Transovarial transmission of novaluron in *Choristoneura rosaceana* (Lepidoptera: Tortricidae)

S.-H.S. Kim,[1] C. Vandervoort, M.E. Whalon, J.C. Wise

Abstract—The obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), has become a significant pest of tree fruit production in North America within the past 25 years. Control of the OBLR has historically relied upon broad-spectrum insecticides like organophosphates, carbamates, and pyrethroids. However, with evidence of resistance development, newer chemistries have been developed to combat this pest. The effects of novaluron, a chitin synthesis inhibitor, were studied to determine if reduced egg hatch occurs after exposure to adults. The transovarial effects of this compound were tested through laboratory bioassays, looking at decreased egg hatch and also presence of novaluron in egg masses following adult exposure. Results from the study demonstrated a decrease in egg hatch after adult exposure. Analysis of egg mass using HPLC also demonstrated novaluron present in the eggs laid by treated adults. Along with the direct ovicidal and larvicidal properties of novaluron, this transovarial activity provides an important contribution to the overall control seen in the field.

Résumé—Au cours des vingt-cinq dernières années, la tordeuse à bandes obliques, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), est devenue un ravageur important des cultures d'arbres fruitiers en Amérique du Nord. La lutte contre la tordeuse à bandes obliques a traditionnellement reposé sur l'utilisation d'insecticides à large spectre, tels que les organophosphates, les carbamates et les pyréthroïdes. Cependant, après la constatation du développement de résistances, de nouvelles molécules chimiques ont été mises au point pour combattre ce ravageur. Nous avons étudié l'effet du novaluron, un inhibiteur de la synthèse de la chitine, pour voir si l'exposition des adultes au produit entraîne une réduction de l'éclosion des œufs. Les effets transovariens du produit ont été vérifiés par des essais en laboratoire qui testaient la réduction de l'éclosion des œufs et la présence de novaluron dans les masses d'œufs après une exposition des adultes. Nos résultats indiquent une réduction de l'éclosion des œufs après une exposition des adultes. L'analyse des masses d'œufs par CLHP (HPLC) révèle la présence de novaluron dans les œufs pondus par les adultes traités. En plus des propriétés ovicides et larvicides directes du novaluron, cette activité transovarienne se révèle être un facteur important dans la lutte globale observée sur le terrain.

Introduction

The obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), has become a significant pest of tree fruit production in North America within the past 25 years. OBLR is a polyphagous insect, usually with two generations per year, which feeds on more than 50 different species (Sanderson and Jackson 1909; Howitt 1993). Larvae are

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external feeders of flowering buds, leaves, and fruit surfaces (Howitt 1993). Early-season damage from overwintering larvae can cause fruit drop or deformed fruit. Late-season damage causes shallow or deep holes on the surface of the fruit (Agnello *et al.* 1996). Control of the OBLR initially relied upon broad-spectrum insecticides such as organophosphates, carbamates, and synthetic pyrethroids (Reissig 1978; Howitt 1993). However, with the elimination of some organophosphates (United States Environmental Protection Agency 2006) and evidence of resistance development (Reissig *et al.* 1986; Carriére *et al.* 1996; Lawson *et al.* 1997; Ahmad *et al.* 2002; Smirle *et al.* 2002), newer chemistries have been developed to combat this pest.

Among the newer control tactics that have been implemented, insect growth regulators (IGR) fit well into Integrated Pest Management (IPM) programs because of their reduced potential for negative environmental and ecological impacts. These compounds work by altering the growth and development of the targeted pests. IGRs have been primarily used to target the larval and egg stages of codling moth (*Cydia pomonella* (Linnaeus)) and other leafroller species (Lepidoptera: Tortricidae) based on the direct toxic effects to these stages seen in other Lepidoptera pests (Ishaaya *et al.* 1996, 1998, 2003). Along with strong toxic effects on eggs or larvae, some IGRs demonstrate sublethal effects on a variety of different pests, including Lepidoptera, Coleoptera, and Diptera (Casa-Giner *et al.* 1999; Knight 2000; Charmillot *et al*. 2001; Cutler *et al*. 2005; Kostyukovsky and Trostanetsky 2006; Pineda *et al*. 2006; Wise *et al*. 2007; Gökçe *et al*. 2009; Kim *et al*. 2011).

Within the IGR class are the chitin synthesis inhibitors that prevent the insect from undergoing proper chitin synthesis after the moulting process (Post *et al*. 1974); however, the exact step at which inhibition occurs is still unclear. Inhibition of chitin may take place at the polymerisation stage of chitin biosynthesis (Hajjar and Casida 1978) or in chitin precursor transport (Nakagawa and Matsumura 1994). The chitin synthesis inhibitors have in some cases shown minimal effects on parasitoids and other natural enemies (Ishaaya 1990; Ishaaya *et al*. 2001). Diflubenzuron is a chitin synthesis inhibitor that has sublethal activity on codling moth resulting in reduced viability of the eggs laid by treated adults (Hoying and Riedl 1980; Elliott and Anderson 1982).

Novaluron, an IGR registered for use in pome and stone fruits, has similar sublethal effects when adult insects are exposed (Cutler *et al.* 2005; Kostyukovsky and Trostanetsky 2006; Wise *et al*. 2007; Gökçe *et al*. 2009; Kim *et al*. 2011). Novaluron is a strong control agent of Lepidoptera primarily by ingestion (Ishaaya *et al*. 2007). It has direct toxic effects on the eggs and larvae of OBLR (Brunner *et al*. 2005); however, sublethal effects from adult exposure have yet to be reported. Delivery of novaluron that results in lowered egg viability can be explained by three possible mechanisms: (a) physiological, (b) transovarial transfer, or (c) horizontal transfer. Although studies with novaluron on physiological mechanisms have yet to be conducted, studies with other benzoylurea insecticides suggest effects on female ovaries and basal oocytes as possible explanations for the reduced egg viability (Soltani and Soltani-Mazouni 1992). Experiments by Gökçe *et al*. (2009) and Kim *et al.* (2011) suggest that the sublethal effects seen in codling moth may be due to transovarial transmission. However, another possible explanation for how novaluron is delivered to OBLR eggs is from horizontal transfer, when previously exposed adults transfer the toxin by tarsal contact with clean eggs, which was seen with another IGR (pyriproxyfen) in mosquitoes (Chism and Apperson 2003).

The objectives of this study were (a) to demonstrate sublethal effects of novaluron on the egg viability of OBLR following adult exposure, and (b) to detect the presence of novaluron in egg masses laid by treated females as evidence for transovarial transmission.

Materials and methods

Chemicals

Novaluron (Rimon 0.83 EC, Chemtura U.S.A. Corporation, Middlebury, Connecticut, United States of America) was prepared according to the recommended field rate (224 g active ingredient ha^{-1}). Latron B-1956® (a spreader and sticker from Dow Agro Sciences LLC, Indianapolis, Indiana, United States of America) was added at 0.038:1 vol:vol to novaluron to fully homogenise the novaluron and water control solutions.

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Insects and bioassay materials

OBLR from a laboratory colony, originally collected from unsprayed apple orchards in southwestern Michigan, United States of America in 2000, were used for the following 2008 experiments. The colony was reared on a modified pinto bean diet (Shorey and Hale 1965) at 23 ± 1 °C, 60% relative humidity, and 16 light: 8 dark hour photoperiod. Pupae were collected, sexed, and placed into 1-L plastic containers until eclosion. Adults were provided with a 10% sucrose solution in a 30 mL soufflé cup with a dental wick protruding from the top.

Adult (one to three days old) OBLR were used. Experiments were replicated six times for the bioassays and four times for the residue studies. Each replicate consisted of five females and five males for the bioassays and 30 males and 30 females for the residue studies. Wax paper was sprayed with either the novaluron or control solution until drip using a handheld sprayer and allowed to fully dry before lining a clean 1-L plastic container. Exposure containers also contained a 30 mL soufflé cup with a dental wick protruding from the lid filled with novaluron solution or the control solution. Adult moths were incubated in exposure containers for three days and then transferred to untreated containers for egg deposition. Egg masses collected for the bioassays were placed into petri dishes sealed with parafilm and incubated at 23 ± 1 °C, 60% relative humidity, and 16 light: 8 dark hour photoperiod for an additional 14 days.

Effects of adult exposure to novaluron on egg survival

Exposure of adult moths was carried out as mentioned above, and then adults were transferred to clean 1-L containers lined with clean wax paper for egg deposition. Moths were allowed to lay eggs for seven days. After the seven-day egg-laying period, each egg masses were photographed to estimate size; the area measurement was taken using NIS Elements software (Nikon Instruments Inc., Melville, New York, United States of America). Since OBLR eggs are laid in a stacked pattern, the numbers of eggs per egg mass was estimated using: number of eggs $= -86.483 + 699.524x$ ($r^2 = 0.90$), where x = egg mass area in cm^2 (Sun *et al.* 2000). Egg masses were incubated as previously stated, and petri dishes were checked daily to count the number of larvae that had hatched.

Effects of novaluron by adult horizontal transfer

Untreated moths were allowed to lay eggs for one to two days before the adults were removed, and the egg masses were counted. Egg masses were photographed and the area was calculated as above. Then five unmated females that had been exposed to novaluron as above were introduced into the containers with the clean eggs. The unmated moths were incubated in the container for seven days and were checked every 12 hours to ensure moths were in contact with the egg masses. After the seven days, the moths were removed, and the egg masses were incubated another seven days to allow for hatch. The egg mass areas were analysed in the same fashion as above after the incubation period was over. Controls for this experiment were carried out in a similar fashion, with Latron B-1956® plus water used as the control for the novaluron solution.

Statistical analysis

The total numbers of larvae emerged from egg masses were compared with the total numbers of eggs. The data collected from both bioassays were used as percent hatch and was normalised using an arcsine square root transformation before being subjected to a paired *t*-test comparing novaluron to the control (SAS 2002). Data was back-transformed for presentation.

Residue profile analysis of eggs laid by treated adults

Adults were exposed to novaluron or a control solution as described above. After the three-day exposure period they were transferred to a clean 1-L plastic container lined with clean wax paper for egg deposition. The moths were allowed to lay eggs for seven days and then were removed from the egg-laying container. Eggs were collected from the wax paper (minimum 0.25 g/repetition × 4 repetitions) and placed into a 25-mL glass vial. Acetonitrile (10 mL) was added and the vials were sent to the Michigan State University (MSU) Pesticide Analytical Laboratory (East Lansing, Michigan, United States of America) for residue analysis.

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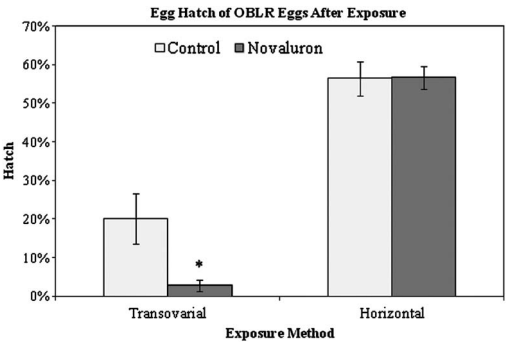
Residue profile analysis of eggs exposed to adult horizontal transfer

Untreated moths were allowed to lay eggs for one to two days before removal. Once the moths were removed, five females exposed to novaluron by the methods described above were introduced into each container with the clean eggs. Moths were incubated in the container for seven days and were checked every 12 hours to ensure that moths were in contact with the egg masses. After seven days, the moths were removed and egg masses were collected (minimum 0.25 g/repetition × 4 repetitions) and placed into a 25-mL glass vial. The vials were prepared and sent to the MSU Pesticide Analytical Laboratory for residue analysis. Controls for this experiment consisted of eggs laid from untreated adults.

Residue analysis

At the MSU Pesticide Analytical Laboratory, each sample vial was homogenised (Model Pro200, ProScientific Inc., Monroe, Connecticut, United States of America), rinsed with three volumes of 20 mL dichloromethane (Burdick & Jackson, Muskegon, Michigan, United States of America) and put through a Na_2SO_4 (EMD, Gibbstown, New Jersey, United States of America) column to remove water. The column was rinsed with two volumes of 20 mL dichloromethane. Solvent volume was reduced to 2 mL by rotary evaporation and placed in a 2.5-mL gas chromatography vial (Supelco®, St. Louis, Missouri, United States of America) prior to analysis. Any remaining particulates were removed by passing the sample through a 0.45 μm filter (Pall Life Sciences, East Hills, New York, United States of America). This occurred after solvent reduction. Novaluron was analysed using GC/MSD (Agilent 6890 Gas Chromatograph with a 5973N MSD) equipped with a Zebron ZB-5 ms 30 m, 0.25 mm I.D. and a 0.25 μm film thickness. For the gas chromatography/mass spectrometry (GC/MSD) analysis settings the oven was held at 115 °C for five minutes with a ramp of 9 °C/minute to 280 °C, followed a ramp of 30 °C/minute to 310 °C. The inlet was held at 200 °C in a pulsed splitless mode with 78 324 Pa and a pulse pressure of 103 421 Pa, a purge flow of 50.0 ml/min of helium gas. The MSD transfer line was held at 285 °C. The mass spectrometer was monitoring for ions 188 and 69 for novaluron. The injector was rinsed three times with acetone and three times with dichloromethane between and also before each injection to eliminate contamination between injections. All samples were quantitated against a standard curve, and recovery data recorded as micrograms of active ingredient per gram (ppm) of egg mass. Limit of detection was 0.001 (ng/g) and limit of quantitation was 0.005 (ng/g).

Fig. 1. Mean percent hatch of obliquebanded leafroller (OBLR) eggs from different exposure methods. The hatch percent (mean ± SEM) with * is significantly different from the control (paired *t*-test, $P < 0.05$). Egg hatch rates are means of six replicates. The presented mean egg hatch data were arcsine square-root transformed before analysis and then back-transformed for presentation.



Results

Effects of adult exposure to novaluron on egg survival

Results obtained from replicated experiments of exposure of adult OBLRs to novaluron demonstrated reduced viability of eggs laid (Fig. 1). Eggs laid by treated adults (transovarial transmission) demonstrated that average egg hatch was 3 ± 2%, which was significantly lower than in the control 20 ± 7% ($t = 2.55$, df = 10, $P = 0.029$).

Effects of novaluron by adult horizontal transfer

Data obtained from the horizontal transmission experiment showed no reduction in egg viability (Fig. 1). The average hatch from the treatment eggs was 57 ± 3%, which did not

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differ from the average of the control 56 ± 4% ($t = -0.03$, df = 10, $P = 0.973$).

Residue profile analysis of egg masses

Analysis of egg masses laid by treated adults using a GC/MS detector produced detectable levels of novaluron present in the eggs (0.034 ± 0.012 ng/g). Eggs collected from the horizontal exposure and control experiments both yielded no detectable levels of novaluron.

Discussion

This study has demonstrated that reduced egg viability occurs following exposure of adult OBLRs to novaluron. Results from the horizontal transfer experiment demonstrate that incidental contact of female moths on previously laid eggs does not transfer sufficient compound to cause sublethal effects resulting in decreased egg viability. The results from the transovarial bioassays along with novaluron residue detection in egg masses demonstrates a high likelihood that the reduced viability occurs due to a transovarial passage of novaluron from adult to the eggs.

A decrease between egg viability of the controls from the two different exposure methods (transovarial and horizontal transmission) was unexpected. These differences may be attributed to exposure of the adults to Latron B-1956® in the controls, for the transovarial transmission experiment. Reinke and Barrett (2007) demonstrated that continuous exposure of the surfactant, Latron B-1956®, to oriental fruit moth resulted in decreased egg viability when compared with the control. Similar results were observed when codling moth adults were exposed to Latron B-1956® (Knight 2000). However, Gökçe *et al.* (2009) demonstrated that no differences in fecundity for codling moth occurred when exposed to the control (water + Latron B-1956®) and novaluron treatment. The effect Latron B-1956® has on the OBLR via ingested versus strictly tarsal contact with dry residues has yet to be studied and may be a cause for the lower egg viability seen between these experiments.

Reduced egg viability after adult exposure to novaluron was not only seen in codling moth and OBLR, but similar results have been reported for Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Alyokhin *et al.* 2008), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Kostyukovsky and Trostanetsky 2006), and plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae) (Wise *et al.* 2007). However, studies with other benzoylurea insecticides have demonstrated different physiological effects of female ovaries and basal oocytes after adult exposure (Soltani and Soltani-Mazouni 1992); thus, there may be multiple mechanisms at work.

Field applications of novaluron should still be timed based upon the direct ovicide or larvicide when targeting oblqiuebanded leafroller. However, the contribution of sublethal activity (reduced egg viability) should be considered in relation to overall population management. This is relevant when targeting OBLR, but also when novaluron is used against other pome fruit pests, such as codling moth, when OBLR adults may be present. The ability of novaluron to retain its potency after an artificial rain treatment of 40 mm/hour at five and 24 hours after treatment allows for control at wetter climates (Ishaaya *et al.* 2001). The sublethal effects, along with minimal effects towards natural enemies (Suh *et al.* 2000; Cloyd and Dickinson 2006), make novaluron a suitable organophosphate alternative in an IPM program. The impending complete phaseout of azinphosmethyl in the United States of America, growing pest resistance to these compounds, and concerns over ecological impacts of the broad-spectrum chemistries have increased impetus to develop newer chemistries to replace the heavily relied upon organophosphates. Education into the most appropriate use and timing of these knowledge-based insecticide tools is crucial for proper and effective control over pests (Wise and Whalon 2009).

Results from this experiment have demonstrated that transovarial transmission is a likely mechanism for the sublethal effects observed. However, further research is needed to fully understand the physiological pathway taken by transovarial transmission of novaluron after adults have been exposed and how the method of exposure influences sublethal effects. Research is needed to investigate whether novaluron is present in the proteins of the chorion surrounding the egg or in the egg itself to determine if novaluron is transovarially transmitted. Also, research into effective timing of a field application is needed to

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fully optimise the effects of novaluron, using both its larvicidal/ovicidal and transovarial effects to control this pest and possibly coincide with other pest species.

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