The activity of Emamectin benzoate against Winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae) in Redmond Linden (*Tilia americana* 'Redmond') trees.

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Abstract

Winter Moth *Operophtera brumata* L. (Lepidoptera: Geometridae) is an introduced defoliator with a broad host range including, shade and flowering trees. These inchworms hatch early, prior to bud break and crawl into dormant tree buds. Neonates feed on leaf primordia, evidenced as shot-hole injury on newly emerged leaves. Unchecked the developing larvae notch, then skeletonize leaf tissues to the mid-rib. Repeated defoliations reduce tree health and may lead to tree decline or death. Injury from Winter Moth (WM) in hardwood trees was first observed in Winchester, MA USA in 2004. Fleisher et. al and Reardon and Webb reported Acephate efficacy against Gypsy moth using stem implants. In this study, we compare the activity of ACE-jet (Acephate 97S) to Emamectin benzoate in *Tilia americana* 'Redmond' (Redmond Linden). Dosages of the insecticides were increased with increasing tree size. Acephate (97 S) was applied at 0.5 to 0.6 g/cm (1.25 to 1.5 g/inch) DBH, in spring 2005 only, by tree injection. Emamectin benzoate was applied at 0.128 to 0.16 g/cm (0.32 to 0.40 g/inch) DBH. Applications of EB were made in spring and fall of 2005 also by stem injection. Trees were rated for the extent of defoliation severity. No choice feeding bioassays were conducted in two consecutive years beginning in 2006. In addition to the canopy defoliation ratings and bioassays, EB leaf residues were conducted in 2007. Both Acephate and Emamectin benzoate provided effective control the first season following application. Both canopy defoliation and no-choice WM feeding assays indicate activity of EB in trees for two years. Only EB provided sufficient residual activity to control WM for two full seasons. Either spring or fall applied EB was effective. This research suggests that one application of Emamectin benzoate by stem injection every two years is sufficient to protect trees from defoliation by Winter moth larvae.

Key Words: Winter moth, Linden, Emamectin benzoate, tree injection

Introduction

Winter moth (WM) is an introduced defoliator with a broad host range including, shade (oaks, maples, linden, ash) and flowering (cherry, apple, pear) trees (U MASS fact sheet). The insects hatch early, prior to bud break and crawl between the bud scales of yet dormant trees to get to the leaf primordia. Neonates feed on leaf primordia, evidenced as shot-holes on newly emerged leaves. Unchecked the developing larvae notch, then completely skeletonize leaf tissues to the mid-rib. Repeated defoliations reduce tree health (Kulman, 1971), increase susceptibility to abiotic agents and may result, ultimately in tree death (U MASS fact sheet). Injury from Winter Moth *Operophtera brumata* L. (Lepidoptera:

Geometridae) in hardwood trees was first observed in Winchester, MA, USA in 2004. No studies to date have been reported using micro-injected Emamectin benzoate (EB) for activity against winter moth, although tree injection techniques have been used to protect trees against other defoliators. In one study, injections of Rubidium was used to mark Gypsy moth which used 4 application methods including, root flare injection, pressurized injection, bole injection and implantation (Fleischer et al., 1989). In that study, root flare injections resulted in the rapid and most persistent accumulation of Rubidium chloride into the foliage. Though not statistically different at 4 weeks, bole injections were the least persistent. In two subsequent studies, Acephate, applied by stem implantation demonstrated control of Gypsy moth *(Lymantria dispar*), (Fleischer et al., 1989, and Reardon and Webb, 1990). In those studies, applications of Acephate were made in early spring, concurrent with leaf expansion and early larval development. Acephate, an organophosphate chemistry is toxic to a broad range of insects including aphids, leaf miners, Lepidopterous larvae, sawflies and thrips, among others (EXTOXNET PIP – Acephate). It is readily soluble in water, but does not persist in plant tissues for very long periods, but rather is subject to metabolic degradation (inchem.org). Therefore, timing of application is based upon pest activity for optimal effectiveness. Emamectin benzoate (4-(*epi*-methylamino)-4-deoxyavermectin B1 benzoate) is a recently introduced Avermectin derivative. Avermectins are the fermentation by-products of a soil actinomycete, *Streptomyces avermitilis*. Technical Emamectin benzoate B1 is a mixture of B1a and B1b homologues in ~90 to 10% ratio. EB is extremely potent against Lepidopterous caterpillars, LC 90's reported between 2-18 ppbs (Syngenta Crop Protection). Argentine et al reported on emamectin benzoate toxicity to five species of Lepidoptera including Beet armyworm, *Spodoptera exigua* (Hübner); tobacco budworm, *Heliothis virescens* (F.); diamondback moth, *Plutella xylostella* (L.); and cabbage looper *Trichoplusia ni* (Hübner) (2002). In that document LC 90 toxicities from formulated EB (Proclaim 0.16 EC from Merck & Co., Inc. Rahway, N.J., USA) was 20.7 for beet armyworm and 11.2 ppbs for tobacco budworm larval feeding. An airbrush was used to apply the test chemistry to foliage prior to conducting larval feeding bioassays. In this study, we (1) compare efficacy of Emamectin benzoate to Acephate applications, (2) consider fall treatment of Emamectin benzoate, and (3) report on the two year efficacy against Winter moth, an exotic defoliator by a basal injection technique.

Methods

Tilia americana 'Redmond' (Redmond Linden) was selected for study. The 18 Lindens selected ranged in size from 18 to 65 cm (7-26") DBH. Trees were grouped in three size classes, which were, <30 (12"), 30-59 (12-23") and 60-89 (24-35") cm upon which increasing dosages were based. ACE-jet (Acephate 97S, Arborjet, Inc. Woburn, MA USA) and Emamectin benzoate 4% (later, TREE-äge, manufactured by Syngenta Crop Protection, Greensboro, NC, USA) were used in these trials. Spring applications were made prior to bud break (between 5/05 – 5/11/05). Fall applications were made on 10/20/2005, at leaf senescence.

Size distribution, treatment and number of replicates in Linden appear in the table, below.

Cm DBH	ACE-jet	EB S	UTC	EB F	Totals
<30	2	1	2	3	8
30-59	2	2	2	3	9
60-89	0	0	1	0	1
Totals:	4	3	5	6	18

Dosages increased with increasing tree diameter (Diameter Breast Height). No injected tree fell into the largest diameter class. Acephate injection solution was prepared by mixing 15 gm 97S Acephate/100 mL de-ionized (DI) water. 3.3 mL of solution per cm DBH was applied to trees up to 30 and cm of tree DBH; and 4.0 mL of solution was applied per cm DBH for trees greater in size. EB was applied at 3.2, or 4.0 mL/cm DBH (8, or 10 mL/DBH") rates. Equal part DI water was used to dilute the EB formulation at tree injection. Grams of Acephate and EB varied according to tree diameter and appear in the table, below.

Grams A.I./cm DBH, mL of formulation and volume of solutions applied for each treatment

Formulation	cm DBH	g/cm DBH	mL/cm	mL/tree
Acephate	<30	0.50	3.3	59-96
Acephate	30-59	0.60	4.0	120-236
Emamectin	<30	0.128	3.2	116-186
Emamectin	30-59	0.160	4.0	240-472

We applied the systemic insecticide treatments into the trunk flare using the Arborjet Tree I.V. The trunk flare is stem tissue located at the base of the tree, typically within 30 cm of the soil. Applications were made at 2.10 kg/cm^2 (30 PSI) of pressure. The number of application sites used was based on one per 15 to 20 cm (6-8") circumference; no less than 4 injection sites were applied in a tree. A 7 mm (9/32") drill bit was used to drill 15 mm (5/8") into the sapwood. The No. 3 Arborplug was then inserted into the drilled hole to create an injection site. An injector needle was then inserted which pierced an internal septum to allow liquid to be administered.

Initial evaluations of spring treated trees were performed 30 DAT. At 30 DAT (6/5/2005) the canopies of the 12 treatments applied were photographed and evaluated for degree of defoliation by WM.

Defoliation Severity

Trees were rated for canopy defoliation severity. This method was particularly useful for large tree evaluation. The severity rating is based on the degree of leaf damaged (shot hole,

notching, skeletonization) and percent of canopy affected (<20, 20-33, >33%). A study of 86 trees in late June 2005, including 4 species of hardwoods, *Acer platanoides* (Norway maple), *A.p*. 'Crimson King', *A.p*. 'Schwedleri' (Schwedler Red Maple), *Acer saccharum* (Sugar maple), *Fraxinus pennsylvanica* 'Marshall's Seedless' (Marshall's Seedless Ash), and *Tilia americana* 'Redmond' (Redmond American Linden) was used to develop the canopy severity rating . In that study *F. pennsylvanica* was not a preferred host; most likely because bud break was later in that population than for the other species studied (Tikkanen et al). Final evaluations were completed in July 2007. The Defoliation Severity Scale developed to assess trees in this study is presented in the table, below.

Canopy Defoliation Severity Ratings

Foliar Symptoms	**% canopy**	**rating**
Minor damage (leaves with shot holes)	<20	1
Intermediate injury (leaves notched)	20-33	2
Severe (leaves skeletonized)	>33	3

In this rating system the overall percent canopy affected is rated using a 3 point scale, but the extent of leaf skeletonized is also taken into account, to quantify leaf damage. By multiplying these, one develops ratings that range from very low severity (of 1) to severe defoliation (of 9).

No Choice Bioassays

No choice feeding bioassays were conducted in 2006 and 2007 on small trees [mean 4.2 cm DBH (10.5")]. Twig samples were collected on 4/27/2006 and forced indoors (70F under metal halide grow lights) for 240 (fall 2005) or 365 (spring 2005) DAT evaluations. 10 larvae from untreated trees were placed on the excised foliage of treated trees in Petri plates. WM mortality was observed over a 48 H period. The no choice bioassay was repeated at 548 and 730 DAT from twig samples were collected on 5/08/07.

EB leaf residue

Leaf samples (from 4 branch samples 60 to 80 cm in length from the mid-canopy, one from each canopy sector) were taken from treated and untreated control trees in August 2007, two years after tree injection. 10 trees were sampled for EB residues, including 3 each of spring 05 and fall 05 EB treatments, 2 Acephate and 2 check trees. Syngenta Crop Science conducted the residue analyses by a modified HPLC florescence technique (as described in Takei et al., 2003) for Linden foliage.

Figure 1 example of severe leaf skeletonization, untreated control tree

Serial Dilutions

Laboratory bioassays were repeated by serial dilutions. Foliage was collected from the untreated Lindens on 4/29/2008 and again on 5/06/2008. On the first collection date, the WM larvae were small, typically 4-5 mm in length (here as, early instars). On the latter date, larvae typically had doubled in size to 9-10 mm in length (i.e., later instars). The excised branches were cut into 15 to 20 cm (6 - 8") lengths. Dilutions of Acephate 97% soluble granules (ACE-jet) and emamectin benzoate 4% ME (TREE-äge) were prepared. Additional cuttings were placed in DI water to serve as untreated controls. The dilutions were based on the concentration of formulated insecticide. The Acephate was prepared using 0.15 g (0.145 g AI) to which sufficient de-ionized water was added to bring the volume to 100 mLs. Serial dilutions were prepared by taking 10 mLs (14.5 mg of AI) and adding sufficient water to once again bring up the volume to 100mLs. This was repeated to prepare 6 serial dilutions. Thus solutions were prepared that provided from 1450 to 0.0145 ppm. Emamectin benzoate was prepared by diluting 10 mLs (0.4 g AI/10 mLs) to bring up the volume of liquid to 100 mLs and likewise diluted as per Acephate solutions. Thus, 400 to 0.004 ppm serial dilutions were prepared. Four replicates were prepared by adding 22 mLs to a 50 mL graduated vial. Uptake was monitored for 18 H and the amount of liquid solution taken up per vial was noted. From this the mg taken up per sample was calculated. After 18 H, the foliage was excised from the twigs and placed on a Petri plate. Three plates per treatment were prepared to which 3 to 4 WM caterpillars were placed. In bioassay 1, larval mortality was assessed in 18, 48 and 120 H. In bioassay 2, the caterpillars were allowed to feed for 72 H, whereupon larval mortality was assessed.

Statistical analyses were conducted in MINI-TAB version 15 (Mini-Tab, Inc. State College, PA USA), significance was accepted when $P<0.05$ with a CI of 95%. Data were converted to arcsine sqrt (x/100) x 57.3 radians prior to ANOVA. Linear regressions of data were conducted when applicable.

Results

Defoliation Severity

Tree canopies were assessed at 30 days after treatment (DAT). Spring treated ACE-jet (N= 4) and EB (N=3) had defoliation ratings (of 1.5 and 1.0. resp.) that were significantly less than the untreated check trees (N=5) or the untreated EB fall 05 trees (N=6), which had defoliation ratings of 6.2 and 5.2, respectively. Linear regression analysis of defoliation severity to grams AI applied was significant (R-sq of 0.399, p = 0.005), indicating that both treatments were protective compared to the untreated trees.

Foliage collected from the treatment trees were also assessed qualitatively for infestation. The foliage of EB and Acephate treatments had little damage, but tiny pin holes in the leaf demonstrated that these trees were infested by WM. No such protection was observed in the neighboring untreated trees (see figure 1). High levels of mortality may be inferred from these observations for the Acephate and EB treatments compared to the untreated check foliage. No such mortality was observed on the untreated controls, rather the larvae fed quite freely. These observations were consistent with the Defoliation Severity Ratings.

Tree canopies were again evaluated in 2006. The Acephate treatments continued to have low defoliation ratings, as did the spring and fall EB treated trees. The untreated check trees were freely predated upon by WM larvae. Tree canopies were next evaluated in 2007 in order to observe evidence of residual activity of treatments. Linear regression of canopy assessments correlated inversely with grams of EB applied in 2005 (R-Sq of 0.607 and p = 0.000). When EB was applied, lower defoliation severity was observed even after 1.5 to 2 years after treatment; whereas when left untreated, higher defoliation severity was observed.

No Choice Bioassays

Larval mortality was first assessed via no-choice bioassay at 240 (fall 2005) or 365 (spring 2005) DAT. WM larvae that fed on the foliage of the EB treated trees died within 24-48 hours. No residual activity was observed at 365 DAT for Acephate. The bioassay was repeated at 548 and 730 DAT. Twig samples were collected on 5/08/07. The results were observed in 48 hours. 80-90% WM mortality was observed in the EB treatments. Very little mortality was observed in the untreated checks or the 2-year old Acephate treatments.

Date	Bioassay of	N	Live	Dead	% Mortality
5/04/06	Controls	10	9	1	10
	Acephate (S 05)	10	10	0	0
	EB (S 05)	10	2	8	80
	EB (F 05)	10	1	9	90

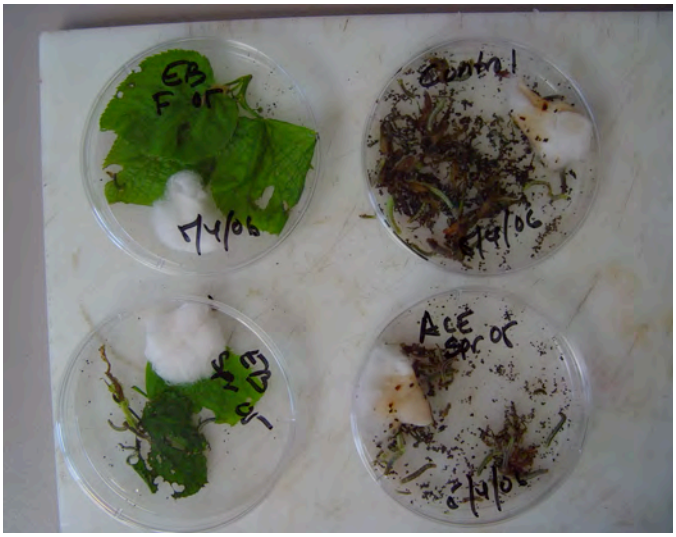

Figure 2 No choice bioassay conducted 5/04/06, clockwise from upper right, complete leaf damage of UTC, and Acephate S 05 (365 DAT); limited consumption of EB S 05 (365 DAT), and least foliage consumed of EB F 05 (240 DAT).

Although Acephate did not protect trees one year after application in bioassay, neither was defoliation observed in that population. Though not chemically protected, they did escape WM infestation in 2006, one year following treatment.

EB leaf residue

The mean PPB in linden foliage recovered was 26.23 for the EB treatments for trees with a mean 37 cm (14.83") DBH, with a range of 3.0 to 66.0 ppbs. Linear regression of Emamectin benzoate ppbs compared to cm DBH (R-Sq of 0.481 and p=0.0126) suggests an inverse relationship between tree size and ppbs of chemistry. These differences may be due to the dilution effects of tree size and/or to losses following seasonal leaf drop.

Serial Dilutions

Serial dilutions were conducted in 2008. After 18, 48 and 120 H on Petri plates, WM mortality was observed to be 16.4, 37.8, and 84.7 for Acephate (mean ppb = 212), and 19.8, 51.8 and 80.9 for EB (mean ppb = 692) and 0, 10.4 and 10.4 for the UTC's. In the 120 H bioassay, larval mortality was >90 in samples that had taken up 40 ppbs of EB. In samples that had taken up 4 ppbs EB, mortality was observed to be >50. Based on these data, bioassay 2 was run for 72 H. After 72H, mortality was >50 for Acephate and EB treatments. There were positive linear regressions for both Acephate and EB treatments comparing ppb to converted mortality (R-Sq values of 0.686 and 0.530, respectively with P values of <0.05).

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Conclusions

Winter moth is an exotic defoliator of hardwood trees. We studied Redmond Linden, a preferential host to the insect. Foliage is damaged early, the first instar larvae crawl into dormant buds to feed on leaf primordia, suggesting a strategy of early systemic treatment. We were interested in evaluating the activity of a tree injection formulation of emamectin benzoate against this defoliator. Assessments included defoliation severity, no choice bioassays and leaf residues.

We first assayed the activity of Emamectin benzoate compared to a insecticide with known activity to Lepidoptera, in this case, Acephate. Spring 2005 treatments were evaluated by severity of defoliation. The first set of treatments were applied on 5/05 and 5/11. At 30 DAT, injury to trees was clearly expressed by overall canopy thinning and extent of leaf area damaged; the larvae had matured to prepupae and feeding ceased. Spring treated ACE-jet (N= 4) and EB (N=3) had defoliation ratings (of 1.5 and 1.0. resp.) that were significantly less than the untreated check trees (N=5) or the not yet treated, EB fall 05 trees (N=6), which had defoliation ratings of 6.2 and 5.2, respectively. Emamectin benzoate was as effective as Acephate in mitigating injury to trees in spring applications.

Acephate, an organophosphate chemistry, does not have long residual activity; therefore was not used in the fall application. Emamectin benzoate was a more likely candidate as suggested by other tree injection research (Takei et. al.). Because feeding occurs very early, within the tree bud on leaf primordia, we wished to mitigate injury with anticipatory applications at leaf senescence. Fall injected EB was effective in protecting trees against WM, as demonstrated by bioassays (90% larvae mortality) and by canopy condition.

Tree defoliation severity was evaluated in June 2007. Linear regression analysis of canopy assessments correlated inversely with grams of EB applied in fall 2005 (R-Sq of 0.607 and p = 0.000). That is, the EB trees were protected while the control trees were not. This analysis suggests that EB protected against larval activity for two years.

In August of 2007, 1 ½ to 2 years following EB treatments, foliage samples were taken. Ten of the eighteen study trees were sampled, including trees that were treated in spring and fall 2005 and the untreated checks. Both smaller diameter and larger diameter trees were assayed. The Acephate and control trees were assayed for EB, these trees had <1.0 ppb of residues. The treated trees on the other hand, had mean residues of 26.3 ranging from 3 to 66 ppbs. A linear regression was run to assess ppb of residue to tree size. Linear correlation suggests a trend of lower residues with increasing tree size. What level of activity might be expected with such minute levels of EB recovered?

A dilution series set up subsequent to the EB residues considered the question. In the first dilution series (early instars), 40 ppbs provided >90% mortality whereas with 4 ppbs, efficacy drop to ~50%. Such minute amounts of chemistry to affect larval mortality and tree protection is remarkable. In the second dilution series (later instars), 600 ppbs were required to provide 50%

mortality [(regression equation, 42.24+ (0.01402xppb EB)]. This is consistent with the increased canopy defoliation in the larger trees observed two years following treatment. This data supports an early treatment strategy and points to the protective effects of EB.

Emamectin benzoate demonstrated activity to Winter moth in either spring or fall applications. The rates used in this study of 0.128 or 0.16 g AI/cm DBH (0.32 or 0.40 g AI/DBH") were effective against WM infestations for two full seasons. Based on the sensitivity of WM larvae to EB, lower rates (e.g., 0.065 and 0.08 g AL/cm) are likely to be effective, particularly in smaller diameter trees (and as suggested by the laboratory dilution assay). This research suggests that one application of Emamectin benzoate by basal stem injection every two years is sufficient to protect trees from defoliation by Winter moth larvae.

Acknowledgements

Thanks to David Cox, PH.D. Syngenta Crop Protection. Western R+D Scientist—Professional Products. 14446 Huntington Rd., Madera, CA 93636 for his review and comments.

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