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# Trichogramma pupae spraying technique development for biocontrol of the European corn borer in sweet corn crops

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## ABSTRACT

The European corn borer, *Ostrinia nubilalis* (Hübner), is the major pest insect of sweet corn in Quebec and around the world. Its presence results in important yield decreases and profit losses for farmers. Currently, chemical insecticides are mainly used to control this pest insect. Chemical hazards for human health and the environment are well documented. Despite government efforts to reduce the use of chemicals in agriculture, pesticides sales have steadily increased over the time. Nevertheless, there are some interesting alternatives to chemicals to control the European corn borer, in particular the use of predator insects. Many research studies have demonstrated the effectiveness of using the trichogramma to successfully control the corn borer. This biological control method is however more expensive and complex than chemical insecticides. The main objective of this research study was to investigate the technical feasibility of spraying trichogramma pupae to facilitate the implementation of this method and reduce the operation cost. For this purpose, many experimental trials using the *Trichogramma ostrinae* were carried out in the laboratory. These trials aimed at finding an adequate aqueous solution to both scatter the pupae in the sprayer tank and stick them to corn leaves once sprayed. A laboratory spraying system was also designed and built to check the viability of trichogramma pupae after being immerged in the spray solution and sprayed. Obtained results showed that the sprayed pupae emergence rate exceeded 80% and was comparable to that of unsprayed pupae. The success of this spraying

technique to control the corn borer in corn crops is highly valuable and could be generalized to other predator insects.

**Keywords:** sweet corn, European corn borer, Trichogramma ostriniae, biocontrol, spraying system, emergence rate.

## INTRODUCTION

The European corn borer, *Ostrinia nubilalis* (Hübner), is the major pest insect of sweet corn in Quebec and around the world (Ferland et al., 2000). In North America and Europe, the European corn borer causes 20% of yield losses (Siegfried and Hellmich, 2012). The European corn borer begins by attacking plant leaves, than dig tunnels in cornstalks. Currently, chemical insecticides are mainly used to control this pest insect. Chemical hazards for human health and the environment are well documented and some alternatives exist (Musser et al., 2006). The use of genetically modified corn (Bt) can efficiently control the corn borer. However, a resistance can be developed by the corn borer in the absence of a non-Bt corn refuge (Pereira et al., 2008; Siegfried and Hellmich, 2012). The biocontrol is another alternative to pesticides. Trichogramma have been used for many decades in sweet corn fields and have been proven to be effective in controlling the European corn borer (Boisclair et al., 2011; Gardner and Giles, 1997). However, field application of trichogramma using tricho cards is more expensive than the use of chemical pesticides. Boisclair et al. (2011) investigated the cost of many corn borer control methods. They indicated that a rate of 400 000 pupae/ha is approximately three times more expensive than the use of permethrine pesticide. Furthermore, the manual distribution of tricho cards is laborious which could not stimulate producers to adopt it. Mechanical pupae distribution would highly facilitate trichogramma spreading in the field and consequently reduce the production cost of this beneficial insect.

An exhaustive literature review revealed that only very few research studies regarding mechanical application methods of trichogramma have been carried out. Few sprayers were tested with trichogramma pupae (Mills et al., 1996; Morrison et al., 1998; Gardner and Giles, 1997). Obtained results showed that the spraying solutions were in general not adequate and had sometimes a negative impact on the sprayed pupae. Different emergence rates ranging from 40% to 92% have been reported (Zimmermann and Wührer, 2010; Mills et al., 1996; Jalali et al., 2005; Kienzle et al., 2012; and Zimmermann and Wührer, 2010). This indicates that it is possible to immerse and spray trichogramma pupae. The main objective of this research study was therefore to investigate the technical feasibility of spraying *Trichogramma ostriniae* pupae to control the European corn borer in sweet corn crops.

## MATERIALS AND METHODS

Laboratory trials were carried out using the *Trichogramma ostriniae* pupae provided by Anatis Bioprotection inc. (Saint-Jacques-le-Mineur, Québec, Canada). Upon their reception, the pupae were kept at $4^{o}$C before using them at the room temperature.

### *Pupae immersion in viscous solutions*

To homogeneously disperse trichogramma pupae in the sprayer tank, viscous solutions have been used. These solutions also helped pupae sticking on the leaves after being sprayed. Drowning resistance of pupae was assessed through immersion trials in two types of viscous solutions (solution 1 and solution 2). Approximately 4 000 pupae were mixed with 400 mL of solution 1. The same procedure was repeated with solution 2. After 15 minutes of immersion, 100 mL was sampled from each solution vessel and pupae were filtrated and rinsed with tap water. Rinsed pupae were then spread in Whatman filtration paper. The same process was repeated after 1 hour, 2 hours, and 3 hours of immersion. Pupae were left at room temperature for 11 days or until the emergence of all surviving trichogramma is completed. A control treatment (not immersed pupae) was also considered for comparison reasons. After emergence, a microscope counting was done to determine total emerged and died pupae. Approximately 400 pupae per treatment were counted. The emergence rate was computed by subtracting the number of died pupae from that of initially immersed pupae.

### *Pupae spraying*

A backpack sprayer was modified by integrating a diaphragm pump and a 12 volt battery. A pressure switch was used instead of a pressure regulator to avoid damaging the trichogramma pupae. Indeed, preliminary spraying tests results revealed a low emergence rate due to the use of a pressure regulator. A five nozzle spraying boom was used with different types of spraying tips to allow adjusting the pressure and the flow rate during spraying tests. Figure 1 shows the sprayer used for the trials.

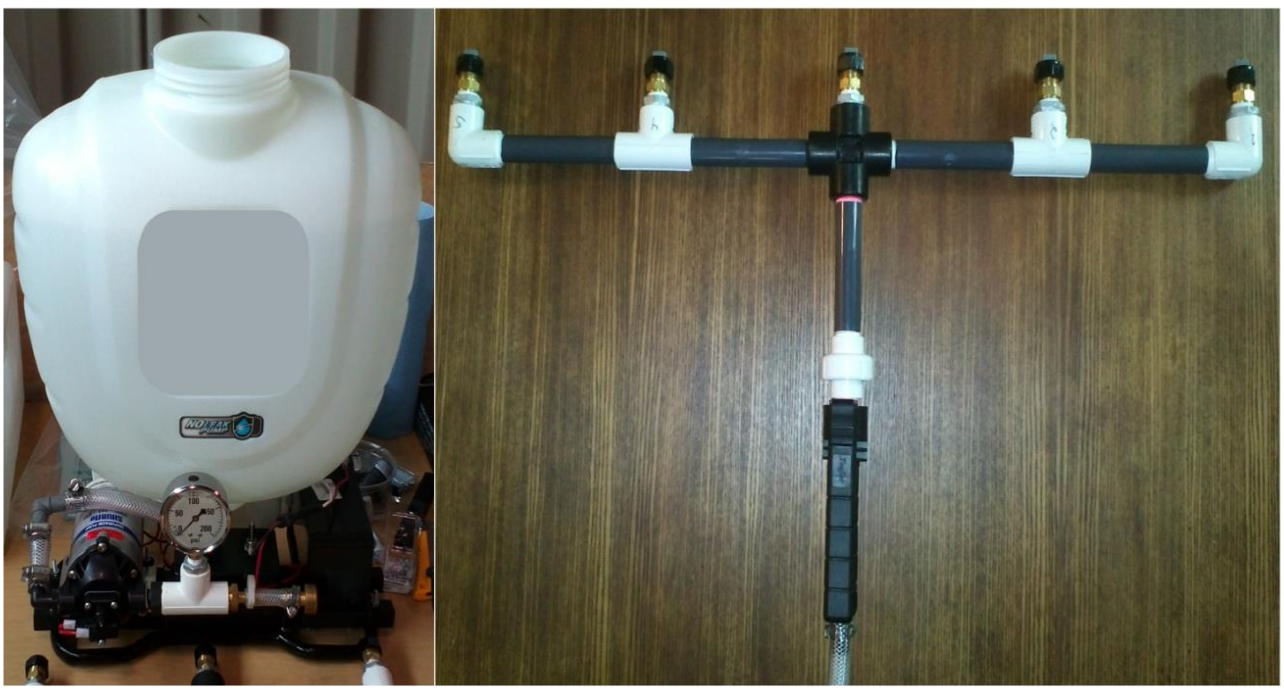

**Figure 1**. Left side: modified backpack sprayer with diaphragm pump and battery. Right side: five nozzles spraying boom.

Spraying impact on pupae were assessed using solution 2 because of it is easy to mix and generates less foam compared to solution 1. Around 200 000 *Trichogramma ostriniae* pupae were mixed with 3 L of solution 2. Two nozzles (nozzle 1 and nozzle 2) were used to adjust the spraying pressure and the flow rate. Samples of the mixture of spray solution and pupae were taken as control from the sprayer tank before spraying. Each treatment was replicated twice.100 mL samples were filtered and rinsed with tap water and left 11 days at room temperature before computing the emerging rate using a microscope.

### *Pupae adhesion on corn leaves*

Preliminary spraying trials on field showed that both aqueous solutions are susceptible to leaching by rain. Pupae adhesion on leaves until emergence is crucial since soil microclimate can be harmful for them. For this purpose, rain resistance of many solutions has been evaluated in the laboratory. A control treatment consisting on pupae immersed in only water was considered. Solution 1 was assessed at a higher concentration to determine the concentration effect on solubility. Finally, a mixture of solutions 1 and 2 was evaluated based on three replicates. Each solution was mixed with pupae and approximately 1 mL of the solution was dropped on corn leaves. The same process was replicated under corn leaves. Pieces of leaf samples were let dry for 24 hours and then a microscope counting was done. After spraying water on the samples to simulate the rain, another count was made to determine the adhesion rate of pupae.

### *Statistical analysis*

An analysis of variance (ANOVA) was performed on the data using the GLM procedure of the SAS software. Quantitative contrasts were done to compare the treatments.

## RESULTS AND DISCUSSION

### *Pupae immersion in viscous solutions*

Obtained results showed a significant difference between dry control and immersed treatments. However, both solution types and immersion time did not have any significant effect on the emergence of pupae. The results of immersion trials are presented in figure 2.

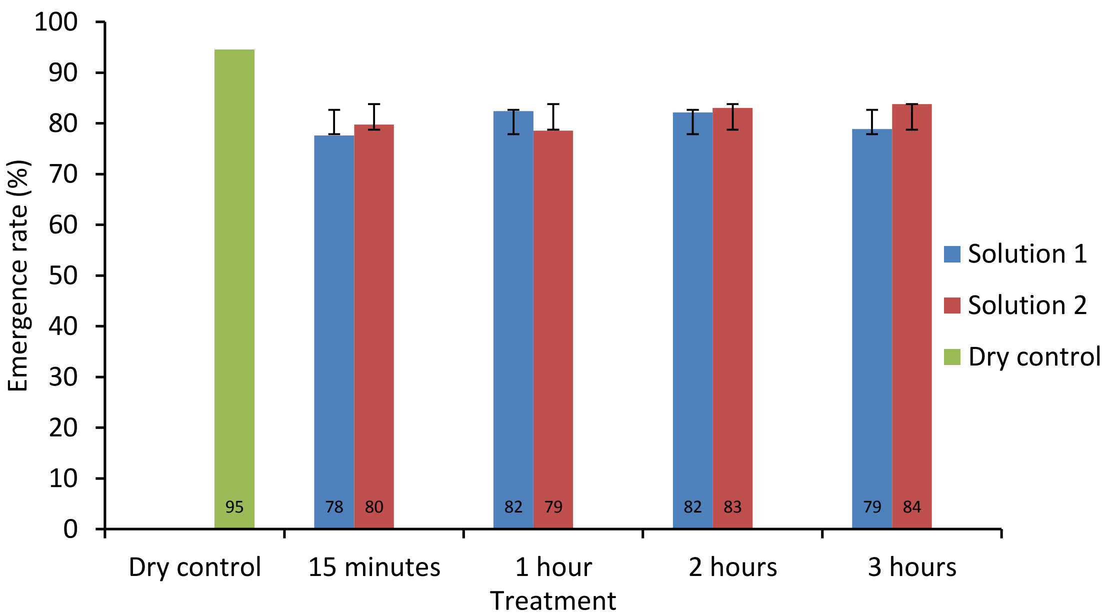


**Figure 2**. Emergence rate of pupae versus immersion time for solutions 1 and 2. Vertical bars represent the standard deviation of the means.

The immersion in both solutions was significantly different than the dry control (Table 1). Solutions 1 and 2 did not have any significant effect on the pupae emergence between 15 minutes and 3 hours of immersion. The emergence rate after 15 minutes of immersion is comparable to those after 1 hour, 2 hours, and 3 hours of immersion for both solutions. The significant effect between dry control and immersed pupae was probably due to filtration and separation before microscope counting.

**Table 1**. Contrasts for immersion trials in solutions 1 and 2

| Contrasts | Df | F | Pr > F |
|---|---|---|---|
| Control vs. solution 1 (15 min) | 1 | 47.81 | <.0001 |
| Control vs. solution 1 (3 hrs) | 1 | 40.99 | <.0001 |
| Control vs. solution 2 (15 min) | 1 | 36.18 | <.0001 |
| Control vs. solution 2 (3 hrs) | 1 | 18.85 | 0.0004 |
| Solution 1 (15 min) vs. solution 2 (15 min) | 1 | 0.81 | 0.3806 |
| Solution 1 (3 hrs) vs. solution 2 (3 hrs) | 1 | 4.25 | 0.0541 |
| Solution 1: 15 min vs. 1 hr to 3 hrs | 1 | 3.18 | 0.0913 |
| Solution 2: 15 min vs. 1 hr to 3 hrs | 1 | 1.04 | 0.3204 |

### *Pupae spraying*

The emergence rates of sprayed pupae are presented in figure 3. These rates indicate that the pump and nozzles used with solution 2 are not harmful to trichogramma pupae.

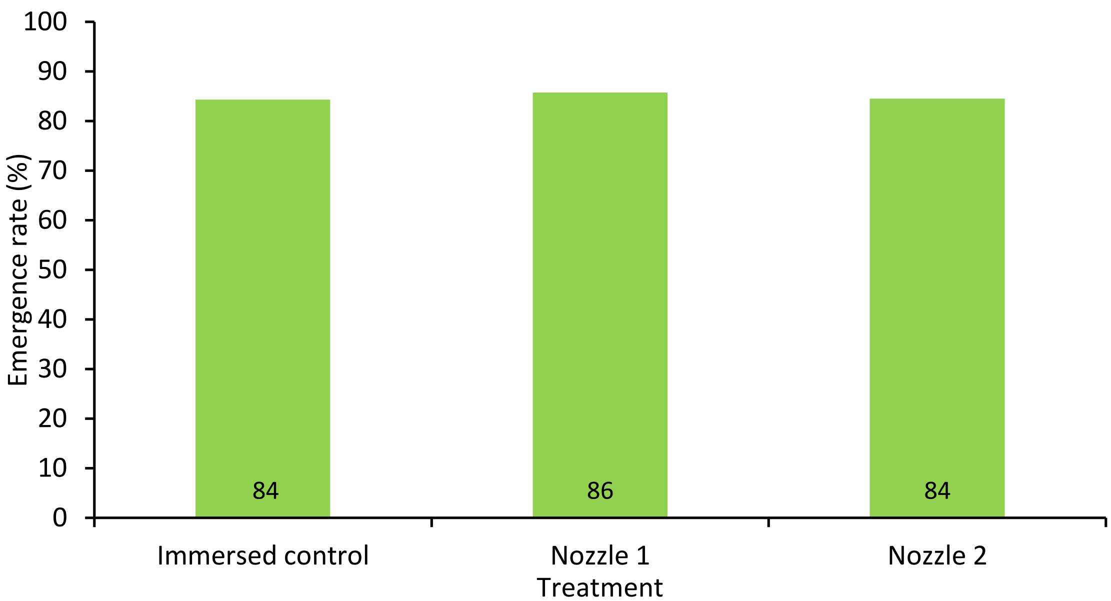


**Figure 3**. Emergence rates of sprayed trichogramma pupae immersed in solution 2 for nozzles 1 and 2.

### *Pupae adhesions on corn leaves*

Figure 4 shows the results of adhesion tests. These results clearly indicate that the side of the leaves did not have a significant effect on the adhesion rate of pupae, in particular when using a mixture of solutions 1 and 2. However, the solution type had a significant effect on the adhesion of pupae.

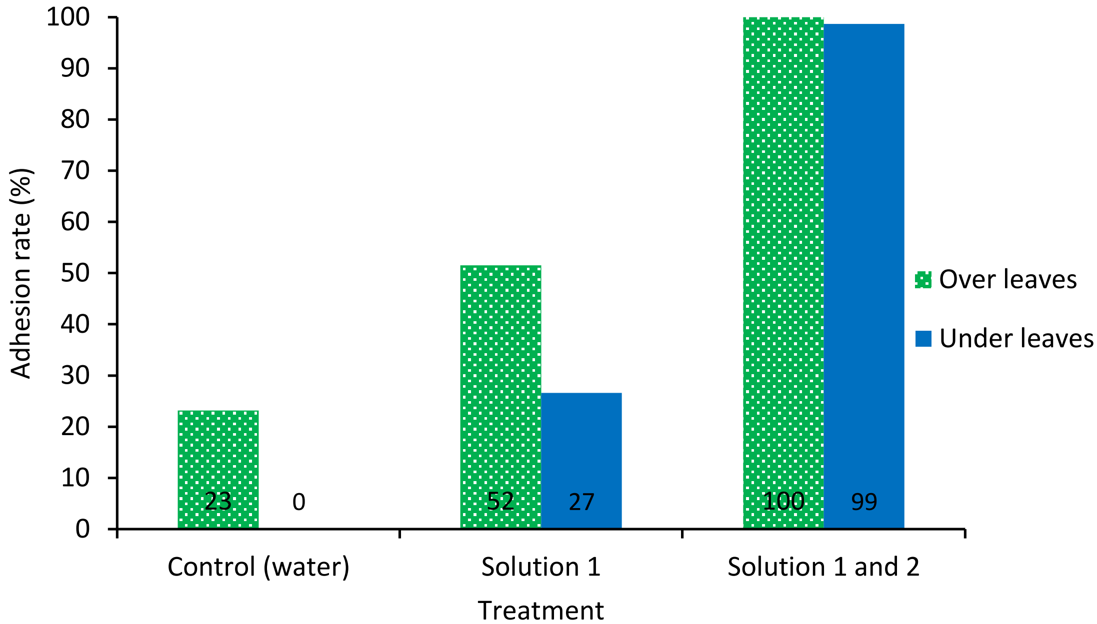


**Figure 4**. Adhesion rates of sprayed pupae in concentrated solution 1 and for the mixture of solutions 1 and 2 over and under corn leaves.

## CONCLUSIONS

Based on the obtained results, the followings conclusions can be drawn:

- The immersion time from 15 minutes to 3 hours in both solutions did not have any effect on the emergence rate of pupae.
- The sprayer with a pressure regulator is not suitable for trichogramma pupae spraying.
- The emergence rate of sprayed pupae is comparable to that of immersed and non-sprayed pupae (control).
- The adhesion of pupae on corn leaves is possible if the spraying solution is rain resistant.
- The mixture of solutions 1 and 2 is the most adequate in terms of adhesion of pupae on corn leaves under rain conditions simulated in the laboratory.


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