The value of ecollogically acceptable insecticide combinations for Colorado Potato Beetle control

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GRADUATE THESIS

Jamie O’Keeffe

Zagreb, July, 2019.

UNIVERSITY OF ZAGREB
FACULTY OF AGRICULTURE

Graduate study:
INTER-EnAgro: Environment, agriculture and resource management

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Jamie O'Keeffe

Mentor: Prof. dr. sc. Renata Bažok

Zagreb, July 2019.

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STUDENT STATEMENT

ABOUT ACADEMIC INTEGRITY

I, **Jamie O'Keeffe**, JMBAG 0178116876, born 27.09.1988. in Vallejo, CA USA, declare that I have independently developed my graduate thesis titled:

THE VALUE OF ECOLLOGICALLY ACCEPTABLE INSECTICIDE COMBINATIONS FOR COLORADO POTATO BEETLE CONTROL

With my signature I declare:

- that I am the only author of this diploma work;
- that all sources of literature used, both published and unpublished, are adequately quoted or paraphrased, and listed in the literature at the end of this work;
- that this academic thesis does not include parts of other thesis written at the Faculty of Agriculture or any other papers written upon completion at other higher education facilities;
- that the electronic version of this diploma work is identical to the paper approved by my mentor;
- that I am familiar with the provisions of the Code of Ethics of the University of Zagreb (Article 19)

In Zagreb, dated _______________ _______________________

Signature of student

UNIVERSITY OF ZAGREB
FACULTY OF AGRICULTURE

REPORT

ABOUT THE GRADE AND DEFENSE OF MASTER THESIS

Master thesis of **Jamie O'Keeffe**, JMBAG 0178116876, titled:

THE VALUE OF ECOLLOGICALLY ACCEPTABLE INSECTICIDE COMBINATIONS FOR COLORADO POTATO BEETLE CONTROL

Defended and graded with the grade: ____________________, dated ___________________ .

Committee:			signature:
1.	Prof. dr. sc. Renata Bažok	mentor	_____________________
2.	Doc. dr. sc. Maja Čačija	member	_____________________
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Contents

Summary

Of the master's thesis - student **Jamie O'Keeffe,** entitled

THE VALUE OF ECOLLOGICALLY ACCEPTABLE INSECTICIDE COMBINATIONS FOR COLORADO POTATO BEETLE CONTROL

Colorado potato beetle is the most notorious and problematic insect defoliator pest of potato and threatens crops in nearly all major potato growing regions. Colorado potato beetle is well known for its ability to develop resistance to chemical insecticides and therefore new and novel treatment methods must be developed and explored. Integrated Pest Management provides the soundest approach to controlling Colorado potato beetle while slowing and preventing resistance development. This work investigated the use of ecologically acceptable insecticide treatments: azadirachtin, spinosad and spinetoram. Reduced dosing and combinational treatments were used to determine if satisfactory efficacy could be achieved while also improving economic results. In 2019 a field trial was conducted with ten treatments and one control. The treatments included the three active ingredients at full and reduced dosing as well as the combination of azadirachtin with spinosad and azadirachtin with spinetoram, both combinations were also carried out at reduced dosing. Efficacy was calculated using the Abbott formula. The results showed that a 50% reduced dose of azadirachtin provided unsatisfactory efficacy results while the full dose provided low to moderate efficacy (47%-84%). Both a 100% full dose and 50% reduced dose of both spinosad and spinetoram provided satisfactory efficacy results (83%-99%), with residual activity of 10-14 days. The 10% reduced dose of both spinosad and spinetoram provided low efficacy results, with the exception of spinosad around days 14-21, where the efficacy improved (75-80%). The combination of a 50% dose of azadirachtin with either a 10% dose of spinosad or a 10% dose of spinetoram only provided moderate efficacy at best, with spinosad (58%-81%) outperforming spinetoram (41%-74%). Both combinational treatments showed the peak efficacy around day 5. Based on the advantages that these treatments offer compared to synthetic chemical insecticides, further work is recommended to determine if these combinational treatments can offer satisfactory efficacy results. The use of 50% reduced dosing of both spinosad and spinetoram is recommended as a treatment method which provides satisfactory efficacy, improved economic results as well positive ecological fate.

Keywords: biological control, *Leptinotarsa decemlineata* Say, Colorado potato beetle, *Solanum tuberosum*, insecticide resistance management, reduced-risk insecticides, integrated pest management (IPM)

1 Introduction

Potato production represents the fifth largest agricultural crop worldwide (FAOStat data 2017). For centuries, potato production and consumption were centered in western countries such as the US and EU but in the past 30 years the production range has increased dramatically into areas such as Asia, Latin America and Africa. With this increased production range, comes increased potato consumption. In just 20 years (1991-2011), worldwide potato consumption increased from 27.35 to 34.64 kg/capita/year, after several decades of hovering around 27 kg capita^{-1} year^{-1} (FAOStat data 2015). This could be in large part due to the expanded growing range of potatoes in the developing world. This increased consumption is seen as a good thing by many, the FAO (2008) states "the potato produces more nutritious food more quickly, on less land, and in harsher climates than any other major crop". In many places where potato is grown, yields are threatened by the infamous insect pest: the Colorado potato beetle.

The Colorado potato beetle (CPB, *Leptinotarsa decemlineata* Say) (Coleoptera: Chrysomelidae) has a long story as an agricultural menace. With over 150 years of history as a pest of potato crops, it's considered the most important and notorious insect defoliator of the potato (Casagrande 1987; Alyokhin 2008, 2009, 2013; Cingel *et al.* 2016). With the increased range of potato production worldwide comes the increased potential for CPB to expand its range as well. CPB has a remarkable plasticity and is able to adapt to a number of biotic and abiotic factors to a degree rarely seen in the world of agricultural insect pests. CPB made quick work out of expanding and colonizing the areas historically known for potato production and it's only logical to expect further expansion into newer growing regions (Worner 1988; Weber 2003).

CPB has a long history of control measures including cultural, physical, mechanical and biological means, even still, synthetic insecticidal control has always been the preferred control measure by growers (Cingel *et al.* 2016). Due to early success with chemical control methods, most growers turn exclusively to broad spectrum, synthetic insecticides to protect their potato crop. The use has grown so prevalent that many growers treat their fields before pest populations have even been discovered. This indiscriminate use of synthetic insecticides has led to resistance problems with CPB. CPB populations have developed resistance to nearly every class of pesticide on the market, leaving some grows with few options for control (Casagrande 1987). The principle of Integrated Pest Management (IPM) has been proposed as a means to combat CPB and reduce resistance problems. IPM uses a multi-thronged approach to deal with pest populations and looks to chemical intervention as a final option. IPM uses all the tools in the plant protection tool box including cultural, physical, mechanical and biological control methods. Implementing IPM into potato production can reduce financial inputs (in the form of fewer insecticides), slow insecticidal resistance development as well as lessen the impacts of synthetic, broad spectrum insecticides on the environment and biodiversity. We can no longer rely on synthetic insecticides alone to tackle CPB problems. New and novel treatment methods must be explored and incorporated into IPM strategies.

Active ingredients such as spinosyns and azadirachtin serve as ecologically acceptable treatment measures which can be incorporated into successful IPM plans.

The hypothesis of this Master thesis is that ecologically acceptable insecticides, azadirachtin, spinosad and spinetoram applied at reduced doses, and in combination at reduced dosing could result with the same efficacy against CPB as the manufacturer recommended full doses. This would result in reducing the amount of insecticides applied, thus improving the economic results and at the same time slow down resistance development.

1.1 Aim

Conduct a field trial to establish the efficacy of reduced dosing and combinations of ecologically acceptable insecticides. Determine residual activity of investigated combinations and evaluate the most acceptable one.

2 Literature Review

2.1 Potato

2.1.1 Systematics and Morphology

The potato is a member of the nightshade family, Solanaceae. All potatoes which are of any economic importance come from the same species, *Solanum tuberosum* L. There are several other potato species cultivated in South America, but this paper will only discuss the common cultivated potato, *S. tuberosum.* Aside from *S. tuberosum*, there are generally six other potato species in cultivation and more than 230 wild species described (Hawkes 1992). The Solanaceae family is comprised of 95 genera and the *Solanum* genus, of which the potato belongs, accounts for the largest and most economically important (Bradeen and Haynes 2011). There are an estimated 1,000-1,700 species within the *Solanum* genus (Bradeen and Haynes 2011). Table 2.1 details the taxonomy of the potato. The Solanaceae family includes several other cultivated food crops, the most common of which being the eggplant or aubergine (*Solanum melongena* L.), tomato (*Solanum lycopersicum* (L.) Karst), and pepper (*Capsicum* spp.) (Bradeen and Haynes 2011). The *Solanaceae* family also includes several common ornamentals such as *Petunia* and *Schizanthus* as well as some species which are more well known for their presence of toxic alkaloids such as *Datura* and *Nicotiana* (Hawkes 1992).

Table 2.1. Potato taxonomy

(Source: Bradeen and Haynes 2011)

Family	Solanaceae
Subfamily	Solanoideae
Tribe	Solaneae
Genus	Solanum L.
Subgenus	Potatoe (G. Don) D'Arcy
Section	Petota Dumortier
Subsection	Potatoe G. Don
Superseries	Rotata Hawkes
Series	Tuberosa (Rydb.) Hawkes
Species	*Solanum tuberosum* L.
Subspecies	tuberosum

In terms of vegetative and flowering pattern, the potato is an herbaceous, annual dicotyledon. Due to the potato's ability to reproduce from tubers, it may also be regarded as perennial, but growing practices treat the crop as an annual. The tuber is the organ of economic importance, which is rich in carbohydrates and grows underground along modified stems called stolons. While potatoes can produce true seed, tubers are used as the dominant propagule (Bradeen and Haynes 2011). Potato leaves are pinnately compound, growing on aboveground stems that tend to be less than 1m long, as displayed in figure 2.2. The size and shape of the leaves can vary greatly depending on temperature and daylength (Steward *et al.*

1981). Potato flowers vary in color from purple to pink to white. They are hypogynous with radial symmetry and joined, five-lobed corollas, as displayed in figure 2.1. The potato fruit is a small spherical berry, inside which contains the true seed which is produced as a result of fertilization. True potato seed is approximately 1-2 mm small, oval shaped and tan in color. Figure 2.2 displays key features of potato morphology.

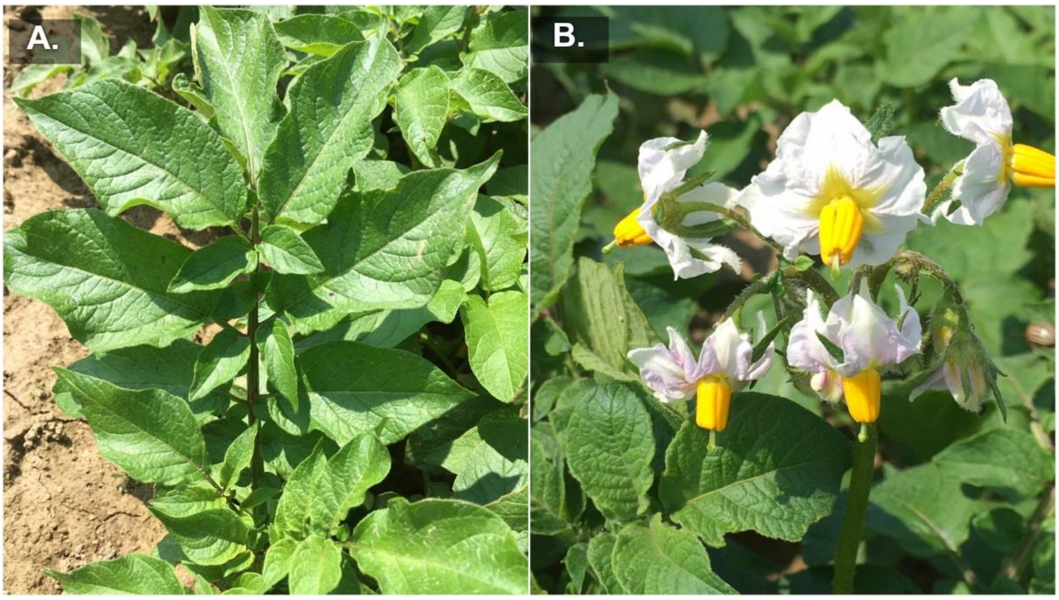


Figure 2.1. a) Potato leaf b) Potato flower

Source: Jamie O'Keeffe

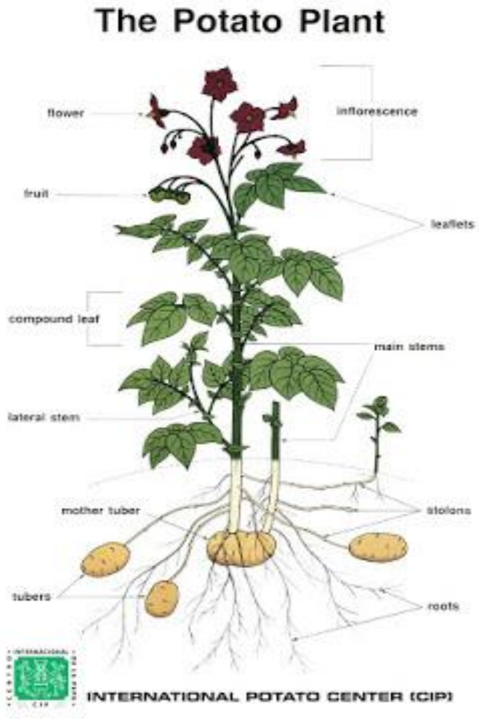


Figure 2.2. Potato plant morphology

Source: International Potato Center

2.1.2 Economic importance

The potato is the fifth largest food crop worldwide, behind only sugar cane, wheat, maize and rice (FAOStat 2017). In 2017, there was 19,302,642 ha harvested worldwide for a total of 388 million metric tons (FAOStat 2017). The potato can produce more calories per hectare than any grain crop and can be grown in many geographic and climatic conditions (Bradeen and Haynes 2011). The high nutrient density, ability for low technology long term storage, scalability for small and large production and widespread growing area explain why the potato has become such an important crop worldwide. The popularity of the potato is considered a major contributing factor to the population boom in Europe during the Industrial Revolution (Bradeen and Haynes 2011). In 60 years spanning the 18th and 19th century, the population of Ireland doubled thanks to the large uptake in consumption of the potato amongst the peasants (Bradeen and Haynes 2011). This heavy reliance of the potato as the primary source of calories ultimately lead to the Irish potato famine of the 1840s brought on by the widespread occurrence of late blight and lack in genetic variability within the potato crop.

Today, China is the number one producer worldwide of potatoes, with over a quarter of total production in 2017 (FAOStat 2017). India, Russia, Ukraine and the U.S. make up the remaining top five producing countries worldwide (FAOStat 2017). The total worldwide value of the 2016 potato harvest was over $92 billion USD, behind the value of only rice and maize for agricultural food crops (FAOStat 2017). The general trend has been a significant rise in production in developing nations and a much slower rise or even decrease in production in more developed nations. Between the years of 1961 to 2017, the data currently available from FAOStat, the US has seen an approximately 50% increase in production (from 13,305,000 tons to 20,017,350 tons), the EU has seen an approximately 50% decrease in production (from 127,073,648 tons to 61,320,170 tons) while the average for developing nations has been an overall nearly 1400% increase in production (from 1,468,966 tons to 21,965,727 tons). This staggering increase in production displays the economic importance that the potato holds in developing nations, most significantly in China and India who have seen a 668% and 1687% production increase respectively since 1961 and sit in the top five of worldwide producers (FAOStat 2017).

2.1.3 Growing practices

The potato can tolerate a wide range of growing conditions. While potato production varies greatly around the world, in general, large scale production looks quite similar in moderate climates: on large plots, as a monoculture and with mechanization in use (Elzebroek and Wind 2008). As previously mentioned, potato is very often planted from seed tubers: small potatoes or pieces of potato which have sprouted. In general, the use of seed tubers for propagation increases the risk of disease and therefore the quality and health of the seed tuber is of utmost importance (Elzebroek and Wind 2008). Disease-free, small tubers grown in vitro are available as well, virtually eliminating the risk of disease transmission from the seed tubers (Elzebroek and Wind 2008). In areas where proper storage for seed tubers and/or disease-free seed tubers are hard to come by, such as Asia and Africa, the use of true potato seed tends to increase (Elzebroek and Wind 2008). For the most part, the use of true seed ensures no transmission of disease and also lowers transport costs due to the light nature of true potato seeds (Elzebroek and Wind 2008)

The length of growing season can vary greatly depending on climatic conditions of the region. Longer growing seasons tend to produce higher yields per hectare. Potato has a relatively poorly developed root system and therefore requires very fertile soils or high levels of fertilization to meet the growing needs. Logically, better nutrient uptake leads to higher yields. Potatoes grow well in a wide range of soil types, apart from very heavy, water-logged soils. Well-aerated, sandy loam, deep soils with a slightly acidic pH provide optimum growing conditions (Elzebroek and Wind 2008). Throughout the growing season, 500-700 mm of rainfall is required and daytime temperatures of 20-25°C with cooler nights are ideal (Elzebroek and Wind 2008). Potatoes are typically planted in rows with in-row spacing of 20-40 cm and inter-row spacing of 75-100 cm (Elzebroek and Wind 2008). Hilling of rows typically occurs in order to prevent tuber exposure to sunlight, which leads to chlorophyll production and a green coloration of tubers. These tubers are inedible from high solanine concentration. Approximately 3 weeks after emergence, tubers start to form underground and after an initial bulking period, tuber growth remains quite constant throughout the growing season (Elzebroek and Wind 2008). Weed control should be conducted pre-emergence via herbicides or mechanical control. Post-emergence weed control is generally taken care of via hilling of rows.

2.1.4 The most important pests

Potatoes are subject to damage from a large number of insect pests including aphids, leafhoppers, psyllids, beetles, wireworms, cutworms, grubs, moths and flies, amongst others. Insect damage can occur as leaf defoliation, tuber attack and vectors of disease transmission. Radcliffe *et al*. (1991) noted that in North America alone there are over 170 species of potato insect pests.

Aphids are considered a serious pest for potatoes worldwide, not so much for the physical damage they cause to the crop but because of their potential as vectors for disease (Hawkes 1992). While they can cause direct plant damage when found in abundance, the real damage comes via the spread of viruses. There are at least 10 viruses which are spread via aphid infestation (Hawkes 1992). The most problematic aphid species is the green peach aphid (*Myzus persicae* Sultz) (Hawkes 1992). The potato leafhopper (*Empoasca fabae* Harris) is a significant problem in the tropics and subtropics, especially North America (Hawkes 1992). Many species of flea beetles are known to cause defoliation damages. In addition to the direct damage caused by flea beetles, feeding wounds allow for the entrance of pathogens such as early blight or bacterial diseases (Hawkes 1992). Soil borne insects such as wireworms and white grubs are known to cause significant tuber damage. Thrips and mites have become of concern in tropical countries with potato production (Hawkes 1992). Other notable insect pests include cutworms, leaf miner flies and the European corn borer. As previously mentioned, no insect pest is more problematic or notorious than the Colorado potato beetle (*Leptinotarsa decemlineata* Say) in potato production. CPBs cause potato damages worldwide and also attack tomato and eggplant crops.

In addition to insect pests, there are also several nematodes which cause great damage worldwide to potato crops. There have been 67 species of nematodes which are reported to associate with potato crops but few cause damages in terms of crop production (Hawkes 1992). Of those which do cause damage, the most harmful are potato cyst nematodes (*Globodera rostochiensis* Wollenweber and *Globodera pallida* Stone*)* (Hawkes 1992). Other species of nematodes which cause significant damages are the root knot (*Meloidogyne spp*.), stubby root (*Trichodorus* and *Paratrichodorus spp*.), root lesion (*Pratylenchus spp*.) and potato rot (*Ditylenchus spp*.) (Hawkes 1992).

2.2 Colorado Potato Beetle

2.2.1 History as an agricultural pest

The Colorado potato beetle (*Leptinotarsa decemlineata* Say) is native to Mexico and was originally observed feeding on several native species from the Solanaceae family, primarily buffalo bur (*Solanum rostratum* Dunal*)* (Casagrande 1987). The exact spread to the US from Mexico in unknown but CPB was first collected in the US by Nattall in 1811 and later collected and described by Thomas Say in 1824, naming it *Doryphora decemlineata* (Casagrande 1987; Alyokhin 2009). It is possible that both CPB and buffalo bur were brought from their native home of southern Mexico by the early Spanish settlers heading northward (Gauthier *et al.* 1981). The first reported serious outbreak on potato crops was observed near Omaha, Nebraska in 1859 (Jacques 1988). Populations likely shifted from weed host plants to agricultural host plants in the mid-1800s due to the establishment of extensive agriculture. East and northward expansion was rapid, reaching the Atlantic coast and Canada in about 15 years (Casagrande 1987). Southern and westward expansion was slower, likely due to lower density of potato crops in the west and south of the US (Casagrande 1987). Eventually, during the second half of the 19th century, several outbreaks were reported on potato crops in Colorado which ultimately lead to the incorporation of the state into its naming (Jacques 1988). Crop devastations were so severe that many farmers chose to stop growing potatoes, leading to scarcity and price increases (Casagrande 1987). At the start of CPB's existence as an agricultural pest, potatoes cost $.50 per bushel, by 1866 they rose to $.75 and by 1873 they peaked at $2.00 per bushel, all thanks to crop devastations caused by CPB and subsequent abandoning of potato growth by many at the time (Casagrande 1987).

CPB was first observed in Europe in England in 1875 and then on continental Europe in Germany in 1877 but quickly eradicated (Alyokhin *et al*. 2013). Several observations are reported across Europe over the next several decades, but eradication and quarantine methods were quite successful at keeping the pest at bay until finally, significant populations were established in France in 1922 (Alyokhin 2009). The rapid spread of the CPB was impressive and by the end of the 20th century, populations had established from North America to Europe and Asia reaching a range of around 16 million km^2 (Weber 2003). While

CPB does have the capability for long range dispersal, it is thought that it's rapid spread is human caused (Alyokhin *et al*. 2013). Factors including the small size of the beetle itself, widespread growth and popularity of the potato and high traffic and movement to and within potato growing areas have all contributed to the problem (Alyokhin *et al*. 2013). CPB was the cause of the first large-scale applications of pesticides in agriculture and likely influenced the widespread uptake in pesticide use throughout the 20th century (Casagrande 1987). Though the spread of CPB has already been extensive over the last 150 years, it is expected for the range to expand even further due to the pest's ability to adapt to locally abundant *Solanum* host species (Horton *et al*. 1988). Currently, CPB has adapted to 20 different host species of solanaceous plants, both wild and cultivated, but its preferred host is the potato *Solanum tuberosum* (Cingel *et al.* 2016). Further spread to temperate areas such as East Asia, India, Australasia and South American is likely to be seen in the future and has already begun today (Worner 1988; Weber 2003)

2.2.2 Taxonomy, Morphology and Life Cycle of Colorado potato beetle

Taxonomy

CPB is a member of the Chrysomelidae family which encompasses leaf beetles and has over 35,000 species described worldwide (Alyokhin *et al.* 2013). Beetles of this family feed on plants at both the larval and adult stages. CPB is included in the *Leptinotarsa* genus and tends to be the most infamous member. A detailed taxonomy can be found in Table 2.2.

Table 2.2. Colorado potato beetle taxonomy

Domain	Eukaryota
Kingdom	Metazoa
Phylum	Arthropoda
Subphylum	Uniramia
Class	Insecta
Order	Coleoptera
Family	Chrysomelidae
Subfamily	Chrysomelinae
Tribe	Doryphorini
Genus	*Leptinotarsa*
Species	*Leptinotarsa decemlineata* Say

Eggs

Eggs are smooth, yellow to orangish and oblong, approximately 1.2-1.8 mm long and 0.8 mm wide (Capinera 2001; EPPO). They are deposited on the underside of leaves in several tidy rows. They are attached with a yellow adhesive which is excreting during the time of laying (Capinera 2001). The eggs remain opaque for the duration of gestation until around 12 hours before hatching, when the embryo becomes visible through the shell (Capinera 2001). Eggs develop at different rates when exposed to different temperatures. Development was a mean of 10.7, 6.2, 3.4 and 4.6 days when incubated at temperatures of 15°, 20°, 24° and 30°C, suggesting optimal development occurs in the temperature range of 24-30°C (Capinera 2001).

Larva

There are 4 instars of larval growth once hatched and they vary in color depending on the age. The first instar is cherry red in color with a shiny black head and color lightens to a more pale-orange color as the larva develops. All instars have two rows of black dots running down either side of their abdomen. Abdomens are large and arched. Larva have 3 sets of legs off their thorax and one proleg of the end of the abdomen. Optimal larval development occurs at 28°C (Capinera 2001).

Pupa

Pupation occurs after the 4th larval instar drops to the soil and burrows 2-5 cm into the soil (Capinera 2001). Larvae form into pupae about two days after burrowing into the soil and optimal pupation temperature is 28°C, which results in 8.8 days of pupation (Ferro *et al.* 1985). Pupae are around 9.2 mm long and 6.4 mm wide, oval and golden to orangish in color (Capinera 2001).

Adult

The adult beetle is highly recognizable with its distinctive black striping. They have oval bodies which are approximately 1.0 cm long by 0.6 cm wide (EPPO), convex backs and they are hard-shelled. Their color is cream to yellowish with 5 black stripes running the length of each wing. The thorax and top of the head have around 10-12 dark spots. Their 3 pairs of legs are lighter at the tops and darker at the tips. Images of the different stages of CPB can be seen in Figure 2.3.

Figure 2.3. Developmental stages of Colorado potato beetle
A. Egg cluster B. Second instar larva C. Pupa D. Adult beetle
Source: A. B. and D. Jamie O'Keeffe C. Whitney Cranshaw, Colorado State University

Life Cycle

CPB displays a facultative diapause and overwinters in the adult stage and emergence from the soil occurs in the spring around the same time of potato emergence. Diapause is induced from short-day photoperiod, temperature and quality of available host plants and terminates in the spring when temperatures reach and exceed 10°C (de Kort 1990; Capinera 2001). Mass emergence from diapause often occurs over the span of 1 to 2 days (EPPO). After emergence, beetles walk or fly to the nearest suitable host. Typically, flight is only used after a few days of unsuccessful searching via walking (Weber 2003). Post diapause flight initiation is also highly regulated by temperature. Caprio and Grafius (1990) reported flight initiation at air temperatures of 15°C and increasing to 100% flight initiation at 20°C. Feeding occurs for 5-10 days and then mating begins, though some females are able to oviposit in the spring from autumn fertilization (Capinera 2001). CBP is a polygamous species, mating with multiple partners over several copulations (Alyokhin 2009). This promiscuity is thought to increase genetic variability and likely contributes to the widespread adaptability of the pest (Alyokhin *et al*. 2013). Edwards and Seabrook (1997) demonstrated that sexually active females produce a sex pheromone which acts as an attractant for males. After mating, oviposition begins 1 to 2 days later with the female laying clusters of eggs, 10-30 at a time, on the underside of leaves in multiple tidy rows (EPPO). Females lay eggs over the period of several weeks until the middle of summer, laying up to 2000 eggs during that time (EPPO). Pregnant females partake in a considerable amount of flying which allows them to distribute their eggs within and between different host areas (Alyokhin *et al*. 2013). From egg to adult, a complete generation occurs in about 30 days (Capinera 2001), and as few as 20.7 days at optimal growing conditions (Ferro *et al* 1985); therefore, multiple generations can occur in one year. The fastest rate of development occurs with temperatures between 25-32°C and it's thought that optimal temperatures for development vary geographically (Alyokhin 2009). Anywhere from one to three generations can occur per year, depending on local climatic conditions (Capinera 2001).

Eggs hatch within 4-12 days provided adequate temperatures are maintained (12°C minimum) (EPPO). After hatching, larvae begin feeding immediately and generally only stop feeding during their moultings, which occur four times over a span of 2-3 weeks (EPPO). Larvae and adults have the ability to thermoregulate depending on their chosen feeding position. Feeding tends to occur on the tops of upper leaves at lower ambient temperatures and lower in the potato canopy as the temperature rises (May 1981). After four instars of larval development, pupation occurs in the top layer of soil. Pupation lasts for 10-20 days and occurs at varying depths (in cm) according to local pedoclimatic conditions (EPPO). Adults emerge from pupation and begin feeding and then depending on the time of emergence, either begin the mating cycle or, if photoperiods are short and temperatures low, they burrow into the soil and begin diapause. Diapause occurs either directly in the host environment, or often CPB head towards field borders or hedgerows to enter diapause (Alyokhin *et al*. 2013).

CPB displays facultative migration when local conditions aren't adequate for survival and can travel considerable distances in search of more favorable conditions or hosts (Alyokhin *et al*. 2013). This ability for migration in combination with multiple behavioral traits such as mating patterns, diapause, and host adaptability allows for a sort of 'bet-hedging' to ensure success from generation to generation (Alyokhin *et al*. 2013). As well, CPB has the ability to distribute its eggs and offspring in both space and time, making it a particularly difficult pest to control (Alyokhin 2008).

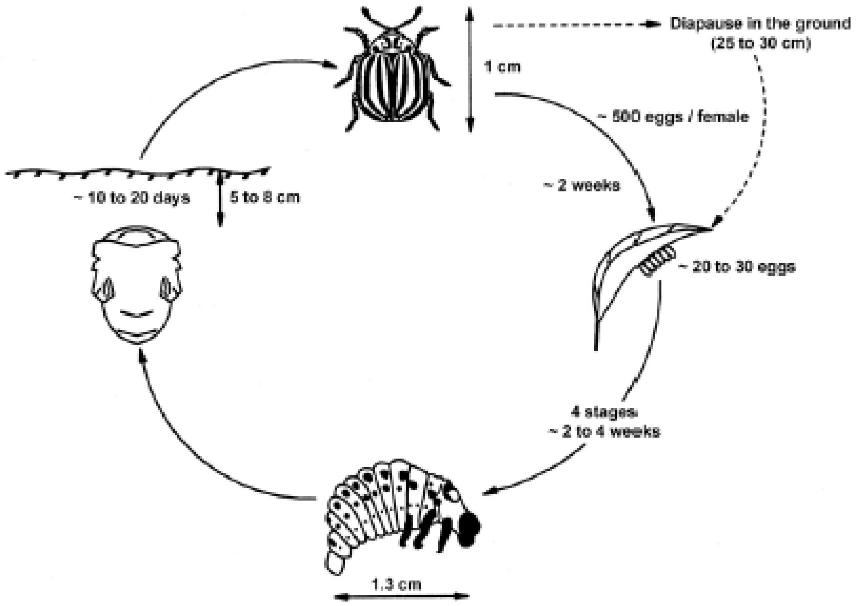


Figure 2.4. Life cycle of Colorado potato beetle
Source: Khelifi 1996 (As cited in Khelifi *et al.*2007)

2.2.3 Damages cause by Colorado potato beetle

CPB is regarded as the most damaging and significant insect defoliator of potato whenever there are established populations present (Alyokhin 2009; Ferro *et al.* 1985). Both adults and larvae consume significant amounts of leaf mass in their life cycle. Ferro *et al* (1985) demonstrated larval feeding rates of 20 cm^2 throughout all instars of the larval stage and a feeding rate of 10 cm^2 day^{-1} during the adult stage at optimal conditions. Even more severe, Logan *et al* (1985) demonstrated cumulative feeding totals up to 40 cm^2 during the four stages of larval development held at optimal growing temperatures around 24-28°C. If leaf defoliation is severe enough, feeding can occur on stems and unearthed tubers, but these represent inferior food sources (Alyokhin 2009). Figure 2.5 shows a severely defoliated potato plant.

Figure 2.5. Defoliation of potato plant by Colorado potato beetle
Source: Jamie O'Keeffe

Potatoes can recover from light to moderate infestations, depending on the growth stage. For example, Cranshaw and Radcliff (1980) demonstrated no impact on yield with early season defoliation of 33% and only a minor reduction in yield with 67% defoliation. Similarly, Wellik *et al.* (1981) found no impact on yield with 29% defoliation. These studies show the remarkable ability of the potato plant to recover from defoliation. Despite this resiliency to moderate levels of defoliation, if infestations are left uncontrolled, significant yield losses can occur. Early and mid-season protection is important as potatoes are most susceptible to damages and yield loss during early growth and bloom, which is when tuber growth is greatly increasing (Capinera 2001). Hare (1980) demonstrated a 64% yield reduction as a result of complete defoliation during the 4th-6th weeks of the growing season. Late season defoliation tends to have no impact on yield. This was demonstrated in studies by Ferro *et al.* (1983) and Zehnder and Evanylo (1989) which both saw no impact on yield when complete defoliation occurred in the final two weeks of growth.

2.3 Integrated control of CPB

2.3.1 Cultural measures

There are a few long established methods of cultural control of CPB, the most common and important of which being crop rotation. The first recommendations for the use of crop rotation as a means of CPB control came as far back as 1872 (Alyokhin 2009). Lashomb and Ng (1984) reported (as cited in Alyokhin 2009) that rotated fields showed reductions of 90% of egg masses compared to non-rotated fields. Wright (1984) demonstrated that fields grown after rye or wheat had early season adult populations at significantly lower densities, 95.8% lower than nonrotated fields. While crop rotation can help with early season reductions in CPB populations, the high mobility of CPB means significant distances are necessary to ensure long season control. A distance of 0.3-0.9km between fields is required in order to maximize the benefit of rotating potato fields (Weisz *et al.* 1994; Weisz *et al.* 1996; *Sexson et al.* 2005). This distance may be difficult for some growers to achieve, even so, crop rotation proves to be the most successful cultural method of control. Even rotating fields on a smaller scale can reduce the need for early season applications of pesticides (Capinera 2001)

Another moderately effective means of control is the alteration of planting dates and use of early or late ripening potato varieties to avoid damages by second generation larvae in the field. Planting later in the spring ensures a later emergence of summer generation adults because overwintered adults won't have a food source to begin mating. These summer generation adults will experience a shorter photoperiod by the time they reach maturity, thereby diapause will be initiated which can reduce second generation larval populations in the field (Alyokhin 2009). Similarly, early plantings can also reduce second generation larval populations. Early potato crops will be harvested from the field at the time of the emergence of second-generation larval populations, therefore reducing their impact on the crop (Alyokhin 2009). This method of altering planting dates might not be feasible for many growers because seasonal and weather patterns limit their flexibility in planting. This also only addressed second-generation larval populations and does nothing to lessen the impact of first-generation larvae and over wintered adults. Finally, Horton and Capinera (1987) discovered that CPB populations can be greatly reduced with the practice of intercropping. CPB is a specialist pest and thrives in the environment of a potato monoculture and so diversifying the field can limit their ability to spread and multiply.

2.3.2 Physical and Mechanical measures

Several physical and mechanical measures have been developed in order to suppress CPB populations in the field. These measures have been developed keeping in mind the CPB's behavior and life cycle. One such measure includes the construction of trenches along the perimeter of growing areas. This measure takes into consideration that a large number of beetles overwinter in the surround vegetation and hedgerows of fields and therefore must travel from the borders of the fields to find their host. Also important is the fact that the CPB generally starts their search for a host by walking and only resorts to flying after several days on unsuccessful searching on foot. Boiteau *et al.* (1994) demonstrated that trenches lined in black plastic with a minimum wall slope of 46° caught and retained 100% of beetles passing through the trench in laboratory settings and 84% in the field. This method could lead to reductions of 47-49% of overwintered adult beetles in the field and 40-90% reductions of second-generation adults compared to fields without such treatment (Boiteau *et al.* 1994). Such trenches could also reduce overwintered adults for the next growing season, intercepting beetles as they head out of the field towards overwintering sites. Such trenches work in conjunction with rotating fields, especially when rotation is only possible at less than optimal distances. Placing a trench in between a previous field with CPB infestation and a new field could help intercept overwintered beetles looking for a new host.

Straw mulch has also shown to be effective at CPB control for several reasons. First, the mulch acts to keep soil temperatures lower in the spring which could thereby delay and limit overwintered beetles' movement (Stoner 1997; Ng and Lashomb 1983). Another effect of straw mulch is the increase in ground predators in mulched areas compared to un-mulched (Vincent *et al.* 2003). Finally, Stoner (1997) demonstrated limited larval migration in plots mulched with straw compared to un-mulched plots. Zehnder and Hough-Goldstein (1990) demonstrated that overwintered adults, egg masses and larvae were all reduced in mulched plots compared to those that weren't mulched. They also found that mulched plots had soil temperatures 2.4°C-3.4°C lower than those without mulch, which could be a major contributing factor in the reduced CPB population. Trap cropping is a method in which a crop is planted in order to lure in and intercept pest populations. A plot of potatoes can be planted in between an overwintered site and a new plot in order to prevent colonization of the main crop (Khelifi *et al.* 2007).

Thermal treatments have also proven effective at controlling CPB. The technique aims to damage or kill the CPB populations while not causing harm to the growing crop. Studies have been conducted to find the threshold of heat with which potatoes can withstand and fully recover. It's been shown that younger plants (10 cm or shorter) can tolerate heat treatments of 175°C and fully recover better than older plants (Duchesne *et al.* 2001). Studies looking at mortality rates of CPB exposed to thermal treatments demonstrated that temperatures from 75°C to 200°C resulted in 100% mortality for all larval instar stages and temperatures above 150°C killed 75% of adult beetles within 2 days; eggs were the most sensitive to heat treatment (Duchesne *et al.* 2001; Pelletier *et al.* 1995). This treatment can be employed at two distinct periods of the growing season: early in the season when young plants can withstand the thermal treatment and late in the season when defoliation is a desirable result for harvesting purposes (Khelifi *et al.* 2007). These methods can be effective at preventing damages in the current growing season as well as reducing populations for the next season.

One novel technique being explored for CPB control is the use of electromagnetic radiation, specifically microwave radiation. According to Khelifi *et al.* (2007), when exposed to microwave radiation, CPB experience a rapid heating that results in mortality at all developmental stages, with energy inputs varying according to the developmental stage of the beetle. Unfortunately, the same microwaves which kill CPB also cause significant, irreversible damage to potato plants and therefore this technology is not currently employed outside of research purposes (Khelifi *et al.* 2007). Various machines have been developed which use pneumatic control to dislodge and suck up CPB at all developmental stages. The machines have demonstrated unimpressive control rates around 50% and more research will need to be conducted in order to improve the technology (Khelifi *et al.* 2007). In addition, many of the negative impacts such as soil compaction from heavy machinery and the impact on beneficial insects will also need to be addressed before it's likely that any of these pneumatic control machines will make it to market. (Khelifi *et al.* 2007).

2.3.3 Biological and biotechnical control

There are several biological and biotechnical methods on the market for controlling CPB populations. In terms of biologically derived active ingredients, spinosad and azadirachtin were the subject of this research project and will be discussed in detail later in this paper. Several microbial or entomopathogenic organisms exist which effectively control CPB populations. *Bacillus thuringiensis* (*Bt*) Berliner is a spore-forming, gram-positive pathogenic bacteria that is known to infect many types of insect pests. *Bt* var. *tenebrionis* (*Btt*) is the specific *Bt* strain that is known to infect the larval stage of CPB (Sporleder and Lacey 2012). Many biotic and abiotic factors affect the efficacy of *Bt* which has limited the uptake in its use commercially, but it is still common within organic farming (Sporleder and Lacey 2012). In 1995, Monsanto introduced its first genetically modified crop, the NewLeaf potato, which was engineered to produce the Cry3A toxin from genes from *Bt* var. *tenebrionis* (*Btt*) in order to stop attacks from CPB (Sporleder and Lacey 2012). Later, the Cry3B toxin was discovered and used as well, resulting in even higher efficacy against CPB attacks (Sporleder and Lacey 2012). Due to public mistrust of transgenic crops, the NewLeaf potato never received much commercial interest and was discontinued in 2001 (Kilman 2001). Despite the early failure of transgenic potatoes to combat CPB attack, interest has been renewed in this area and new *Bt* varieties are in development (Cingel *et al.* 2016). In recent years, RNA interference, gene silencing via double stranded RNA, has been explored for possible control of CPB but research is still in the early stages and commercial use of RNAi is far in the future, if at all (Cingel *et al.* 2016).

Beauveria bassiana (Bals.) Vuill is a pathogenic fungus which is the longest standing microbial treatment for CPB and is known to control several other potato pests (Sporleder and Lacey 2012). *B. bassiana* enters the insect host via the cuticle and produces a wide range of toxic metabolites and has the benefit of persisting in the soil after host mortality (Sporleder and Lacey 2012). Avermectins are fermentation products of a naturally occurring actinomycete found in the soil called *Streptomyces avermitilis* (Burg *et al*.) Kim and Goodfellow, which cause nervous system paralysis in nematodes and insects (Sporleder and Lacey 2012). Avermectins have been used for CPB control but resistance has already been detected (Christiane *et al*. 2003).

Many studies have been conducted to test the efficacy of entomopathogenic nematodes (EPN) for CPB control. EPNs are parasites which obligately associate with symbiotic bacteria. There are two genera of EPNs which infect insect pests: *Steinernema* and *Heterorhabditis*. After entering a host, the EPN releases the symbiotic bacteria which are ultimately responsible for the host's death (Sporleder and Lacey 2012). EPNs can live for several reproductive cycles inside a dead host and once all the nutrients have been consumed they can enter the soil and persist for months without a host (Sporleder and Lacey 2012).

Several field and laboratory studies have shown EPNs to be effective at CPB control (Berry *et al.* 1998; Kepenekci *et al.* 2015; Trdan *et al.* 2009).

There are several natural enemies of CPB known, including lady beetles, stink bugs, flies, and arthropods. The most successful and commonly used in CPB control will be described here. *Myiopharus aberrans* Townsend and *Myiopharus doryphorae* Riley are two species of parasitic tachinids that seem to be specialists of CPB (Weber 2012). They larviposit into CPB larvae, preferring the second and third instar, and are capable of overwintering in adult beetles and emerge in spring after the beetle exits diapause (López *et al*. 1997). *M. aberrans* also larviposits directly into adult CPB early and late in the season (Weber 2012; López *et al*. 1997). *Lebia grandis* Hentz is a ground beetle predator of both CPB eggs and larvae and Chaboussou (1939) discovered that it is also a parasitoid of CPB pre-pupae and pupae (as cited by Weber 2012). *L. grandis* is thought to be a strong predator of CPB and though it's rearing in the lab is difficult, conservation efforts should be considered of natural populations in the field, especially with regards to the use of non-selective insecticides (Weber 2012; Weber *et al.* 2006).

Perillus bioculatus Fabricius and *Podisus maculiventris* Say are predatory stink bugs of CPB eggs and larvae. *P. maculiventris* is a generalist predator and *P. bioculatus* is considered more of a CPB specialist. Cloutier and Bauduin (1995) demonstrated a large reduction of CPB eggs in field trials after *P. bioculatus* release but Tipping *et al.* (1999) argue that large-scale rearing for commercial growth is not economically feasible. Finally, *Coleomegilla maculate* De Geer is lady beetle and is a widely studied non-specialized predator which feeds on CPB eggs and early stage larvae. *C. maculata* tend to overwinter near corn fields and therefore a rotation of potato after corn leads to high populations (Weber 2012; Hazzard *et al.* 1991). A common limitation with parasitic and predatory species of CPB is the difficulty with introductions on a large scale. Laboratory rearing and wide-spread release tend to not be economically just and therefore this approach might be better suited for smaller production and greenhouse growing. Figure 2.6 displays some of the natural predators of CPB.

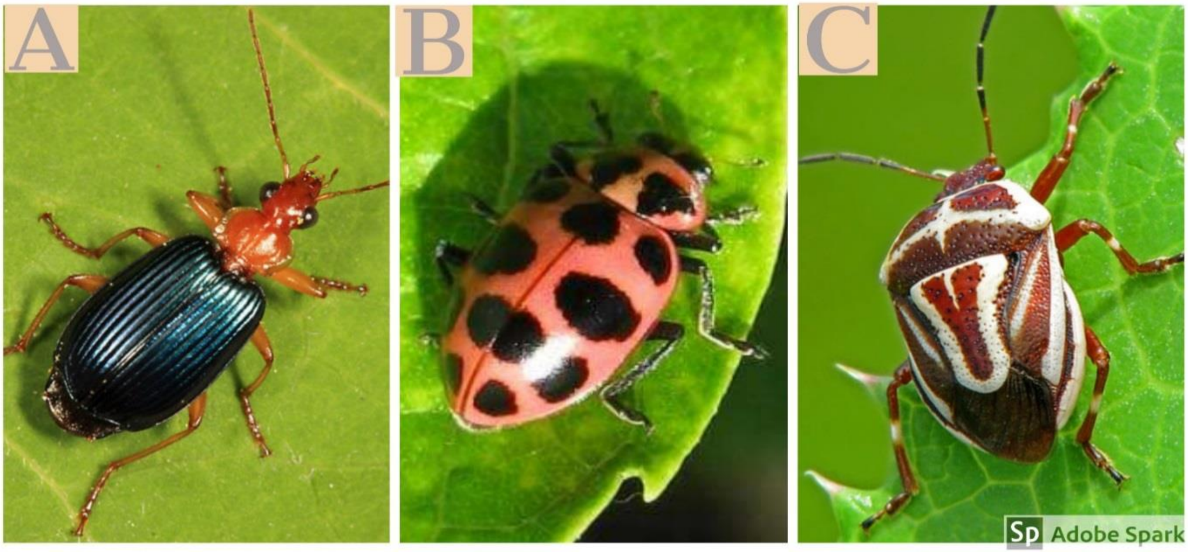


Figure 2.6. Three natural predators of Colorado potato beetle
A. *Lebia grandis* B. *Colomegilla maculata* C. *Perillus bioculatus*
Source: A. BugGuide.net Tom Murray B. Perdue University C. TerryThormin.com

2.3.4 Synthetic insecticides

Despite all previously mentioned methods for CPB control, synthetic insecticides still remain the most common treatment strategy for commercial growers (Cingel *et al.* 2016). This heavy reliance on synthetic insecticidal treatment has led to CPB developing resistance to nearly every class of insecticide that it's been exposed to. There're currently 56 active ingredients reported which CPB populations have shown resistant against (Mota-Sanchez and Wise 2019). CPB has many mechanisms for resistance which it employs including reduced pesticide penetration, target site mutation, behavioral changes, increased insecticide excretion and enhanced metabolism aided by various enzymes (Alyokhin *et al.* 2008). Only in recent decades have people started considering the implications of indiscriminate insecticide use and more attention is being put towards alternative methods of control as well as an integrated approach of many control methods, so called Integrated Pest Management (IPM).

CPB's history with synthetic insecticides started in 1874 with the use of Paris green (Casagrande 1987). Paris green is a paint pigment containing copper arsenate which displayed insecticidal properties and was widely used for CPB control on potatoes for several decades after it's discovered effects (Casagrande 1987). Grower's largely ignored the recommended non-chemical control methods suggested by entomologists, such as crop rotation, and many raised concerns about the health hazards and environmental impact (Casagrande 1987). Gauthier *et al.* (1981) notes that arsenical insecticides remained the primary control method for CPB until the late 1940s, with a shift towards lead arsenate and calcium arsenate in the early 1940s (as cited in Casagrande 1987). DDT was introduced in 1945 and was so effective at CPB control that arsenical insecticides were largely abandoned (Casagrande 1987). Despite early evidence of resistance developing to arsenical insecticides, the problem was avoided all together with the advent of DDT, a chlorinated hydrocarbon (Casagrande 1987). Casagrande

(1987) also notes that insecticidal resistance was not a recognized phenomenon yet at that time and therefore early signs of its presence with arsenical insecticides were largely ignored. Resistance to DDT started to develop just 7 years after use began and in as few as 14 beetle generations in some places (Gauthier *et al*. 1981). After DDT came dieldrin, another chlorinated hydrocarbon, which failed just 3 years later and growers in the Northeast of the US were forced to switch active ingredients every few years in order to stay ahead of resistance problems (Casagrande 1987). Next came organophosphates and carbamates and both eventually failed within different CPB populations. In the 1970s, pyrethroids were introduced and provided adequate control for several years until resistance began to develop as well (Kuhar *et al.* 2012).

By the 1990s it became apparent that CPB was a super-pest, capable of developing resistance to nearly any active ingredient it was exposed to. That being said, not every beetle population has developed resistance to each and every active ingredient which have failed at some point. Many studies note though, that cross resistance and multiple resistance are both prevalent problems (Alyokhin 2008). Aside from the obvious problem of resistance, other problems arose from the heavy and frequent application of synthetic broad-spectrum insecticides to control CPB populations. Secondary pests also began to cause problems as natural predators were decimated in the field (Metcalf 1980).

More recently, neonicotinoids have played an important role in CPB control. First introduced in Europe in 1990 and registered for potato protection in the US in 1996 (Kuhar *et al.* 2012), neonicotinoids are the most common insecticide used for CPB control on potatoes (Kuhar *et al.* 2012). They act as a neurotoxin and can be translocated from the soil to the plant tissue as a systemic insecticide. Most growers use them as seed treatment or in the seed furrow at planting, providing long-term protection without the need of foliar applications (Kuhar *et al.* 2012). Not only do neonicotinoids provide control for CPB, they're also capable of controlling a wide variety of potato pests, making them a powerful tool for potato growers (Huseth *et al.* 2014). As with all classes of insecticides introduced for CPB control, neonicotinoids are not without problems. Resistance has developed in CPB populations and concerns have been raised on the impacts of neonicotinoid use on non-target species, specifically pollinators and bees (Kuhar *et al.* 2012; Huseth *et al.* 2014). This problem with non-target species lead the EU to ban three major neonicotinoid active ingredients (imidacloprid, clothianidin and thiamethoxam) at the end of 2018 (PAN Europe 2018).

Two insect growth regulators exist on the market for CPB control: Novaluron and Cyromazine. Both chemicals act as chitin synthesis inhibitors and impact the larval growth stage (Kuhar *et al.* 2012). Both products show high success rates for control with novaluron providing 85% mortality of the 2nd instars 5 weeks after treatment (Cutler *et al.* 2005) and cyromazine providing 90% mortality of larvae (Abbott and Thetford 1992). These products can provide important alternatives to the standard applications of broad-spectrum insecticidal treatment.

It’s clear to see that CPB has a remarkable ability to develop resistance to all types of synthetic control measures. This is why it’s important for growers to use an integrated approach, incorporating methods of cultural, physical, mechanical and biological control into their pest management schemes. Also important is the practice of rotating active ingredients, giving the beetle less chance to develop resistance to one product. With the CPB’s unique ability to thrive despite adversity, using a diverse approach is the best strategy to combat the CPB.

3 Materials and Methods

3.1 Description of the used insecticides

Azadirachtin

The first active ingredient which was used was azadirachtin, which contained 10 g l^{-1} active ingredient was used for this field trial. The recommended dosing for treatment of Colorado potato beetle on potato is 2.5l ha^{-1}. Azadirachtin is a tetranortriterpenoid (limonoid) compound found in the neem tree (*Azadarichta indica* A. Juss), within the leaves and seeds. Azadirachtin acts as an antifeedant for CPB, causes mortality and also can act as an insect growth disruptor by blocking morphogenic hormones (Zehnder and Warthen 1998; Mordue and Blackwell 1993; Trisyono and Whalon 1999). Growth regulator properties are most effective on eggs and early instars, so application timing is important (Trisyono and Whalon 1999; Kowalska 2007). Extracts from neem are known to have low mammalian toxicity and are less toxic to many natural enemies and predators (Schmutterer 1997). Azadirachtin tends to provide moderate efficacy (Kuhar *et al.* 2012; Zehnder and Warthen 1998). In one study, Marčić and Perić (2009) obtained 53.5−83.5% mortality of CPB and noted that the antifeedant properties reduced defoliation significantly. Igrc *et al.* (2006) reported 54-88% efficacy of neem extract using the full recommended dosing. Products containing azadirachtin are approved for organic and ecological production because of their biological origins.

Spinosad

Spinosad was the second active ingredient used, containing 240 g l^{-1} active ingredient. The recommended dosing for treatment of Colorado potato beetle on potato is 0.15 l ha^{-1}. Spinosad contains a mixture of various compounds called spinosyns, with the major components of Spinosad being spinosyn A and spinosyn D, which have the highest insecticidal activity. Spinosyns are a product of fermentation from the soil dwelling actinomycete bacteria, *Saccharopolyspora spinosa* Mertz and Yao. In numerous laboratory and field trials, Spinosad provides very high efficacy rates against CPB, typically around 95%-100% (Bret *et al.* 1997; Igrc *et al.* 1999, 2006; Marčić and Perić 2009). Spinosad has low toxicity for mammals and beneficial insects and displays low persistence in the environment (Bret *et al.* 1997). Spinosad impacts nicotine acetylcholine receptors and excites the central nervous system, causing muscle contractions and tremors, ultimately leading to paralysis and death (Kuhar *et al.* 2012; Salgado 1998). Despite the involvement of the nicotine receptor, it's mode of action is distinct from neonicotinoids and all known insecticides (Crouse *el al.* 2007). Spinosad is also approved for certified organic production in many countries, including the US and EU. Spinosad is effective against a number of agricultural pests including Lepidoptera, Diptera, Thysanoptera, termites, ants, and of course, some Coleoptera species (Dripps *et al.* 2008).

Spinetoram

Finally, spinetoram containing 120 g l^{-1} active ingredient was used also. The recommended dosing for treatment of Colorado potato beetle on potato is 0.3 l ha^{-1}. Spinetoram also contains spinosyns and therefore its properties are quite similar to spinosad. After the discovery of spinosad, Dow Chemicals set about discovering and creating new spinosyn molecules with insecticidal properties (Dripps *et al.* 2008). The outcome was the creation of spinetoram, which contains spinosyns J and L, resulting in a semisynthetic insecticide. Due to the largely similar molecular structures between spinosyns A and D and spinosyns J and L, spinetoram controls the same pest groups as spinosad and carries the same toxicological and environmental attributes (Dripps *et al.* 2008). Spinetoram also shows improved residual activity compared to spinosad (Dripps *et al.* 2008). Efficacy against CPB is similarly high to spinosad and control is even superior to spinosad with some pest groups (Dripps *et al.* 2008).

3.2 Description of the field

The field trial was located at the experimental station Maksimir at the Faculty of Agriculture in Zagreb. The field was planted with 20 rows of Tiamo variety potatoes, approximately 55 m long. The planting depth and density was as follows: 15 cm deep, 30 cm in-row spacing and 50 cm inter-row spacing. The soil type is slightly acidic clay soil. The field was previously planted with maize in 2016 and 2017 and was fallow in 2018. Potatoes were never grown on this plot before. A few days prior to planting, fertilization was conducted with a mix of NPK 7-20-30 (100 kg ha^{-1}) and NPK 15-15-15 (50 kg ha^{-1}) being used. Approximately 10-14 days after planting, herbicide treatment was conducted using Sencor SC 600 (0.6 l ha^{-1}) and a second herbicide treatment of Basagran 480 (2 l ha^{-1}) was conducted on May 8th.

3.3 Project implementation

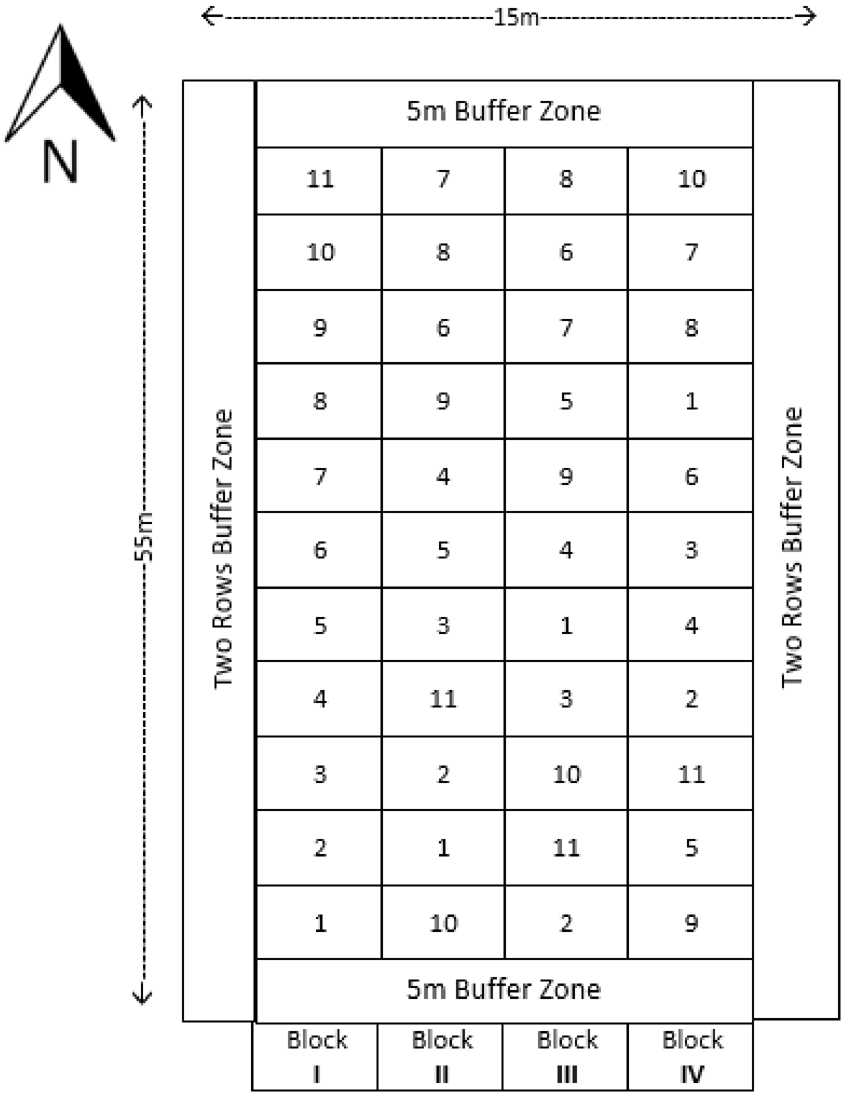
The field trial took place in 2019 at the experimental station Maksimir on an 800 m^2 with 20 rows of potatoes planted April 3rd and maintained until early June. Due to heavy rains throughout May, the trial start date was delayed by several weeks. The study field was divided into four blocks (I, II, III, and IV), each containing 4 rows (3 m wide) while two rows were left as a border on each side (20 rows total). Inside each block 11 treatments were randomized using a randomized block design, which is detailed in table 3.1**.** Dosing rates were carried out at varying amounts of 100 %, 50 % and 10 % of recommended dosage based on manufacturer recommendations. The length of each plot was 4 m, with each plot covering 12 m^2 and there were four replications per treatment (48 m^2 total per treatment).

The day before insecticidal spraying took place, June 5th, CPB larvae were counted and plants were marked until 100 larvae were identified. Making the baseline 100 larvae for each plot. Some plots had as few as 1 marked plant and other plots had over 10 marked plants in order to identify the starting 100 larvae. Additionally, the natural infestation of CPB was only moderate on this particular field and therefore larvae were collected from an adjacent field and deposited on some plots with low infestation rates in order to obtain the 100 larvae needed for the reference point on each plot.

Insecticidal treatment was carried out on June 6th, when CPB larvae were present, according to the randomized plot determined in advance. Spraying was conducted using a high-pressure sprayer called Euro-Pulvé, delivering 300 l ha^{-1}. Figure 3.1 shows the field trial randomized plot. After spraying, larvae counts occurred on the previously marked plants on days 2, 5, 7, 10, 14 and 21. Efficacy was established according to the count of surviving larvae on each plot.

Table 3.1. Insecticidal Treatments

Treatment number	Insecticidal treatment and recommended dosage (l ha^{-1})	Percentage of Recommended Dosage (%)	Resultant dosage (l ha^{-1})
1	Azadirachtin 2.5	50	1.25
2	Azadirachtin 2.5	100	2.5
3	Spinosad 0.15	10	0.015
4	Spinosad 0.15	50	0.075
5	Spinosad 0.15	100	0.15
6	Spinetoram 0.3	10	0.03
7	Spinetoram 0.3	50	0.15
8	Spinetoram 0.3	100	0.3l
9	Azadirachtin 2.5 + Spinetoram 0.3	50 + 10	1.25 + 0.03
10	Azadirachtin 2.5 + Spinosad 0.15	50 + 10	1.25 + 0.015
11	Untreated Control	N/A	N/A



Block I	Block II	Block III	Block IV
11	7	8	10
10	8	6	7
9	6	7	8
8	9	5	1
7	4	9	6
6	5	4	3
5	3	1	4
4	11	3	2
3	2	10	11
2	1	11	5
1	10	2	9

Figure 3.1. Randomized Plot Scheme of Field Trial

3.4 Statistical analysis

Number of larvae per treatment was analyzed using ANOVA. Duncan Multiple Range Test (DNMT) was used to determine the differences among the treatments (including untreated control) between the mean values of larvae per treatment. Based on average number of larvae per treatment and untreated control the efficacies of insecticides were calculated by using the Abbott formula (Abbott 1925).

$$\text{Corrected \%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) * 100$$

Calculated efficacies were analyzed using ANOVA to determine the differences among insecticide treatments. Duncan Multiple Range Test (DNMT) was used to determine the differences amongst the means of treatments. Results were analyzed by the use of ARM 9® software, (Gylling Data Management 2019).

4 Results

Table 4.1 shows the results of the field trial expressed as average number of total surviving larvae per treatment, from the starting baseline of 100 larvae. Tables 4.2, 4.3 and 4.4 show the results of the field trial expressed as percentage efficacy of each insecticidal treatment (azadirachtin, spinosad and spinetoram) at varying percentage of recommended dosage (10%, 50% and 100%). Tables 4.5 and 4.6 show the results of the field trial expressed as percentage efficacy as a comparison of combinational treatments (azadirachtin with spinosad and azadirachtin with spinetoram) vs. the insecticidal treatments on their own. Figures 4.1 and 4.2 show a graphical representation of tables 4.5 and 4.6, which compare individual insecticidal treatments next to the combined treatments of azadirachtin with spinosad and azadirachtin with spinetoram.

Table 4.1. Average number of CPB larvae after insecticidal treatment

Treatment	Dose ($l\ ha^{-1}$)	Average N° of living CPB larvae plot^{-1} after treatments–days after application					
		2	5	7	10	14	21
Azadirachtin	1.25	56,56a	58,90a	75,63ab	51,53ab	64,50ab	20,39bc
Azadirachtin	2.5	49,70ab	28,42ab	34,55b-e	24,48a-d	22,50c	13,66c
Spinosad	0.015	36,17ab	49,53a	55,15a-d	32,13a-d	24,75bc	26,55bc
Spinosad	0.075	2,54c	0,98c	3,46f	4,62d	31,50bc	64,03ab
Spinosad	0.15	10,60bc	4,74bc	7,02ef	10,20bcd	26,75bc	21,89bc
Spinetoram	0.03	57,12a	56,79a	63,37abc	47,62abc	51,25bc	55,20ab
Spinetoram	0.15	10,93bc	10,83bc	10,22ef	12,67bcd	28,75bc	32,02bc
Spinetoram	0.3l	9,72bc	8,13bc	7,36ef	5,96cd	18,00c	26,08bc
Azadirachtin + Spinetoram	1.25 + 0.03	54,54a	20,98ab	21,76c-f	33,83a-d	29,25bc	46,50abc
Azadirachtin + Spinosad	1.25 + 0.015	32,90ab	13,04bc	17,61def	15,81a-d	28,50bc	28,44bc
Untreated Control	NA	77,56a	53,75a	106,19a	104,64a	99,00a	92,59a

Means followed by same letter or symbol in the column do not significantly differ (P=.05, Duncan's New MRT).

Table 4.2. Efficacy of azadirachtin at 50% and 100% of recommended dosage

Treatment	Dose (l ha^{-1})	Efficacy of treatments–days after application					
		2	5	7	10	14	21
Azadirachtin	1.25	38.62a	18.95a	49.93a	63.01a	54.23b	56.11a
Azadirachtin	2.5	46.68a	72.00a	65.45a	71.72a	77.27a	84.37a

Means followed by same letter or symbol in the column do not significantly differ (P=.05, Duncan's New MRT).

Table 4.3. Efficacy of spinosad at 10%, 50% and 100% of recommended dosage

Treatment	Dose (l ha^{-1})	Efficacy of treatments–days after application					
		2	5	7	10	14	21
Spinosad	0.015	68.39b	30.09a	64.89a	60.64a	75.00a	79.54a
Spinosad	0.075	97.24 a	98.93a	96.80a	94.45a	68.18a	42.33a
Spinosad	0.15	98.74a	92.65a	90.16a	88.18a	72.98a	72.51a

Means followed by same letter or symbol in the column do not significantly differ (P=.05, Duncan's New MRT).

Table 4.4. Efficacy of spinetoram at 10%, 50% and 100% of recommended dosage

Treatment	Dose (l ha^{-1})	Efficacy of treatments–days after application					
		2	5	7	10	14	21
Spinetoram	0.03	51.87a	23.60a	40.27b	60.50a	67.16a	37.73a
Spinetoram	0.015	84.92a	80.53a	89.24a	83.00a	70.96a	61.99a
Spinetoram	0.3	87.13a	85.93a	88.79a	94.16a	81.82a	67.12a

Means followed by same letter or symbol in the column do not significantly differ (P=.05, Duncan's New MRT).

Table 4.5. Comparison of efficacies of individual treatments and combined treatments – azadirachtin and spinosad

Treatment	Dose (l ha^{-1})	Efficacy of treatments–days after application					
		2	5	7	10	14	21
Azadirachtin	1.25	38.62a	18.95a	49.93b	63.01a	54.23b	56.11a
Spinosad	0.015	68.39a	30.09a	64.89ab	60.64a	75.00a	79.54a
Azadirachtin + Spinosad	1.25 0.015	58.17a	77.18a	81.46a	75.89a	71.21a	64.96a

Means followed by same letter or symbol in the column do not significantly differ (P=.05, Duncan's New MRT).

Table 4.6. Comparison of efficacies of individual treatments and combined treatments – azadirachtin and spinetoram

Treatment	Dose (l ha^{-1})	Efficacy of treatments–days after application					
		2	5	7	10	14	21
Azadirachtin	1.25	38.62a	18.95a	49.93a	63.01a	54.23a	56.11a
Spinetoram	0.03	51.87a	23.60a	40.27a	60.50a	67.16a	37.73a
Azadirachtin + Spinetoram	1.25 0.015	41.77a	73.73a	71.40a	67.64a	70.45a	47.98a

Means followed by same letter or symbol in the column do not significantly differ (P=.05, Duncan's New MRT).

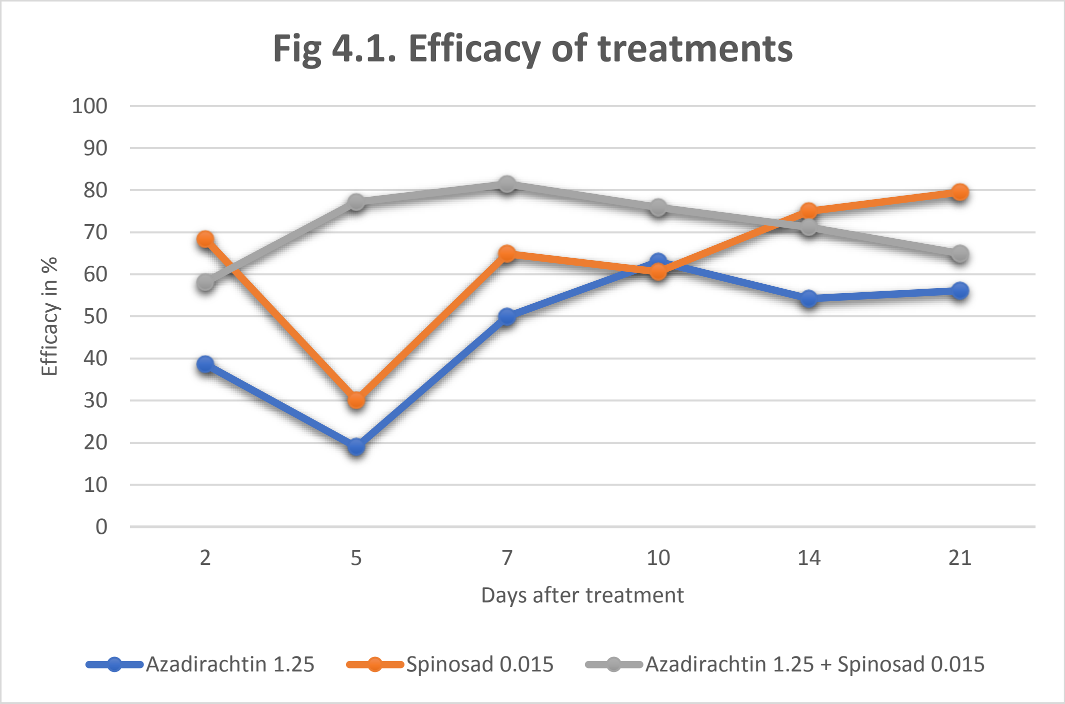


Figure 4.1. Comparison of efficacies of the treatments against CPB larvae – Azadirachtin (1.25 l ha^{-1}), Spinosad (0.015 l ha^{-1}) and Azadirachtin (1.25 l ha^{-1}) + Spinosad (0.015 l ha^{-1})

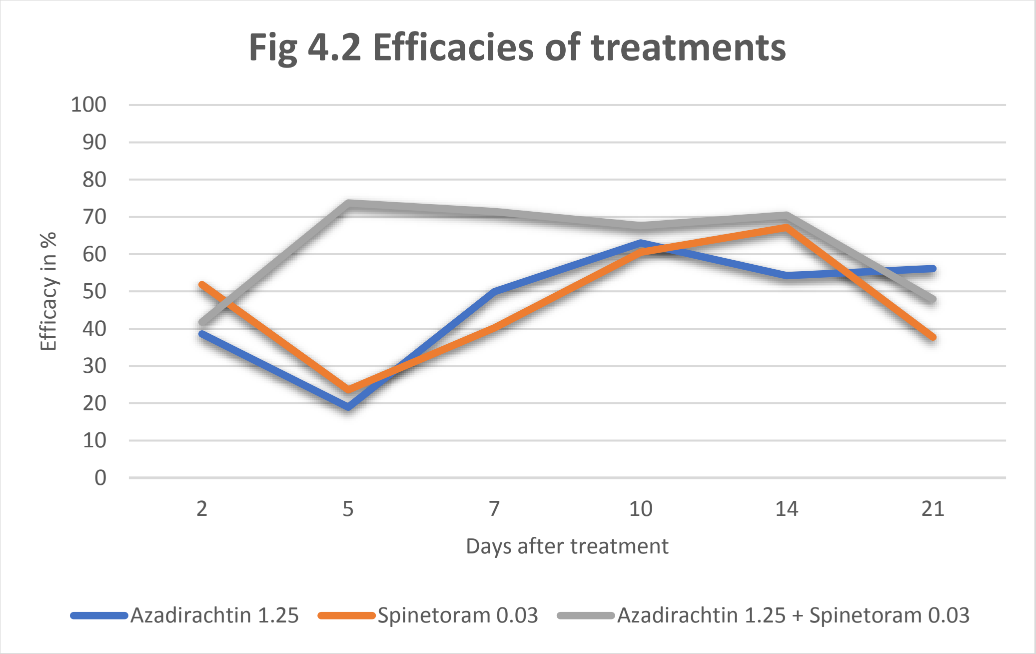


Figure 4.2. Comparison of efficacies of the treatments against CPB larvae – Azadirachtin (1.25 l ha^{-1}), Spinetoram (0.03 l ha^{-1}) and Azadirachtin (1.25 l ha^{-1}) + Spinetoram (0.03 l ha^{-1})

All insecticidal treatments reduced the average number of larvae per plot when compared to the untreated control, with significant reductions seen on all treatments except for the 10% dosage of spinosad and spinetoram, where only moderate reductions were seen. Overall, the half and full doses of spinosad showed the highest efficacy rates with residual activity of 10-21 days, depending on the treatment.

The efficacy of azadirachtin was clearly higher for the full dosage of 2.5 l ha^{-1} compared to the half dosage of 1.25 l ha^{-1}. The full dosage efficacy ranged from 65%-85% starting from 5 days after treatment and continuing through the end of the trial at 21 days after treatment. The half dosage treatment showed a maximum efficacy of 63% 10 days after insecticidal spraying.

For the series of spinosad treatments, both the 50% and 100% treatments showed very high efficacy results, 94-99% and 88-99% respectively, with 10 days of residual activity, after which the efficacy rates began to drop off. For the 10% treatment, low to moderate efficacy was observed, with a peak of 80% at 21 days after treatment.

For the series of spinetoram treatments, efficacy results were similar for the 50% and 100% treatments, as well as residual activity. The full dosage treatment saw efficacy rates between 82-94% with residual activity lasting 14 days and the half dosage treatment saw efficacy rates between 80-89%, lasting 10 days. The 10% dosage treatment did not exceed 67%.

The combination of a 10% dosage of spinosad (0.015 l ha^{-1}) and a 50% dosage of azadirachtin (1.25 l ha^{-1}) certainly showed some additive efficacy properties, but only starting 5 days after treatment and residual activity only lasting till around 10 days after treatment.

The combined treatment reached a peak efficacy of 81% at 7 days after treatment. Similarly, the combination of a 10% dosage of spinetoram (0.03 l ha^{-1}) with a 50% dosage of azadirachtin showed additive efficacy properties when comparing the treatments individually, though this effect was rather short lived. The combined treatment showed moderate efficacy rates starting 5 days after treatment and lasting till around 14 days after treatment, with a peak efficacy of 74% at 5 days after treatment.

5 Discussion

The efficacy of a full dose of azadirachtin peaked at 84%, 21 days after treatment and the efficacy of the half dose peaked at 63%, 10 days after treatment. This indicates that the lower dosing has shorter residual activity, as well as lower efficacy. These efficacy levels match other studies conducted, which obtained efficacy levels between 53.5-88% (Marčić and Perić 2009; Igrc *et al*. 2006). There are conflicting results amongst the literature as to the expected residual activity of treatments of azadirachtin. One study found the residual activity lasted only around 7 days (Igrc *et al.* 2006), which would require multiple treatments throughout the potato growing season. Our results showed the highest efficacy from the full dose treatment at 3 weeks after treatment, suggesting the known antifeedant properties of azadirachtin played a role in eventual mortality of the CPB larvae. Baumgart *et al*. (1997) claimed a single full does treatment of azadirachtin was sufficient for control, which is more in line with our results. Because of the antifeedant properties of azadirachtin, it would be beneficial to analyze defoliation damage along with efficacy, but that was not the focus of this study. Schrod *et al*. (1996) (as cited in Igrc *et al.* 2006) noted that efficacy of azadirachtin treatments would also be influenced by defoliation levels, and not just CPB mortality.

The results of spinosad treatments of 50% and 100% of the recommended dose showed high efficacies for the first 10 days after treatment, ranging from 88-99%. This is consistent to results from other studies (Bret *et al*. 1997; Igrc *et al.* 1999, 2006; Marčić and Perić 2009). The 50% dose achieved consistently higher efficacy results than the 100% dose starting from 5 days after treatment. It should be noted that one of the replicates of the 100% spinosad treatment had significantly higher larvae counts compared to the other three replicates. This is possibly due to the fact that spinosad does not possess ovoidal effects towards CPB eggs (Sharif and Hejazi 2014), therefore it is possible a cluster of eggs hatched shortly after treatment, increasing the larvae count for this plot. Efficacy results would likely be calculated at higher values for the 100% treatment had this data not skewed the results. If this was the case, then efficacy of the 100% treatment would be expected to be equivalent or higher than the 50% treatment. Efficacy dropped significantly around day 14 for both treatments (50% and 100%), indicating low residual activity. This is consistent with results found by Igrc *et al.* 2006, who also saw efficacies drop around day 10 or 14, depending on the experimental year. The opposite result was seen with the treatment of 10% of recommended dose. Efficacy appears to increase over time with a maximum efficacy of 80% reached at day 21 after treatment. It is suspected that this result was caused by early defoliation on these treatment plots, thereby encouraging the CPB to move onto other plants as food sources became scarce. Heavy defoliation was witnessed in these plots towards the middle and end of the trial. This could lead to false efficacy results simply due to beetle and larvae migration and not necessarily to mortality.

The 50% and 100% spinetoram treatments also provided high efficacy, though not at levels seen in the spinosad plots. The 100% treatment peaked at 94% efficacy 10 days after the trial and dropped significantly in the third week of the trial. The 50% treatment peaked at 89% 7 days after the trial began. While the efficacies were still relatively high, they did not reach the levels achieved by the spinosad treatments, suggesting spinosad is more suited for

CPB control than spinetoram. Though the two active ingredients function with the same mode of action, it is possible that the spinosyns A and D found in spinosad induce mortality at higher rates than spinosyns J and L found in spinetoram. The 10% treatment provided the least control, showing that a clear dose response was present. The 10% treatment showed the highest efficacies around 10-14 days after treatment, with a maximum efficacy of 67% 14 days after treatment, providing only low control levels.

For the combined treatment of a 50% dose of azadirachtin and a 10% dose of spinosad, the efficacy was higher than the individual treatments from 5-10 days after treatment, with the peak efficacy reaching 81% at day 7 after treatment. Starting with day 14 until day 21, the 10% spinosad treatment appears to outperform the combinational treatment, achieving an efficacy 15% greater than the combined treatment on day 21. This could be due to the previously mentioned explanation of high defoliation on the 10% spinosad treatment plots. The combined treatment really outperforms the individual treatments on day 5. The efficacy of the combined treatment was 77% and the sum of the individual treatments only totaled 39%. It appears this combination provides a synergism which boosts efficacy to a level higher than the sum of individual treatments around day 5. The same result was not witnessed for any of the other efficacy calculations, even on days 7 and 10 when the combined treatment offered superior control than either one of the individual treatments, but still fell short of exceeding the sum of individual treatment efficacies.

The combination of a 10% dose of spinetoram and 50% dose of azadirachtin did not perform as well as the combination of azadirachtin with spinosad, achieving only moderate efficacy results. With a peak of 74% on day 5 and providing residual activity until around day 14. A similar result was witnessed (with the spinosad and azadirachtin combination) on day 5, that the combined treatment's efficacy (74%) far exceeded the sum of individual efficacies (43%). Again, this was the only time this was witnessed. On days 7, 10 and 14, the combined treatment provided superior control to either one of the individual treatments but did not provide the synergistic effect seen on day 5.

The results show that the addition of low doses of spinosad or spinetoram to half doses of azadirachtin improved the efficacy of azadirachtin alone between days 5-14 after treatments. As the only major improvement in efficacy from the combinational treatment was seen on day 5 after treatment, it is unclear if the resultant efficacy of combination was simply from the presence of spinosyns in the combination, or if it was due to some synergistic effect between the spinosyns and azadirachtin. Igrc *et al.* (2006) points out that a possible impairment of the combination of azadirachtin and spinosyns is the fact that azadirachtin acts as an antifeedant and spinosyns are highly active via ingestion, therefore the antifeedant result from azadirachtin would halt the potential impact of spinosyn ingestion. The author recommends further studies on these combinations, specifically the addition of higher doses of spinosyn containing products with azadirachtin. Perhaps around the levels of 20-30% of recommended doses with the same 50% dose of azadirachtin. If high efficacy results were seen with these dosing rates, this could be a viable option for implementation into an IPM program for CPB control. While also achieving desirable economic results in the form of less active ingredients used. The results of this study suggest that the combination of 50%

azadirachtin with 10% spinosad provide moderate efficacy, and with short residual effects. More effective are the half doses of spinosad or spinetoram, which provided moderate to high efficacy. These treatments could easily be incorporated into an IPM system while also providing a satisfactory economic outcome. This same recommendation was made by Igrc *et al.* (2006), suggesting that the manufacturer's recommended dosage is higher than necessary to achieve CPB control. Reducing the dosing will also help to prevent or delay populations from developing resistance to the active ingredients spinosyns.

The modes of action of spinosyns and azadirachtin are different from each other, which allows for slowed development of resistance when used in combination. Spinosyns act by allosterically binding to the nicotinic acetylcholine receptor (NACHR) causing hyperexcitation of the nervous system. The specific molecular mode of action for azadirachtin is still unknown but there are several symptoms of exposure including multiple mechanisms of antifeedancy, growth regulation and sterility; none of which are similar to the mode of action of spinosyns. This method of combining treatments with unique modes of action is an important tool when considering any IPM strategy. While a result of synergism would be the most desired outcome when considering combinational treatments, even the result of increased efficacy of azadirachtin with small additions of spinosyns can be considered a positive outcome.

The need for new and novel treatment methods for CPB control is more important now than ever, especially with the latest development of resistance to neonicotinoids. Active ingredients such as spinosyns and azadirachtin are highly biodegradable, offer low mammalian toxicity and pose little threat to beneficial organisms in the field. Overall, they are far more ecologically sound treatment options than classical synthetic insecticides and should be considered when developing any IPM strategy to tackle CPB infestations.

6 Conclusion

- Low efficacy against CPB larvae was initially observed from a full dose treatment of azadirachtin, with efficacy improving over time and increasing to moderate efficacy three weeks after treatment.
- Unsatisfactory efficacy was achieved from a half dose treatment of azadirachtin.
- Satisfactory efficacy was achieved with one full dose treatment of spinosad and spinetoram, with spinosad out-performing spinetoram.
- Half dose applications of spinosad and spinetoram also performed well, with the half dose of spinosad achieving very high efficacy and the half dose of spinetoram achieving moderately high efficacy.
- The combination of a 10% dose of spinosyn containing product with a 50% dose of azadirachtin achieved unsatisfactory efficacy initially but improved to low-to-moderate efficacy between days 5 to 14, depending on the treatment. The 10% addition of spinosad performed better than the 10% addition of spinetoram.
- Reduced dosing of spinosyn containing products proved to be a viable option for incorporation into an IPM program.
- Further studies should be conducted on the combination of azadirachtin and spinosyns to see if improved efficacy can be achieved.

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Biography

Jamie O'Keeffe was born in Northern California on Sept. 27th, 1988. She completed high school with honors in 2006 and enrolled in university at San Diego State University. In 2011, she graduated with honors from SDSU with a Bachelor of Science degree in Chemistry as well as a Bachelor of Arts degree in Spanish. In the years following completion of university, she travelled and worked in the States and Europe, meeting her husband along the way. As well, Jamie developed a strong interest and passion for local food movements and sustainable agriculture, working on a small urban farm for two years in Northern Indiana. In 2017, Jamie and her husband moved to Zagreb and Jamie enrolled in the Master program InterEnAgro: Agriculture, Environment and Resource Management at the Faculty of Agriculture. Ultimately, Jamie plans to obtain land and own and operate a small-scale, diversified organic vegetable farm selling to the local community. In addition to agriculture, Jamie also has strong interest in craft beer, music and travel. She speaks Spanish at a conversational level.