PERGAMON

Journal of Stored Products Research 39 (2003) 205–212

Journal of STORED PRODUCTS RESEARCH

www.elsevier.com/locate/jspr

Efficacy of a volatile formulation of hydroprene (Pointsource™) to control *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae)☆

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Accepted 25 August 2001

Abstract

Three- and 4-week-old *Tribolium castaneum* (Herbst), the red flour beetle, and *Tribolium confusum* (du Val), the confused flour beetle, were exposed at five different temperature–relative humidity (r.h.) combinations to a volatile formulation of the insect growth regulator (IGR) hydroprene (called Pointsource™). Typical effects associated with IGR exposure, such as arrested larval growth, morphological deformities in adults, twisted and deformed wings, and incomplete adult emergence were produced in both species. *Tribolium castaneum* and *T. confusum* were susceptible to Pointsource™, but *T. castaneum* appeared to be the more susceptible species. More 3-week-old larvae of both species were arrested in that stage compared to the 4-week-old larvae. Nearly all of the 3- and 4-week-old *T. castaneum* larvae that were able to complete development to the adult stage quickly died after they emerged and were grossly morphologically deformed. In contrast, some emerged adult *T. confusum* remained alive after they emerged and were not deformed in any manner or had only twisted and incomplete wings. A greater percentage of larvae of both species were arrested in the larval stage and more adults died after they emerged in exposure studies conducted at 32°C, 75% r.h. as compared with 32°C, 30% r.h., but the reverse was true for exposures conducted at 27°C. Pointsource™ appears to have excellent potential for use in controlling *Tribolium* species within indoor facilities. Published by Elsevier Science Ltd.

Keywords: Volatile hydroprene; Control; *Tribolium castaneum*; *Tribolium confusum*

☆This paper reports the results of research only. Mention of a proprietary chemical or a trade name does not constitute a recommendation or endorsement by the US Department of Agriculture.

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0022-474X/02/$ - see front matter Published by Elsevier Science Ltd.

PII: S0022-474X(01)00054-6

1. Introduction

The insect growth regulator (IGR) hydroprene (Gentrol) is labeled as an aerosol and as a general surface treatment to control stored-product insects inside food warehouses and other storage facilities. IGRs do not kill adult insects, instead they eliminate the infestations by inhibiting development of immatures and reducing adult emergence. Exposure of *Tribolium castaneum* (Herbst), the red flour beetle, and *Tribolium confusum* (du Val), the confused flour beetle to IGRs can often result in arrested larval growth, and morphological defects in immatures and emerged adults (Williams and Amos, 1974; Amos et al., 1977). Similar morphological deformities in adult cockroaches can occur as a result of larval exposure (King and Bennett, 1988). Twisted and incomplete wings are common symptoms of exposure to hydroprene, and deformed adult cockroaches can be flightless and sterile (King and Bennett, 1989; Atkinson et al., 1992; Short and Edwards, 1992; Reid and Bennett, 1994).

Tribolium castaneum and *T. confusum* are common pests found in indoor food storage facilities. Many research studies in which IGRs have been tested against these species and other indoor stored-product pests have involved mixing the chemical with insect diet and seeding the diet with eggs (Oberlander et al., 1997). With this technique, the IGR is often effective because the earlier an insect is exposed to IGRs in the larval life cycle, the less likely it is to reach the adult stage. However, this may not accurately simulate exposure under actual field conditions. Also, there is little published information regarding the susceptibility of late-instar larvae to IGRs, or the effects of temperature and humidity on the efficacy of IGRs.

Pointsource™ is a volatile formulation of hydroprene that is being marketed for cockroach control inside residential structures. It is dispensed from a wick, and is activated simply by pressing two tabs on the wick. Information on the label states that it will give control of cockroaches for up to 3 months. The objectives of the following experiment were to determine: (1) the susceptibility of late-instar larvae of *T. castaneum* and *T. confusum* to Pointsource™; and (2) the effects of temperature and relative humidity (r.h.) on product efficacy.

2. Materials and methods

Replicated trials were conducted in walk-in climate-controlled environmental chambers (3.69 m length, 1.85 m diameter, 2.46 m height) at the following five temperature–r.h. combinations: 22°C–75% r.h., 27°C–30% r.h., 27°C–75% r.h., 32°C–30% r.h., and 32°C–75% r.h. The two levels of r.h. were chosen to represent the extremes of the normal range that would occur in an indoor facility. However, a treatment of 22°C, 30% r.h. was not included because of the possibility of prolonged development at this temperature–r.h. combination. For each trial, the exposure arenas consisted of plastic storage boxes with dimensions of $61.0 \times 40.6 \times 15.24$ cm. For each temperature–r.h. combination, ten 3-week-old and ten 4-week-old *T. castaneum* larvae were put in separate 100×15 mm Petri dishes lined with filter paper and containing about 500 mg of flour; then the procedure was repeated for both 3- and 4-week-old *T. confusum* larvae. The four dishes containing the two age groups of each species were put inside the plastic boxes, which were in turn placed inside the environmental chamber. Larvae of both species were obtained from pesticide-susceptible colonies reared at 27°C, 60% r.h.; at these conditions, both species normally reach the pre-pupal stage by 5 weeks, and emerge as adults a week later.

The Pointsource™ wick was taped to the underside of the lid of the plastic storage box and activated by pressing the tabs, then the lid was attached to the box. Four individual replicate treated boxes were set on shelves inside the chamber, along with an untreated control box. An additional control box was set in a second chamber at the same environmental conditions. After 2 weeks, the containers were checked weekly for 4 weeks to determine if exposure to the IGR would cause a delay in adult emergence, and the number of larvae, pupae, live emerged adults, and emerged adults that had died were recorded each week. Live adults were classified as having either wing deformities (twisted and crooked wings) or gross morphological deformities (wings that could not be folded or were missing, unsclerotized portions of the exoskeleton, and deformed body parts).

The data were analyzed using the ANOVA and the GLM procedures of the Statistical Analysis System (SAS Institute, 1987). The number of larvae and pupae were tabulated each week, and insect species, the age of the larvae (3-week-old versus 4-week-old), and the environmental conditions at which the larvae were exposed were main effects. Data for the weekly observations was a repeated measure. The numbers of dead and living adults were totaled for weekly observations, and analyses for number of dead emerged adults as a percentage of total adult emergence, the percentage of those dead adults that were deformed, and the percentage of deformed live emerged adults were performed with insect species, age of larvae, and environmental conditions of exposure as main effects. Means for each response variable at the five environmental conditions were separated using the Waller–Duncan k-ratio t-test.

3. Results

At each exposure condition, total adult emergence of 3- and 4-week untreated control larvae of each species exposed in the same chamber as the treatments and in the second chamber was virtually 100%. None of the live emerged adults from either set of controls were deformed in any manner, and there was no difference ($P \geqslant 0.05$) in adult emergence between the control box held in the same chamber as the treatment replicates and the control box held by itself in the second chamber. Data for controls were therefore eliminated from further statistical analysis.

Main effects larval age ($F = 2088.7$, df$=1,60$) and environmental condition ($F = 312.5$, df$=4,60$) and the repeated measure week ($F = 22.2$, df$=3,180$) were all significant ($P<0.05$) for the percentage of individuals that remained in the larval stage, but species was not significant ($F = 0.4$, df$=1,60$, $P = 0.51$). All interactions except species $\times$ age, environmental condition $\times$ week, and environmental condition $\times$ species $\times$ larval age $\times$ week were also significant ($P<0.05$). Except for trials conducted at 32°C, 30% r.h., a greater percentage of the 3-week-old *T. castaneum* larvae were still in the larval stage at the conclusion of the test as compared to the 4-week-old larvae (Table 1). The percentage of exposed 3-week-old *T. castaneum* larvae that remained in that stage gradually declined with each weekly observation, but at each week there were fewer larvae at 27°C, 30% r.h. compared to the other environmental conditions. Most of the 4-week-old larvae were able to complete development, and there were no significant differences

Table 1
Percentage (mean ± SEM) of 3- and 4-week-old *Tribolium castaneum*, red flour beetle, and *T. confusum*, confused flour beetle, failing to molt after 2–5 weeks exposure to Pointsource™ at five temperature–relative humidity (r.h.) combinations[a]

Species	Initial age of larvae (weeks)	Temperature–r.h. condition	Percentage molt failure—weeks of exposure			
			2	3	4	5
T. castaneum	3	22°C–75% r.h.	72.5 ± 11.1a	62.5 ± 14.3a	60.0 ± 16.8a	60.0 ± 16.8a
		27°C–30% r.h.	55.0 ± 15.0a	50.0 ± 12.2a	47.0 ± 15.0a	45.0 ± 13.2a
		27°C–75% r.h.	70.0 ± 17.8a	50.0 ± 10.8a	45.0 ± 10.4a	40.0 ± 10.8a
		32°C–30% r.h.	45.0 ± 9.6a	15.0 ± 11.9b	12.0 ± 9.5b	7.0 ± 4.8b
		32°C–75% r.h.	83.5 ± 14.3a	45.5 ± 8.6a	45.0 ± 8.6a	45.0 ± 8.6a
	4	22°C–75% r.h.	25.0 ± 8.6a	10.0 ± 4.1a	7.5 ± 4.7a	7.5 ± 4.7a
		27°C–30% r.h.	15.0 ± 8.6a	15.0 ± 8.6a	15.0 ± 8.6a	15.0 ± 8.6a
		27°C–75% r.h.	10.0 ± 4.1a	10.0 ± 4.1a	10.0 ± 4.1a	10.0 ± 4.1a
		32°C–30% r.h.	7.0 ± 7.5a	5.0 ± 5.0a	2.0 ± 2.5a	2.5 ± 2.5a
		32°C–75% r.h.	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
T. confusum	3	22°C–75% r.h.	92.5 ± 4.8a	92.5 ± 4.8a	92.5 ± 4.8a	92.5 ± 4.8a
		27°C–30% r.h.	70.0 ± 21.2a	75.0 ± 11.9ab	72.5 ± 13.8ab	72.5 ± 13.8ab
		27°C–75% r.h.	5.0 ± 2.9b	5.0 ± 2.9c	5.0 ± 2.9c	2.5 ± 2.5c
		32°C–30% r.h.	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
		32°C–75% r.h.	65.0 ± 10.4a	62.5 ± 8.6b	62.5 ± 8.6b	62.5 ± 8.6b
	4	22°C–75% r.h.	35.0 ± 8.7a	25.0 ± 11.9a	25.0 ± 11.9a	22.5 ± 11.1a
		27°C–30% r.h.	10.0 ± 4.1b	10.0 ± 4.1b	10.0 ± 4.1b	10.0 ± 4.1b
		27°C–75% r.h.	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
		32°C–30% r.h.	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b
		32°C–75% r.h.	7.5 ± 7.5b	5.0 ± 5.0b	5.0 ± 50b	0.0 ± 0.0b

[a] Means for percentage molt failure within temperature-humidity combinations (columns) for each species and larval age that are followed by the same letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan *k*-ratio *t*-test).

among the environmental conditions ($P \geqslant 0.05$) with respect to the percentage of individuals remaining in the larval stage.

The percentage of 3-week-old *T. confusum* larvae that failed to molt to the pupal and adult stages was relatively constant throughout the weekly observations, and most of the larvae exposed at 27°C, 75% r.h. and 32°C, 30% r.h. were able to complete development. When 4-week-old *T. confusum* larvae were exposed, most were able to complete development, with more larvae remaining in that stage when exposed to Pointsource™ at 22°C and 75% r.h. compared to the other four environmental conditions.

At the conclusion of the test, pupation of both species was virtually 100%, therefore pupae were eliminated from further statistical analysis. The next analysis variable, the percentage of dead adults as a proportion of total adult emergence, was significant ($P < 0.05$) for main effects species ($F = 138.3$, df = 1, 47) and environmental condition ($F = 24.5$, df = 4, 47) but not for larval age ($F = 0.8$, df = 1, 47, $P = 0.37$). All interactions except species × age were also significant ($P < 0.05$). Nearly all of the 3- and 4-week-old *T. castaneum* larvae that were able to complete development to the adult stage quickly died after they emerged (Table 2), and all were grossly

Table 2
Percentage (mean ± SEM) of dead *Tribolium castaneum* and *Tribolium confusum* adults as a percentage of the total number of adults that were able to emerge after being exposed to Pointsource™ as larvae under five sets of environmental conditions[a]

Species	Initial age of larvae (weeks)	Percentage mortality after emergence from environmental condition				
		22°C–75% r.h.	27°C–30% r.h.	27°C–75% r.h.	32°C–30% r.h.	32°C–75% r.h.
T. castaneum	3	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	4	100 ± 0.0a	97.2 ± 2.8a	100 ± 0.0a	100 ± 0.0a	97.5 ± 2.5a
T. confusum	3	[b]	100 ± 0.0a	68.0 ± 5.1b	25.0 ± 11.9c	89.8 ± 11.1ab
	4	100 ± 0.0a	95.0 ± 5.0a	2.7 ± 2.7c	33.1 ± 16.4b	67.5 ± 11.1ab

[a] Means for percentage mortality after emergence for 3- and 4-week-old larvae of each species at each environmental condition (rows) that are followed by the same letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan *k*-ratio *t*-test).
[b] No adults emerged under these conditions.

morphologically deformed in some manner. No 3-week-old *T. confusum* larvae emerged as adults when exposed as larvae at 22°C and 75% r.h., and all 3-week-old larvae exposed at 27°C and 30% r.h. died after they emerged. Fewer adults died after they emerged in the trial conducted at 32°C and 30% r.h. compared to the trials at 27°C and 32°C, 75% r.h. When 4-week-old *T. confusum* larvae were exposed at 22°C, 75% r.h. and 27°C, 30% r.h., nearly all of the emerged adults quickly died (100% and 95.5%, respectively) and all were deformed (Table 2). Only 2.7% of the adults that emerged from larvae exposed at 27°C and 75% r.h. died, and more dead adults were found from larvae exposed at 32°C, 75% r.h. compared to 32°C, 30% r.h.

The next analysis variable, the percentage of live adults that exhibited wing deformities, as a percentage of total adult emergence, was significant ($P < 0.05$) for main effects species ($F = 38.5$, $\text{df} = 1,47$) and environmental condition ($F = 8.8$, $\text{df} = 4,47$) but not for larval age ($F = 0.1$ $\text{df} = 1,47$, $P = 0.8$), and all interactions except environmental condition × larval age and species × larval age were also significant ($P < 0.05$). All *T. castaneum* adults died after emergence and there were no live adults with either wing or gross morphological deformities. Except for 4-week-old larvae exposed at 27°C, 30% r.h., the number of live emerged *T. confusum* adults with wing deformities ranged from 0% to 16%, and the only significant difference occurred at that environmental condition (Table 3). The percentage of adults with gross deformities, as a percentage of total adult emergence, was also significant with respect to main effects species ($F = 7.1$, $\text{df} = 1,47$) and environmental condition ($F = 3.3$, $\text{df} = 4,47$) but not for larval age ($F = 0.1$, $\text{df} = 1,47$, $P = 0.8$). No interactions were significant ($P \geqslant 0.05$). Adult *T. confusum* emerging from 4-week-old larvae exhibited gross morphological deformities when exposed at 32°C, 30% r.h. (Table 3).

4. Discussion

The presence of morphological deformities has been positively correlated with sterility and other reproductive effects when last-instar nymphs of *Blattella germanica* (L.), the

Table 3
Percentage (mean ± SEM) live emerged *T. confusum* adults that exhibited either wing deformities or gross morphological deformities as a result of exposure as larvae to Pointsource™ under five sets of environmental conditions[a]

Initial age of larvae (weeks)	Environmental conditions[b]	Percentage adults deformed	
		Wing deformities	Gross deformities
3	22°C–75% r.h.	—[b]	—
	27°C–30% r.h.	—	—
	27°C–75% r.h.	16.1 ± 5.4a	7.5 ± 7.5a
	32°C–30% r.h.	15.0 ± 8.7a	0.0 ± 0.0a
	32°C–75% r.h.	0.0 ± 0.0a	11.1 ± 11.1a
4	22°C–75% r.h.	—	—
	27°C–30% r.h.	2.5 ± 2.5b	—
	27°C–75% r.h.	33.0 ± 7.2a	15.0 ± 6.4a
	32°C–30% r.h.	8.1 ± 2.7b	0.0 ± 0.0b
	32°C–75% r.h.	7.5 ± 2.5b	12.5 ± 7.5a

[a] Means for percentage deformed *T. confusum* adults within environmental condition (columns) for each larval age that are followed by the same letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan *k*-ratio *t*-test).
[b] No live emerged adults were found under these conditions.

German cockroach, were exposed to hydroprene (King and Bennett, 1989). Treatment during this last stadium was associated with physiological and morphological effects that were most pronounced when treatment occurred early in the stadium (King and Bennett, 1991). Although the sensitivity at various stages of development was not determined in this experiment with 4-week-old *T. castaneum* and *T. confusum* larvae, both species would normally be in this final larval stage for about a week when reared at 27°C, 60% r.h. Morphological effects were also produced when these last-instar beetle larvae were exposed to the volatile formulation of hydroprene.

Additional published studies of *B. germanica* show that hydroprene had volatile activity even when it was applied as a liquid spray to various surfaces (King and Bennett, 1988). Third-instar nymphs were exposed at maturity to the atmosphere above various surfaces that had been treated with 18.9 mg (AI)/m^2 hydroprene or 78.6 mg (AI)/m^2 fenoxycarb. No volatile effects were observed for fenoxycarb, and although survival of nymphs exposed to hydroprene was not affected, morphological effects in nymphs and adults and reproductive effects in adults occurred with all treated surfaces.

Insect growth regulators are generally more toxic to the earlier nymphal stages of *B. germanica* (King and Bennett, 1988). In most studies with stored-product insects, eggs or adults have been exposed to treated grain or treated diet, and suppression of the F1 generation was measured (Oberlander et al., 1997). There are little if any published data regarding the susceptibility of different larval ages or stages. Results of the current study showed that for both *T. castaneum* and *T. confusum*, more 3-week-old larvae remained in that stage compared to the 4-week-old larvae when exposed to volatile hydroprene. However, there was no significant effect due to larval age in the adult characters that were assessed.

In this experiment *T. castaneum* appeared to be more susceptible to Pointsource™ than *T. confusum*. Virtually all of the emerged adult *T. castaneum* quickly died even if they were able to complete emergence, and all of these dead adults were grossly deformed. In contrast, not all of the emerged adult *T. confusum* died shortly after they emerged, and some of these emerged live adults had only slight wing deformities or were not deformed in any manner. Similar results were obtained in a previous study in which 4-week-old *T. castaneum* and *T. confusum* larvae were exposed for varying time intervals on concrete treated with the label rate of the liquid formulation of hydroprene (Gentrol), or continuously exposed on concrete treated with different concentrations of hydroprene (Arthur, 2001).

Pointsource™ may effectively control *T. castaneum* and *T. confusum* in confined spaces inside food storage facilities; however, environmental conditions may affect control. More larvae of both species were arrested in the larval stage and more adults died after they emerged in exposure studies conducted at 32°C, 75% r.h. compared to 32°C, 30% r.h., but the reverse was true for exposures conducted at 27°C. Similar temperature effects were obtained in which 4-week-old *T. castaneum* and *T. confusum* larvae were exposed on concrete treated with the Gentrol formulation of hydroprene (Arthur, 2001). In these tests, the percentage of arrested larvae, dead adults, and deformed live adults was greatest at 32°C, 75% r.h. compared to 32°C, 30% and 57% r.h. As temperature increases, larvae would be expected to complete development in a shorter time, thereby decreasing the time in which they are exposed to the chemical. Conversely, it is also possible that as temperature increases, more of the hydroprene is being dispensed from the wick.

Acknowledgements

I thank C.K. Hoernemann for excellent technical assistance with this study. I also thank Wellmark International for providing product and financial support. I also thank R.T. Arbogast and C. Cannon for reviewing the paper prior to journal submission.

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