THE EFFECT OF FULFILL® (PYMETROZINE) ON GREEN PEACH APHIDS (*MYZUS PERSICAE* SULZER) IN COMPARISON TO CURRENT APHICIDES, AND THE ROLE OF ADJUVANTS FOR USE WITH THIS APHICIDE

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

Master of Science

in the Department of Biology

Faculty of Science

University of Prince Edward Island

Sarah Elizabeth Stewart

Charlottetown, P. E. I.

October, 2002

SIGNATURE PAGES

iii-iv

REMOVED

ABSTRACT

Fulfill® 50 WG is a new highly selective aphicide that contains the active ingredient pymetrozine. Pymetrozine has a unique mode of action. Instead of acting as a general toxin, this aphicide inhibits feeding in aphids which consequently leads to starvation. This aphicide is currently being considered for registration in Canada. The main objectives of this study were: 1) to verify that aphids cease feeding after exposure to Fulfill®, 2) to determine, using scanning electron microscopy, whether Fulfill® causes any morphological changes to the aphid proboscis, 3) to compare the efficacy of Fulfill® with the currently used insecticides, Monitor® and Pirimor® under PEI conditions and 4) to determine which adjuvant, if any, increases the efficacy of Fulfill®.

All pesticide applications occurred in the field with a tractor mounted sprayer. In both the efficacy comparison and adjuvant field studies, plots were replicated four times and three gauze bags with 30 green peach aphids/bag were placed onto plants in each plot prior to spraying. In the lab studies, aphids were placed onto leaves that were sprayed in the field, then were brought to the lab and placed in vials of water. In all studies, aphid survival was monitored daily to determine aphicide efficacy. Videos of aphid feeding behaviour after exposure to Fulfill® were also recorded in lab conditions.

This research was conducted during 2001 and 2002. In 2001, potato plants were severely water stressed due to drought conditions. Leaves were wilted and plant growth was greatly reduced. In contrast, plants received ample rainfall in 2002 and were healthy. The activity of Fulfill® under these two conditions was very different. During 2001, Fulfill® failed to provide aphid control that was equivalent to Monitor® or

Pirimor®. However, Fulfill® provided excellent aphid control in 2002, possibly due to altered leaf physiology with abundant water. In 2001, when leaves that were water stressed in the field were removed and brought to the lab where they were given ample water, Fulfill® offered excellent aphid control. This study found that drought conditions limit the activity of Fulfill®.

Although the Fulfill® label recommends using a penetrating adjuvant during application, this study found no evidence to corroborate this claim. Citowett Plus® , LI 700® and Superior 70 Oil® were all used as adjuvants during this study. These adjuvants did not increase the efficacy of Fulfill® under typical PEI conditions, and only Superior 70 Oil® slightly increased the efficacy of Fulfill® during drought conditions.

When aphids in the lab were exposed to Fulfill® by feeding on treated plants, they did not resume feeding after their initial exposure to this pesticide. Some aphids were observed attempting to feed by pressing their proboscis against the leaf; however, stylets never penetrated the leaf surface. Between 1-5 days following exposure to Fulfill®, aphids starved to death. No aphids were observed giving birth after 24 hours following exposure to Fulfill®. The SEM component of this study found no evidence that the visible external morphology of the proboscis of aphids is altered after exposure to Fulfill®.

ACKNOWLEDGMENTS

I would like to sincerely thank my co-supervisors, Dr. Christian Lacroix and Dr. Robert Coffin, for their guidance and endless support during the duration of this project. They both made time for me in their busy schedules whenever I needed them and this was greatly appreciated. Thank you as well to my committee member Dr. Donna Giberson for statistical advice and helpful comments on the manuscript. I would like to offer my thanks to the staff of the Research Division at Cavendish Farms (Michelle Burns, Wendy Burns, Norah Dyment, Kelley Peters) for their help with many aspects of the project. Thank you to Joni Kemp, William MacDonald, and Fraser Clark for help with fieldwork. They all know the joys of squashing potato beetles in the hot sun! Thank you to Dr. Gilles Boiteau for supplying an aphid culture and to Dr. James Kemp for supplying material for aphid bags. I would also like to thank Dr. James Kemp, Dr. Rick Peters and Dr. Nola Etkin for serving on my examination committee. Thank you as well to Harold Wright, Tara McCaughey and David Thompson of Syngenta Crop Protection for their advice and helpful comments during this project. Finally, thank you to NSERC, Cavendish Farms, Syngenta, the UPEI biology club, and the Harvey Moore Wildlife Fund for funding for this project.

TABLE OF CONTENTS

LIST OF TABLES

---

* Refers to a reference from a company monograph, government website or university website.

LIST OF FIGURES

Chapter 1: Introduction and Literature Review

1.0 INTRODUCTION

As integrated pest management (IPM) programs become standard practice in agriculture, new highly selective pesticides that have minimal impact on non-target organisms are needed to replace broad action toxic formulas (National Research Council 2000). This is particularly important in Prince Edward Island (PEI) where such a high proportion of the province's land is used in potato production. There is a large risk to the Island environment when large quantities of highly toxic pesticides are used on the over 4050 hectares of potatoes planted each year (Statistics Canada 2000).

Some of the major pests of potatoes are aphids, such as the green peach aphid, *Myzus persicae* (Sulzer) (Insecta, Homoptera), and the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Insecta, Homoptera) (Dixon 1973; Šutić et al. 1999). Aphids are major virus vectors, spreading viruses such as potato leafroll virus (PLRV) and potato virus Y (PVY) among potato plants (Parry 1990). Currently on PEI, aphicides such as Monitor® and Pirimor® are used to control aphid populations. However, these aphicides are very toxic (*Zeneca 1997; *EXTOXNET 2001) and are unsuitable for use in integrated pest management programs. There is also evidence that aphids are developing resistance to current chemicals rendering these chemicals ineffective for aphid control (Parry 1990; Bohmont 2000).

The Prince Edward Island potato industry requires new, selective aphicides, with

---

*Refers to a reference from a company monograph, government website or university website.

low environmental impact, to control aphid populations and to limit virus spread. Fulfill® 50 WG is one such pesticide that is currently being considered for registration in Canada. Fulfill® is currently registered for use in the United States and in Europe (Harold Wright, Technical Crop Manager, Syngenta Crop Protection Canada, 140 Research lane, Guelph Ontario, N1G 4Z3, personal communication). Instead of acting as a general toxin, Fulfill® inhibits the cibarial valves, food pump and salivary pump of aphids, which results in starvation (Harrewijn and Kayser 1997; *Novartis Crop Protection 1998). Although aphids take up to several days to die of starvation, they do not feed, and therefore do not spread potato viruses (Flückiger et al.1992; Harrewijn and Kayser 1997; *Glogoza 2000). However, doubts about the consistency of this mode of action were raised by an American entomologist at the 2001 Northeast Potato Technology Forum in Charlottetown (Sarah Stewart, personal observation). There is also uncertainty as to whether Fulfill® causes morphological changes to aphid mouthparts (Harold Wright, personal communication). Although the American Fulfill® label contains a recommendation to use an adjuvant with Fulfill®, there is no recommendation as to which adjuvant products maximize the efficacy of Fulfill® (*Novartis Crop Protection 1999a). Adjuvants are commonly added to pesticides to increase the efficacy of chemical products (Tadros 1984; Van Emden 1989). However, using the wrong adjuvant can limit pesticide efficacy and even damage foliage (*Hock 1994; Bohmont 2000). The low impact of Fulfill® on beneficial organisms makes it a suitable pesticide for IPM programs (Flückiger et al.1992; Follas and Blanc 1995).

The objectives of this project are 1) to verify that aphids cease feeding after

exposure to Fulfill®, 2) to determine whether Fulfill® causes any visible morphological changes to the mouthparts of aphids, 3) to determine the efficacy of Fulfill® in controlling *Myzus persicae* under PEI field and laboratory conditions in comparison to the current insecticides Pirimor® and Monitor® and 4) to determine which adjuvant, if any, increases the efficacy of Fulfill®.

1.1 LITERATURE REVIEW

1.1.1 Aphids

Aphids are hemimetabolous insects that are classified in the Division Neoptera, Subdivision Paraneoptera, Order Homoptera, and Family Aphididae (Poule and Gentilli 1997). Aphids feed almost exclusively on plant phloem sap (Dixon 1973; Dixon 1998) making those that feed on agricultural plants the most significant group of plant virus vectors in agriculture (Dixon 1973; Eastop 1983; Jones 1987). There are over 4000 species of aphids worldwide (Dixon 1998). Aphids exhibit polymorphism in their life cycles with both alate (winged) and apterous (wingless) forms present within the same species (Dixon 1973; Blackman 1974; Peters 1987; Dixon 1998). Parthenogenesis and telescoping of generations are also significant characteristics of the aphid life cycle (Dixon 1973; Blackman 1974; Dixon 1998).

1.1.1.1 Diagnostic Morphological Characteristics

All aphids have their mandibles and maxillae modified as piercing stylets which facilitate phloem feeding (Dixon 1973; Blackman 1974). The two pairs of stylets work closely together and are held within a dorsal groove in the elongated labium called the proboscis (Figure 1.0) (Dixon 1973; Matthews 1981; Blackman 1974). At the labial tip the groove becomes a tube which directs stylet movement. The maxillary stylets run side by side in the groove forming two narrow canals: a central canal for sap ingestion and a smaller canal for saliva secretion from the aphid into plant tissues. The mandibular stylets surround the maxillary stylets, function as support structures for the

maxillary stylets, and aid in the penetration of plant tissues (Blackman 1974). Mandibular stylets are approximately 0.04 μm in diameter and their small size facilitates penetration of plant tissues (Dixon 1973). Mechanoreceptors are located along the length of the mandibular stylets (Matthews 1981). When inactive, the stylets remain inside the labial groove on the labium which extends posteriorly underneath the thorax (Blackman 1974).

The compound eyes of alate aphids are larger and have more ommatidia (lenses) than the compound eyes of apterous aphids. An ocular tubercle, consisting of three additional lenses, is located behind each compound eye in both morphs. In addition, alate aphids also have three ocelli (simple eyes) (Blackman 1974).

Aphids have three pairs of legs, the hind legs being the longest of the three (Figure 1.1). Tarsi (the last leg sections) are two segmented and end in claws which facilitate movement on plants (Blackman 1974).

Alate aphids have two pairs of wings and the hind wings are smaller than the front wings (Figure 1.1). The hind wings have hooks which latch into a groove in the front wings and enable the wings to work together during flight. Aphid wings have one longitudinal main vein and there is a stigma at the base of the front wing veins (Figure 1.1) (Blackman 1974). The aphid body bears spiracles, siphunculi, and cauda. Each of the first seven abdominal segments has a pair of breathing spiracles and the thorax contains two pairs of spiracles. The prothorax and abdomen contain lateral tubercles which are also found dorsally on the head, thorax and abdomen. Siphunculi and cornicles are also located dorsally on the abdomen, usually on the fifth

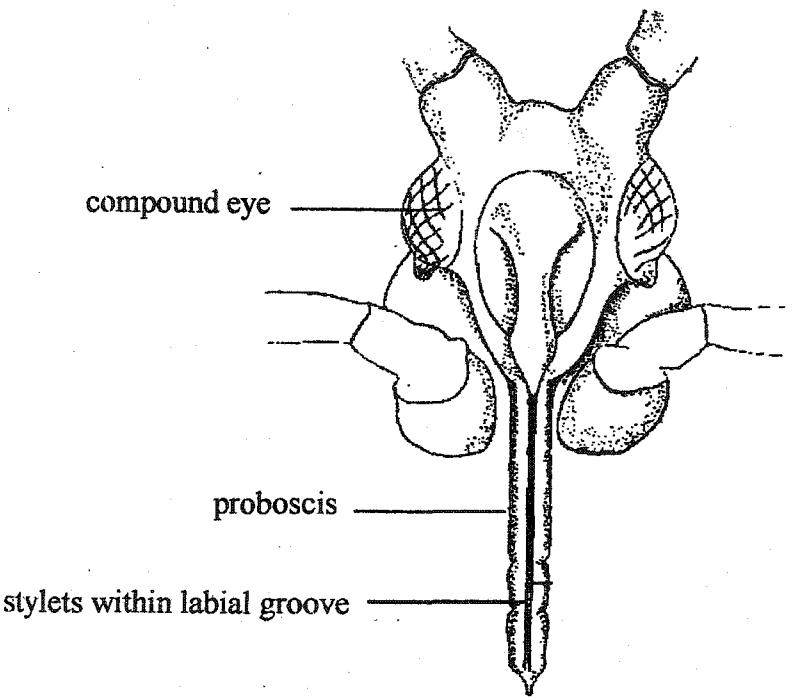


Figure 1.0. External anterior view of aphid head (Modified from Blackman 1974).

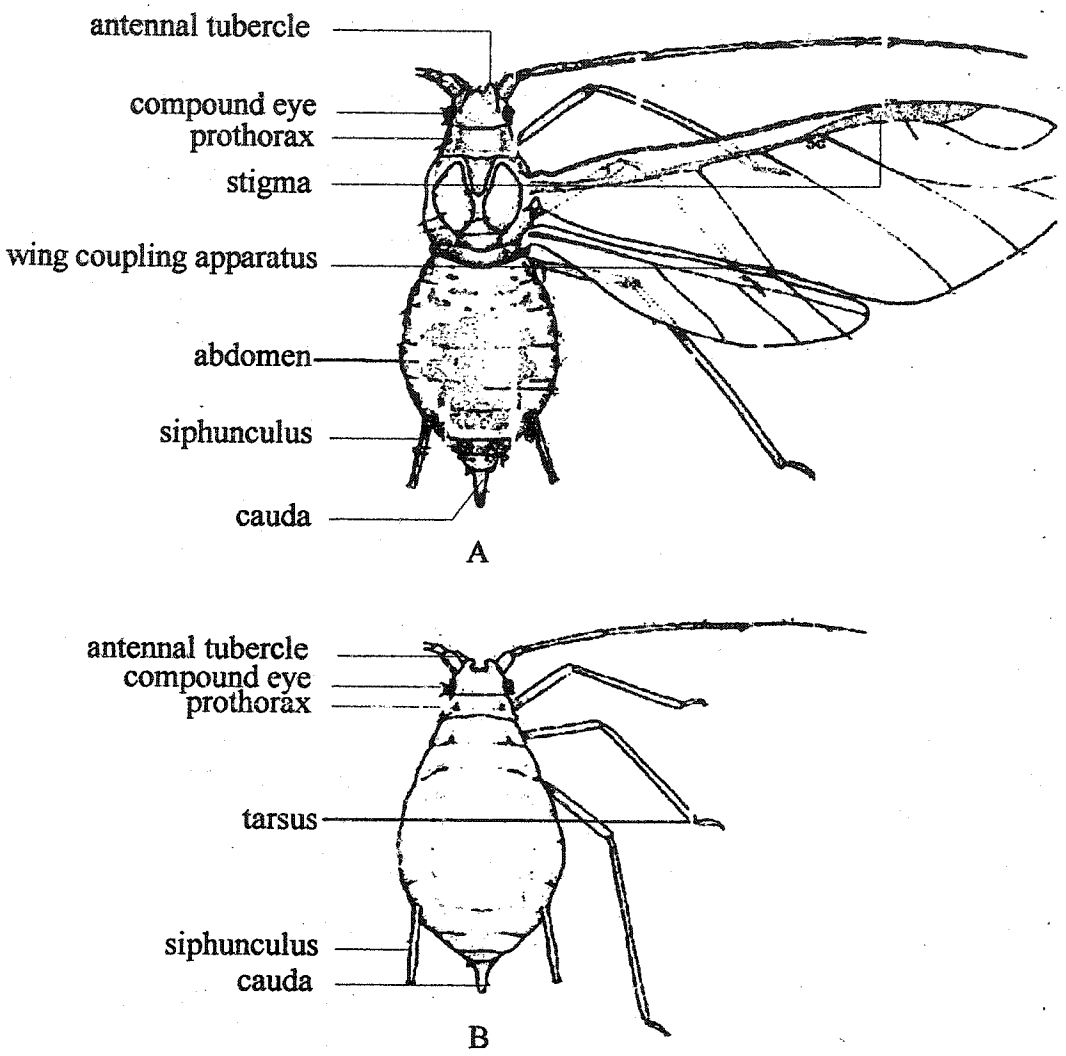


Figure 1.1. External features of an aphid. A: Dorsal view of alate female. B: Dorsal view of apterous female. (Modified from Blackman 1974)

or sixth abdominal segments (Figure 1.1). The shape and length of siphunculi are features which distinguish different species. Aphids have a modified ninth abdominal tergite called the cauda which projects outward above the anus (Figure 1.1). The shape and size of the cauda also helps to distinguish between different aphid species as well as between adult aphids and nymphs (Blackman 1974).

1.1.1.2 Life Cycle

Aphids can reproduce sexually or parthenogenetically, depending on the species and the time of year, and may give birth to live young or to eggs. There are three basic life cycles that aphids can follow: the holocycle, anholocycle or androcycle (Dixon 1973; Blackman 1974; Peters 1987; Dixon 1998). The cycles differ in the presence or absence of a sexual stage. For example, in the holocycle, aphids depend on sexually produced eggs to overwinter, whereas in the anholocycle no sexual phase occurs and aphids overwinter in sheltered conditions. During the androcycle, aphids overwinter both as eggs produced sexually and as aphids produced parthenogenetically in sheltered conditions (Dixon 1973; Blackman 1974; Peters 1987; Dixon 1998). All three life cycles are very dependant on parthenogenesis, the production of an individual from a single unfertilized ovum (Blackman 1974; Dixon 1998). Aphids are also viviparous, which means that they give birth to live young (Dixon 1973; Blackman 1974).

Aphids undergo thelytokous parthenogenesis, which means that only diploid female individuals are produced during parthenogenesis (Blackman 1974). Female aphids have one pair of sex chromosomes (XX), whereas male aphids have only one sex

chromosome (XO). When a male aphid is produced, the two X chromosomes form a bivalent chromosome which then separates, with one half entering the polar body and the other remaining in the oocyte. During the first meiotic division, the X chromosome in the oocyte remains in the middle of the spindle so that during the second meiotic division, one sperm cell contains the X chromosome while the other contains no sex chromosome and subsequently degenerates (Figure 1.2) (Dixon 1973; Blackman 1974; Dixon 1998).

The holocycle includes an egg stage which allows the aphid to overwinter in harsh conditions. In the spring, a female apterous aphid, called the fundatrix, hatches from the egg. The fundatrix produces other female aphids parthenogenetically, and these offspring, called fundatrigeniae, are also apterous. The fundatrigeniae, responding to feeding and environmental cues, produce a mixture of alate and apterous female aphids parthenogenetically. More alate aphids are produced with each subsequent generation. The last generation of aphids living on plants of the primary host species (fall/winter host species) are all alate and become the migrants, who leave primary host plants in search of plants of the secondary host species (spring/summer host plants). The migrants colonize secondary hosts and then produce apterous aphids parthenogenetically on the new host. These apterous aphids then also produce other female aphids parthenogenetically with more alates produced in each generation. This ensures that alate aphids are present when conditions on the new host plant deteriorate and the aphids must move to a new plant. Once autumn arrives, declining day length triggers the production of male aphids and a group of female aphids called the gynoparae which

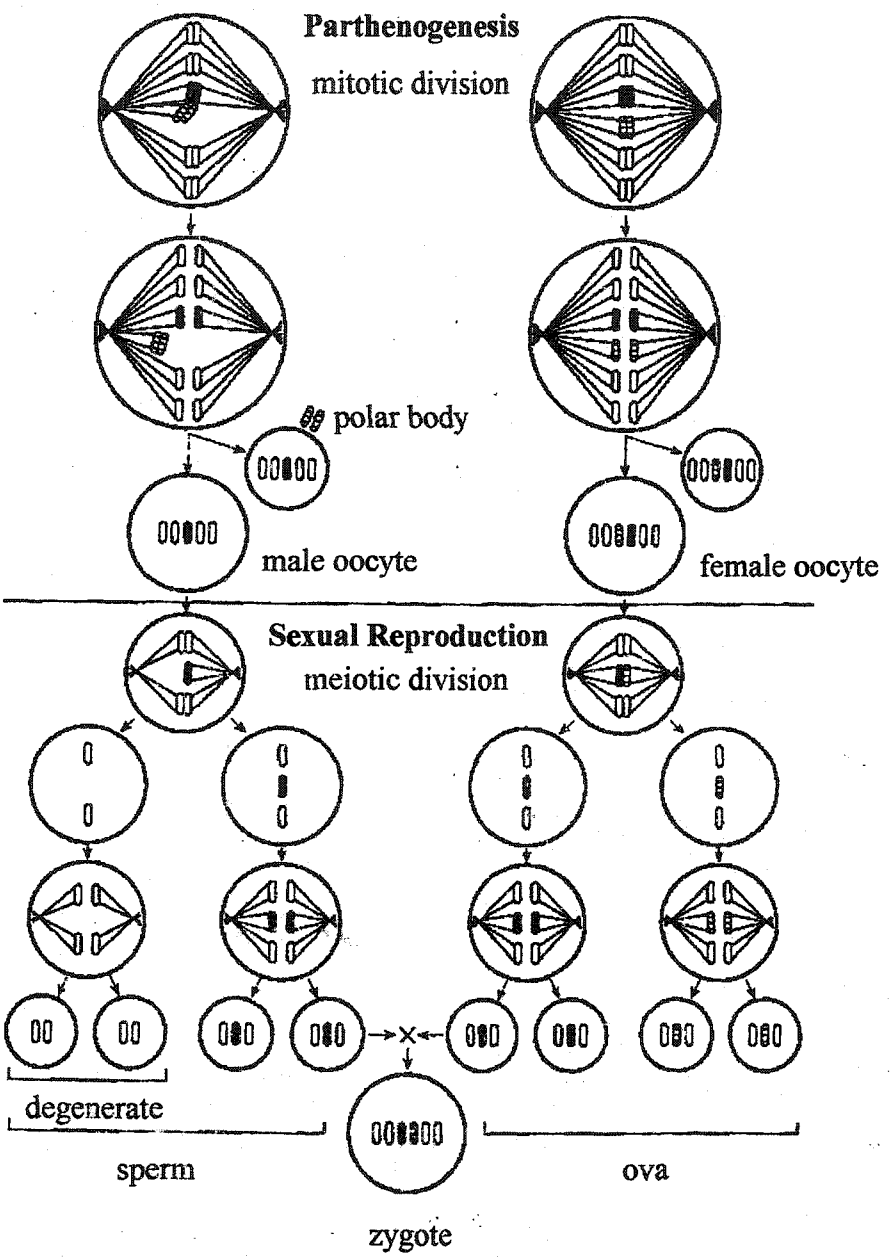


Figure 1.2. Aphid chromosomes during parthenogenesis and sexual reproduction, showing the chromosomes during mitotic divisions that lead to the development of male (XO) and female (XX) oocytes and during meiotic divisions in male and female aphids that lead to the development of sperm and ova from germ cells carrying an X chromosome (Black chromosome represent X chromosomes) (Modified from Blackman 1974).

return to the primary host. The gynoparae produce the female aphids parthenogenetically that will mate with the males. These female aphids are called oviparae and are the only sexual females in the aphid life cycle. All other female aphids are referred to as virginoparae. The eggs produced by the oviparae remain on the primary host until conditions in the spring induce hatching (Dixon 1973; Blackman 1974; Peters 1987; Dixon 1998). Each ovipara produces approximately four to six eggs an hour after mating. Males mate with several females during their short life span (Blackman 1974). Some aphids feed on only one host species and are termed autoecious or monophagous. Aphid species that fluctuate between different spring and autumn host species are called heteroecious or polyphagous (Dixon 1973; Blackman 1974).

The two other possible life cycles for aphids to follow are the anholocycle and the androcycle. Aphids following the anholocycle do not undergo any sexual reproduction but are solely parthenogenetic. Anholocyclic aphids overwinter in sheltered conditions and are most common in tropical regions. In aphids undergoing the androcycle, both holocyclic and anholocyclic life cycles are possible. Depending on environmental conditions such as relative humidity, day length and host plant condition, aphids will either follow the sexual life cycle or the parthenogenetic life cycle during the winter period (Dixon 1973; Peters 1987).

Parthenogenesis has many benefits for aphids. For example, ova do not have to be fertilized so embryos are able to develop in parthenogenetic aphids before they reach maturity. This greatly speeds up the reproductive process (Dixon 1973; Blackman 1974; Dixon 1998). By giving birth to live young, aphids also decrease the time- and energy-

consuming egg stage. Aphids also save time and energy by reproducing without the costs associated with mate selection and mating. These characteristics allow for a very short generation time which enables aphids to reproduce very quickly and on a large scale (Blackman 1974). Aphids reach maturity between one to two weeks after birth (Blackman 1974; Dixon 1998; *Radcliffe 1998a) and the life cycle includes four instars (Dixon 1973; Blackman 1974; Dixon 1998). Aphid nymphs are morphologically different from adults. They have shorter antennae, shorter legs, and shorter siphunculi (Blackman 1974). A single female can produce up to 30 nymphs per generation and can have nine generations per season (Dixon 1973). High fecundity along with the short generation time give aphids the ability to reach high population levels in a short period of time.

Since aphid populations are so large and aphids reproduce so quickly, rare mutations occur on a regular basis (Dixon 1998). Due to the large reproductive capacity of each individual aphid, mutations pass quickly through populations (Dixon 1998). Mutation, mitotic recombination and variability in genetic expression are all factors which contribute to variations in aphids produced through parthenogenesis (Blackman 1974). The sexual reproduction that occurs on an annual basis further generates variability within each species (Dixon 1998).

There are many environmental cues that determine whether apterous or alate aphids are produced. Overcrowding, impaired host plant health and increased host plant age are all factors which induce the production of alate individuals. This gives aphids the capability to fly away to new host plants when conditions deteriorate on the existing

host plant. Short days and low temperatures also induce the production of alate aphids so that in autumn alate aphids are present to fly back to primary hosts and produce gynoparae and male aphids for sexual reproduction (Dixon 1973; Blackman 1974; Peters 1987; Dixon 1998).

1.1.1.3 Feeding

Aphids feed on plant phloem by inserting their stylets into plant tissue (Dixon 1973; Blackman 1974; Peters 1987; Dixon 1998). Most aphids are host specific and feed only on certain species or families of plants (Van Emden 1989). Aphids prefer to feed on the underside of leaves and are most commonly found along leaf veins. Before feeding, aphids probe the leaf surface gathering chemical and physiological information about the plant (Blackman 1974; Matthews 1981; Dixon 1998). Sensilla on both the proboscis and the antennae are involved in this process (Dixon 1998). It is believed that secondary plant substances serve as indicators for aphids in the recognition of suitable host plants for feeding (Dixon 1973). Aphids may spend less than 60 seconds probing the leaf surface before feeding (Dixon 1973; Matthews 1981; Dixon 1998).

Once an aphid has ceased probing the leaf surface and is ready to commence feeding, it inserts its stylets down through the plant tissues, passing between cells until the phloem is reached. This process may take anywhere from minutes to days. Both the mandibular and maxillary stylets work together as one needle-like structure. The aphid secretes saliva through the salivary canal in the maxillary stylets which gels and forms a sheath around the penetrated area and the stylets. This sheath gives the stylets increased

support in the plant tissue (Dixon 1973; Matthews 1981; Dixon 1998). The saliva also contains the enzyme pectinase, which helps to break bonds between cell walls facilitating the penetration of the stylets (Dixon 1973). Once the phloem is reached, the maxillary stylets penetrate the sieve tube (Matthews 1981).

Aphids feed continuously (Dixon 1998), even while giving birth (Blackman 1974). Continuous sucking action by the aphid would require huge expenditures of energy, but most of the sap enters aphid stylets passively without any sucking action from the aphid (Dixon 1973; Blackman 1974; Dixon 1998). Plant phloem sap is typically under 15-30 atmospheres of pressure. This is enough pressure to force sap into the narrow aphid stylet without any pulling action from the aphid. Dilator muscles in the aphid's pharynx control a piston valve, which regulates the flow of sap entering the aphid. If the pressure the plant sap is under becomes lower than the pressure in the aphid's alimentary canal, the dilator muscles in the aphid's cibarial pump contract, expanding the diameter of the pump lumen. This larger diameter lowers the pressure within the pump, which causes the esophageal valve to close and the esophagus to collapse, preventing regurgitation from the midgut (Dixon 1998). By closing the piston valve and relaxing the dilator muscles of the cibarial pump, liquid can flow from the oesophagus into the stomach. If the piston valve remains open, liquids can be forced back out through the stylets (Dixon 1998). Aphids can also lower the concentration of sugars in their stomach by diluting phloem sap with liquid from the hindgut. The large aphid stomach can therefore be used to dilute phloem sap instead of storing food (Dixon 1998). If necessary, aphids can actively suck phloem sap from plants by using their

cibarial food pump. This is particularly important when feeding on wilted plants with a low sap pressure (Dixon 1973; Blackman 1974).

Not all plant tissues are palatable for aphid feeding so aphids use colour, among other cues, to discriminate between different tissues. Aphids respond to the yellow tones in young or senescent tissues, which are often yellower and are more nutritious than mature tissues (Dixon 1973). Pubescent tissues are not desirable for aphid feeding as the hairs interfere with aphid probing. Growing stems and leaves are most often the organs of choice for aphids (Dixon 1998).

Phloem sap is high in sugar and water but contains only low levels of the amino acids that are vital for aphid growth and development (Dixon 1973; Dixon 1998). Sucrose represents 10-25% of the total sap concentration (Blackman 1974). To derive enough amino acids from phloem sap, aphids must consume extremely large quantities of sap (Dixon 1973; Dixon 1998). Excess nutrients are excreted by aphids in the form of honeydew (Dixon 1973). The sugars excreted by aphids are not in the disaccharide form of sucrose, but are rather trisaccharides such as melezitose and glucosucrose (Blackman 1974). Aphids have an invertase enzyme in their gut that catalyzes the conversion of sucrose to trisaccharides. Trisaccharides are molecularly larger than disaccharides and are not absorbed in the gut as easily as disaccharides (Blackman 1974). This conversion allows excess sucrose to be excreted from the body.

Aphids do not have malpighian tubules (tubules involved in the excretion of metabolic waste in most insects) but they secrete excess nitrogen waste in the honeydew. The large quantities of water that aphids continuously consume is also

thought to dilute the internal concentrations of ammonia to tolerable levels. This high water intake also helps to continuously flush ammonia out of the aphid gut (Dixon 1973). Aphids also contain symbionts in their gut that recycle excess nitrogen (Dixon 1973; Dixon 1998).

The most common aphid symbiont is a bacterium called *Buchnera aphidicola* (Munson et al. 1991). These bacterial symbionts supplement the diet of aphids by synthesizing vitamins, and amino acids (Blackman 1974). Symbionts are most numerous in aphids prior to maturity. Once an aphid reaches maturity, the number of symbionts in its gut is greatly reduced. This may indicate that symbionts are responsible for the production of certain amino acids which are particularly important during development (Dixon 1998).

1.1.1.4 Virus transmission

Aphids are the vectors responsible for the transmission of many agriculturally important viruses (Dixon 1973; Blackman 1974; Eastop 1983; Peters 1987; Parry 1990; Bernard et al. 1993; Šutić et al. 1999). The constant probing and feeding exhibited by aphids make them efficient vectors in the spread of viruses from plant to plant. Aphids transmit two types of viruses: persistent, also known as circulative, and non-persistent also known as stylet borne (Blackman 1974; Matthews 1981; Eastop 1983; Parry 1990). There are more non-persistently spread plant viruses than persistently spread ones (Blackman 1974).

Non-persistent viruses such as PVY can be acquired by aphids during the brief

probing period that occurs prior to feeding (Dixon 1973; Blackman 1974; Matthews 1981; Eastop1983; Parry 1990). Aphids carry the virus particles on the tip of their stylets (Dixon 1973; Blackman 1974). After acquiring a non-persistent virus, aphids can immediately transmit it to other plants (Dixon 1973; Matthews 1981; Parry 1990). However, the ability of the aphid to transmit the virus is lost after a few hours (Dixon 1973; Matthews 1981; Parry 1990). If an aphid moults after becoming infected, it loses the ability to transmit the virus because the virus is shed with the exoskeleton of the stylets (Dixon 1973; Blackman 1974; Matthews 1981). Another factor that contributes to the non-persistent nature of these viruses is that aphids lose some of the viral particles each time they probe into plant tissue (Matthews 1981).

Persistent viruses can only be acquired by aphids during extended periods of feeding on sieve elements of phloem tissue in plants (Dixon 1973; Blackman 1974). Therefore, the transmission of persistent viruses, such as PLRV, takes a few hours (Dixon 1973; Blackman 1974). Once an aphid has acquired a persistent virus, it is not able to transmit the virus to other plants until the virus has passed through the aphid gut into the haemolymph where it can then be secreted in the aphid's saliva (Dixon 1973; Matthews 1981; Parry 1990). This latent period can last up to several hours (Dixon 1973; Blackman 1974; Matthews 1981; Parry 1990). However, once an aphid has acquired a persistent virus it is able to transmit the virus for the rest of its life (Dixon 1973; Blackman 1974; Parry 1990).

Different species of aphids are able to transmit different types of both persistent and non-persistent viruses (Blackman 1974; Matthews 1981). In fact, an aphid can carry

more than one type of virus at a time (Dixon 1973). Aphids can also vary in the level of efficiency with which they transmit plant viruses (Blackman 1974; Matthews 1981). This variation in the efficiency of transmission can depend on the fact that some aphid species move around and probe more than other species, or it may be attributed to different structural components of the mouthparts in different species (Blackman 1974; Matthews 1981). This variation in efficiency in virus transmission may also be related to the viral strain (Matthews 1981).

Both PVY and PLRV severely reduce the yield of infected potato plants (*Diamond et al. 1996; *Diamond and Sturz 1997; Šutić et al. 1999). In some cases, yields can be reduced by up to 80% (*Diamond et al. 1996). Symptoms of PLRV include pale and leathery leaves, an upwards rolling of leaves and net necrosis in tubers (*Diamond and Sturz 1997; Šutić et al.1999). Symptoms of PVY include stunted plants and necrotic, brittle leaves (*Bernard et al. 1993). PLRV can have serious consequences for processing growers as net necrosis in tubers make potatoes undesirable for processing. Both PLRV and PVY have serious implications for seed growers as there is a limited level of virus allowable in tubers grown for the seed industry.

1.1.1.5 Predators

Aphids have many natural predators that help to control their population levels. These predators include insects, fungi, and parasites. However aphids are not completely defenseless against their predators. Aphids can kick smaller predators when provoked, or can promptly leave the plant when approached by a predator. Some aphids

also guard against predators by producing poisonous substances within their body. Most aphids feed facing the petiole on leaves or facing down on the stem so that they can see predators as quickly as possible. The most effective avoidance behaviour used by aphids is their continuous movement from plant to plant. This movement allows aphids to avoid many of their predators (Dixon 1973).

Ladybird beetles, such as *Adalia bipunctata* Linnaeus (Insecta, Coleoptera), are common insect predators of aphids (Dixon 1973; Blackman 1974; Dixon 1998). One ladybird beetle can eat up to 100 aphids per day (Blackman 1974). Lacewings, such as *Chrysopa carnea* Stephens (Insecta, Neuroptera), are also common predators of most aphid species, as are hoverfly larvae, including *Syrphus vitripennis* Meigen (Insecta, Diptera) (Dixon 1973; Blackman 1974; Dixon 1998).

Parasitoid wasps (Insecta, Hymenoptera) also prey on aphids. The wasps that commonly parasitize aphids belong to the families Aphidiidae and Aphelinidae (Blackman 1974) and include *Aphelinus flavus, Dyscritulus planiceps, Trioxys cirsii* and *Monoctorus pseudoplatani* (Insecta, Hymenoptera) (Dixon 1973; Dixon 1998). These wasps often pierce the skin of aphids and deposit an egg within the aphid's body. Once the egg hatches, the larva consumes the aphid from the inside and spins a cocoon around itself and the aphid. The larva emerges from the cocoon when it is ready to pupate (Blackman 1974).

Fungi, such as *Entomophthora aphidis*, are also natural enemies of aphids. These fungi flourish and spread quickly throughout crops under warm and humid weather conditions. Aphids infected by fungi often appear brown and fluid-filled before dying

(Hall 1974; Blackman 1974).

1.1.1.6 *Myzus persicae*

1.1.1.6.1 Diagnostic Morphological Characteristics: The green peach aphid, *Myzus persicae*, is 1.2-2.5 mm in length at maturity and has an egg-shaped body (Bernard et al. 1993). The cornicles are swollen at their tips and the antennal tubercles are prominent and extend inwards. Apterous green peach aphids are a pale translucent green colour while alate aphids have a dark brown to black head and thorax and a dark patch on the dorsal side of their abdomen (Bernard et al. 1993).

1.1.1.6.2 Life Cycle: Green peach aphids are heteroecious (Blackman 1974; Dixon 1998) and alternate between peach trees, *Prunus persica,* or other *Prunus* sp. during the fall/winter, and potato, *Solanum tuberosum,* in the spring/summer (Eastop 1983; Bernard et al. 1993). Other vegetable crops in the Compositae, Chenopodiaceae, Cruciferae and Leguminosae families can be secondary hosts for this aphid species but the potato plant is the favored summer host. Green peach aphids have a worldwide distribution and the climate of each region has a large impact on the life cycle followed by aphids (Bernard et al. 1993). All three types of aphid life cycles (holocycle, anholocycle, androcycle) are possible in this species. In some regions all three life cycles occur simultaneously. In regions with cold winters, the holocycle occurs almost exclusively (Peters 1987).

In eastern Canada, the green peach aphid is found in small numbers compared to other aphid species (Bernard et al. 1993). However, it is so efficient at transmitting viruses that even in small numbers it is considered an important virus vector in potato (*Diamond et al. 1996; *Radcliffe 1998a; *Thomas et al. 2000). Green peach aphid populations increase in eastern Canada during mid August when aphids arrive on air currents from the United States and when they migrate from greenhouse plants that have been shipped from the United States. Green peach aphids do not overwinter outdoors in any form in eastern Canada. However, green peach aphids may overwinter in heated greenhouses (Bernard et al. 1993; *Diamond et al. 1996).

Green peach aphids reach maturity in 7-11 days (Guldemond et al. 1998; *Radcliffe 1998a). Individuals of this species of aphid are very fecund. Each aphid has the ability to produce up to 5 offspring a day for as long as one month (*Radcliffe 1998a).

1.1.1.6.3 Feeding: Like other aphids, green peach aphids feed exclusively on the phloem of plants. In potatoes, they are most commonly found feeding on the underside of older leaves at the base of the plant near the soil (*Radcliffe 1998a).

1.1.1.6.4 Virus transmission: The green peach aphid, *Myzus persicae*, is a vector for approximately 120 plant viruses (Eastop 1983) and is the most efficient vector of the two most important potato viruses, PLRV and PVY (Jones 1987; Parry 1990; Bernard et al. 1993; *Diamond et al. 1996; *Diamond and Sturz 1997; *Suranyi et al. 1999;

*Thomas et al. 2000). Green peach aphids are considered to be more of a dispersed species than an aggregating one (Jones 1987; Guldemond et al. 1998), and it is their frequent movement from plant to plant and constant probing that makes them such an efficient virus vector in potatoes (Blackman 1974). Insecticidal control is recommended for seed producers when aphid levels reach 3 aphids per 100 lower leaves (*Radcliffe 1998a).

1.1.1.7 *Macrosiphum euphorbiae*

1.1.1.7.1 Diagnostic Morphological Characteristics: The potato aphid, *Macrosiphum euphorbiae*, is larger than the green peach aphid and measures 1.7 to 3.6 mm in length. The body of this insect has an elongated shape, and apterous aphids are medium green in colour with a darker green colouring along the middle of the back. In comparison, alate aphids have a dark green to brown head and thorax (Bernard et al. 1993).

1.1.1.7.2 Life Cycle: Potato aphids are a heteroecious species. Plants in the Rosaceae family serve as the primary host and potatoes or herbaceous weeds serve as the secondary host. Aphids of this species can overwinter both as females produced parthenogenetically and as eggs. Long nights trigger the production of winged male and female aphids which will return to the winter host. The winged female aphids then produce aphids parthenogenetically that will reproduce sexually with the males and in turn produce eggs. Females, which are produced parthenogenetically and which remain

on the summer host are also produced during long night conditions (Lamb et al. 1997). Following the hatching of eggs, several generations of aphids are produced on the primary host before alate aphids migrate to secondary hosts. Potato aphids are most numerous in Atlantic Canada during the month of July (Bernard et al. 1993). Potato aphid populations increase in response to warm temperatures and periods of low precipitation (Lamb et al. 1997).

1.1.1.7.3 Feeding: Potato aphids feed exclusively on the phloem of plants. These aphids prefer feeding on leaves in the middle to upper portion of the plants (Bernard et al. 1993).

1.1.1.7.4 Virus transmission: Although potato aphids are the most numerous aphid species on potato in Atlantic Canada, they are very inefficient vectors of potato viruses (Bernard et al. 1993). Potato aphids usually only transmit potato virus Y in tobacco crops. Transmission of PVY in potato by potato aphids is extremely rare (Bernard et al. 1993). Tamada and Harrison (1981) found that potato aphids are only 2% as efficient as green peach aphids in spreading potato leaf roll virus. Roberts and Harrison (1979) found 10-30 times more PLRV in green peach aphids than potato aphids. Potato aphids are such poor transmitters of viruses that control is only recommended when numbers of this aphid reach 50 aphids per three compound leaves (Bernard et al. 1993). However, high densities of this aphid on potato crops can lead to leaf wilting and to a reduction in tuber yields (Bernard et al. 1993).

1.1.2 Chemical Control

Insecticides are the most important and efficient method of aphid control in potatoes (*Difonzo et al. 1996; *Radcliffe 1998b). However, current insecticidal controls are becoming less useful due to an increase in aphid resistance (Cancelado and *Radcliffe 1979; Moores et al. 1994; *Radcliffe 1998b; Bohmont 2000). This predicates the need for new products to ensure a sufficient diversity of aphicides for the efficient control of aphids. It also highlights the importance of delaying aphid resistance to new aphicides by limiting their use, and by using non-chemical controls such as crop borders when possible (Woodford et al. 1983; Bohmont 2000; Foster et al. 2000; National Research Council 2000).

Many insecticides used today are also highly toxic to non-target organisms. Many of these insecticides are broad spectrum, contact, neurotoxic pesticides, which are very harmful to non-target organisms in the surrounding environment. Insecticides currently being developed for use in IPM programs should have a narrow range of activity, be specific to target organisms, and be systemic rather than contact in their mode of action. These qualities would limit the negative effects of pesticide use on non-target organisms in the surrounding environment. These qualities are highly desirable in IPM programs (Horn 1988; National Research Council 2000)

Boad spectrum pesticides can also reduce aphid control indirectly. For example, applications of insecticidal treatments that control aphid predators, and fungicides that kill fungal organisms which control aphid populations, can cause aphid populations within a field to flare up (*Radcliffe 1998b; *Suranyi 1999). This places further stress

on aphid control methods.

1.1.2.1 Monitor®

Although green peach aphids are developing resistance to many aphicides, Monitor® continues to provide consistent control in many areas (Harding 1973; Bacon et al. 1976; Hanafi et al. 1989; *Radcliffe 1998a; *Radcliffe 1998b; *Mowry et al. 2000). However, Monitor® is one of the most toxic organophosphorous pesticides and has many negative effects on the surrounding environment (*EPA 1999). In the United States, Monitor® is considered toxic enough to be classified as a "Restricted Use Pesticide" for potato crops (*EPA 1999; *EPA 2000; *EXTOXNET 2001). Monitor® also has a strong sulfur smell which makes it undesirable for use in residential areas (*Bayer 1997).

The active ingredient in Monitor® is methamidophos (*Bayer 1997; *EPA 1999; *EPA 2000; *EXTOXNET 2001). Methamidophos controls aphids by inhibiting acetylcholinesterase action which results in death (*Bayer 1997; *EPA 1999; *EPA 2000; *EXTOXNET 2001). Monitor® is also effective in controlling flea beetles, mites, leafhoppers, worms, potato tubeworms and army worms (*EXTOXNET 2001). This product can also be used on crops other than potatoes including, cotton, broccoli, tobacco, sugar beets, celery, hops and corn (*EXTOXNET 2001).

Applications of Monitor® have serious implications for the ecological integrity of surrounding areas. Honey bee foraging and pollination by bees is seriously reduced when bees are exposed to methamidophos (Gary and Lorenzen 1989; Moores et al. 1994). Methamidophos is also highly toxic to beneficial organisms including other

insects (*EPA 2000). This chemical is also highly toxic to birds (*Bayer 1997; *EPA 2000; *EXTOXNET 2001, as well as to many aquatic organisms (*EPA 2000; *EXTOXNET 2001). These toxic effects to beneficial organisms are detectable even when Monitor® is used as recommended on the pesticide label (*EPA 2000). Even at concentrations as low as 0.22 ng/l, methamidophos is toxic to larval crustaceans (*EXTOXNET 2001). Mammals are also very sensitive to the effect of methamidophos on acetylcholinesterase activity (*EPA 2000).

1.1.2.2 Pirimor®

Harding (1973) and Woodford et al. (1983) reported effective control of green peach aphids with Pirimor®. Additional research has shown that Pirimor® is even effective against aphids which have already developed resistance to most organophosphorous pesticides (*Cornell University 2001). However, there is also evidence that this chemical is losing much of its ability to control green peach aphids (Cancelado and *Radcliffe 1979; Moores et al. 1994). Other aphid species such as *Aphis gossypii* have already developed resistance to Pirimor® (Martin and Workman 1997; Villatte et al. 1999).

Pirimor® is a carbamate aphicide with pirimicarb as its active ingredient (*Zeneca 1999; *Cornell University 2001). Although pirimicarb is also an acetylcholinesterase inhibitor, this chemical is less toxic to most non-target organisms than Monitor® (*Cornell University 2001). Pirimor® is very toxic to aquatic organisms (*Zeneca 1999); however, due to both photolysis and degradation via microbial

metabolism, Pirimor® has a very short half life. This means that there is a low risk for leaching of the pesticide in soil, which diminishes the risk for contamination of both surface and ground water (*EPA 1999).

Pirimor® exhibits fast acting control via translaminar and local systemic activity in plants (*Zeneca 1997; *Cornell University 2001). This pesticide is registered for use on crops such as potatoes, tobacco, peppers, lettuce, asparagus, sweet corn, peas, asparagus, apples, strawberries and peaches to control many aphid species including both the potato aphid and the green peach aphid (*Zeneca 1997).

1.1.2.3 Fulfill®

Pymetrozine is the active ingredient in a new aphicide called Fulfill® (Kristinsson 1994). Pymetrozine belongs to a new class of chemical pesticides called pyridine azomethines (Kristinsson 1994; Harrewijn and Kayser 1997) and is efficient in controlling aphids on tobacco, potatoes and many other tuber and corm vegetables (Flückiger et al. 1992; *Novartis Crop Protection 1999a; *Novartis Crop Protection 1999b). Fulfill® has a much lower toxicity than Monitor® and Pirimor® and is considered harmless to beneficial organisms. These characteristics make Fulfill® a desirable product for use in Integrated Pest Management systems (Flückiger et al. 1992; Follas and Blanc 1995).

Fulfill® does not exert a general toxic effect on aphids but instead causes antifeedant activity on insects with sucking mouthparts (Flückiger et al. 1992; Kristinsson 1994; Harrewijn and Kayser 1997). Pymetrozine targets nervous system regulation of the cibarial valves, the food pump and the salivary pump (Harrewijn and

Kayser 1997). Concentrations of pymetrozine in aphid haemolymph as low as 10^{-4} M are high enough to almost completely inhibit probing and larvapositioning (Harrewijn and Kayser 1997). Immediately following pymetrozine exposure, aphids cease probing and consequently stop feeding (Flückiger et al. 1992; Harrewijn and Kayser 1997). At low doses, the antifeedant activity of Fulfill® is reversible (Harrewijn and Kayser 1997). At higher doses, Fulfill® induces irreversible changes in feeding behaviour (Kristinsson 1994; Harrewijn and Kayser 1997). In these situations, starvation occurs within two to five days (Flückiger et al. 1992; Harrewijn and Kayser 1997). Aphids exposed to pymetrozine have been observed holding their proboscis in an altered manner. However, morphological changes to the feeding parts of aphids after exposure to Fulfill® have yet to be investigated (Harold Wright, personal communication). Fulfill® is unique in its mode of action. There are no other aphicides on the market that inhibit aphid feeding without paralyzing the aphids (Harrewijn and Kayser 1997). Although the activity of Fulfill is primarily systemic, there is also evidence that contact activity contributes to the efficacy of Fulfill® (Flückiger et al. 1992).

Pymetrozine penetrates plant cuticles during the drying of the spray mixture (Wyss and Bolsinger 1997a). Pymetrozine also exhibits translaminar movement in plant leaves. Consequently, aphids feeding on the underside of the leaf are affected even when Fulfill® is only applied to the upper surface of leaves (Flückiger et al. 1992). Pymetrozine is also translocated throughout the plant via the phloem and to a minimal extent by the xylem. This vascular system of plants is particularly efficient in transporting pymetrozine from the older treated leaves to new leaves which emerge after

pymetrozine exposure (Wyss and Bolsinger 1997b). However, aphids feeding on older leaves show higher mortality than those feeding on newly emerged leaves, possibly due to the fact that concentrations of pymetrozine are higher in the older leaves which were initially treated with the aphicide (Wyss and Bolsinger 1997a). Pymetrozine absorbed by the stem or root during foliar application does not appear to contribute to the systematic activity of the aphicide (Wyss and Bolsinger 1997b). The residual activity of pymetrozine persists for approximately two weeks following application (*Novartis Crop Protection 1998). The movement of pymetrozine in the tissues of different plant species has been shown to be very different (Wyss and Bolsinger 1997a). Fulfill® has not exhibited any phytotoxic effects on any of the crops on which it was tested (Bedford et al. 1998; *Novartis Crop Protection 1998; *Novartis Crop Protection 1999a).

Pymetrozine is believed to have a minimal environmental impact due to its low toxicity (Flückiger et al. 1992). Fulfill® is rated as practically non-toxic to fish, bees, birds and many beneficial insects and is only slightly toxic to *Daphnia sp.* (Flückiger et al. 1992; *Novartis Crop Protection 1998). Due to the low toxicity of pymetrozine to beneficial organisms and its minimal environmental impact, the United States Environmental Protection Agency has given Fulfill® 50WG a reduced risk status (*Novartis Crop Protection 1998). Table 1 is a comparison of the toxicity of Monitor®, Pirimor® and Fulfill®.

Since pymetrozine belongs to a new class of chemicals, aphids have not yet developed resistance to this chemical. In fact, pymetrozine shows excellent control of aphid species which are already resistant to organophosphate, carbamate, and pyrethroid

Table 1.0 Comparison of the toxicity of Fulfill® (Flückiger et al. 1992; *Novartis Crop Protection 1999b) Monitor® (*EXTOXNET 2001) and Pirimor® (*Zeneca 1999; *Cornell University 2001)

Hazard Indicator	Fulfill®	Monitor®	Pirimor®
Avian Oral LD50	>2000 mg/kg	8-11 mg/kg	8-17 mg/kg
Fish LC50	>100 mg a.i. /l	25-100 mg/l	29-55 mg/l
Honey bee LD50	>100 μg/bee	highly toxic	N/A
Mammalian Oral LD50	>5820 mg/kg	16-21 mg/kg	100-200 mg/kg
Mammalian Dermal LD50	>2000 mg/kg	50 mg/kg	>1000 mg/kg
Mammalian Inhalation LD50	>1.8 mg/l air	9 mg/kg	300 mg/m^3/6hr
Mammalian Eye Irritation	Slight	Strong	Slight
Mammalian Skin Irritation	Negative	Slight	Slight
Mammalian Skin Sensitization	Negative	Negative	Negative
Mammalian Mutagenicity	Negative	Weakly	Negative
Mammalian Neurotoxicty	Negative	Positive	Positive
Mammalian Carcinogenicity	Positive	Negative	Negative
Mammalian Teratogenicity	Negative	Weakly	Negative

insecticides (Flückiger et al. 1992; Kristinsson 1994). To ensure that pymetrozine remains effective in aphid control, measures must be taken to manage applications appropriately in order to delay the development of resistance. The current label rate of application for Fulfill® in potatoes is 196 grams/hectare with a maximum of two applications per season (*Novartis Crop Protection 1998; *Novartis Crop Protection 1999a; *Glogoza 2000; *Mowry et al. 2000). It is also recommended that Fulfill® be applied with an adjuvant in order to facilitate maximum absorption of the chemical by the plant (*Novartis Crop Protection 1998; *Novartis Crop Protection 1999a). Fulfill® should not be applied through an irrigation system (*Mowry et al. 2000). A seven day period should elapse between applications and a 14 day period should separate the last application date from the time of harvest (*Novartis Crop Protection 1999a; *Glogoza 2000).

1.1.2.4 Adjuvants

Although the American Fulfill® label includes a recommendation for the addition of a penetrating adjuvant during application, there is no recommendation, nor any documented data, as to which adjuvants maximize the efficacy of this product (*Novartis Crop Protection 1999a). Syngenta did not have to provide any evidence that adjuvants increase the efficacy of Fulfill® to include this recommendation on its label, since adjuvants are not registered products in the United States (*Baysinger and DeFelice 2000; *Petroff 2000). In Canada, adjuvants are required by law to be registered. For an adjuvant to receive Canadian registration, there must be evidence that

the adjuvant either increases the efficacy of the pesticide, or increases the spectrum of activity of the pesticide (*PMRA 1993). Before Fulfill® is registered in Canada with a label recommending the addition of an adjuvant, Syngenta must provide evidence that the activity of Fulfill® is increased by the addition of an adjuvant during application. These data are very important as using the wrong adjuvant can limit pesticide efficacy and even damage foliage (*Hock 1994; Bohmont 2000).

Adjuvants are commonly used in the pesticide industry because they increase pesticide coverage on foliage and thus increase pesticide efficacy (Tadros 1984; Van Emden 1989). Adjuvants act by altering the surface tension of spray droplets after application (Hancock 1984; Tadros 1984). Water has a high surface tension, which means that pesticide spray droplets often remain beaded on foliage rather than evenly spread over plant surfaces (*Petroff 2000).

Surfactants are a type of adjuvant with a molecular configuration consisting of a hydrophilic head and a lipophilic tail. It is the hydrophilic head that attracts the surfactant to the pesticide droplet and the protruding lipophilic tail that decreases the surface tension of the spray droplet (Hancock 1984; Ottewill 1984; Cross 1987; Bohmont 2000; *Petroff 2000). While surfactants can be anionic, cationic or non-ionic depending on the charge they carry, non ionic surfactants are the type of adjuvant used most frequently in the pesticide industry (Ottewill 1984; *Hock 1994; Bohmont 2000). Non-ionic formulations do not carry any electric charge and are based on alcohol and fatty acid components (Ottewill 1984; Cross 1987; *Petroff 2000). Non-ionic surfactants are the most effective in enhancing systemic pesticide penetration of leaf cuticles

(*Hock 1994).

There are many types of adjuvants including penetrating adjuvants, spreader-sticker adjuvants and mineral oils. Penetrating adjuvants are surfactants which are formulated specially to increase the penetration of pesticides through plant cuticles (*Hock 1994). This increased penetration is particularly important for systemic pesticides which depend on the translocation of the active ingredient through the plant for effective control (Bohmont 2000). Spreader-stickers are another type of surfactant adjuvant that increases the adhesion of pesticides to foliage. This increased adhesion not only increases the quantity of pesticides which is absorbed by the plant but also decreases the amount of pesticide which is lost through evaporation, ultraviolet light degradation, and rainfall (*Hock 1994; Bohmont 2000). Mineral oils act in a similar manner as penetrating adjuvants and increase the penetration of pesticides through plant cuticles (Pree et al. 1996; Horowitz et al. 1997; *Hock 1994). Citowett Plus®, LI 700®, and Superior 70 Oil® are all commonly used adjuvants in the pesticide industry.

Citowett Plus® is a water-soluble non-ionic spreader-sticker adjuvant which is composed of octylphenoxypolyethoxyethanol (*BASF 1998). Citowett Plus® increases the adhesion of pesticides to plant cuticles and increases the wettability of the pesticide to dusty, waxy and hairy plant surfaces (*BASF 1998). The increased adhesion provided by Citowett Plus® decreases the amount of pesticide which drips off foliage following application thus increasing the pesticide's efficacy (*BASF 1998).

LI 700® is a non- ionic, penetrating, translocating surfactant and pH adjuster which is composed of phosphatidylcholine, methylacetic acid and alkyl polyoxyethylene

ether (*Loveland Industries 2002). The label for this adjuvant recommends use with pesticides that require wetter/spreader or adjuvant oil. LI 700® is also appropriate for use with hard or alkaline water where a pH adjustment is needed to prevent alkaline hydrolysis (*Loveland Industries 2002).

Superior 70 Oil® is a commonly used mineral oil surfactant comprised of 97% light and heavy paraffinic distillates and 3% emulsifier (*Belau 2001). In addition to being used as adjuvants with pesticides, mineral oils such as Superior 70 Oil® are also commonly used to reduce the spread of non-persistant viruses, such as PVY, in potato crops (*Diamond et al. 1996). Mineral oils reduce the spread of viruses by interfering with the transmission of viral particles from aphid stylets to plants during probing. Viral particles are washed off of the stylets of aphids during probing activity (Gibson et al. 1984; *Suranyi 1999). Superior 70 Oil® is toxic to aquatic organisms and should not be used near water (*Belau 2001).

Chapter 2: Laboratory Studies- Aphid Behaviour After Exposure to Fulfill® and SEM Morphological Study

2.0 INTRODUCTION

Fulfill® 50 WG is a new, highly selective aphicide that is currently being considered for registration in Canada. This product is already registered for use in the United States and in Europe. Fulfill® has a low impact on beneficial organisms, which makes it a suitable pesticide for Integrated Pest Management programs (Flückiger et al. 1992). Fulfill® contains the active ingredient pymetrozine (Kristinsson 1994) which belongs to a new class of chemical pesticides called the pyridine azomethines (Kristinsson 1994; Harrewijn and Kayser 1997). Fulfill® does not exert a general toxic effect on aphids but instead invokes antifeedant activity (Flückiger et al. 1992; Kristinsson 1994; Harrewijn and Kayser 1997). Pymetrozine targets nervous system regulation of the cibarial valves, the food pump and the salivary pump (Harrewijn and Kayser 1997). Immediately following pymetrozine exposure, aphids cease probing and consequently stop feeding (Flückiger et al. 1992; Harrewijn and Kayser 1997). In these cases, starvation occurs within two to five days (Flückiger et al. 1992; Harrewijn and Kayser 1997).

It is the antifeedant activity of Fulfill® that makes this product an effective aphicide, because it is through probing and feeding on plants that aphids pick up and transmit potato viruses (Blackman 1974; Matthews 1981; Eastop 1983; Parry 1990). Even though aphids live for a few days following exposure to Fulfill®, they do not feed and are therefore incapable of spreading viruses to potato plants (Harrewijn and Kayser 1997). Controlling aphid populations is important to the potato industry, as aphids are

the vectors responsible for the transmission of many agriculturally important viruses such as PVY and PLRV (Dixon 1973; Blackman 1974; Eastop 1983; Peters 1987; Parry 1990; Bernard et al. 1993; Šutić et al. 1999).

Although the antifeedant activity of Fulfill® has been described by Flückiger et al. (1992), Harrewijn and Kayser (1997), and *Novartis Crop Protection (1998), doubts about the consistency of this mode of action were raised at the Northeast Potato Technology Forum in Charlottetown, 2001 (Sarah Stewart, personal observation). Before Fulfill® is recommended to potato farmers on Prince Edward Island, it is important to verify whether or not aphids cease feeding after exposure to this chemical. There is some speculation that aphids exposed to pymetrozine hold their proboscis in an altered manner (Harold Wright, personal communication). However, Syngenta is uncertain whether this is due to morphological changes to the feeding parts of aphids induced by pymetrozine, or whether it is a symptom of pymetrozine's effect on the cibarial valves, the food pump and the salivary pump (Harold Wright, personal communication).

The aphid proboscis is a modified labium which contains the mandibular and maxillary stylets within a labial groove that runs anteriorly along the proboscis (Dixon 1973). The aphid proboscis is segmented and is attached to the aphid head in front of and in between the first coxae (Dixon 1998). When inactive, the stylets remain inside the labial groove of the proboscis which hangs underneath the thorax parallel to the

---

* Refers to a reference from a company monograph, government website or university website.

aphid body (Blackman 1974). While feeding, the proboscis moves to a position perpendicular to the aphid body, and the stylets protrude from the end of the proboscis into plant tissue (Dixon 1973). The last segment of the labium is smaller than the others and during feeding is pushed back into the other larger segments (Dixon 1973). Tactile receptors are found at the end of the proboscis and these sensilla aid in finding leaf veins where aphids prefer to feed (Dixon 1998). A labial palp extends over the first section of the labial groove where the proboscis extends outwards from the aphid body.

The objectives of this study are: 1) to verify that aphids cease feeding after exposure to Fulfill® even though they may remain alive for several days and 2) to determine, using Scanning Electron Microscopy, whether Fulfill® induces any visible morphological changes to the proboscis of aphids.

2.1 MATERIALS AND METHODS

2.1.1 Aphids

Green peach aphids, *Myzus persicae*, were obtained from Agriculture and Agri-food Canada, Potato Research Centre, Fredericton courtesy of Dr. Gilles Boiteau. Potato aphids, *Euphorbiae macrosiphum*, were collected from a potato field in New Annan Prince Edward Island. Aphids were maintained on potato leaves in petri dishes, or on potato plants which were kept in a growth chamber set at 21° C, 80% humidity, and on a light/dark schedule of 14/10 hours.

2.1.2 Spraying

A three-point Hardi sprayer with flat fan nozzles and 2 atm pressure using 40.5 litres of water/hectare/spray volume was used to apply Fulfill® aphicide to Shepody and Russet Burbank potatoes in a field in New Annan, Prince Edward Island (46° 25' 89" N; 63° 40' 53" W). Fulfill® was applied at the label rate of 196 grams/hectare. Citowett Plus®, a non-ionic spreader sticker was used as an adjuvant in some treatments (Table 2.0), and was added at the rate of 1 litre/1000 litres of spray volume. LI 700® was used as an adjuvant in other treatments (Table 2.0), and was applied at the label rate of 5 litres/1000 litres of spray volume. No rainfall occurred within at least 12 hours of any application.

2.1.3 Feeding Evaluation (2001)

Three trials of this experiment were conducted during 2001, and each trial

Table 2.0. Treatment groups for all three trials in lab feeding evaluation during 2001.

Trial	Variety	Treatment	Leaflets	Aphids/Leaflet
Trial 1	Shepody	Control	3	10
	Shepody	Fulfill® Citowett®	3	10
	Russet Burbank	Control	3	10
	Russet Burbank	Fulfill® Citowett®	3	10
Trial 2	Shepody	Control	3	10
	Shepody	Fulfill® LI700®	3	10
	Russet Burbank	Control	3	10
	Russet Burbank	Fulfill® LI700®	3	10
Trial 3	Shepody	Control	3	10
	Shepody	Fulfill®	3	10
	Shepody	Fulfill® LI700®	3	10
	Russet Burbank	Control	3	10
	Russet Burbank	Fulfill®	3	10
	Russet Burbank	Fulfill® LI700®	3	10

included a variety of treatments (Table 2.0). Forty-eight hours following treatment with Fulfill® aphicide, three leaflets were collected from each of the treatment groups. The leaflets were maintained in florist water picks during transport to the lab. In the lab, leaflets were placed in vials filled with water and each leaflet was then placed in a separate petri dish. Ten aphids were placed in each petri dish. Petri dishes were placed randomly in clear tupperware containers which were kept in a growth chamber set at 21° C, 80% humidity, and on a light/dark schedule of 14/10 hours. Each petri dish was checked every 24 hours for seven days. The number of aphids feeding, giving birth, walking on the leaf, walking on the dish, and the number of dead aphids was evaluated each day. An aphid was considered to be feeding if its stylet had penetrated the leaf. Aphid behaviour after exposure to Fulfill® was monitored in all treatments by observing aphids through a microscope during the feeding evaluation trials. Aphids were observed immediately following exposure to Fulfill® and at random intervals throughout the experiment. Videos were also recorded of aphids using the Pixera® system to monitor aphid behaviour after exposure to Fulfill® . These videos were then viewed to observe aphid behaviour after exposure to Fulfill®.

2.1.4 Statistical Analysis

Daily patterns in aphid abundance were evaluated by plotting the total number of aphids that were alive in the treatment groups on each day. One-way analysis of variance (ANOVA) was used to compare the number of feeding aphids among treatment groups on day two and day seven as well as to compare aphid survival among treatment

groups. In cases where the data were not normal, and could not be normalized by transformations, but all other assumptions of the ANOVA were met, the non-parametric equivalent of the one-way ANOVA, the Kruskal-Wallis test was used (Sprent1989; Dytham 1999). In cases where ANOVA or Kruskal-Wallis tests indicated that there was a significant level of variance among groups, t-tests and Mann-Whitney- U tests (the non-parametric equivalent of the t-test) were used to determine which treatment groups were significantly different from one another (Dytham 1999). Bonferonni corrections were performed for all multiple comparisons to avoid the possibility of inflating the type 1 error (Milton 1992; G.E. Dallal, Chief Statistician, Tufts University, 711 Washington Street, Boston Massachuset, 02111-1524, personal communication). In cases where data were normal but groups had an unequal variance, one-way ANOVA was performed using Dunnett T3 post-hoc tests (Dunnett 1980).

2.1.5 Proboscis Morphology Comparison (2001)

Fifteen green peach aphids (5 aphids/leaf) were placed on potato leaves that had been collected from the field after the crop had been treated with Fulfill®. Twenty five other aphids (5 aphids/leaf) were placed in petri dishes on potato leaves that were not treated with Fulfill®. Aphids were also allowed to feed on these leaves. Aphids on the Fulfill® treated leaves were collected from the petri dishes after they were either dead, or exhibiting antifeedent behaviour from exposure to Fulfill® via feeding. Aphids feeding on the control leaves were also collected at the same time. A group of four aphids were placed in a petri dish without any leaves and were collected once they had

starved to death. To ensure that these aphids died from starvation and not dessication, moist filter paper was placed in the petri dish. This group was included to ensure that the process of starvation itself did not induce any morphological changes in aphid mouthparts. The aphids that had starved to death using this method were mixed in with the control group before being examined.

All aphids from each group were fixed in formelin-acetic-acid-alcohol and were then dehydrated in a graded ethanol series from 70% to100%. Specimens were critical point dried using CO_2 as the transitional fluid in a model 28 000 LADD® critical point dryer. Specimens were mounted on stubs, grounded with silver paint, then coated with 300 Angstroms of gold- palladium using a Denton Vacuum Desk II® sputter-coater. Specimens were examined with a Cambridge Stereoscan 604® scanning electron microscope, and thermal prints of the digital SEM images were acquired using SEMICAPS® software and produced using a Mitsubishi P67U® video copy processor.

The morphology of the proboscis for each aphid specimen was examined under SEM and was compared visually for morphological disparities from each other and from the morphological descriptions found in the literature. The following characteristics were investigated for each specimen: whether the stylets were protruding from the proboscis, whether the labial palp was elevated or closed, and whether there were any obvious visual deformities in the proboscis.

2.2 RESULTS

2.2.1 Feeding Evaluation (2001)

In all of the experiments in this section, there were no significant differences in either feeding or aphid survivorship on Shepody or Russet Burbank leaves, so data from the two potato varieties were combined into one group.

2.2.1.1 Trial One (Fulfill® Citowett®): By day two of the experiment, the aphid control of Fulfill® was evident. There were only a small number of aphids feeding in the Fulfill® treated group, whereas most aphids in the control group were still feeding at this time. Only three of the 55 living aphids were feeding on leaflets sprayed with Fulfill® Citowett® at this point compared to 114 of the 123 living aphids feeding on the untreated control leaflets (Figure 2.0). The difference between feeding in control and Fulfill® Citowett® treatment groups was statistically significant (Kruskal- Wallis $p<0.05$) as was the difference in the number of living aphids in control and Fulfill® Citowett® treatment groups (ANOVA; $p<0.05$).

By day seven, Fulfill® offered complete aphid control. There were no aphids observed feeding on the Fulfill® Citowett® treated leaves and 631 aphids feeding on the untreated control leaves (Figure 2.0). This difference was statistically significant (Kruskal-Wallis; $p<0.05$). By day seven, there were two living aphids on the Fulfill®Citowett® treated leaves and 631 living aphids on the untreated control leaves. This difference was also statistically significant (ANOVA; $p<0.05$).

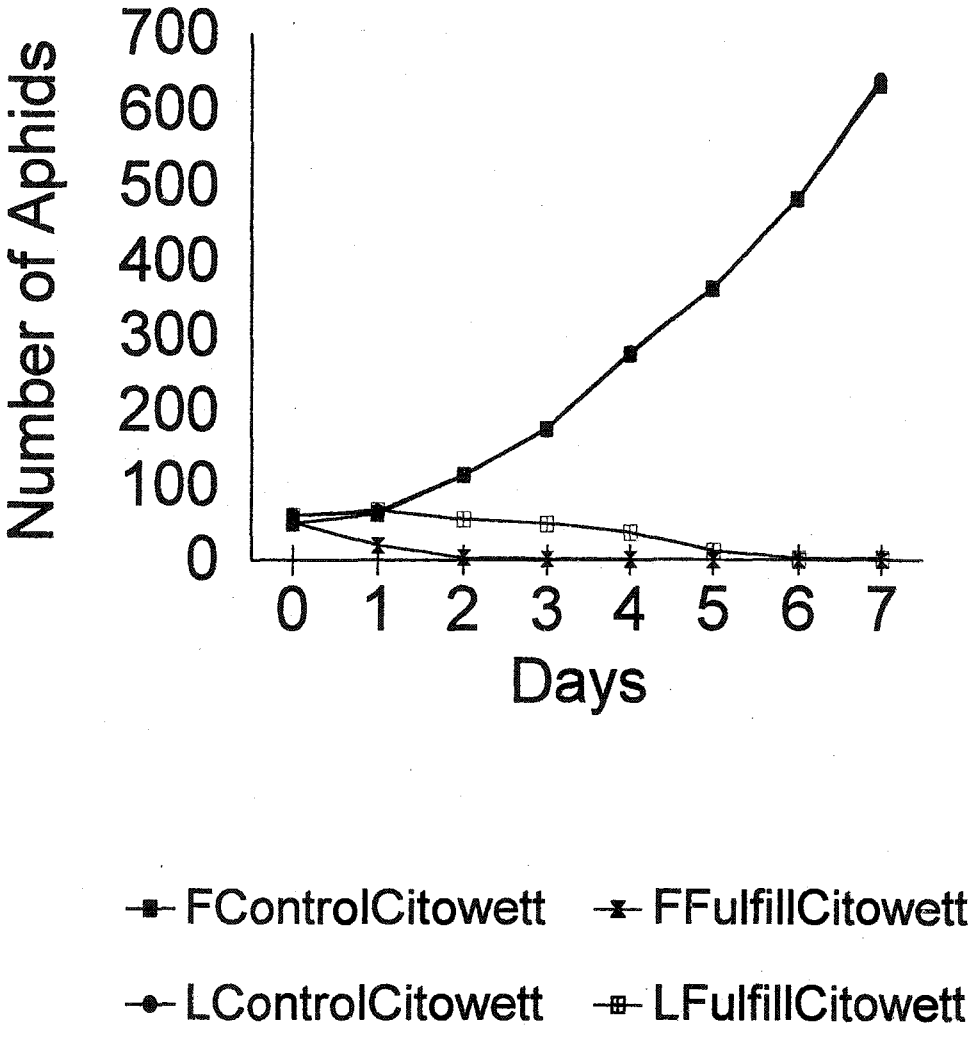


Figure 2.0. Trial 1. Lab based feeding evaluation study. A comparison of the feeding (F) and number of living (L) aphids in control and Fulfill® Citowett® groups. Note that the 'FControlCitowett' and the 'LControlCitowett' lines overlap. All living aphids in the control group were feeding.

2.2.1.2 Trial Two (Fulfill® LI700®): By day two there were very few aphids feeding on the Fulfill® treated leaflets. All 10 living aphids were feeding on the Fulfill® LI700® treated leaves and all 66 living aphids were feeding on the untreated control leaves (Figure 2.1). This difference was statistically significant (ANOVA; $p<0.05$).

By day eight Fulfill® offered complete aphid control. There were no aphids observed feeding on the Fulfill® LI700 treated leaves. However, there were still 12 living aphids remaining in this group. In the control groups, 388 of the 535 living aphids were observed feeding on the untreated control leaves (Figure 2.1). The difference in feeding between control and Fulfill® LI700® groups was statistically significant (Kruskal-Wallis; $p<0.05$) as was the difference in the number of living aphids between control and Fulfill® LI700® groups (ANOVA; $p<0.05$).

2.2.1.3 Trial Three (Fulfill® and Fulfill® LI700®): The same trend was seen again in trial three. On day two, 122 of the 126 living aphids in the control group were feeding. At the same time, only 4 of the 42 living aphids in the Fulfill® group were feeding while only 1 of the 38 living aphids in the Fulfill® LI700® group were feeding. The number of aphids feeding in the control group was significantly higher than in the Fulfill® and Fulfill® LI700® groups (ANOVA; Dunnett T3; $p<0.05$). There was no significant difference between aphid feeding in Fulfill® and Fulfill® LI700 treatments (ANOVA; Dunnett T3; $p>0.05$) (Figure 2.2).

On day seven, all 588 living aphids in the control group were feeding. All 3 of the living aphids in the Fulfill® group were feeding on day seven while there were no

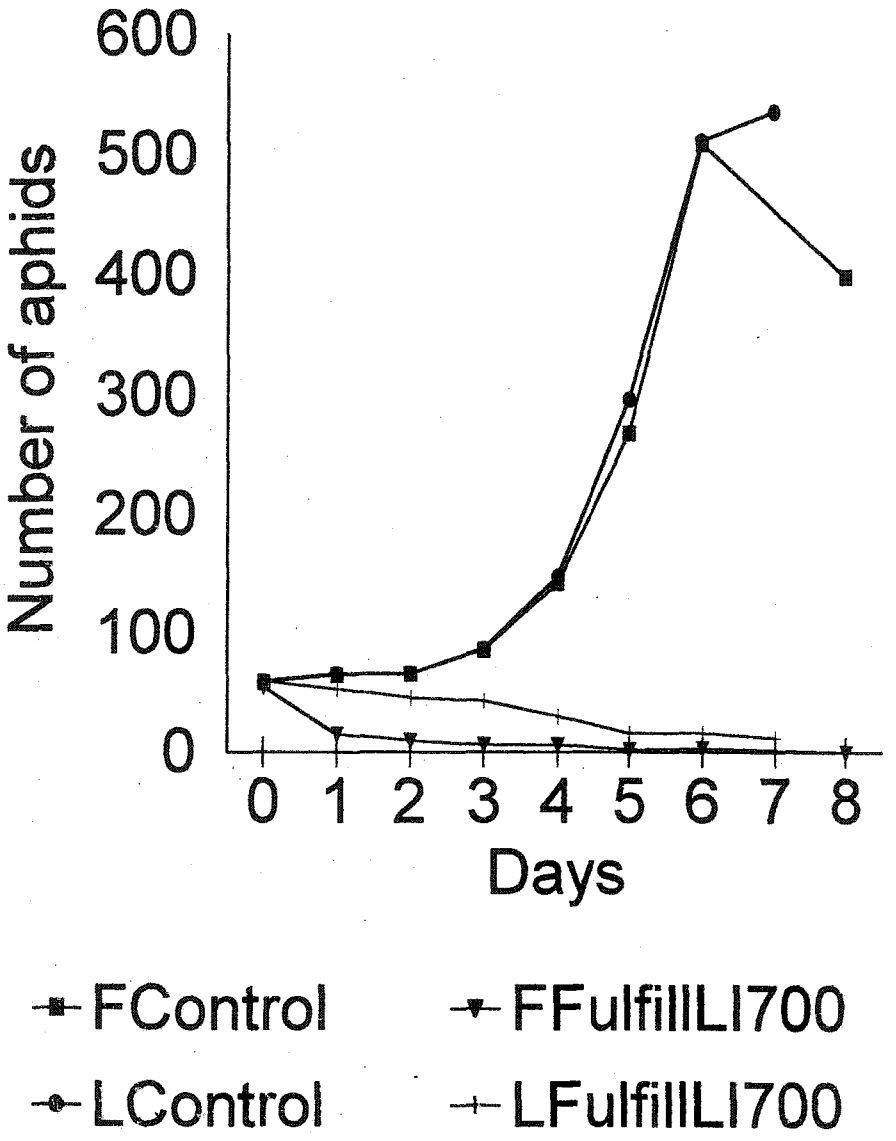


Figure 2.1. Trial 2. Lab based feeding evaluation study. A comparison of the number of feeding (F) and living (L) aphids in control and Fulfill® LI700® treatment groups.

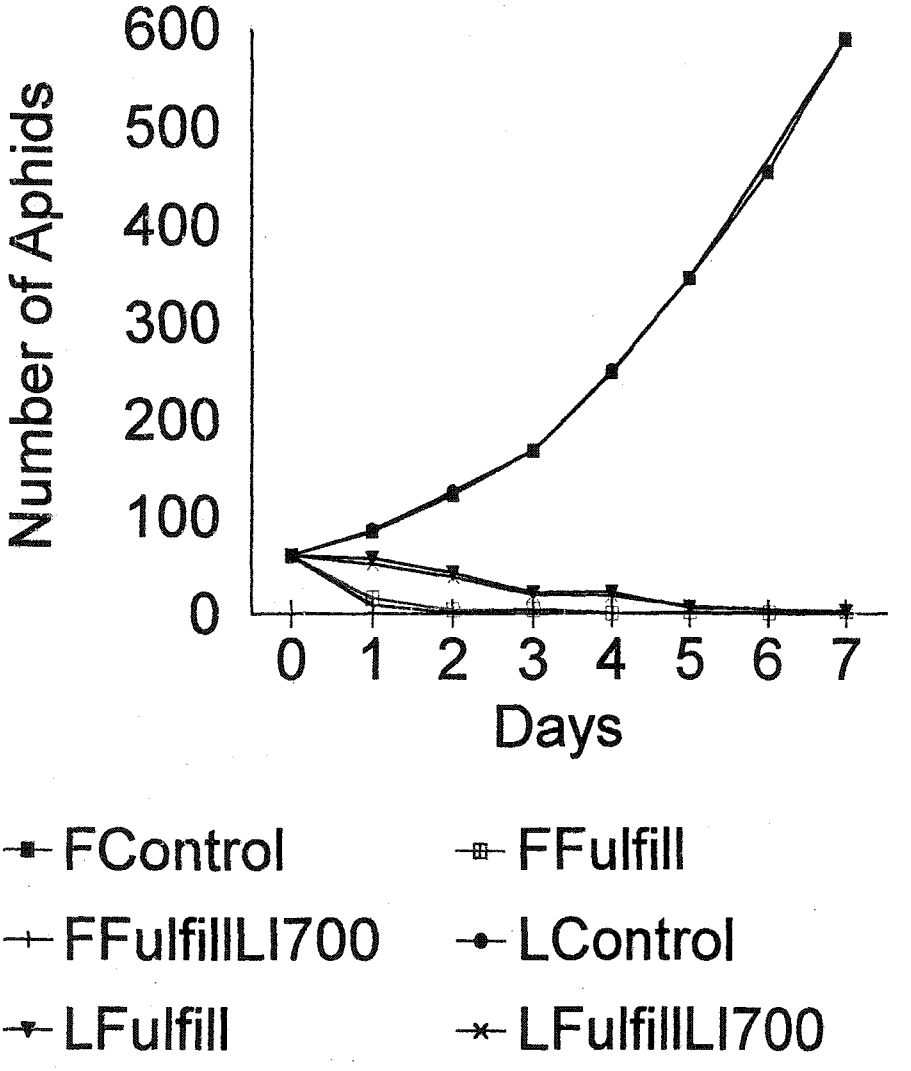


Figure 2.2. Trial 3. Lab based feeding evaluation study. A comparison of the number of feeding (F) and living (L) aphids in control, Fulfill® and Fulfill® LI700® groups. Note that 'FControl' and 'LControl' results overlap (nearly all living aphids in the control group were feeding). The 'FFulfill' and "FFulfillLI700' lines also overlap as do the 'LFulfill' and the 'LFulfillLI700' lines due to the similar modes of action of these treatment groups.

living aphids in the Fulfill® LI700® group. Aphid feeding in the control group was significantly higher than in the Fulfill® and Fulfill® LI700® treatments (ANOVA; Dunnett T3; $p<0.05$). There was no significant difference between aphid feeding in Fulfill® and Fulfill® LI700® groups (ANOVA; Dunnett T3; $p>0.05$) (Figure 2.2).

2.2.1.4 Aphid Behaviour (2001): Aphids began twitching after feeding on leaves treated with Fulfill® as early as two hours following initial exposure to the chemical. Twitching involved apparent uncontrollable movement of legs and antennae. Exposure to Fulfill® also decreased the locomotory movement of aphids; many of them stopped walking and moved slightly only if prodded. When aphids in the Fulfill® treated groups did walk, they appeared disoriented and moved in an unsteady manner. Some aphids appeared to remain in a feeding position with stylets facing the leaf after Fulfill® exposure. However, closer examination revealed that stylets were not penetrating the surface of the leaf. Aphids were not observed feeding after their initial exposure to Fulfill®. No aphids were observed holding their proboscis in an altered manner. Two aphids gave birth after one day on a Fulfill® treated leaf. However, no Fulfill® treated aphids were observed giving birth after this time period.

2.2.2 Proboscis Morphology Comparison (2001)

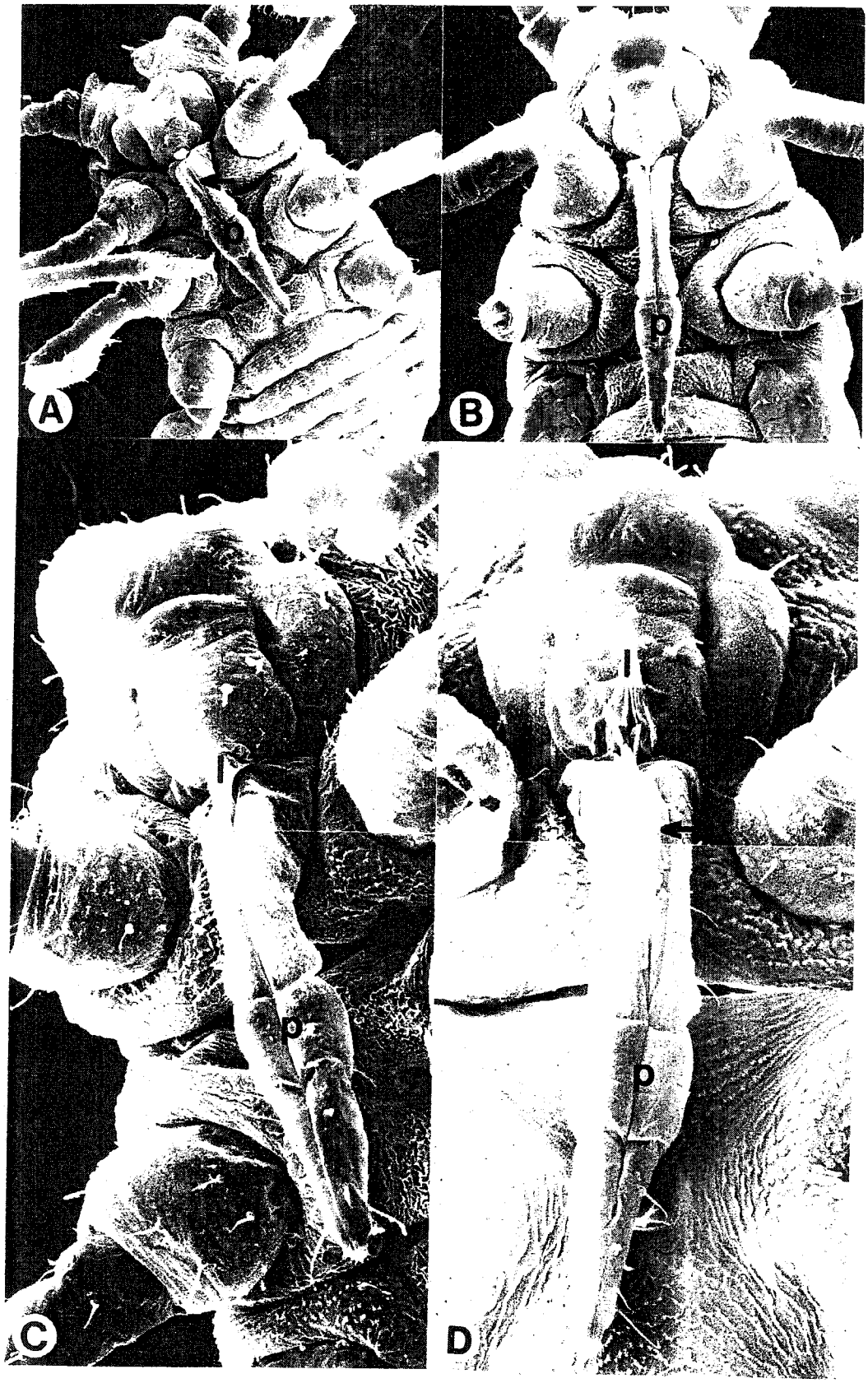
In both the control and Fulfill® groups, the labial palp was either resting against the proboscis as shown in Figure 2.3 C, or was in an upright position where the palp and the proboscis were not touching as shown in Figure 2.3 D (Table 2.1). In all cases, the

proboscis looked like that described in the literature: the most proximal segment was the shortest and widest, the second segment was the longest, the third segment was short and wider than the second segment, and the most distal segment was the narrowest and tapered towards the tip. In every proboscis that was observed, the stylets did not extend out of the labial groove at the tip. However, in some aphids in both groups the stylets protruded from the labial groove of the second segment as shown in Figure 2.3 A and D (Table 2.1). There were no visual deformities in the proboscis of any of the control and Fulfill® aphids (Table 2.1).

Table 2.1. Proboscis characteristics in aphids exposed to Fulfill® and in control aphids.

	elevated labial palp	Stylets protruding from proboscis	visual deformities
Fulfill Aphids (n=15)	5	6	0
Control Aphids (n= 29)	8	12	0

Figure 2.3. (Figure on page 52) Examples of aphid proboscises in control and Fulfill® treated groups. **A.** Aphid from control group with stylets (arrowheads) protruding from the proboscis (p) 200X. **B.** Fulfill® treated aphid with the proboscis (p) in resting position 200X. **C.** Aphid from the control group with the labial palp (l) resting on the proboscis (p) 500X. **D.** Fulfill® treated aphid with the labial palp (l) raised, and stylets (arrowhead) protruding from the proboscis (p) 500X.



2.3 DISCUSSION

2.3.1 Feeding Evaluation (2001)

Feeding inhibition occurred consistently in aphids exposed to Fulfill®Citowett®, Fulfill® LI700®, and Fulfill® less than 24 hours following exposure. After their first exposure to Fulfill®, aphids did not resume feeding. This verifies other findings reported in the literature (Flückiger et al. 1992; Harrewijn and Kayser 1997). Mortality lagged behind feeding inhibition. This was expected due to the mode of action of Fulfill®.

2.3.2 Aphid Behaviour (2001)

The twitching and disorientation exhibited by aphids after exposure to Fulfill® via feeding is consistent with findings reported by researchers at *Novartis Crop Protection (1998b). The decreased mobility exhibited by aphids after exposure to Fulfill®, in particular the limited walking, was also observed in aphids following exposure to the nicotynil insecticide imidacloprid (Boiteau and Osborn 1997). This limited movement is generally associated with exposure to a poison (Boiteau and Osborn 1997). Even though the action of Fulfill® has been attributed solely to its antifeedant properties and it is not considered a general toxin, the behaviour exhibited by aphids after exposure to Fulfill® in this study may indicate that pymetrozine does have a general toxic effect on aphids in addition to its antifeedant properties.

2.3.3 Proboscis Morphology Comparison (2001)

There were no visual disparities in the external morphology of the aphid

proboscis between control and Fulfill® treated groups. Some aphids in both groups had raised labial palps or stylets protruding from the second segment of the proboscis. Since these observations were reported in both groups, it is probable that these changes are associated with the methods used to prepare specimens for SEM. Since aphids in both the control and Fulfill® treated groups died from starvation before being fixed, this starvation may also have contributed to these morphological changes. The morphology of all proboscises observed in this study were similar to the descriptions of Dixon (1973), Blackman (1974) and Dixon (1998).

Although reports from Syngenta, the manufacturer of Fulfill®, claim the proboscis of aphids may look different after exposure to Fulfill® (Harold Wright, personal communication), this study did not produce any evidence to support this claim. Aphid behavior after exposure to Fulfill® did not reveal any evidence that aphids hold their proboscis in a different manner. This does not support the theory that the effect of Fulfill® on the food pump, cibarial valves and salivary pump may alter the external morphology of aphid mouthparts.

2.4 CONCLUSION

Fulfill® provided excellent aphid control under controlled laboratory conditions. Although some aphids remained alive for several days, they were not observed feeding after their initial exposure to Fulfill®. Farmers and crop scouts will need to be informed about the unique mode of action of Fulfill®. They must understand that although aphids may remain alive on their crops for several days after the application of this aphicide, aphids will not probe plants and therefore will not spread potato viruses.

This study did not find any evidence that Fulfill® induces morphological changes in the mouthparts of aphids. Since only the external morphology of the proboscises were examined in this study, it is possible that internal changes took place after exposure to Fulfill®. However, internal changes were not investigated in this study. It is likely that the antifeedant activity of Fulfill® is attributed almost exclusively to neural inhibition of the cibarial valves, food pump and salivary pump.

Chapter 3: Field Studies: Efficacy Comparisons and the Use of Adjuvants

3.0 INTRODUCTION

As integrated pest management programs become standard practice in agriculture, new highly selective pesticides which have minimal impact on beneficial organisms, will be necessary to replace broad action toxic formulas (Horn 1988; National Research Council 2000). This is particularly important in Prince Edward Island where such a high proportion of land is used in potato production. The Island's environment is under increased risk from the large quantities of highly toxic pesticides applied on the more than 4050 hectares of potatoes planted each year in Prince Edward Island (Statistics Canada 2000).

Some of the major pests of potatoes are aphids, such as the green peach aphid, *Myzus persicae*, and the potato aphid, *Macrosiphum euphorbiae* (Dixon 1973; Šutić et al. 1999). Aphids that colonize potato crops feed on plant phloem by inserting their stylets into plant tissue (Dixon 1973; Blackman 1974; Peters 1987; Dixon 1998). This probing activity from plant to plant makes aphids a major virus vector, spreading viruses such as potato leaf roll virus (PLRV) and potato virus Y (PVY) between plants (Parry 1990).

Currently on Prince Edward Island, insectides such as Monitor® and Pirimor® are being used to control aphid populations. Monitor® has the active ingredient methamidophos, which inhibits acetylcholinesterase action and results in death (*Bayer

---

* Refers to a reference from a company monograph, government website or university website.

1997; *EPA 1999; *EPA 2000; *EXTOXNET 2001). Pirimor® is also an acetylcholinesterase inhibitor with the active ingredient pirimicarb (*Zeneca 1999; *Cornell University 2001). Even though they have been very effective, these aphicides are also very toxic (*Zeneca 1997; *EXTOXNET 2001) and are not suitable for integrated pest management programs. There is also evidence that aphids are developing resistance to current chemicals, rendering the chemicals ineffective for aphid control (Parry 1990; Bohmont 2000). The Prince Edward Island potato industry requires a new, selective aphicide, with low environmental impact, to control aphid populations and to limit the spread of potato viruses.

Fulfill® 50 WG is one such pesticide that is currently being considered for registration in Canada. This product is currently registered for use in the United States and in Europe. Due to the low toxicity of Fulfill® to non-target organisms, Fulfill® received accelerated review by the United States Environmental Protection Agency (Robert Coffin, Plant Pathologist, Cavendish Farms Research Division, New Annan Prince Edward Island, personal communication). Fulfill® contains the active ingredient, pymetrozine (Kristinsson 1994) which belongs to a new class of chemical pesticides called the pyridine azomethines (Kristinsson 1994; Harrewijn and Kayser 1997; Wyss and Bolsinger 1997a; Wyss and Bolsinger 1997b). Fulfill® is not reported to exert a general toxic effect on aphids but instead induces antifeedant activity on this sucking insect (Kristinsson 1994; Harrewijn and Kayser 1997). Pymetrozine targets the operation of the nervous system regulation of the cibarial valves, the food pump and the salivary pump, and leads to starvation (Harrewijn and Kayser 1997; *Novartis Crop

Protection 1998). The low impact of Fulfill® on beneficial organisms makes it a suitable pesticide for IPM programs (Flückiger et al. 1992; Follas and Blanc 1995).

Although the American Fulfill® label contains a recommendation for the addition of a penetrating adjuvant during application, there is no recommendation, nor any documented data as to which adjuvants maximize the efficacy of Fulfill® (*Novartis Crop Protection 1999a). Syngenta did not have to provide any evidence that adjuvants increase the efficacy of Fulfill® to include this recommendation on its label, since adjuvants are not registered products in the United States (*Baysinger and DeFelice 2000; *Petroff 2000). In Canada, adjuvants are required by law to be registered. For an adjuvant to receive Canadian registration, there must be evidence that the adjuvant either increases the efficacy of the pesticide, or increases the spectrum of activity of the pesticide (*PMRA 1993). For Syngenta to register Fulfill® in Canada with a label recommending the use of adjuvants with this aphicide, they must provide evidence that adjuvants increase the efficacy of Fulfill® or increase its spectrum of activity.

Adjuvant data is very important as using the wrong adjuvant can limit pesticide efficacy and even damage foliage (*Hock 1994; Bohmont 2000). To date, only two studies mention the addition of an adjuvant during application of Fulfill®. Bedford et al. (1998) used a wetting adjuvant with the tradename 'Codacide®' produced by Microcide Ltd, while Sweeden and McLeod (1997) used the surfactant 'Kinetic®' which is manufactured by Setre Chemical Company. It is important to note that neither of these studies involved potato plants. Wyss and Bolsinger (1997a), found that pymetrozine is

distributed differently in the tissues of different plant species, so the activity of pymetrozine in different species is not necessarily comparable.

Adjuvants are commonly used in the pesticide industry because they increase pesticide coverage on foliage and thus increase pesticide efficacy. Adjuvants act by altering the surface tension of spray droplets after they settle on plants. (Tadros1984; Van Emden 1989; Bohmont 2000). Penetrating adjuvants are surfactants that are specially formulated to increase the penetration of pesticides through plant cuticles (*Hock 1994; Bohmont 2000). This increased penetration is particularly important for systemic pesticides which depend on the penetration and translocation of the compound through the plant (Bohmont 2000). Mineral oils are another type of adjuvant that increase penetration of pesticides through plant cuticles (*Hock 1994; Pree et al. 1996; Horowitz et al. 1997).

LI 700® is a commonly used surfactant. It is a non ionic, penetrating, translocating surfactant and pH adjuster consisting of phosphatidylcholine, methylacetic acid and alkyl polyoxyethylene ether (*Loveland Industries 2002). The label for this adjuvant recommends use with pesticides that require wetter/spreader or adjuvant oil. LI 700 is also appropriate for use with hard or alkaline water when a pH adjustment is needed to prevent alkaline hydrolysis (*Loveland Industries 2002).

Superior 70 Oil® is an emulsifiable mineral oil that is commonly used as an adjuvant (*Belau 2001). Like most mineral oils, Superior 70 Oil® is 97% light and heavy paraffinic distillates and 3% emulsifiers (*Hock 1994; *Belau 2001). Superior 70 Oil® is reported to increase the penetration of some pesticides through the plant cuticle (*Belau

2001).

Although Citowett Plus® was used during lab experiments, it was not included in the field experiment. The Fulfill® label recommends using a penetrating surfactant during application (*Novartis Crop Protection 1999a), and since Citowett Plus® is a spreader sticker (*BASF 1998), it was not included in this experiment for that reason.

The objectives of this experiment are 1) to compare the efficacy of Fulfill® in controlling green peach aphids to the current aphicides Monitor® and Pirimor® under Prince Edward Island field conditions and 2) to determine which adjuvant, if any, enhances the efficacy of Fulfill®.

3.1 MATERIALS AND METHODS

3.1.1. Aphids

Green peach aphids, *Myzus persicae*, were obtained from Agriculture and Agri-food Canada, Potato Research Centre, Fredericton courtesy of Dr. Gilles Boiteau. Potato aphids, *Euphorbiae macrosiphum*, were collected from a potato field in New Annan Prince Edward Island. Aphids were maintained on potato leaves in petri dishes, or on potato plants which were kept in a growth chamber set at 21° C, 80% humidity, and on a light/dark schedule of 14/10 hours.

3.1.2 Efficacy Comparison Study (2001)

A potato field in New Annan, Prince Edward Island (46° 25' 89" N; 63° 40' 53" W) was divided into 24 plots. Each plot was 9 m long and consisted of two rows of Shepody and two rows of Russet Burbank. Shepody potatoes were planted at 25.4 cm intervals whereas Russet Burbank potatoes were planted at 45.7 cm intervals. The distance between rows was the standard spacing of 91.44 cm. A distance of 3.6 m separated each plot to minimize any spray drift. Experimental plots were arranged in a randomized block design involving six treatments that were replicated four times. Planting occurred during late May in both 2001 and 2002.

A three point Hardi sprayer with flat fan nozzles and 2 atm pressure using 40.5 litres of water/hectare/spray volume was used to apply aphicides. Treatments included Fulfill® plus LI 700, Monitor®, Pirimor®, Superior 70 Oil®, Superior 70 Oil® plus Fulfill®, and a control group that was not sprayed with any insecticide. LI 700®, a non-

ionic penetrating surfactant, was used as an adjuvant to Fulfill®. LI 700® was applied at the label rate of 5 litres/1000 litres of spray volume. Fulfill® was applied at the label rate of 196 grams per hectare. Monitor® was applied at the mid-rate of 2 litres per hectare. Pirimor® was applied at the mid-rate of 500 grams per hectare. Superior 70 Oil® was applied at the rate of 2% of spray volume (0.9 litres per hectare).

Before spraying began, three aphid bags were tied around leaflets approximately 2/3 of the way up of three randomly selected plants in each of the 24 treated plots. Aphid bags were constructed from pollination bags with a plastic window added to one side of the bag (Figure 3.0). Bags were approximately 6 cm wide and 11 cm long. Bags were only attached on leaflets of plants located in the two inside rows. The outside rows of each variety acted as a guard row for each plot. Each aphid bag contained 30 aphids which were randomly selected from the lab culture. Aphids were transferred into bags using a small paint brush. Each bag was checked daily for nine days to determine the total number of living aphids remaining in each bag.

3.1.2.1 Natural Aphid Population (2001): In addition to counting the number of aphids in each bag during the efficacy field experiment, five lower leaves per plot were randomly chosen each day and were searched for aphids that had moved into the field, as is commonly expected during July in Prince Edward Island. This was to measure the effect that each aphicide had on aphids moving naturally into the field.

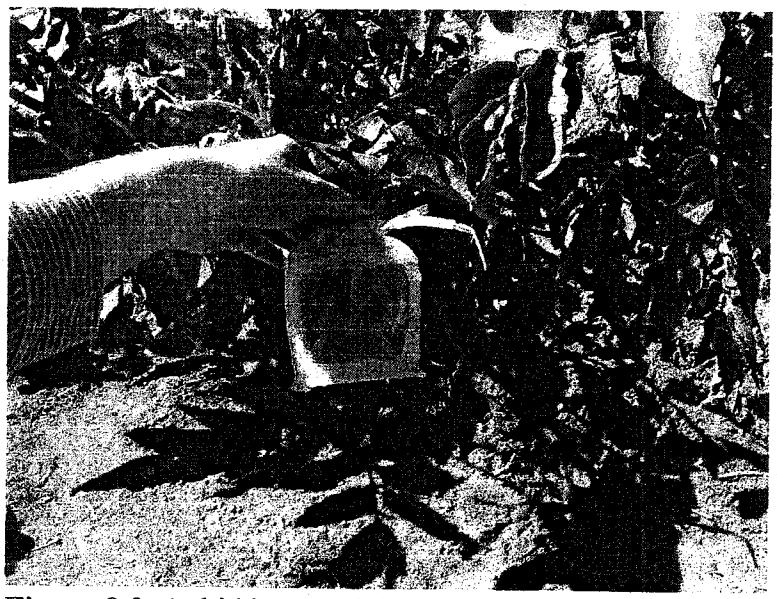


Figure 3.0. Aphid bag used during field studies.

3.1.3 Complementary Field Study (2001)

Approximately two weeks following the spraying of the efficacy comparison study, a smaller field experiment was designed to address the concern that the plastic window in the aphid bags used during the efficacy comparison study were reducing the amount of Fulfill® coming into contact with both the aphids and the leaves. Three of the control plots used in the efficacy comparison study were used for this complementary field experiment. One plot was not sprayed with any insecticide, one plot was sprayed with Fulfill® without an adjuvant, and one sprayed with Fulfill® and the adjuvant LI 700®. In each plot, six aphid bags were placed on each variety before plots were sprayed. Three of these bags had a clear plastic window on one side (Fulfill® ^{baggedwindow}) (like the bags used in the field efficacy comparison study) whereas the other three bags had no window (Fulfill® ^{baggednowindow}). The efficacy of Fulfill® with these two types of bags was then compared to ensure that Fulfill® was penetrating through the bags, even though a plastic window was covering the basal side of the leaf. Thirty aphids were placed in each bag prior to spraying. Bags were checked daily over a seven day time period to evaluate aphid survivorship in each treatment.

In both the Fulfill® and the Fulfill® $^{LI700®}$ plots an additional three bags were used during this experiment. In these plots, three leaves were randomly selected and 30 aphids were placed on each leaf prior to spraying (Fulfill® ^{direct}). Approximately one hour following spraying, these leaves were covered with aphid bags. This was done to determine whether Fulfill® would exhibit greater control of aphids that had direct contact with Fulfill® as well as exposure via feeding. Bags were checked daily for seven

days to determine the total number of living aphids in each bag.

3.1.4 Complementary Lab Study (2001)

A lab experiment was conducted to complement the efficacy comparison study. This lab study was designed to investigate the possibility that aphid bags were a significant factor in limiting the activity of Fulfill® in the efficacy comparison study. In the lab study, leaves were sprayed in the field, and then transported to the lab where aphids were placed on them. Three leaves in each of the treatment groups (control, Fulfill®, Fulfill® $^{LI700®}$) were covered with aphid bags during spraying. Forty-eight hours following treatment with Fulfill® aphicide, bags were removed and these leaves were collected. Leaves sprayed directly with Fulfill® were also collected from each of the treatment groups. The aphid control exerted by Fulfill® in bagged (Fulfill® ^{bagged}) and non-bagged (Fulfill® ^{notbagged}) leaves was compared to determine the effect of the bags on the efficacy of Fulfill®. Leaflets were maintained in florist water picks during transport to the lab, until they were placed in vials filled with water and then placed in separate petri dishes. Fifteen aphids were placed in each petri dish. Petri dishes were placed in clear tupperware containers which were kept in a growth chamber set at 21° C, 80% humidity, and on a light/dark schedule of 14/10 hours. Each petri dish was checked at 24 hour intervals for seven days. The number of aphids feeding, giving birth, walking on the leaf, walking on the dish, and the number of dead aphids was evaluated each day. An aphid was considered to be feeding if its stylet was penetrating the surface of the leaf.

3.1.5 Adjuvant Field Study (2002)

A potato field in New Annan, Prince Edward Island (46° 25' 89" N; 63° 40' 53" W) was divided into 24 plots. Each plot was 6 m long and contained four rows of Shepody potatoes planted at 25.4 cm intervals. The distance between rows was the standard spacing of 91.44 cm. A distance of 3.6 m separated each plot. Experimental plots were arranged in a randomized block design involving six treatments that were replicated four times. Treatments included Fulfill®, LI 700®, Superior 70 Oil®, Fulfill® and LI 700®, Fulfill® and Superior 70 Oil®, and a control group that was not sprayed with any insecticide. A three point Hardi sprayer with flat fan nozzles and 2 atm pressure using 40.5 litres of water/hectare/spray volume was used to apply Fulfill® and the adjuvants. LI 700® was applied at the label rate of 5 litres/1000 litres of spray volume. Fulfill® was applied at the label rate of 196 grams per hectare. Superior 70 Oil® was applied at the rate of 2% of spray volume.

Before spraying began, three aphid bags were tied around leaflets approximately 2/3 of the way up the plant in each of the 24 treated plots. Aphid bags were constructed from pollination bags with a plastic window added to one side of the bag (Figure 3.0). Bags were only attached on leaflets of plants located in the two inside rows. The outside rows of the plots acted as a guard row for each plot. Each aphid bag contained 30 aphids which were randomly selected from the lab culture. Each bag was checked daily for eight days to determine the total number of living aphids remaining in each bag.

3.1.5 Statistical Analysis

Daily patterns in aphid abundance were evaluated by plotting the total number of aphids that were alive in the aphids bags in all treatments on each sample day. One-way ANOVA was used to compare aphid survival among treatment groups at the end of the experiment. In cases where the data were not normal and could not be normalized by transformations, but all other assumptions of the ANOVA were met, the non-parametric equivalent of the one-way ANOVA, the Kruskal-Wallis test was used (Sprent 1989; Dytham 1999). In cases where ANOVA or Kruskal-Wallis tests indicated that there was a significant level of difference between the groups, t-tests and Mann-Whitney-U tests (the non-parametric equivalent of the t-test) were used to determine which treatment groups were significantly different from one another (Dytham 1999). Bonferonni corrections were performed for all multiple comparisons to avoid the possibility of inflating the type 1 error (Milton 1992; G.E. Dallal, personal communication). In cases where data were normal but groups had an unequal variance, one-way ANOVA tests were performed using Dunnett T3 post hoc tests (Dunnett 1980).

3.2 RESULTS

3.2.1 Efficacy Comparison Study (2001)

There was no significant difference between aphid survivorship in Shepody and Russet Burbank varieties so data from the two groups were combined into one data set (Kruskal-Wallis; $p > 0.05$). There were also no significant differences in aphid survivorship among bags from the same plot and among different plots of the same treatment. These groups were also combined (Kruskal-Wallis, Mann-Whitney U Test; $p > 0.05$).

Treatments in this study resulted in varying degrees of aphid control in the following gradient from most effective to least effective treatment: Monitor®, Pirimor®, Fulfill® Superior Oil®, Superior 70 Oil®, Fulfill® LI700®, and control. The number of aphids in the control group decreased from 360 to 229 during the nine day field experiment. The number of living aphids in bags exposed to Monitor® decreased from 360 to 0 after six days. After nine days, aphid numbers decreased from 360 to 14 in the Pirimor® group, decreased from 360 to 169 in the Superior 70 Oil® group, decreased from 360 to 210 in the Fulfill® LI700® group and decreased from 360 to 85 in the Fulfill® Superior Oil® group (Figure 3.1). Monitor®, Pirimor® and Fulfill® Superior Oil® treatment groups all had significantly lower aphid survivorship than the control group (Mann-Whitney U with Bonferronni corrections; $p < 0.05$). Monitor® and Pirimor® treatment groups also had significantly lower aphid survivorship than the Fulfill® LI700® group (Mann-Whitney U with Bonferronni corrections; $p < 0.05$).

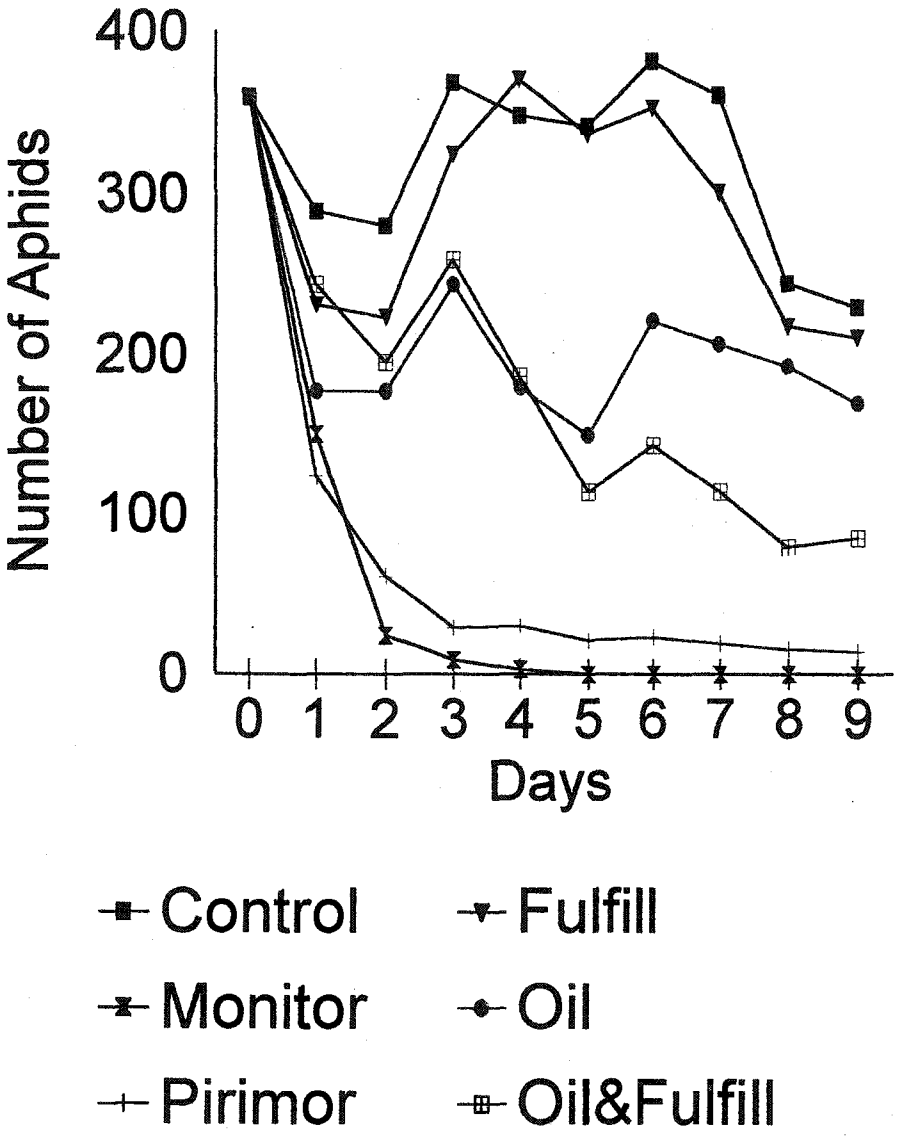


Figure 3.1. Efficacy Comparison Study: Total aphid survivorship in plots after treatment with Monitor, Fulfill+LI700, Pirimor, Fulfill+Superior Oil, Superior Oil and compared to the control plots.

3.2.1.1 Natural Aphid Population (2001)

The natural aphid population in the field fluctuated daily among treatments. By day seven, all plots, including the control, had three or fewer aphids. The largest number of aphids per plot was 26 on day two in the Superior 70 Oil® plot (Figure 3.2).

3.2.2 Complementary Field Study (2001)

During the complementary field study, Fulfill® and Fulfill® LI700® offered excellent aphid control in both the 'baggedwindow' and 'baggednowindow' groups while aphids in the control 'baggedwindow' and 'baggednowindow' groups increased exponentially. Aphids in the control^{baggedwindow} group increased from 180 to 476 over the seven day test period while the Fulfill®^{baggedwindow} group decreased from180 to 5 and the Fulfill®LI700®^{baggedwindow} group decreased from 180 to 40 (Figure 3.3). The differences in aphid survival among these three groups was not statistically significant possibly due to large variations within treatments and small sample sizes (ANOVA; Dunnett T3; $p > 0.05$). During this same time period, aphids in the control^{baggednowindow} group in the aphid bag without windows increased from 180 to 267 and aphids in the Fulfill® LI700®^{baggednowindow} group decreased from 180 to 36. There was no significant difference in aphid survival between the 'baggedwindow' and 'baggednowindow' groups (Figure 3.4) (ANOVA; Dunnett T3; $p > 0.05$).

Fulfill®'s efficacy did not increase when aphids were directly sprayed with Fulfill®. Aphids in the group which was sprayed directly with Fulfill®^{direct} and the group sprayed directly with Fulfill® LI700®^{direct} both decreased from 90 to 0 after seven days

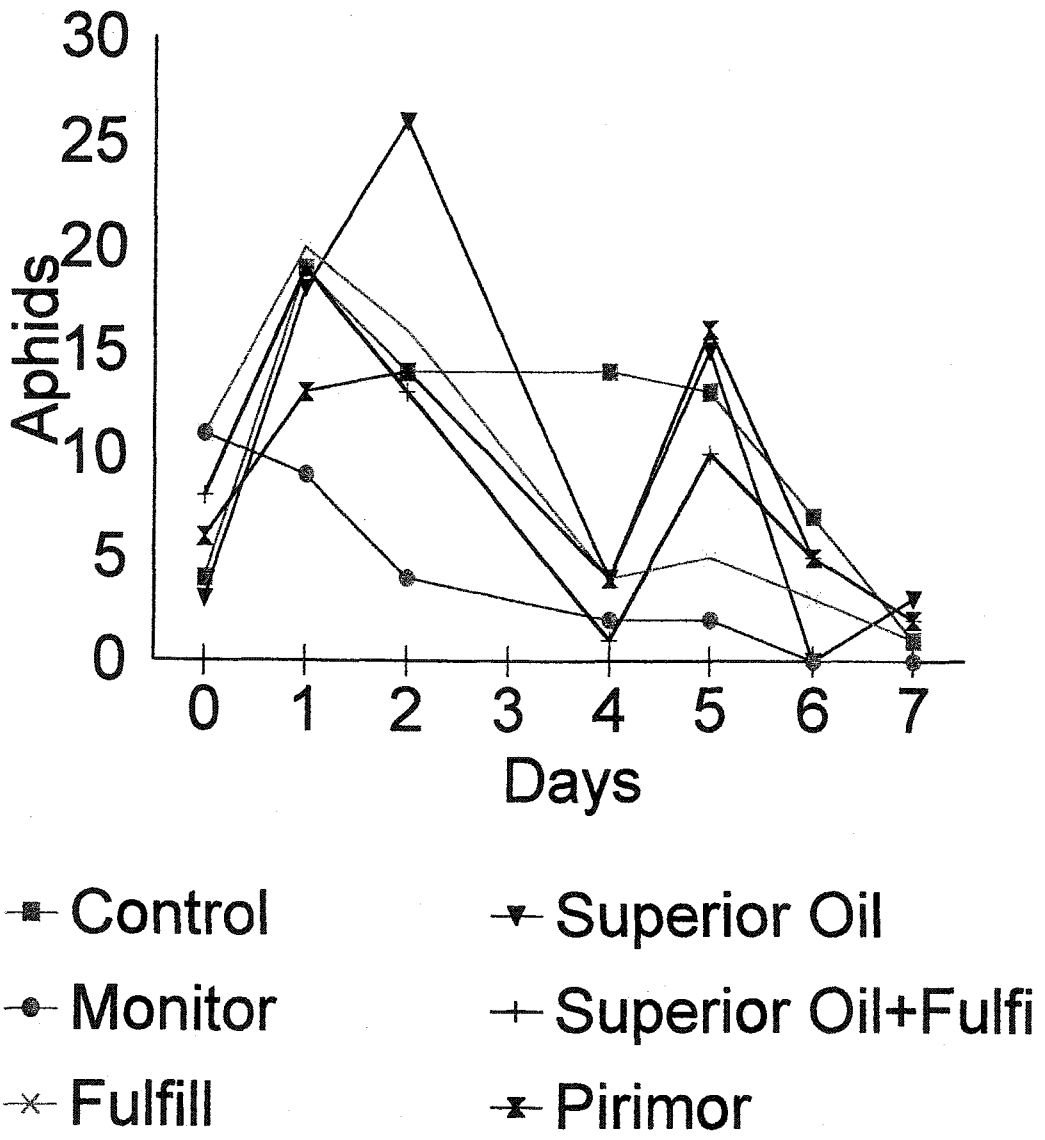


Figure 3.2. Natural aphid population on five leaflets per plot within treatment plots in field experiment.

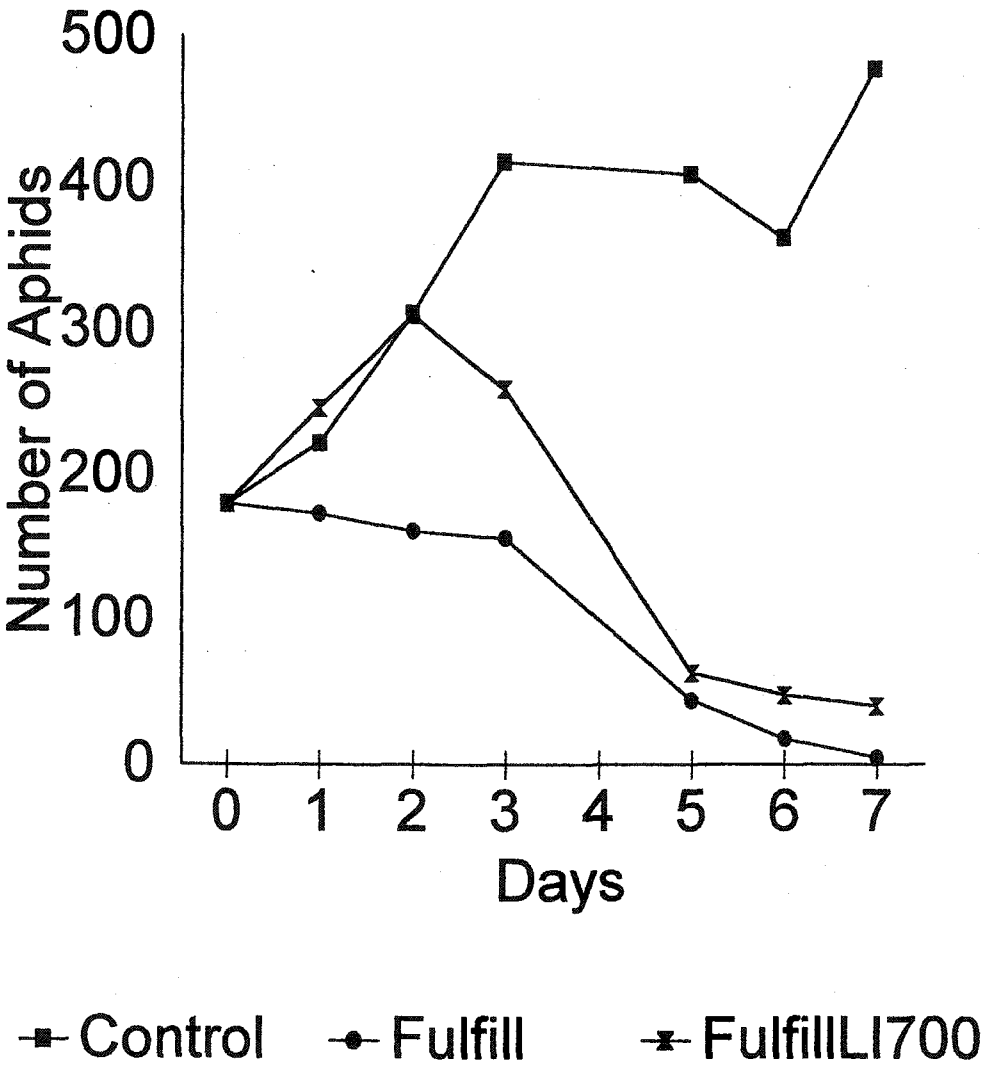


Figure 3.3. Complementary Field Study. Total number of living aphids in control plots and in plots treated with Fulfill and Fulfill^{LI700}.

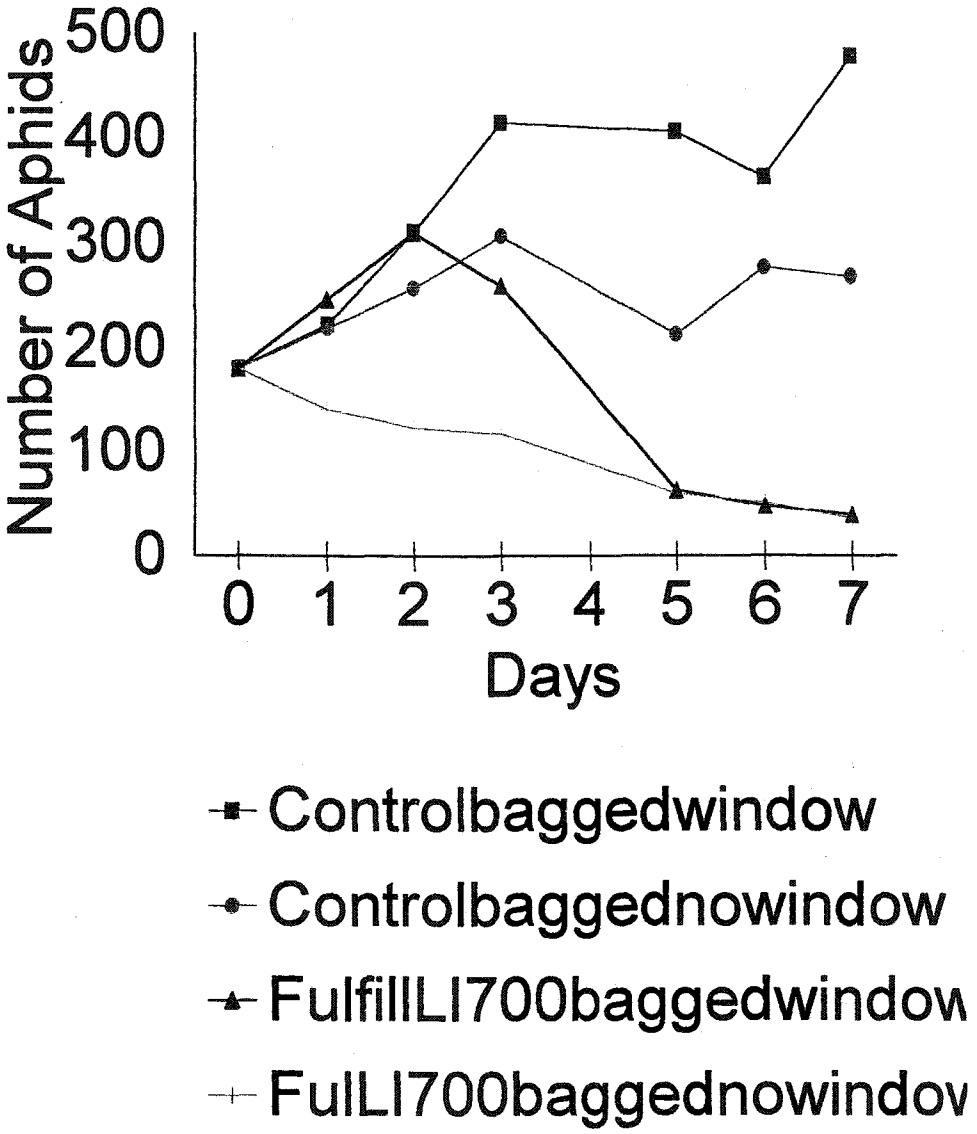


Figure 3.4. Complementary Field Study. The effect of the plastic window on aphid bags to the efficacy of Fulfill. Controlbaggedwindow and FulfillLI700baggedwindow aphids were contained within an aphid bag with a plastic window while controlbaggednowindow and FulfillLI700baggednowindow were contained within an aphid bag without a window.

(Figure 3.5). These results were not significantly different from the Fulfill®baggedwindow treatments that were bagged before being sprayed (ANOVA; Dunnett T3; $p > 0.05$).

3.2.3 Complementary Lab Study (2001)

Aphids in the control group increased from 60 to 588 during the seven day period, while aphids in the Fulfill® group decreased from 60 to 3 and aphids in the Fulfill®LI700® group increased from 60 to 98. Aphids in the Fulfill®bagged group decreased from 60 to 1 while aphids in the Fulfill®LI700® bagged group decreased from 60 to 19. There was no significant difference in the number of living aphids in the Fulfill® and Fulfill®bagged groups (ANOVA; Dunnett T3; $p>0.05$). There was also no significant difference between the number of living aphids in the Fulfill® LI700® and Fulfill® LI700® bagged groups (Figure 3.6) (ANOVA; Dunnett T3; $p>0.05$).

3.2.4 Adjuvant Field Study (2002)

In all treatments but LI 700®, there were no significant differences in aphid survivorship among bags from the same plot and among different plots of the same treatment. These groups were therefore combined into one data set (Kruskal-Wallis, Mann-Whitney U Test; $p > 0.05$).

The number of aphids in the control group and in the adjuvant groups increased exponentially over the eight day field experiment. The number of aphids in the control group increased from 360 to 607 during the eight day field experiment. The number of living aphids in bags exposed to LI 700® increased from 360 to 712 after eight days and

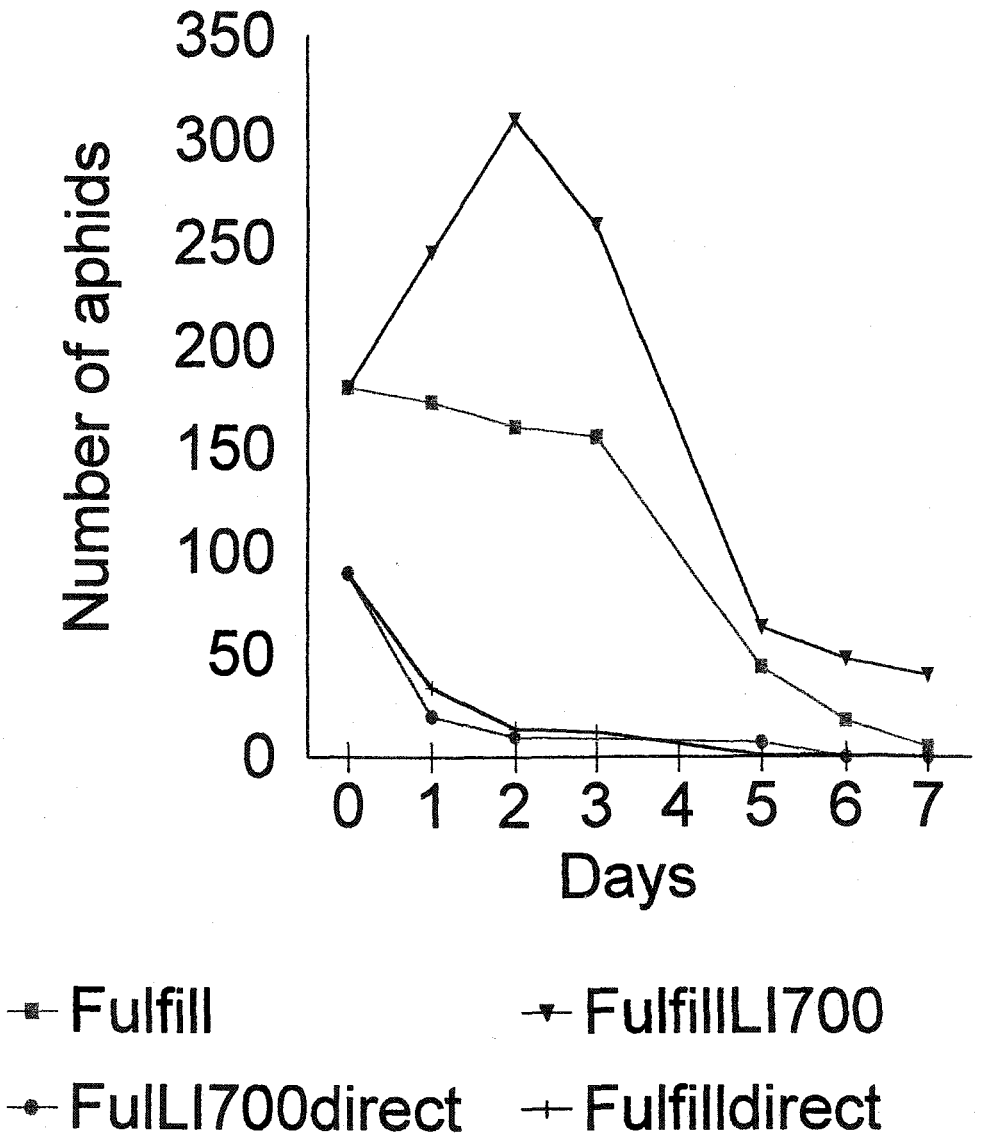


Figure 3.5. Complementary Field Study: Comparison of the efficacy of Fulfill® in aphids sprayed directly with Fulfill® and Fulfill®LI700® (Fulfilldirect and FulLI700direct) and those contained within an aphid bag during spraying (Fulfill and FulfillLI700).

from 360 to 520 in the Superior 70 Oil® group.

In this experiment, Fulfill® offered excellent aphid control regardless of the type of adjuvant used. Aphid numbers decreased from 360 to 15 in the Fulfill® group, decreased from 360 to 18 in the Fulfill® LI700® group and decreased from 360 to 29 in the Fulfill® Superior Oil® group after eight days (Figure 3.7). Fulfill®, Fulfill® LI700® and Fulfill® SuperiorOil® treatment groups all had significantly lower aphid survivorship than the control group (ANOVA; Dunnett T3 $p< 0.05$). There were no significant differences in aphid survivorship among Fulfill®, Fulfill® LI700® and Fulfill® SuperiorOil® treatment groups (ANOVA; Dunnett T3; $p> 0.05$).

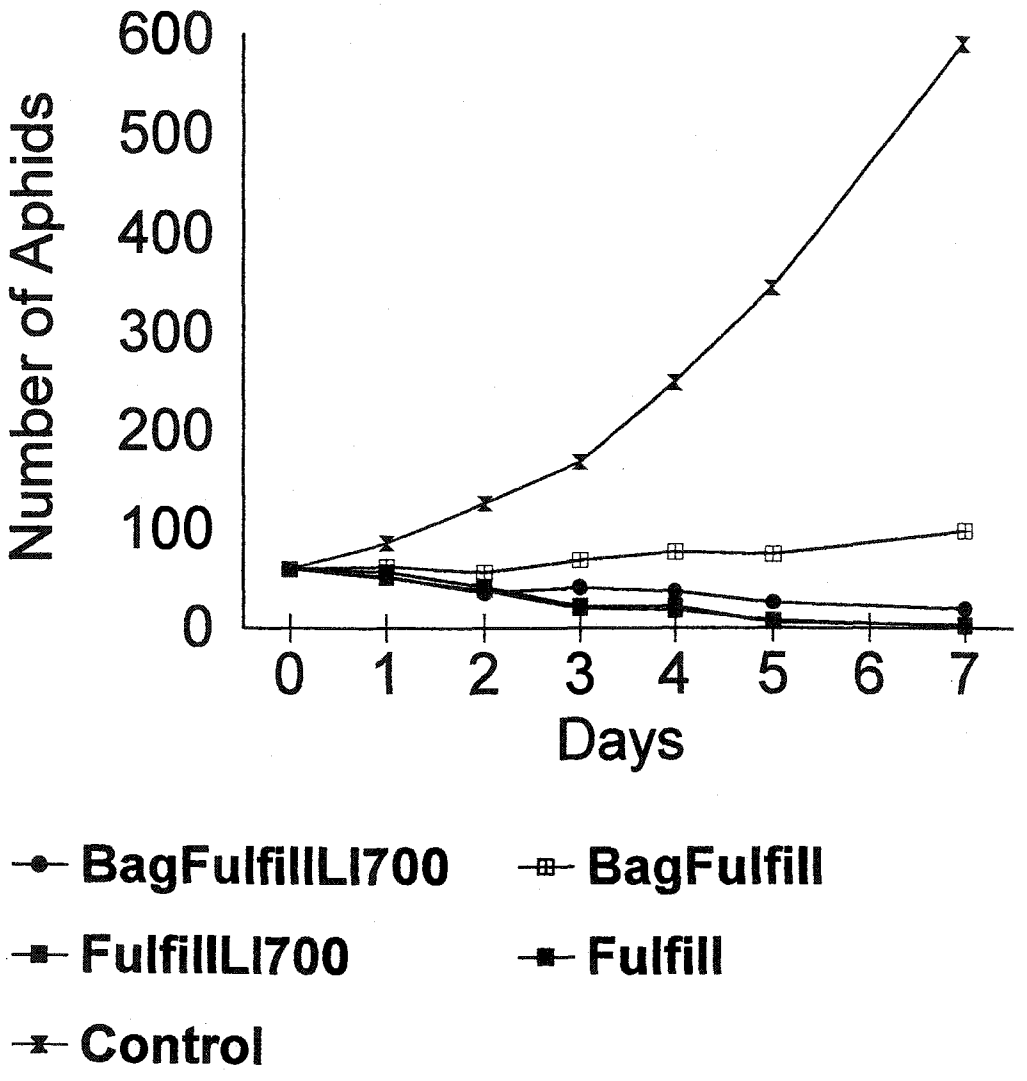


Figure 3.6. Complementary Lab Study: Effect of Bag and Adjuvant on Aphid Survivorship. 'BagFulfillLI700' and 'BagFulfill' refer to aphid survival on leaves that were covered with an aphid bag during spraying. 'Fulfill' and 'FulfillLI700' refer to aphid survival on leaves that were sprayed directly with Fulfill®.

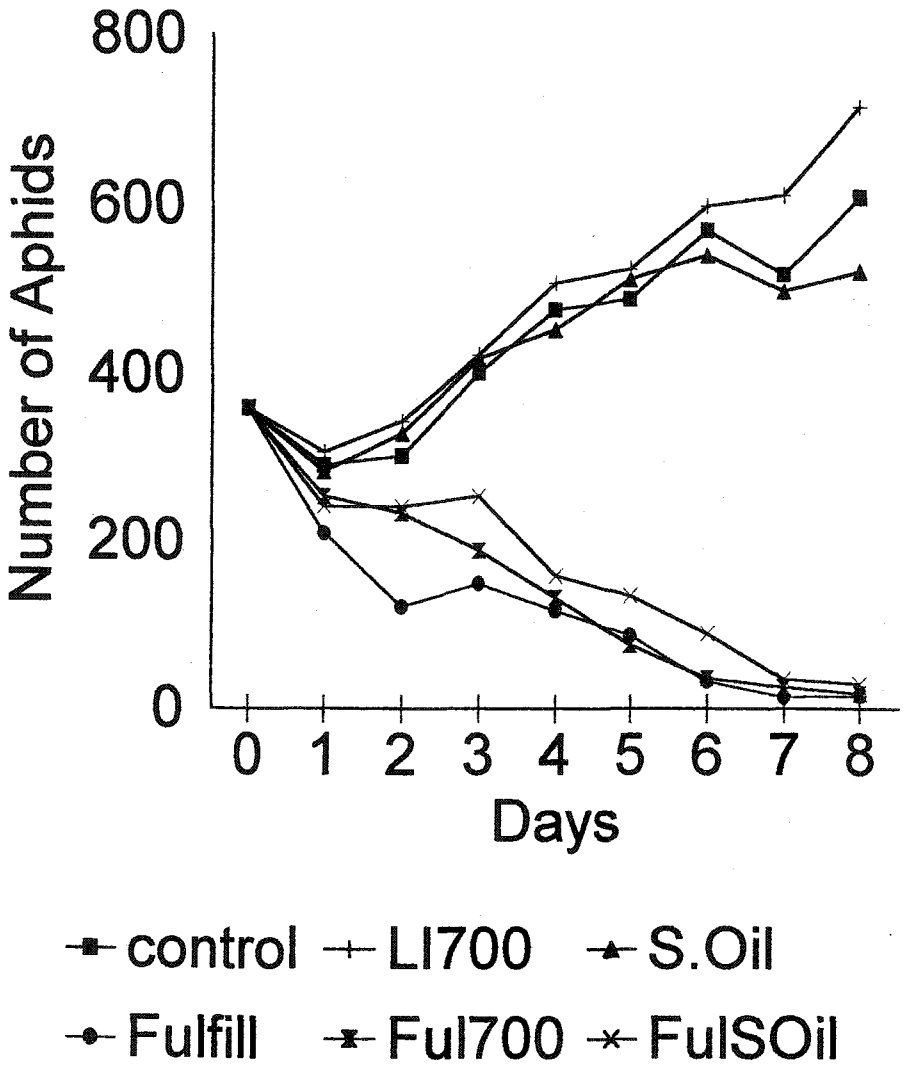


Figure 3.7. Adjuvant Field Study. Aphid survival in plots treated with LI 700®, Superior 70 Oil® (S.Oil), Fulfill®, Fulfill® with LI 700® (Ful700) and Fulfill® with Superior 70 Oil® (FulSOil), as well as in the control group.

3.3 DISCUSSION

3.3.1 Overview

Fulfill® offered excellent aphid control in every study except for the efficacy comparison study. The efficacy comparison study took place during a year of drought conditions (Environment Canada 2002) and this factor likely limited the efficacy of Fulfill®. Fulfill® is a systemic pesticide that relies on penetration into the plant and translocation throughout the plant to be effective (Wyss and Bolsinger 1997a; Wyss and Bolsinger 1997b). Both translocation and penetration are decreased when plants are under drought stress (Kramer 1969; Itai and Benzioni 1976; Sutcliffe1979). Adjuvants did not significantly increase Fulfill®'s efficacy in any of the experiments. The aphid bags used in this study did not significantly effect the efficacy of Fulfill®.

3.3.2 Efficacy Comparison Study (2001)

In the field efficacy comparison study, treatments exhibited varying degrees of aphid control in the following gradient from most effective to least effective treatment: Monitor®, Pirimor®, Fulfill® Superior Oil®, Superior 70 Oil®, Fulfill® LI700®, and control. Both Pirimor® and Monitor® are cholinesterase inhibitors (*Bayer 1997; *Zeneca 1997) and have shown excellent aphid control in many studies (Harding 1973; Bacon et al. 1976; Woodford et al. 1983; Hanafi et al. 1989; *Radcliffe 1998b; *Mowry et al. 2000; Cornell 2001). Pirimor® and Monitor® are also more toxic than Fulfill® and act primarily through direct contact (*Cornell University 2001; *EXTOXNET 2001). Unexpectedly, Fulfill® LI700® did not exhibit aphid control that was significantly greater than the control

group. Unlike Monitor® and Pirimor®, which are contact insecticides, Fulfill® is a systemic aphicide that depends primarily on exposure during feeding to exert aphid control. To be effective, this systemic aphicide must penetrate the plant cuticle and move into the xylem and phloem where it is translocated throughout the plant (Wyss and Bolsinger 1997a; Wyss and Bolsinger 1997b).

During the test period (2001), the plants in our test plots were extremely stressed due to drought conditions. Drought has a large impact on both plant cuticles and translocation within a plant. These two changes within plants may have limited the activity of Fulfill® during this field trial. The efficiency of chemical uptake in plants depends greatly on the nature of the plant cuticle (Martin and Juniper 1970). The cuticle is a protective layer surrounding the epidermal cells of leaves. It is comprised of cutin, suberin, waxes and tannins (Martin and Juniper 1970). The plant cuticle therefore, acts as the first barrier to chemicals, including pesticides, that are applied to plants (Martin and Juniper 1970). Water stress triggers hormonal changes which directly induces changes in cuticle permeability to water and solutes (Itai and Benzioni 1976). Further, with respect to leaf morphology, water stress generally causes a reduction in leaf area and an increase in cutinization and leaf thickness (Kramer 1969). These typical changes induced in the cuticle of plants by drought stress are thought to have limited penetration of the chemical into the study plants, thereby reducing the efficacy of the product. The penetration of aqueous solutions through stomates is insignificant in comparison to their penetration through the cuticle (Martin and Juniper 1970). Consequently, the closure of stomates during drought is not thought to have greatly influenced the penetration of

Fulfill® into our plants.

Translocation of Fulfill® through the phloem has a major impact on the systemic action of the compound (Wyss and Bolsinger 1997b). During a period of water stress, plants show a reduction in translocation (Kramer 1969; Itai and Benzioni 1976; Sutcliffe 1979). This reduction in translocation could also have limited the movement of Fulfill® through the plant, thereby limiting its efficacy. Boiteau et al. (1985) reported a decrease in efficacy of a systemic insecticide due to a lower concentration of the pesticide within potato leaves. This low concentration of the insecticide in leaves was attributed to limited pesticide uptake during a period of water stress (Boiteau et al. 1985).

Superior 70 Oil® was not expected to control aphids, because Superior Oil is used as an adjuvant to increase penetration of pesticides, but does not offer aphicidal activity on its own (*Belau 2001). Oil sprays are also used to prevent the spread of PVY (*Diamond et al. 1996). Oils interfere with the transmission of viral particles from aphid stylets to plants during probing by "washing" virus particles off of stylets (Gibson et al. 1984).

The combination of Fulfill® and Superior 70 Oil® did reduce aphid numbers to levels that were significantly lower than in the control group. One of the changes induced by water stress in cuticles, is an increase in lipophilic areas (Lee-Stadelmann and Stadelmann 1976), which would facilitate increased penetration of Fulfill® and Superior 70 Oil® through the cuticle. Mineral oil additives have had synergistic effects when administered with insecticides in many other studies by increasing penetration through plant cuticles (Treacy et al. 1991; Pree et al. 1996; Horowitz et al. 1997).

Gibson and Rice (1986) reported similar results and postulated that increased insecticide efficacy might also be attributed to an increase in the penetration of the insecticide into the aphid itself. In this field study, Superior 70 Oil® was a much more effective adjuvant than LI 700®, which was unexpected since the Fulfill® label recommends using a penetrating adjuvant during application (*Novartis Crop Protection 1999a).

3.3.2.1 Natural Aphid Population (2001)

In contrast to the field efficacy experiment, no differences were found among treatments in the natural aphid population. The natural aphid population of resident aphids in our test plots was extremely low during the entire field experiment, even though some aphids were imported from other fields. The natural population of aphids was too low to make any comparisons of aphid control among treatment groups. Further, Ladybird beetles were present in very high numbers within our test plots. These beetles appeared to be a major factor in the decimation of the natural aphid population in our field experiments.

3.3.3 Complementary Field Study (2001)

During this field study, variations within the control group made statistical analyses difficult; however, the trend is very apparent. In contrast to the efficacy comparison study, both Fulfill® and Fulfill® LI700® gave excellent control against aphids. The air temperatures during the complementary field study were much cooler and precipitation was higher than during the efficacy comparison study (Environment

Canada 2002). Under these conditions, translocation would have been occurring at a higher rate than during the efficacy comparison study. This may explain the difference in efficacy for Fulfill® between the efficacy comparison study and the complementary field study.

According to our results, the adjuvant LI 700® did not increase the efficacy of Fulfill®. This was unexpected since the Fulfill® label recommends using a penetrating adjuvant (*Novartis Crop Protection 1999a). Although the Fulfill® label makes this recommendation, no data was found in published reports or from *Novartis Crop Protection which document the improved efficacy of Fulfill® with the use of a penetrating adjuvant.

When aphids were placed on leaves, sprayed directly with Fulfill® and Fulfill®LI700® and then covered with an aphid bag, aphid control took place at a faster rate than when aphids were covered with the aphid bag prior to spraying. However, by the end of the seven day period, Fulfill® and Fulfill® LI700® gave similar results in terms of aphid control whether leaves were bagged or not prior to spraying. Therefore, although Fulfill® did work primarily by exposure via feeding, contact action did slightly increase the rate of action of this chemical. This is consistent with findings by Flückiger et al. (1992).

After the efficacy comparison study, there was some concern that the window on the aphid bag facing the underside of the leaf was shielding the leaf from pesticides during spraying and that this limited the efficacy of Fulfill®. However, by the end of the complementary field study, Fulfill® LI700® exhibited the same aphid control whether the

aphids were in a bag with a window during spraying or in a bag without a window. Fulfill® LI700® acted at a faster rate on the aphids bags without a window but in the end, the level of control was the same. This demonstrates that Fulfill® does move translaminarly through potato leaves as was reported by Flückiger et al. (1992) and *Novartis Crop Protection (1999a).

3.3.4 Complementary Lab Study (2001)

To ensure that the aphid bags themselves weren't contributing to the low efficacy of Fulfill® during the efficacy comparison field study, aphids were placed on leaves that had been covered with aphid bags before or after spraying in the field. There were no significant differences between aphid control on leaves bagged before or after spraying. This demonstrates that the bags were not a contributing factor to the low efficacy displayed by Fulfill® during the efficacy comparison study.

3.3.5 Adjuvant Field Study (2002)

During the adjuvant field study, Fulfill®, Fulfill® LI700®, and Fulfill® Superior Oil® exhibited excellent aphid control. Plants in the adjuvant field study were not water stressed and were much healthier than plants in the efficacy comparison trial (2001). This provides further evidence that drought conditions during the efficacy comparison trial were limiting the efficacy of Fulfill®. Under normal field conditions for PEI, such as those experienced during the adjuvant field study in 2002, aphid control by Fulfill® was comparable to the control offered by Monitor® and Pirimor® in the efficacy

comparison trial during 2001. Since Fulfill® is much less toxic to other organisms than Monitor® and Pirimor® (*Novartis Crop Protection 1998; *Cornell University 2001; *EXTOXNET 2001), it is recommended that Fulfill® be used instead of these two chemicals for aphid control when plants are not under water stress.

The adjuvants LI 700® and Superior 70 Oil® did not increase the efficacy of Fulfill®. This was surprising since Syngenta, the manufacturer, recommends using a penetrating adjuvant with Fulfill® (*Novartis Crop Protection 1999a). However, not all adjuvant formulations increase pesticide efficacy, as was seen by French II et al. (1992) with the pesticide chlorpyrifos. Since one of the best qualities of Fulfill® is its low toxicity to beneficial organisms, using an adjuvant that is toxic to fish and aquatic organisms, such as Superior 70 Oil® (*Belau 2001), would lessen the benefits of using Fulfill® as an aphicide. This is especially true under conditions where adjuvants do not increase the efficacy of Fulfill®. However, Fulfill® applied with an adjuvant still has a lower toxicity to non-target organisms than Monitor® or Pirimor® (*Novartis Crop Protection 1998; *Belau 2001; *Cornell University 2001; *EXTOXNET 2001). Based on the results of this study, Fulfill® should only be applied with an adjuvant under drought conditions.

3.4 CONCLUSION

During drought conditions, Fulfill® failed to exhibit aphid control that was equal to the current insecticides Monitor® and Pirimor®. Changes in the plant cuticle and a decreased rate of translocation induced by drought conditions are believed to be factors that limited the efficacy of Fulfill® in this study. Under field conditions where plants

were not under drought stress, Fulfill® provided excellent aphid control. Although the Fulfill® label recommends using a penetrating adjuvant, the penetrating adjuvant used in this study did not increase the efficacy of Fulfill®. Superior 70 Oil® only acted synergistically with Fulfill® when plants were under drought stress. Based on the results of this study, Fulfill® should be applied with an adjuvant only during drought conditions.

Chapter 4: Summary and Conclusions

4.0 SUMMARY AND CONCLUSIONS

4.0.1 Aphid Behaviour

When aphids were exposed to Fulfill® by feeding on treated plants, they did not resume feeding after their initial exposure to this pesticide. Some aphids were observed attempting to feed by pressing their proboscis against the leaf; however, stylets never penetrated the leaf surface. Between 1-5 days following exposure to Fulfill®, aphids starved to death. No aphids were observed giving birth after 24 hours following exposure to Fulfill®. This behaviour is consistent with that described in the literature (Flückiger et al. 1992; Harrewijn and Kayser 1997; *Novartis Crop Protection 1998; *Glogoza 2000). Aphids also exhibited signs of disorientation after exposure to Fulfill® including twitching, tremors and slow movement. This behaviour may indicate that Fulfill® does exert a slight general toxic effect on aphids in addition to its antifeedant properties, as these symptoms are generally associated with exposure to a poison (Boiteau and Osborn 1997).

4.0.2 SEM Morphological Study

Although reports from Syngenta, the company manufacturing Fulfill®, speculate that the aphid proboscis appears different after exposure to Fulfill® (Harold Wright, personal communication), this study has not produced any evidence to support this claim. The literature also does not attribute the antifeedant action of Fulfill® to morphological changes in the proboscis. Instead, the antifeedant activity of Fulfill® in aphids is attributed to the effect of the chemical on the regulation of the cibarial valves,

the food pump and the salivary pump by the nervous system (Harrewijn and Kayser 1997; *Novartis Crop Protection 1998).

4.0.3 Efficacy Comparison Study

Fulfill® exhibited erratic control under field conditions. In the first field experiment (2001), which occurred under drought conditions, Fulfill® failed to offer any aphid control. However, Monitor® and Pirimor® offered excellent aphid control during this same period. The probable reason for this pattern is that Fulfill® is a systemic pesticide that depends on translocation of the chemical into and throughout the plant to offer aphid control. During drought conditions translocation does not take place efficiently. Monitor® and Pirimor® are both able to kill aphids on contact, so these chemicals remain effective during drought conditions. Fulfill® offered excellent aphid control in situations where water stress was not a problem. Due to the low toxicity of Fulfill® to other organisms in comparison to Monitor® and Pirimor®, Fulfill® should be used as an aphicide when plants are not under water stress. However, when plants are under water stress, it is advisable to use alternate means to control aphids.

4.0.4 Adjuvants

Although the Fulfill® label recommends using a penetrating adjuvant during application, both lab and field studies found no evidence to corroborate this claim (*Novartis Crop Protection 1999a). When leaves sprayed with Fulfill® in the field were transported back to the lab and exposed to aphids, Fulfill® exerted excellent aphid control whether it was used without an adjuvant, or with LI 700® or Citowett Plus®. Under these conditions, adjuvants did not increase the efficacy of Fulfill®. In the field,

Superior 70 Oil® only acted synergistically with Fulfill® when plants were under drought stress. LI 700® did not increase the efficacy of Fulfill® under drought conditions nor under normal PEI field conditions. Since one of the best qualities of Fulfill® is its low toxicity to beneficial organisms (Flückiger et al. 1992; Follas and Blanc 1995), using an adjuvant which is toxic to fish and aquatic organisms, such as Superior 70 Oil® (*Belau 2001), would lessen the benefits of using Fulfill® as an aphicide. This is especially true under conditions where adjuvants do not increase the efficacy of Fulfill®. However, Fulfill® applied with an adjuvant still has a lower toxicity to non-target organisms than Monitor® or Pirimor®. Based on the results of this study, an adjuvant should only be used with Fulfill® under drought conditions.

5.0 WORKS CITED

Bacon, O.G., V.E. Burton, D.L. McLean, R.H. James, W.D. Riley, K.G. Baghott and M.G. Kinsey. 1976. Control of the green peach aphid and its effect on the incidence of potato leafroll virus. Journal of Economic Entomology 69: 410-414.

*BASF AG. 1998. Citowett Plus Label.

*Bayer. 1997. Monitor Material Safety Data Sheet.

*Baysinger, J. and M. DeFelice. 2000. Regulation of adjuvants. Pioneer Crop Management.www.pioneer.com/usa/crop_managem...gronomics/spray_adjuvants_regulation.htm.

Bedford, I.D., A.Kelly, G.K. Banks, D. Fuog and P.G. Markham. 1998. The effect of pymetrozine, a feeding inhibitor of Homoptera, in preventing transmission of cauliflower mosaic caulimovirus by the aphid species *Myzus persicae* (Sulzer). Annals of Applied Biology 132: 453-462.

*Beleau, D. 2001. Superior 70 Oil Material Safety Data Sheet. Platte Chemical Company.

Bernard, G., S.K. Asiedu and P. Boswall Eds. 1993. Atlantic Canada Potato Guide. Publication 1300/93. Agdex# 257/13.

Blackman, R. L. 1974. Aphids. Ginn: London.

Bohmont, B.L. 2000. The Standard Pesticide User's Guide 5th ed. Prentice- Hall: New Jersey.

Boiteau, G., R.R. King and D. Levesque. 1985. Lethal and sublethal effects of aldicarb on two potato aphids (Homoptera: Aphidae): *Myzus persicae* Sulzer and *Macrosiphum euphorbiae* (Thomas). Journal of Economic Entomology 78: 41-44.

Boiteau, G. and W.P.L. Osborn. 1997. Behavioural effects of imidacloprid, a new nicotinyl insecticide, on the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Homoptera, Aphididae). The Canadian Entomologist 129: 241-249.

---

*Refers to a reference from a company monograph, government website or university website.

Cancelado, R.E. and E.B. Radcliffe. 1979. Action threshold for green peach aphid on potatoes in Minnesota. Journal of Economic Entomology 72: 606-609.

*Cornell University. 2001. Pirimicarb (Pirimor) Chemical Profile 4/85. www.pmep.cce.cornell.edu/profiles/inse...te/pirimicarb/insect-prof-pirimicarb.html

Cross, J. 1987. Introduction to nonionic surfactants. Nonionic Surfactants: Chemical Analysis. J. Cross (Ed). Marcel Dekker Inc: New York.

*Diamond, J.F., A.V. Sturz, P.W. Boswall, T.L. DeHaan and M.K. Keenan. 1996. Potato Virus Y. Factsheet of the Prince Edward Island Department of Agriculture and Forestry. Agdex 164/94. www2.gov.pe.ca/af/agweb/library/factsheets/potviry.asp.

*Diamond, J.F. and A.V. Sturz. 1997. Potato Leafroll Virus. Factsheet of the Department of Agriculture and Forestry. Agdex#161/93.

*Difonzo, C.D., D.W. Ragsdale and E.B. Radcliffe. 1996. Integrated management of PLRV and PVY in seed potato, with emphasis on the Red River Valley of Minnesota and North Dakota.Univeristy of Minnesota. www.ipmworld.umn.edu/chapters/difonzo.htm.m

Dixon, A.F.G. 1973. Biology of Aphids. Edward Arnold Publishers Limited: London.

Dixon, A.F.G. 1998. Aphid Ecology 2nd ed. Chapman and Hall: London.

Dunnett, C.W. 1980. Pairwise multiple comparisons in the unequal variance case. Journal of the American Statistical Association 75: 796-800.

Dytham, C. 1999. Choosing and Using Statistics: A Biologist's Guide. Blackwell Science Ltd: Oxford.

Eastop, V.F. 1983. Biology of Aphid Virus Vectors. Plant Virus Epidemiology: The Spread and Control of Insect-Borne Viruses. R.T. Plumb and J.M. Thresh Eds. Blackwell Scientific Publications: Oxford.

*Environment Canada. 2002. Average Rainfall for PEI (O'Leary) 1992-2001 and Average Daytime Temperature for PEI (O'Leary) 1992-2001). Environment Canada Halifax.

*EPA. 1999. Methamidophos Summary. www.epa.gov/pesticides/op/methamidophos/metham_summ.htm

*EPA. 2000.Overview of the revised methamidophos risk assessment. www.epa.gov/pesticides/op/methamidophos/metham_overview.htm.

*EXTOXNET. 2001. Methamidophos. www.ace.ace.orst.edu/info/extoxnet/pips/methamid.htm.

Flückiger, C.R., H. Kristinsson, R. Senn, A. Rindlisbacher, H. Buholzer and G. Voss. 1992. CGA 215'944- A novel agent to control aphids and whiteflies. Brighton Crop Protection Conference- Pests and Diseases: 43-50.

*Follas, G. and S. Blanc. 1995. Control of aphids in tomatoes and brassicas with pymetrozine. Proceedings of the 47th New Zealand Plant Protection Conference. www.hortnet.co.nz/publications/nzpps/proceedings/94/94_125.htm.

Foster, S.P., I. Denholm and A.L. Devonshire. 2000. The ups and downs of insecticide resistance in peach-potato aphids (*Myzus persicae*) in the UK. Crop Protection 19: 873-879.

French II, N.M., S.B. Ramaswamy, D.B. Smith & D. Paroonagian. 1992. Effect of three adjuvants on coverage, persistence, and efficacy of ground-applied chlorpyrifos for suppression of cotton aphid (Homoptera: Aphididae). Journal of Economic Entomology 85: 1347-1355.

Gary, N.E. and K. Lorenzen. 1989. Effect of methamidophos on honey bees (Hymenoptera: Apidae) during alfalfa pollination. Journal of Economic Entomology 82: 1067-1072.

Gibson, R.W., J.A. Pickett, G.W. Dawson, A.D. Rice and M.F. Stribley. 1984. Effects of aphid alarm pheromone derivatives and related compounds on non- and semi-persistant plant virus transmission by *Myzus persicae*. Annals of Applied Biology 104: 203-209.

Gibson, R.W. and A.D. Rice. 1986. The combined use of mineral oils and pyrethroids to control plant viruses transmitted non- and semi-persistently by *Myzus persicae*. Annals of Applied Biology 109: 564-472.

*Glogoza, P. 2000. 2000 field crop insect management. North Dakota State University. www.ext.nodak.edu/extpubs/plantsci/pests/e1143w16.htm

Guldemond, J.A., W. J. van den Brink and Eefje den Belder. 1998. Methods of assessing population increase in aphids and the effect of growth stage of the host plant on population growth rates. Entomologia Experimentalis et Applicata 86: 163-173.

Hall, I.M. 1974. Pathogens of aphids. Perspectives in Aphid Biology. Ed. A.D. Lowe. Auckland: The Entomological Society of New Zealand Inc.

Hanafi, A., E.B. Radcliffe and D.W. Ragsdale. 1989. Spread and control of potato leafroll virus in Minnesota. Journal of Economic Entomology 82: 1201-1206.

Hancock, R.I. 1984. Macromolecular surfactants. Surfactants. T.F. Tadros (Ed.). Academic Press: New York.

Harding, J.A. 1973. Green peach aphids: Field trials with newer insecticides on cabbage and spinach. Journal of Economic Entomology 66: 459-460.

Harrewijn, P. and H. Kayser. 1997. Pymetrozine, a fast-acting and selective inhibitor of aphid feeding. *In- situ* studies with electronic monitoring of feeding behaviour. Pesticide Science 49: 130- 140.

*Hock, W.K. 1994. Agrichemical Fact Sheet #18: Horticultural Spray Adjuvants. Penn State University. www.pested.psu.edu/fact10.html.

Horn. D.J. 1988. Ecological Approach to Pest Management. The Guilford Press: New York.

Horowitz, A.R., Z. Mendelson and I. Ishaaya. 1997. Effect of abamectin mixed with mineral oil on the sweetpotato whitefly (Homoptera: Aleyrodidae). Journal of Economic Entomology 90: 349-353.

Itai, C. and A. Benzioni. 1976. Water stress and hormonal response. Water and Plant Life: Problems and Modern Approaches. Eds. O.L. Lange, L. Kappen and E.D. Schulze. New York: Springer- Verlag.

Jones, A.T. 1987. Control of virus infection in crop plants through vector resistance: a review of achievements, prospects and problems. Annals of Applied Biology 11: 745-772.

Kramer, P.J. 1969. Plant and Soil Water Relationships: A Modern Synthesis. New York: McGraw-Hill.

Kristinsson, H. 1994. Pymetrozine- A new insecticide. Advances in the chemistry of insect control III. Ed. G.G. Briggs. Essex:: Royal Society of Chemistry.

Lamb, R.J., L.L. Wise and P.A. MacKay. 1997. Photoperiodism and seasonal abundance of an aphid, *Macrosiphum euphorbiae* (Thomas), in oilseed flax. The Canadian Entomologist 129: 1049-1058.

Lee-Stadelmann, O.Y. and E.J. Stadelmann. 1976. Cell permeability and water stress. Water and Plant Life: Problems and Modern Approaches. Eds. O.L. Lange, L. Kappen and E.D. Schulze. New York: Springer-Verlag.

*Loveland Industries Inc. 2002. LI 700 Label.

*Loveland Industries Inc. 2001. LI 700: A unique penetrating, translocating and acidifying agent.

Martin, J.T. and B.E. Juniper. 1970. The Cuticles of Plants. St. Martin's Press: New York.

Martin, N.A. and P.J. Workman. 1997. Melon aphids (*Aphis gossypii*) resistance to pesticides. Proceedings of the New Zealand Plant Protection Society.

Matthews, R.E.F. 1981. Plant Virology. 2nd Ed. Academic Press: New York.

Milton, J.S. 1992. Statistical Methods in the Biological and Health Sciences 2nd ed. Mcgraw-Hill: New York

Moores, G.D., G.J. Devine and A.L. Devonshire. 1994. Insecticide-insensitive acetylcholinesterase can enhanc esterase-based resistance in *Myzus persicae and Myzus nicotianae.* Pesticide Biochemisty and Physiology 49: 114-120.

*Mowry, T., K. Pike, D. Ragsdale, G. Reed, A. Schreiber and B. Stolz. 2000. Integrated pest management program for green peach aphid/potato leaf roll virus for Pacific Northwest potatoes. Potato Country. www.freshcut.com/article.cfm?id=790&magazine=2&userdate=0

Munson, M.A., P. Baumann, M.A. Clark, L. Baumann, N.A. Moran, D.J. Voegtlin and B.C. Campbell. 1991. Evidence for the establishment of aphid-eubacterium endosymbiosis in an ancestor of four aphid families. Journal of Bacteriology 173: 6321-6324.

National Research Council. 2000. The Future Role of Pesticides in US Agriculture. Washington: National Academy Press.

*Novartis Crop Protection Inc. 1998. Fulfill Treatment Bulletin. Novartis Crop Protection Inc. www.cp.us.novartis.com/products/cro...rochures/fulfill_tech_bulletin/index.shtml

*Novartis Crop Protection Inc. 1999a. Fulfill insecticide label.

*Novartis Crop Protecion Inc. 1999b. Fulfill Material Safety Data Sheet.

Ottewill, R.H. 1984. Introduction. Surfactants. T.F. Tadros (Ed.) Academic Press: New York.

Parry, D.W. 1990. Plant Pathology in Agriculture. Cambridge University Press: Cambridge.

Peters, D. 1987. Spread of viruses in potato crops. Viruses of potatoes and seed potato production 2nd Ed. J.A. de Bokx and J.P.H. Van der Want (Eds.) Pudoc: Wageningen.

*Petroff, R. 2000. Pesticide Adjuvants and Surfactants. Montana State University. www.Scarab.msu.montana.edu/extension/agadj002.htm.

*PMRA. 1993. Registration Requirements for adjuvant products. Agriculture and Agri-Food Canada. Dir93-15.

Poule. R.W. and P. Gentilli (Eds.) 1997. Nomina Neartica Insecta: A Checklist of the Insects of North America. Non-holometabolous Insects: Volume 4. Rockville: Entomological Information Services.

Pree, D.J., A.B. Stevenson and E.S. Barszcz. 1996. Toxicity of pyrethroid insecticides to carrot weevils: Enhancement by synergists and oils. Journal of Economic Entomology 89: 1254-1261.

*Radcliffe, T. 1998a.Control of green peach aphid. Aphid Alert No.2. University of Minnesota. www.ipmworld.umn.edu/aphidalert/alert2.htm#Key.

*Radcliffe, T.1998b. Insecticidal control of green peach aphid. Aphid Alert No. 4. University of Minnesota. www.ipmworld.umn.edu/aphidalert/alert4.htm.

Roberts, I.M. and B.D. Harrison. 1979. Detection of potato leafroll and potato mop-top viruses by immunosorbent electron microscopy. Annals of Applied Biology 93: 289-297.

Sprent, P. 1989 Applied Nonparametric Statistical Methods. Chapman and Hall: New York.

Statistics Canada. 2000. Canadian potato production. Agricultural division statistical bulletin catalogue no. 22-008-UB.

Sutcliffe, J.F. 1979. Plants and Water. 2nd ed. Edward Arnold Publishers Ltd: London.

*Suranyi, R., T. Radcliffe, D. Ragsdale, I. MacRae and B. Lockhart. 1999. Aphid Alert: A research/outreach initiative addressing potato virus problems in the northern midwest USA. World Potato Congress. Wysiwyg://173/http://www.potatocongress.org/articles/september99.htm.

Šutić, D.D., R.E. Ford and M.T Tošić. 1999. Handbook of Plant Virus Diseases. CRC Press: New York.

Sweeden, M.B. and P.J. McLeod. 1997. Systemic toxicity and field efficacy for imidacloprid, pymetrozine, and triazamate against *Myzus persicae* (Homoptera: Aphididae) on spinach. Journal of Agricultural Entomology 14: 421-433.

Tadros, T.H. 1984. Some applications of surfactants in biological systems. Surfactants. T.F. Tadros (Ed.). Academic Press: New York.

Tamada, T. and B.D. Harrison. 1981. Quantitative studies on the uptake and retention of potato leafroll virus by aphids in laboratory and field conditions. Annals of Applied Biology 98: 261-276.

*Thomas, P.E., K.S. Pike and G.L. Reed. 2000. Research understanding green peach aphid flights and potato leaf roll disease in the Columbia Basin. Potato Country. www.freshcut.com/article.cfm?id=705magazine=2&userdate=2000-02-01.

Treacy, M.F., J.H. Benedict, K.M. Schidt and R.M. Anderson. 1991. Mineral oil: Enhancement of field efficacy of a pyrethroid insecticide against the boll weevil (Coleoptera: Curculionidae). Journal of Economic Entomology 84: 659-663.

Van Emden, H.F. 1989. Pest Control. 2nd ed. Edward Arnold: London.

Villatte, F., D. Auge, P. Touton, R. Delorme and D. Fournier. 1999. Negative cross-sensitivity in insecticide-resistant cotton aphid, *Aphis gossypii* Glover. Pesticide Biochemistry and Physiology 65: 55-61.

Woodford, J.A.T., B.D. Harrison, C.S. Aveyard and S.C. Gordon. 1983. Insecticidal control of aphids and the spread of potato leafroll virus in potato crops in eastern Scotland. Annals of Applied Biology 103; 117-130.

Wyss, P. and M. Bolsinger. 1997a. Plant-mediated effects on pymetrozine efficacy against aphids. Pesticide Science 50: 203-210.

Wyss, P. and M. Bolsinger. 1997b. Translocation of pymetrozine in plants. Pesticide Science 50: 195- 202.

*Zeneca Agro. 1997. Pirimor 50DF Label.

*Zeneca Agro 1999. Pirimor 50DF Material and Safety Data Sheet.