XVII^th World Congress of the International Commission of Agricultural and Biosystems Engineering (CIGR)

Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)
Québec City, Canada June 13-17, 2010

TEST BENCH FOR MECHANICAL DISTRIBUTION OF PREDATORS TO CONTROL INSECT PESTS

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CSBE101390 – Presented at Section III: Equipment Engineering for Plant Production Conference

ABSTRACT In agriculture, chemical insecticides are widely used to protect crops from insect pests. Over the years, some insects like the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), succeed in developing resistance to most of the registered chemical insecticides. In these cases, heavy applications of chemical insecticides to control this insect pest become ineffective on a long-term basis and can lead to serious health and environmental problems. The use of natural enemies to control the Colorado potato beetle represents an interesting alternative to chemical means. However, hand release of predators is not feasible at a large scale in the field. It is time consuming and even not conceivable. The main objective of this research study was to design and build a test bench to investigate the technical feasibility of mechanically releasing predators. The test bench consisted in a vertical chain conveyor mounted on two vertical shafts driven by an electric motor. Since the predators are very small and fragile, they were placed into a specially designed container to preserve their physical integrity. At one end of the container, a trap held closed with a hinge spring was installed. The container is then mounted on the chains using a sliding support. When crossing a plastic rail, the trap opens to allow predators dropping on the ground. Trials using this test bench showed that a carrier material is required since most of the predators cling firmly to the walls of the container and hence remain inside. When mixed with a carrier material, all the predators are successfully released. The success of this mass release system of predators will be highly valuable for the biological control of insect pests in many crops such as potato, strawberry, lettuce, etc.

Keywords: Insect pests, chemical insecticides, biological control, natural enemies, mass release, mechanical distributor.

INTRODUCTION AND LITERATURE REVIEW The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is the major pest insect of potato crops, *Solanum tuberosum* (L.), in Canada (Howard et al., 1994; Boiteau et al., 1992; Hare, 1980, Cloutier et al., 2002), in the United States (Radcliffe et al., 1993), and in Europe (Jolivet, 1991). To date, the use of chemical insecticides remains the most popular method to control the CPB populations (Weisz et al., 1994). However, heavy reliance on chemical insecticides could result in health and environmental problems. Moreover, most of the registered chemicals are presently not efficient to manage this important potato pest on a long term basis because of its high adaptability (Forgash, 1981; Martel 1987; Boiteau et al. 1987; Whalon et al. 1993; Randall, 1999). Currently, strong resistance to imidacloprid based insecticide, Admire ®, has been reported in Canada and in several American states (Mota-Sanchez et al., 2006; Alyokhin et al., 2007). Admire ® was introduced about 10 years ago and has been widely used to control all stages of the CPB. To date, most of the developed alternatives to chemical insecticides have not been efficient enough or difficult to apply on a commercial scale.

The natural enemy of the CPB, the two-spotted stink bug *Perillus bioculatus* (Fab.) (Heteroptera: Pentatomidae), is among the most specialized insect predators (Saint-Cyr and Cloutier 1996; Hough-Goldstein et al., 1993; Cloutier et al., 2002) with potential to attack eggs, all larval instars, and even adults. The biological control of the CPB using *P. bioculatus* has been proven to be one of the most promising alternatives to chemical insecticides at a small scale and in green houses (Bellows, 1993; van Lenteren, 1993; Cloutier et al., 2002). For a more precise management and to save on the treatment cost, Cloutier and Bauduin (1995) confirmed the pre-emptive strategy that would consist of releasing *P. bioculatus* nymphs as soon as spring CPB egg mass density is at the highest point. Therefore, it is possible to estimate the potential damage to potato crops by first generation larvae and adults resident CPB. This period normally appears at the end of June in Quebec and only one mass release at a rate of 2 to 4 2^{nd} instar nymphs per plant would be necessary depending on the infestation level of CPB (Cloutier and Bauduin, 1995; Cloutier and Jean, 1998).

Recent study of de Ladurantaye (2008) showed the importance of optimizing the proper storage and transport techniques from the production/rearing facility until their field distribution. Transportation over long distances and variable temperatures make the storage of suitable stage predators essential until they are released in the field. Storage temperatures in the range of 9 to 15°C for a maximum of eight continuous days will slow down the predator larvae's metabolism and activity without apparent stress. Their growth is therefore paused which is desirable to maintain them at the proper development stage for mass release in the field. De Ladurantaye (2008) results also showed that it is possible to keep the nymphs in darkness making their storage and transport easier by using common delivery boxes and refrigerated chambers once delivered at the farm. However, the scientific literature on potentiel distribution technics of this predator in the field at a large scale is almost inexistant. The use of biological control at a commercial scale requires the development of an efficient mechanical distributor of predators which currently represents a major issue.

OBJECTIVE The main objective of this research study was to develop a test bench to mechanically release 2^{nd} instar nymphs of *P. bioculatus* in row crops such as potato plants. The predator distribution device must allow releasing the predators at a specific rate while preserving their physical integrity.

MATERIALS AND METHODS ***Podisus maculiventris*** The predatory pentatomid, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae), was used instead of the *Perillus bioculatus* for avaibility convenience. The eggs were directly collected from a colony maintained on a mealworms (*Tenebrio molitor*) diet from the insect producer, The Bug Factory, Vancouver Island, Canada. These eggs were mixed with vermiculite in small containers and then shipped by air in a box with newspaper and cold pack to keep them cool. At their arrival at the laboratory located at the Department of Soil Science and Agri-Food Engineering, Université Laval, QC, Canada, the eggs were placed in ventilated plastic Petri dishes (1 x 5 cm) over layers of damp paper toweling. Each plastic Petri dish was placed in normal room air conditions: a temperature of 22 ± 1°C, a RH of 50 ± 10%, and a photoperiod of 16:8 h (light: dark). To prevent desiccation, they were gently sprayed daily with distilled water. The larvae were raised with water soaked tampon until larval stage 2 and then transfered in different containers ready for the experimentations. To prevent cannibalism between lavae of stage 2, they were feed with mealworms.

Laboratory test bench release design Based on the field experimentations of Cloutier and Jean (1998) and Cloutier and Bauduin (1995), the mechanical distributor was designed for source point release of predators in potato fields. The final design is a test bench using special mounted containers (Fig. 1) that will release all the pre-counted predator larvae at once. Depending on their prey surching or walking capacity for field dispersion and based on the work of Lachance and Cloutier (1997), the amount of predator larvae per container and the distance between the release points in the field has to be ajusted. For all the trials, the container was specifically designed for predators such as the *P. Maculiventris* 2nd instar nymphs. Compared to eggs and 1st instars, 2nd instar nymphs would be more suitable for mass release because of their immediate predation potential on egg clusters and small CPB instars, as well as the predator dispersal capabilities in the field (Lachance and Cloutier, 1997; Cloutier, 1997). Older instars or adults have been found to quickly quit the release point or even the field instead of searching for prey. Thin wood chips and popcorn were choosen as carrier material because they are biodegradable, light, and non-compact to give all the freedom that the young predators need to be confortable.

Figure 1. Specially designed acrylic container filled up with a carrier material (thin wood chips in this case), a water soaked tampon, and 50 *P. Maculiventris* 2nd instar nymphs.

Experimental design and procedure A complete randomized factorial design was used to test two factors: (1) predator distribution technique (PDT): mechanical or manual release, and (2) predator carrier materiel (PCM): thin wood chips or pop corn, for a total of four factor combinations (2 PDT x 2 PCM). Every combination was repeated three times. To simulate farm delivery, 500 ml of the carrier materiel along with one water soaked tampon and the *P. maculiventris* 2nd instar nymphs were placed into an aerated container 24h before the trials and kept in the same room air conditions. Thereafter, the basic design consisted of transfering all the container content into a specially designed container (Fig. 1) that could be fixed to the chain conveyor on the test bench (Fig. 2). The observation unit consisted of this special container each one containing 50 nymphs and each unit being randomly assigned among treatment combinations. Every container did one complete loop on the conveyor before hitting the yellow release device (Fig. 2) that opens the bottom trap and shake all the content down in a big tray placed underneath the predator release zone (Fig. 3). On Figure 2, the container is at the starting point and will be driven for a complete loop before hitting the release device. Dropped predators were counted directly in the tray after each trial to compute the release rate. The remaining nymph predators in the container after the release were not used for further trials, as well as all the other nymphs (i.e. for each trial, a new group of 50 nymphs was used). The survival rate of dropped predators was observed twice after the release, one and seven days after the trials with the same water/food combination as before the trials. Manual release of the young nymphs was done by picking them up from the container and dropping them in the tray using a small brush (Fig. 4).

The operating speed of the chain conveyor was 0.30 m/s and the opening time in the release zone was about one second. Before each trial, the conveyor was activated for 15 minutes to experience the young nymphs a stress before being released. This also allowed simulating the transportation conditions that would occur before the field distribution process.

Figure 2. Specifically designed acrylic container mounted on a chain conveyor of the laboratory test bench to simulate the mechanical release of predators in potato crops. Diagonal notches were done in both sides of the release tracks to shake down the nymph predators that would remain inside the container.

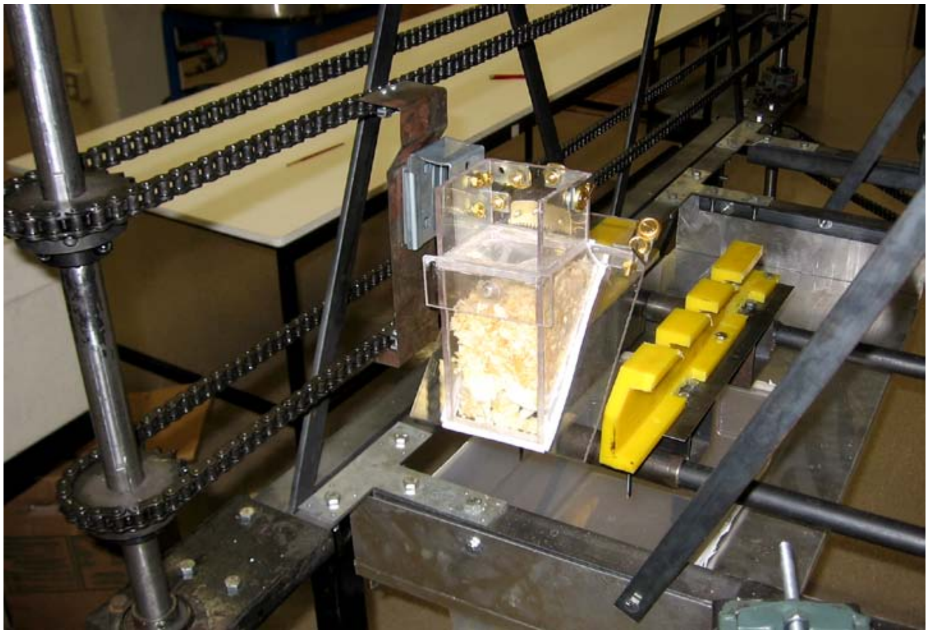

Figure 3. Overview showing the container, the yellow release system, and the tray underneath the release zone in which fall down the carrier material and the predators.

Figure 4. Handling technique used during the manual release of *P. Maculiventris* 2nd instar nymphs to delicately collect and drop them in the original the tray.

Statistical analysis An ANOVA was performed on the predator release rate data using the Proc GLM procedure (SAS Institute, 2005). For the predator survival rate after release data, a repeated measures analysis of variance model was used. For this purpose, the MIXED procedure with the statement "repeated" and the variance-covariance structure, which minimizes the Akaike criterion, was used (SAS Institute, 2005).

RESULTS AND DISCUSSION Carrier material release efficiency Thin wood chips or popcorn had no significant effect on the release efficiency of the *P. maculiventris* 2nd instar nymphs ($F_{1,9} = 0.18$, $P = 0.6823$) (Table 1). The average rate of release with both carrier materials was about 94.2 ± 3.8%, which is suitable for field mass release. This suggests that the mechanical distributor is adaquate for mass release of young predators regardless of the carrier material.

Table 1. ANOVA results for the carrier material release efficiency

Source of variation	D.F.	F Values	Pr > F
Bloc	1	4.42	0.0648 NS
Carrier material	1	0.18	0.6823 NS
Error	9		

(NS): not significant.

Effect of mechanical release on nymph survival The release technique as well as the carrier material had no significant effect on the survival of the predator nymphs ($F_{1,20}$ = 0.46, P = 0.5065 and $F_{1,20}$ = 2.17, P = 0.1561, respectively) (Table 2). This suggests that the mechanical release system using a container filled with the appropriate carrier material would not hurt the young nymphs and is as efficient as the manual release using a brush. The mechanical distributor could therefore be safely used to release young predators in the field. However, the popcorn tended to soften more quikly when water soaked tampon was placed for a while in the container. The popcorn became sticky making the release process less efficient.

For both release techniques, only the time after release has a high significant effect on the survival of the nymph predators ($F_{1,20}$ = 61.97, P <0.0001) (Table 2). One day after the release, the average survival rate for both carrier materials was 98.6 ± 2.1% (Fig. 5). Seven days after the release, the average survival rate dropped to about 92.9 ± 4.9% (Fig. 5) which is still very acceptable for a commercial use of this predator release technique.

Table 2. ANOVA results for the survival rate of nymphs after mechanical release

Source of variation	D.F.	F values	Pr > F
Distribution technique	1	0.46	0.5065 NS
Carrier material	1	2.17	0.1561 NS
Distribution technique*carrier material	1	0.34	0.5663 NS
Time	1	61.97	<0.0001
Distribution technique*time	1	0.01	0.9340 NS
Carrier material*Time	1	2.41	0.1359 NS
Distribution technique*carrier material*time	1	1.02	0.3247 NS
Error	20		

(NS): Non significant

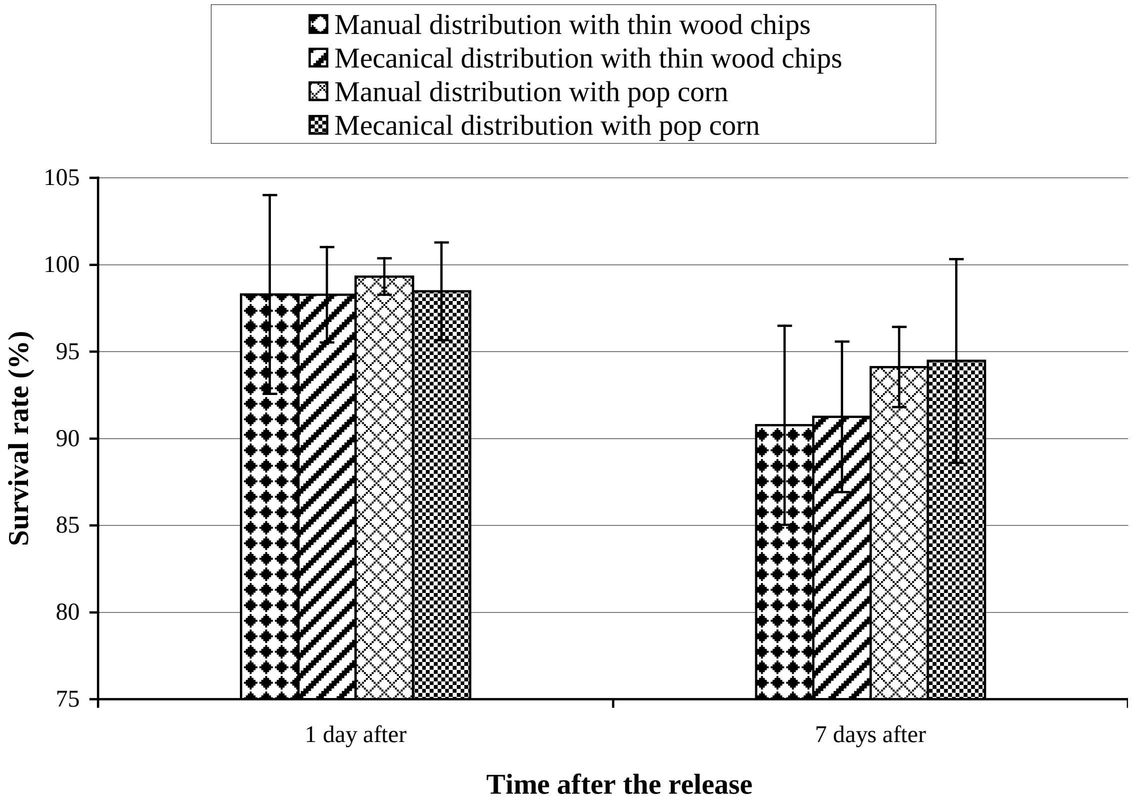

Figure 5. Predator survival rates after their release according to the distribution method and the carrier material used.

CONCLUSIONS Based on the results of our experiments, the following conclusions were drawn:

- The mechanical distributor is efficient as it allowed releasing a high rate of young predators;

- The high survival rate after the mechanical release shows that the distributor has no adverse effect on the predators. These survival rates are comparable to those obtained with the manual release using a brush;

- Both carrier materials (thin wood chips and popcorn) are suitable for mechanical release of young predators as the efficiency of release and the survival rate obtained with these two materials are comparable.

Observations made during the trials indicated the need of mixing young predators with a carrier material to prevent them from remaining attached to the container side walls. In this context, the container should be completely filled with the mixture leaving no choice for the predators than to grip to the carrier material.

RECOMMANDATIONS For comparison reasons, further trials should be conducted on the mechanical release of *Perillus bioculatus* instead of *Podisus maculiventris*. More investigation should also be carried out on the predaceous performance (i.e. dispersal and predation rate) following the mechanical release to provide additional insight into potential effects which will be helpful in improving the design and consequently maximizing the use of this beneficial predator in controlling the CPB.

Acknowledgements The authors gracefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada (NSERC).

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