Field evaluation of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) and *T. brassicae* as biocontrol agents of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), in fresh market sweet corn

Elsa Etilé[1], Paula Cabrera[1], Josée Boisclair[2], Daniel Cormier[2], Silvia Todorova[3], and Éric Lucas[1]

Received 2023-01-19; accepted 2024-06-28

PHYTOPROTECTION 104 : 35-46

The efficiency of two *Trichogramma* species as biocontrol agents against the European corn borer (ECB) *Ostrinia nubilalis* (Lepidoptera: Crambidae) was investigated under field conditions. Five inundative releases of *Trichogramma ostriniae* and *T. brassicae* (Hymenoptera: Trichogrammatidae) were done in sweet corn plots. *Ostrinia nubilalis* sentinel egg masses were placed on corn plants in order to compare the efficiency of the two *Trichogramma* species. The parasitism rate of *O. nubilalis* egg masses by *T. ostriniae* was 13% higher than egg masses parasitized by *T. brassicae*. Likewise, the percentage of eggs parasitized per mass by *T. ostriniae* was significantly higher following two of the five releases. Attacked egg masses showed an average of 29% of eggs parasitized by *T. ostriniae,* versus 14% of eggs parasitized by *T. brassicae.* The rate of parasitoid emergence from sentinel egg masses was also significantly higher for *T. ostriniae* than for *T. brassicae*. Damages to plants due to ECB did not differ significantly in presence of the two parasitoid species. However, plants in plots receiving parasitoids showed less damage than plants in control plots. Overall, *T. ostriniae* was clearly more efficient than *T. brassicae* to parasitized egg masses of *O. nubilalis* in sweet corn.

Keywords: egg parasitoid, oophagous, inundative releases, endoparasitoid, biological control.

[Évaluation de *Trichogramma ostriniae* (Hymenoptera : Trichogrammatidae) et *T. brassicae* en tant qu'agents de lutte biologique contre la pyrale du maïs, *Ostrinia nubilalis* (Lepidoptera : Crambidae), dans le maïs sucré]

L’efficacité de deux espèces de *Trichogramma* pour lutter contre la pyrale du maïs, *Ostrinia nubilalis* (Lepidoptera: Crambidae), a été évaluée dans des conditions de terrain. Cinq lâchers de *Trichogramma ostriniae* et de *T. brassicae* (Hymenoptera: Trichogrammatidae) ont été faits dans des parcelles de maïs sucré. Des masses d’œufs sentinelles d’*O. nubilalis* ont été placées sur les feuilles de plants de maïs afin de comparer l’efficacité des deux espèces de trichogrammes. Le pourcentage de masses d’œufs d’*O. nubilalis* parasitées par *T. ostriniae* a été 13 % supérieur aux masses parasitées par *T. brassicae*. Parallèlement, le pourcentage d’œufs sentinelles parasités par masse par *T. ostriniae* a été significativement supérieur durant deux des cinq lâchers. Par masse parasitée, *T. ostriniae* a parasité 29 % des œufs, tandis que *T. brassicae* a parasité 14 % des œufs. Le taux d’émergence des parasitoïdes, des masses d’œufs sentinelles, a été significativement supérieur pour *T. ostriniae* que pour *T. brassicae*. Les dommages aux plantes à la récolte n’ont pas été significativement différents suite aux lâchers des deux espèces de parasitoïdes. Toutefois, les parcelles traitées avec les parasitoïdes ont montré moins de dommages que les parcelles témoins. Globalement, *T. ostriniae* a été clairement plus efficace que *T. brassicae* pour parasiter les œufs d’*O. nubilalis.*

Mots-clés : parasitoïde des œufs, oophage, lâchers inondatifs, endoparasitoïde, lutte biologique.

1. Laboratoire de lutte biologique, Département des sciences biologiques, Université du Québec à Montréal, 141 avenue du Président-Kennedy, Montréal (Québec), Canada. H2X 3X8. ✉ elsa.etile@gmail.com; cabrera.paula@uqam.ca; lucas.eric@uqam.ca
2. Institut de recherche et de développement en agroenvironnement, 335 rang des Vingt-Cinq Est, Saint-Bruno-de-Montarville (Québec), Canada J3V 0G7. ✉ josee.boisclair@irda.qc.ca; daniel.cormier@irda.qc.ca
3. Anatis Bioprotection, 278 rang Saint-André, Saint-Jacques-le-Mineur (Québec), Canada J0J 1Z0. ✉ stodorova@anatisbioprotection.com

INTRODUCTION

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), is one of the most damaging insect pests of sweet corn in North America and Europe (Blandino *et al.* 2010; Mason 1996). Larvae of the insect dig holes and galleries into the leaves and stalks of corn plants. However, damages inflicted to the ears are the most problematic for sweet corn growers because they are sold to consumers. Control of ECB is particularly challenging because the larvae enter the plant and hide within 2 to 3 days after hatching, giving a very short window for a phytosanitary intervention targeting the larvae.

In North America, the use of *Bt* corn, a transgenic variety expressing the *Bacillus thuringiensis* (Bacillales: Bacillaceae) protein against ECB, has widely controlled this pest. Nevertheless, due to consumer concerns regarding the use of transgenic crops, only 0.3% of commercially planted sweet corn is transgenic in Quebec and most producers rely on insecticides to control ECB (Gagnon *et al*. 2017).

In Southern Quebec (Canada), two races of *O. nubilalis* overlap during the season, the univoltine E race, and the bivoltine Z race (McLeod *et al*. 1979; Smith *et al*. 2015). As a result, ECB larvae may be active in the fields from mid-June to mid-October each year (Jean and Boisclair 2009). Eggs of the E race can be found between mid-June and early August, whereas eggs of the Z race are laid between late-May and early-July for the first generation and between late July and mid-September for the second generation. Consequently, *O. nubilalis* eggs could be found in sweet corn fields from late-May to mid-September, for a period of about 15 weeks. Fresh market sweet corn producers may apply up to three insecticide applications per season against ECB infestations (Duval *et al*. 2018).

Among natural enemies, some species have been reported attacking the ECB. For the predators, the lady beetle *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), and the minute pirate bug *Orius* spp. (Hemiptera: Anthocoridae) have been recorded to attack eggs and early instars (Coll and Bottrell 1992; Musser and Shelton 2003). The predaceous mites, *Trombidium fuliginosum* (Hermann) and the red-winged blackbirds, *Agelaius phoeniceus*, have also been identified to feed, respectively on eggs and larvae (Bendell *et al.* 1981; Hergula 1930). Moreover, populations of ECB larvae can be subject to pathogenetic infestations (Phoofolo *et al*. 2001). Nevertheless, these natural enemies do not reduce ECB infestation levels to below economic thresholds.

Various biological control methods for ECB have been tested, such as the application of entomopathogenic microorganisms like *Beauveria bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales) or *Nosema pyrausta* (Paillot) (Microspora: Nosematidae) (El-Sheikh and El-Shami 2017; Zimmermann *et al*. 2016). Among tested biocontrol agents, egg parasitoids *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) demonstrated a level of parasitism reaching more than 75% for ECB management (Camerini *et al*. 2018; Hassan 1981; Hoffmann *et al*. 2006; Qian *et al*. 1984; Razinger *et al.* 2016). In Europe, *Trichogramma brassicae* (Bedzenko) has been implemented successfully for several decades (Andow 2019). Efforts to transfer this technology for ECB management to North America started in the mid-90s (Andow *et al.* 1995). Inundative releases using *T. brassicae* often reduced both the density of the larvae and the frequency of stalk damage. Nevertheless, the variability between release rates and rates of egg parasitism generated inconsistent control (Andow *et al.* 1995). An evaluation of augmentative releases of *T. brassicae* for suppression of ECB in sweet corn in Iowa (Qian *et al.* 1984) demonstrated that even with higher release doses than those recommended in Europe (400,000 parasitoids per hectare), levels of parasitism remained lower (maximum of 54%) compared with those reported in Europe (maximum of 93%) (Phoofolo 1997).

In Quebec, Canada, *T. brassicae* has been commercially available and released in sweet corn fields for more than 20 years (Gagnon *et al*. 2017). Yet, another candidate for the control of *O. nubilalis* in Quebec is *Trichogramma ostriniae* (Pang & Chen), which has given encouraging results recently, with a high level of parasitism (Etilé *et al*. 2011; Gagnon *et al*. 2017; Gardner *et al*. 2013; Gauthier *et al*. 2019; Hoffmann *et al*. 2002). In the late '90s, it was introduced in the USA from China, where it proved effective against *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae), the Asian corn borer (Chen and Chiu 1986; Hirai 1995; Wang *et al.* 1984; Zhang 1988; Zhang *et al.* 2010). Studies have demonstrated this parasitoid to be highly effective at controlling ECB in sweet corn at different levels: search and dispersion efficiency (Gardner *et al*. 2012; Wright *et al.* 2001); parasitism efficiency (Hoffmann *et al.* 1998, 2002; Mason 1996), ECB mortality (Kuhar *et al.* 2002) and corn damage reduction (Etilé *et al*. 2011; Wright *et al.* 2002). Furthermore, in field conditions, this species also appeared to be more efficient at finding ECB egg masses than *Trichogramma nubilale* (Ertle and Davis), another agent for ECB control in Iowa (USA) (Wang *et al.* 1999). Furthermore, under laboratory, *T. ostriniae* demonstrated its capacity to successfully parasitize aging ECB eggs until the "blackhead" stage (when ECB larvae are within 24 hours before emergence) (Hoffmann *et al.* 1995), its ease to be reared commercially (Hoffmann *et al.* 2001), its good resistance to cold storage (Pitcher *et al.* 2002; St-Onge *et al.* 2014). Also, *T. ostriniae* females don't lose fitness after several generations reared on factitious hosts, such as *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) or *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) (Hoffmann *et al*. 2001).

Both *T. brassicae* and *T. ostriniae* are commercially available for the control of ECB. Previously, local suppliers recommended five releases, at one-week interval, at a dose of 250,000 *T. brassicae* wasps per hectare (Jean 2017). New York *T. ostriniae* suppliers recommend 3-4 releases at a dose of 74,000 wasps per hectare or more (IPM Labs Inc. 2020). A lower dose used by New York suppliers is explained by the results obtained by Hoffmann *et al*. (2002), conducting inoculative releases early in the corn-growing cycle. Thus, in Quebec, the use of *T. ostriniae* instead of *T. brassicae*, or the combination of both biocontrol agents, could possibly reduce the number of wasps needed and hence the costs associated to this practice.

The purpose of this study was to compare the efficacy of *T. brassicae* and *T. ostriniae* inundative field releases for the control of *O. nubilalis* in fresh market sweet corn in southern Quebec, to determine the potential of *T. ostriniae* as a suitable species for organic farming or integrated pest management programs. Both species were evaluated on parasitism efficiency, and their ability to reduce the damage caused by *O. nubilalis*.

MATERIALS AND METHODS

Insect material

Pupae of ECB were shipped from Agricultural Research, Inc., Lamberton, Minnesota, USA. On arrival, pupae were sexed (Gelman and Hayes 1982) and an equal number of male and female pupae were placed into inflated plastic bags (23 °C, 70% RH, 16L:8D). Plastic bags allow easy manipulation of the moths and facilitate the collection of egg masses. Adults usually started to emerge three to five days later, and egg masses were collected every day by cutting the piece of plastic bag around each egg mass. Afterwards, they were stored into a 6 °C, 60% RH refrigerator for a maximum of five days before being used as sentinel eggs. Sentinel eggs on the piece of plastic bag were placed in the field at each release and retrieved after three days to evaluate parasitism rates in the field.

Trichogramma brassicae and *T. ostriniae* wasps were obtained from Para-Bio (St-Augustin-de-Desmaures, Quebec, Canada) and IPM Laboratories, Inc. (Locke, New York, USA), respectively. They were shipped as pupae inside "ready-to-use" cards (commonly called Trichocards) containing around 8,000 sterilized and parasitized *E. kuehniella* eggs, glued on the trichocards.

Field bioassays

Field releases were conducted at Institut de recherche et de développement en agroenvironnement (IRDA) research farm in Saint-Hyacinthe (72.56 °W; 45.39 °N), Quebec, Canada. Twelve sweet corn plots (40 × 50 m each) were seeded on May 22, 2008, with the variety *Temptation*. Plots were separated by 150 m, to minimize *Trichogramma* dispersion to other plots. Corn rows were seeded 0.75 m apart, and plants were distanced 0.3 m along the rows. The experiment was conducted according to a completely randomized design with the following treatments:

a) Five *T. ostriniae* releases at a rate of 160,000 parasitoids/ha,
b) Five *T. brassicae* releases at a rate of 160,000 parasitoids/ha
c) The control, with no parasitoids released.

Each treatment was replicated four times.

Dates for releases were chosen as suggested by the local phytosanitary monitoring network, according to corn plants development and ECB activity monitoring (Jean 2017). The first release was made when at least 50% of corn plants reached the V6 developmental stage (4-6 fully developed leaves). Subsequent releases took place every 10 days (June 21, July 1, 11, 21, and 31).

Four trichocards per plot were placed in the centre of each treated plot, to create a central release point. All trichocards were prepared so that half the parasitoids emerged in less than 24 h and the other half had a delayed emergence (3-4 days later). Consequently, two cohorts of *Trichogramma* were released, thus maximizing the duration of the wasps' activity in the field.

Twelve sentinel egg masses, consisting of an ECB egg mass laid in the laboratory on a piece of plastic bag (18 ± 0.47 eggs/mass in average) were placed in each plot on plants located at 5, 10, and 15 m from the central *Trichogramma* release point, in a X pattern. Each egg mass was attached to a leaf located at the middle portion of the corn plant with a foldback clip (15 mm). Two cohorts of sentinel egg masses were installed in the field, early in the morning, successively and at the same point. The first one was placed the day following trichocards installation, and the second one, four days later, to guarantee fresh egg masses when the parasitoids emerged. These two sentinel egg cohorts allowed us to evaluate the parasitism levels from the two *Trichogramma* cohorts.

Four days after installation, all sentinel egg masses were retrieved from the field, placed into plastic cups (diameter 85 mm, height 120 mm) (Solo Cup©, IL, USA) and incubated in an environmental chamber (27 °C, 60% RH, 16L:8D). The number of parasitized eggs per egg mass was counted after four days and the number of egg masses lost due to predation or other causes (wind, rain) was recorded for each plot (24.8% on average; 357 out of 1440 sentinel egg masses in total).

All parasitized egg masses were maintained in the environmental chamber until parasitoid emergence was over. To assess the percentages of parasitoid emergence, the total number of emerged adults was divided by the total number of parasitized host eggs (blackened eggs). This assessment was appropriated, because one trichogram adult emerged per ECB egg (unpublished).

All trichocards were retrieved after 10 days in the field. *Trichogramma* emergence was evaluated on approximately 250 parasitized *E. kuehniella* eggs per trichocard, by counting the number of emergence holes on blackened eggs. In addition, three control trichocards were put in an environmental chamber the day of each release (27 °C; 60%; 16L:8D). After 10 days, these cards were evaluated for the percentage of emergence using the same method as the field trichocards. Furthermore, the sex-ratio and the proportion of brachypterism of the emerged wasps were assessed out of 250 individuals.

Damage at harvest was assessed by counting all *O. nubilalis* larvae and signs of damage on tassels, stalks, and ears on 100 plants observed in a group of five plants in 20 stations.

Meteorological data were obtained from the station Saint-Liboire integrated into the CIPRA software (Bourgeois *et al*. 2005).

Statistical analysis

On-farm parasitoid release quality

Percentage of parasitoid emergence, sex-ratio and brachypterism were compared between fields receiving *T. brassicae* and fields receiving *T ostriniae* using Student's t-test on the average proportion of individuals emerging from field trichocards and control trichocards, following angular transformation. All statistical analyzes were carried out using JMP 8 (SAS Institute, Inc.).

Parasitism efficiency

As per Wang and Ferro (1998), we used the number of egg masses parasitized in each plot to indicate the percentage of egg masses located by the two *Trichogramma* species. An egg mass was considered located by a wasp no matter how many eggs were parasitized in it. The proportions of egg masses parasitized over the whole season and at each of the five releases were compared among the three treatments. The data were initially analyzed using a logistic regression model considering distance (5, 10 or 15 m), cohort (first (a) and second (b) cohort), relase (releases 1 to 5) and treatment (*T. ostriniae, T. brassicae* and control) as parameters. Because no effect of the distance or cohort factors was detected, data were pooled for both cohorts as well as for the three distances. The significance of a variable was determined by using the likelihood-ratio test. Analyses of variance were performed, and post hoc Tukey's HSD was used to evaluate differences among treatments considering dates of *Trichogramma* release.

To evaluate parasitism efficiency at each release, we compared the proportion of parasitized sentinel eggs in each treatment with a non-parametric Kruskal-Wallis test. Differences among groups were assessed using the Wilcoxon each pair test (Goos and Meintrup 2016).

Furthermore, the number of parasitized eggs per female released in the field was calculated (no of eggs parasitized / (no parasitoids released × % emergence × % females emerged)). This allowed us to evaluate how much of the parasitism was attributable to individuals' activity rather than to their abundance. These data were compared among plots receiving *Trichogramma* only by Mann-Whitney-Wilcoxon tests.

Finally, we assessed how females exploited the egg masses comparing the average proportion of parasitized eggs in a mass. This was evaluated by performing a Kruskal-Wallis test between the two treatment groups on the average proportion of parasitized eggs in a mass, following an angular transformation.

Damage at harvest

Percentage of damaged tassels, stalks and ears per treatment, as well as mean number of larvae per plant were compared between treatments by two-way ANOVAs followed by HSD Tukey test. Student's t-tests were used to compare damage to stalks between *Trichogramma* and control plots, and to compare damage to stalks between both *Trichogramma* species.

Analyses were conducted using JMP software (JMP®, Version 14.0.0 SAS Institute Inc., Cary. NC, 1989-2021).

Table 1. Emergence rate, sex-ratio and percentage of brachypterism of *Trichogramma ostriniae* and *T. brassicae* from field release and control trichocards

Release date	Field trichocards		Control trichocards					
	Emergence (% ± SE)		Emergence (% ± SE)		♀ / (♂ + ♀)		Brachypterism (% ± SE)	
	T. ostriniae	*T. brassicae*	*T. ostriniae*	*T. brassicae*	*T. ostriniae*	*T. brassicae*	*T. ostriniae*	*T. brassicae*
June 21	65.7 ± 9.2	51,3 ± 6.7*	64.6 ± 8.3	44.9 ± 2.2*	0.67	n.d	17 ± 26.2	7 ± 1.6
July 1	55.2 ± 11.2	42.9 ± 8.4*	66.4 ± 11.9	30.5 ± 29.8	0.69	0.32	6 ± 1.3	54 ± 51.6
July 11	66.9 ± 6.4	42.5 ± 7.7*	61.1 ± 3.5	59.4 ± 2.6	0.68	0.53	5 ± 1.3	12 ± 3.1
July 21	73.6 ± 5.5	55.9 ± 4.9*	69.1 ± 1.8	54.6 ± 4.9*	0.60	0.49*	35 ± 56.1	66 ± 40.3
July 31	--	--	76.9 ± 5.7	48.9 ± 5.5*	0.69	0.46*	4 ± 1.4	19 ± 10.1

* Indicates a significant difference between the two species (α = 0.05) compared with Student's t-test on the global proportion of emergence evaluated on field and control trichocards, sex-ratio and proportion of individuals presenting brachypterism, all following angular transformation.

-- The percentage of emergence could not be evaluated on field cards for July 31 because the trichocards were unexploitable due to mould.

RESULTS

Parasitoid quality

The percentage of emergence from trichocards used for the field releases ranged from 55.2 to 73.6% for *T. ostriniae* and from 42.5 to 55.9% for *T. brassicae* and it was significantly higher for *T. ostriniae* at all releases (Table 1).

Control trichocards kept in environmental chambers showed emergences ranging from 61.1 to 76.9% for *T. ostriniae* and from 30.5 to 59.4% for *T. brassicae* (Table 1).

Emergence rate was significantly higher for *T. ostriniae* at the first ($t_{2.27} = 3.90$; $P = 0.0496$), fourth ($t_{2.54} = 4.85$; $P = 0.0225$), and fifth ($t_{3.99} = 5.93$; $P = 0.0045$) release date.

Globally, populations emerging from *T. ostriniae* trichocards were more female-biased than the ones emerging from *T. brassicae* trichocards (Table 1). However, this difference was only significant at the fourth ($t_{3.38} = 4.68$; $P = 0.0141$) and fifth ($t_{3.21} = 4.17$; $P = 0.022$) release date. At each release date, brachypterism was not significantly different between the trichograms species (Table 1).

Parasitism efficiency

Both the treatment ($\chi^2 = 66.58$; d.f. = 2; $P < 0.0001$) and the date of release ($\chi^2 = 132.94$; d.f. = 4; $P < 0.0001$) had a significant positive effect on the overall proportion of egg masses located by parasitoids. There was also a significant interaction between the two factors ($\chi^2 = 22.81$; d.f. = 8; $P = 0.0036$). Therefore, proportions of egg masses parasitized are first presented pooled over the whole season (Fig. 1A) and then separately for each release (Fig. 1B). More sentinel egg masses were parasitized in plots receiving parasitoids compared to control plots and significantly more egg masses were parasitized in plots receiving *T. ostriniae* (40.6%) compared to *T. brassicae* (27.2%) ($q = 2.79$; $P < 0.05$) (Fig. 1A).

The pattern of parasitism observed through the season was similar to both species (Fig. 1B). The percentage of parasitism initially increased and then decreased. For *T. ostriniae*, the peak of parasitism was reached at the fourth release (July 21) with an average of 72% masses parasitized. For *T. brassicae,* the peak was reached at the third release (July 11) with an average of 58% masses parasitized. Most of the difference in parasitism rates between the two species was noted at the fourth release, where *T. ostriniae* parasitized 45% more egg masses than *T. brassicae* (Fig. 1B).

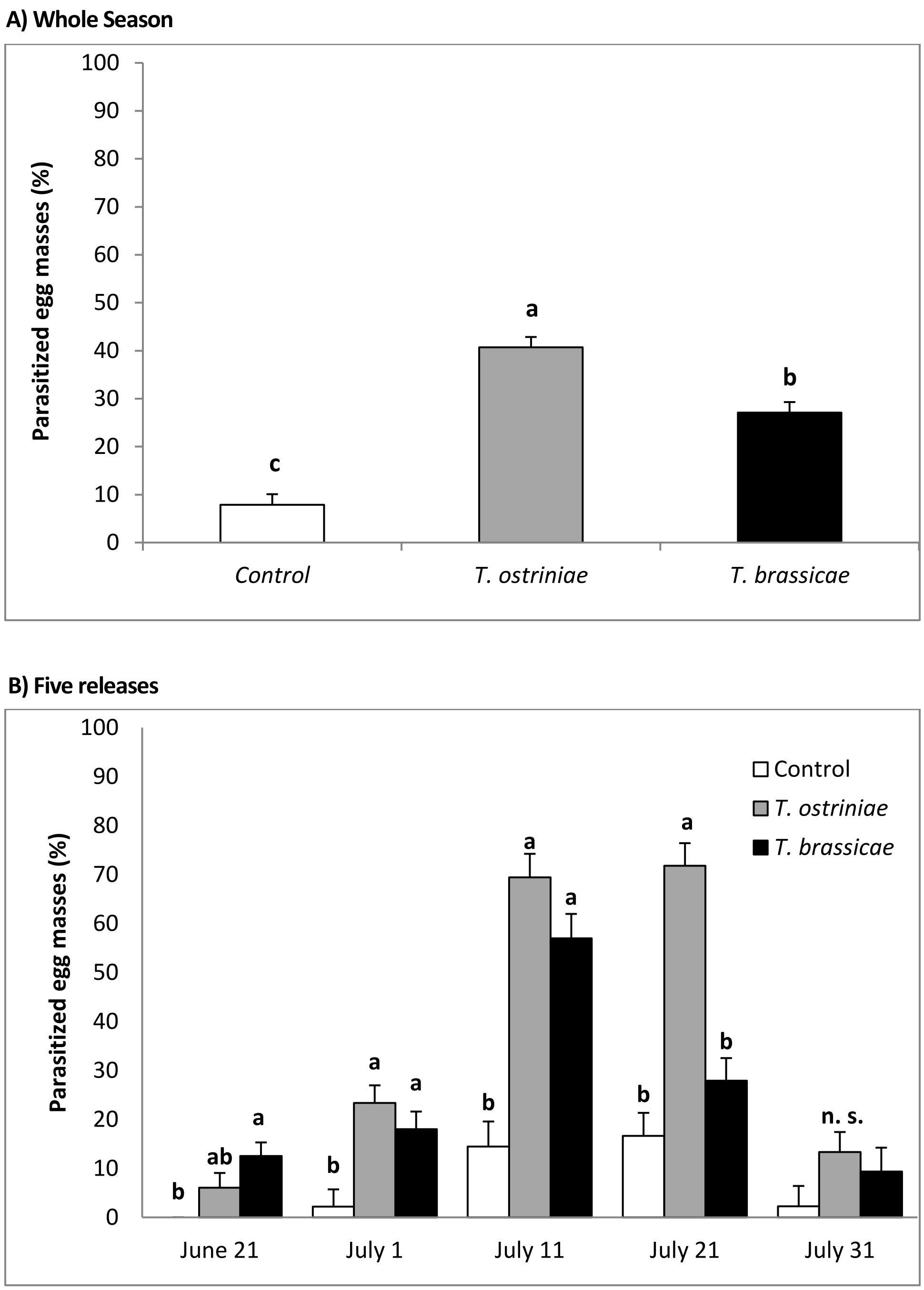

Figure 1. Percentage (mean ± SE) of sentinel *O. nubilalis* egg masses parasitized by *T. ostriniae* and *T. brassicae* for (A) the whole season and (B) five release dates. Different letters above the bars indicate significant differences between treatments based on logistic regression (a) and ANOVA ($\alpha = 0.05$) performed on the percentage of egg masses parasitized within each parasitoid release (b).

On the 1,231 recovered egg masses (14.5%), the proportion of eggs parasitized per egg mass was significantly higher for *T. ostriniae.* On average, *T. ostriniae* females parasitized 29% of an egg mass, while *T. brassicae* females parasitized 14% of an egg mass ($\chi^2_{(1)} = 120.17$; $P < 0.0001$) (Fig. 2A). However, the percentage of egg masses entirely parasitized was not different between the two species (*T. ostriniae*: 9.3%; *T. brassicae*: 7.2%; $\chi^2_{(1)} = 0.33$; $P = 0.5657$).

From the second to the fifth releases (July 1 to 31), the percentage of parasitized eggs per egg mass by *T. ostriniae* was higher than *T. brassicae* (Fig. 2B) but these differences were significant only after the third ($X^2_{(2)} = 16.42$; $P = 0.0003$) and fourth releases ($X^2_{(2)} = 15.67$; $P = 0.0004$) (Fig. 2B).

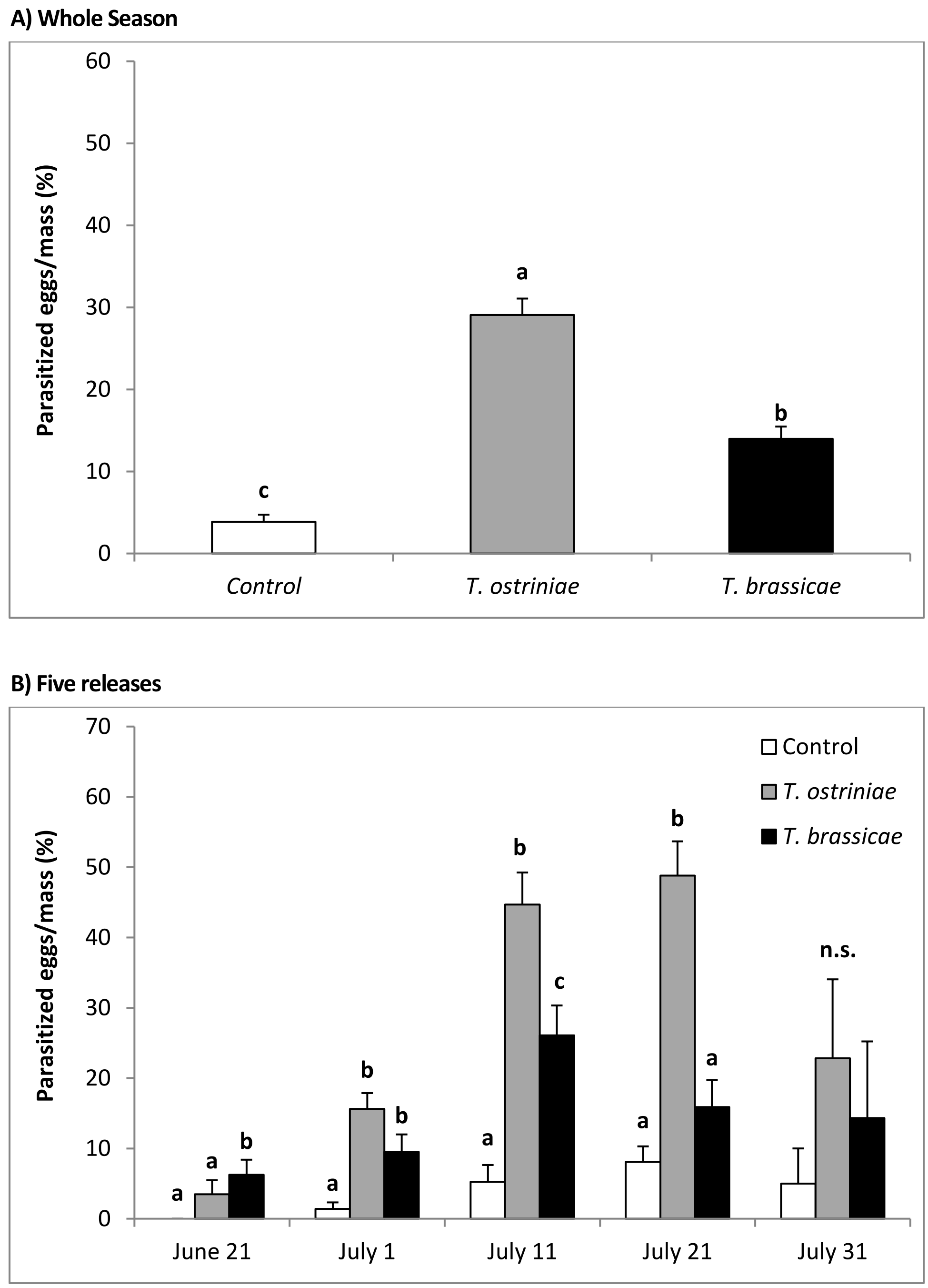

Figure 2. Percentage (mean ± SE) of sentinel *O. nubilalis* eggs parasitized per egg mass on the total number of recovered egg masses for (A) the whole season and (B) five release dates. Different letters above the bars indicate significant differences between treatments based on Kruskal-Wallis tests (α = 0.05).

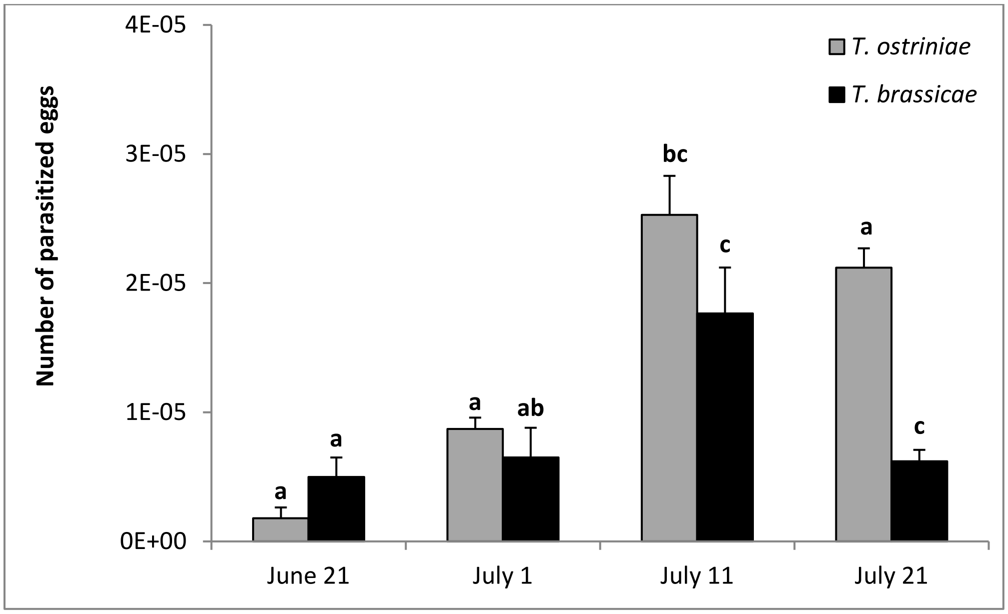

Figure 3. Number (mean ± SE) of sentinel *O. nubilalis* eggs parasitized per released female after releases 1-4 (data adjusted for the emergence rate). Release 5 is not presented because no emergence rate was available. Different letters above the bars indicate significant differences between groups based on Kruskal-Wallis tests (α = 0.05).

Finally, when adjusting the data with the emergence rates to calculate the number of eggs parasitized per released female (calculated) for *T. ostriniae* was significantly higher than *T. brassicae* at the fourth release (Fig. 3) (June 21 through July 11; $P > 0.05$; July 2[1]: $P = 0.0018$).

Damage at harvest

There were significantly less ECB larvae per plant in plots treated with parasitoids compared to control plots ($F_{2,9} = 9.02$; $P = 0.0001$) but there were no significant differences between plots treated with *T. ostriniae* and *T. brassicae* (Fig. 4).

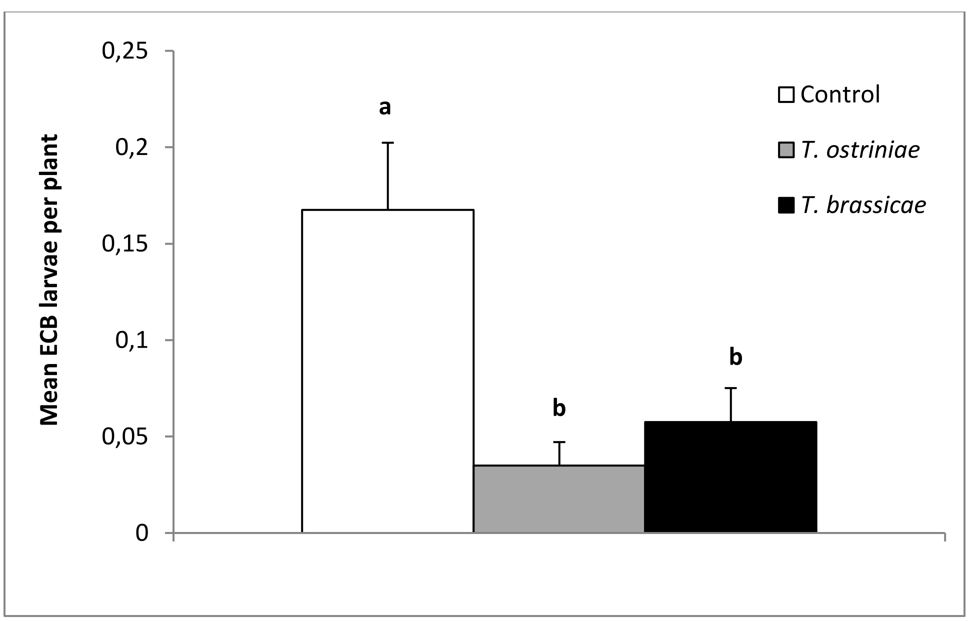

Figure 4. Mean number of ECB larvae per sweet corn plant (mean ± SE). ANOVA followed by HSD Tukey test (α = 0.05).

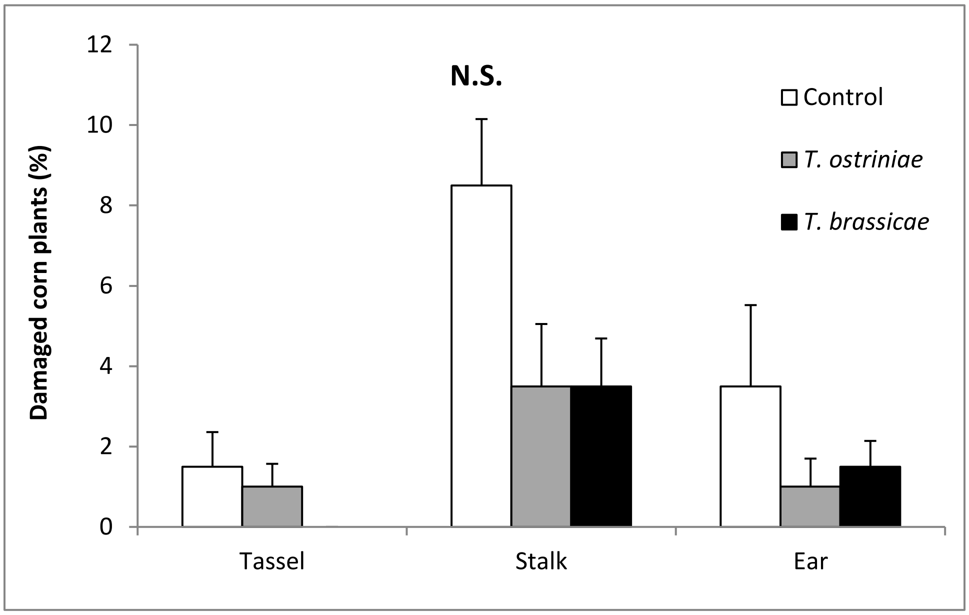

Figure 5. Percentage (mean ± SE) of sweet corn plants damaged at the tassel, stalk and ear. ANOVA followed by HSD Tukey test (α = 0.05).

The amount of damage inflicted to corn tassels was less than 2% and not different among the three treatments ($F_{2,9}$ = 1.62, P = 0.2507). Damage to stalks was not significantly different among treatments ($F_{2,9}$ = 3.79, P = 0.0640). There were significantly less damaged stalks in plots treated with *Trichogramma* compared to control plots ($t_{4.87}$ = -2,645; P = 0.0468) but there were no significant differences between *T. brassicae* and *T. ostriniae* ($t_{5.62}$ = 0; P = 1.0). Damage inflicted to corn ears was not significantly different among the three treatments ($F_{2,9}$ = 1.05; P = 0.3892) (Fig. 5).

The proportion of plants without any damage caused by *O. nubilalis* was higher in plots treated with *Trichogramma* ($t_{4.53}$ = 2.32; P = 0.0365) but the difference between the two parasitoid species was not significant ($t_{4.58}$ = -0.65; P = 0.728).

DISCUSSION

The results of the present study indicate that *T. ostriniae* performed better than *T. brassicae* under field conditions. Parasitism of ECB egg masses by female parasitoids in plots treated with *T. ostriniae* was 13% higher than in those treated with *T. brassicae*. The percentage of parasitized egg masses, over the whole season, showed the same trend. Furthermore, the percentage of parasitized sentinel ECB eggs per egg mass, by *T. ostriniae,* was significantly higher than the percentage of parasitized sentinel ECB eggs per egg mass by *T. brassicae* after two of the five releases. Similarly, over the whole season, the percentage of parasitized eggs per mass, by *T. ostriniae*, was higher than the percentage of parasitized eggs per mass by *T. brassicae*. In any case, the parasitism was higher for *T. ostriniae* than *T. brassicae*, which may be explained by different hypotheses.

The first hypothesis to explain the performance of *T. ostriniae* is linked to the quality of the populations released in the field. Parasitoid release quality was similar in the field and in the laboratory and generally higher for *T. ostriniae*. However, a key point is the fact that *T. ostriniae* showed significantly higher emergence rates (+ 18%) on both field and control trichocards and a stronger female-biased sex-ratio at the last two releases. While brachypterism was high for *T. ostriniae*, it was not different than brachypterism of *T. brassicae*. Emergence rate, sex-ratio and presence of brachypterism in *Trichogramma* have all been recognized as key elements to evaluate the quality of parasitoid species (van Lenteren and Bigler 2010). In our study the difference in the emergence rate may be due to a natural emergence rate lower for *T. brassicae* than for *T. ostriniae,* or to a reduced quality associated with the production, handling or transportation of *T. brassicae* to our laboratory. Cerutti and Bigler (1995) reported that low emergence rate could be associated with exposition to a long-range transportation. However, *T. brassicae* was obtained from a company close to our laboratory, suggesting that long-range transportation was not the major factor affecting low emergence rate.

A second hypothesis that should be considered is that environmental conditions may have detrimentally affected more *T. brassicae*. However, since laboratory results provide the same pattern as field results, we must discard the environmental effect on emergence rates. Then, in the field, taking into consideration the pattern of parasitism observed over the season, an increase followed by a decrease of parasitism was observed. Such pattern is typical of *Trichogramma* activity in corn fields (Hoffmann *et al.* 2002; Wang *et al.* 1997, 1999) and it is primarily due to changing weather conditions and plant architecture within the season (Klug and Meyhöfer 2009; Wang *et al.* 1997). This pattern was similar to both species. Nevertheless, we suggest that *T. ostriniae* was significantly more efficient than *T. brassicae* during the ten-day period of the fourth release, possibly due to weather conditions. In fact, *T. brassicae* walking activity is negatively affected by temperatures below 20 °C (Suverkropp *et al.* 2001). On the other hand, *T. ostriniae* seems to be more tolerant to cold temperatures, since a decrease in activity has been noticed only below 17 °C (Wang *et al.* 1997). Conversely, low walking activity at low temperatures can be a major constraint for the success of *T. brassicae* as a biological control agent in temperate countries, because cool weather leads to unacceptably low levels of field parasitism for this species (Lessard and Boivin 2012; Pak and van Heiningen 1985). During the ten-day period corresponding to the fourth release in our study (July 21 to 31), the average daily temperature ranged from 18.8 to 29.5 °C. However, the minimum daily temperature ranged between 18.4 and 28.8 °C and during 76 h, temperatures were below 20 °C. Consequently, cool weather may have been a limiting factor to the efficiency of parasitism by *T. brassicae*. For example, it is possible that, after a cold night, *T. ostriniae* was able to start its searching activity earlier than *T. brassicae*, therefore gaining a longer period of activity over the day.

A third hypothesis is a superior biological efficiency of *T. ostriniae* over *T. brassicae*. Egg mass location, expressed as the proportion of parasitized egg masses over the total number of masses recovered from the field, was considered by Bin and Vinson (1991) to be a measure of host finding efficiency, that is, habitat and host location by the parasitoid. Consequently, it is a major quality for natural enemies. In the present study, females *T. ostriniae* were globally better than *T. brassicae* at finding egg masses. Furthermore, *T. ostriniae* parasitized more egg masses over the whole season, and more specifically at the fourth release. *Trichogramma ostriniae* also parasitized more eggs per egg mass than *T. brassicae* during July 1 to 31 releases and this difference was statistically significant at third and fourth releases. Thus, *T. ostriniae* is more efficient at finding egg masses and exploiting egg mass than *T. brassicae*. Consequently, *T. ostriniae* is better at preventing *O. nubilalis* larvae from emerging from egg masses than *T. brassicae*.

From a biocontrol perspective, both parasitoid species effectively reduced the ECB damage compared to the control. In fact, the percentage of damaged corn ears in control plots was lower than the 5% tolerated by fresh market sweet corn producers, but parasitism also occurred in control plots. The significant differences in parasitism rates between the two *Trichogramma* species over the season were not reflected in the amount of damage at the end of the growing season. Considering the higher performance of *T. ostriniae* compared to *T. brassicae* in terms of parasitism, we suggest that the lack of plant damage differences between plots treated with *T. ostriniae* and *T. brassicae* could be explained by a low percentage, less than 10%, of fully parasitized egg masses by both *Trichogramma* species. Although *T. ostriniae* is known to be highly efficient, rarely 100% parasitism of eggs per egg mass is observed in a field situation (Gardner *et al*. 2007; Hoffmann *et al*. 2002; Kuhar *et al*. 2002). In the case of lepidopteran species such as *O. nubilalis*, even one emerging larva is enough to potentially infest the corn cob, hence making it unacceptable for the fresh market. Although *T. ostriniae* was more efficient than *T. brassicae* at exploiting an ECB egg mass, none of them were better at parasitizing an egg mass entirely. *T. ostriniae* fully parasitized 9.3% of all the egg masses retrieved from the field while *T. brassicae* parasitized 7.2% of the masses. This could be explained by the influence of several factors on the female parasitoid decision, such as the physiological state of the female, its egg laying potential, its age, and environmental circumstances (Hirose *et al*. 1976; Konopka *et al*. 2018; Rahimi-Kaldeh *et al*. 2018). Nevertheless, based on optimal foraging behaviour, a partial resource used by parasitoids is a way to avoid superparasitism and multiparasitism. In other words, to be stronger competitors, females must dedicate more time to assess the quality of egg clusters than parasitizing eggs. Thus, more time is invested in selecting, on average, one future egg cluster, which optimizes future gain (Montovan *et al*. 2015).

Regarding the dose of parasitoids used in this study, we released 160,000 parasitoids/ha, which is an intermediary dose between doses used by Quebec suppliers, five releases at a dose of 250,000 *T. brassicae* wasps per hectare (Jean 2017), and New York suppliers, 3-4 releases of *T. ostriniae* at a dose of 74,000 parasitoids/ha (IPM Labs Inc. 2020). As mentioned in the introduction section, the later use a lower dose compared to Quebec suppliers based on results from Hoffmann *et al*. (2002). These authors demonstrated that a low density of 75,000 females/ha was enough to assure viable reproductive populations of *T. ostriniae* in sweet corn fields with the potential to contribute to control ECB in IPM programs. Using an intermediary dose, we wanted to assess the respective performance of *T. ostriniae* and *T. brassicae*. In another study, three mass releases of 90,000 *T. ostriniae* per hectare were successful by reducing the ECB larval population and damages (Gagnon *et al.* 2017).

Concluding, the present study demonstrates clearly that any experiment with *Trichogramma* species must include an evaluation of the emergence rate both in the laboratory and in the field. Thus, our results suggest that *T. ostriniae* is a better biocontrol agent for ECB on sweet corn than *T. brassicae*. Besides, it has been demonstrated that releases of *T. ostriniae* are cost-effective to control ECB in Quebec (Gagnon *et al*. 2017). Moreover, utilization of parasitoids is an environmentally sound option to chemical methods and safe for human health. Due to several parameters favouring *T. ostriniae* as a potential biocontrol agent, even if we did not demonstrate a beneficial impact at the harvest level, *T. ostriniae* is now mass-produced and released together with *T. brassicae* in Quebec sweet corn fields. In future studies we plan to modify the frequency and dose of parasitoids released per season and the way to release them, especially in large sweet corn fields, such as the use of drones as described by Martel *et al*. (2021).

ACKNOWLEDGEMENTS

We would like to thank Stéphane Dupuis, Lee French, IPM Laboratories and Anatis Bioprotection Inc. for supplying the insects. We also thank Dr. Stephen Gaimari for molecular *Trichogramma* species identification. This research was supported by the Programme de soutien à l'innovation en agroalimentaire of the ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec.

REFERENCES

Andow, D.A. 2019. Integrating biological control in IPM systems. Pages 71-86 *in* D.A. Andow (ed.), Ecological interactions and biological control. CRC Press, Boca Raton, FL, USA. doi:10.1201/9780429041686-4

Andow, D.A., G.C. Klacan, D. Bach, and T.C. Leahy. 1995. Limitations of *Trichogramma nubilale* (Hymenoptera: Trichogrammatidae) as an inundative biological control of *Ostrinia nubilalis* (Lepidoptera: Crambidae). Environ. Entomol. 24: 1352-1357. doi:10.1093/ee/24.5.1352

Bendell, B.E., P.J. Weatherhead, and R.K. Stewart. 1981. The impact of predation by red-winged blackbirds on European corn borer populations. Can. J. Zool. 59: 1535- 1538. doi:10.1139/z81-208

Bin, F., and S.B. Vinson. 1991. Efficacy assessment in egg parasitoids (Hymenoptera): proposal for a unified terminology. Pages 175-180 *in* E. Wajnberg (ed.), *Trichogramma* and other egg parasitoids: 3rd international symposium,

San Antonio, TX, USA, September 23-27, 1990. Institut national de la recherche agronomique, Paris, France.
Blandino, M., A. Peila, and A. Reyneri. 2010. Timing clorpirifos + cypermethrin and indoxacarb applications to control European corn borer damage and fumonisin contamination in maize kernels. J. Sci. Food Agric. 90: 521-529. doi:10.1002/jsfa.3850
Bourgeois, G., N. Beaudry, D. Plouffe, G. Chouinard, R. Audet, and G. Deaudelin. 2005. Forecasting pests in field crops using real-time weather information: the CIPRA network in Quebec. Acta Hort. 674: 303-304. doi:10.17660/ActaHortic.2005.674.35
Camerini, G., S. Maini, and M. Riedel. 2018. *Ostrinia nubilalis* parasitoids in Northern Italy: past and present. Biol. Control 122: 76-83. doi:10.1016/j.biocontrol.2018.03.011
Cerutti, F., and F. Bigler. 1995. Quality assessment of *Trichogramma brassicae* in the laboratory. Entomol. Exp. Appl. 75: 19-26. doi:10.1111/j.1570-7458.1995.tb01905.x
Chen, C.C., and S.C. Chiu. 1986. Studies on the field releases of *Trichogramma chilonis* and the factors affecting its activity. J. Agric. Res. China 35: 99-106.
Coll, M., and D.G. Bottrell. 1992. Mortality of European corn borer larvae by natural enemies in different corn microhabitats. Biol. Control 2: 95-103. doi:10.1016/1049-9644(92)90032-9
Duval, B., I. Couture, C. Leblanc, and J.-P. Légaré. 2018. Pyrale du maïs [Fiche technique]. Réseau d'avertissements phytosanitaires – Maïs sucré. Available online [https://www.agrireseau.net/documents/Document_97138.pdf].
El-Sheikh, M.F., and I.A. El-Shami. 2017. Isolation and virulence of *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) isolates from hibernated *Ostrinia nubilalis* (Hb.) and *Sesamia cretica* Led. from maize stalks. J. Plant Prot. & Pathol. 8: 549-553. doi:10.21608/jppp.2017.46604
Etilé, E., J. Boisclair, D. Cormier, S. Todorova, and É. Lucas. 2011. Evaluation of two biological control strategies against the European corn borer (*Ostrinia nubilalis*) in southern Quebec fresh sweet corn. Pages 614-620 *in* 4e conférence internationale sur les méthodes alternatives en protection des cultures. Évolution des cadres réglementaires européen et français. Nouveaux moyens et stratégies innovantes. Lille, France, March 8-10, 2011. Association française de protection des plantes (AFPP).
Gagnon, A.-È., C. Audette, B. Duval, and J. Boisclair. 2017. Can the use of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) to control *Ostrinia nubilalis* (Lepidoptera: Crambidae) be economically sustainable for processing sweet corn? J. Econ. Entomol. 110: 59-66. doi:10.1093/jee/tow293
Gardner, J., M.P. Hoffmann, S.A. Cheever, A.J. Seaman, P. Westgate, and R.V. Hazzard. 2007. Large-scale releases of *Trichogramma ostriniae* to suppress *Ostrinia nubilalis* in commercially grown processing and fresh market sweet corn. J. Appl. Entomol. 131: 432-440. doi:10.1111/j.1439-0418.2007.01198.x
Gardner, J., M.G. Wright, T.P. Kuhar, S.A. Pitcher, and M.P. Hoffmann. 2012. Dispersal of *Trichogramma ostriniae* in field corn. Biocontrol Sci. Techn. 22: 1221-1233. doi:10.1080/09583157.2012.723676
Gardner, J., T.-H. Yong, S.A. Pitcher, and M.P. Hoffmann. 2013. Overwintering of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) within target and non-target host eggs. Biocontrol Sci. Techn. 23: 367-380. doi:10.1080/09583157.2012.761175
Gauthier, P., M. Khelifi, A. Dionne, and S. Todorova. 2019. Technical feasibility of spraying *Trichogramma ostriniae* pupae to control the European corn borer in sweet corn crops. Appl. Eng. Agric. 35: 185-192. doi:10.13031/aea.12833
Gelman, D.B., and D.K. Hayes. 1982. Methods and markers for synchronizing maturation of fifth-stage larvae and pupae of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 75: 485-493. doi:10.1093/aesa/75.5.485
Goos, P., and D. Meintrup. 2016. Statistics with JMP: hypothesis tests, ANOVA and regression. John Wiley & Sons, Chichester, United Kingdom. 656 pp.
Hassan, S.A. 1981. Mass-production and utilization of *Trichogramma*: 2. Four years successful biological control of the European corn borer. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent, 46: 417-427.
Hergula, B. 1930. On the mortality of the eggs and young larvae of *Pyrausta nubilalis*. Acta Soc. Ent. 3-4: 44-60.
Hirai, K. 1995. Selection of effective *Trichogramma* species for controlling Asian corn borer in a sweet corn field. Pages 119-124 *in* E. Wajnberg (ed.), *Trichogramma* and other egg parasitoids: 4th international symposium, Cairo, Egypt, October 4-7, 1994. Institut national de la recherche agronomique, Paris, France.
Hirose, Y., H. Kimoto, and K. Hiehata. 1976. The effect of host aggregation on parasitism by *Trichogramma papilionis* Nagarkatti (Hymenoptera: Trichogrammatidae), an egg parasitoid of *Papilio xuthus* Linne (Lepidoptera: Papilionidae). Appl. Entomol. Zool. 11: 116-125. doi:10.1303/aez.11.116
Hoffmann, M.P., P.R. Ode, D.L. Walker, J. Gardner, S. van Nouhuys, and A.M. Shelton. 2001. Performance of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) reared on factitious hosts, including the target host, *Ostrinia nubilalis* (Lepidoptera: Crambidae). Biol. Control 21: 1-10. doi:10.1006/bcon.2000.0912
Hoffmann, M.P., S.A. Pitcher, S.A. Cheever, J. Gardner, J.E. Losey, T.P. Kuhar, C.A. Laub, and R.R. Youngman. 2006. Efficacy of inoculative releases of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) against European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae) in field corn. Biol. Control 36: 345-349. doi:10.1016/j.biocontrol.2005.10.008
Hoffmann, M.P., A.J. Seaman, J. Gardner, and S.A. Chenus. 1998. Early season establishment of *Trichogramma ostriniae* for season-long suppression of European corn borer in sweet corn. Pages 143-146 *in* 1998 Progress report to the New York State integrated pest management program. Available online [https://hdl.handle.net/1813/55121].
Hoffmann, M.P., D.L. Walker, and A.M. Shelton. 1995. Biology of *Trichogramma ostriniae* (Hym.: Trichogrammatidae) reared on *Ostrinia nubilalis* (Lep.: Pyralidae) and survey for additional hosts. Entomophaga 40: 387-402. doi:10.1007/BF02373727
Hoffmann, M.P., M.G. Wright, S.A. Pitcher, and J. Gardner. 2002. Inoculative releases of *Trichogramma ostriniae* for suppression of *Ostrinia nubilalis* (European corn borer) in sweet corn: field biology and population dynamics. Biol. Control 25: 249-258. doi:10.1016/S1049-9644(02)00105-6
IPM Labs Inc. 2020. Sweet corn growers. Manage European corn borer. Use ECB's natural enemy. IPM Labs Inc, Locke, NY, USA. 2 pp.

Jean, C. 2017. Les trichogrammes dans le maïs; lutte contre la pyrale du maïs. Para-Bio, St-Augustin-de-Desmaures, Quebec, Canada. Available online [http://www.parabio.ca/brochure.pdf].

Jean, C., and J. Boisclair. 2009. Les insectes nuisibles et utiles du maïs sucré : mieux les connaître. Institut de recherche et de développement en agroenvironnement, Québec, Québec, Canada. 92 pp. Available online [https://www.agrireseau.net/agriculturebiologique/documents/Guide_complet_P.pdf].

Klug, T., and R. Meyhöfer. 2009. Performance of two *Trichogramma brassicae* strains under greenhouse and field conditions for biocontrol of the silver Y moth in spinach cultures. J. Pest Sci. 82: 73-79. doi:10.1007/s10340-008-0224-y

Konopka, J.K., D. Poinapen, T. Gariepy, and J.N. McNeil. 2018. Understanding the mismatch between behaviour and development in a novel host-parasitoid association. Sci. Rep. 8: 15677. doi:10.1038/s41598-018-33756-6

Kuhar, T.P., M.G. Wright, M.P. Hoffmann, and S.A. Chenus. 2002. Life table studies of European corn borer (Lepidoptera: Crambidae) with and without inoculative releases of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae). Environ. Entomol. 31: 482-489. doi:10.1603/0046-225X-31.3.482

Lessard, E., and G. Boivin. 2012. Effect of low temperature on emergence, fecundity, longevity and host-feeding by *Trichogramma brassicae*. BioControl 58: 319-329. doi:10.1007/s10526-012-9493-8

Martel, V., R.C. Johns, L. Jochems-Tanguay, F. Jean, A. Maltais, S. Trudeau, M. St-Onge, D. Cormier, S.M. Smith, and J. Boisclair. 2021. The use of UAS to release the egg parasitoid *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) against an agricultural and a forest pest in Canada. J. Econ. Entomol. 114: 1867-1881. doi:10.1093/jee/toaa325

Mason, C.E. 1996. European corn borer: ecology and management. Iowa State University, Ames, IA, USA.

McLeod, D.G.R., C. Ritchot, and T. Nagai. 1979. Occurrence of a two-generation strain of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), in Quebec. Can. Entomol. 111: 233-236. doi:10.4039/Ent111233-3

Montovan, K.J., C. Couchoux, L.E. Jones, H.K. Reeve, and S. van Nouhuys. 2015. The puzzle of partial resource use by a parasitoid wasp. Am. Nat. 185: 538-550. doi:10.1086/680036

Musser, F.R., and A.M. Shelton. 2003. Predation of *Ostrinia nubilalis* (Lepidoptera: Crambidae) eggs in sweet corn by generalist predators and the impact of alternative foods. Environ. Entomol. 32: 1131-1138. doi:10.1603/0046-225X-32.5.1131

Pak, G.A., and T.G. van Heiningen. 1985. Behavioural variations among strains of *Trichogramma* spp.: adaptability to field-temperature conditions. Entomol. Exp. Appl. 38: 3-13. doi:10.1111/j.1570-7458.1985.tb03491.x

Phoofolo, M.W. 1997. Evaluation of natural enemies of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Iowa State University, Ames, IA, USA.

Phoofolo, M.W., J.J. Obrycki, and L.C. Lewis. 2001. Quantitative assessment of biotic mortality factors of the European corn borer (Lepidoptera: Crambidae) in field corn. J. Econ. Entomol. 94: 617-622. doi:10.1603/0022-0493-94.3.617

Pitcher, S.A., M.P. Hoffmann, J. Gardner, M.G. Wright, and T.P. Kuhar. 2002. Cold storage of *Trichogramma ostriniae* reared on *Sitotroga cerealella* eggs. BioControl 47: 525-535. doi:10.1023/A:1016533116798

Qian, Y-Q., R-L. Cao, and G-Z. Li. 1984. Biology of *Trichogramma ostriniae* and evaluation of its effectiveness in controlling corn borer on spring corn. Acta Entomol. Sin. 27: 287-293.

Rahimi-Kaldeh, S., A. Ashouri, A. Bandani, and S.A.M. Hasani. 2018. Maternal age influence on diapause induction in sexual and asexual *Trichogramma brassicae*. Int. J. Pest Manag. 64: 345-351. doi:10.1080/09670874.2017.1422569

Razinger, J., V.P. Vasileiadis, M. Giraud, W. van Dijk, Š. Modic, M. Sattin, and G. Urek. 2016. On-farm evaluation of inundative biological control of *Ostrinia nubilalis* (Lepidoptera: Crambidae) by *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) in three European maize-producing regions. Pest. Manag. Sci. 72: 246-254. doi:10.1002/ps.4054

St-Onge, M., D. Cormier, S. Todorova, and É. Lucas. 2014. Comparison of *Ephestia kuehniella* eggs sterilization methods for *Trichogramma rearing*. Biol. Control 70: 73-77. doi:10.1016/j.biocontrol.2013.12.006

Smith, J.L., T.S. Baute, and C.E. Mason. 2015. Pheromone races of *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) infesting grain corn in Manitoba, Ontario, and Québec provinces of Canada. J. Entomol. Soc. Ont. 146: 41-49.

Suverkropp, B.P., F. Bigler, and J.C. van Lenteren. 2001. Temperature influences walking speed and walking activity of *Trichogramma brassicae* (Hym., Trichogrammatidae). J. Appl. Entomol. 125: 303-307. doi:10.1046/j.1439-0418.2001.00546.x

van Lenteren, J.C., and F. Bigler. 2010. Quality control of mass reared egg parasitoids. Pages 315-340 *in* F.L. Cônsoli, J.R.P. Parra and R.A. Zucchi (eds.), Egg parasitoids in agroecosystems with emphasis on *Trichogramma*. Springer, Dordrecht, Netherlands. doi:10.1007/978-1-4020-9110-0_12

Wang, B., and D.N. Ferro. 1998. Functional responses of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) to *Ostrinia nubilalis* (Lepidoptera: Pyralidae) under laboratory and field conditions. Environ. Entomol. 27: 752-758. doi:10.1093/ee/27.3.752

Wang, B., D.N. Ferro, and D.W. Hosmer. 1997. Importance of plant size, distribution of egg masses, and weather conditions on egg parasitism of the European corn borer, *Ostrinia nubilalis* by *Trichogramma ostriniae* in sweet corn. Entomol. Exp. Appl. 83: 337-345. doi:10.1046/j.1570-7458.1997.00189.x

Wang, B., D.N. Ferro, and D.W. Hosmer. 1999. Effectiveness of *Trichogramma ostriniae* and *T. nubilale* for controlling the European corn borer *Ostrinia nubilalis* in sweet corn. Entomol. Exp. Appl. 91: 297-303. doi:10.1046/j.1570-7458.1999.00496.x

Wang, C.-L., H.X. Wang, C.M. Gui, and H. Lu. 1984. Studies on the control of the Asian corn borer, *Ostrinia furnacalis* (Guenee), with *Trichogramma ostriniae*. Pages 268-273 *in* P.L. Adkisson and S. Ma (eds.), Proceedings of the Chinese Academy of Sciences – United States National Academy of Sciences Joint symposium on biological control of insects, Beijing, China, September 25-28, 1982. Science Press, Beijing, China.

Wright, M.G., M.P. Hoffmann, S.A. Chenus, and J. Gardner. 2001. Dispersal behavior of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae) in sweet corn fields: implications for augmentative releases against *Ostrinia nubilalis* (Lepidoptera: Crambidae). Biol. Control 22: 29-37. doi:10.1006/bcon.2001.0948

Wright, M.G., T.P. Kuhar, M.P. Hoffmann, and S.A. Chenus. 2002. Effect of inoculative releases of *Trichogramma ostriniae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn. Biol. Control 23: 149-155. doi:10.1006/bcon.2001.0993

Zhang, F., D. Babendreier, Z.-Y. Wang, K.S.Il., L. Zheng, Y.C. Pyon, S.-X. Bai, K. Song, J.O. Ri, M. Grossrieder, and U. Kuhlmann. 2010. Mass releases of *Trichogramma ostriniae* increase maize production in DPR Korea. J. Appl. Entomol. 134: 481-490. doi:10.1111/j.1439-0418.2010.01512.x

Zhang, Z.L. 1988. *Trichogramma* spp. parasitizing the eggs of Asian corn borer *Ostrinia furnacalis* and its efficacy in Beijing suburbs. Colloques de l'INRA 43: 629-632.

Zimmermann, G., A.M. Huger, G.A. Langenbruch, and R.G. Kleespies. 2016. Pathogens of the European corn borer, *Ostrinia nubilalis*, with special regard to the microsporedium *Nosema pyrausta*. J. Pest Sci. 89: 329-346. doi:10.1007/s10340-016-0749-4