Residual and sub-lethal effects of an attracticide formulation on *Choristoneura rosaceana* (Harris), *Pandemis pyrusana* Kearfott, and *Cydia pomonella* (L.) males (Lepidoptera: Tortricidae)

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Abstract

A bioassay was developed to evaluate effects on *Choristoneura rosaceana* (Harris), *Pandemis pyrusana* Kearfott, and *Cydia pomonella* (L.), males exposed to an attracticide loaded with 6% permethrin and aged under field conditions. High levels of knock down (>97%) at 1 h, and mortality (> 94%) at 24 h after exposure to fresh attracticide were observed among the three species. Mortality and knock down decreased linearly as the attracticide aged and was greater than 50% even with attracticide aged 30 days. Leg autotomy was observed in all three species, being a novel response for both leafroller species. Leg autotomy reached maximum values (62.7%, *C. rosaceana*; 41.2%, *C. pomonella*; and 40.5%, *P. pyrusana*) with males exposed to fresh or attracticide aged 10 days, increasing significantly between 1 and 24 h after exposure. Leg autotomy was highly variable over time with more than 50% of males dropping at least one leg but less than 3% dropping up to 4 among tortricid species. Most autotomized legs were from the second and third pair. The zone of abscission was the junction between the trochanter and femur in all individuals. Most leg autotomized males died.

Keywords: Bioassay; Knock down; Leg autotomy; Permethrin

1. Introduction

Modern attracticides are baits that combine the use of synthetic pheromones and a contact insecticide to attract then kill the target species (Charmillot et al., 2000). This technology represents a specific alternative to conventional pesticides (Hofer and Angst, 1995). Attracticides have been reported for several species of Lepidoptera, e.g. *Cydia pomonella* (L.) (Tortricidae) and *Pectinophora gossypiella* (Saunders) (Gelechiidae) (Charmillot et al., 1996, 2000; Curkovic and Brunner, 2003).

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Lethal effects of the attracticide are proportional to the insecticide concentration and exposure time (De Souza et al., 1992). Time-dependent mortality depends on insecticide persistence in the formulation. To improve their residual activity, attracticide formulations include ultraviolet (UV) screeners to protect the pheromone from degradation. Encapsulated suspensions (De Souza et al., 1992) and greases (Charmillot et al., 1996, 2000) have been used as attracticide formulations.

Sub-lethal effects can be as important as direct mortality if intoxication reduces the ability of male moths to search for females or the fecundity of females that mate with intoxicated males (Haynes et al., 1986). Insecticides used in attracticide formulations must act quickly by contact, be highly toxic, and non-repellent

(De Souza et al., 1992; Downham et al., 1995). Pyrethroids have a fast knock down and cause high mortality, even at low concentrations, while having the least repellent effect among several types of insecticides evaluated against *Spodoptera littoralis* (Boisduval) adult males (De Souza et al., 1992), and are the preferred insecticides in attracticide formulations used against *P. gossypiella* and *C. pomonella* (Hofer and Angst, 1995; Charmillot et al., 1996). However, some pyrethroids (e.g. permethrin) have repellent effects on mosquitoes (Diptera: Culicidae).

The response from adult moths to insecticides has not been studied in many species (Moore and Tabashnik, 1989; Sun et al., 2000). Some methods described to evaluate insecticidal activity on adult moths are residual films (Chen et al., 1985), topical application (Riedl et al., 1985; Clark and Haynes, 1992), and flight tunnel tests (Haynes et al., 1986). However, these methods do not control insect exposure, use chilling treatments or anesthesia (CO_2), and/or are difficult to use with relatively small species. The vacuum method described by Busvine (1964) avoids the potentially negative physiological effects anesthesia and low temperatures could have on test organisms (Schumacher et al., 1997) and provides an easy handling system, even with small individuals. The research presented here describes a bioassay methodology and the evaluation of the residual and sub-lethal effects of an attracticide on males of *Choristoneura rosaceana*, *Pandemis pyrusana*, and *C. pomonella*.

2. Materials and methods

2.1. Attracticide

The attracticide formulation used (Sirene®CM) was provided by Novartis, Basel, Switzerland. It is a paste containing 0.16% codlemone, 6% permethrin plus inert ingredients; UV-screen, carbon, sticker, and thickener (Phillip Kirsch, IPM Technologies, Portland, OR, pers. comm.). The delivery system was a plastic container with 100 g of formulated product with an applicator valve that released drops of ≈50 μl of product. The attracticide was stored in the dark at 0–1 °C until use. We did not test attracticide without insecticide.

2.2. Insects

Pupae of *C. rosaceana* and *P. pyrusana* were obtained from colonies maintained at the Washington State University Tree Fruit Research and Extension Center (WSU-TFREC), Wenatchee, WA, USA. Pupae of *C. pomonella* were obtained from a mass rearing facility at the USDA-ARS laboratory in Wapato, WA, USA. The rearing conditions were 23 ± 2 °C and 64 ± 5% RH and 26 ± 3 °C and 55 ± 6% RH at Wenatchee and Wapato, respectively. The photoperiod regime in both Wapato and Wenatchee was 16:8 (L:D) h using fluorescent lights. All pupae were sexed, washed in 5% bleach solution, dried off, placed in plastic cups, and kept under the rearing conditions described above. Upon emergence, adult males were provided with a honey water solution via cotton wicks. One to 3-day old males of the three species were used in bioassays since preliminary results show 99% survival (Curkovic, 2004) and reproductive maturity at this age (Delisle, 1995).

2.3. Attracticide aging

One hundred attracticide drops were applied to branches of apple trees in an unsprayed orchard at the WSU-TFREC during the summer of 1998, and aged under field conditions. Drops were protected from contact by moths or other large organisms by a wire mesh screen ($0.6\times0.6\,cm^2$ hardware cloth). A sample of attracticide (20 drops) was randomly collected every 10 days for a total period of 40 days. The aged attracticide drops were stored at less than −14 °C. Prior to use in bioassays the aged samples were placed at 24 ± 1 °C for 15 min.

2.4. Adult moth bioassay

The vacuum method was adapted to evaluate residual effect of attracticide formulations. The method allowed control of moth exposure. A 30 ml plastic cup (S-100, Prairie Packaging, Bedford Park, IL) was placed on the tip of the vacuum nose (Bioquip #2820) and a 10 ml BD syringe (Becton Dickinson and Co., Franklin Lakes, NJ) with its needle tip cut and covered by a piece of mesh screen was introduced through the cup. The method allowed the handling of individual moths rapidly and without injury (Curkovic, 2004). The tip of the vacuum device was brought close to the dorsal side of the moth over the thorax until air suction held it firmly in place. The moth was then allowed a brief contact, ≈1 s/moth, of one or more legs with the attracticide (Fig. 1). This method was thought to provide a good mimic of behavior observed in the field for *C. pomonella*, where males contact the attracticide with their legs and then fly away immediately after contact (Curkovic, 2004). Similar behavior has been observed with *Epiphyas postvittana* (Walker) by Brockerhoff and Suckling (1999). Males handled using the vacuum method but not exposed to the attracticide were the control treatment. After contact with the attracticide each moth was placed individually in a transparent plastic cup (30 ml) under rearing conditions described above and supplied with honey water solution via a cotton wick.

Fig. 1. Male *Choristoneura rosaceana* handled with vacuum and contacting an attracticide aged droplet.

2.5. Evaluation of attracticide effect

Knock down, mortality and leg autotomy, the self-amputation of one or more legs, were recorded at 1 and 24 h after exposure of moths to an attracticide treatment. Moth mortality was characterized by individuals with no, or very weak, movement of appendages and an inability to assume an upright position. Knock down was characterized as an individual unable to maintain an upright position for more than 5 s. These individuals usually ended up on their backs but were able to actively move appendages with strong but erratic movements. For individuals showing leg autotomy register of the number of legs dropped, leg pair, and zone of abscission was recorded. Mortality and knock down data (%) were analyzed by linear regression (Minitab, 2000) and leg autotomy by χ^2 test and an adaptation of SNK test for multiple comparisons of proportions ($p = 0.05$, Zar, 1996).

3. Results and discussion

Males handled with the vacuum method but not exposed to the attracticide formulation (control treatment) did not die and presented less than 5% of individuals knocked down, therefore no corrections on mortality and knock down were performed. Leg autotomy at 1 and 24 h after exposure to attracticide was also below 5% for both, *C. pomonella* and *P. pyrusana*, but ranged between 5% and 8% for *C. rosaceana* males at 1 and 24 h. Among males exposed to the formulation, data for knock down (1 h) and mortality (24 h after treatment) were correlated with the time the attracticide was aged in the field. Maximum response was obtained using fresh attracticide (0 days in the field) where all species showed nearly 100% mortality. Charmillot et al. (2000) and Suckling and Brockerhoff (1999) also found high mortality on *C. pomonella* and *E. postvittana* males, respectively, exposed to a fresh attracticide formulation. In our study, the correlation between mortality and attracticide (Fig. 2) age was best explained by a linear relationship for the three species ($r^2 = 0.86$ to 0.94 and p-values $= 0.004$ to 0.015). Mortality decreased slowly (slopes ranging from -1.58 to -1.25) when moths were exposed to aged droplets of attracticide, approaching 50% with attracticide aged 30 days in the field, probably due to enhanced persistence of the insecticide because of the UV-screen (Quisumbing and Kydonieus, 1989). Charmillot et al. (2000) also found insecticidal activity decreasing slowly over time for a similar formulation. Knock down (1 h evaluation) did show a more erratic tendency in *C. rosaceana* exposed to attracticide aged 40 days (Fig. 3). The r^2 ranged from 0.64 to 0.87 and p-values ranged from 0.014 to 0.066 for the three species. Knock down will limit male abilities to find females and mate, removing them from the reproductive population (De Souza et al, 1992).

Leg autotomy was observed in the three species (Tables 1–3), being a novel response for *C. rosaceana*

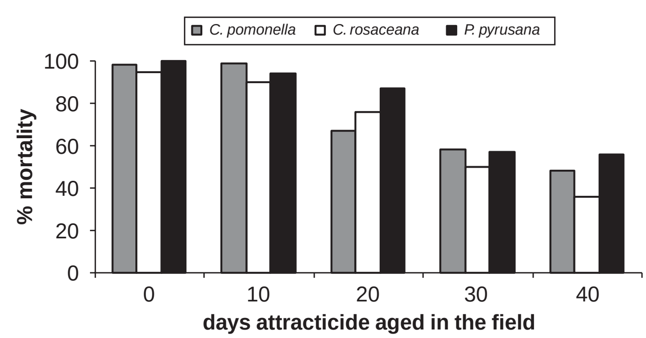

Fig. 2. Mortality (%) of *Cydia pomonella*, *Choristoneura rosaceana*, and *Pandemis pyrusana* male moths exposed to attracticides aged for different periods (days) in the field.

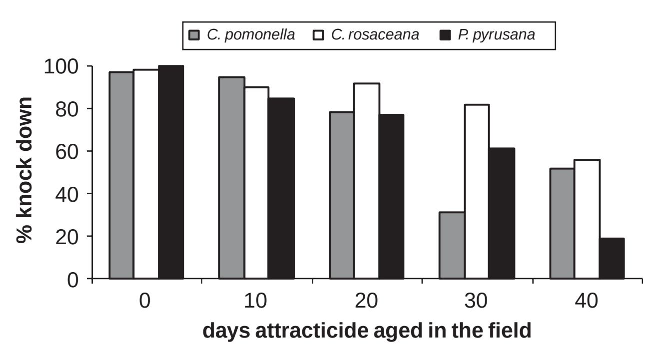

Fig. 3. Knock down (%) of *Cydia pomonella*, *Choristoneura rosaceana*, and *Pandemis pyrusana* male moths exposed to attracticides aged for different periods (days) in the field.

Table 1
Number (#) of *C. rosaceana* males dropping legs after exposure to attracticide droplets aged in the field

Choristoneura rosaceana	Days droplets were aged in the field				
Legs autotomized (#)	0	10	20	30	40
1	36 a	16 a	18 a	8 a	5 a
2	22 a	7 a	10 a	2 a	4 a
3	7 a	3 a	3 a	0 a	3 a
4	3 a	1 a	0 a	0 a	1 a
Total males autotomizing legs	68 a	27 ab	31 a	10 b	13 ab
N (males treated)	110	58	50	38	25

Same letter within a row indicates no significant differences between attracticide ages, χ^2 and SNK test ($p = 0.05$).

Table 2
Number (#) of *P. pyrusana* males dropping legs after exposure to attracticide droplets aged in the field

Pandemis pyrusana	Days droplets were aged in the field				
Legs autotomized (#)	0	10	20	30	40
1	25 a	39 a	15 a	10 a	8 a
2	16 a	11 a	4 a	2 a	1 a
3	7 a	3 a	3 a	0 a	1 a
4	2 a	2 a	1 a	0 a	1 a
Total males autotomizing legs	50 a	55 a	23 a	12 a	11 a
N (males treated)	112	131	54	36	23

Same letter within a row indicates no significant differences between attracticide ages, χ^2 and SNK test ($p = 0.05$).

Table 3
Number (#) of *C. pomonella* males dropping legs after exposure to attracticide droplets aged in the field

Cydia pomonella	Days droplets were aged in the field				
legs autotomized (#)	0	10	20	30	40
1	15 a	9 a	5 a	5 a	4 a
2	2 a	5 a	3 a	0 a	1 a
3	0 a	0 a	1 a	0 a	0 a
4	0	0	0	0	0
Total males autotomizing legs	17 a	14 a	9 a	5 a	5 a
N (males treated)	45	34	31	23	16

Same letter within a row indicates no significant differences between attracticide ages, χ^2 and SNK test ($p = 0.05$).
No test was conducted for the 4 autotomized legs category.

and *P. pyrusana*. It was also reported on *C. pomonella* males exposed to attracticides by Krupke (1999). Insecticide induced leg autotomy has been observed in several Lepidoptera (*Sitotroga cerealella* (Olivier), *Plutella xylostella* (L.), *P. gossypiella*) (Moore et al., 1989). In this study we did not test attracticide without insecticide in order to evaluate the possible physical stress on legs contacting the sticky substance, but new experiments indicate that this is not an important factor causing leg autotomy. Only attracticide loaded with permethrin causes significant autotomy (Curkovic, Brunner, and Brown, in preparation). Leg autotomy consists in an externally induced self-amputation of legs (Moore and Tabashnik, 1989). In our data, leg autotomy increased significantly between 1 and 24 h, reaching a maximum of 62.7% (dropping one or more legs/individual) in *C. rosaceana* males exposed to fresh material, whereas up to 42% and 40.5% males were observed dropping at least one leg in *C. pomonella* and *P. pyrusana*, respectively. Moore et al. (1992) observed up to 52% leg autotomy in *Plutella xylostella* adult males exposed to fenvalerate residues. Ortego and Bowers (1996) found never over 60% autotomy in *Schistocerca americana* (Drury) nymphs injected (in abdomen or left hind leg femur) or topically applied with an ecdysone agonist insecticide. They also found leg autotomy after topical application of deltamethrin at low concentrations (1 μg/g) whereas it occurred at low rates or very slowly on exposure to organophosphate or organochlorine insecticides. Leg autotomy is a chemically induced response, occurring on exposure to one of several different chemical compounds, including different types of insecticides and toxins (Moore et al., 1992; Ortego and Bowers, 1996). Autotomy is reported to be a reflexive action controlled by metathoracic ganglia in *S. americana* (Ortego and Bowers, 1996). In our study, leg autotomy was observed as soon as 2–3 min after contact with an attracticide in some individuals. Autotomy was also a relatively quick response in *S. americana* (Ortego and Bowers, 1996). Leg autotomy in *C. rosaceana*, *P. pyrusana*, and *C. pomonella* matches the description given by Moore and Tabashnik (1989).

In our experiments, leg autotomy showed an erratic behavior over time. The number of individuals dropping legs decreased within treatments, i.e. age of the attracticide (see Tables 1–3). However, no statistical differences were observed among individuals dropping any particular number of legs along with the age of the attracticide (13 out of 14 analyses, Tables 1–3), except for the total number in *C. rosaceana* (Table 1). It is possible that leg autotomy was not dependent on the concentration of permethrin in attracticide, but this hypothesis needs further research in order to be confirmed.

All individuals self-amputated their legs at the trochanter–femur joint as was also observed with *P. xylostella* (Moore and Tabashnik, 1989). The distributions of the proportion of males showing autotomy indicate that, among all males belonging to the three species, 8.4–10.7% dropped legs belonging to the first pair, 23.9–57% to the second, and 18.3–43.6% to the third pair. It is unclear whether dropped legs or others were those contacting previously the attracticide. Ortego and Bowers (1996) found that *S. americana* mostly

(48%) dropped the leg that was injected with an ecdysone agonist insecticide. In our study most males dropping legs were knocked down (1 h) and almost all of them die (24 h), suggesting that leg autotomy, within the time-frame evaluated, does not protect males from effects of the pesticide.

In nature, leg autotomy is a mechanism to escape from predators or sticky substrates; to stop hemorrhage, or to reduce spread of a toxicant into the body of some arachnids and insects (Randall, 1981; Eizner and Camazine, 1983; Carlberg, 1986). However, these later hypotheses need to be tested for the three species evaluated in our study.

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