Host finding and parasitism efficiency of obliquebanded leafroller egg masses by *Trichogramma minutum* after inundative releases in apple orchards

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Abstract

A local strain of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) was mass produced and released weekly in 0.25-ha plots of four commercial apple orchards to evaluate the level of parasitism in sentinel egg masses of the obliquebanded leafroller, *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae). Sentinel eggs were attached to foliage, either on release trees or at mid-distance between two release trees, and were renewed every 3-4 days. Other sentinel eggs were placed in adjacent plots (approx. 20 m from the release plots). The incidence of parasitism and number of eggs parasitized within each mass were evaluated. In dwarf tree orchards, parasitism was lower on sentinel egg masses placed at mid-distance between two release trees than on release trees; however, parasitism was more uniform in semi-dwarf tree orchards. The detection of parasitoid activity in the adjacent plots, even upwind, showed that under repetitive releases at a high release rate, many parasitoids travelled or were transported more than 20 m from their release site. The occurrence of parasitism and the number of parasitized eggs per mass were higher in release plots than in adjacent plots, but partial parasitism of host egg masses generally occurred, even in close proximity to release sites. In addition, even if host finding efficiency was higher, parasitized egg masses in release plots were not more heavily parasitized than those in adjacent plots. These results suggest that interference between females or detection of previously parasitized hosts may prevent additional oviposition in the same egg mass by conspecifics, and that the level of this response is less pronounced for females that travelled further from their emerging sites before encountering a host.

Keywords: biological control agent, egg parasitoid, partial parasitism, dispersal, tortricid

INTRODUCTION

The obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris) is an important pest of apples and is widely distributed in temperate North America. In Quebec (Canada), some populations have developed resistance to pyrethroid and organophosphate insecticides (Carriére et al., 1996), making control of this pest difficult. *Trichogramma* spp. have been used as biological agents of many lepidopterous pests in different crops (Smith, 1996; Mills, 2010) and their use in orchards may be an appropriate reduced-risk strategy to achieve adequate control of OBLR. However, many factors can affect the success of biological control by egg parasitoids, such as dispersal, searching ability, host acceptance, and parasitism efficiency (Hassan, 1994). These factors may be influenced by the crop (size and complexity of the plant) as well as the target host (whether eggs are laid in clusters or singly) (Smith, 1996; Vinson, 2010). To determine the suitability of *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) for *C. rosaceana* control in apple orchards, a local strain of this parasitoid was released weekly in plots of dwarf and semi-dwarf tree orchards. The purpose of this study was to evaluate the level of parasitism of OBLR sentinel egg masses located on release trees, on non-release trees and in adjacent plots with no releases, and to compare parasitoid dispersal in the two types of orchard.

MATERIALS AND METHODS

Sites

Parasitoid releases were conducted in four commercial orchards located in the Laurentians (QC, Canada). The orchards consisted either of dwarf or semi-dwarf apple trees at different planting densities depending on rootstock (Table 1). Inundative releases were made in 0.25-ha plots and two identical size plots with no *T. minutum* releases were selected on each side of the release plots. Within each orchard, all plots contained the same cultivar and received the same treatments and management practices during the trial.

Table 1. Tree spacing and planting density in the four commercial orchards of the trial.

Orchard parameter	Dwarf trees (high density)		Semi-dwarf trees (medium density)	
	Orchard 1	Orchard 2	Orchard 3	Orchard 4
Distance between trees (m)	2.0	1.75	4.0	4.0
Distance between rows (m)	4.5	4.0	7.0	5.5
No. trees ha^{-1}	1111	1429	357	455

Parasitoid releases

A local strain of *T. minutum* collected in 2002 in a Quebec orchard was mass reared on *Ephestia kuehniella* (Zeller) eggs in a Plexiglas rearing box as described by McGregor et al. (2000). Parasitized eggs were glued on folded cardboard (trichocard) containing mixed-aged parasitoids (two emerging cohorts) for releases in the field. Eleven weekly releases were made from mid-June to late August at a rate of 1.0-1.3 million parasitoids ha^{-1} week^{-1}. Trichocards were hung on 40 release trees evenly distributed in the release plots (Figure 1) (equivalent to 160 release trees ha^{-1}). Based on the estimated number of parasitized eggs, the sex ratio, and the adult emergence, approximately 4000 females were released each week on each release point.

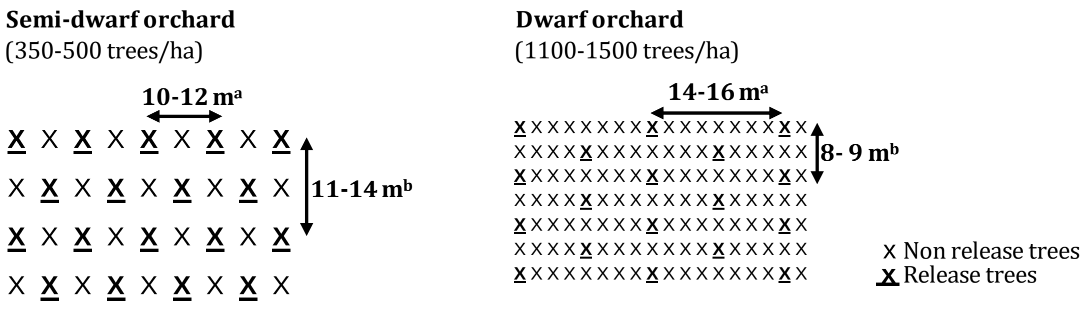

Figure 1. General layout of release trees in dwarf and semi-dwarf apple tree orchards, and mean distances between two release trees within[a] and between[b] rows.

Parasitism measurements

To assess parasitism, OBLR sentinel egg masses obtained from a laboratory colony were used. The colony was established with larvae collected in apple orchards and maintained on a bean-based artificial diet. Male and female moths were placed inside plastic bags held in a rearing chamber (20°C; 60% RH; 16 L:8 D). Sections of plastic bag with egg masses attached were collected daily and held at 7°C (max. 7 d) until use in the field. Sentinel egg masses were attached to foliage placed on five trees in the middle of each release plot. Egg masses were placed either on a release tree or at mid-distance between two release trees. An identical layout of five egg masses was used at the center of the two adjacent plots located upwind or downwind of the release plots (Figure 2). The sampling areas in plots with no releases were separated from the border of the release plots by a minimum distance of 20 m. Sentinel egg masses were renewed every 3-4 days and recovered eggs were incubated in growth chambers (22°C; 60% RH; 16 L:8 D) before being assessed for parasitism.

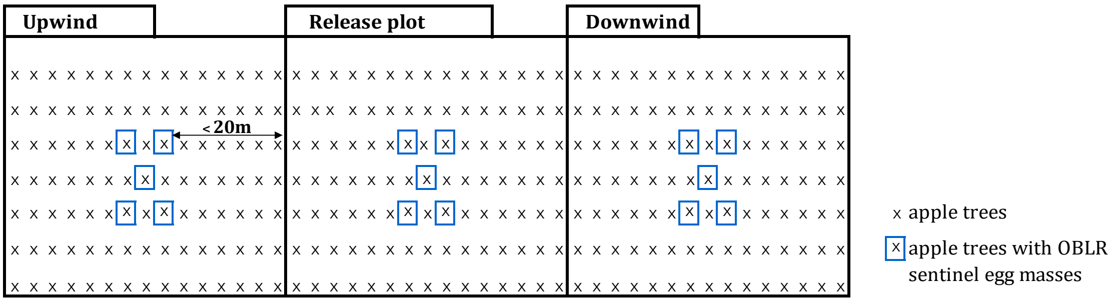

Figure 2. General layout of sentinel obliquebanded leafroller (OBLR) egg masses in the release and adjacent plots.

Analysis

Three parameters were defined to evaluate the activity of parasitoids: 1) occurrence of parasitism within a mass; 2) number of parasitized eggs per egg mass; and 3) number of parasitized eggs per parasitized egg mass. To determine whether there were differences between the two orchard types (dwarf and semi-dwarf apple trees) in overall levels of parasitism among egg masses located on release tree or at mid-distance between two release trees, and on egg masses located in adjacent plots with no *T. minutum* releases, the data were analyzed by two-way ANOVAs (rootstock and release tree; rootstock and plot) with a split-plot design, assigning 'rootstock' as the whole plot factor. Data were arcsine or square root transformed when required.

RESULTS

Comparisons within the release plot

Sentinel egg masses on trees in which parasitoids were released were not more frequently parasitized than those placed at mid-distance between two release trees ($F_{1,2}$=5.28; P=0.1484) (Figure 3a). In dwarf apple tree orchards, females parasitized significantly fewer eggs per egg mass on non-release trees than on release trees ($F_{1,2}$=28.69; P=0.0331) (Figure 3b). A significant interaction between 'rootstock' and 'release tree' was also observed when parasitism levels were calculated from parasitized egg masses only ($F_{1,2}$=26.83; P=0.0353) (Figure 3c).

Comparisons with adjacent plots

For all parameters, interactions between 'rootstock' and 'plot' were not significant (ANOVA; df=2,4; P>0.05), thus pooled data for dwarf and semi-dwarf apple tree orchards are presented (Table 2). The occurrence of parasitism ($F_{2,4}$=13.32; P=0.0171) and number of parasitized eggs per egg mass ($F_{2,4}$=14.35; P=0.0150) were higher in the release plots than in the adjacent plots. Parasitized egg masses from the release plots were not more heavily parasitized than those in adjacent plots ($F_{2,4}$=4.49; P=0.0950).

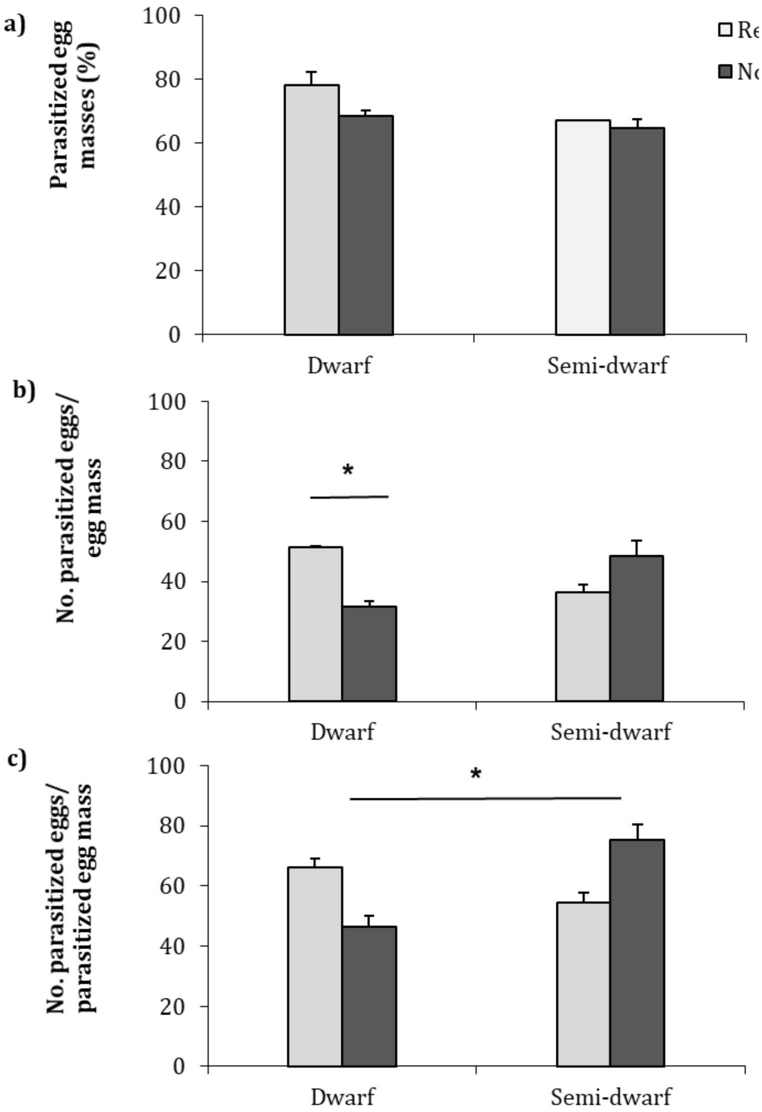

Figure 3. Occurrence of parasitism (a), and number of parasitized eggs on each *Choristoneura rosaceana* egg mass (b) and on parasitized egg mass only (c), located on release trees and at mid-distance between two release trees. Asterisk indicates significant differences (ANOVA, α=0.05). Means±SE. Data were arcsine or square root transformed when required.

Table 2. Occurrence of parasitism and number of eggs parasitized in each *Choristoneura rosaceana* sentinel egg mass in plots receiving repeated inundative releases of *Trichogramma minutum* as well as in adjacent plots.

Plot	Parasitized egg masses (%)	No. eggs parasitized per	
		egg mass	parasitized egg mass
Release	70.1±2.5 a	42.5±0.8 a	60.8±1.1 a
Upwind adjacent	42.3±6.0 b	29.5±5.7 b	69.9±8.3 a
Downwind adjacent	33.7±5.6 b	19.2±3.2 b	57.1±1.6 a

Values are mean±SE. Different letters within each column indicate significant differences (ANOVA, α=0.05). Data were arcsine or square root transformed when required.

DISCUSSION

The occurrence of parasitism on sentinel egg masses expressed as the percentage of parasitized egg masses can be considered to be a measure of host finding efficiency by egg parasitoids (Wang et al., 1999). The average fecundity of *T. minutum* is about 20-30 eggs per female for a 24-h period (Yu et al., 1984a; Cormier, unpublished data). OBLR egg masses usually contain more than 200 eggs (Chapman and Lienk, 1971), thus more eggs than a single *T. minutum* female can parasitize., The number of parasitized eggs per mass is also a measure of host finding efficiency because a higher number of eggs should be parasitized if a mass is visited by more than one female. The efficacy of parasitoids can further be evaluated by comparing levels of parasitism within egg masses calculated from only parasitized masses and defined as parasitism efficiency.

In our study, female parasitoids successfully located egg masses; more than 70% of sentinel egg masses were parasitized. However, only partial parasitism generally occurred, even in close proximity to release sites from which approximately 4,000 female parasitoids emerged. The effect of previous parasitism by conspecifics or interference between females (Lawson et al., 1997; McGregor et al., 2000; Vinson, 2010) may explain the partial parasitism in the release plots. Detection of previously parasitized hosts by female parasitoids may have prevented additional oviposition in these egg masses and this response might have a negative impact on the success of the biological control by *T. minutum.* In adjacent plots with no *T. minutum* releases, sentinel egg masses were less frequently localized than those in the release plots, but, surprisingly, similar numbers of eggs were laid in the egg masses visited by at least one female. Thus, even if host finding efficiency was higher in release plots, higher parasitism efficiency was not achieved. This suggests that each ovipositing female that reached an egg mass in the non-release plots might have deposited more eggs than females that found an egg mass near their emerging site. A greater urge of older females to lay eggs (Godfray, 1994) after travelling distances up to 20 m might explain their greater propensity to deplete their egg supply when a host was encountered.

Many studies report that the distance trichogram adults disperse from a release point rarely exceeds 20 m in orchards (Yu et al., 1984b; McDougall and Mills, 1997), and that dispersal is significantly affected by wind direction (Glenn and Hoffman, 1997; Cossentine and Jensen, 2000; Chapman et al., 2009). The activity of parasitoids detected in the adjacent plots, even upwind, indicates that under repetitive releases at a high release rate, many parasitoids travelled or were transported more than 20 m from their release site.

The distance of sentinel eggs from a release tree is known to have a significant influence on parasitism with percent parasitism values of 30% or less being reported in orchards at distances of 10-15 m from the release tree (McDougall and Mills, 1997; McGregor et al., 2000; Hegazi et al., 2012). In our study, release sites were spaced no more than 16 m apart, and with this high number of release sites, sentinel egg masses placed at mid-distance between release trees were not more frequently parasitized than those on release trees. However, parasitism was more uniform in semi-dwarf apple tree orchards than in dwarf apple tree orchards. This suggests that the dispersal of *T. minutum* may have been influenced by the size and density of apple trees. Several studies have shown that the dimensional volume of a crop and the complexity of the environment may affect the ability of *Trichogramma* spp. to disperse, search and locate hosts (Smith, 1988, 1996; Lawson et al., 1997; Gingras and Boivin 2002). The difference in parasitism patterns observed in dwarf and semi-dwarf apple tree orchards in this study might also be a result of the general arrangement of release trees in the two orchard types. In dwarf apple tree orchards, the release tree closest to an egg mass placed at mid-distance between two release trees was located on an adjacent row rather than on the same row (Figure 1). Our results suggest that *Trichogramma* spp. dispersal is lower between rows than within rows, as was observed by Smith (1996) and McDougall and Mills (1997). Consequently, as parasitoids appear to avoid open areas, this illustrates the need for more release trees in dwarf apple tree orchards, and the importance of releasing parasitoids on each row to achieve uniform parasitism in orchards.

CONCLUSIONS

- In dwarf apple tree orchards, parasitism was lower at mid-distance between two release trees because *Trichogramma* spp. disperse less successfully between rows than within rows. More release sites are required in high-density plantings compared to medium-density plantings, and *T. minutum* need to be released along every row.
- Despite high host-finding efficiency by *T. minutum* females, only a low level of parasitism was observed within egg masses in plots with inundative releases.
- Partial parasitism of OBLR egg masses indicates that females disperse sooner from egg masses in which previously parasitized eggs are encountered. The level of this response was less pronounced for females that travelled long distances from their emerging sites before encountering a host.
- This behaviour should have a negative impact on the success of OBLR population control by *T. minutum* in apple orchards, and therefore *Trichogramma* spp. releases should be used in combination with other control methods.

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