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Integrated Control of Colorado Potato Beetle and Potato Virus Y Using Mineral Oil

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INTEGRATED CONTROL OF COLORADO POTATO BEETLE AND POTATO VIRUS Y USING MINERAL OIL

By

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An Abstract of the Thesis Presented
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Colorado potato beetle (*Leptinotarsa decemlineata*) (CPB) and Potato Virus Y (PVY) are two of the most damaging pests attacking potato crops. CPB can cause significant defoliation to potato fields and is difficult to control using insecticides because its populations rapidly develop insecticide resistance. PVY, which is transmitted non-persistently by aphids, can result in yield loss and rejection of seed potato lots. Due to its rapid mode of transmission, insecticides are often ineffective at curtailing the spread of the virus. Thus, an integrated pest management (IPM) approach is essential for both CPB and PVY control.

Mineral oil is a product used to reduce PVY transmission in potato fields. However, there is little information available about other effects that oil may have on insect pests of potato. To better understand how mineral oil affects potato pests, we performed a series of experiments testing the effects of oil on mortality, behavior, and development of aphids and Colorado potato beetles. Oil was harmful to aphids, acting as a contact insecticide, causing high levels of residual

mortality to nymphs, and inducing avoidance of oil-treated foliage. Colorado potato beetles were also negatively affected by oil. Additionally, oil acted synergistically with the entomopathogenic fungus *Beauveria bassiana*; CPB larvae were killed more rapidly when sprayed with both products compared to when sprayed with *B. bassiana* alone. Based on these results, mineral oil has potential for expanded use in potato IPM programs.

The epidemiology of PVY is complex and poorly understood. We constructed a spatially-explicit, agent-based simulation model to improve understanding of the factors affecting PVY spread. According to the results of the model, initial inoculum and vector transmission efficiency are both important. The model also showed that aphids that do not colonize potato spread PVY more effectively than potato-colonizing aphids. Field size did not affect PVY spread. The results emphasize the importance of both planting clean seed to keep virus levels low as well as treating fields with mineral oil to effectively reduce transmission efficiency of aphid vectors. In addition, control should focus on reducing spread by non-colonizing aphids rather than on attempting to eliminate colonizing aphid populations.

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CHAPTER 1

BIOLOGY AND CONTROL OF POTATO PESTS

1.1. Potato

Potato (*Solanum tuberosum*) (Solanales: Solanaceae) has a long history as one of the most important crops in the world. Potatoes were first domesticated in the Andes region from the wild species *Solanum brevicaule* around 10,000 years ago (Spooner et al. 2005, Ames and Spooner 2008). The potato was brought to Europe in the 1500s, where it became a staple crop (Spooner et al. 2005). In the 1800s, Irish diets were heavily reliant on potato, so much so that the Irish potato famine became a devastating event. Potatoes became widely consumed in large part because they are calorie-dense and nutrient-rich compared to other staple crops (Navarre et al. 2014). Today, potato remains a critical part of diets worldwide.

The majority of potato crops are grown through vegetative propagation rather than true seed (Davidson and Xie 2014). Growing potato from true seed has a number of disadvantages. Potatoes take several months to grow in a greenhouse, then must be transplanted in the field, making the whole process lengthy and labor-intensive. Thus, potatoes are generally grown from tubers referred to as seed potatoes. The process of growing seed potatoes starts with the production of nuclear seed, which is grown *in vitro* using tissue culture, then in greenhouses (Davidson and Xie 2014). This nuclear seed is then transplanted into the field to produce foundation seed, which is used to grow future seed lots. These then become certified seed, which is planted to grow potatoes for consumption. To ensure high-quality seed, seed potato production is heavily regulated. Seed potatoes are grown in a limited generation system; seed cannot be produced beyond a certain number of years. Seed potatoes are also subjected to strict

certification processes to maintain low disease levels; if disease incidence exceeds these levels in a given year, the lot will be rejected and cannot be sold as seed.

In Maine, potato is the most economically important crop, accounting for over $142 million in income in 2016 (USDA 2016). The state ranks fifth in the United States in potato acreage. About two-thirds of Maine potatoes are used in processing (Maine Potato Board 2016). Seed potato is also important, accounting for about 20% of the state's potato acreage annually. The rest is sold as table stock. Potato is an integral component of Maine's economy. Thus, it is critical to use sustainable production methods to ensure the Maine potato industry remains strong in the long term.

Potato crops are attacked by a variety of insect pests that can reduce yield and serve as a significant cost to growers to control. Colorado potato beetle (*Leptinotarsa decemlineata*) (Coleoptera: Chrysomelidae) consumes potato leaves and is one of the most important pests of potato (Alyokhin 2009). Aphids (Hemiptera: Aphididae) can colonize potato plants, but are primarily harmful as virus vectors (Radcliffe and Ragsdale 2002). Aphids transmit viruses such as Potato Leafroll Virus and Potato virus Y, which cause significant yield loss. Control of these and other pests is imperative so growers can maintain profitable potato farms.

1.2. Integrated Pest Management

Pesticide use has been the major control method in crops worldwide for decades. However, this has created issues, such as harmful non-target effects (Desneux et al. 2007) and increased resistance among pests (Whalon et al. 2008). Thus, there has been a strong effort among pest control professionals to promote integrated pest management (IPM). IPM involves using a knowledge-based approach to pest management, combining multiple techniques to control pests (Smith and Allen 1954, Prokopy 1993). Barzman et al. (2015) list eight components

of IPM: prevention and suppression of pests using cultural methods; monitoring pests; decision-making about timing of control actions based on thresholds; use of non-chemical methods whenever possible; using selective rather than broad-spectrum pesticides; limiting pesticide use; employing anti-resistance strategies such as using multiple pesticide types; and evaluation of control methods. IPM requires significant knowledge of a growers' specific system, and must be adaptable. However, if done well, IPM programs can create effective and sustainable pest suppression and minimize negative impacts on the environment.

Unfortunately, there has been limited adoption of major components of IPM in potato (Alyokhin 2009). Many potato growers are still heavily reliant on insecticides as the primary method of control. As a consequence, insecticide resistance has become widespread in Colorado potato beetle populations (Huseth et al. 2014, Alyokhin et al. 2015). Thus, it is necessary to improve alternative control strategies and encourage growers to use more IPM practices.

1.3. Potato Virus Y

Potato virus Y (PVY) is an aphid-vectored virus that infects potatoes and related plants. PVY is a (+)-sense single-strand RNA virus belonging to the genus *Potyvirus*, in the family Potyviridae. PVY virus particles are filamentous and flexuous. They have a length of 730-740 nm and a width of 11-12 nm. The genome, which is 9.7 kb long, encodes for several proteins. These include coat proteins, movement proteins, and HC-Pro, which has a variety of functions, including attachment to the mouthparts of the aphid vector (Quenouille et al. 2013).

PVY is the most damaging virus infecting potato, and one of the most economically important plant viruses in the world (Gray et al. 2010, Scholthof et al. 2011). The virus can stunt plant growth and cause foliar symptoms, including mosaic, chlorosis, and necrosis (Gray et al. 2010). Some strains of the virus cause potato tuber necrotic ringspot disease (PTNRD), which

renders infected tubers unmarketable. In severe cases, PVY can cause yield losses of up to 80% (Quenouille et al. 2013). PVY is of particular importance to seed potato growers. Seed potato lots must be kept at low PVY levels or the entire lot will be rejected by seed certification programs. If not controlled, PVY can build up over time in seed potato lots, resulting in severe epidemics.

PVY is a complex of several different strains. Historically, the most common strain has been PVY^{O}. This strain mainly causes foliar symptoms in potato (Lorenzen et al. 2006). Another major strain is the tobacco veinal necrosis strain, PVY^{N}. While this is a severe disease in tobacco, symptoms tend to be relatively mild in potato. However, several new strains have emerged as a result of recombination between PVY^{O} and PVY^{N}. One of these recombinants, PVY^{NTN}, is of particular importance due to its ability to cause PTNRD, while having few visible foliar symptoms. With mild foliar symptoms, it is more likely the plant will be missed when removing infected plants. PVY^{O} is rapidly being displaced by recombinant strains such as PVY^{NTN} (Lorenzen et al. 2006, Lacomme et al. 2014). This has created new challenges for growers and has led to the reemergence of PVY as a major issue in potato.

PVY is vectored by various species of aphids (Hemiptera: Aphididae). Green peach aphid (*Myzus persicae* Sulzer) and potato aphid (*Macrosiphum euphorbiae* Thomas) are the two most common potato colonizing species in Maine, and both are vectors of PVY. Green peach aphid in particular is important as the most efficient vector of PVY (Al-Mrabeh et al. 2010). Aphids that do not colonize potato are also important vectors, despite transmitting the virus less efficiently (Robert et al. 2000, Steinger et al. 2015). At least 65 species of aphids are potential vectors of PVY (Pelletier et al. 2012), making it impossible to focus control on only a few species.

Because it is a non-persistently transmitted virus, PVY virus particles attach to the aphid's stylet when an aphid probes an infected plant (Nault 1997, Gray and Banerjee 1999). Due to this mode of transmission, there is no latent period after virus acquisition, so that the aphid is immediately able to transmit the virus. Aphids also do not need to feed on the plant for an extended period to acquire or transmit the virus. Instead, the virus is spread through probes of the plant lasting under a minute. On the other hand, aphids are only viruliferous for a short time, and can lose the virus after probing five plants or less (Bradley and Rideout 1953).

This mode of transmission is in contrast to persistent viruses such as Potato Leafroll Virus (PLRV), another important virus infecting potato. Persistent viruses enter the gut of the aphid vector, where they may either replicate (propagative) or not (circulative) (Nault 1997, Gray and Banerjee 1999). This means persistent viruses require a latent period ranging from a few hours to several days after acquisition before the vector can transmit the virus. The aphid must also feed on a plant for several hours before acquiring the virus. Once viruliferous, a vector is capable of retaining the virus for an extended period of time, sometimes for the remainder of its life. In potato, PLRV is primarily transmitted by potato-colonizing aphids, particularly the green peach aphid (Radcliffe and Ragsdale 2002). Controlling vector populations within the field is often an effective way to control PLRV. However, dynamics of non-persistently transmitted PVY differ greatly; thus, PVY must be managed differently from PLRV.

Since PVY is transmitted in a non-persistent manner, aphid species that do not colonize potato have a particular importance. Significant PVY spread can occur despite the absence of potato colonizing aphids (Kirchner et al. 2011). Even when potato colonizers are present, their activity may not be correlated with PVY spread (Steinger et al. 2015). Non-colonizers can spread the virus quickly, as they will visit a greater number of potato plants than colonizing aphids.

Because non-colonizing aphids do not consider potato to be a suitable host, they will probe multiple plants in a field, whereas colonizing aphids will readily settle on a potato plant (Boquel et al. 2014). The non-persistent nature of PVY transmission allows for rapid virus spread in the host-searching process, as sustained feeding is not necessary for an aphid to acquire the virus and there is no latent period before a vector becomes viruliferous. Population dynamics can also explain the importance of non-colonizing aphids. In some areas, non-colonizing aphids are more abundant than colonizing aphids early in the season, when the plant is most vulnerable to virus infection (Kirchner et al. 2011). The importance of non-colonizing aphids creates a challenge for PVY control, as vector suppression within the field is often insufficient to prevent the spread of PVY.

Chemical control of PVY vectors has been met with limited success. Insecticides can be effective at lowering potato-colonizing aphid populations within the field, which reduces PVY spread by these vectors (Martín-López et al. 2006). However, this often does not result in acceptable PVY control (MacKenzie et al. 2017). Insecticides fail to control PVY because the virus can be spread quickly before the aphids are killed (Alyokhin et al. 2002). Insecticides may even increase PVY spread by increasing aphid movement in response to the spray (Lowery and Boiteau 1988). Given these limitations, insecticides cannot adequately control PVY on their own.

Seed certification programs have long been the most effective way to keep PVY under control (Gray et al. 2010). In these programs, government bodies set tolerance levels for the proportion of plants allowed to be infected with PVY in potato fields grown for seed (Davidson and Xie 2014). To ensure low virus levels, fields are scouted by inspectors searching for viral symptoms throughout the growing season. Roguing, which is the term used to describe the

removal of virus-infected plants from the field, is an important part of this process. When virus-infected plants are found, growers can rogue to lower virus levels prior to the next inspection. At the end of the season, post-harvest testing is performed to determine whether virus is present at acceptable levels. The levels are set by each individual seed potato growing region, and vary based on seed class. In Maine, first-year seed lots grown from nuclear seed cannot contain more than 0.1% virus-infected plants (Department of Agriculture Conservation and Forestry 2016). Foundation seed must remain under 0.5% infection, while seed in the certified class is limited to 5% of plants with PVY. If lots do not meet these limits, the lot will be downgraded or rejected.

The emergence of new PVY strains with milder foliar symptoms has led to the increased adoption of molecular diagnostic methods in seed certification programs. Enzyme-linked immunosorbent assay (ELISA) is one such method. ELISA is relatively quick and easy. However, it is not able to distinguish between recombinant strains such as PVY^{NTN} and $PVY^{N\text{-}Wi}$ (Kogovsek and Ravnikar 2013). Polymerase chain reaction (PCR) is generally more effective and can accurately determine whether or not a lot should be rejected at the end of the season. However, using PCR for roguing for within-season control is impractical. Thus, roguing remains less effective when harder to detect strains are present, increasing the likelihood of rejection.

Cultural practices can be used to reduce the probability of PVY being introduced into or spread throughout the field. Potential sources of inoculum should be removed to avoid PVY introduction. This includes volunteer plants (potato plants emerging from tubers left unharvested during previous years) (Gray et al. 2010), as well as weeds in and around the field (Cervantes and Alvarez 2011). Planting crop borders around potato fields is another strategy to reduce the probability of PVY introduction into the field (Boiteau et al. 2009). Crop borders serve as a virus sink – aphids preferentially land on the borders, then lose the virus while probing the plant

before entering the field. Mulches, particularly straw mulch, can also be used to repel aphids from landing in the field (Kirchner et al. 2014). None of these practices on their own can sufficiently control PVY, but all are potentially useful tools in IPM programs.

1.4. Colorado Potato Beetle

Colorado potato beetle (CPB) has long been one of the most important pests of potato. The beetle can cause significant defoliation, resulting in major yield losses (Ferro 1983). CPB are highly fecund; females can lay hundreds of eggs over their lifetimes (Harcourt 1971), leading to rapid population increases. Thus, it is critical for potato growers to keep CPB under control. Unfortunately, sustainable CPB management is very difficult.

Chemical control is the most common method used to control CPB. This is problematic because the beetle has the tendency to rapidly develop resistance to virtually every insecticide used against it (Alyokhin 2009). The beetle's life history allows resistance to emerge and spread quickly throughout populations (Alyokhin et al. 2015). The high fecundity of CPB females means that offspring of individuals carrying resistance alleles will be present in large numbers. Multiple generations per season and the tendency for females to mate with several males increases genetic diversity. Additionally, as a specialist on toxin-heavy solanaceous plants, the beetles are quick to develop resistance to toxins (Ferro 1993). To achieve sustainable control, the use of methods besides synthetic insecticides is critical.

One alternative to conventional insecticides is the use of fungal insecticides such as *Beauveria bassiana* (Hypocreales: Clavicipitaceae). *B. bassiana* has some advantages over synthetic insecticides. *B. bassiana* has a sophisticated mode of action, as it kills insects through physical growth and the release of multiple enzymes and compounds (Inglis et al. 2001). This is in contrast to most synthetic insecticides, which generally target one specific site. Thus, there is a

lower chance of insects developing resistance to *B. bassiana* (Kraaijeveld and Godfray 2008, Dubovskiy et al. 2013). *B. bassiana* also has little environmental impact, unlike many synthetic insecticides (Strasser et al. 2000). These advantages have led some growers to use *B. bassiana* to control CPB. While *B. bassiana* can kill CPB, it has considerable limitations. *B. bassiana* generally causes lower beetle mortality than synthetic insecticides (Hajek et al. 1987, Lacey et al. 1999). *B. bassiana* requires the right environmental conditions, such as sufficient moisture, to be effective. The fungus also takes several days to kill beetles, which means that larvae could molt and lose the fungus before they are killed (Inglis et al. 2001). A more effective method may involve combining *B. bassiana* with other insecticides. For example, *B. bassiana* has been shown to act synergistically with insecticides based on *Bacillus thuringiensis* delta endotoxin, leading to improved CPB control (Wraight and Ramos 2005). Incorporating *B. bassiana* and other biological insecticides into potato IPM could create more sustainable control and mitigate issues with resistance.

Several cultural control methods can help suppress Colorado potato beetle populations. Crop rotation with a non-host crop can reduce the amount of overwintering beetles colonizing the field (Wright 1984). Straw mulch can help lower Colorado potato beetle abundance, possibly by interfering with beetle movement (Zehnder and Hough-Goldstein 1990) or increasing natural enemy abundance (Brust 1994). Trap cropping can also help reduce the amount of pesticide used by attracting beetles to a small area of the field (Martel et al. 2005). Including these practices in IPM programs reduces exposure of CPB to pesticides, delaying the development of resistance.

Biological control can also help in Colorado potato beetle management. CPB is attacked by a variety of natural enemies. These include predators such as several species of carabids, particularly *Lebia grandis* (Szendrei et al. 2010), and pentatomids *Perillus bioculatus* and

Podisus maculiventris (Ferro 1994). Potato beetle larvae can also be parasitized by some tachinid and hymenopteran parasitoids. Unfortunately, natural enemies cannot control Colorado potato beetle on their own, and costs of rearing effective natural enemies make inundative releases prohibitively expensive (Ferro 1994). Still, an effort should be made to conserve natural enemies in potato fields through cultural practices and the use of biorational insecticides, as doing so can help reduce CPB populations (Patt et al. 1997).

1.5. Mineral Oil

Mineral oil is a petroleum-based product which has a variety of uses in pest management (Davidson et al. 1991). Mineral oils are composed of a blend of various hydrocarbons, primarily paraffins, and often include a surfactant. Oil has been applied to crops for over a century, but its use was relatively limited due to concerns over phytotoxicity. However, in the last few decades it has reemerged as a promising tool with the advent of less harmful formulations. Oil has been used as an insecticide in some systems. Mineral oil acts as an insecticide primarily against small, soft-bodied pests such as aphids, mites, and scales (Herron et al. 1995, Martín-López et al. 2006, Kraiss and Cullen 2008). Oil can also serve as an ovicide, as has been shown in some species of lepidopteran pests (Riedl et al. 1995, Taverner et al. 2012). The mechanism behind the insecticidal activity of oil is unclear. Oil can kill some insects through suffocation, caused by blocking the insect's spiracles (Davidson et al. 1991). Oil can also penetrate the cuticle upon contact and cause cellular damage (Najar-Rodríguez et al. 2008). The efficacy and mode of action may depend on the oil formulation and the pest species (Najar-Rodríguez et al. 2008), so a clearer understanding of the mechanism could improve its use as an insecticide.

Mineral oil can contribute to pest control in other ways aside from acting as a contact insecticide. Oil can be used as a synergist with other products, including fungal insecticides such

as *B. bassiana* (Akbar et al. 2005). Mineral oil can also affect pest behavior; treatment with oil has caused repellency (Liu et al. 2006) and reduction in oviposition (Riedl et al. 1995). Treating plants with oil may reduce the release of volatiles involved in host plant location (Mensah et al. 2005). Mineral oil is also used to reduce transmission of non-persistent viruses. Oil interferes with both virus acquisition by aphids and inoculation of plants probed by viruliferous vectors (Bradley et al. 1962, Wróbel 2007). Oil works against non-persistent viruses by reducing the ability of the virus particle to attach to the aphid stylet (Wang and Pirone 1996, Boquel et al. 2013).

Mineral oil has several benefits in IPM programs. Its activity against non-persistent viruses is not known to occur in any other product. As an insecticide, oil is promising because there is a low chance of insects developing resistance to it. The physical mode of action by which oil kills insects makes it more difficult for insects to develop resistance (Najar-Rodríguez et al. 2008). Indeed, there are no known cases of resistance to mineral oil among any insect (Vincent et al. 2003). Mineral oil is also considered a biorational insecticide due to its low impact on natural enemies. Oil is relatively safe compared to many synthetic insecticides due to its low amount of residue left (Davidson et al. 1991) and reduced toxicity to important natural enemy groups such as lady beetles (Kraiss and Cullen 2008), parasitoids (Urbaneja et al. 2008), and minute pirate bugs (Biondi et al. 2012).

In potato, mineral oil has often been used to reduce the spread of PVY (Al-Mrabeh et al. 2010). The efficacy of mineral oil at reducing PVY spread in the field is well-demonstrated (Bradley et al. 1966, Boiteau and Singh 1982, Kirchner et al. 2014). Oil consistently reduces PVY incidence. However, the extent of PVY control can vary. On its own, mineral oil is not always sufficient for reducing PVY to acceptable levels (Hansen and Nielsen 2012). Oil may

work best in combination with other tools such as additional insecticides (MacKenzie et al. 2017) or crop borders (Boiteau et al. 2009). Still, mineral oil remains an integral component of many PVY control programs.

The efficacy of mineral oil against PVY in potato was demonstrated in a recent paper by MacKenzie et al. (2017). In this study, oil was sprayed alone and with a variety of insecticides to test how well it reduced PVY spread. Alone, insecticides did not reduce PVY incidence relative to the untreated control. Oil on its own lowered PVY below the control levels in only one of the two years. However, oil and insecticides used in combination were consistently able to suppress the spread of PVY, regardless of the oil dose and frequency of insecticide sprays. These results support previous work by the group that correlated use of mineral oil, with or without insecticides, with reduced PVY incidence among New Brunswick potato growers (MacKenzie et al. 2014, 2016). Taken together, these studies demonstrate that mineral oil can be used on a large scale in potato, especially when used with insecticides.

Despite the frequent use of mineral oil in potato, there has been relatively little investigation into other effects oil sprays may have in potato IPM. Mineral oil could be used to help control aphids. This could provide an additional level of PVY suppression, while also contributing to the control of other aphid-vectored potato viruses such as Potato Leafroll Virus (PLRV). Mineral oil has been studied as an insecticide against aphids in other systems (Herron et al. 1995, Najar-Rodríguez et al. 2008). While oil consistently showed some insecticidal activity, the extent to which it killed aphids can vary. Oil residues may also have lethal and/or sublethal effects on aphids. Some studies have shown that aphids were more likely to avoid oil-treated foliage (Ameline et al. 2009), while others observed no effect of oil on host plant selection or feeding behavior (Vanderveken 1968, Najar-Rodríguez et al. 2007b). Oil residues may increase

mortality among aphids (Ameline et al. 2009), but could increase fecundity of survivors (Martoub et al. 2011). A better understanding of these effects would help determine the extent to which oil sprays can control aphids in potato.

In addition to aphids, oil sprays could potentially affect other insect pests such as Colorado potato beetle. No information is currently available on possible effects of mineral oil on CPB. If effective, oil could be compatible with CPB control strategies that utilize biological control. Mineral oil has been shown to have no effect on the stink bug *Perillus bioculatus*, a predator of Colorado potato beetle (Hough-Goldstein and Keil 1991). Oil could also potentially be useful as a synergist with microbial insecticides such as *B. bassiana* to increase their efficacy against CPB (Akbar et al. 2005). Research into these areas will allow growers to make informed decisions about the use of mineral oil not just against PVY, but as part of a comprehensive IPM program.

1.6. Present Study

The studies described in this thesis aimed to improve potato IPM, in particular the use of mineral oil in IPM programs. Our more specific objectives were to test the effects of mineral oil on mortality, development, and behavior of aphids and Colorado potato beetles. We also investigated whether oil could act as a synergist with *B. bassiana* by improving lethality of the fungus against CPB. In addition, a simulation model was constructed to improve knowledge on the dynamics of PVY spread. The model was used to better understand which components of PVY epidemiology to target for control.

CHAPTER 2

LETHAL AND SUBLETHAL EFFECTS OF MINERAL OIL ON POTATO PESTS

2.1. Introduction

Mineral oil is a petroleum-based product which has been used in pest management for over a century (Davidson et al. 1991). Mineral oil is used in a variety of ways, often as an insecticide. It is used primarily against small, soft-bodied insects such as aphids, mites, and scales (Herron et al. 1995, Najar-Rodríguez et al. 2008, Urbaneja et al. 2008), although it can kill other species such as some lepidopteran larvae (Mensah et al. 2005). While oil can be effective, it often results in lower mortality than synthetic insecticides (Karagounis et al. 2006, Bahlai et al. 2010).

The mechanism by which mineral oil kills insects is not clear, although several hypotheses exist. Mineral oil can act by blocking the exposed insect's spiracles, resulting in suffocation (de Ong et al. 1927). There is also evidence to suggest that oil can penetrate the cuticle and damage nerve cells (Najar-Rodríguez et al. 2008).

In recent years, there has been a greater interest in mineral oil due to its compatibility with integrated pest management (IPM). Mineral oil is considered a biorational insecticide and has a reduced impact on natural enemies compared to most synthetic insecticides (Fernandez et al. 2005, Kraiss and Cullen 2008, Biondi et al. 2012).

In addition to direct toxicity, mineral oil can have sublethal effects on insect pests. Oil treatment on plants has been shown to reduce oviposition in some lepidopteran pests (Mensah et al. 2005, Liu et al. 2006), as well as host acceptance in fruit flies (Nguyen et al. 2007). Oil can also be used as a synergist with other insecticides (Martín-López et al. 2006). Mineral oil has been used to help improve cuticle penetration by the entomopathogenic fungus *Beauveria*

bassiana, resulting in higher red flour beetle mortality (Akbar et al. 2005). It is uncertain how widespread these effects are, but there is potential to discover new opportunities to use oil.

Mineral oil is also used to reduce the spread of non-persistent viruses. Oil reduces spread by disrupting the acquisition and transmission of virus particles between plants and aphid vectors (Bradley et al. 1962). The exact mechanism by which this happens is unclear, but the main hypothesis is that it physically interferes with the attachment of the virus particles to the aphid's stylet (Wang and Pirone 1996, Boquel et al. 2013). Mineral oil can also affect aphid probing behavior, although likely not to a large enough extent to account for all protection (Ameline et al. 2009). Oil also may induce plant defenses that help protect against PVY infection (Khelifa 2017). Regardless of the mechanism, mineral oil provides effective control against non-persistent viruses.

In potato, mineral oil is used to control the spread of Potato virus Y (PVY). It is especially important in seed potatoes, where tolerance for PVY infection is very low (Radcliffe and Ragsdale 2002). Oil has frequently been shown to reduce the spread of PVY (Bradley et al. 1966, Boiteau and Singh 1982, Kirchner et al. 2014). Relatively few reliably effective options exist for PVY control (Davidson et al. 2013), making mineral oil an essential tool for many seed potato growers.

Despite its frequent use in potato, there is little information on whether mineral oil sprays can help to control other potato pests. The effects of mineral oil on aphids have been studied, but a clear understanding of these effects is still lacking. Aphids (Hemiptera: Aphididae) are important pests of potato due to their status as vectors of important plant viruses such as PVY and Potato Leafroll Virus (PLRV) (Radcliffe and Ragsdale 2002). Mineral oil is known to act as an insecticide against aphids, although its complex mode of action may lead to variations in its

efficacy based on oil formulation or aphid species (Najar-Rodríguez et al. 2007a). Possible sublethal effects of oil have also been investigated. Host finding by potato aphids, *Macrosiphum euphorbiae*, was impaired by a masking effect of oil on foliage (Ameline et al. 2009). However, in another study, host selection by winged morphs of melon aphid, *Aphis gossypii*, was not affected (Najar-Rodríguez et al. 2007b). Oil residues appear to be toxic to aphids, but oil volatiles may increase fecundity, which could account for cases where aphid populations are not reduced by oil sprays (Martoub et al. 2011). Overall, mineral oil appears to be a promising option to help control aphids, although more information is needed to understand how it can be used in the best possible way.

The effects of mineral oil on Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) (CPB), have not been investigated. CPB is one of the most important insect pests in potato, capable of rapid population increase and major potato defoliation (Alyokhin 2009). CPB is difficult to control due to its tendency to rapidly develop resistance to virtually any insecticide used against it. Thus, there is a constant need to develop new control strategies. If effective, mineral oil may be a promising option for CPB control for several reasons. Due to its physical mode of action, there is believed to be a reduced risk of insects developing resistance against oil (Najar-Rodríguez et al. 2008). In addition, mineral oil may be compatible with biological control of CPB; oil has been shown to have low toxicity to a predator of CPB, *Perillus bioculatus* (Hemiptera: Pentatomidae) (Hough-Goldstein and Keil 1991). If mineral oil sprays can help to reduce CPB populations, it could lessen the overuse of pesticides sprayed to control CPB.

This paper describes a series of experiments studying the effects of mineral oil on aphids and Colorado potato beetle. The experiments were done on two species of aphids: green peach

aphid, *Myzus persicae,* and potato aphid, *Macrosiphum euphorbiae*. These species are two of the most common potato-colonizing aphids and differ in their size and behavior (Alyokhin and Sewell 2003).

2.2. Materials and Methods

2.2.1. Insects

Aphids used in these experiments were reared in laboratory colonies. The colonies originated from individuals collected from potato fields at Aroostook Experimental Farm, Presque Isle, ME, during the summers of 2015 and 2016. Colonies were restarted annually at the beginning of each summer, and new field-collected aphids were added throughout the field season. Green peach aphids (*Myzus persicae*) and potato aphids (*Macrosiphum euphorbiae*) were maintained in separate colonies in a growth chamber (Series 33 Controlled Environment Chamber, Percival Scientific Inc., Perry, IA) at 20°C with a 16:8 h L:D photoperiod. Aphid colonies were kept in enclosures made from transparent plastic jars (ca. 1,900 ml) with holes covered in fine mesh for airflow. Each jar contained a vial filled with water with an inserted potato leaf cut from greenhouse-grown potato plants (cv. Superior).

Colorado potato beetles used in these experiments were also obtained from laboratory colonies. Colonies were founded by adults collected from potato fields at Aroostook Experimental Farm. Colonies were restarted at the start of each summer, and refreshed with new field-collected beetles throughout each of the two summers of the study. Beetles were kept in wood and fine mesh cages (50 x 50 x 90 cm) in a research greenhouse. Beetles were fed potted potato plants (cv. Superior) grown in the same greenhouse. Eggs were collected and kept in a growth chamber (Percival Scientific Inc., Perry, IA) at 20°C with a 16:8 h L:D photoperiod until larvae hatched, at which point they were returned to the cages.

2.2.2. Mineral Oil

The mineral oil used in this study was JMS Stylet-Oil (JMS Flower Farms Inc., Vero Beach, FL). A concentration of 3% (v/v) oil in water was used in all experiments, as this is the maximum recommended field rate for oil treatment in seed potatoes. Water was used as a control in all experiments.

2.2.3. Aphids

2.2.3.1. Insecticidal Properties of Oil

Potato leaflets (cv. Superior) were taped onto the bottoms of Petri dishes (90 x 15 mm). Five wingless adult aphids were put into each dish. For each aphid species, sixteen dishes, eight per treatment, were set up at one time and treated as a statistical block in the subsequent analysis. Dishes were sprayed with either mineral oil or water using a Burkard computer-controlled spraying apparatus (Burkard Scientific, Hertfordshire, UK) at 10 psi. Sprayed aphids were transferred using a fine hair paintbrush to new dishes with an unsprayed leaflet and a damp paper towel in the bottom. Aphids were kept in an environmental chamber (Percival Scientific Inc., Perry, IA) at 20°C with a 16:8 L:D photoperiod for 24 h. At the end of the period, the number of nymphs and dead adult aphids were counted. The above procedure was repeated eight times, resulting in a total of 64 replications per treatment for each species.

All analyses reported in this paper were done in R Studio (R Core Team 2016), unless specified otherwise. Before analysis, data were tested for normality using a Shapiro-Wilk test. Non-normal data ($p<0.05$) were subjected to rank transformation for analysis. For each species, mean aphid mortality and number of nymphs per dish were analyzed by two-way ANOVA, with treatment and block as main effects.

2.2.3.2. Repellant and/or Antifeedant Properties of Oil

Forty Petri dishes (90 x 15 mm), twenty for each species, were lined with a damp paper towel on the bottom. Potato leaflets (cv. Superior) were dipped for 1 s in either mineral oil or water and allowed to dry for 30 min. Once oil formulation dried, two leaflets were placed in each dish, one on either side. In the choice experiment, each dish received one oil-treated and one water-treated leaflet. In the no-choice experiment, both leaflets in each dish received the same treatment; half the dishes received oil-treated leaflets, the other half received water-treated leaflets. Five wingless adult aphids were put into the center of each dish with a fine hair paintbrush. Dishes were placed in a growth chamber (Percival Scientific Inc., Perry, IA) at 20°C at a 16:8 L:D photoperiod for 24 h. After that, the number of aphids feeding on each leaflet was counted. Numbers of dead aphids on each leaflet and numbers of nymphs on each leaf were also recorded. The experiment was repeated five times, resulting in 100 replications per treatment in the choice bioassay and 50 replications per treatment in the no-choice bioassay for each species. The data were analyzed separately for each species by two-way ANOVA, with treatment and block considered to be main factors as described above.

2.2.3.3. Effects on Survivorship and Reproduction

Twenty-four hours before the experiment, 100 adults of each species were placed into separate enclosures similar to those used to rear aphids and allowed to reproduce. Four enclosures were prepared for the experiment. In each enclosure, a large potato leaf placed in a vial of water mixed with Floralife Cut Flower Preservative (Floralife Inc., Burr Ridge, IL). Leaves were dipped in either mineral oil or water for 1 s, then left to dry for 30 min. When leaves were dry, 50 first-instar nymphs produced by the adults in the prepared enclosures were placed onto the leaves of each cage with a fine hair paintbrush. Two cages per species were used

at one time, one for each treatment. Cages were kept in a growth chamber (Percival Scientific Inc., Perry, IA) at 20°C with a 16:8 h L:D photoperiod. Floralife vials were refilled as needed. Cages were checked after 9 days, and the number of live adult aphids was recorded. This procedure was replicated four times, resulting in four replications per treatment for each species. To analyze the effects of treatment, one-way ANOVAs were performed on the mean number of aphids surviving after 9 d for each species.

2.2.4. Colorado Potato Beetles

2.2.4.1. Repellant and/or Antifeedant Properties of Oil

Choice and no-choice bioassays were performed to test possible repellant and/or antifeedant properties of mineral oil on Colorado potato beetle following the same protocol as described above for the aphid experiment. One adult, one fourth-instar larva, or ten first-instar larvae were placed in the center of each dish, equidistantly between the two leaflets. For the choice assay, twenty dishes of each tested life stage were used. For the no-choice assay, ten dishes containing oil-treated leaflets and ten dishes containing water-treated leaflets for each stage were used. Dishes were placed in an environmental chamber (Percival Scientific Inc., Perry, IA) for 24 h at 20°C with a 16:8 h L:D photoperiod. This procedure was repeated five times for each life stage, resulting in 100 replications for choice assays and 50 replications for no-choice assays.

To assess feeding, leaflet area was measured at the beginning and at the end of each trial using a leaf area meter (LI-3100, LI-COR Inc, Lincoln, NE). The difference between the two measurements was used as an estimate of leaflet consumption. In addition, the number of beetles on each leaflet was counted. Preliminary trials indicated that in the absence of beetle feeding, oil treatment itself did not affect leaflet area during the experimental period.

Mean numbers of first instars residing on leaflets were compared among the treatments using two-way ANOVA as described for the aphid experiments above, with treatment and block treated as main effects. Proportions of adults and fourth instars found on oil- and water-treated leaves were compared using logistic regression. Foliage consumption was analyzed using two-way ANOVA of mean leaf area loss.

2.2.4.2. Effects of Oil on Development

Four potted potato plants (cv. Superior) were placed into each of two wooden frame cages (50 x 50 x 90 cm; see above). Plants in one cage were sprayed with oil, while plants in the other cage were sprayed with water. Pairs of cages were treated as statistical blocks. All plants were allowed to dry for 30 min before being put in cages. One hundred newly hatched potato beetle larvae were placed onto foliage in each cage with a fine hair paintbrush. The cages were left in an experimental greenhouse and the beetles were allowed to grow and feed, with plants being replaced as needed. Replacement plants were treated with oil or water as described above. When all beetles had burrowed into the soil inside pots to pupate, foliage was clipped and pots were monitored daily for adult emergence. Adults were collected and weighed on a microbalance (Ohaus Corporation, Parsippany, NJ). Total number of beetles emerging, adult weight, and development time from first instar to adulthood were recorded. This procedure was replicated five times. Mean number of beetles emerging per cage was analyzed by one-way ANOVA. Mean adult weight per beetle and mean number of days to develop to adulthood per beetle were compared using two-way ANOVA, with treatment and block as main factors.

2.2.4.3. Interaction Between Mineral Oil and *Beauveria bassiana*

A spray assay was done to test whether spraying beetles with a combination of mineral oil and *Beauveria bassiana* would increase beetle mortality compared to each of the treatments

applied alone. Single potato leaflets (cv. Superior) were taped to the bottom of each of 32 Petri dishes (eight per treatment). Five first instar Colorado potato beetle larvae were placed on the leaflet of each dish with a fine hair paintbrush. Dishes were sprayed with either water, 3% mineral oil, Mycotrol ESO (BioWorks Inc, Victor, NY) suspended in water at a concentration of $2x10^{10}$ conidia/mL, and Mycotrol ESO ($2x10^{10}$ conidia/mL) mixed with a 3% water solution of mineral oil. Applications were made with a garden sprayer (Roundup Multi-Purpose Sprayer, The Fountainhead Group Inc., New York Mills, NY). To prevent cross-contamination, each treatment solution was prepared in a separate sprayer. Sprayers were calibrated to deposit a 2 mL fine mist spray in each trial. After spraying, leaflets and beetles were transferred to new Petri dishes lined with a damp paper towel at the bottom. Larvae were reared in a growth chamber (Percival Scientific Inc., Perry, IA) at 20°C with a 16:8 h L:D photoperiod for nine days and were checked daily for mortality. The above procedure was repeated five times.

To confirm infection by *B. bassiana*, dead larvae were collected each day and reared in 85 x 125 mm 48-well plates (Corning Inc., Corning, NY). Beetles were sterilized by dipping in zephiran chloride, then rinsing twice with distilled water before being transferred to the plates. To grow the fungi, plates were kept at 100% humidity by putting plates in a Tupperware container with wet paper towels, and then kept in darkness in a growth chamber at 22°C. Beetles were checked daily for *B. bassiana* sporulation.

To analyze beetle mortality and sporulation of *B. bassiana* among beetle cadavers over time, three-way repeated measures ANOVA was ran using SAS (PROC MIXED, SAS Institute 2012). Treatment with oil, treatment with *B. bassiana*, and block were used as main factors.

2.3. Results

2.3.1. Aphids

2.3.1.1. Insecticidal Properties of Oil

Spraying aphids with oil caused greater mortality than spraying aphids with water. Green peach aphids were significantly more likely to be killed when sprayed with oil compared to the control. Treatment with oil did not affect the number of nymphs born per surviving adult. Potato aphids also were significantly more likely to be killed by oil than by water, and treatment with oil did not affect the number of nymphs for that species (Table 2.1).

Table 2.1. Number of adult aphids dying and number of nymphs being produced by surviving aphids sprayed with the oil formulation or water. Data are presented as mean ± standard error, along with ANOVA results.

			ANOVA		
	Oil	Water	F	df	p
GPA					
No. adults dying	1.45 ± 0.17	0.016 ± 0.06	76.2	1, 119	<0.001
No. nymphs born	1.38 ± 0.0.14	1.3 ± 0.13	0.09	1, 114	0.76
PA					
No. adults dying	1.41 ± 0.18	0.31 ± 0.09	37.0	1, 119	<0.001
No. nymphs born	0.41 ± 0.09	0.56 ± 0.12	0.53	1, 100	0.47

2.3.1.2. Repellant and/or Antifeedant Properties of Oil

In the choice bioassay, treatment did not affect green peach aphid location. However, mortality was significantly greater among green peach aphids located on leaflets treated with oil than green peach aphids on water-treated leaflets. Treatment did not affect nymph production by surviving green peach aphid adults (Table 2.2).

Potato aphids in the choice bioassay were significantly less likely to choose oil-treated leaflets than water-treated leaflets. Treatment with oil did not affect mortality. Potato aphid nymph production was reduced among surviving adults on oil-treated leaflets (Table 2.2).

In the no-choice bioassay, green peach aphids exposed to the oil treatment were significantly less likely to be found on leaflets than those in the water treatment. Oil treatment also increased mortality of green peach aphids on leaflets. Nymph production by surviving adults was not significantly affected by oil treatment (Table 2.2).

Potato aphids in the no-choice bioassay were significantly less likely to be found on leaflets in the oil treatment compared to the control. Mortality among potato aphids on oil-treated leaflets was significantly higher. Similarly, nymph production by potato aphids was also significantly lower for surviving adults in the oil treatment (Table 2.2).

Table 2.2. Number of aphid adults choosing a leaflet, aphids dying on leaflets, and nymphs produced by surviving adults on leaflets treated with the oil formulation or water. Data are presented as mean ± standard error, along with ANOVA results.

	Choice Assay					No-Choice Assay				
			ANOVA					ANOVA		
	Oil	Water	F	df	p	Oil	Water	F	df	p
GPA										
Adults	1.4 ± 0.1	1.8 ± 0.1	3.7	1, 194	0.057	1.2 ± 0.1	2.2 ± 0.1	45.7	1, 94	<0.001
Mortality	0.3 ± 0.1	0.1 ± 0.04	6.3	1, 194	0.013	0.34 ± 0.1	0.1 ± 0.03	18	1, 94	<0.001
Nymphs	0.98 ± 0.2	0.92 ± 0.1	0.02	1, 135	0.9	1.3 ± 0.2	1.7 ± 0.3	1.7	1, 81	0.17
PA										
Adults	0.7 ± 0.1	2.2 ± 0.2	69.5	1, 194	<0.001	0.98 ± 0.1	1.6 ± 0.1	15.9	1, 94	<0.001
Mortality	0.22 ± 0.1	0.2 ± 0.04	1.1	1, 194	0.3	0.2 ± 0.05	0.02 ± 0.0	10.3	1, 94	0.002
Nymphs	0.15 ± 0.1	0.68 ± 0.2	9.9	1, 108	0.002	0.36 ± 0.2	0.78 ± 0.2	5.9	1, 76	0.02

2.3.1.3. Effects on Survivorship and Reproduction

After nine days, oil treatment significantly reduced the number of surviving green peach aphids compared to the control. Potato aphid survival was also significantly lower on oil-treated leaves than water-treated leaves after nine days (Table 2.3).

Table 2.3. Number of aphids surviving on oil-treated or water-treated leaves after nine days. Data are presented as mean ± standard error, along with ANOVA results.

			ANOVA		
Species	Oil	Water	F	df	p
GPA	0 ± 0	20 ± 0.8	25.3	1, 6	0.002
PA	4.8 ± 0.6	21.3 ± 2.6	39.1	1, 6	<0.001

2.3.2. Colorado Potato Beetle

2.3.2.1. Repellant and/or Antifeedant Properties of Oil

In the choice bioassay, adults fed significantly less on leaflets treated with oil than on leaflets treated with water (Table 2.4). However, treatment did not have an effect on adult location (X^2=2.97, df=1, p=0.09). Twelve out of 100 were located on the oil-treated leaflet, while 21 out of 100 were on the water-treated leaflet.

Treatment did not affect feeding damage by first instars in the choice bioassay (Table 2.4). First instars were significantly less likely to be found on leaflets treated with oil than on leaflets treated with water (Table 2.5).

Fourth instar feeding was not significantly affected by treatment in the choice bioassay (Table 2.4). Treatment also did not affect location of fourth instars (X^2=0.023, df=1, p=0.88). Of the 100 larvae tested, 31 were on the oil-treated leaflet and 32 were on the water-treated leaflet.

In the no-choice bioassay, treatment did not affect feeding by adult beetles (Table 2.4). Adult location also was not affected by treatment with oil (X^2=0.047, df=1, p=0.83). Fifteen out of 50 beetles in the oil treatment were found on leaflets, while 16 of 50 in the water treatment were found on leaflets.

Feeding by first instars was not affected by treatment with oil in the no-choice bioassay (Table 2.4). However, larvae were less likely to be found on leaflets when exposed to oil-treated leaflets than those exposed to water-treated leaflets (Table 2.5).

Treatment with oil did not affect feeding by fourth instars in the no-choice bioassay (Table 2.4). Treatment also did not affect fourth instar location (X^2=1.17, df=1, p=0.28). Thirty-two out of 50 larvae receiving the oil treatment were found on leaflets; 37 out of 50 receiving the water treatment were on a leaflet.

Table 2.4. Leaflet area loss (cm^2) due to beetle feeding in choice and no-choice bioassays. Data are presented as mean ± standard error, along with ANOVA results.

	Choice Assay					No-Choice Assay				
			ANOVA					ANOVA		
	Oil	Water	F	df	p	Oil	Water	F	df	p
Adult	2.7 ± 0.3	3.8 ± 0.3	9.48	1, 194	0.002	2.9 ± 0.3	3.5 ± 0.3	30.5	1, 194	0.037
1st Instar	1.2 ± 0.1	1.1 ± 0.1	0.09	1, 194	0.77	1.3 ± 0.2	1.0 ± 0.1	2.8	1, 194	0.096
4th Instar	3.5 ± 0.3	3.9 ± 0.3	0.36	1, 194	0.55	4.2 ± 0.3	3.6 ± 0.3	0.11	1, 194	0.74

Table 2.5. Location of first instar beetle larvae in choice and no-choice experiments. Data presented as mean ± standard error, along with ANOVA results.

			ANOVA		
	Oil	Water	F	df	p
Choice	2.7 ± 0.24	4.57 ± 0.25	29.3	1, 194	<0.001
No-Choice	6.7 ± 0.28	7.7 ± 0.2	6.9	1, 94	0.01

2.3.2.2. Effects of Oil on Development

Treatment with oil did not significantly affect number of beetles surviving to adulthood. Adult beetles which fed on oil-treated plants weighed significantly less than those which fed on water-treated plants. Beetles reared on oil-treated plants also took significantly longer to develop into adults than those from water-treated plants (Table 2.6). Neither block nor the interaction between block and treatment affected beetle weight (block F=3.5, df=1, 304, p=0.06; interaction F=0.0, df=1, 304, p=0.98) and speed of development (block F=1.3, df=1, 304, p=0.26; interaction F=0.04, df=1, 304, p=0.83), suggesting lack of confounding effects of keeping measured beetles in the same cages.

Table 2.6. Numbers of emerging adult beetles, adult weights, and development time for beetles raised on oil-treated or water-treated potato plants. Data are presented as mean ± standard error, along with ANOVA results.

			ANOVA		
	Oil	Water	F	df	p
Emerged adult beetles	26.6 ± 7.5	35 ± 8.4	0.56	1, 8	0.48
Adult weight (g)	0.099 ± 0.002	0.11 ± 0.002	16.7	1, 304	<0.001
Development time (days)	30.4 ± 0.3	28.7 ± 0.17	48.2	1, 302	<0.001

2.3.2.3. Interaction Between Mineral Oil and *Beauveria bassiana*

Treatment with oil did not affect beetle mortality (F=3.12, df=1, 156, p=0.079). Beetles treated with *B. bassiana* showed significantly greater beetle mortality compared to beetles not treated with the fungus (F=28.5, df=1, 156, p<0.0005). No significant interaction between oil and *B. bassiana* was detected (F=0.46, df=1, 156, p=0.5). However, interaction between oil, *B. bassiana*, and day since treatment was significant (F=2.29, df=24, 1248, p=0.0004), with beetles treated with oil and *B. bassiana* together dying sooner than beetles sprayed with *B. bassiana* alone (Figure 2.1).

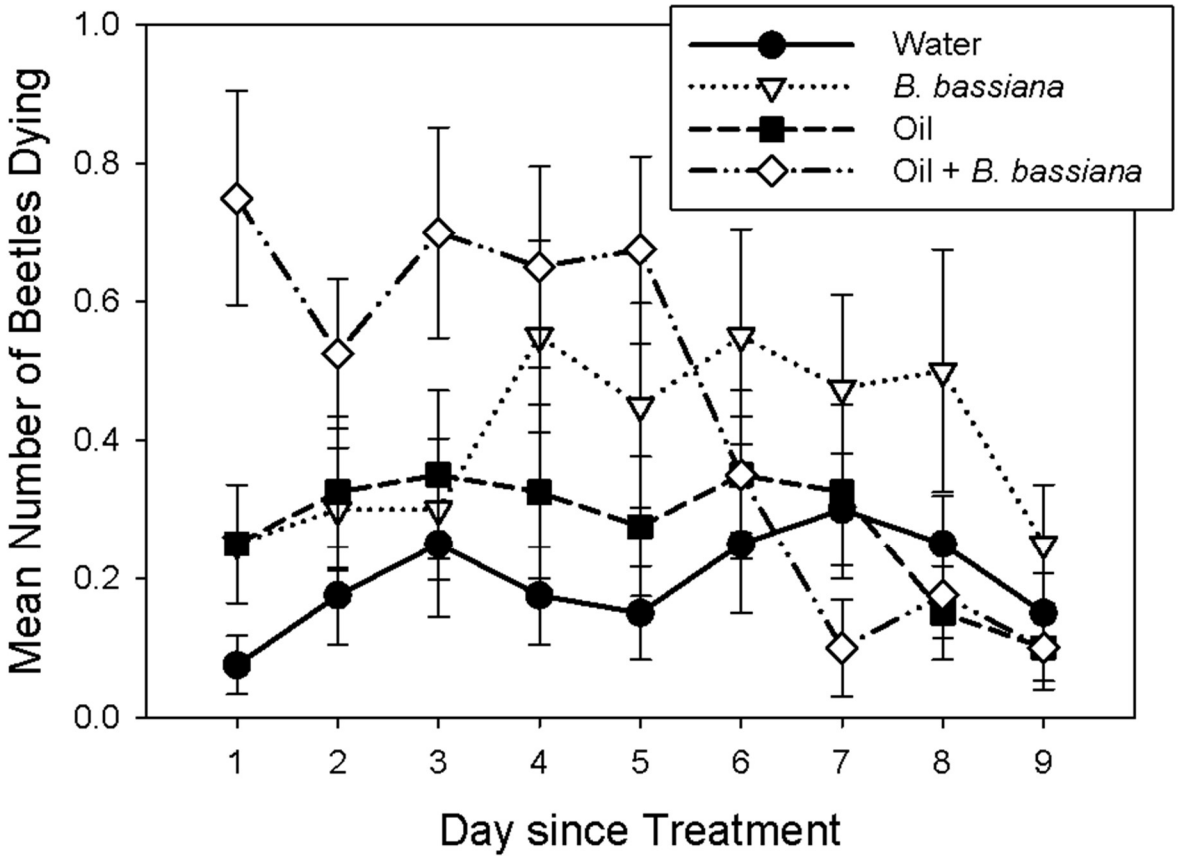

Figure 2.1. Number of beetles dying over the nine-day period following treatment with oil and/or *B. bassiana*. Data are presented as means per Petri dish ± standard errors.

Number of sporulating cadavers was not affected by oil spray (F=0.55, df=1, 155, p= 0.4601). Treatment with *B. bassiana* caused significantly greater sporulation compared to treatments without *B. bassiana* (F=90.17, df=1, 155, p<0.0001). The interaction between oil and *B. bassiana* was not significant (F=0.05, df=1, 155, p= 0.8246). Similar to mortality data, interaction between oil, *B. bassiana*, and day since treatment was significant (F=4.08, df=27, 1404, p<0.0001). Cadavers of beetles treated with oil and *B. bassiana* together sporulated sooner than those of beetles sprayed with *B. bassiana* alone (Figure 2.2).

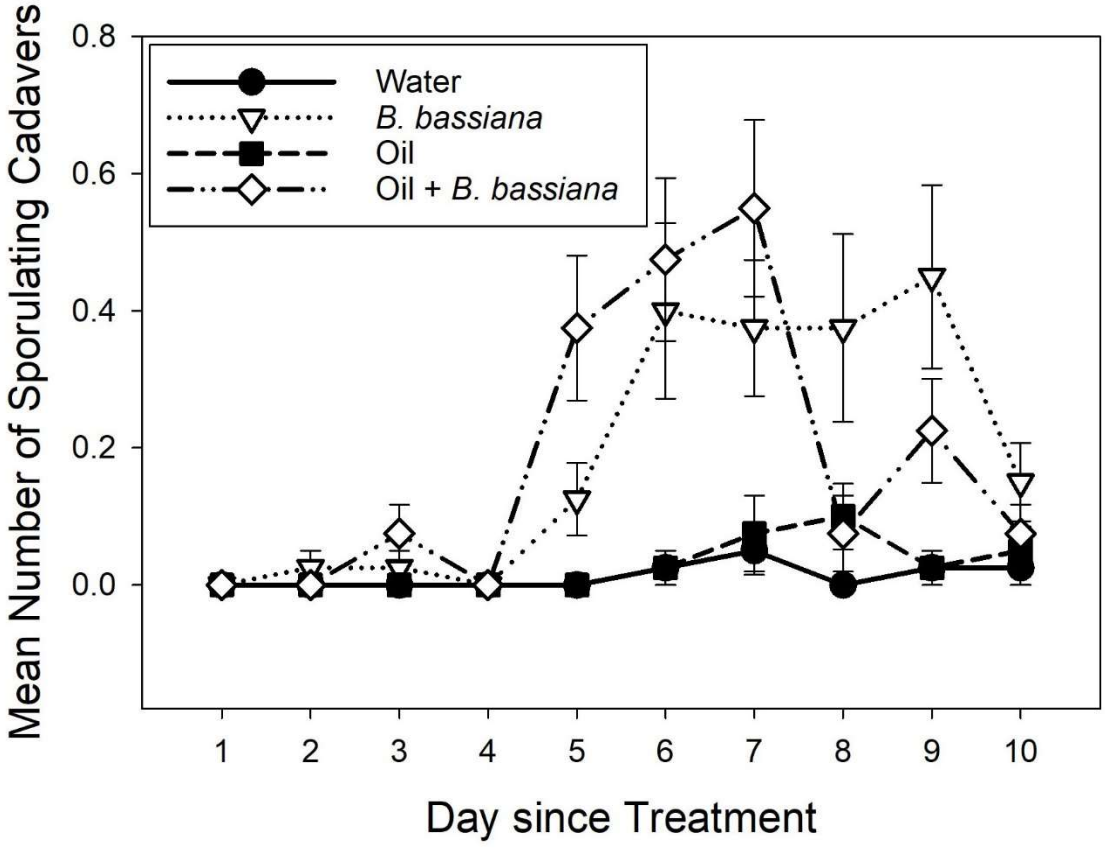

Figure 2.2. Number of beetle cadavers sporulating over the nine-day period following treatment with oil and/or *B. bassiana*. Data are presented as means per Petri dish ± standard errors.

2.4. Discussion

Mineral oil has been used as an insecticide to control aphids in various crops, although the mode of action is unclear (Najar-Rodríguez et al. 2008). In the present study, this contact insecticidal activity was demonstrated. However, mortality was only about 30% for both species tested. This is lower than in similar experiments conducted by other researchers, where aphid mortality ranged from 75% to over 95% after 24 hours (Martín-López et al. 2006, Najar-Rodríguez et al. 2007a, Kraiss and Cullen 2008). One possible explanation is the use of different mineral oils between experiments. Mineral oil comes in a variety of formulations with different chemical blends, and this affects the activity of the oil (Najar-Rodríguez et al. 2007a). The present study used JMS Stylet-Oil, while the cited studies did not. The variation could also be due to the methods used. Each study used a different sprayer, and it is possible that affected droplet size and coverage. Different aphid species also have different susceptibility to oil (Herron et al. 1995). However, green peach aphids have previously shown higher vulnerability to oil (Martín-López et al. 2006). Furthermore, both species used in the present study were equally affected by oil; therefore, variation in oil susceptibility among species likely does not completely account for differences in results.

Mineral oil residues on foliage also negatively impacted aphids. In both the choice and no-choice assays, potato aphids showed a strong preference for leaflets without oil. This is consistent with results reported by Ameline et al. (2009), who attributed this effect to oil masking the potato foliage. Surprisingly, when given a choice, green peach aphids did not preferentially colonize untreated leaflets. The difference in biology between the two species could possibly help explain this result. Potato aphids are more mobile than green peach aphids (Alyokhin and Sewell 2003); therefore, they may have had a higher propensity for moving from

an initially colonized oil-treated leaflet onto an untreated leaflet. On the other hand, green peach aphids may have had a difficulty making their way to an untreated leaflet. Indeed, green peach aphids found on oil-treated leaflets were more likely to be dead compared to those found on untreated leaflets. Green peach aphids were less likely to be found on oil-treated leaflets in the no-choice bioassay compared to the untreated leaflets. Therefore, there was some avoidance of oil by green peach aphids that moved short distances from treated leaflets to the untreated parts of Petri dishes, even though they may not have made their way to the untreated leaflets.

Mineral oil exposure has been shown to increase aphid fecundity among survivors despite decreased survival (Martoub et al. 2011). In the present study, we were unable to observe any effect on aphid development because very few aphid nymphs survived on the oil-treated leaflets, with none of the green peach aphids surviving to adulthood. This seems encouraging, as the high level of mortality of apparently more vulnerable early instars could offset any increase in reproduction and maintain good aphid control. The results of the choice and no-choice assays also did not provide evidence for increasing fecundity due to oil applications. Nymph production was either not affected or significantly lower among aphids on oil-treated leaflets. This does not mean that the possibility of oil increasing aphid reproduction should be ignored. In the field, aphids are likely to be exposed to lower oil concentrations because of incomplete coverage and/or environmental degradation of the material (Najar-Rodríguez et al. 2007a, Al-Mrabeh et al. 2010). As a result, they may acquire only sublethal doses of the active ingredient, leading to its hormetic effect on their reproduction (Cohen 2006). Overall, though, mineral oil appears promising for aphid control, with contact mortality, feeding deterrence, and residual toxicity all negatively impacting aphids.

The effects of mineral oil on Colorado potato beetles had not been tested prior to the present study. Our results showed that oil residues can reduce beetle damage to potato plants, although this effect depends on the life stage. First instars were the most susceptible. Although they would still feed on oil-treated foliage, first instars showed a preference for leaflets without oil. Adult and fourth instar beetles appeared to be less affected by oil treatment. Adults did feed less on oil-treated leaflets when given a choice, but not when provided only oil-treated leaflets. Fourth instars did not change feeding behavior in either assay. This is not surprising because this life stage is the most voracious in the Colorado potato beetle life cycle (Ferro et al. 1985). Therefore, oil effects were likely not strong enough to be detected in our assays.

While the ability of first instars to survive to adulthood was not affected, adults emerging from oil-treated plants weighed less and took longer to develop compared to the adults grown on the untreated plants. Weight is related to a variety of life history traits, such as fecundity, in insects (Honek 1993, Chown and Gaston 2010). Therefore, feeding on potato plants treated with mineral oil could result in reduced adult fitness, thus reducing beetle populations and potentially providing at least some level of crop protection in the field.

The synergy between mineral oil and *B. bassiana* is a potentially important consideration for potato IPM. *B. bassiana* is an appealing tool for IPM due to its reduced environmental impact (Roy and Pell 2000) and lower risk of developing resistance compared to most conventional insecticides (Dubovskiy et al. 2013). Its use against Colorado potato beetles, however, has been limited because it is generally not as effective as many synthetic insecticides (Wraight et al. 2009). Therefore, finding ways to increase its efficacy could improve its adoption by farmers. Combining *B. bassiana* and mineral oil increased red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae), mortality compared to *B. bassiana* alone (Akbar et al. 2005),

possibly due to more efficient cuticle penetration by the fungus. In our study, overall mortality did not increase when *B. bassiana* was combined with oil. However, in the combined treatment beetle larvae died earlier. This is important, as one of the issues limiting more widespread *B. bassiana* use is its tendency to take several days to kill the targeted pests. Some caution should be used, as killing insects too quickly could reduce the likelihood of secondary infections (Klinger et al. 2006). Still, growers who spray oil to protect plants against PVY may be able to add *B. bassiana* to their oil sprays and control Colorado potato beetles at the same time.

In summary, mineral oil appears to be a good fit for many potato IPM programs. Its efficacy against PVY spread is well-established, and is a primary driver of its use by commercial potato growers. In addition, its negative effects on aphids could further curtail the spread of PVY and other viruses. Oil could also help reduce Colorado potato beetle populations, and could be combined with *B. bassiana* to help the fungus control the beetles more effectively. As a result, adopting mineral oil is likely to improve crop protection for IPM-practicing potato growers.

CHAPTER 3

SIMULATION MODEL OF POTATO VIRUS Y SPREAD

3.1. Introduction

Potato Virus Y (PVY) is the most economically important virus infecting potato (Gray et al. 2010, Scholthof et al. 2011). PVY is transmitted non-persistently by aphids (Hemiptera: Aphididae). Therefore, the virus is acquired in brief probes even in the absence of sustained feeding (Gray and Banerjee 1999). The virus has a complex epidemiology, with over 65 species of aphids serving as potential vectors (Pelletier et al. 2012). Because of this, control of the disease is difficult, and few reliable control methods are available (Davidson et al. 2013). Efficacy of control strategies can vary significantly from year to year or between different locations. Understanding the dynamics driving PVY spread could help clarify which factors should be the focus of control efforts.

PVY can be transmitted both by potato-colonizing aphids and aphids which do not colonize potato plants. However, the relative importance of potato colonizers and non-colonizers is uncertain. On one hand, colonizing aphid populations are able to build up within potato fields, and these aphids often have high transmission efficiencies (Al-Mrabeh et al. 2010). On the other hand, non-colonizers will likely probe more plants within a potato field, leading to more opportunities to transmit the virus (Boquel et al. 2014). Individual species can also differ in characteristics such as transmission efficiency and propensity to move among plants, which could also affect PVY spread (Boquel et al. 2011, 2014). However, many characteristics related to vector behavior are nearly impossible to measure (Ferriss and Berger 1993), making it difficult to know which species are most responsible for spreading the virus. This is unfortunate

because having a good idea of which vector species are epidemiologically important could inform decisions about which species to target for control.

Mineral oil is a tool used by many potato growers to reduce the spread of PVY. Mineral oil hinders the attachment of the virus particle to the aphid's mouthparts, thus reducing transmission efficiency by aphid vectors (Wang and Pirone 1996, Boquel et al. 2013). The ability of mineral oil to reduce PVY spread has frequently been demonstrated (Bradley et al. 1966, Boiteau and Singh 1982, Kirchner et al. 2014). While oil consistently lowers PVY incidence in the field, the magnitude of control can vary considerably from year to year, and oil is often insufficient for keeping PVY at economically acceptable levels on its own (Hansen and Nielsen 2012, MacKenzie et al. 2017). It is likely that the variation can be explained by outside factors, such as inoculum levels and vector abundance (MacKenzie et al. 2017). A clearer understanding of these processes could help optimize the use of mineral oil along with other management strategies.

Early-season virus inoculum is a major cause of PVY epidemics. Because of this, a common control strategy is limiting the amount of inoculum in the field. This can be done through seed certification programs, in which tolerance limits for the amount of PVY allowed in seed potato lots are set by government agencies (Davidson et al. 2013). Rouging, or the physical removal of plants showing PVY symptoms, can be used to lower virus inoculum in fields during the growing season (Radcliffe and Ragsdale 2002). However, the spread of strains showing milder foliar symptoms has made these strategies more difficult, thus increasing virus inoculum in seed potato lots and causing more of them to be rejected (Lorenzen et al. 2006, Lacomme et al. 2014).

Field size is another factor that could affect how PVY is spread among potato plants. Field size can vary considerably among different potato-growing regions (Alyokhin et al. 2015). Some techniques, such as planting more susceptible cultivars in the middle of fields surrounded by resistant cultivars (Davidson et al. 2013), are more effective in small plots. Smaller fields also have a greater edge to area ratio, which could be relevant as migrating aphids arriving from surrounding vegetation are more likely to land on the edges of fields (Radcliffe and Ragsdale 2002). However, little information is available on how exactly field size affects the dynamics of PVY spread.

Simulation modeling is a tool with a variety of applications in the study of plant disease epidemiology. Modeling is beneficial because it allows the user to test variables which are extremely difficult to manipulate in laboratory or field experiments (Ferriss and Berger 1993). Models can also synthesize complex epidemiological information and clarify the factors influencing disease spread.

Several approaches have been used to model plant disease. Many models have been constructed for specific diseases to forecast their epidemics based on field-collected data. For example, one such model simulated the spread of barley yellow dwarf virus (BYDV), a persistently transmitted virus of cereals (Kendall et al. 1992). That model was able to predict virus spread well based on aphid trap counts and environmental data. Another model, EPIVIT, was developed to simulate degeneration of potato lots due to viruses (Bertschinger et al. 1995). That model used temperature and aphid trap counts to project virus spread over time. EPIVIT predicted overall virus infection well, but did not distinguish between different viruses or transmission types. Disease-specific models can be very useful for informing pest management decisions, such as timing of control measures. However, they require gathering a large amount of

data, which is very labor-intensive (Kendall et al. 1992). They are also specific to a certain set of conditions, and often focus on finding correlations between disease levels and environmental factors rather than on understanding basic mechanisms underlying disease epidemiology.

Simulation models can also be used to draw more general conclusions about dynamics of plant diseases. For example, Jeger et al. (1998) constructed a model based on models used in human disease epidemiology. That model used a series of linked equations into which parameters related to vector population dynamics and transmission processes were inputted. The model was able to demonstrate how viruses with different modes of transmission responded differently to management techniques. Madden et al. (2000) expanded on the model by incorporating vector immigration, and further investigated how the factors affecting disease spread varied by transmission class. That model was instrumental for showing broad patterns among different types of plant viruses. However, its use is limited by the fact that it focuses on broad epidemiological patterns rather than on specific characteristics of a certain disease or vector.

Spatially-explicit, agent-based simulation modeling is one approach that can be used to incorporate biological and behavioral characteristics of different vectors. This approach involves constructing a virtual field with plants represented by cells in a matrix. The field contains agents, representing vectors, which move among the cells. Currently, few spatially-explicit simulation models have been made to measure the spread of plant viruses. One which does exist was constructed by Ferriss and Berger (1993). That model allowed the user to manipulate a range of input variables to simulate how plant disease can spread within a field. However, it had a number of limitations, including a restricted field size and vector population. Additionally, that model was designed to show only very general patterns among all arthropod-vectored plant viruses. A

more specific model was later constructed to simulate rice tungro virus disease (RTVD), which is semi-persistently transmitted by leafhoppers (Holt and Chancellor 1996). That model was able to determine different scenarios in which rouging could be an effective tool to control RTVD.

In the present study, we built a spatially-explicit model to simulate PVY spread among potato plants. Our major objectives were to gain insights into possible effects of transmission efficiency, initial inoculum levels, vector behavior, and field size on disease levels at the end of a simulated growing season. Clarifying the relationships between the various factors influencing PVY spread may have important implications for designing integrated pest management plans targeting virus reduction in commercial potato fields.

3.2. Materials and Methods

3.2.1. Model Description

The model constructed for the present study was a spatially-explicit model simulating PVY spread in a potato field. The field was set up as a square grid of cells that represent potato plants. Aphids occupied plants within the field; multiple aphids could be residing on the same plant. Aphids moved among plants as the model ran. Plants existed in two states: virus-infected or uninfected. Likewise, aphids could be either viruliferous or aviruliferous. The computer program to run the model was written by Dr. Hongchun Qu (Chongqing University of Posts and Communications) in the computer programming language GAMA, version 1.7 (Grignard et al. 2013).

Each replication of the model included a series of simulations, with each simulation representing one week in a field season. A simulation consisted of a set number of movement steps; each movement step was a single movement of every aphid in the field (Figure 3.1). Once all movement steps in a simulation were complete, the next simulation began with the same field

as in the end of the previous simulation, but with a different group of aphids. Each simulation could have a different group of aphids with different parameters. This process continued until the desired number of simulations was reached.

Parameters inputted into the model for the field and aphid community are listed in Table 3.1. Field parameters were field size and initial proportion of virus-infected plants. Field size was represented by a number of cells in a square grid. Aphid parameters were population size, transmission efficiency (i.e., the probability of a viruliferous aphid transmitting the virus when it landed on an uninfected plant), number of probes before a viruliferous aphid lost the virus, number of plants an aphid visited, and maximum number of spaces the aphid moved in a single movement step. Number of plants visited by each aphid was determined based on a normal distribution within one standard deviation of the set mean. Number of spaces moved during each movement step was randomly selected from a uniform distribution ranging from one to the set maximum. The model allowed for multiple aphid species with different parameters to be present at the same time.

Table 3.1. List of parameters included in the model.

Parameter	Value	Distribution	Units
Field size	170x170 – 762x762	Constant	Potato plants spaced at 30 cm from each other
Initial inoculum	0.001 – 0.2	Constant	Proportion of plants infected
Aphid population size	10,000	Constant	Individuals per simulated week
Transmission efficiency	0.01 – 0.75	Constant	Probability of probe resulting in successful virus transmission
Plants visited before losing virus	3	Constant	Number of plants that a viruliferous aphid can infect
Number of plants visited	2 (colonizers); 10 (non-colonizers)	Normal	Plants visited per simulated week
Distance moved per movement step	1-50	Uniform	Number of plants moved per movement step

At the beginning of each replication, virus-infected plants were randomly distributed throughout the field, and aphids were randomly placed on plants. Aphids then moved following the algorithm shown in Figure 3.1. During each movement step, each aphid moved to a random plant within the field. The maximum distance of that move was determined by the parameter set for that species prior to running the model. If the plant where the aphid landed was infected, the aphid became viruliferous. If the aphid was viruliferous and the plant was uninfected, the plant had a chance of becoming infected depending on the transmission efficiency of the aphid. Each aphid repeated this process until it settled on a plant after the specified number of plants visited for that species, until it reached the field edge and left the field, or until the simulation ended. Viruliferous aphids lost their ability to transmit viruses after landing on three uninfected plants (Bradley and Rideout 1953, Wrobel 2007). Once all replications had finished, the model tallied and recorded the proportion of infected plants at each movement step.

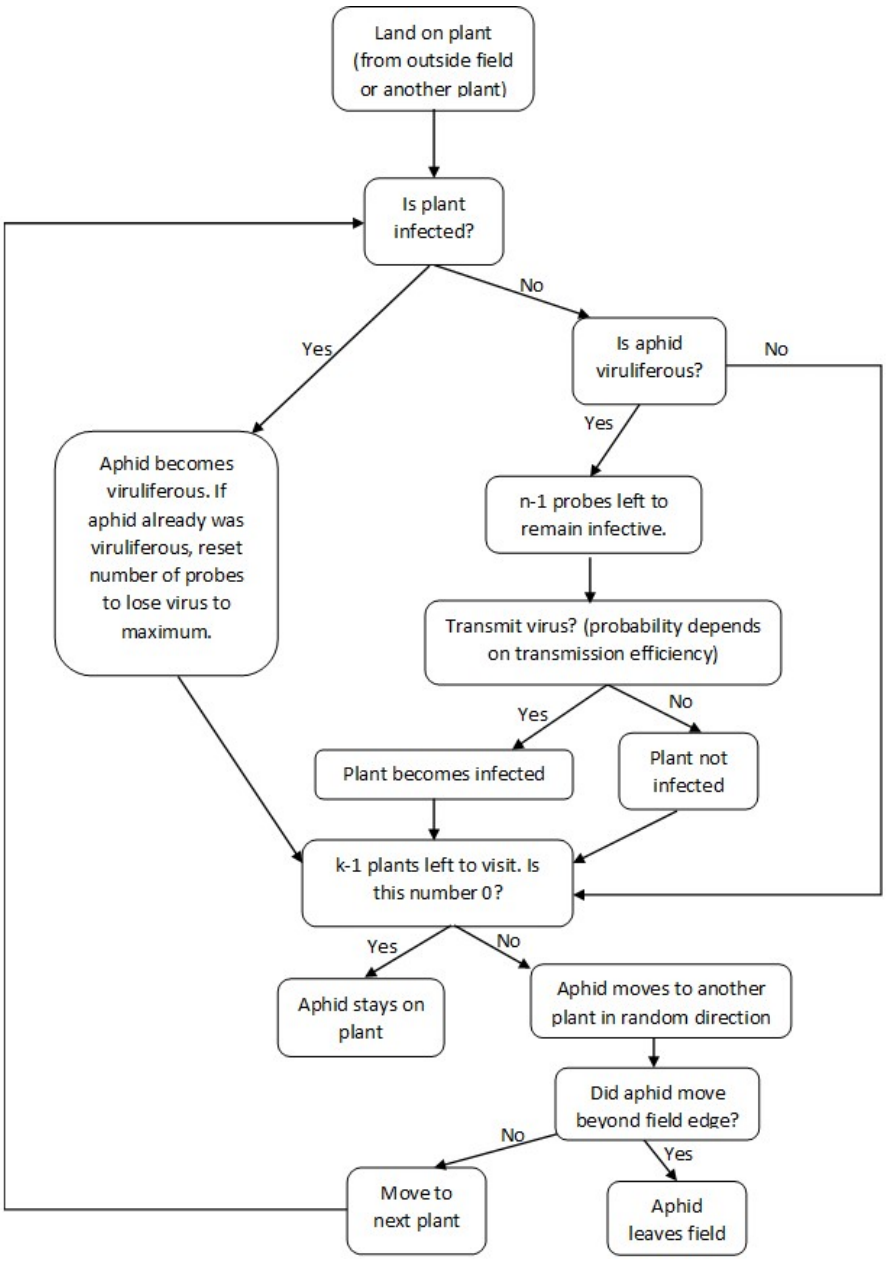

Figure 3.1. Algorithm of aphid movement and virus transmission in the simulation model.

3.2.2. Transmission Efficiency vs. Initial Inoculum

A series of runs was performed with different levels of initial inoculum and transmission efficiency to test their relative importance in the spread of PVY. Virus spread was tested at initial inoculum levels (defined as proportions of infected plants at the beginning of the first run before any transmission by aphids; see above) of 0.001, 0.005, 0.01, 0.05, and 0.1. At each inoculum level, five aphid transmission efficiencies were tested: 0.01, 0.1, 0.25, 0.5, and 0.75. The field size was kept constant at 660 x 660 cells to approximate a 12 ha potato field. Fourteen simulations were run per replication, with each simulation representing a week during the field season. Each simulation consisted of fifteen movement steps. The aphid population was kept constant at 10,000 winged individuals belonging to the same species during each week. Aphids visited ten plants per week. Each transmission efficiency by initial inoculum level scenario was replicated 30 times.

3.2.3. Colonizing vs. Non-Colonizing Aphids

Another series of runs was used to compare the relative importance of potato-colonizing aphids and non-colonizing aphids in spreading PVY. Three treatments were tested in this experiment. The first treatment represented colonizing aphids, specifically green peach aphids, which are the most efficient PVY vectors among potato colonizers. Transmission efficiency was set at 0.71 (Piron 1986). Green peach aphids were set to visit a mean of two plants per simulated week. Ten thousand aphids were used for each simulation. The second treatment consisted of non-colonizing aphids. In that treatment, twenty different non-colonizing potato aphid species were represented (Table 3.2). Transmission efficiencies for each species were taken from Al-Mrabeh et al. (2010). Species were selected to get a range of genera and transmission efficiencies. When multiple values were reported, the maximum transmission efficiency was

used. All non-colonizing species visited a mean of ten plants per simulated week. Five hundred individuals of each species were used, totaling 10,000 winged aphids per week. The third treatment represented both colonizing and non-colonizing aphids present within the field together. Five thousand green peach aphids were included in the simulation using the same parameter settings as in the first treatment. In addition, 250 winged individuals of each non-colonizing aphid species used in the second treatment were included. The experiment was performed at three initial inoculum levels: 0.01, 0.05, and 0.2. As in the previous experiment, field size was set at 660 x 660 cells. Fifteen movement steps were used per simulation (week); fourteen simulations were done per replication. Each treatment was replicated 30 times per initial inoculum level.

Table 3.2. List of non-colonizing aphid species represented in the model, with corresponding transmission efficiencies expressed as probability of probes resulting in successful virus transmission. Transmission efficiencies compiled by Al-Mrabeh et al. (2010).

Species	Transmission efficiency
Acyrthosiphon pisum	0.14
Aphis fabae	0.24
Aphis glycines	0.75
Aphis gossypii	0.31
Aphis sambuci	0.12
Brachycaudus helichrysi	0.125
Capitophorus eleagni	0.2
Cavariella aegopodii	0.04
Cryptomyzus galeopsidis	0.174
Diuraphis noxia	0.07
Hyalopterus pruni	0.139
Hyperomyzus lactucae	0.174
Metopolophium dirhodum	0.03
Myzus cerasi	0.032
Phorodon humuli	0.35
Rhopalosiphum maidis	0.015
Rhopalosiphum padi	0.115
Sitobion avenae	0.018
Sitobion fragariae	0.101
Uroleucon spp.	0.083

3.2.4. Field Size

A final series of runs was used to test the effects of field size on PVY spread. Field sizes used were 100 x 100 plants (~0.4 ha field), 330 x 330 plants (~3 ha field), 539 x 539 plants (~8 ha field), 660 x 660 (~12 ha field), and 762 x 762 (~16 ha field). Transmission efficiencies for the non-colonizing aphids from the previous experiment were used (Table 3.2). Aphid population sizes per week were adjusted to maintain a constant population density of 833.3 winged aphids per hectare. Consequently, aphid populations for each week were as follows: 33 per species for 0.4 ha plots; 125 per species for 3 ha plots; 334 per species for 8 ha; 500 per species for 12 ha plots; and 667 per species for 16 ha plots. The experiment was run at initial inoculum levels of 0.01, 0.05, and 0.2. All other parameters were the same as in the previous experiment. Each replication consisted of fourteen simulated weeks, with fifteen movement steps per week. For each field size, 30 replications were performed per inoculum level.

3.2.5. Statistical Analysis

For each experiment, a two-way ANOVA was performed to test the effect of each variable tested in that experiment on both final PVY inoculum and proportional increase, defined as the proportion of PVY at the end of the season compared to the initial inoculum level. When a significant interaction was found, one-way ANOVAs were performed. For final proportion infected, one-way ANOVAs were run testing the effects of each variable (i.e., transmission efficiency, colonization behavior, or field size) on final proportion infected at each initial inoculum level. For proportional increase, one-way ANOVAs were ran testing the effects of initial inoculum on proportional increase for each individual treatment. Tukey's test was performed when one-way ANOVA results were significant. All analyses were performed in R (R Core Team 2016). Increase in PVY inoculum over time was analyzed by fitting the data to three-

parameter exponential curves using TableCurve 2D (Systat Software 2002). Linear curves were also fit; however, exponential curves were a stronger fit in all cases based on the Akaike Information Criteria. Best-fit equations, R^2 values, and significance of fit based on ANOVA were obtained from the analyses.

3.3. Results

3.3.1. Transmission Efficiency vs. Initial Inoculum

Increasing transmission efficiency ($F=1933$, $df=4, 740$, $p<0.001$) and initial inoculum ($F=14876$, $df=1, 740$, $p<0.001$) each significantly increased the proportion of plants infected with PVY at the end of a simulated field season (Table 3.3). The interaction between the two factors was significant ($F=1379$, $df=4, 740$, $p<0.001$). Increasing transmission efficiency significantly increased final infection rates at all initial inoculum levels.

Table 3.3. Proportion of plants infected with PVY at the end of a simulated field season at each transmission efficiency and initial inoculum level. Data presented as mean ± SD, along with ANOVA results. Means followed by the same letters within columns are not significantly different from each other ($p<0.05$) based on Tukey's test.

Transmission Efficiency	Initial Inoculum				
	0.001	0.005	0.01	0.05	0.1
0.01	0.0011 ± 0.0001a	0.0053 ± 0.0001a	0.011 ± 0.0001a	0.053 ± 0.0004a	0.11 ± 0.0004a
0.1	0.0019 ± 0.0001b	0.0093 ± 0.0002b	0.018 ± 0.0003b	0.089 ± 0.0006b	0.17 ± 0.0007b
0.25	0.004 ± 0.0003c	0.02 ± 0.0005c	0.039 ± 0.0008c	0.17 ± 0.0015c	0.3 ± 0.0016c
0.5	0.01 ± 0.0005d	0.048 ± 0.001d	0.092 ± 0.0017d	0.35 ± 0.002d	0.54 ± 0.002d
0.75	0.018 ± 0.001e	0.086 ± 0.002e	0.16 ± 0.003e	0.52 ± 0.002e	0.7 ± 0.002e
F	5398	36762	55512	450021	951211
df	4,145	4,145	4,145	4,145	4,145
p	<0.001	<0.001	<0.001	<0.001	<0.001

Transmission efficiency ($F=23174$, $df=1, 740$, $p<0.001$) and initial inoculum ($F=449.6$, $df=4, 740$, $p<0.001$) also significantly affected the proportional increase in virus inoculum over a simulated field season (Table 3.4). The interaction between transmission efficiency and initial inoculum was significant ($F=653.5$, $df=4, 740$, $p<0.001$). Increasing initial inoculum did not affect the proportional increase at a transmission efficiency of 0.01. However, greater initial inoculum levels showed lower proportional increase at all other transmission efficiencies.

Table 3.4. Proportional increase in virus inoculum over a simulated field season at each transmission efficiency and initial inoculum level. Data presented as mean ± SD, along with ANOVA results. Means followed by the same letters within columns are not significantly different from each other ($p<0.05$) based on Tukey's test.

	Transmission Efficiency				
Initial Inoculum	0.01	0.1	0.25	0.5	0.75
0.001	1.07 ± 0.05a	1.87 ± 0.09a	4.02 ± 0.28a	9.89 ± 0.47a	18.4 ± 1.1a
0.005	1.07 ± 0.02a	1.86 ± 0.05a	3.94 ± 0.1ab	9.61 ± 0.21b	17.1 ± 0.36b
0.01	1.06 ± 0.02a	1.84 ± 0.03a	3.89 ± 0.08b	9.24 ± 0.17c	16 ± 0.26c
0.05	1.06 ± 0.007a	1.78 ± 0.01b	3.46 ± 0.03c	7.02 ± 0.04d	10.3 ± 0.05d
0.1	1.06 ± 0.004a	1.7 ± 0.007c	3.05 ± 0.02d	5.35 ± 0.02e	7.02 ± 0.017e
F	0.63	68.4	264.1	1926	2675
df	4,145	4,145	4,145	4,145	4,145
p	0.643	<0.001	<0.001	<0.001	<0.001

Proportion of infected plants increased at an exponential rate (Figure 3.2; $p<0.05$ for all fitted models). The rates of increase got progressively steeper with decreasing initial inoculum and increasing transmission efficiency. Exponential curves explained the majority of variation except at the lowest transmission efficiency with the lowest initial inoculum level.

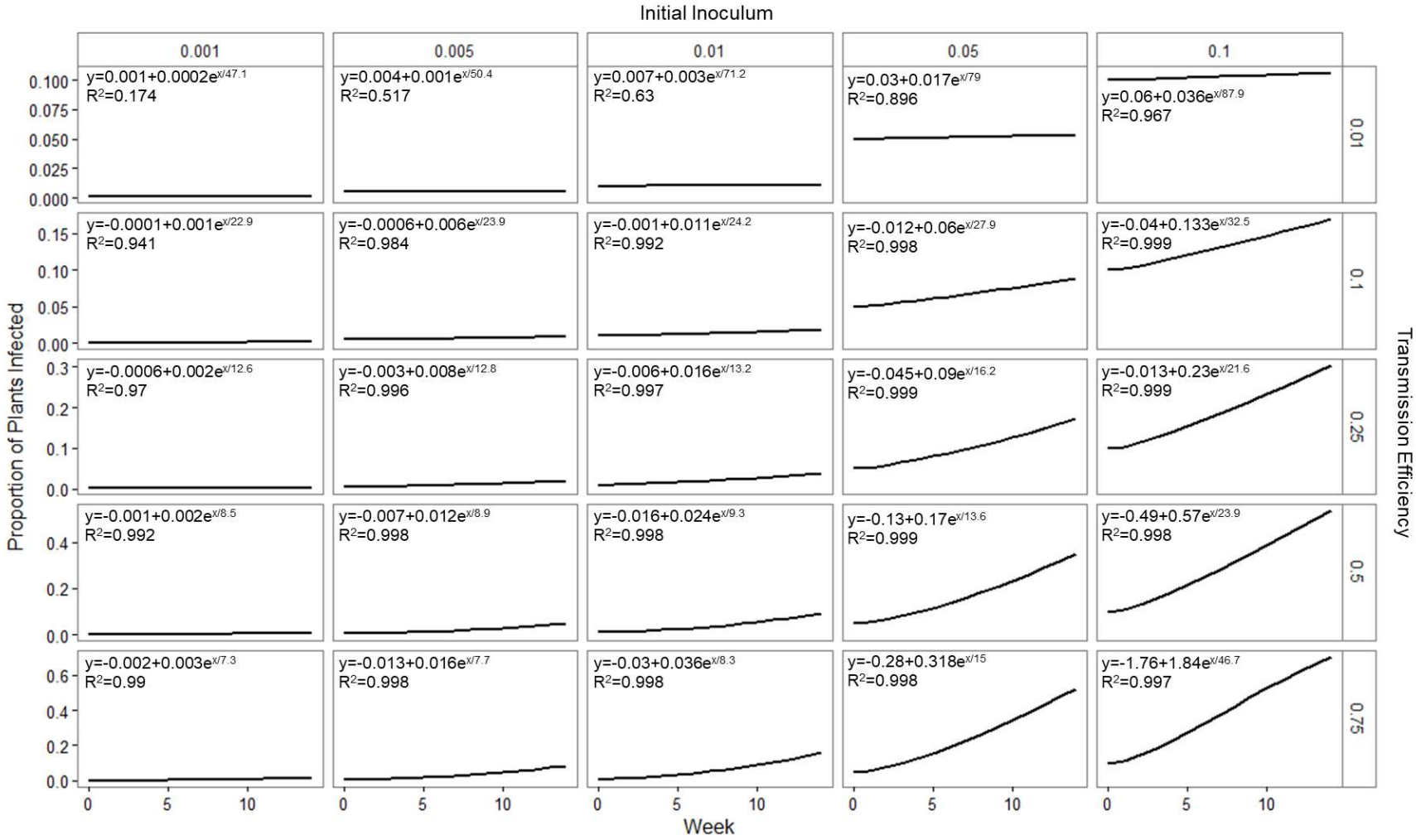

Figure 3.2. Increase in proportion of infected plants throughout a simulated growing season at each transmission efficiency and initial inoculum level. Exponential equation and R^2 value for each curve are shown.

3.3.2. Colonizing vs. Non-Colonizing Aphids

Aphid colonizing behavior ($F=3622$, $df=2, 264$, $p<0.001$) and initial inoculum ($F=168346$, $df=1, 264$, $p<0.001$) significantly affected final proportion of infected plants at the end of a simulated growing season (Table 3.5). Their interaction was also significant ($F=2052$, $df=2, 264$, $p<0.001$). Activity of non-colonizing aphids led to significantly greater final number of infected plants compared to the activity of colonizing aphids and to the activity of a mix of colonizing and non-colonizing aphids at all initial inoculum levels.

Table 3.5. Proportion of plants infected with PVY at the end of a simulated field season at each colonization type and initial inoculum level. Data presented as mean ± SD, along with ANOVA results. Means followed by the same letters within columns are not significantly different from each other ($p<0.05$) based on Tukey's test.

Colonization Behavior	Initial Inoculum		
	0.01	0.05	0.2
Colonizers	0.013 ± 0.0002a	0.063 ± 0.0005a	0.24 ± 0.0007a
Non-Colonizers	0.024 ± 0.0004b	0.11 ± 0.001b	0.37 ± 0.001b
Both	0.018 ± 0.0004c	0.086 ± 0.0006c	0.3 ± 0.001c
F	7892	34173	111294
df	2,87	2,87	2,87
p	<0.001	<0.001	<0.001

Aphid colonizing behavior ($F=39505$, $df=2, 261$, $p<0.001$) and initial inoculum ($F=4497$, $df=2, 261$, $p<0.001$) also significantly affected the proportional increase in inoculum throughout the season (Table 3.6). The interaction between colonizing behavior and initial inoculum was also significant ($F=1045$, $df=4, 261$, $p<0.001$). Increasing initial inoculum significantly lowered proportional increase for colonizing aphids, non-colonizing aphids, and a combination of colonizers and non-colonizers.

Table 3.6. Proportional increase in virus inoculum over a simulated field season at each colonization behavior and initial inoculum level. Data presented as mean ± SD, along with ANOVA results. Means followed by the same letters within columns are not significantly different from each other ($p<0.05$) based on Tukey's test.

Initial Inoculum	Colonization Behavior		
	Colonizers	Non-Colonizers	Both
0.01	1.28 ± 0.02a	2.39 ± 0.04a	1.79 ± 0.04a
0.05	1.25 ± 0.01b	2.25 ± 0.02b	1.72 ± 0.01b
0.2	1.21 ± 0.004c	1.84 ± 0.01c	1.52 ± 0.005c
F	248.8	3134	1198
df	2,87	2,87	2,87
p	<0.001	<0.001	<0.001

In each treatment, the proportion of infected plants increased exponentially over time (Figure 3.3; $p<0.05$ for all fitted models). At each initial inoculum level, non-colonizing aphids caused PVY to be spread at a faster rate compared to colonizing aphids. The equations explained most of the variation in all cases.

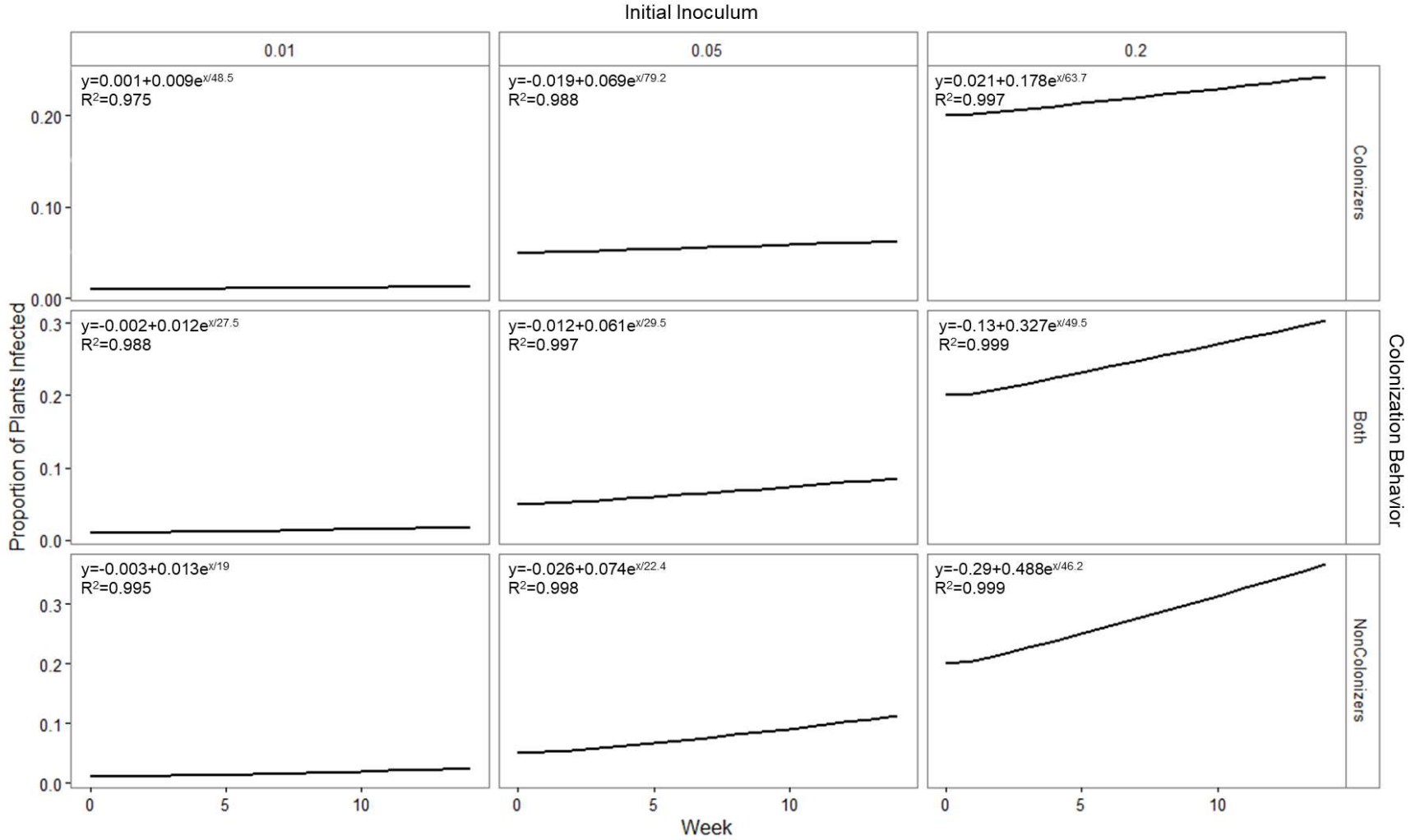

Figure 3.3. Increase in proportion of infected plants for each colonization type and initial inoculum level. Exponential equation and R^2 value for each curve are shown

3.3.3. Field Size

Field size did not significantly affect final proportion of infected plants at the end of the simulated field season ($F=1.358$, $df=4, 440$, $p=0.25$) (Table 3.7). Increasing initial inoculum, however, did result in a significant increase in final virus infection level ($F=16090$, $df=1, 440$, $p<0.001$). The interaction between field size and initial inoculum was not significant ($F=0.596$, $df=4, 440$, $p=0.67$).

Table 3.7. Proportion of plants infected with PVY at the end of a simulated field season at each field size and initial inoculum level. Data presented as mean ± SD.

	Field Size (ha)				
Initial Inoculum	0.4	3	8	12	16
0.01	0.024 ± 0.002	0.024 ± 0.001	0.024 ± 0.0006	0.024 ± 0.0004	0.024 ± 0.0004
0.05	0.11 ± 0.003	0.11 ± 0.002	0.11 ± 0.001	0.11 ± 0.001	0.11 ± 0.0007
0.2	0.36 ± 0.005	0.37 ± 0.002	0.37 ± 0.001	0.37 ± 0.001	0.37 ± 0.0009

Each treatment in the field size experiment showed an exponential increase (Figure 3.4; $p<0.05$ for all fitted models). At each inoculum level, rates of increase were similar regardless of field size. The curves explained the majority of the variation in the data.

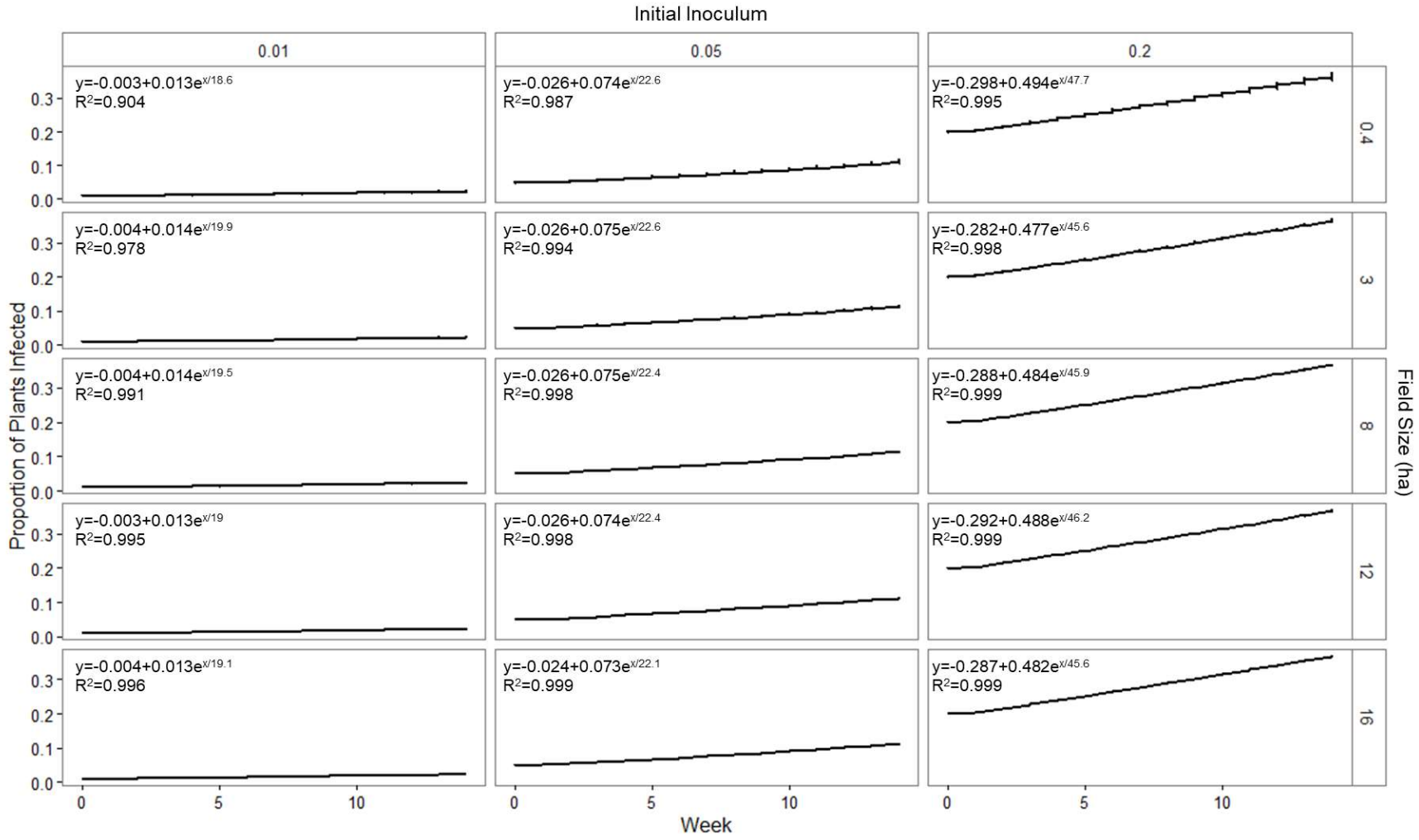

Figure 3.4. Increase in proportion of infected plants at each field size (ha) and initial inoculum level. Exponential equation and R^2 value for each curve are shown.

3.4. Discussion

Our simulations confirmed that planting seed with lower PVY incidence would result in lower virus infection at the end of the season. However, greater transmission efficiency of aphid vectors also significantly increased final virus infection levels regardless of initial inoculum. With enough efficient vectors, significant PVY spread could occur even when initial virus inoculum was low. This emphasizes the importance of using mineral oil, which essentially reduces the transmission efficiency of aphids probing oil-treated plants (Wang and Pirone 1996, Boquel et al. 2013). This is consistent with recent field studies, which demonstrated that PVY can be effectively controlled in the field using mineral oil (MacKenzie et al. 2014, 2016, 2017), especially when used in combination with insecticides. However, in those studies virus reduction was less consistent than suggested in our model. In the field, incomplete coverage may be an issue (Boiteau et al. 2009), and virus spread may be higher or lower depending on aphid population size or vector species present. Still, oil does effectively reduce aphid transmission efficiency and contribute to PVY control.

Results from the curve-fitting analyses supported the importance of reducing transmission efficiency and maintaining low virus levels to reduce PVY spread. While all curves showed exponential increase, rates of increase grew dramatically with increasing transmission efficiency. In addition, when both initial inoculum and transmission efficiency were kept low, the models were a poor fit and curves remained relatively flat throughout the simulated growing season. It is likely that virus levels under those conditions were, in large part, stochastically driven. Similarly, MacKenzie et al. (2014) identified interaction between initial inoculum and vector activity as an important parameter affecting PVY spread on commercial potato fields.

The proportional increase in the incidence of infected plants between the beginning and the end of the field season supported similar conclusions. While the final viral incidence was higher when initial inoculum was large, at higher transmission efficiencies the ratio of final to initial number of infected plants decreased with increasing virus levels at the start of the season (Table 3.4). Thus, highly efficient vectors spread the virus rapidly even when initial inoculum levels were low. At the lowest transmission efficiency, initial inoculum did not affect proportional increase, and virus spread was small regardless of amount of PVY present.

In the model, non-colonizing aphids played a greater role in the spread of PVY compared to colonizing aphids. This adds to a growing body of empirical evidence suggesting that potato colonizers may be less important than non-colonizers in PVY epidemiology. In one study, significant PVY infection was observed despite a lack of colonizing aphids (Kirchner et al. 2011). Another study showed that PVY spread was not correlated with green peach aphid abundance, even when green peach aphids were present in high numbers (Steinger et al. 2015). Our model may help to explain the importance of non-colonizers. Colonizing aphids will settle on a potato plant when searching the field. Thus, even if they acquire the virus, they may not leave the infected plant to spread it to other plants. This is in contrast to non-colonizing aphids; each individual is more likely to land on another plant and potentially infect it. While colonizing green peach aphids are much more efficient vectors than non-colonizing aphids, they will have little opportunity to spread the virus compared to non-colonizers. As discussed earlier, transmission efficiency is still important, at least with regards to non-colonizing species. Non-colonizing aphids with very low efficiencies may have little impact on PVY spread even when initial inoculum is relatively high (see graph in the upper right corner on Figure 3.2); these inefficient vectors may therefore be less critical to target for control. However, even efficient

potato colonizers appear to account for relatively little PVY spread. Behavioral differences could also exist among individual species within groups, making some individual species more important than other species. The model assumed that all non-colonizers had the same propensity for movement; in reality, this is uncertain. Factors such as plant health could also affect the results; this may have varying effects on different species (Boquel et al. 2010). Regardless, non-colonizing aphids still appear to have a greater impact on PVY spread in most cases.

Based on this information, it may be more important that growers focus their control on reducing virus spread by non-colonizers rather than trying to control colonizing aphids within the field. That is not to say that colonizing aphids should be completely ignored; they are important vectors of persistent viruses, and did cause some PVY spread in the model. Still, methods such as reducing initial inoculum and using mineral oil may provide more effective PVY control than simply reducing colonizing aphid populations. This could help explain why insecticides are often ineffective at suppressing PVY in the field by themselves (MacKenzie et al. 2017). These sprays primarily target colonizing aphids. Non-colonizers coming from outside the field, which are responsible for most of the PVY spread, are unaffected. Insecticides still may increase efficiency of oils, possibly through incapacitating aphid vectors (MacKenzie et al. 2017).

Field size did not have a significant impact on virus spread. The basic dynamics driving PVY spread appeared to be consistent regardless of field size. However, certain caution should be exercised when extrapolating these findings to field conditions. It is possible that some additional factors could be present in the field that could impact disease spread differently at fields of different sizes. For instance, the tendency for aphids to land more frequently on the edges of fields (Radcliffe and Ragsdale 2002) was not accounted for in this model. Additionally, field size could affect the spatial arrangement of disease spread even when the overall disease

incidence is comparable. Field dimensions are also likely important. All simulations in the present study were run on a square grid; different field shapes would have different edge to area ratios, which could affect results. Overall, though, the underlying factors driving PVY epidemiology are likely to be similar regardless of field size, and many control methods should be equally effective in both small and large fields.

The model described in the present study adds another tool to the study of plant disease epidemiology. This model is similar in design to a previous model constructed by Ferriss and Berger (1993). However, our model is capable of testing a wider variety of parameters. As it focuses on PVY, it can also examine disease-specific factors. The model could potentially be applied to other non-persistently transmitted viruses as well.

Similar to our model, EPIVIT (Bertschinger et al. 1995) identified differences among aphid species as important factors in virus spread in potato. However, it did not provide specific detail on which aphids may be important, nor did it distinguish between different viruses. The present model demonstrated that non-colonizing aphids with high transmission efficiencies are the most important in spreading PVY.

Our model also has the potential to test many additional factors not examined in the present study. For instance, aphid population was estimated and kept constant throughout the simulated growing period. In reality, seasonal variations in aphid populations are likely to impact disease spread.

The utilized model does have some limitations. In particular, it relies on parameters for which exact measurements are nearly impossible to obtain. On one hand, this is beneficial because it allows hypotheses to be tested which could not be studied in laboratory or field experiments. However, this also makes validating the model extremely difficult. Thus, results of

the model are more useful as estimates showing general patterns rather than exact predictions. It is, therefore, unlikely to be useful in disease forecasting. Still, our model is an effective theoretical tool which can improve understanding of PVY epidemiology.

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