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Reduced Brood Production of Southern Pine Beetles by Diflubenzuron

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SUMMARY

Treating the female southern pine beetle (Dendroctonus *frontalis* Zimmerman) with the insect growth regulator diflubenzuron will decrease the hatch of eggs deposited in the first 2 dm of egg gallery. Treatment of males had no effect. Surface applications of 1 to 10 μg of diflubenzuron per female beetle and exposure to artificial diets with 100 to 1000 μg/g of diflubenzuron inhibited initial egg hatch, resulting in a larvae-free segment of gallery. The duration of the inhibiting effects depended on the level of initial exposure. Neither fecundity nor the length of gallery excavated were affected. Application of 0.3 percent diflubenzuron to host logs did not appear to have any inhibiting effects on eggs or emerging brood.

Additional keywords: Dimilin®, egg hatch, ovicide, insect growth regulator, bark beetles.

INTRODUCTION

Synthetic insect growth regulators offer potential alternatives to non-specific pesticides in the control of bark beetles without the hazards often associated with these pesticides. Diflubenzuron', a synthetic insect growth regulator, has the potential for affecting adult beetle populations by interfering with the hatch of eggs deposited by treated females (Grosscurt 1977, McGregor and Kramer 1976, McLaughlin 1977, Moore and Taft 1975, Novak and Sehnal 1978). This effect is usually short-lived after exposure is terminated (Grosscurt 1977, Novak and Sehnal1978, Schroeder et al. 1976).

The proposed method for application of diflubenzuron to control bark beetles is to treat the bark surface of potential host trees and, subsequently, .to reduce emerging adult brood populations. Because only a small portion of the life cycle of bark beetles is spent outside the inner bark microenvironment,. applications of diflubenzuron to bark surfaces must deliver an effective dose to the adult beetles as they crawl over and penetrate the bark. Application of diflubenzuron to host logs before beetle attack. has been shown to reduce the number of larvae produced per gallery for the spruce beetle, *Dendroctonus rufipennis* Kirby (Sahota and lbaraki 1980).

This report summarizes the effects of diflubenzuron administered to adult southern pine beetles *(Dendroctonus frontalis* Zimmerman) directly by surface application to the beetles and indirectly by absorption from artificial diets or host logs.

MATERIALS AND METHODS

All southern pine beetles used in the experiments were reared from naturally infested bolts of loblolly pine *(Pinus taeda* L.) which had been placed in 50 liter rearing cans in the laboratory at 21" to 24°C. To minimize uncontrolled matings, adult beetles were collected at 2 to 3 hour intervals as recommended by Covington (1970). Beetles were immediately segregated by sex and stored for up to 3 days at 4°C to accumulate an adequate supply of virgin females.

Uninfested pine bolts used for surface treatment and for inoculating with treated beetles were cut from loblolly pines growing near the edge of abandoned beetle infestations on the Kisatchie National Forest in Central Louisiana. After treatment, all bolts were placed in screened cages in the laboratory to prevent attacks by stray beetles.

'Other names include Dimilin®, TH 6040, and 1-(4-chlorophenyl)-3-(2,6-difluorobenzyl)-urea. This paper reports the results of research only. Mention of a pesticide, or use of a trade or proprietary name does not constitute a recommendation for use by the US. Department of Agriculture nor does it imply registration under FIFRA as amended.

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Surface Treatment of Adult Beetles

Technical grade diflubenzuron (99 percent active ingredient) was dissolved -in AR grade acetone to prepare test solutions containing 0.0, 0.01, 0.1, 1.0, and 10 μg diflubenzuron/μl. Two doses of 0.5 μl of test solution or acetone only were applied 1 minute apart to each beetle's ventral abdomen using a 25 μl Hamilton syringe mounted in a Hamilton 50-stop repeating dispenser. For each test solution the following three treatment combinations were used: a) both sexes treated; b) females treated, males untreated; and c) males treated, females untreated. An additional check with both males and females untreated was also included, resulting in a total of 16 treatment combinations.

Sixteen beetle pairs for each treatment combination were inoculated into holes drilled into uninfested bolts cut from a single loblolly pine. Holes (2 to 3 mm diameter, 1 dm apart) were drilled diagonally through the outer and inner bark. The female was inserted first and was immediately followed by the male. The entrance hole was covered with a piece of outer bark and taped.

Treatment With Artificial Diets

In a preliminary experiment, the addition of the dye Calco Oil Red N-1700 to the diet resulted in red eggs being laid by treated females, showing that ingested fat soluble compounds like diflubenzuron can be deposited in the eggs of treated females. This experiment was done to check if adults could absorb diflubenzuron as they crawled over or ingested treated materials.

An artificial diet (modified from Bridges 1979) was prepared to provide 0, 10, 100, and 1000 μg diflubenzuron per gram of a moist diet. Twenty newly emerged beetles of the same sex were kept in petri dishes containing 6 grams of the moist diet for 7 days. Beetles were carefully removed from the media and only those that could walk were used. Diet-fed females were paired with untreated males and diet-fed males were paired with untreated virgin females. Untreated, newly emerged males and females served as an additional check treatment. Beetle pairs (12 to 24 pairs per treatment combination) were inoculated into uninfested bolts as in the previous experiment. The experiment was replicated 4 times using bolts from 12 loblolly pines.

Surface Treatment of Bolts

This experiment was designed to show if application of diflubenzuron to bark surfaces before beetle attack could deliver to adults a dosage that would be sufficient to inhibit egg hatch or affect subsequent brood emergence and longevity.

Diflubenzuron (WP-25) was suspended in water at concentrations of 0.4, 1.5, and 3.0 grams of active ingredient per liter and sprayed on uninfested pine bolts to the drip point (approximately 5 ml/dm^2). Unsprayed bolts and bolts sprayed only with water served as check treatments. The treated and check bolts were placed inside screened cages and newly emerged beetles of both sexes were introduced daily over a two week period. Approximately 5 beetles per dm^2 of bark surface were placed in each cage. Thirty-five days after initial treatment, half of the bolts were placed in 50 liter rearing cans to collect emerging brood and the other half was examined for attacks, gallery construction, and egg hatch.

To determine the effects of diflubenzuron on gallery construction and egg hatch, bolts were shaved to expose the individual galleries. Each gallery was measured to determine overall gallery length, the distance to the first larval mine, and the distance to the last recently hatched larvae. For each 5 cm segment of gallery beginning from the entrance hole, the number of egg niches, larval mines, and eggs were counted. Percentage of egg hatch was determined for the 1st, 2nd, and 3rd dm of gallery up to the portion of gallery containing only recently hatched larvae or freshly laid eggs.

Brood adults emerging from surface-treated bolts were collected every other day until emergence was complete. Brood emergence per dm of bark surface, time of peak brood emergence, and brood longevity were determined for each bolt. Beetle longevity was the mean number of days until 50 percent of the starved brood kept in petri dishes at 23°C had died.

For all treatments, data was analyzed by one- or two-way analysis of variance and tested for significant differences (α = 0.05 and 0.01) among treatment means and Duncan's New Multiple Range Test and used to separate significantly different treatment means (α = 0.05). The arcsin transformation was applied to all percentages before analysis.

RESULTS

Surface Treatment of Adult Beetles

Surface treatment of adult beetles with a known amount of diflubenzuron demonstrated the sensitivity of the beetle to the test compound and formed the basis for evaluating the effectiveness of the more indirect methods for absorbing the compound (table 1). Diflubenzuron was not acutely toxic at rates up to 10 μg per beetle as judged from the number of pairs excavating egg galleries. Neither diflubenzuron nor acetone alone had a significant effect on the number of egg niches per dm of gallery nor the length of gallery excavated during a 24-day period when compared to newly emerged untreated pairs.

Diflubenzuron at dosages of 1 or 10 μg per beetle caused a significant inhibition in egg hatch in the 1 st and 2nd dm of

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Table 1.-*Southern pine beetle gallery excavation and brood production 24 days after surface treatment of adults with diflubenzuron in acetones*

Sex of treated beetles	Diflubenzuron concentration per beetle	Galleries produced	Average gallery length	Egg niches per dm of gallery+	Egg hatch per dm gallery			Initial larvae-free gallery segment
					1st dm	2nd dm	3rd dm	
	μg	number	dm	number	percent			cm
Both	10.0	7	3.06	30.4	1.8	8.3	37.9	21.1
	1.0	6	2.40	24.2	0.0	5.2	31.3	18.1
	0.1	4	2.80	30.5	36.3	61.0	54.7	6.4
	0.01	6	3.22	26.7	57.7	61.5	54.5	5.3
	0.0	5	2.58	27.5	45.8	55.7	49.3	6.1
Females	10.0	7	2.80	26.9	11.5	36.4	41.0	15.8
	1.0	4	3.75	29.0	6.5	30.5	50.8	15.4
	0.1	7	3.27	31.4	42.7	51.9	47.5	7.2
	0.01	5	2.80	24.5	48.4	70.6	58.1	5.1
	0.0	9	2.99	31.0	57.3	63.7	57.7	5.1
Males	10.0	8	2.91	26.1	55.0	73.1	70.2	4.9
	1.0	13	3.60	28.0	54.3	63.9	57.7	5.1
	0.1	9	2.83	26.1	53.5	54.0	55.9	4.0
	0.01	5	3.80	27.5	42.9	72.9	62.0	6.0
	0.0	5	3.00	28.4	53.4	68.9	73.5	6.2
Neither		9	3.36	24.1	46.2	70.4	68.9	4.7
Source	df:				ANOVA F values			
Sex (S)	2	(‡)	0.52	0.27	6.52**	10.82**	2.42	11.59**
Dosage (D)	4		0.25	0.55	5.69**	7.11**	0.49	12.27**
S × D	8		0.39	0.74	2.42	2.91**	0.31	4.05**

"Treatment values are means from all galleries out of 16 inoculations for each treatment combination. Two way analysis of variance was performed and tested for significance at $\alpha = 0.05$ (*) and $\alpha = 0.01$ (**).

†The first decimeter of gallery had approximately 25 percent fewer egg niches than the average for the entire gallery.

*A chi-square test showed no significant differences between treatments in the number of pairs excavating galleries ($\chi^2 = 7.095$, 8 df).

gallery for treated females mated with either treated or untreated males. Although few eggs hatched in the 1 st dm of gallery at these dosages, egg hatch was normal by the 3rd or 4th dm of gallery. Crosses between untreated females and treated males did not result in a reduction in egg hatch, indicating the effect is not mediated through the males.

Increasing the diflubenzuron dosage to females significantly increased the initial larvae-free gallery segment, but treatment of males had no effect. Egg niches normally begin within 0.2 to 0.3 dm of the entrance hole (Van Sambeek and Kile 1981), thus it appears that diflubenzuron is quickly absorbed by the female and is deposited in the first few eggs that are laid, causing them not to hatch. Length of the initial larvae-free gallery segment would appear to be an excellent parameter for evaluating the effect of diflubenzuron on southern pine beetle reproduction.

Treatment With Artificial Diets

Female beetles tunnelled extensively in the artificial diet with or without diflubenzuron. Male beetles, however, tunnelled very little during the 7 days on the diet, but many were still active and were capable of mating with virgin females when inoculated into bolts. Exposure of either sex to diets with or without diflubenzuron had no significant effect on the percent of beetles excavating galleries (i.e. attacks), the average gallery length after 24 days, or the number of egg niches per dm of gallery (table 2).

The highest amount of diflubenzuron in the diet-fed females reduced the ability of eggs to hatch (table 2). Female beetles placed on diets containing 1000 μg diflubenzuron per gram of moist diet deposited significantly fewer viable eggs in the 1 st and 2nd dm of egg gallery. The length of the initial larvae-free gallery segment was significantly increased by females which fed on diets containing either 100 or 1000 μg diflubenzuron per gram.

Surface Treatment of Bolts

Results from this experiment showed no significant differences due to diflubenzuron on length of gallery excavated, egg niches per dm of gallery, or egg hatch in any of the first 3 dm (table 3). Even the best parameter for assessing diflubenzuron effects, i.e. length of initial larvae-free gallery, was essentially equal to that observed in other experiments (Van Sambeek and Kile 1981). Similarly, diflubenzuron had no effect on the number of days to peak brood emergence or mean longevity of starved emerging brood (table 4).

Bolts sprayed with 3 grams per liter of diflubenzuron had the highest brood production per dm^2 of bark surface and were apparently more heavily attacked initially than bolts in the other treatments. Brood production near the ends of the galleries and from late attacks was greatly reduced due to heavy fungal contamination originating at the ends of the bolts. Bolts with the earliest peak brood emergence tended to produce the most brood beetles.

DISCUSSION

Diflubenzuron had no direct effect on adult southern pine beetles, whether topically applied or ingested. However, Richmond et al. (1978) reported that topical application of 5 μg diflubenzuron per female reduced both the number of galleries and the number of eggs laid during the first 5 days following treatment. Application of 10 μg per female showed no effect in this study on either the number of galleries or egg-niche excavation during a 24-day period. The extended length of the study period could account for the discrepancy in the egg-niche response,, but diflubenzuron generally does not affect the production of eggs (Grosscurt 1977).

In these experiments, as in experiments with other coleopteran beetles, the primary effect of diflubenzuron was egg hatch inhibition. Brood production inhibition has also been recently demonstrated for the spruce bark beetle *(Dendroctonus rufipennis)* following ingestion of diflubenzuron by adults from both artificial diets and surface-treated bolts (Sahota and Ibaraki 1980). The spruce beetle is apparently more sensitive than the southern pine beetle to diflubenzuron because feeding on diets containing 200 μg per gram of diet for 2 days completely inhibited egg hatch along the entire gallery. In most Coleoptera, the effect of an initial application of diflubenzuron is short lived, and continued exposure is necessary for control (Grosscurt 1977, Novak and Sehnal 1978). In this study, the first eggs laid by diflubenzuron-treated females did not hatch, although the last eggs laid hatched normally. For the southern pine beetle, diflubenzuron can significantly reduce egg hatch in the first and second dm of gallery, but may not for the entire gallery. Galleries produced under laboratory conditions were usually 3 dm or more long after 24 days, but galleries produced under field conditions average only 1 to 1.5 dm in length (Thatcher and Pickard 1964). Diflubenzuron has the potential to reduce brood production of the southern pine beetle in the field.

Table *2.-Southern pine beetle gallery excavation and brood production following exposure to diets containing diflubenzuron'*

Sex of treated beetles	Diflubenzuron concentration in moist diet	Pairs inoculated	Attacks	Average gallery length	Egg niches per dm of gallery*	Egg hatch per dm gallery: 1st dm	2nd dm	3rd dm	initial larvae-free gallery segment
	μg/g	number	percent	dm	number	percent			cm
Females	1000	70	48.2	3.04	18.5	20.9	32.8	39.9	8.6
	100	71	51.1	3.03	20.2	33.5	54.3	58.2	6.6
	10	77	50.0	2.87	23.5	40.5	54.0	66.0	4.7
	0	70	40.9	3.19	22.5	46.6	53.0	57.3	2.6
Males	1000	63	61.0	2.96	22.2	46.7	55.8	57.8	3.5
	100	59	55.8	3.05	21.8	47.7	61.1	63.8	3.2
	10	61	51.0	3.02	20.5	49.4	58.9	61.0	2.6
	0	63	55.0	3.32	24.2	54.9	63.8	59.3	1.8
Neither	.....	74	51.1	3.41	23.2	51.5	59.5	59.7	2.8
Source	df:		ANOVA F values						
Sex (S)	1		2.79	0.04	0.37	15.48**	9.65**	1.79	15.19**
Dosage (D)	3		0.32	0.25	0.64	3.86	3.31*	2.78	5.35**
S × D	3		0.43	0.04	0.76	1.26	1.23	1.55	1.70

†Treatment values are means of four replications of entire experiment with 12 to 24 beetle pairs inoculated/treatment combination/replicate. Two way analysis of variance was performed and tested for significance at $\alpha = 0.05$ (*) and $\alpha = 0.01$ (**).

*The first decimeter of gallery had approximately 25 percent fewer egg niches than the average for the entire gallery.

Table *3.-Southern pine beetle galley excavation and brood production 35 days after surface-treatment of bark with a diflubenzuron solution to the drip point*§

Diflubenzuron concentration in water	Galleries examined †	Attacks per dm^2 bark	Average gallery length	Egg niches per dm of gallery*	Egg hatch per dm gallery: 1st dm	2nd dm	3rd dm	Initial larva-free gallery segment
g/liter	number	number/dm^2	dm	number	percent			cm
3.0	45	1.06	3.67	30.7	76.3	87.6	86.7	1.9
1.5	43	0.76	4.03	29.3	66.0	77.0	71.0	3.2
0.4	42	0.61	4.23	31.3	74.7	73.3	75.3	1.9
0.0	45	0.77	4.27	30.0	71.0	82.3	32.3	2.4
		ANOVA F values						
		1.68	0.79	0.52	0.29	2.21	1.98	0.59

"Treatment values are means of three replications of entire experiment. One way analysis of variance was performed and tested for significance at $\alpha = 0.05$.

†The first decimeter of gallery had approximately 25 percent fewer egg niches than the average for the gallery.

*Only galleries without heavy fungal contamination were examined to measure gallery parameters.

Table 4.-*Southern pine beetle brood emergence and longevity from diflubenzuron surface treated bolts*[§]

Diflubenzuron concentration in water	Emerging brood	Days to peak emergence	Brood longevity
g/liter	*number/dm²*	*days*	*days*
3.0	7.96 a	47.7	3.17
1.5	2.42 b	55.5	2.73
0.4	3.75 b	50.4	3.30
0.0	4.27 b	52.9	3.52
Untreated	5.32 b	48.9	2.62
	..... *ANOVA F values* . .		
	5.504*	2.277	0.667

[§]Treatment means values in the same column not sharing the same letter are significantly different at $\alpha = 0.05$ (*) according to Duncan's New Multiple Range Test. ANOVA was done on treatment means for three replications of entire experiment.

Exposure of males to diflubenzuron produced no significant changes in the percentage of eggs hatching or the initial larvae-free gallery segment. Sahota and Ibaraki (1980) also found no significant changes in brood production following treatment of male spruce bark beetle males.

McLaughlin (1978) and Moore et al. (1978) found that changes observed following treatment of males resulted from a direct uptake by the female during mating or contact within the microenvironment.

Unfortunately, the spraying of bolts with diflubenzuron provided no evidence for an increase in length of the initial larvae-free gallery or a reduction in the percentage of eggs hatching. Sahota and Ibaraki (1980) reported that using a 1 percent diflubenzuron spray containing the adjuvant, Savol®, reduced the number of spruce bark beetle larvae produced per gallery by 55 percent and that in 42 percent of the galleries none of the eggs hatched. Grosscurt (1977) and McLaughlin (1977) found that the diflubenzuron formulation, the type of carrier, and the amount of exposure to the carrier-diflubenzuron solution can markedly change the rate of absorption or effective concentration.

This study demonstrated that brood production for the southern pine beetle can be reduced in the laboratory by diflubenzuron. Before field experimentation is attempted, however, the use of better carriers or adjuvants should be explored to improve absorption of the compound by the adult beetles as they move around in the bark microenvironment.

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