Insect Growth Regulator Impact on Fecundity and Fertility of Adult Tufted Apple Bud Moth, *Platynota idaeusalis* Walker[1]

Clayton T. Myers[2] and Larry A. Hull

Penn State University, Fruit Research and Extension Center, Biglerville, PA 17307 USA

J. Entomol. Sci. 38(3): 420-430 (July 2003)

Abstract The tufted apple bud moth, *Platynota idaeusalis* Walker, is a major pest of apples in the eastern United States. Resistance to conventional insecticide chemistries has made this pest difficult to control. The insect growth regulators (IGRs) tebufenozide and methoxyfenozide, which are target-specific to lepidopteran pests, have been shown to have high efficacy against *P. idaeusalis* larvae. These compounds are known to affect adult moths in related tortricid species, but the effects of exposure on adult *P. idaeusalis* are unknown. This study investigated the effects of adult exposure on the fecundity and fertility of *P. idaeusalis.* Both tebufenozide and methoxyfenozide significantly reduced the fecundity and fertility of female *P. idaeusalis* adults that were exposed to dry films of formulated IGR residue. Female fecundity and fertility also were reduced when untreated or treated females mated with treated males. Contrary to other related studies, there was no difference in fecundity/fertility reduction efficacy between tebufenozide and methoxyfenozide. Thus, because tebufenozide and methoxyfenozide have reproductive activity against adults, such compounds may bring significant benefits to integrated pest management (IPM) of tree fruits.

Key Words Tufted apple bud moth, *Platynota idaeusalis* Walker, tebufenozide, methoxyfenozide, ecdysone agonist, reproductive effects, sublethal effects

The tufted apple bud moth, *Platynota idaeusalis* Walker (Lepidoptera: Tortricidae), is a major pest of apples in Pennsylvania (Hull et al. 1997b). Fruit damage is caused by the feeding of larvae from both generations on the surface of the fruit. Although larvae from the second generation that hatch in late July to late August and feed on the fruit until harvest have been shown to cause the majority of damage, both generations can cause significant feeding injury to fruit (Knight and Hull 1987). The most economically damaging injury is inflicted on apples destined for the fresh market. The 'pin-hole' feeding of *P. idaeusalis* reduces packing quality and aesthetic appearance, causing low market value.

While *P. idaeusalis* damage is superficial, high levels of injury are also economically damaging to processing fruit. Feeding damage can put injured apples into a lower grade by causing premature ripening, water loss, and infectious bacterial/fungal rots while the apples are kept in cold storage (Knight and Hull 1987). Apples with too much *P. idaeusalis* damage can only be used for making cider or juice (Knight and Hull 1987). Also, with the increase in problematic internal Lepidoptera (i.e., codling

[1]Received 30 August 2002; accepted for publication 15 October 2002.
[2]Direct offprint requests (email: ctm134@psu.edu).

moth and Oriental fruit moth) in the mid-Atlantic region, processing apples are increasingly scrutinized for the presence of live larvae, including *P. idaeusalis* in bins of harvested apples (Hull et al., unpubl. data).

Effective control of *P. idaeusalis* has been complicated over the years by the development of resistance to conventional insecticides. Organophosphate (OP) resistance levels of up to 40-fold have been documented in several *P. idaeusalis* populations in Pennsylvania (Hull et al. 1997b). *Platynota idaeusalis* also has developed resistance to the carbamate insecticide, methomyl (Hull et al. 1997a). Methomyl was more widely used in the 1980's and 90's to combat OP-resistant *P. idaeusalis* populations (Hull et al. 1997b). Locally, *P. idaeusalis* populations have been found that possess 10-fold to 15-fold reduction in susceptibility to methomyl (Hull et al. 1997a). In addition, methomyl is much more toxic to several species of beneficial insects found in orchards, in comparison with the 'softer' OP compounds (Hull et al. 1997b). Increased use of methomyl has, in some cases, led to increases in field populations of spider mite pests and has increased the need for more applications of acaricides, a costly practice that is counter to the goals of apple IPM strategies. This problem, coupled with the discovery of methomyl resistance in *P. idaeusalis,* has led researchers to investigate the efficiency of more novel and narrow-spectrum insecticide chemistries in order to combat this damaging pest (Biddinger and Hull 1995, Biddinger et al. 1996).

Tebufenozide (RH-5992, Confirm®) and methoxyfenozide (RH-2485, Intrepid®), which were developed by the Rohm and Haas Company (now Dow AgroSciences, Indianapolis, IN), are members of a novel group of chemicals known as ecdysone agonists, a subclass of insect growth regulators (IGRs). IGRs constitute a novel insecticide group that target the insect endocrine system. In contrast to conventional insecticide classes (i.e., OPs, carbamates, pyrethroids, etc.), IGRs are not neurotoxins, but rather act as hormone agonists/antagonists and thus disrupt insect endocrine physiology leading to developmental and reproductive abnormalities in insects. Chemically, tebufenozide and methoxyfenozide are bisacylhydrazine ecdysteroid agonists (Dhadialla et al. 1998). These are nonsteroidal compounds that mimic the insect hormone ecdysone (20-hydroxyecdysone) and cause premature molting in lepidopteran larvae. Binding with ecdysone receptors in lepidopteran larvae leads to deformity and eventually death of the insect (Smagghe and Degheele 1994, Retnakaran et al. 1995).

While ecdysone is common to all insects, tebufenozide and methoxyfenozide have been shown to be specific in activity to the ecdysone receptors of Lepidoptera and, thus, are mostly non-toxic to insects of other orders, including beneficial predators and parasitoids in various ecosystems (Brown 1996, Smagghe and Degheele 1994, 1995, Butler et al., 1997). Within Lepidoptera, these compounds exhibit very high activity and efficacy against a variety of economic pests in various agricultural and natural systems (Chandler et al. 1992, Charmillot et al. 1994a, Butler et al. 1997, Trisyono and Chippendale 1997, 1998).

Both tebufenozide and methoxyfenozide are efficacious against several tortricid pests in field trials. While methoxyfenozide is more broad-spectrum in larval activity than tebufenozide, both have been shown to give excellent control of *P. idaeusalis* (Hull 1995, 1997, 2000, Hull and Krawczyk 1998, Hull and Myers 2001) and several related leafroller pests (Hull and Krawczyk 1998, Brunner et al. 1999, Hull 2000, Hull and Myers 2001).

In addition to direct larval toxicity, tebufenozide and methoxyfenozide have sub-

lethal effects on lepidopteran adults and larvae, as well as direct ovicidal activity against some species. *Platynota idaeusalis* larvae fed tebufenozide in artificial diet had lower adult fecundity than untreated individuals, or individuals treated with sublethal doses of some other insecticides. This indicates a sublethal reproductive effect of these compounds (Biddinger and Hull 1999). Tebufenozide also induces molting and causes a reduction in the pre-emergence period in diapausing codling moth, *Cydia pomonella* L., larvae (Sauphanor et al. 1999). European corn borer larvae exposed to tebufenozide had smaller pupal weights, slower larval growth rate, and lower rates of adult emergence (Trisyono and Chippendale 1997).

Most IGR studies with *P. idaeusalis* have examined effects on larvae. Little work has been done to investigate the effects of tebufenozide and methoxyfenozide exposure on adults. Charmillot et al. (1994b) showed that adult exposure to tebufenozide and methoxyfenozide caused significant reductions in fecundity, fertility, and egg viability of the grape pests, *Lobesia botrana* Denis & Schiffermüller and *Eupoecilia ambiguella* Hübner. The same study also showed that these IGR compounds had a significant ovicidal effect on the same pests. Adult exposure to tebufenozide residues caused a significant reduction of fecundity and fertility in codling moth, both in the lab and the field (Sun and Barrett 1999, Knight 2000). Redbanded leafroller, *Argyrotaenia velutinana* Walker, and obliquebanded leafroller, *Choristoneura rosaceana* Harris, adults exposed to tebufenozide and methoxyfenozide residues also had reduced fecundity and fertility, confirming the reproductive effect of these compounds on adults across several tortricid species (Sun et al. 2000). The goal of this study was to investigate the reproductive and possible chemosterilizing effects of exposure to tebufenozide and methoxyfenozide residues on adult *P. idaeusalis.*

Materials and Methods

Insects. Adult moths for this experiment were obtained from a laboratory-reared colony of a local field strain of *P. Idaeusalis,* collected the previous summer from commercial orchards in Adams Co., PA, as egg masses and larvae. Larvae were kept in the lab for 1 yr and reared through approximately 5 generations, with some overlap. The insects were reared on a meridic lima bean-based diet (Meagher 1985) in growth chambers maintained at 22 to 25°C, on a photoperiod of 16h:8h (light:dark). Larvae were placed as neonates into 28 mL plastic diet cups that were each provisioned with approximately 5 to 7 mL of diet. Five neonate larvae were placed into each cup to feed freely and develop. Individuals were sexed as pupae and then segregated by sex to prevent premature mating after adult eclosion.

Bioassays. Plastic Petri dishes (5 cm diam × 1 cm) were treated with a 0.5 mL aliquot of a treatment suspension of either tebufenozide (RH-5992 70WP) or methoxyfenozide (RH-2485 80WP) that approximated the label dilute rate for apple (90 ppm tebufenozide, 45 ppm methoxyfenozide). Treatment suspensions also contained a 300 ppm solution of the adjuvant Tween®-20 (Atlas Chemical Co., Alexandria, Egypt, polyoxy ethylene sorbitan monolaurate) that aided in uniform distribution of the treatment mixture over the entire Petri dish. The formulated insecticide and Tween-20 were mixed with distilled water to the appropriate concentrations. Untreated control dishes were only treated with 0.5-mL aliquots of a 300 ppm Tween-20 solution. After treatment, all dishes were air dried under a fume hood while being periodically swirled to ensure uniform deposition of treatment residue. After the dishes were air dried, they

were transferred to a refrigerator for later use. All dishes were used within 5 days of treatment.

Newly-emerged moths (<36 h) were placed into Petri dishes (2 ♂ / dish or 1 ♀ / dish) with the appropriate treatment residue. After 24 h of exposure, the moths were removed and placed into 1-L cardboard mating chambers. Each chamber consisted of a 12-cm cardboard cylinder, approximately 10 cm in diam. The ends of the cylinder were covered with mesh screen, which was held on by cardboard rings that fit over the end of the cylinder. Each chamber was provisioned with a small cotton wick that contained a 10% honey solution and a small piece of wax paper as an oviposition medium. One female and two male moths were placed in each chamber to mate until female death. Honey solutions on the cotton wick were replenished as needed, and the wax paper medium was replaced daily.

There were three treatment groups for each insecticide (tebufenozide or methoxyfenozide), with 25 replicates (mating groups) each as follows: untreated male × treated female, treated male × untreated female, and treated male × treated female. Additionally, there was one control group: untreated male × untreated female.

Egg masses were collected daily from the mating chambers until the death of the female moth. Egg masses were cut from the wax paper and were placed into labeled, untreated, 5-cm plastic Petri dishes that were stored in a growth chamber as above. Eggs were allowed to hatch inside each Petri dish, where they were left for subsequent counting.

Fecundity/fertility measurements. Fecundity was measured four ways: (1) calculating the percentage of females that oviposited within each treatment, (2) counting the total number of egg masses laid per female, (3) counting the total number of eggs laid per female, and (4) measuring egg mass size by counting the number of eggs in each egg mass. The latter two steps were done by visually counting the number of eggs in each egg mass under a stereomicroscope. For some replicates, hatched egg shells (stained with red food coloring) were counted after neonates had hatched. After some work was completed, we determined that staining did not increase the speed of counting, and thus it was ceased. Either method is reliable, as both stained and unstained eggs are easily discernable under a stereomicroscope, and egg shells are not consumed by neonate larvae.

Fertility was measured two ways: (1) calculating the mean larval hatch percentage for each replicate (i.e., the number of hatched larvae divided by the number of eggs), and (2) counting the total number of live larvae per female.

Statistical analysis was done using a one-way analysis of variance, using a completely randomized design. Means were separated using Fisher's protected least significant difference (LSD) test, using an alpha value of 0.05 (Abacus Concepts 1989). In a one case, raw data were transformed using a square root function in order to stabilize variance.

Results

Fecundity. Both tebufenozide and methoxyfenozide significantly reduced adult *P. idaeusalis* fecundity following 24 h of exposure to a dry film residue (Table 1). Exposure significantly impacted the percentage of females laying eggs ($F = 9.184$, df = 168, $P = 0.0001$). Female moths that were exposed to residues of tebufenozide or methoxyfenozide were less likely to oviposit than untreated females. Also, untreated females that mated with exposed males were less likely to oviposit than those mated

Table 1. Effects of tebufenozide and methoxyfenozide residues on *P. idaeusalis* fecundity

Treatment	n	% Females laying eggs (±SE)	Mean egg masses laid per female (±SE)	Mean total eggs laid per female (±SE)*	n**	Mean number of eggs laid per egg mass (±SE)
Untreated–both	25	80 (8.2)a	2.32 (0.35)a	97.5 (17.7)a	58	42.0 (3.8)a
Tebufenozide–Female	25	12 (6.6)c	0.28 (0.17)d	18.3 (10.6)cd	7	65.4 (7.3)a
Tebufenozide–Male	25	44 (10.1)b	1.04 (0.28)bc	44.9 (15.4)bc	26	43.2 (6.9)a
Tebufenozide–both	25	16 (7.5)c	0.24 (0.12)d	11.1 (6.8)d	6	46.3 (10.6)a
Methoxyfenozide–Female	25	28 (9.2)bc	0.40 (0.15)cd	24.0 (8.7)cd	10	60.1 (11.2)a
Methoxyfenozide–Male	25	44 (10.1)b	1.40 (0.38)b	70.9 (21.7)b	35	50.6 (7.5)a
Methoxyfenozide–both	25	8 (5.5)c	0.08 (0.06)d	1.0 (0.7)d	2	12.0 (4.0)a

Means with different letters are significantly different (a = 0.05), as determined by Fisher's Protected LSD.

* Analysis of square-root transformed data.

** n = total number of egg masses for each treatment (i.e., number of replicates in analysis).

with untreated males. Treated females that were mated with treated males were less likely to oviposit than untreated females that mated with treated males. There was no significant difference between tebufenozide and methoxyfenozide regarding the percentage of females laying eggs.

Females exposed to either tebufenozide or methoxyfenozide laid fewer egg masses than untreated females ($F = 11.260$, df = 168, $P = 0.0001$). Untreated females that mated with exposed males likewise laid fewer egg masses than those mated with untreated males, but laid more egg masses than treated females that mated with either treated or untreated males. There was no difference between the impact of tebufenozide and methoxyfenozide on the number of egg masses laid.

Exposed females also laid fewer total eggs than untreated females ($F = 8.459$, df = 168, $P = 0.0001$). Untreated females that mated with treated males laid fewer eggs than those mated with untreated males. In the methoxyfenozide treatment, untreated females mated with treated males laid more eggs than treated females that mated with either treated or untreated males. There was no impact on the number of eggs laid.

Egg mass size (number of eggs laid per egg mass) was unaffected by exposure of either sex to tebufenozide or methoxyfenozide ($F = 1.25$, df = 137, $P = 0.2854$). However, overall decreases in fecundity led to very low N values for some treatments, making it difficult to draw a firm conclusion.

Fertility. Hatch percentage of *P. idaeusalis* eggs was unaffected by adult exposure to either tebufenozide or methoxyfenozide ($F = 1.554$, df = 51, $P = 0.1800$, Table 2). As with measurement of egg mass size, low N values for some treatments make it difficult to strongly support this conclusion. Most importantly, exposure to either compound significantly ($F = 4.444$, df = 168, $P = 0.0003$) reduced the number of live neonates per female (Table 2). This number represents each female's living offspring potential. Treated females (mated with either a treated or untreated male) did not differ significantly from untreated females that mated with treated males. There was no significant difference between similar pairing treatments of tebufenozide and methoxyfenozide on *P. idaeusalis* fertility.

Discussion

Both tebufenozide and methoxyfenozide are highly efficacious against *P. idaeusalis* and also have sublethal reproductive effects on *P. idaeusalis* adults. Our data indicate that adult *P. idaeusalis* females exposed to tebufenozide or methoxyfenozide had significantly lower fecundity and fertility than unexposed adults. Additionally, untreated females that mated with treated males had lower fecundity and fertility than untreated females that mated with untreated males, indicating a reproductive effect on males. These results are in agreement with the previous research reported on related tortricid species, including *L. botrana, E. ambiguella* (Charmillot et al. 1994b), *A. velutinana, C. rosaceana* (Sun et al. 2000), *C. pomonella* (Sun and Barrett 1999), and larvae of *P. idaeusalis* (Biddinger and Hull 1999).

The observed reductions in fecundity and fertility in our study were the result of exposed females laying fewer egg masses (Table 1). Because neither egg mass size nor hatch percentage significantly varied among treatments (Tables 1, 2), most of the variation in number of eggs and number of neonates produced per female can be attributed to laying fewer egg masses. This indicates a reduction in the number of 'egg-laying episodes' among treated individuals.

Table 2. Effects of tebufenozide and methoxyfenozide residues on *P. idaeusalis* fertility

Treatment	n*	% of eggs hatching (±SE)	n	Mean number of live larvae per female (±SE)
Untreated–Both	20	35.2 (6.8)a	25	44.6 (11.9)a
Tebufenozide–Female	3	22.5 (11.6)a	25	5.0 (3.6)bc
Tebufenozide–Male	11	38.4 (7.7)a	25	23.3 (9.8)b
Tebufenozide–Both	4	10.7 (9.7)a	25	2.6 (2.5)bc
Methoxyfenozide–Female	7	27 (12.5)a	25	8.0 (4.4)bc
Methoxyfenozide–Male	11	15 (6.8)a	25	19.0 (10.8)bc
Methoxyfenozide–Both	2	0 (0.0)a	25	0.0 (0.0)c

Means with different letters are significantly different (a = 0.05), as determined by Fisher's Protected LSD.
* n = the number of females laying eggs (i.e., the number of replicates in analysis).

In studies by Sun et al. (2000), *A. velutinana* and *C. rosaceana* adults exhibited significant reductions in fecundity and fertility after continuous exposure of both male and female adults to residues of tebufenozide or methoxyfenozide on apple leaves and mating cages. Exposure for 24 h of either male or female moths to tebufenozide or methoxyfenozide resulted in reductions in fecundity for *C. rosaceana* while only female exposure to methoxyfenozide reduced fecundity in *A. velutinana*. Reduction in *A. velutinana* egg fertility occurred with both male and female exposure to methoxyfenozide, but only with female exposure when using tebufenozide. Conversely, *C. rosaceana* fertility was significantly reduced by female exposure to methoxyfenozide, or by either male or female exposure to tebufenozide.

Cydia pomonella adults also exhibited significant reductions in fecundity and fertility after 1 h of female exposure to tebufenozide, and after 24 h of male exposure to tebufenozide (Knight 2000). Continuous exposure of both sexes of *C. pomonella* to either tebufenozide or methoxyfenozide also resulted in significant reductions in fecundity and fertility (Sun and Barrett 1999). Significant reductions in *C. pomonella* egg hatch were observed on apple foliage in the field when moths were exposed to tebufenozide residues for 24 h or when adults and eggs were exposed for 10 d (Knight 2000). *Cydia pomonella* fecundity and fertility were significantly reduced after female exposure to tebufenozide for 24 h, or after either male or female exposure to methoxyfenozide (Sun and Barrett 1999).

In contrast to work by Charmillot et al. (1994b), we observed no differences in fecundity and fertility reductions between tebufenozide and methoxyfenozide. In most cases, methoxyfenozide-exposed moths had numerically lower fecundity and fertility, but these differences were not statistically significant. Sun and Barrett (1999) and Sun et al. (2000) observed that methoxyfenozide exposure led to greater reductions in hatch percentage than tebufenozide, but found no differences between the two compounds on fecundity (number of eggs laid per female). In our study, not a single viable larva hatched from any of the methoxyfenozide female* methoxyfenozide male treatments (Table 2). However, this result was not statistically different from exposure of both males and females to tebufenozide. The relative concentration difference between methoxyfenozide and tebufenozide (90 ppm tebufenozide vs 45 ppm methoxyfenozide, 2-fold difference) used in this study was consistent with previous research (Charmillot et al. 1994b, Sun and Barrett 1999, Sun et al. 2000).

In contrast to our results, other researchers, who were working with related tortricid species (Sun and Barrett 1999, Sun et al. 2000, Knight 2000) observed marked reductions in the hatch percentage of eggs after adult exposure to tebufenozide or methoxyfenozide. While we observed marked reductions in egg number and in the number of live neonates per female, we did not observe significant differences in hatch percentage among any of the treatments (Table 2). This may be due to differences in the concentrations (use of dilute concentrations of both compounds) used in this study compared to others. For this experiment, moths were exposed to dishes treated with 90 ppm tebufenozide and 45 ppm methoxyfenozide. This is in contrast to Sun and Barrett (1999) and Sun et al. (2000) who exposed moths to 360 ppm tebufenozide and 180 ppm methoxyfenozide and Knight (2000) who used 370 ppm tebufenozide. This amounts to a four-fold difference in concentration which may have had a significant impact on fertility efficacy.

The mechanisms for this reduction in fecundity and fertility in female moths are thought to be due to interference with oogenesis and possibly resorption of eggs (Smagghe and Degheele 1994). Clearly, ecdysone receptors constitute an important

component of the cascade of physiological events involved with reproductive development. The point of disruption in such a cascade, however, is unknown. For male moths, it has been proposed that IGRs may interfere with spermatogenesis and/or reduction of sperm mobility in males (Biddinger and Hull 1999), but this hypothesis remains unconfirmed. Reduction in sperm mobility may explain reductions in hatch percentage and female fertility, but would not explain reductions in fecundity, as female moths are capable of ovipositing unfertilized eggs. Others have speculated that tebufenozide or methoxyfenozide could be transferred to female moths by males during copulation, thus causing fecundity/fertility reductions in the female (Biddinger, pers. comm.). Very little is known about how these compounds are taken up by adults, as neither compound has a high level of contact activity.

Clearly, exposure to residues of tebufenozide or methoxyfenozide can significantly impact oviposition success of *P. idaeusalis,* and, thus, may impact population dynamics in the field. Any such chemosterilant activity could provide added control of *P. idaeusalis* populations in the field if applications were targeted to adults. Some workers speculate that beyond the larvicidal effects, applications of tebufenozide against tortricids could have a lasting 'carry-over effect' that is exhibited in reduced fecundity and fertility and modified behavior of surviving individuals (Cadogan et al. 2002). Beyond an initial population knockdown, reduction of the ensuing brood via fertility reduction would provide an added benefit for long-term management of tortricid pests.

Additional research is needed on the molecular mechanisms of tebufenozide and methoxyfenozide receptor-binding in adult stages. It has been well documented that these chemicals bind to ecdysone receptors in larvae and induce a premature and lethal molt. However, the target site in adults (and the subsequent binding affinity kinetics, etc.) are unknown. If a different suite of receptors is bound in adults (or the binding chemistry differs), there would be evidence of a second mode-of-action for these compounds. Multiple active sites are not uncommon, especially with insecticides that mimic natural insect hormones, which can be recycled in different capacities over the course of holometabolous insect development.

Additionally, more field work needs to be done to verify that the chemosterilant activity of tebufenozide and methoxyfenozide against *P. idaeusalis* translates into field efficacy. If reduction of larval populations could be accomplished by targeting adults, it could possibly allow for reductions of insecticide inputs against *P. idaeusalis* later in the season. Further elucidation of efficacy may allow apple growers to add yet another control tactic to the arsenal for controlling *P. idaeusalis,* thus adding to the sustainability of apple IPM programs in the eastern United States.

Acknowledgments

The authors thank Greg Krawczyk and David Biddinger (PSU Department of Entomology) for assistance with methodology as well as manuscript review, Dorothy Dunning (West Virginia University Department of Biology) for advice and suggestions, and laboratory assistants Seth Althoff and Brian Pitzer for their assistance with chamber maintenance and data collection.

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