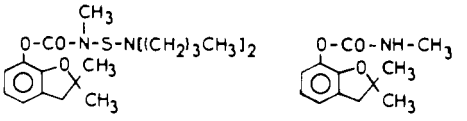

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Figure 1. Studied structures: carbosulfan (1) and carbofuran (2).

Field Plots. On the reforestation site, Marshal/SuSCon granules were applied at a rate 0.8 g of ai/plant at planting time in April 1987, in the planting hole before the 3-year-old Douglas firs were placed.

Assessments and Sampling. *Nursery.* Each plot contained 100 trees, and samples of 5 trees per dose were randomly collected 7, 14, 21, 28, and 150 days after treatment. Samples were stored in plastic bags at −20 °C until analyzed.

Field Site. Samples of 5 trees were randomly collected on a subplot of 50 trees treated at 0.8 g of ai/plant 18, 24, and 30 months after treatment. Samples were stored in plastic bags at −20 °C for transport.

Residue Analysis. *Sample Preparation.* In the laboratory, roots and branches were discarded and the remaining stems were frozen (−20 °C). For each sample of five young Douglas firs, stems were weighed. The stems of two medium-weight plants were directly analyzed, and the stems of the three remaining plants were separated into a lower part (LP), where most *Hylobius* damages occur, and an upper part (UP) including the terminal bud.

Extraction Procedure. The extraction procedure is similar to the one used by Leppert *et al.* (1983) for low-moisture crops and takes into account their modification for acidic crops (Leppert *et al.*, 1984). Subsamples (3–20 g) were cut in small pieces and blended with a Waring Blendor for 3 min in 100 mL of a 9:1 (v/v) mixture of methanol/pH 8 phosphate buffer. The ground stem was macerated for 10 min and then filtered through Büchner funnels lined with ashless paper. The filter cake and the filter paper were again blended for 3 min with a second portion of the extraction mixture. The blending jars and the filter cakes were rinsed with 50 mL of blending solvent. The volume of each sample was adjusted to 300 mL with the blending solvent. Aliquots of 100 mL were transferred in a 250-mL separatory funnel and diluted with 100 mL of borax buffer (0.0052 M) containing 15 g of sodium chloride. The sample was extracted three times with

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Table 1. Residues of Carbofuran in Douglas Firs Treated in the Nursery at 100 g of ai/m²

part of plant[a]	residue level, ppm				
	7 days	14 days	21 days	28 days	150 days
LP	0.40 (0.11)	5.15 (0.55)	16.24 (3.44)	20.71 (0.36)[b]	17.90 (7.50)
UP	0.57 (0.09)	2.64 (0.78)	11.86 (3.78)	14.34 (3.15)	14.15 (2.27)
WS	0.63 (0.06)	3.23 (0.04)	7.20 (0.40)	7.73 (0.20)	18.61 (1.04)

[a] LP, lower part or attackable zone; UP, upper part; WS, whole stem. [b] Mean value for two plants; for three plants the values are 14.97 (9.94).

50 mL of methylene chloride; each portion of methylene chloride was filtered through anhydrous sodium sulfate, and the total volume per aliquot was noted. This extract could be partially concentrated or directly used for chromatographic injection. No cleanup was necessary. Under these conditions carbosulfan and carbofuran were coextracted.

Gas Chromatography Analysis. A Hewlett-Packard 5890 gas chromatograph equipped with a nitrogen-selective detector was used in a splitless mode of injection (Ripley and Braun, 1983). The operating parameters were as follows: carrier gas, nitrogen (1 mL/min); column, 25 m × 0.32 mm fused silica column with HP-1 film (0.17 μm); injector temperature, 250 °C; initial oven temperature program, 40 °C for 1 min, raised 200 °C at a rate of 35 °C/min, and maintained at this temperature for 4 min; second plateau at 240 °C reached at 20 °C/min and maintained for 5 min. Chromatograms and quantification were performed using a Spectra-Physics Chromjet integrator. Peak areas of responses in samples were compared to external standards of carbosulfan and carbofuran. The standard molecules were provided by Dr. Ehrenstorffer GmbH Co. and 3-hydroxycarbofuran by FMC Corp., Brussels, Belgium.

RESULTS

Carbosulfan was not detected in the different parts of the stem, except in some low-weight Douglas firs. As Umetsu *et al.* (1980) reported high rates of decomposition of carbosulfan into carbofuran under acidic conditions, a study was conducted on the root system of plants sampled on the field site (pH of the soil, 5.5). Roots were washed with water, prepared, and analyzed as previously described for the stems. After 28 days, the carbosulfan level ranged between 3.70 and 5.22 ppm for an 8.0-g treatment of Marshal/SuSCon per plant. The corresponding levels of carbofuran were 27.0 and 42 ppm. After a flash washing of the roots with methanol, only carbofuran was found at a level of 34 ppm. Carbosulfan did not seem to penetrate into the roots. This fact confirmed the results of analysis of the aerial parts of young Douglas firs. Traces of carbosulfan may then be due to a contamination at sampling time, and protection of the plants against *Hylobius* may then be attributed to carbofuran.

Partition of Carbofuran in Douglas Firs vs Time of Contact with Marshal/SuSCon Granules. *Nursery Study.* Observed levels of carbofuran are shown in Table 1. Standard deviation of the whole-stem samples is lower than that of the partitioned stems, since they have approximately the same growth stage (weight and spread). According to the root spread and to the spatial distribution of granules around the root system, standard deviation in carbofuran uptake will be large. Levels for 28 days have the same range as for 150 days for both upper and lower parts (Table 1). For the whole stem, the increase rate of carbofuran over time is related to plant growth.

Field Study. The controlled release of carbosulfan from Marshal/SuSCon granules occurs over a 2-year period (Cooper *et al.*, 1986). The level of carbofuran in plant tissue was thus determined over time (Table 2). The observed concentration of carbofuran in the stem decreased with time. This may in part be due to the growth of the plant since the granules deliver the same quantity of insecticide. Despite this, field trial results after 18 months show that some damage by the insect was tolerated by the plant in relation to the increased size of the plant (Lemperiere *et al.*, 1989).

Table 2. Residues of Carbofuran in Douglas Firs in the Field with a Marshal/SuSCon Formulation (10% ai)

part of plant	residue level, ppm		
	18 months	24 months	30 months
LP	6.54 (2.76)	2.39 (0.74)	0.69 (0.04)
UP	10.24 (3.72)	4.65 (2.84)	1.39 (0.02)
WS	6.61 (3.16)	3.52 (0.21)	0.93 (0.04)

Table 3. Quantities of Carbofuran Recovered per Plant at Different Times after Treatment According to the Weight of Each Sample

time	treatment	part of plant	range, μg	median, μg
5 months	100 g of ai/m²	LP	93.1–187.9	124.7
		UP	33.5–61.3	42.8
		WS	141.6–236.3	179.5
18 months	8 g/plant	LP	113.2–173.9	143.6
		UP	32.5–74.0	53.3
		WS	134.4–267.2	200.8
24 months	8 g/plant	LP	72.5–98.7	85.6
		UP	32.2–52.0	42.1
		WS	121.6–152.8	129.4

Table 4. Residues of Carbofuran in Douglas Firs of the Nursery 28 Days after Treatment at Different Rates

part of plant	residue level, ppm, at application rate of		
	25 g of ai/m²	50 g of ai/m²	100 g of ai/m²
LP	7.18 (4.55)	7.69 (4.13)	14.97 (9.94)
UP	6.04 (2.64)	4.58 (2.32)	14.34 (3.15)
WS	9.89 (3.18)	9.21 (8.03)	7.73 (0.20)

Table 3 gives the quantity of carbofuran per plant according to the weight of each plant. The results are similar for the 5- and 18-month samples and show a slow decrease after 24 months. For the 30-month samples, the fall in carbofuran content (LP, 7.9 μg; UP, 8.9 μg; WS, 16.5 μg) could be attributed to the exhaustion of the active ingredient in the matrix. The progressive exhaustion of carbosulfan in the granules could be reflected by a decreasing intake of carbofuran in the plant and a possible decreasing concentration of carbosulfan in the soil.

Dose Effect. The dose effect is dependent on the standard deviation of the samples in relation with the inhomogeneity of distribution of the granules in the soil. After 28 days, the higher treatment rate (100 g of ai/m²) led to a significant difference in the level of carbofuran in the plant when compared with the low treatment rates (Table 4). This difference was also reflected in results from the field study, where the treatment at 0.8 g of ai/plant provided good protection when compared with treatments at 0.2 and 0.4 g of ai/plant.

DISCUSSION AND CONCLUSION

In this study, plants treated with Marshal/SuSCon granules were protected from 3 weeks after treatment up to 24 months and carbofuran level was estimated to be stable during this period, in relation with a continuous absorption of the active ingredient present in the soil, the growth of the plant, and its potential metabolizing activity. The effective concentration in Douglas firs seems to be

dependent on the available concentration of active ingredient (carbosulfan or carbofuran) in the soil and is obtained for rates as 100 g of ai/m^2 or 0.8 g of ai/plant. Due to the mode of action of the granules, there is a need for the root system to forage through the whole treated area for a full efficacy of uptake of carbofuran, and a high coefficient of variance can result in the observed residue levels in both nursery and field plots.

Nursery. The plant carbofuran uptake rate is quite fast, and effective concentrations are reached within 3 weeks (Table 1). The high levels observed in Douglas firs are consistent with those observed in Mugho pine by Pree and Saunders (1973, 1974), where a high concentration was noted in the 24-day needle samples and where metabolism of carbofuran seems to be slower than in other plants. An analytical study done in the same conditions on the roots showed that a typical xylemic translocation pathway of carbofuran may be suspected since the concentration is decreasing from the roots to the upper part (roots, 30–40 ppm; LP, 20 ppm; UP, 14 ppm for 28 day samples).

Field. The observed levels of carbofuran in the young Douglas firs are corroborated by the field results demonstrating an efficient protection of the young trees 24 months after treatment (Lemperiere and Julien, 1989). The decrease in carbofuran concentration may be due a metabolic pathway of carbofuran, which cannot be the same as for a "one-dose" application since the plant is in constant contact with the carbosulfan–carbofuran complex. The age of the plant may also affect the way the plant responds to the presence of these insecticides and changes the metabolic pathways proposed by Umetsu *et al.* (1980) and by Pree and Saunders (1973).

Investigation of 3-hydroxycarbofuran, which could be considered another insecticidally active molecule and a major metabolite of carbofuran, was undertaken using the 18-month samples and the analytical method of Nelsen and Cook (1983). This metabolite could not be detected at a significant level, suggesting that the metabolism of carbofuran in Douglas fir could be different from that occurring in other plants as mentioned by Pree and Saunders (1974). These authors proposed *N*-(hydroxymethyl)carbofuran (2,3-dihydro-2,2-dimethyl-1,7-benzofuranyl *N*-(hydroxyethyl)carbamate), which can be metabolized into carbofuran phenol, as the major metabolite. However, carbofuran phenol could not be detected under our chromatographic conditions since our gas chromatograph was not set up to detect it. Although we could not confirm the *N*-(hydroxymethyl)carbofuran pathway in this study, it is postulated as the major breakdown product of the carbofuran residues in these plant samples.

As residue levels are constant 5–18 months after treatment, showing a slow decrease after 24 months and ensuring good protection of the young trees over the critical period of establishment of the plantation, it is of major environmental interest to use this long-term controlled-release formulation in forestry.

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