European Corn Borer (*Ostrinia nubilalis*) in Potatoes (*Solanum tuberosum*) and the use of *Trichogramma* Species as an Agent for Biological Control

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ABSTRACT

The European corn borer (*Ostrinia nubilalis*, ECB) has been observed at pest levels in potato crops on PEI since 1987 and is now considered a primary pest of potatoes in the Maritimes. ECB egg masses are currently used as an indicator of the level of ECB infestation in potatoes, with crop scouts using 2 egg masses per ten plants as an action threshold. Research plots using different artificially created ECB egg mass levels were established at the Harrington Research Farm using two potato varieties, Russet Burbank and Shepody. While the relationship between increasing ECB egg mass levels and larvae numbers is clear and significant ($p<0.05$), the effect of increasing ECB egg mass levels on tuber yields is less clear. There is evidence that the control threshold can be raised to 4 egg masses per ten plants in both Russet Burbank and Shepody potatoes without affecting tuber yields.

There is a significant linear relationship between ECB larval holes and larval tunnels ($R^2 = 0.83$-0.93, $p<0.001$), as well as between ECB larval holes and larvae ($R^2 = 0.70$-0.74, $p<0.001$) for both Shepody and Russet Burbank potatoes. Regression equations were calculated for these two relationships which make it possible to estimate ECB damage, in the form of tunnels, and ECB larval levels from the number of ECB larval holes in potato stems.

Trichogramma wasps are internal parasites of primarily lepidopteran insect eggs, which makes them attractive as biological control agents of insect pests such as the European corn borer. Three species of *Trichogramma, T. brassicae,*

T. pretiosum, and *T. minutum* were tested as biocontrol agents of European corn borer in potatoes both in laboratory choice tests and in small scale trials in potato fields. Mated *T. brassicae* females showed some interest in parasitizing ECB eggs when given a choice between them and the eggs of the facultative host, *Ephestia kuehniella*, although they still preferred the eggs of *E. kuehniella* 2 to1 over ECB eggs. In an initial trial in organic research plots *T. brassicae* showed a significant ($p<0.05$) reduction of ECB larval holes, tunnels, and larvae, but these results were not repeated in field and cage trials in organic and conventionally managed potato fields.

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Allan, Alice, Elizabeth, and Mary, who never once complained about the time my schooling was taking away from them. I have been at University for the majority of your lives, time to get a job!

You've traveled this long

You just have to go on

Don't even look back to see

How far you've come

Though your body is bending

Under the load

There is nowhere to stop

Anywhere on this road

Anywhere on this Road

-Lhasa

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CHAPTER 1

Introduction to Europe corn borer (*Ostrinia nubilalis*) in Potatoes (*Solanum tuberosum*) and the use of *Trichogramma* Species as a Biological Control

1.1 Introduction

The potato (*Solanum tuberosum*) is the single most important farm crop on Prince Edward Island (PEI). It contributed about 44% percent of the total farm cash receipts on PEI in 2005, and PEI production made up about 20% of Canada's total potato production (Statistics Canada, 2006). Therefore any pest that reduces potato yields on PEI is of concern to farmers. The European corn borer (*Ostrinia nubilalis*, ECB), which has a long history as a pest in corn, is also known as an occasional pest in potatoes, usually when populations are high in corn (Hodgson, 1928). Since 1987 the importance of ECB as a pest in potatoes on PEI has been steadily growing, in part because the Colorado potato beetle has been so well controlled. In 2004 it was decided that ECB had become one of the primary pests of potatoes in the Maritimes and Maine (ECB Working Group, Fredericton, 2004). While the rise of ECB as a pest in potatoes is relatively new in this region, in North Carolina the spring generation of a bivoltine population has displayed an oviposition preference for potatoes over corn for many years (Anderson et al., 1984). European corn borer infestations, however, do not always result in the reduction of the quantity and/or quality of the potato tubers (Stewart,1992; Nault et al., 2001; Bray,1961; Kennedy, 1983) and it is not clear at what level of ECB infestation economic damage occurs.

1.2 Review of the Literature

History of European corn borer as an agricultural pest

The European corn borer (*Ostrinia nubilalis*) is a moth native to northwest Africa, limited areas of southeastern Asia, and Europe. It was first described by Hübner in 1796 (Hudon et al., 1989, Munroe, 1976). Original host plants in Europe for this species included mugwort (*Artemesia vulgaris*), wild hops (*Humulus lupulus*), and wild hemp (*Cannabis sativa*) (Hudon and LeRoux, 1986a). ECB began to infest corn (*Zea mays*) soon after corn was imported from the new world and it quickly became a serious pest in Europe, giving the species its common name (Hudon and LeRoux, 1986a; Thomas et al. 2003). One reason why ECB became such a serious pest was that corn seems to offer increased protection from the natural predators of ECB over historic host plants (Thomas et al., 2003).

ECB was introduced to North America in the first decades of the 20th century at several locations, probably in shipments of broom corn (*Sorghum bicolor*) from Europe, and it rapidly became a serious pest in corn in North America (Hudon et al., 1989; Hudon and LeRoux, 1986a; Smith, 1920; Hodgson, 1928). In Canada, broom corn was imported to factories around Hamilton, Toronto and Montreal for the manufacture of brooms (Smith, 1920). European corn borer became established in southern Ontario in the early 1920s resulting in a 75% reduction in corn acreage by the late 1920s. The moth spread across the country from there and was first reported in New Brunswick in 1925, in Quebec in 1926, in Nova Scotia in 1929, in Manitoba in 1948 and Saskatchewan in 1950.

By 1986 the geographical distribution of ECB extended from Newfoundland through the Maritime provinces to Alberta in Canada, as well as throughout 40 U.S. states (Munroe, 1976; Hudon and LeRoux, 1986a; Palaniswamy et al., 1990).

Host Plants

While ECB is primarily a pest in corn, it is also known to infest > 200 species of plants in North America for at least part of its life cycle (Hodgson, 1928). These host plants include both cultivated and non-cultivated plants. Commercially-grown crops, besides corn, which have occasionally been found to be infested by ECB include potatoes (*Solanum tuberosum*), hops (*Humulus lupulus*), cabbage (*Brassica oleracea*), beans (*Vicia faba*), eggplant (*Solanum melongena*) and tomatoes (*Lycopersicon esculentum*) (Kuhar et al., 2003; Hudon and LeRoux,1986a; Hodgson, 1928) .

Life History

European corn borers are classified into two genetically distinct populations with one showing a univoltine life cycle with a compulsory diapause and the second capable of multiple generations with a facultative diapause. The populations with a facultative diapause which are located in northern Europe and northern North America may have only one generation per year, while in southern areas these populations may have up to three or four generations per year (Thomas et al., 2003). Historically, ECB has been considered to be univoltine on PEI (Stewart, 1994; Dornan and Stewart, 1995a), however, in 2006 and 2007 a second generation of adult moths was observed at the end of August and the beginning of September. The moths (Figure 1.1) on PEI usually emerge from the previous years potato stems in early summer (Dau-Schmidt, personal observation).

Egg Stage

Females begin to lay eggs approximately two days after mating (Hudon and LeRoux,1986b). ECB eggs are white and less than 1 mm in diameter. They are usually deposited in masses of 1-150 eggs, with the flat eggs overlapping each other like fish scales (Hudon and LeRoux, 1986a). Egg masses (Figure 1.2) are usually deposited on the undersides of leaves and on the stems of host plants (Hodgson, 1928; Dornan and Stewart,1995b).

Larval Stage

European corn borer larvae enter the stem of the host plant shortly after hatching, tunneling inside the stems and consuming tissue as they mature (Figure 1.3; Hodgson, 1928; Hudon et al., 1989). Once inside the host plant stem, the larvae are protected from insecticide applications and all but very specialized predators (Sorenson et al., 1995; Stewart, 1992; Thomas et al., 2003). European corn borer larvae pass through five instars (Hudon and LeRoux, 1986a), and on PEI they usually reach maturity by the end of August.

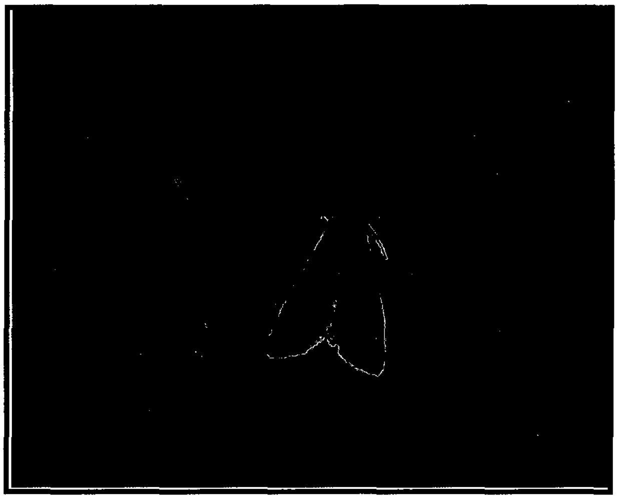


Figure 1.1: Adult European corn borer moths. The male (~14 mm) is on the left and the female (~17 mm) is on the right.

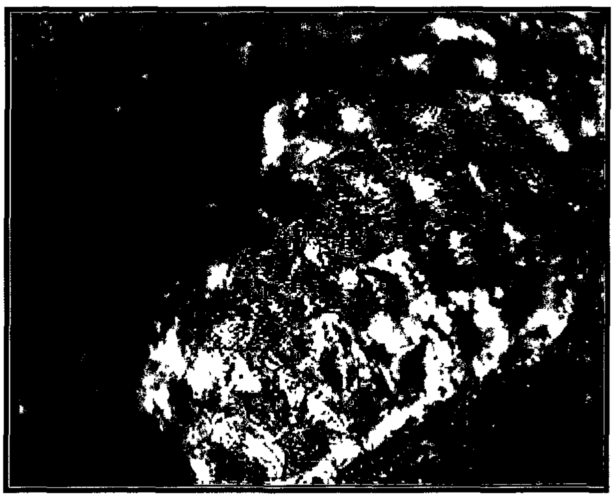


Figure 1.2: A European corn borer egg mass at the blackhead stage. Each egg is slightly less than 1 mm.

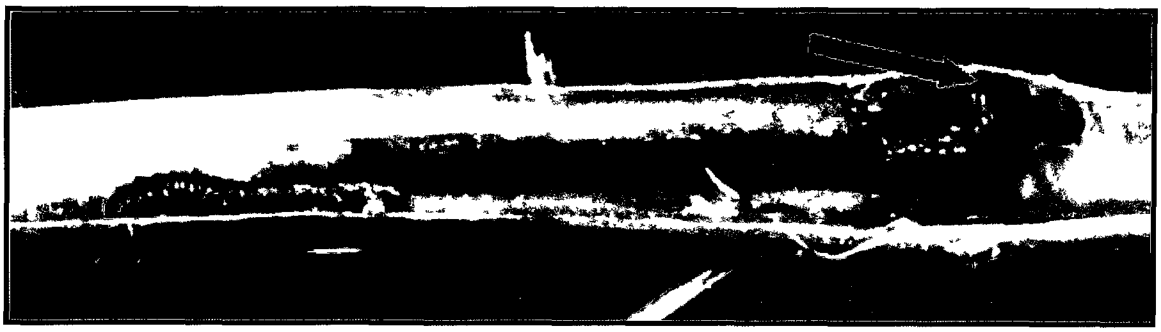


Figure 1.3: A European corn borer larva (~4 mm) within its tunnel. The entrance hole is at the right (arrow).

The fifth instar larvae enter diapause in the fall in northern areas and overwinter in the host plant stalks (Thomas et al., 2003; Hudon and LeRoux, 1986b). In the spring, in response to warmer temperatures, the larvae pupate within the stalks and the mature moths emerge from the host plant stems starting the cycle again. As stated before, a second generation of adult ECB moths was observed flying on PEI at the end of the summer in 2006 and 2007 indicating that, as a result of longer, warmer summers, some larvae are not entering diapause, but are pupating and giving rise to a second generation. The larvae hatching from the eggs laid by these moths would not have enough time to complete the larval stages before winter and probably do not survive.

1.3 European Corn Borer in Potatoes

In 1928 the potato was included on a list of 34 “more important hosts” for ECB in the New England states (Hodgson, 1928). Potatoes were listed as being an occasional host for ECB eggs and adults, while being a frequent host for larvae and pupae. At that time the potato plant was not considered to be a host for “hibernation” (Hodgson, 1928). One of the first instances of serious ECB infestation in potatoes was documented in Connecticut in 1936 by Turner and Zappe (1937). ECB damage in potatoes was first documented on PEI in 1987 (Dornan and Stewart, 1995a).

European corn borer larvae bore into the potato stems feeding primarily on the pith, but also on xylem and phloem tissue, restricting the nutrient flow (Kennedy and Anderson, 1980). Infested plants become susceptible to wind damage, wilting, and infection by pathogens. However, reports on the direct effect of ECB stem damage on potato tuber yields have been mixed. Stewart (1992) found that low infestation rates did not affect potato yields on P.E.I., but that high infestation rates could reduce the quality and quantity of commercially grown potatoes. Bray (1961) found that the number of vines destroyed as a result of ECB damage did not result in significant reductions in the yield of U.S. no.1 potatoes. Nault and Kennedy (1996), as well as Nault et al. (2001), found that levels of ECB larval stem damage as high as 50-100% sometimes resulted in a reduction of tuber yield or quality, but other times did not. That high levels of ECB infestation does not always result in tuber yield reductions may be due to intrinsic or extrinsic factors at the time of infestation. A variety of factors can contribute to the effect of ECB damage in potatoes, including soft rot, the phenological stage of the plant, the type of potato, the presence of other insects, temperature, water, stress, and soil fertility (Kennedy 1983). On PEI, stem breakage by the wind and feeding birds, such as crows and sea gull that tear open the potato stems to get to the ECB larvae are other factors that may increase the effect of ECB damage. However, even the contribution of these factors is not clear. One study found that the phenological stage of the potato plants at the time of ECB damage was not a factor in whether or not a yield reduction was seen (Nault et al., 2001). Another study found no clear evidence of ECB damage directly causing a reduction in total yields or in U.S. no.1 yields of spring grown potatoes, but that the most significant, although minor, factor resulting in potato yield reduction was bacterial soft rot. There was a small positive relationship between ECB damage and bacterial soft rot (Kennedy, 1983).

Action Threshold

With such an uncertain relationship between ECB infestation and any reductions in tuber yields, it is difficult for farmers to know when to implement control measures for this pest. The action threshold is the pest density at which control measures must be taken to prevent the pest population from increasing to or beyond the economic injury level. The economic injury level is the pest density at which the economic value of the crop loss prevented by pest control methods is equal to the cost of those methods. The action threshold occurs at a lower pest level than the economic injury level because the pest population can still increase after the control action has been initiated (Norris et al., 2003). There have been difficulties in proposing an action threshold for ECB because no clear relationship has been found between the number of adult ECB males caught in pheromone traps in potato fields (the current monitoring method) and the larval damage in that field (Dornan and Stewart, 1995a). However, the larval density can be related to the peak number of egg masses per stem (Dornan and Stewart, 1995a). Given this, the number of egg masses per plant could be a good predictor of potential ECB damage in potatoes if the larval numbers can be related to yield losses. Based on a preliminary study, Stewart (1992) proposed that the action threshold may be between a seasonal peak of 0.11 and 0.30 egg masses per stalk (one to three egg masses per 10 plants), but that more research was required. Currently, farmers in Atlantic Canada are advised to begin pesticide applications when ECB egg masses reach the level of two egg masses per 10 plants ©. Noronha, AAFC, Charlottetown, Personal Communication). This is more of a control threshold than an action threshold.

European Corn Borer Mortality and Natural Predators

Few studies have evaluated the natural mortality of ECB in the field. In Québec, natural overall ECB mortality in corn was found to range from 96.8% to 99.4% with an average of 98.7% (Hudon and LeRoux, 1986c). The pest population could be maintained with a survival rate as low as 1.3%, and population increases will occur when the survival rate is above this level (Hudon and LeRoux, 1986c). Therefore, control measures that reduce the survival rate below 1.3% should result in reductions in the ECB population.

One major source of natural mortality in ECB is natural predators, which can play an important role in reducing the ECB populations. Predators of ECB eggs in North America include the egg parasitoid *Trichogramma minutum* (Hymentoptera), the lady beetles *Coleomegilla maculata, Harmonia axyridis,* and *Coccinella septempunctata* (Coleoptera), the minute pirate bug *Orius insidiosus* (Hemiptera), lacewings (*Chrysopa* spp.; Neuroptera), and syrphid fly larvae (Diptera) (Hudon and LeRoux, 1986c; Kuhar et al., 2002).

Of the natural egg predators, *Trichogramma spp.* are of particular interest as biological control agents. *Trichogramma minutum*, because it is a native species and a natural predator of ECB, would appear to be a good choice as a biological control of ECB. In the field, its life cycle is poorly synchronized with ECB eggs as it is naturally present mainly at the end of the ECB egg laying period (Hudon and LeRoux, 1986c). However, because *T. minutum* or other *Trichogramma* species can be easily reared in production facilities, they could be good candidates as biocontrol agents of ECB through inundative releases at the appropriate time in the ECB life cycle.

Monitoring for European Corn Borer

European corn borer monitoring on PEI is carried out mainly by the province and by crop scouts. The Provincial Department of Agriculture monitors flying ECB adults in June. When flying adults are found in provincial pheromone traps or are reported to the province by growers, a notice is put on the provincial pest hotline. Crop scouts then begin to monitor specific potato fields by examining samples of 10 plants for ECB egg masses from at least four or five locations in the field. The current control threshold is two egg masses per 10 plants. If that threshold is reached in a particular field, the grower implements control measures. Current ECB control measures usually consists of insecticidal sprays such as Novaluron™ or Spinosad™ which have a lower toxicity for non-target organisms, and which are effective ECB eggs just prior to hatching, as well as the newly hatched ECB larvae (Boiteau and Noronha, 2007; PEI Agriculture, Fisheries and Aquaculture, 2007).

1.4 *Trichogramma* spp. as Biocontrol Agents

The genus *Trichogramma* is made up of about 180 species of parasitic wasps (Hymenoptera). All of these species are very tiny and are usually smaller than 0.2 mm. They are all internal parasites of insect eggs. Collectively they parasitize the eggs of several hundreds of other species of insects, predominantly lepidopteran species (butterflies and moths). Individual *Trichogramma* species are not usually species-specific, but will parasitize a wide variety of Lepidopteran eggs which makes them attractive for use in the biological control of many insect pests (Hassan, 1994; Pinto, 1998).

The very qualitites which make *Trichogramma spp.* attractive as biocontrol agents raises the possibility that they could spread beyond the agricultural fields and parasitize non-target lepidopteran insects. *Trichogramma spp.* were found to parasitize non-target lepidopteran eggs in the lab, and under caged and field conditions (Babendreier et al., 2003a; Babendreier et al.,2003b; Kuhar et al., 2003). However, under caged and field conditions, *Trichogramma spp.* displayed reduced searching efficiency outside of the test areas, as well as reduced non-target host acceptance and low parasitism rates of non-target host eggs (Babendreier et al., 2003c; Babendreier et al., 2003d; Kuhar et al., 2003). This lead to the conclusion that releases of *Trichogramma spp.* as a biocontrol would have minimal effects on non-target lepidopteron insects.

Trichogramma species have become very important in the biological control of lepidopteran pests. The most commonly used species, such as *T. brassicae, T. minutum, and T. pretiosum* are mass-reared in large commercial production facilities on facultative host eggs, usually belonging to the Angoumois grain moth *Sitotroga cerealella* or the Mediterranean flour moth *Ephestia kuehniella.* The *Trichogramma* wasps are shipped to customers as pupae inside parasitized eggs from one of these two species (Smith, 1996).

The quality (effectiveness) of commercially reared *Trichogramma* species can vary among suppliers, as well as among shipments (Lundgren and Heimpel, 2003; Hassan and Zhang, 2001). One factor that can affect the quality of commercially available *Trichogramma* is the host they are reared on. For example, Hoffman et al. (2001) found that on average, wasps reared on *E. kuhniella* lived a shorter number of days and the females parasitized fewer eggs than those reared on other hosts. He concluded that *E. kuhniella* eggs were poor hosts for *Trichogramma* production. The effectiveness of a *Trichogramma* species as biocontrol agents in the field also depends on a wide variety of factors such as the searching behaviour of the *Trichogramma* female, host preference, and the ability to adapt to local environmental conditions (Hassan 1994). Plant structure can also influence the ability of *Trichogramma* females to find host eggs: Females had more success finding host eggs on plants with a simple structure than on plants with a more complicated structure (Lukianchuk and Smith, 1997; Gingras et al., 2002).

Control of ECB in commercial crops using *Trichogramma*

Biocontrol of lepidopteran pests with *Trichogramma spp.* has been investigated in a wide variety of crops (Oatman and Platner, 1971; Burbutis and Koepke, 1981; Smith, 1996; Kuhar et al., 2003; Kuhar et al., 2004; Wang and Shipp, 2004).The most research and the most consistently positive results for ECB have been in corn (Smith, 1994). In a variety of studies *Trichogramma* species released in corn plots resulted in parasitism rates ranging from 40% to 89%, a 78.3% reduction of larvae in field corn plots and a 61-93% increase in

ECB mortality (Orr and Landis, 1995; Kuhar et al., 2002; Berger, 1991; Prokrym et al., 1992; Losey et al., 1995; Wang et al., 1999). Given the success in corn, it is important to investigate using *Trichogramma* spp. as a biological control of ECB in potatoes.

1.5 Objectives

The growing importance of ECB as a pest of potatoes on PEI resulted in a demand for more research into the local ECB populations, particularly into the effects ECB infestations on the potato yield and into novel methods of control. The primary objective of my project was to examine the relationship between levels of ECB infestation and the quality and quantity of tuber yields, as well as to examine the relationship between the number of egg masses and ECB infestation levels. My practical goal was to determine a more accurate control threshold for ECB infestation in potatoes and a more appropriate monitoring tool. My working hypothesis was that the true action threshold is higher than the current control threshold of 2 egg masses/10 plants. The secondary objective of my project was to evaluate several commercially reared *Trichogramma* species and to identify which, if any, are suitable to be used as biocontrol agents of ECB in potatoes. Given that *Trichogramma* spp. are an effective biological control of ECB in corn, my working hypothesis was that *Trichogramma* spp. would be effective biocontrol agents against ECB in potatoes.

CHAPTER 2

Investigating the effectiveness of *Trichogramma* (Hymenoptera: Trichogrammatidae) as a biocontrol agent for the European corn borer (*Ostrinia Nubilalis*) in Potatoes

2.1 Introduction

The European corn borer (*Ostrinia nubilalis*, ECB) is a nonnative polyphagous moth, the larvae of which tunnel inside the stems of host plants consuming tissue as they mature (Hudon et al., 1989). Once inside the stem they are protected from most insecticide applications (Stewart, 1992) and all but very specialized predators (Thomas et al., 2003). Historically the European corn borer has been an important pest in corn, but since 1987 it has become an important pest in potatoes in the Atlantic provinces and Maine (Dornan and Stewart, 1995b). Because of the stem boring behaviour of the larvae the most vulnerable stage in this pest's life cycle in North America is the egg stage, making it a possible target for egg parasitoids such as species of *Trichogramma* (Hymenoptera: Trichogrammatidae).

Trichogramma wasps (Figure 2.1) are tiny (<0.2mm) internal parasites of the eggs of other insects, primarily those from the order Lepidoptera. They have been studied as a biocontrol agent of insect pests for more than 100 years and have been commercially reared for this purpose for nearly 70 years (Smith, 1996). While their use has resulted in varying degrees of pest control in many types of crops, *Trichogramma* species have been consistently successful in the control of ECB in corn (Stinner, 1977; Pinto and Stouthamer, 1994; Smith, 1996; Knutson, 1998). A few species of *Trichogramma* wasps, that have been reared in the lab, have been investigated as biological control agents of ECB and the

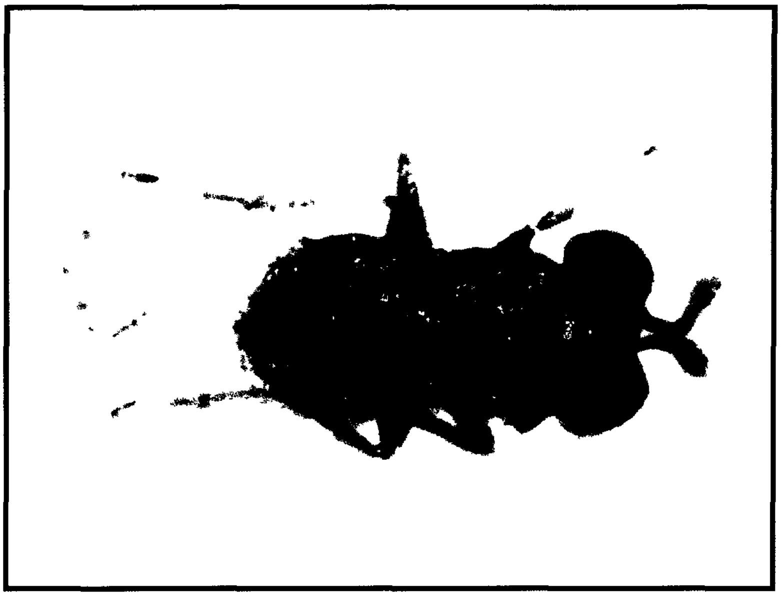


Figure 2.1: Trichogramma wasp (<0.2mm)

potato tuber moth (*Phthorimaea operculella*) in potatoes with positive results (Kuhar et. Al, 2004; Saour, 2004).

Individual *Trichogramma* species can differ as to their preferred insect host, their ability to find potential hosts on crop plants, and in their response to the environmental conditions found in an agricultural field (Hassan, 1994). When choosing a *Trichogramma* species for use in a biological control program, a species must be chosen which finds the target host and the agricultural habitat acceptable. A *Trichogramma* species should be tested on the target host and the specific agricultural crop in lab tests and in small field tests before being released in large scale field trials (Hassan, 1994).

All three of the *Trichogramma* species examined in this study have characteristics which indicate they have potential as ECB biocontrol agents in potatoes. *T. brassicae* has been shown to be effective against ECB in corn under agricultural conditions (Smith, 1996; Hassan and Zhang, 2001; Hoffman et al., 2001), but has not previously been tested in potatoes. *Trichogramma pretiosum* has also not been tested on potatoes, but was effective in the biological control of *Heliothis zea*, *Trichoplusia ni*, and *Manduca sexta* in field tomatoes (Oatman and Platneri, 1971) and *Keiferia lycopersicella* in greenhouse tomatoes (Wang and Shipp, 2004). The tomato is a close relative of the potato with a similar plant structure, suggesting that this *Trichogramma* species should not be deterred by the potato plant structure. The other *Trichogramma* species examined in this study, *T. minutum*, is a natural predator of ECB in corn in

Quebec (Hudon and Laroux, 1986c). As well, *T. minutum*, and *T. pretiosum* both occur naturally in Canada (Pinto, 1998).

The purpose of this study was to investigate the effectiveness of using commercially reared *Trichogramma* wasps as biocontrol agents of ECB in potatoes.

2.2 Methods

The effectiveness of *Trichogramma* control of European corn borer was tested in two ways: by releasing wasps into several potato fields on Prince Edward Island and by conducting lab trials on *Trichogramma* oviposition preference between ECB and the facultative host *Ephestia kuehniella* (Mediterranean Flour Moth). All three species of *Trichogramma* pupal cards and the *Ephestia kuehniella* eggs used in this study were purchased from Natural Insect Control (3737 Netherby Road, Stevensville, Ontario, Canada, L0S 1S0).

***Trichogramma* Release Studies**

Trichogramma wasps were released in fields with different management histories. The fields in these studies included an organic research field, two commercial organic fields, and a non-organic conventionally managed research field (Table 2.1). One card of approximately 3,500 *Trichogramma* pupae was placed in a 4oz plastic take-out container and covered with a lid into which a screen had been inserted (Figure 2.2). When the *Trichogramma* wasps were to be released the containers were hung from the potato plants using twist ties

Table 2.1: Summary of *Trichogramma* Release Studies

Field	Trial Number	Location	Type of Cultivation	Release Dates	Test Plots	Comments
Field "HO"	1 (2004)	Harrington, P.E.I.	Research Organic	July 22, 29 August 5	1 plot *T. brassicae* 1 plot *T. pretiosum* 1 plot Control	-release rate of 65,000 wasps/ha
Field "D"	2 (2004)	Winsloe, P.E.I.	Commercial Organic	July 22, 29 August 5	3 plots *T. brassicae* 3 plots Control	-release rate of 200,000 wasps/ha
Field "L"	2 (2004)	Springfield P.E.I.	Commercial Organic	July 22	3 plots *T. brassicae* 3 plots Control	-release rate of 200,000 wasps/ha -abandoned
Field 345	2 (2004)	Harrington, P.E.I.	Research Conventional	July 29 August 5	3 plots *T. brassicae* 3 plots Control	-release rate of 200,000 wasps/ha -investigation moved from Field "L"
Field 345	3 (2005)	Harrington, P.E.I.	Research Conventional	July 28 through August 2	4 plots *T. brassicae* 4 plots *T. pretiosum* 4 plots *T. minutum*	wasps released within 2x2x4 m cages

Figure 2.2: Trichogramma Release Containers

that had been stapled to the containers. While a smaller container containing a wick soaked in honey water was originally attached to the side of the container as a food source for the wasps, the use of honey water was discontinued because it attracted ants which consumed non-emergent pupae.

In the first trial, two species of *Trichogramma, T. brassicae* and *T. pretiosum*, were each released in separate plots on July 22, 29 and August 5 in an organic research field (Field "HO") (Table 2.1) when the wild moths were flying. The *Trichogramma* wasps were released at a rate of 65,000 wasps/ha into 23 X 30 m plots, separated by a 50m buffer zone. A third plot was maintained as a control, with no wasp release (Table 2.1). Sample stems were collected August 24. The effectiveness of ECB control was evaluated by comparing larval holes, tunnels and larvae from the stems of 30 potato tops collected from each *Trichogramma* release plot and 20 tops from the control top. The holes were counted directly, then the stems were dissected to determine the number of tunnels and larvae within stems.

In the second trial a single species, *T. brassicae*, was released in two commercial organic fields (Fields "D" and "L")(Table 2.1). However, because the potato plants were defoliated by Colorado potato beetles and late blight had been discovered in a neighbouring field, the study was terminated in Field "L" after one *Trichogramma* release on July 22. That trial was moved to a conventionally managed commercial sized field at the Harrington Agricultural Research Farm (Field "345"). This led to a single trial in an organic commercial field (Field “D”), and a similar trial in a conventionally managed field (Field 345). In Field 345 Admire® was applied in furrow at planting to control the Colorado potato beetle and aphids, and all plots were fertilized with 15:15:15 N:P:K. The wasps were released at a rate of 200,000 wasps/ha into 0.2ha plots with 30m buffer zones. The wasps were released on July 22 in Fields “D” and “L” and on July 29 and August 5 in Fields “D” and 345. When the potatoes reached maturity, approximately 110 days for Shepody and 130 days for Russet Burbank (CFIA, 2005a and 2005b), 16 potato tops were collected from each test plot. The number of larval holes, tunnels, and larvae in each potato stem and each plant was counted and recorded as before.

***Trichogramma* preference studies**

Three *Trichogramma* species; *T. brassicae, T. pretiosum*, and *T. minutum*, were tested in laboratory and in field trials to determine their preference for ECB eggs when compared to those of their facultative host, *Ephestia kuehniella*. Fresh ECB egg masses were collected from lab reared moths. Artificial egg masses of approximately the same size as the ECB egg masses were created by glueing 0.02g *E. kuehniella* eggs to wax paper using diluted egg white. Single mated females were offered a choice between one ECB egg mass and one mass of *E. kuehniella* eggs in individual bottles following the method of Hassan (1994). The female wasp’s position in the study arena (20ml glass bottles) was checked every 45 minutes for a total of 12 observations. Each female was scored as being in contact with the ECB egg mass, in contact with the *E.*

kuehniella mass of eggs, or in contact with no egg mass. Each *Trichogramma* species was evaluated using a total of 50 females. The egg masses were then incubated at 16:8 L:D, 27°C day, 18°C night, and 75% RH until the eggs had clearly reached either the black head stage (indicating the presence of ECB embryos) or had turned entirely black (indicating the presence of *Trichogramma* pupae). The egg masses were examined using a dissecting microscope to determine the number of parasitised eggs.

The field preference trials (Trial 3, Table 2.1) consisted of placing individual cards of parasitised *E. kuehniella* eggs into 2x2x4m cages located in a conventionally tilled research field at the Harrington Research Farm. Buffer zones of 80m were established between cages. On July 28, one card, ca. 3000 wasps, of one of three *Trichogramma* species; *T. brassicae, T. pretiosum*, and *T. minutum*, was placed in each cage. The *Trichogramma* wasps were just emerging when the cards were placed. At the same time, 10 ECB egg masses (collected from lab reared moths) were pinned to every second potato plant in each of the two rows of plants in each cage. These were allowed to remain undisturbed until August 2. Then the egg masses were removed and incubated at 16:8 L:D, 27°C day, 18°C night, and 75% RH until the eggs had clearly reached either the black head stage or had turned entirely black. The egg masses were examined using a dissecting microscope to determine the rate of parasitism. The potato field in this trial had been treated with Admire® in furrow at planting and was sprayed with Bravo® approximately weekly after late blight was detected on PEI No pesticides were sprayed during the test period.

Statistical Analyses

The effect of *Trichogramma* releases was determined by comparing the rates of infestation by ECB among *T. brassicae, T. pretiosum,* and control treatments (Trial 1). Infestation rates were evaluated by comparing the number of larval holes, larval tunnels and larvae per potato plant using a Kruskal-Wallace test. Post-hoc testing was then carried out using Mann-Whitney U-tests with a Bonferroni corrected significance level of 0.017 to avoid error inflation from multiple testing.

The effect of a higher infestation rate of *T. brassicae* on ECB infestation was evaluated by comparing the number of larval holes, larval tunnels and larvae per plant between treatment and control plots in Field 345 (Trial 2) using ANOVA. The remaining commercially managed organic field (Field "D") had such low levels of ECB infestation (< 1 ECB larva/plot) that *Trichogramma* parasitism could not be evaluated.

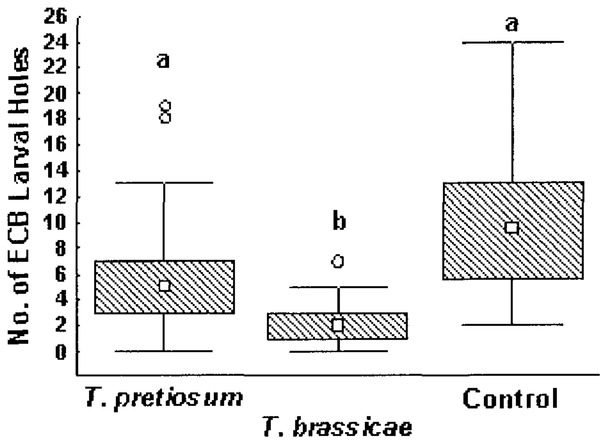
The preference treatment results were summarized and assessed visually.

2.3 Results

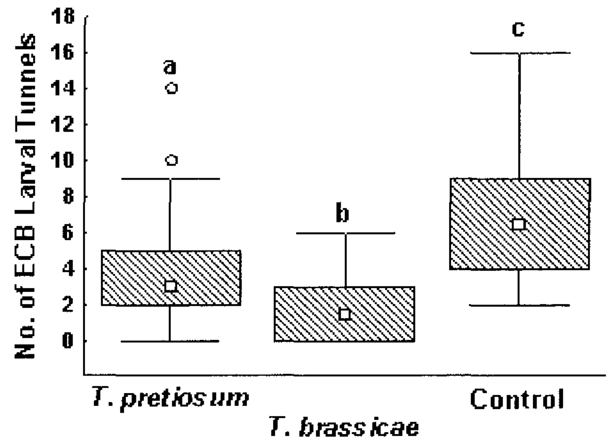
Trichogramma Release Studies

The initial results from the organic test plots (Field "HO"), where two parasitoid species were released, showed that *Trichogramma* could reduce ECB infestation, but results varied between the two species. Potatoes grown in plots where *T. brassicae* had been released had significantly lower ECB infestation as measured by the number of larval holes, larval tunnels or larvae than *T. pretiosum* or the control plots ($p<0.017$) (Figure 2.3 A, B,& C). In the plots where *T. pretiosum* were released, ECB infestation only differed from the control for the number of larval tunnels which were lower than the control, but higher than the potatoes in *T. brassicae* plots ($p<0.017$) (Figure 2.3 B).

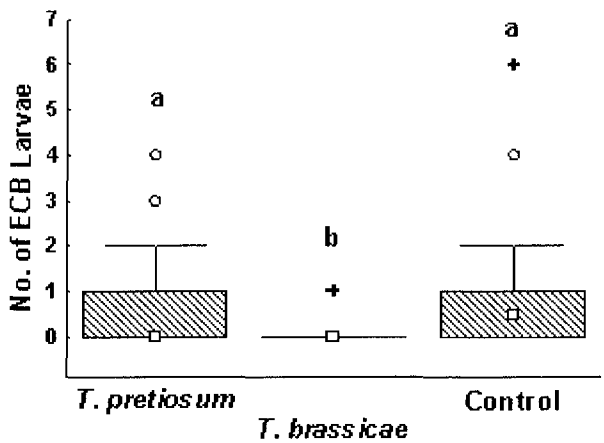
The release of *T. brassicae* in Field 345, the conventionally managed field, did not result in any significant differences between ECB infestation in the control plots and the treatment plots. Field 345 had a high ECB infestation rate with an average of nearly 50 larvae per plot, but there was no statistical difference in larval holes, larval tunnels, or larvae between the control and the treatment plots ($p>0.05$) (Figure 2.4 A, B &C), or in total number or weight of tubers ($p>0.05$) (Figure 2.5 A & B).



A) Average number of larval holes per plant



B) Average number of larval tunnels per plant



C) Average number of larvae per plant

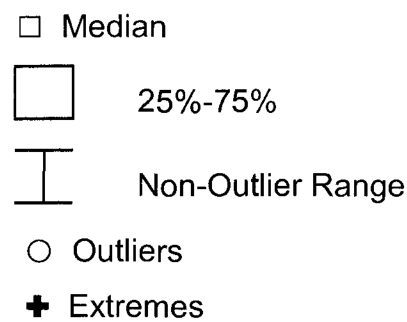
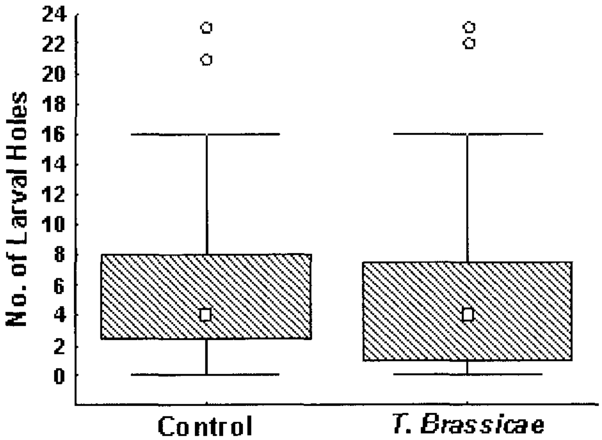
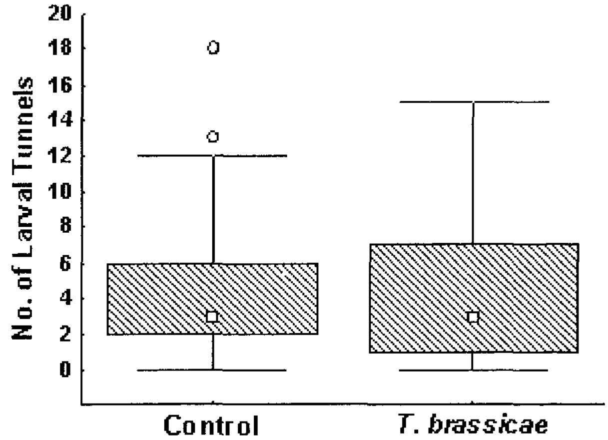


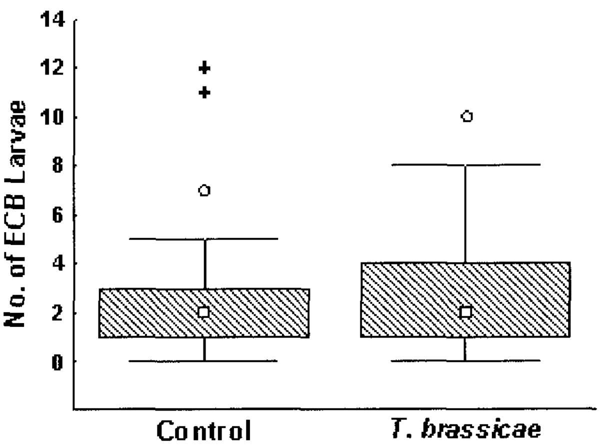
Figure 2.3: Average number of ECB larval holes (A), larval tunnels (B) and larvae (C) per potato plant found in an organic research field where *T. brassicae* and *T. pretiosum* were released in 2004. There is a significant ($p<0.017$) difference between bars with different letters.



A) Average number of larval holes per plant.



B) Average number of larval tunnels per plant.



C) Average number of larvae per plant.

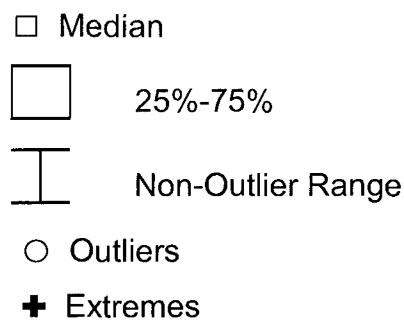
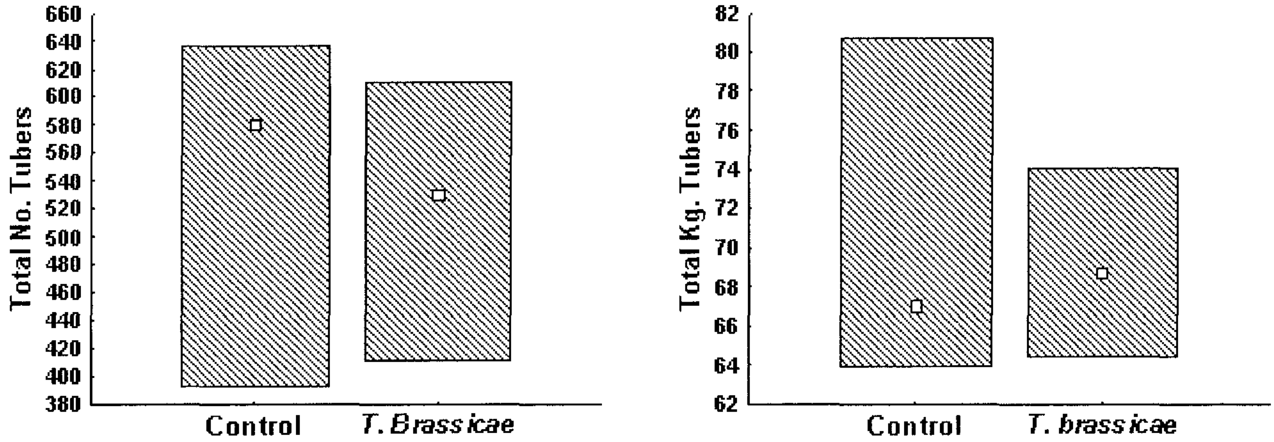


Figure 2.4: Average number of ECB larval holes (A), larval tunnels (B) and larvae (C) per potato plant in a conventionally managed, commercial size field where *T. brassicae* was released in 2004. There was no significant difference ($p>0.05$).



A) Total number of tubers

B) Total weight of tubers

☐ Median

☐ 25%-75%

Figure 2.5: Total number (A) and weight (B) of tubers in a conventionally managed, commercial size field where *T. brassicae* was released in 2004. There was no significant difference ($p>0.05$).

Trichogramma Preference Studies

When given a choice between eggs from ECB and from *Ephestia kuehniella* in laboratory trials, all three *Trichogramma* species showed a preference for *E. kuehniella* eggs (Table 2.2). *Trichogramma brassicae* showed the most interest in ECB eggs, but still preferred *E kuehniella* eggs two to one *T. pretiosum* and *T. minutum* showed essentially no interest in ECB eggs. In caged plots in a commercial sized field (Field 345, Trial 3), no parasitism was observed except for one egg mass by *T. brassicae.*

2.4 Discussion

In this study, *T. brassicae* was the only species to show any effectiveness against ECB, and only in the organic test plots. The results of the preference experiments also indicate that *T. brassicae* was the only species that would parasitize the ECB when given a choice of hosts suggesting that one of the reasons for the low parasitism rate by *T. pretiosum* and *T. minutum* may have been the oviposition preference of the female wasps for *E. kuhniella* eggs over ECB eggs. Losey and Calvin (1995) found that *T. pretiosum* and *T. minutum* could parasitize and develop in ECB eggs, but in this study only 5% of *T. pretiosum* and 0% of *T. minutum* females actually parasitized ECB egg masses. Since *T. brassicae* parasitized 34% of the egg masses in the preference trials, it appears that something else could be interfering with the ability of *T. brassicae* wasps to parasitize ECB egg masses in commercial sized fields.

Table 2.2: Summary of the results of trials where *Trichogramma* females from three species were given the choice of two types of insect egg masses, European corn borer (ECB) and *Ephestia kuehniella* (*EK*). The table shows the number of *Trichogramma* females either on an ECB egg mass or on an *Ek* mass of eggs, or not on any egg mass ("Off Egg"). The percentages of total are shown in the parentheses. The table also shows the total number and percentages of individual eggs parasitized by each species of *Trichogramma* for each potential host species.

***Trichogramma* Species**	**Total Number of Females (percent)**			**Total No. of Parasitized Eggs (percent)**	
	On ECB Mass (%)	On *Ek* *Mass* (%)	Off Egg Mass (%)	ECB (%)	*Ek* (%)
T. brassicae	69 (12.1)	199 (34.9)	302 (53.0)	97 (34.4)	185 (65.6)
T. minutum	20 (3.8)	102 (19.2)	410 (77.1)	51 (5.2)	932 (94.8)
T. pretiosum	6 (1.1)	242 (42.6)	320 (56.3)	0 (0)	1446 (100)

One factor that may effect *Trichogramma* parasitism is plant structure. Plant characteristics can affect the ability of the *Trichogramma* wasps to find potential hosts because *Trichogramma* wasps tend to search for potential hosts by walking (Andow and Prokrym, 1990; Schmidt, 1994). Andow and Prokrym (1990) identified three major components of plant structure which are important to searching parasitoids: the plant size or surface area, the variation among plant parts (structural heterogeneity), and the connectivity of parts or plant form (structural complexity). All three of these characteristics differ between corn plants and potato plants, so *Trichogramma* species which control ECB in corn may not do so in potatoes. The variation among plant parts (structural heterogeneity) especially affects the searching of *Trichogramma* wasps. For example, trichomes on potato leaves can inhibit *Trichogramma* movements (Romeis et al., 1998) and as the number and complexity of trichomes increases, the number of turns the wasp takes goes up and the walking speed goes down (Keller, 1987), reducing the ability of the *Trichogramma* wasp to find potential hosts. The connectivity of parts or the plant form (structural complexity) also reduces the ability of *Trichogramma* wasps to find potential host eggs (Andow and Prokrym, 1990; Gingras et al., 2002). While corn has a single stem, the potato can have one to more than ten stems, with secondary stalks branching off of those.

The relative success in this study with *T. brassicae* suggests that this species may be successful in reducing ECB in potatoes if the problems with the structure of the potato plant can be overcome. While there were positive results controlling lepidopteran pests using *T. pretiosum* in tomatoes, a plant with a similar structure to the potato plant, *T. pretiosum* did not show much interest in ECB eggs in the lab. Future studies should concentrate on *T. brassicae* and possibly evaluate higher release densities of the parasite in commercial size fields.

CHAPTER 3

Determining a New Control Threshold for the European Corn Borer (*Ostrinia nubilalis)* in Potatoes

3.1 Introduction

The European corn borer (*Ostrinia nubilalis*; ECB) is a moth native to northwest Africa, limited areas of southwestern Asia, and to Europe. It was first described by Hübner in 1796 (Hudon et al. 1989). European corn borer was a relatively innocuous insect found in host plants such as mugwort (*Artemesia vulgaris*), wild hops (*Humulus lupulus*), and wild hemp (*Cannabis sativa*) (Hudon and LeRoux 1986a). When corn (*Zea mays*) became established in Europe, ECB quickly became a serious pest of this crop because corn provided more protection from predators than the native host plants (Thomas et al. 2003; Hudon and LeRoux 1986a). ECB was in turn introduced to North America from Europe in the early decades of the 20th century in shipments of broom corn (*Sorghum bicolor* (L.)) and rapidly became a serious pest in corn in North America (Hudon et al. 1989; Hudon and LeRoux 1986a; Smith 1920; Hodgson 1928).

While ECB is primarily a pest in corn, it can be found in other crops, including potatoes. In 1928, Hodgson included the potato in a list of 34 “more important hosts” for ECB in the New England states. He stated that the stems were frequently attacked and that the potato plant was frequently infested, but that it was strictly a food host and was not, at that, time known to be a host for “hibernation”. ECB damage to potatoes was first observed on PEI in 1987 (Dornan and Stewart 1995a, 1995b). At this time it was discovered that ECB was present in potatoes during all of its life stages.

European corn borer larvae bore into the potato stems and damage the pith, xylem and phloem (Kennedy and Anderson 1980). Infested plants become susceptible to wind damage, wilting, and infection by pathogens (Kennedy 1983). Once inside the potato stem, the larvae are protected from most insecticide applications and all but very specialized predators (Sorenson et al. 1995; Stewart 1992; Thomas et al. 2003).

There is no clear relationship between ECB damage and tuber production. Several studies, including one on PEI, have found no reductions in potato yield with ECB infestation. (Stewart 1992, Kennedy, 1983, Bray, 1961), though some researchers have found that yields may be affected at very high infestation numbers (Stewart 1992). The response of potatoes to ECB infestation may be mixed even within individual studies. For example, a study by Nault et al. (2001) showed reduced yields were only seen in 5 out of 14 experiments when ECB infestation numbers ranged from 50 to 90% of stems injured. Several biotic and abiotic factors contribute to the effect of ECB damage in potatoes, such as the phenological stage of the plant, the variety of potato, the presence of other insects and disease organisms, growing temperatures, water stress, and soil fertility (Kennedy, 1983). Kennedy reported the most significant factor resulting in yield reduction was bacterial soft rot, which correlated with ECB damage. This relationship may stem from the ECB larval feeding pattern: larvae feed in several locations over the course of their development and tend to abandon soft rot infected stems, thereby spreading soft rot throughout the crop (Anderson et al., 1981). More investigation needs to be done on the relationship of disease with larval infestation in potatoes in the Maritimes.

Currently, crop scouts on PEI use an control threshold of two ECB egg masses per ten plants to advise farmers when to treat for ECB infestations. The primary objective of my project was to examine the relationship between levels of ECB infestation, and the quality and quantity of tuber yields in two varieties of potatoes commonly grown on PEI., as well as to examine the relationship between the number of egg masses and ECB infestation levels. My practical goal was to determine a more accurate control threshold for ECB infestation in potatoes. My working hypothesis was that the true action threshold is higher than the current control threshold of 2 egg masses/10 plants.

In the course of this study it became apparent that collecting data on the levels of ECB infestation was very labour intensive, involving long hours slicing open potato stems. This led to another objective, to determine a method by which ECB infestation levels could be estimated using the larval entry holes. While the larvae themselves are hidden inside the stems, the larval holes can be easily seen on the surface of the stem. If the larval holes could be used to estimate the level of ECB infestation, the amount of work needed to determine ECB infestation levels could be reduced. It could even be possible to estimate the level of ECB infestation in the fields without removing the potato tops.

3.2 Methods

Relationship between the levels of initial ECB egg mass infestation and tuber yields.

Four replications of a set of research plots were established at the Harrington Research Farm (Field "630") using two potato varieties commonly grown on PEI, Shepody and Russet Burbank. This resulted in a total of 16 test plots in 2004 and 20 test plots in 2005 for each potato variety, giving 32 and 40 test plots total for 2004 and 2005 respectively. Each of the plots was four rows wide (approximately 3.7m) and 7.6m long with 20 potato plants in each row. The two outer rows were designated as guard rows and the two centre rows were designated as sample rows. Two potato varieties commonly grown on PEI were evaluated: Russet Burbank and Shepody. Admire® was applied in furrow at planting to control the Colorado potato beetle and aphids, and all plots were fertilized with 15:15:15 N:P:K.

Treatments consisted of four infestation levels in 2004: 0, 2, 2.5, and 3 egg masses per 10 plants, and five infestation levels in 2005: 0, 2.5, 3, 4 egg masses per 10 plants and a natural infestation level (Table 3.1). In 2005 a treatment level was added where the moths were allowed free access to the potato plants (Table 3.1). This resulted in an infestation level of greater than 5 egg masses per 10 plants. The four repetitions of each treatment were assigned to plots for each potato variety using a random block design. With the exception of the open access treatment (Treatment 5) in 2005, treatment levels were established by pinning ECB egg masses collected from potato plants in

Table 3.1: ECB egg mass treatment levels 2004 and 2005

Year	Infestation per 10 plants				
	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
2004	0 egg masses	2 egg masses	2.5 egg masses	3 egg masses	
2005	0 egg masses	2.5 egg masses	3 egg masses	4 egg masses	>5 egg masses

neighboring fields or, ECB egg masses deposited on wax paper sheets by ECB moths reared in field cages and in lab rearing cages (Figure 3.1).

When the potatoes in the plots reached maturity, the tops from eight plants in each sample row (sixteen plants in total) were collected from each test plots. The planting, sample, and harvest dates for each year and potato variety are outlined in Table 3.2. Each stem of the sample tops was dissected and the number of ECB holes, tunnels and larvae were recorded for each stem and each plant. Sample stems not immediately cut were stored at 4°C to prevent larval movement and stem degradation until they could be dissected. This was successful as no larvae were found outside of the stems. The remaining potato tops were killed by two applications of diquat. The potato tubers in the two sample rows were harvested and graded mechanically into the following tuber classes of increasing tuber weight size: Culls, Canada #1 small, Canada #1, Canada #1 large, and, in the Shepody variety, Jumbo (Table 3.3).

In 2004 the Harrington Research Farm experienced a massive ECB outbreak that began about July 17. As a result it became obvious that wild egg masses had to be removed from the test plots in order to try to maintain the egg mass treatment levels, rather than by adding egg masses to the plots. As soon as the experimental infestation numbers were thought to be established, the centre two sample rows were covered with permeable fabric covers on July 28-29 to prevent wild moths from laying more eggs in the those rows (Figure 3.2) . The plots were left covered until the wild ECB moths stopped flying and then the covers were removed on August 19.

Figure 3.1: A European corn borer egg mass on wax paper is pinned to the underside of the leaf of a potato plant.

Table 3.2: Planting, sampling, and harvest dates for Shepody and Russet Burbank potatoes, Field 630, for 2004 and 2005.

Year	Variety	Planting Date	Stem Sampling Dates	Top-Kill Dates	Harvest Date
2004	Shepody	May 17	Sept 1	Sept 14 Sept 22	Sept 28
	Russet Burbank	May 17	Sept 22	Sept 17 Sept 30	Oct 12
2005	Shepody	May 31	Sept 20	Sept 20	Oct 6
	Russet Burbank	May 31	Sept 27	Oct 3	Oct 14

Table 3.3: Tuber classes for the Shepody and Russet Burbank Varieties of Potatoes.

Variety	Tuber Classes				
	Culls	**Canada #1 small**	**Canada #1**	**Canada #1 large**	**Jumbo**
Shepody	<41.5 g	41.5 g - 112.9 g	113.0 g - 339.9 g	340.0 g - 509.9 g	≥510.0 g
Russet Burbank	<33.2 g	33.2 g - 85.5 g	85.6 g - 434.6 g	≥434.7 g	

In 2005 on July 5-6, all four rows of the test plots were covered with permeable fabric (Figure 3.2) just prior to the emergence of the wild moths on about July 10. The open access plots (Treatment 5) were left open to ovipositing wild moths. One half of the egg masses need to establish the treatment levels were pinned in each plot from July 12-15 and the other half from July 18-19. The covers were left in place until August 4 when the wild moths had stopped flying.

Tubers were collected from both sample rows for all analyses, except for the Russet Burbank plots in 2005, where one sample row did not receive the correct amount of fertilizer. In that case only a single sample row was used for the analyses. For all trials, potato tuber yields (overall yield, and numbers and weights of potatoes in specific grades) were compared among treatments using ANOVA (Genstat, Version 10). Individual treatment differences were compared using Fishers LSD multiple comparison test (Genstat, version 10) when significant treatment differences were detected using ANOVA. Non-normal data were transformed using a square root transformation prior to analysis, as required.

Tubers were collected from both sample rows for all analyses, except for the Russet Burbank plots in 2005, where one row did not receive the correct amount of fertilizer. In that case only, a single sample row was used for the analyses.

Figure 3.2: The research field at the Harrington Research Farm showing the research plots and the permeable cloth covers over the centre two sample rows in 2004.

Figure 3.3: The research field at the Harrington Research Farm showing the research plots and the permeable cloth covers covering complete plots 2005.

Larval infestation numbers (number of larval holes, larval tunnels, and larvae per stem or plant) were related to potato tuber yields (overall yield, and numbers and weights of potatoes in specific grades) using Pearson correlations if the data were normal or could be transformed, or Spearman correlations if the data were non-normal and could not be transformed (Statistica, version 6).

Estimation of ECB larval infestation using ECB egg masses

Egg mass numbers were compared to the resultant larval infestation numbers (number of larval holes, larval tunnels, and larvae per stem or plant) using ANOVA, to determine how the numbers of egg masses related to actual larval infestation. Individual treatment differences were compared using Fishers LSD multiple comparison test (Genstat, version 10) when significant treatment differences were detected using ANOVA. Non-normal data were transformed using square root transformation prior to analysis, as required.

Estimation of ECB larval infestation using ECB larval holes

The use of larval holes to estimate larval infestation numbers was evaluated through regression analysis (Statistica, version 6) using combined data from 2004 and 2005. The number of larval holes was related to the numbers of larvae per stem and per plant, and to the numbers of larval tunnels per stem and per plant. Plants that did not have any larval holes were excluded from the analysis, since they did not contain larvae or tunnels. Non-normal data were transformed as necessary to meet the assumptions of regression analysis (i.e. error normality, residual plotting).

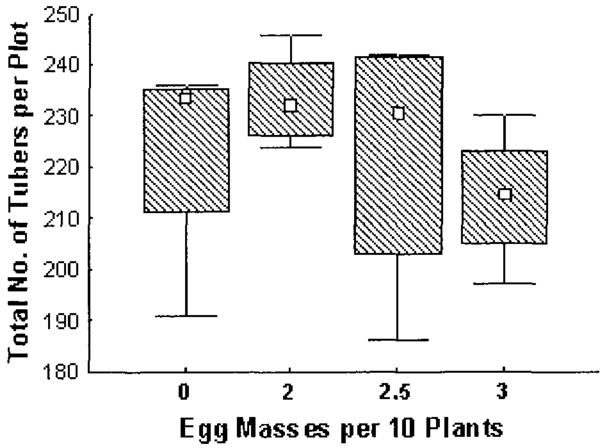
3.3 Results

The relationship between the levels of initial ECB egg mass infestation and tuber yields

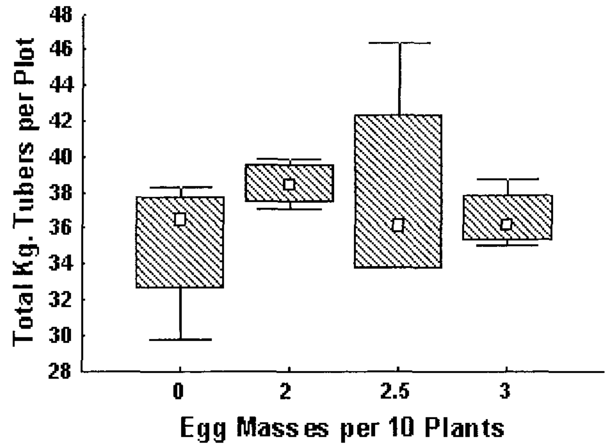
There were no significant differences (ANOVA, $p>0.05$) among the different egg mass treatments in the numbers and weights of tubers for either variety of potato for either year for the experimental treatment levels of 0-4 egg masses/10 plants. (Figures 3.4-3.5). In 2005, Russet Burbank potatoes grown at high infestation levels (>5 egg masses/10 plants) showed significantly higher yields than the other treatments (Figure 3.5 C & D) (ANOVA, $p<0.05$), a pattern which was not seen in the Shepody potatoes (Figure 3.4 C & D). These differences in the Russet Burbank potatoes were a result of increases in the numbers and weights of the smaller classes of tubers, i.e. in the culls, the Canada #1 small and the Canada #1 classes (Figure 3.6 A, B, & C). There was also a trend towards a reduction in the Canada #1 large tubers in the open access plots, but this was not significant (Figure 3.6 D).

Estimation of ECB larval infestation using ECB egg masses

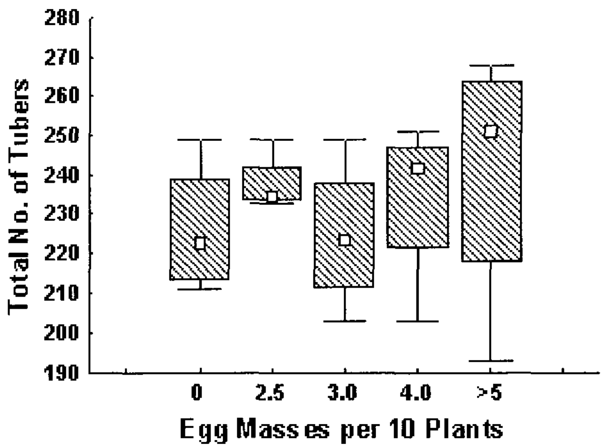
In 2004, the experimental egg mass treatment levels were not maintained and the larval infestation numbers did not show a consistent pattern with the intended egg mass number for either variety of potato. This resulted in larval infestation numbers of 4-10 ECB larvae per plant (70-150 larvae per plot) and 8-12 ECB larvae per plant (130-180 larvae per plot) (Russet Burbank and Shepody varieties respectively) (Figures 3.6 A & B, 3.7 A & B) compared to the <25 larvae per plot (<1 larva/plant) in 2005 (Russet Burbank and Shepody



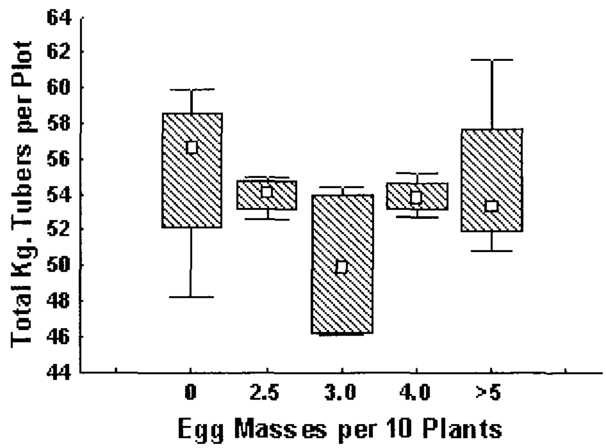
A) Total number of tubers/plot, 2004.



B) Total weight tubers/plot. 2004.



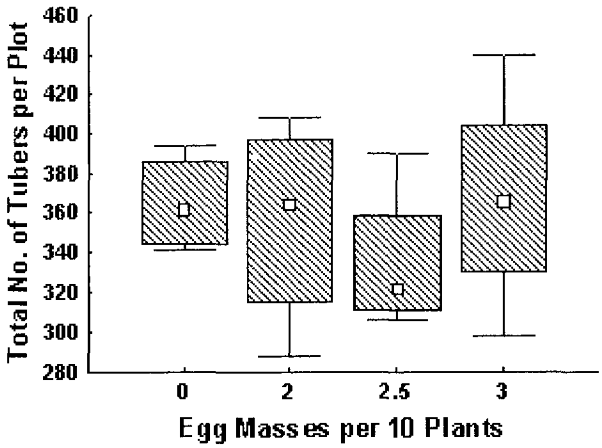
C) Total number of tubers/plot, 2005.



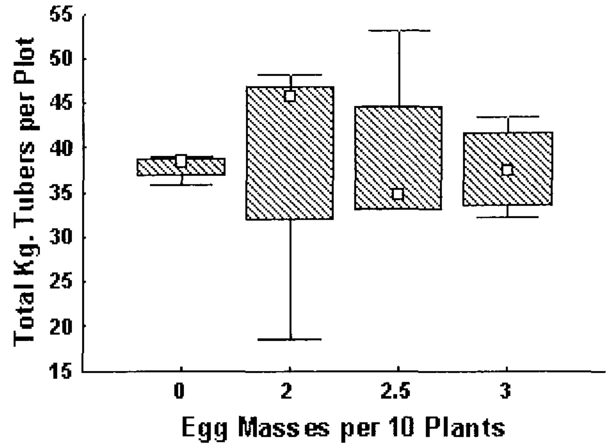
D) Total weight tubers/plot. 2005.

□ Median ▭ 25%-75% ⌶ Non-Outlier Range

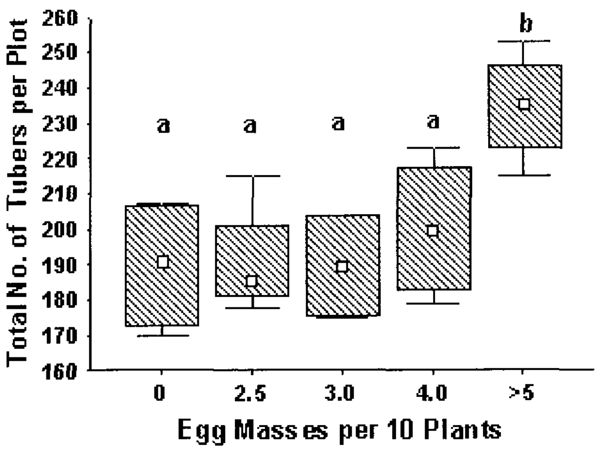
Figure 3.4: Total tuber yield per plot of Shepody potatoes for five experimental ECB egg mass levels over two years. There is no significant relationship (p>0.05).



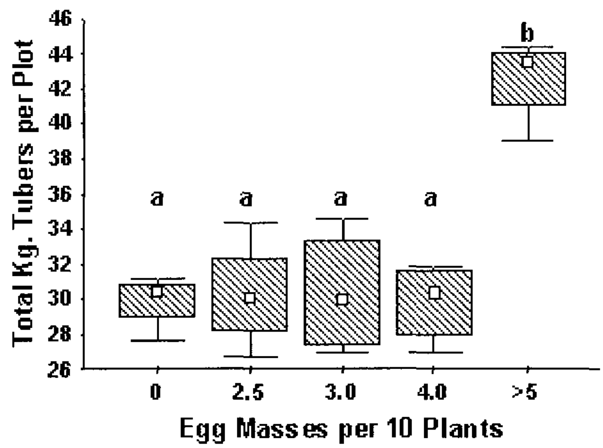
A) Total number of tubers/plot, 2004.



B) Total weight tubers/plot. 2004.



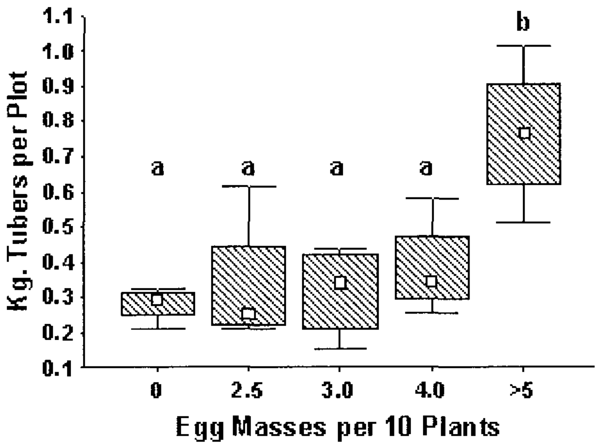
C) Total number of tubers/plot, 2005.



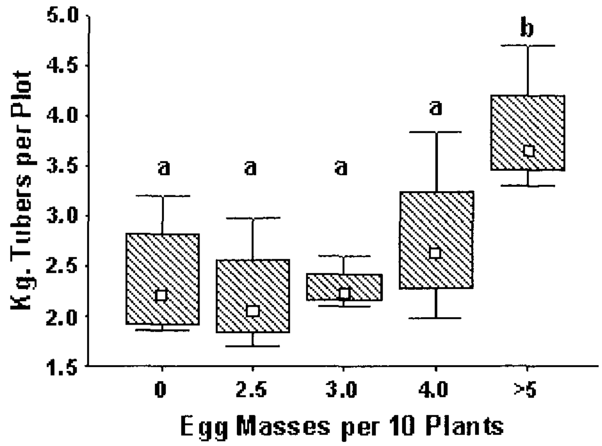
D) Total weight tubers/plot. 2005.

□ Median 25%-75% Non-Outlier Range

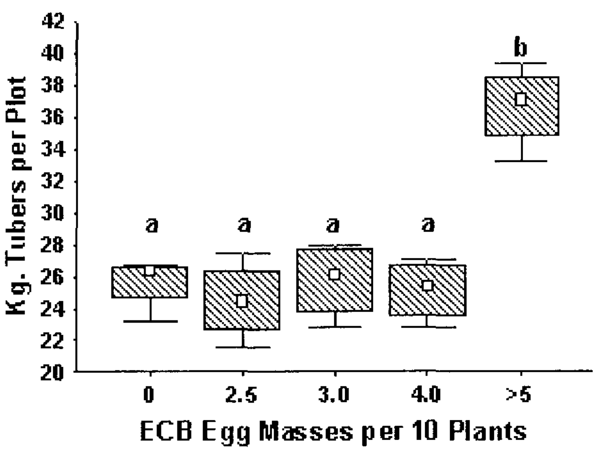
Figure 3.5: Tuber yield per plot for Russet Burbank potatoes for five experimental ECB egg mass levels over two years. There is a significant ($p<0.05$) difference between bars with different letters. There is no significant difference ($p>0.05$).



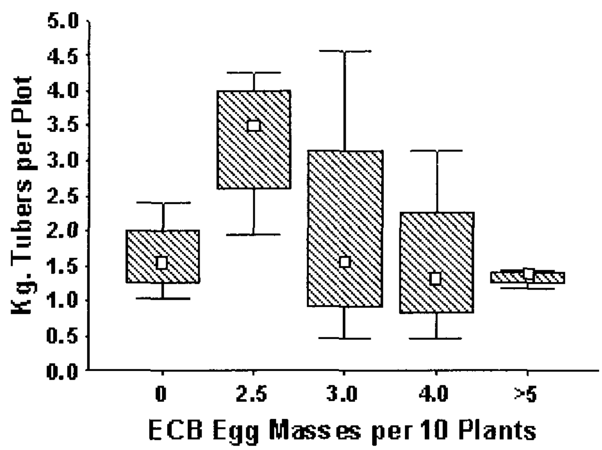
A) Total kilograms of cull tubers/plot.



B) Total kilograms of Canada#1 small tubers/plot.



C) Total kilograms of Canada #1 tubers/plot.



D) Total kilograms of Canada#1 large tubers/plot.

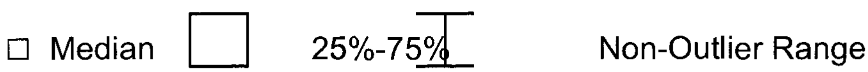


Figure 3.6: Tuber yield per plot for four tuber classes of Russet Burbank potatoes for five experimental ECB egg mass levels. There is a significant ($p<0.05$) difference between bars with different letters.

varieties). In 2004 only the centre two sample rows were covered and the wild moths could oviposit in the outer two guard rows. Either the wild moths oviposited in the centre two sample rows before they were covered, or the growing ECB larvae in the outer rows could have spread into the centre two sample rows after the covers were removed.

In 2005, the larval infestation number showed a significant pattern with egg mass number (ANOVA, $p<0.05$; Figures 3.7 C & D). The larval infestation numbers for the control plots (0 egg masses per 10 plants) for both potato varieties were significantly lower (Fishers LSD, $p<0.05$) than the larval infestation numbers for all other egg mass levels. There was no difference (Fishers LSD, $p>0.05$) between larval infestation numbers among the three experimental egg mass levels (2.5, 3, and 4 egg masses per 10 plants; Figures 3.7 C & D).The open plots, where the wild moths were allowed to oviposit (>5 egg masses per 10 plants), had larval infestation numbers significantly higher (Fishers LSD, $p<0.05$) than any of the other experimental egg mass levels (Figures 3.7 C & D).

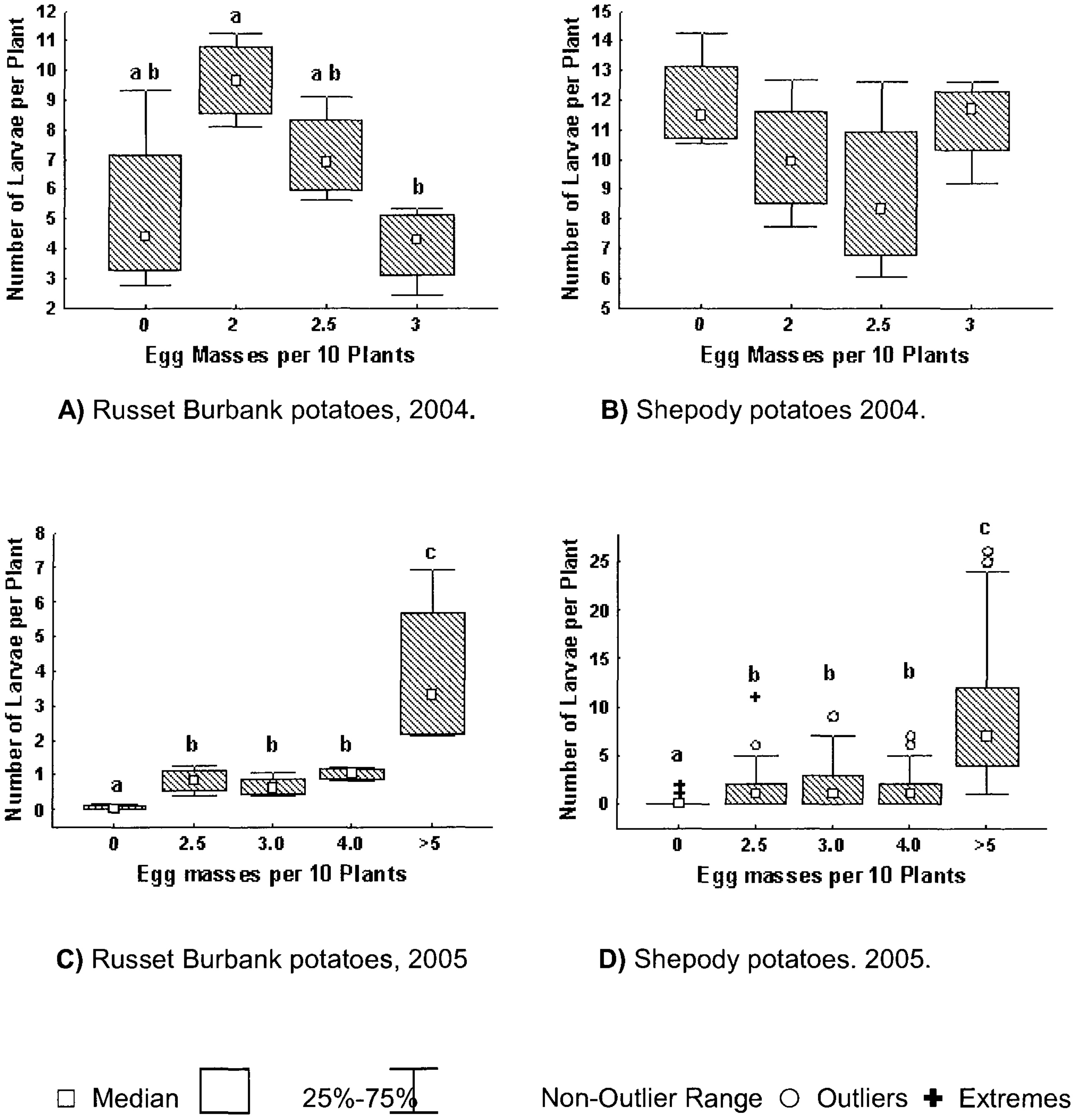


Figure 3.7: Number of larvae per plant for Shepody and Russet Burbank potatoes for five experimental ECB egg masses levels. There is a significant ($p<0.05$) difference between bars with different letters.

Estimation of ECB iarval infestation using ECB larvai holes

The number of larval holes was significantly related to both the number of larvae and the number of larval tunnels (both per plant and per stem) for both varieties in both 2004 and 2005 (regression analysis, $p<0.001$, using transformed data where necessary). The between-year patterns were similar so data from potato plants or stems exhibiting larval holes from 2004 and 2005 were combined (Figures 3.8 - 3.9). The regression equations for each grouping are shown below. The regression equations make it possible to estimate the number of larvae and larval tunnels in a plant or a stem by counting the number of larval holes on the surface of the stems. When estimating infestation levels on a per plant basis, 10 larval holes would result in 8 tunnels and 4-5 larvae per each Shepody potato plant, and 7-8 tunnels and 3 larvae per Russet Burbank potato plant. When estimating infestation levels on a per stem basis, 5 larval holes would result in 4-5 tunnels and 3 larvae per stem for Shepody potatoes, and 4 tunnels and 2 larvae per stem for Russet Burbank potatoes.

Regression Equations:

Shepody: per plant

tunnels = 0.72(±0.18)*(holes+1.5) + 0.48(±0.06)*√(holes+1.5) - 1.42(±0.20)

r^2= 0.93

larvae = 0.41(±0.20)*(holes+1.5) + 0.41(±0.08)*√(holes+1.5) - 1.40(±0.28)

r^2= 0.74

Russet Burbank: per plant

tunnels = 0.62(±0.22)*(holes+ 1.5) + (0.44(±0.17)*√(holes+ 1.5)) - 1.42(±0.24)

r^2= 0.83

larvae = 0.24(±0.17)*(holes+ 1.5) + (0.53(±0.10)*√(holes+ 1.5)) - 1.21(±0.33)

r^2= 0.70

Shepody: per stem

tunnels = 0.79(±0.02)*holes + 0.23(±0.15)

r^2= 0.92

larvae = 0.47(±0.02)*holes + 0.10(±0.01)

r^2= 0.73

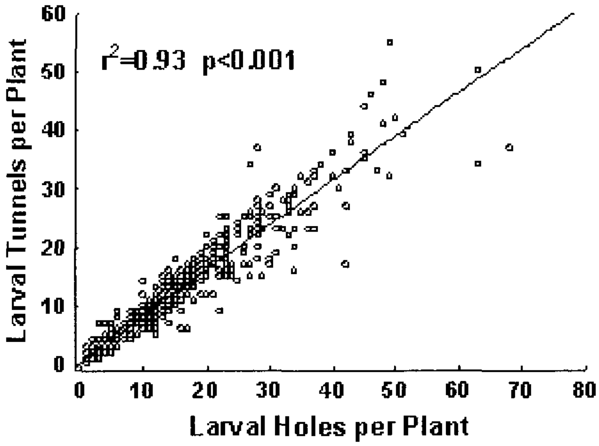
Russet Burbank: per stem

tunnels = 0.69(±0.03)*holes + 0.11(±0.13)

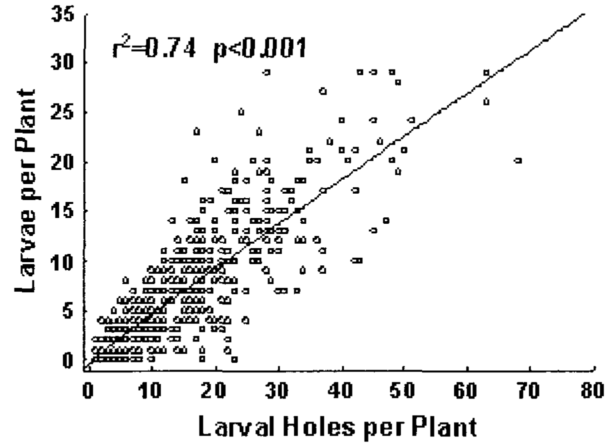
r^2= 0.83

larvae = 0.34(±0.02)*holes + 0.05(±0.10)

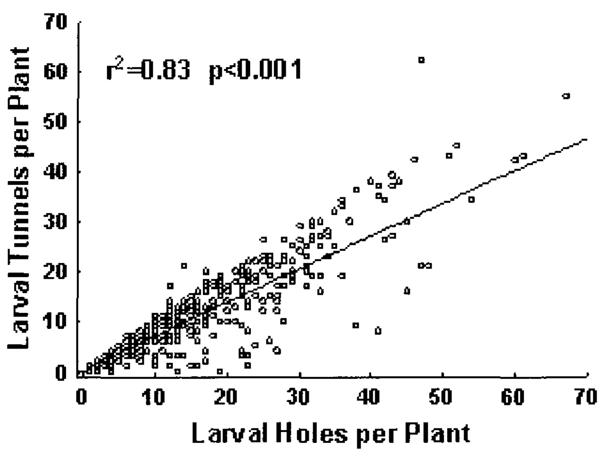
r^2= 0.70



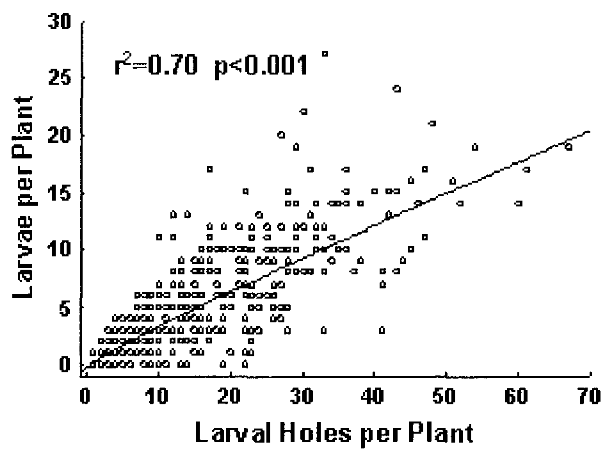
A) Shepody potatoes, 2004 and 2005



B) Shepody potatoes, 2004 and 2005.

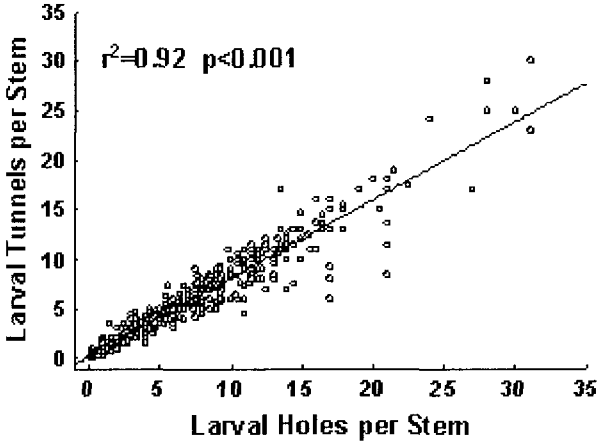


C) Russet Burbank potatoes, 2004 and 2005.

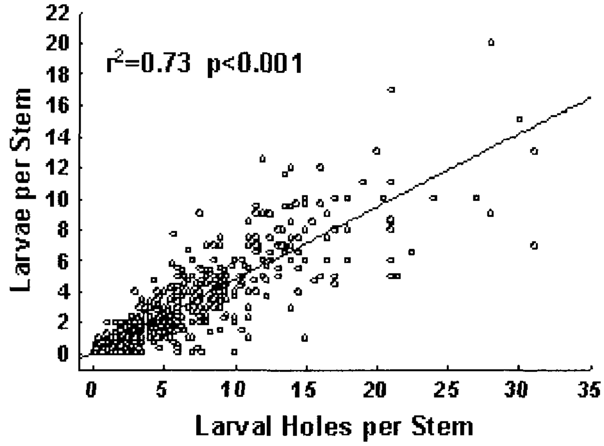


D) Russet Burbank potatoes, 2004 and 2005

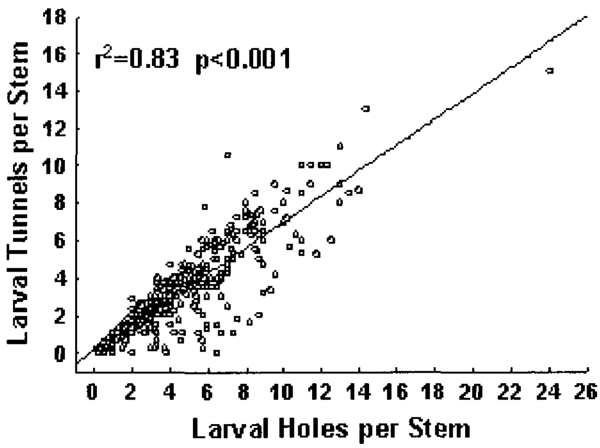
Figure 3.8: The relationship between ECB larval holes and both larval tunnels and larvae per plant for Shepody and Russet Burbank potatoes for 2004 and 2005 combined.



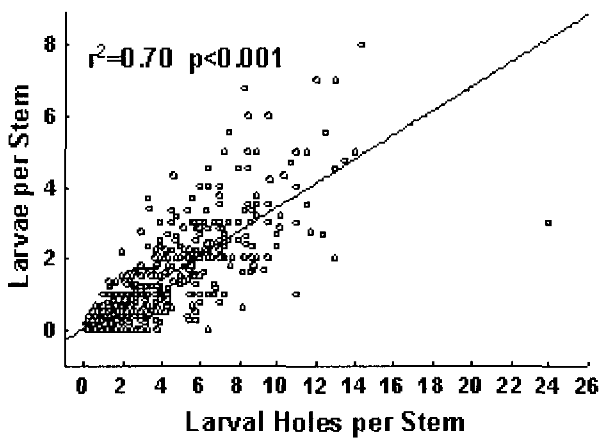
A) Shepody potatoes, 2004 and 2005



B) Shepody potatoes, 2004 and 2005.



C) Russet Burbank potatoes, 2004 and 2005._



D) Russet Burbank potatoes, 2004 and 2005

Figure 3.9: The relationship between ECB larval holes and both larval tunnels and larvae per stem for Shepody and Russet Burbank potatoes for 2004 and 2005 combined.

3.3 Discussion

The results of this study indicate that while the two potato varieties, Shepody and Russet Burbank, differed in some of their responses to different levels of ECB egg mass abundance, the apparent differences between years is probably an artifact of the experimental conditions. Despite attempts to restrict the access of the wild moths, the plots in the 2004 trials, even the plots with an expected trial level of 0 egg masses per 10 plants, had very high levels of ECB larval infestation. This indicates that either oviposition from wild moths occurred in these plots or that larvae later entered these plots after the covers were removed. The level of larval infestation for both varieties of potatoes in 2004 was similar to that in the open access treatment in 2005.

In 2005, the year that it could be assured that larval infestation numbers were based only on the treatment levels, larval infestation numbers did increase with increasing numbers of egg masses, but they did not differ among any of the experimental treatment levels (2.5 to 4 egg masses/ 10 plants). Part of the reason for the lack of difference in larval infestation numbers in the experimental treatment plots could be the differences in the numbers of eggs in individual egg masses. ECB egg masses can contain from 1 to 150 eggs (Hudon and LeRoux 1986b). Mid-range egg masses were selected for pinning, but it was impractical to ensure that the egg masses were exactly uniform in size. As well, the purpose of this study was to develop an control threshold which could be used by the crop scouts in the field where ECB egg masses are not uniform. Therefore, based on larval numbers alone, the control threshold for both potato varieties can be raised to at least 4 egg masses/10 plants.

In 2005 there was a large increase in larval numbers in the open access plots (Treatment 5) for both varieties. However, there was no corresponding decrease in tuber yields at the higher infestation levels for either variety and an apparent significant increase in potato tuber yields in the Russet Burbank variety, though this increase took place in the smaller classes of tubers. This is consistent with other studies (Nault et al., 2001; Nault and Kennedy, 1996; Stewart, 1992; Kennedy, 1983; Bray,1961) that found that ECB infestation did not always negatively affect potato tuber yields. Therefore, based on the tuber yields of the Shepody and Russet Burbank potatoes, the current control threshold can be increased without affecting potato yields. Since levels at or below 4 egg masses per 10 plants did not affect yield or infestation numbers, the control threshold for both potato varieties can be raised to 4 egg masses per10 plants. However, even though it is apparent that potatoes can accommodate higher numbers of ECB larvae without affecting tuber yields, other factors, such as a prolonged egg laying period, stem breakage, and/or disease, may affect the plant's ability to compensate for ECB larval damage. In that case producers may want to consider a lower control threshold.

The differences between the responses of the two potato varieties can be explained by the differences in between the two varieties. Shepody potatoes are a mid-season variety, maturing in 90-110 days (CFIA, 2005a), with thick upright stems which are more prone to being knocked over in the wind, particularly if previously damaged by ECB larvae. Because they are a mid-season variety, Shepody potatoes are more likely to be close to or reach maturity before the growing ECB larvae reach a size where they can cause severe damage. On the other hand, Russet Burbank potatoes are a late to very late maturing potato (CFIA, 2005b) with thinner vine-like stems which tend to lay on the ground. Because of this, wind damage is not as much of a problem.

Late season potato varieties, such as Russet Burbank, can withstand higher levels of defoliation, as high as 70% (Ziems et. al, 2006), without a subsequent reduction in tuber yields. They may even produce higher tuber yields. However, yields of larger sized tubers are more likely to be negatively affected by defoliation than yields of the smaller, less desirable, sized tubers (Ziems et. al, 2006; Cranshaw and Radcliffe, 1980). Increased tuber yields may be the result of increased branching due to the removal of the apical shoot as a result of ECB larval damage and the release of the apical dominance. It could also be a result of the removal of shaded foliage at the bottom of the plant, creating a more optimal leaf area for the plant (Cranshaw and Radcliffe, 1980).

It is also possible that the differences in the classes of tubers were caused by other factors than ECB damage. Although there were significant relationships between larval infestation numbers and the yields of tubers in certain size classes, the correlations were weak ($r^2 \leq 0.40$) (Table 3.4), accounting for less than half of the variation for the most part. A lot of factors go

Table 3.4: Correlations between the number of tubers in different tuber classes and the of the number of egg masses and larvae.

Russet Burbank Potatoes 2005		
Number of Tubers in Class	**Number of Egg Masses**	**Number of Larvae**
Culls	$r^2 = 0.40$ p=0.003	$r^2 = 0.31$ p=0.01
Canada #1 Small	$r^2 = 0.40$ p=0.003	$r^2 = 0.20$ p=0.045
Canada #1	$r^2 = 0.40$ p=0.003	$r^2 = 0.28$ p=0.02
Canada #1 Large	no correlation	no correlation
Total	$r^2 = 0.38$ p=0.003	$r^2 = 0.34$ p=0.008

into the overall development of potato tubers including the presence of soft rot, the phenological stage of the plant, the type of potato, the presence of other insects, temperature, water stress, and soil fertility (Kennedy 1983). ECB larval damage is only one factor among many. Given a good growing year, adequate nutrients, and adequate moisture, potatoes seem to be able to accommodate a certain amount of ECB damage. This is one reason why, historically, there has been no clear and consistent relationship between ECB damage and tuber production.

Current monitoring methods for ECB include monitoring adult males (to determine when the moths are flying) and using crop scouts to count egg masses on potato plants in the field ©. Noronha, Agriculture Agri-foods Canada, personal communication).The results of this study indicate that egg masses are not necessarily good indicators of larval infestation numbers, at least at low egg mass numbers, since no differences in larval infestation numbers were observed between 2.5 and 4 egg masses per plant. A better indicator may be larval holes on the stems of the potato plant, since these were strongly correlated to both the number of larvae and larval tunnels. In this study, 4 egg masses per 10 plants resulted in about 1 larva/plant for both varieties, which corresponds to 2-3 larval holes per plant for the Shepody variety and 3-4 larval holes for the Russet Burbank variety. The problem is that the larval holes are apparent only after ECB infestation has occurred. Using larval holes to monitor ECB infestations will be more useful to agricultural researchers who will be able to use larval holes to estimate the level of larval infestation or the level of crop damage (in the form of larval tunnels) without having to dissect potato stems, which is very labour intensive.

European corn borer is a poorly understood pest in potatoes on PEI. Overall, this research shows that the current control threshold of 2 egg masses per 10 plants could be raised to 4 egg masses per 10 plants without affecting potato tuber production. However, more work is needed to determine the actual infestation levels that cause consistent reduction in potato tuber yields on PEI, especially relative to other factors that affect tuber yield such as soil moisture, nutrients, wind conditions and disease. A new method is also proposed which would allow researchers to monitor ECB infestation levels in PEI potato fields using ECB holes in the stems and which could support the work to pin down an action threshold for ECB in potatoes.

CHAPTER 4

Conclusions

The infestation of potato plants by the European corn borer has become a persistent issue for the potato growers in the Maritimes. Part of the difficulty with this pest is that it is not clear what level of infestation consistently results in tuber yield reductions. As well, growers must try to assess the level of ECB infestation at the egg stage, before the actual infestation by the larvae, the plant damaging stage, has occurred. This is because control measures must be applied before the hatching ECB larvae enter the potato stems.

The most vulnerable stage of the European corn borer's life cycle in North America is the egg stage. *Trichogramma* wasps are one potential biological control option which targets the ECB egg stage. The initial small successes with *T. brassicae* in the test plots and in the lab suggests that there may be a place for *T. brassicae* in the control of ECB in potatoes. However, in order to develop *T. brassicae* as a control option, it is important to identify what might be interfering with the ability of *T. brassicae* females to find ECB egg masses in the field. One important part of this work would be to examine how *T. brassicae* females search for egg masses on potato plants to see if the potato plants are interfering with the female's ability to search and if so, how.

Potato plants seem to be resilient when it comes to ECB infestation. In this study the potato plants were able to withstand relatively high ECB infestation levels without suffering a reduction in tuber yields. Some of the potato plants, the Russet Burbanks, even produced an increase in the number of tubers in response to ECB infestation, though much of this increase was focussed in the smaller less desirable tuber classes.

When a significant relationship was demonstrated between potato tuber yield and ECB infestation levels in this study, the level of ECB infestation accounted for less than half of the variation in tuber yields. Given good growing conditions, with adequate moisture and nutrients, and with good disease and pest control, it would appear that potato plants can withstand a fairly large ECB infestation without an appreciable reduction in tuber yields. This study has shown that the control threshold can be raised to 4 ECB egg masses per 10 plants at a minimum and that the monitoring of larval holes can be used to monitor ECB infestations in potatoes. Because of the variable response of potato plants to ECB infestation, it would be important to repeat this study using an expanded set of test infestation levels to confirm these results and to push the known infestation levels higher to see if there is a point at which a consistent reduction in tuber yields occurs.

Appendix 1

In 2004, a separate trial investigating ECB biocontrol using *Trichogramma* wasps was carried out in a conventionally managed field of Russet Burbank potatoes at the Harrington Agricultural Research Farm (Field 345). Six 0.2ha plots were established in this field. When the *Trichogramma* trial was done and the potato tubers were mature, 16 potato tops were collected from each test plot and the stems were dissected to determine the number of ECB larval holes, larval tunnels and larvae within the stems. The remaining potato stems were killed by two applications of diquat. The potato tubers in the sample plots were harvested and graded mechanically into the following tuber classes of increasing tuber size: Cull, Canada #1 small, Canada #1, and Canada #1 large. There were no significant differences between test plots in the *Trichogramma* trial, but when the average number of larvae per plant was correlated with tuber class, it was found that as the number of larvae increased, the number and weight of Canada#1 large tubers increased (Table 5.1).

This relationship held true for the other Russet Burbank test plots grown in 2004 (Field 630, previously discussed in this thesis; Table 5.1). As well, in both Russet Burbank fields (Fields 345 and 630) in 2004 the Canada #1 small class of tubers showed the opposite pattern (Table 5.1). They decreased in number and weight as the average number of larvae per plant increased. In contrast, the Russet Burbank potatoes grown in 2005 (Field 630, previously discussed in this thesis) increased in number and weight as the average number of larvae per plant increased (Table 5.2).

Table A.1: Correlations between selected tuber classes and the average number of ECB larvae per plant in potatoes in 2004.

Field	Potato Variety	Tuber Class	Relationship with the Avg. No. of Larvae per Plant	Correlation
345	Russet Burbank	number of Canada #1 small tubers	negative	r^2=0.66 p=0.049
345	Russet Burbank	weight of Canada #1 small tubers	negative	r^2=0.66 p=0.051
345	Russet Burbank	number of Canada #1 large tubers	positive	r^2=0.88 p=0.006
345	Russet Burbank	weight of Canada #1 large tubers	positive	r^2=0.87 p=0.006
630	Russet Burbank	number of Canada #1 small tubers	negative	r^2=0.27 p=0.039
630	Russet Burbank	weight of Canada #1 small tubers	negative	r^2=0.29 p=0.032
630	Russet Burbank	number of Canada #1 large tubers	positive	r_s^2=0.41 p=0.008
630	Russet Burbank	weight of Canada #1 large tubers	positive	r_s^2=0.36 p=0.015

Table A.2: Correlations between selected tuber classes and the average number of larvae per plant in Russet Burbank potatoes, 2005.

Field	Potato Variety	Tuber Classes	Relationship with the Avg. No. of Larvae per Plant	Correlation
630	Russet Burbank	total number tubers	positive	r^2=0.38 p=0.004
630	Russet Burbank	total weight of tubers	positive	r^2=0.43 p=0.002
630	Russet Burbank	number of Canada #1 tubers	positive	r^2=0.35 p=0.006
630	Russet Burbank	weight of cull tubers	positive	r^2=0.40 p=0.002
630	Russet Burbank	weight of Canada #1 small tubers	positive	r^2=0.31 p=0.010
630	Russet Burbank	weight of Canada #1 of tubers	positive	r^2=0.29 p=0.014

In a study investigating the effect of artificial defoliation on potato tuber yields Cranshaw and Radcliffe (1980) found that there were long periods of the growing season during which potato plants can almost completely compensate for defoliation, particularly the late season potato varieties such as Russet Burbank. Definite yield reductions were only evident when defoliation took place during the middle of the growing season when the potato tubers were initiated and rapidly bulking up. When the defoliation occurred early or later in the growing season, later maturing cultivars, such as Russet Burbank, were able to compensate for that injury and at times actually produce increased tuber yields. Reductions in tuber yields were also more likely to occur when defoliation was not uniform, but was scattered over the potato plant (Cranshaw and Radcliffe, 1980). On PEI the European corn borer larvae do not reach a large enough size to cause serious damage to the stem of the potato plant until August, near the end of the growing season. As well, their feeding patterns tend to result in the uniform removal of foliage, stem by stem. As a result, it is possible that the Russet Burbank potato plants would be able to compensate for the ECB damage and may, as Cranshaw and Radcliffe (1980) showed, even produce an increase in yield.

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