Effects of two reduced-risk insecticides on the egg parasitoid *Trichogramma minutum* in apple orchards

Daniel Cormier[1], Paula Cabrera[2], Marc Fournier[2], Franz Vanoosthuyse[1], Éric Lucas[2]
[1]*Research and Development Institute for the Agri-Environment (IRDA), 335 rang des Vingt-Cinq Est, Saint-Bruno-de-Montarville, J3V 0G7 (Québec), Canada;* [2]*Département des Sciences Biologiques, Université du Québec à Montréal, C.P. 8888 Succ. Centre-Ville, Montreal, H3C 3P8 (Quebec), Canada*

Abstract: *Trichogramma minutum* Riley is an egg parasitoid found in North American apple orchards where it contributes to the biological control of several lepidopteran pests including the codling moth, *Cydia pomonella* (L.). To control this major pest, reduced-risk insecticides have been commonly applied in Quebec orchards for several years. We investigated the compatibility of these insecticides with the biological control agent *T. minutum* in field and laboratory experiments. In an experimental orchard, codling moth eggs were exposed to parasitism a few days prior or after the application of either novaluron, chlorantraniliprole or water (control). For each egg cohort, the emergence of *Trichogramma* adults from the codling moth eggs was significantly lower for novaluron than chlorantraniliprole and control. In laboratory experiments, we evaluated the effects of these insecticides on the development of the immature stages of *T. minutum*. Eggs were treated 24 and 120 h after parasitism. Results indicated that the development of *Trichogramma* from the egg to pupal stage was not affected by the treatment. Similar results were observed for their development from the pupal to adult stage. The results suggest that the inhibition of chitin synthesis associated with novaluron did not affect the development of immature stages of *T. minutum* but may affect specialized structures such as mandibles used by *Trichogramma* during the emergence process.

Key words: Trichogrammatidae, Tortricidae, parasitic wasp, apple pest, integrated pest management, side effects

Introduction

Trichogramma minutum Riley (Hymenoptera: Trichogrammatidae) is the most abundant egg parasitoid found in Canadian apple orchards. It contributes to the biological control of several lepidopteran pests, including the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Yu *et al*., 1984). To control this major pest, reduced-risk insecticides have been commonly applied in Québec, Canada, year after year (Chouinard *et al*., 2014). Reduced-risk insecticides may have a negative impact on natural enemies (Cabrera *et al*., 2014) and they may affect indirectly immature stages of parasitoids (Cônsoli *et al*., 1998; Schneider *et al*., 2004).

We investigated the susceptibility of immature stages of *T. minutum* to two reduced-risk insecticides, novaluron and chlorantraniliprole, in field and laboratory experiments.

Material and methods

Insect rearing

Our laboratory rearing of codling moths originated from an adult population (Okanagan-Kootenay Sterile Insect Release Program, Osoyoos, BC, Canada). Adults were reared in plastic bags and colonies were maintained in rearing chambers at 23 °C, 60% RH and 16 L:8 D. The egg parasitoid *T. minutum* were obtained from Anatis Bioprotection Inc. (Saint-Jacques-le-Mineur, QC, Canada) and reared at the same environmental conditions.

Insecticides

Insecticide selection was based on the products known to be toxic to codling moths typically used by apple growers in Québec. The two reduced-risk insecticides tested included the growth regulator novaluron (Rimon® 10 EC (Makhteshim Agan of North America, Raleigh, NC, United States) 93-100 g a.i./ha) and chlorantraniliprole (Altacor® 35 WG (DuPont Canada, Mississauga, ON, Canada) 50.75 g a.i./ha).

Experimental apple orchard

At the experimental apple orchard of the Research and Development Institute for the Agri-Environment located in Saint-Bruno-de-Montarville, QC, Canada, codling moth eggs were obtained by placing 15 codling moth couples per sleeve, which were placed over fruit-bearing shoots from ca. 15 PM to 9 AM the following day. Codling moths and sleeves were removed and codling moth eggs were subjected to parasitism by feral populations of *T. minutum* for a period of three days as shown in Figure 1. Three cohorts of eggs were established based on the parasitism period, whether prior or after the application of insecticides or water (control). Eggs of cohorts 1 and 3 were exposed to parasitism either prior or after insecticide applications, respectively, whereas eggs of cohort 2 were exposed for one day prior and after applications. Insecticides were applied until drip point using a handgun sprayer delivering ca. 2500 l/ha. After an exposure to parasitism for a period of three days, apples with sprayed eggs were removed, brought to the laboratory where numbers of parasitized eggs with an emergence exit hole were recorded. Data were analyzed using one-way ANOVAs for each cohort, followed by a HSD Tukey test for mean separation ($\alpha = 0.05$) (Steel & Torrie, 1980).

Laboratory experiments

Parasitized eggs where obtained by introducing one hundred *T. minutum* adults in a two liter plastic bag containing about 1000 codling moth eggs. After 24 h, eggs were removed and groups on 30 eggs were glued with mucilage on small pieces of cardboard. Half of the cardboards containing 24 h parasitized eggs were treated with the reduced-risk insecticides or water (control) using a Potter tower (2 mg/cm^2 aqueous insecticide deposit). The other cardboards were similarly treated, but 120 h after parasitoid-egg contact, when parasitoids were expected to be at the pupal stage. After treatment applications, cardboards containing codling moth eggs were placed in a growth chamber at 25 °C, 70% RH and 16L:8D. The parasitoid development from egg to pupa was evaluated by recording the number of host eggs turning black eight days after parasitoid-egg contact, and the development from pupa to adult was evaluated by recording the number of parasitized eggs with an emergence hole twelve days after parasitoid-egg contact as shown in Figure 2. One-way ANOVAs were performed for each cohort ($\alpha = 0.05$) (Steel &Torrie, 1980).
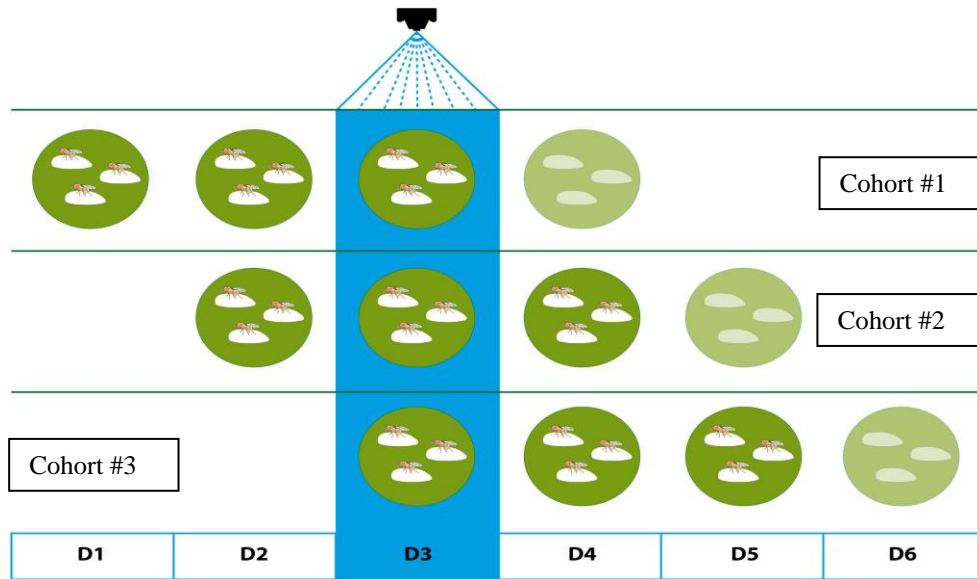
Figure 1. Experimental layout of field trials. Each cohort represents the period where codling moth eggs were exposed to natural parasitism by *Trichogramma minutum* in the apple orchard in relation to the insecticide application (blue rectangle).
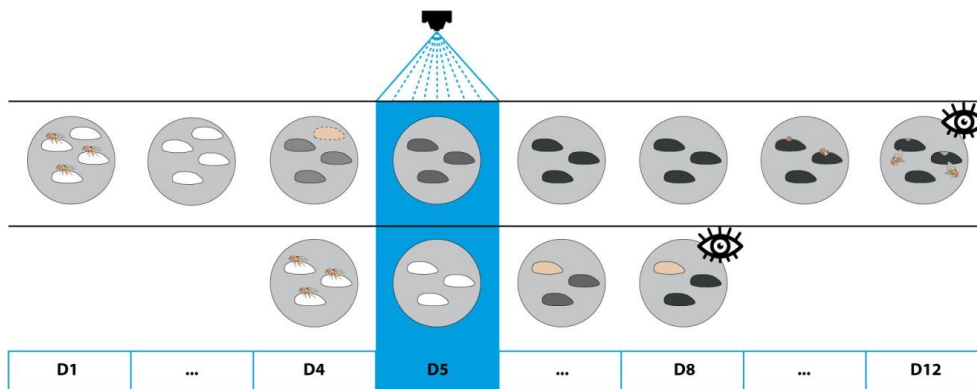

Figure 2. Experimental layout of laboratory bioassays. Codling moth eggs were exposed to *Trichogramma minutum* for 24 h. Insecticides were then applied (blue rectangle) on the eggs with a Potter tower either 120 h (top) or 24 h (bottom) after parasitism. Eggs were observed at adult emergence (top) or once host eggs had blackened (bottom).

Results and discussion

Experimental apple orchard

For each egg cohort, the emergence of *Trichogramma* adults from the codling moth eggs was significantly lower for novaluron than chlorantraniliprole and control ($F_{2,4} = 15.56$, $P < 0.0001$, $F_{2,4} = 9.63$, $P = 0.0002$, $F_{2,4} = 19.57$, $P < 0.0001$, respectively for cohorts #1, 2 and 3) as shown in Figure 3.
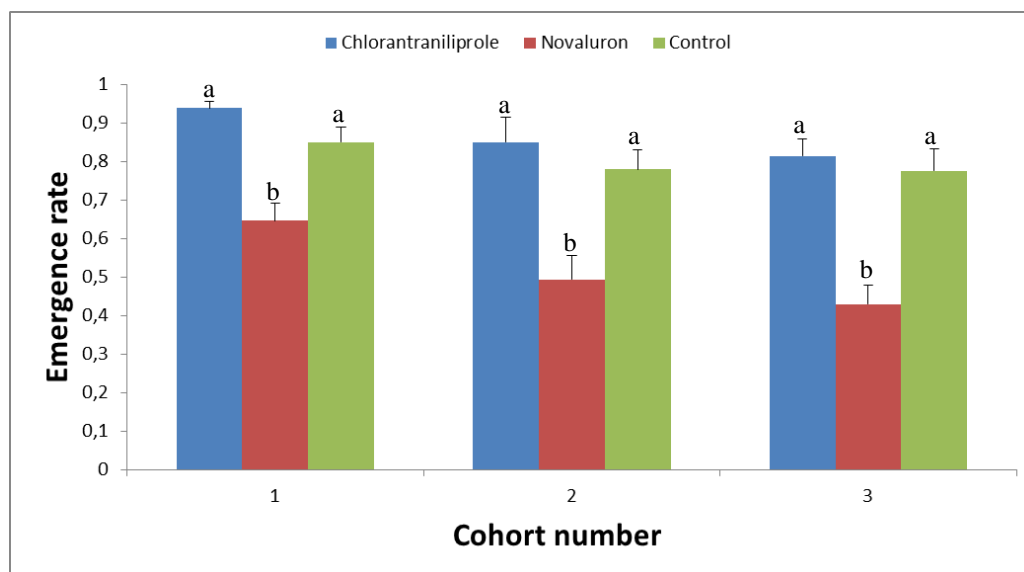
Figure 3. Emergence rate (mean ± SE) of indigenous *Trichogramma* adults from codling moth eggs exposed to parasitism for a few days prior (cohorts #1 and 2) or after (cohorts #2 and 3) the application of either novaluron, chlorantraniliprole or water (control).

Laboratory experiments

As shown in Table 1, the development of *Trichogramma* eggs into pupae was not affected by either insecticide ($F_{2,6} = 0.25$, P = 0.7865). Similar results were observed for the development of pupae into adults ($F_{2,6} = 0.15$, P = 0.8654).

Table 1. Percentage (mean ± SE) of *Trichogramma minutum* eggs successfully developing into pupae and *T. minutum* pupae into adults after insecticides were applied 24 and 120 h after parasitism, respectively.

Treatments	Eggs to pupae	Pupae to adults
Chlorantraniliprole	94.44 ± 2.22a	94.44 ± 2.94a
Novaluron	96.67 ± 3.33a	92.22 ± 4.01a
Control	96.67 ± 1.93a	92.22 ± 2.94a

In both field trials and laboratory bioassays, chlorantraniliprole did not affect the parasitoid development and adult emergence. This reduced-risk insecticide could be compatible with *Trichogramma* spp. but, extensive sublethal studies should be performed before concluding to the safety of the insecticide. On the other hand, novaluron affected negatively the parasitoid emergence in field trials but did not affect the development of the parasitoid from egg to pupa or from pupa to adult in laboratory bioassays. We believe that the inhibition of chitin synthesis associated with novaluron may have been involved during the larval development of the parasitoid and that it affected the adult emergence, as observed on *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) (Bastos *et al*., 2006). We showed that codling moth eggs treated at the parasitoid pupal stage did not affect the parasitoid emergence. However, although treating codling moth eggs at the parasitoid egg

stage did not affect the development of the parasitoid, it affected the parasitoid emergence. We hypothesize that the mandibles, a chitinous structure, used by *Trichogramma* spp. during their emergence were not strong enough to chew through the host chorion. Malformed mandibles and partially sclerotized mandibles following the application of an insect growth regulator has already been observed in Lepidoptera and Diptera (Aller & Ramsay, 1988; Darvas *et al.*, 1992). Insecticide applications during the larval stage of the parasitoid and observations of the development of the parasitoid from egg to adult emergence, along with a detailed analysis of the mandibles, have to be performed in order to validate this hypothesis.

Acknowledgements

References

Aller, H. E. & Ramsay, J. R. 1988: RH 5849, a novel insect growth regulator with a new mode of action. Brighton Crop Prot. Conf. Pests Dis. 2: 511-518.

Bastos, C. S., de Almeida, R. P. & Suinaga, F. A. 2006: Selectivity of pesticides used on cotton (*Gossypium hirsutum*) to *Trichogramma pretiosum* reared on two laboratory-reared hosts. Pest Manag. Sci. 62: 91-98.

Cabrera, P., Cormier, D., Fournier, M. & Lucas, É. 2014: Lethal effects of two reduced risk insecticides on *Harmonia axyridis* and *Coleomegilla maculata* (Col., Coccinellidae) following two routes of exposure. IOBC-WPRS Bull. 103: 41-45.

Chouinard, G., Cormier, D., Bergeron, K. & Philion, V. 2014: Guide to foliar treatments of apple trees 2014-2015. Centre de Référence en Agriculture et Agroalimentaire du Québec, Québec, Canada.

Cônsoli, F. L., Parra, J. R. P. & Hassan, S. A. 1998: Side-effects of insecticides used in tomato fields on the egg parasitoid *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). J. Appl. Entomol. 122: 43-47.

Darvas, B., Polgár, L., Tag El-Din, M. H., Eröss, K. & Wing, K. D. 1992: Developmental disturbances in different insect orders caused by an ecdysteroid agonist, RH 5849. J. Econ. Entomol. 85: 2107-2112.

Schneider, M. I., Smagghe, G., Gobbi, A., & Viñuela, E. 2004: Toxicity and pharmacokinetics of insect growth regulators and other novel insecticides on pupae of *Hyposoter didymator* (Hymenoptera: Ichneumonidae), a parasitoid of early larval instars of lepidopteran pests. J. Econ. Entomol. 96: 1054-1065.

Steel, R. G. D. & Torrie, J. H. 1980: Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York, USA, 633 pp.

Yu, D. S. K., Hagley, E. A. C. & Laing, J. E. 1984: Biology of *Trichogramma minutum* Riley collected from apples in southern Ontario. Environ. Entomol. 13: 1324-1329.