SYSTEMIC DELIVERY OF INSECTICIDES IN BLUEBERRIES FOR CONTROL OF BLUEBERRY STEM GALL WASP, *HEMADAS NUBILIPENNIS*

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ABSTRACT

SYSTEMIC DELIVERY OF INSECTICIDES IN BLUEBERRIES FOR CONTROL OF BLUEBERRY STEM GALL WASP, *HEMADAS NUBILIPENNIS*

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Blueberry stem gall wasp (*Hemadas nubilipennis*) is a pest of highbush blueberry and can pose a challenge to control with foliar sprays due to adult activity during bloom and larval development within the plant tissues. In this thesis, systemic delivery of insecticides in blueberry bushes was evaluated using three application methods on potted bushes, in blueberry shoot bioassays, and on a commercial blueberry farm. Each study aimed to evaluate impact on gall and gall wasp development, along with active ingredient residue delivery to plant tissues. I hypothesized that if the insecticide moved systemically within the blueberry vascular system to areas where the blueberry stem gall wasp larvae are developing, then the insecticide will kill the larvae leading to a reduction in gall formation and number of surviving adults per gall. In the potted bush study, applications were made by crown injection, soil drench and foliar sprays. Imidacloprid, flupyradifurone, and spirotetramat were recovered in shoot and leaf tissues, however there was no evidence of inhibited gall or gall wasp development. In the shoot bioassays, imidacloprid and spirotetramat were found to have the greatest potential for control of blueberry stem gall wasp. Active ingredient recovery in bioassay gall tissue revealed the concentration needed to get moribund/ lethal larval response. The on-farm study indicated there was successful movement of imidacloprid and flupyradifurone using chemigation, however, the impact on blueberry stem gall wasp was not great enough to provide control.

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CHAPTER 1: INTRODUCTION

HEMADAS NUBILIPENNIS (HYMENOPTERA: PTEROMALIDAE)

Discovery and History

The blueberry stem gall wasp (*Hemadas nubilipennis* Ashmead) was named and described in North America by Dr. W. H. Ashmead in 1887, who believed that *H. nubilipennis* to be a parasite or guest fly of the galls forming on lowbush blueberry plants, *Vaccinium angustifolium* (Driggers 1927). Dr. W. H Ashmead thought that gall formation on blueberry bushes was formed by the wasp *Solenozopheria vaccinia* (Driggers 1927). Even though later studies found this to be incorrect, Dr. W. H. Ashmead was the first to publish the description of the gall found blueberry bushes in the *Transactions of the American Entomological Society* in 1887 (Driggers 1927). His description was "reniform, pithy gall on the stem or branches of *Vaccinium corymbosum* and *Vaccinium pennsylvanicum,"* (Driggers 1927). Further research was done by B. F. Driggers (1927) who saw the lack of knowledge about this gall maker on blueberry bushes to be a restriction to providing control. Through Driggers' experiments a previously unidentified species was found to be the inducer of the galls described by Dr. W. H. Ashmead.

B.F. Driggers conducted a rearing experiment with 400 galls collected from highbush blueberry, *Vaccinium corymbosum,* in the winter of 1925 continuing into spring 1926 (Driggers 1927). He found *H. nubilipennis* to be the most numerous species to emerge, and that emergence was at the same time as the blueberry bushes were pushing out new vegetation (Driggers 1927). The other species that were found emerging from the gall

include *Decatoma* sp., *Eurytoma solenozopheria* Ashm., *Ormyrus vacciniicola* Ashm., and *Eupelmus* sp. (Driggers 1927). However, no *S. vaccinia* were found to be emerging from the galls (Driggers 1927). *O. vacciniicola* were found to emerge at two different timings: during the emergence of all the other species and in the late growing season (Driggers 1927).

B. F. Driggers conducted an observation of the five chalcidoids in a cage with young blueberry shoots at the New Jersey Agricultural Experiment Station (Driggers 1927). He saw *H. nubilipennis* oviposition into the young shoots repeatedly by inserting and removing the ovipositor while moving up the stem (Driggers 1927). The line of oviposition marks was about an inch long (Driggers 1927). These shoots were dissected, and eggs were found (Driggers 1927). No other species were observed with oviposition activity into the new shoots, but *Decatoma* sp. were found to oviposit in the same shoot that *H. nubilipennis* had previously laid eggs into (Driggers 1927). To further test which species of chalcidoids was forming the gall, B. F. Driggers placed ten nursery blueberry plants in five cages (Driggers 1927). The first cage did not receive any of the chalcidoids and was used as a control (Driggers 1927). The second and third cage received *H. nubilipennis* and ten days later the third cage received *Decatoma* sp., *E. solenozopheria*, and *O. vacciniicola* (Driggers 1927). The fourth cage received *E. solenozopheria* and *O. vacciniicola* (Driggers 1927). The fifth cage received *Decatoma* sp. (Driggers 1927). These plants were observed throughout the growing season (Driggers 1927). The plants in the control cage were found to be free of galls, along with the plants in the 4th cage that were only exposed to *E. solenozopheria*, and *O. vacciniicola* (Driggers 1927). Galls formed on all the bushes in cages two and three (Driggers 1927). Oviposition damage was observed prior to the addition of *Decatoma* sp., *E. solenozopheria*, and *O. vacciniicola* to cage three (Driggers 1927). There were two globular

galls found on one plant in cage five, exposed to *Decatoma* sp. (Driggers 1927). B. F. Driggers concluded that *H. nubilipennis* was the species capable of forming the "reniform, pithy" galls on blueberry bushes and that the other species were either parasites or "guest flies" (Driggers 1927). B. F. Driggers suggested that *Decatoma* sp. may be capable of forming galls on blueberry bushes but not at the magnitude of *H. nubilipennis*; but he also could not rule out that other insects may have caused the galls to form (Driggers 1927).

A few years later in Maine, L. C. McAlister and W. H. Anderson (1932) conducted a study with *H. nubilipennis* on lowbush blueberries. Galls were collected from lowbush blueberry plants in April and the abundance of each species of wasp emerging was recorded each day (McAlister and Anderson 1932). McAlister and Anderson (1932) observed that *H. nubilipennis* had peak emergence while blueberry plants were growing quickly and producing new vegetation. They also found no *S. vaccinia* to be emerging from the galls (McAlister and Anderson 1932). With the wasps that emerged, a similar study to Driggers was conducted involving cages, blueberry gall dwelling species, and blueberry plants. There were eleven cages that received different combinations of species emerging from the galls (McAlister and Anderson 1932). These combinations included *H. nubilipennis* with or without *E. solenozopheria* and *Decatoma* sp., and some cages received the "guest fly" species without *H. nubilipennis* (McAlister and Anderson 1932). There was also a designated check cage that received no wasp (McAlister and Anderson 1932). Oviposition activity by *H. nubilipennis*, and by a single *E. solenozopheria* on a bush that was not exposed to *H. nubilipennis* was observed but no gall was formed (McAlister and Anderson 1932). The bushes exposed to *E. solenozopheria* Ash. and *Decatoma* sp., or not exposed to any

wasp resulted in no galls forming (McAlister and Anderson 1932). Only blueberry bushes exposed to *H. nubilipennis* formed galls (McAlister and Anderson 1932).

The parallel results of oviposition activity and gall formation of *H. nubilipennis* found by McAlister and Anderson (1932), and Driggers (1927) indicate that *H. nubilipennis* was the species capable of forming the galls on blueberry bushes, and the other species required the initiation of the gall by *H. nubilipennis*. These other species including *E. solenozopheria, Decatoma* sp., and *O. vacciniicola* can be considered inquilines. Inquilines may be parasitoids or cohabitants of gall and plant tissue, utilizing the resources the gall provides like food and protection (Roskam 1992; Stebbins 1910). Inquilines of the blueberry gall can emerge at the same time or just before or after the *H. nubilipennis* is actively laying eggs into shoots (Shorthouse et al. 1990). This means the inquiline eggs could be with *H. nubilipennis'* eggs or freshly hatched larvae (Shorthouse et al. 1990). Shorthouse and colleagues believed that parasitoids do not impact the size or shape of the gall because the parasitoids do not kill the larvae until the gall is mature (Shorthouse et al. 1990). They hypothesize that the parasitoids use a delayed growth tactic which has been seen in other parasitoid scenarios (Shorthouse et al. 1990). These inquilines were observed emerging in greater numbers than *H. nubilipennis* in a study conducted in Byron Bog in Southwestern Ontario (Judd 1959). Emergence of chalcidoids from 39 galls resulted in only two galls to have *H. nubilipennis*, while *O. vacciniicola* and *E. solenozopheriae* emerged in greater numbers from more galls (Judd 1959). This is likely due to successful parasitism of the gall wasp.

Natural History

H. nubilipennis is thought to be secondarily phytophagous which means this species' ancestors fed on or parasitized insect life stages after earlier ancestors were plant feeders (Malyshev 1968, as cited in West and Shorthouse 1989). Ancestors of *H. nubilipennis* were most likely targeting insects within the blueberry tissues for parasitism but when this host became unavailable the larvae may have been forced to feed upon the blueberry tissue (Malyshev 1968). With this change, *H. nubilipennis* has gained control of the plant tissue development for its own advancement (West and Shorthouse 1989, Malyshev 1968). The native host for *H. nubilipennis* is the lowbush blueberry (West and Shorthouse 1989).

H. nubilipennis is a chalcid wasp belonging to the superfamily Chalcidoidea (Yoshimoto 1984). Wasps in this superfamily are holometabolous which means they complete all stages of metamorphosis – egg, larva, pupa, and adult (Yoshimoto 1984). Although those stages are well agreed upon in the literature, whether mating is required for the development of the wasp is not. McAlister and Anderson (1932) tested whether female *H. nubilipennis* were parthenogenic by separating females from males as they emerged and exposing them to blueberry plants in cages. Oviposition was observed and there was slight swelling when unmated females were exposed to blueberry shoots, but after later dissection of the shoot there were no larvae found. It was concluded that either no eggs were laid or the eggs were infertile (McAlister and Anderson 1932). In 1990, Shorthouse and colleagues suggest that males are rare and females were capable of producing viable off-spring without mating (Shorthouse et al. 1990). No mating was observed in Shorthouse's 1990 study and galls formed from oviposition of unmated

females. However, in the studies following this chapter, mating was observed. Whether it is necessary for viable eggs, that was not tested and is still not well agreed upon.

A Note on Gall Development

The formation of an insect gall involves an "interspecific association" where both the insect and the plant play important roles for the success of the insect (Rohfritsch and Shorthouse 1982). Although the outcome for the two individuals is not equal. The insect has much to gain from the plant such as shelter and nourishment, in return the plant's energy and resources are redirected from fruiting and foliar expansion to benefit the insect (Rohfritsch and Shorthouse 1982; Shorthouse et al. 2005). The insect gall inducer gains control of this region by causing the plant to create a gall as a "separate entity" with its own polarity and symmetry (Rohfritsch 1992).

Raman and colleagues define a successful gall system as having four key characteristics: appropriate host-plant timing, specialized colony/ovipositional behavior of the insect, timely response of growth of gall tissues, and separation of the gall from the host benefits which is around the time the insect reaches maturation and is ready to leave the gall (Raman et al., 2005). The blueberry stem gall wasp meets all four of these characteristics causing it to be very successful in blueberry fields with abundant shoot expansion.

Researchers are unclear on the specific mode of action that allows the blueberry stem gall wasp to form a gall (Rohfritsch and Shorthouse 1982). Some insect larvae have "growth-promoting substances" in their saliva which may cause the plant to produce nutritive cells lining the gall chambers, similar to the gall wasp chamber lining, indicating

that feeding is initiating the gall (Rohfritsch and Shorthouse 1982). Other ways insect galls can form are from mechanical action, chemical secretions, or wounding, and sometimes a combination of these actions. For cynipid wasps, along with sawflies and weevils, oviposition activity alone can stimulate gall initiation (Raman et al. 2005). It is likely that there are many factors leading to the development of the blueberry stem gall wasp gall.

Life Cycle

Adult *H. nubilipennis* emerge from galls formed in the previous growing year when the plant is growing rapidly, usually around the time of blueberry bloom (Isaacs et al. 2020). Adult wasps measure only 2-3 millimeters in length (Isaacs et al. 2020). Females lay eggs into expanding shoots as quickly as within minutes of exiting the gall (West and Shorthouse 1989). A female gall wasp first taps the surface of the expanding shoot with her antennae then begins to oviposit 5-15 mm below the tip of the shoot facing the apex, moving a few millimeters up the shoot each time to lay an egg (Shorthouse et al. 1986, West and Shorthouse 1989). Each time she inserts her ovipositor into the shoot a channel is created, and an egg is deposited at the end (Shorthouse et al. 1986). The egg is primarily deposited into the pith of the shoot but may be in contact with vascular tissue and cortex near one end of the egg (West and Shorthouse 1989). Oviposition on the same shoot happens until 12 to 15 eggs are laid; and the distance of the oviposition was recorded to be 1.27 cm to 2.54 cm long (McAlister and Anderson 1932). When the female *H. nubilipennis* is done laying eggs, it was observed that she attempts to prevent the shoot tip from further expansion by using her ovipositor to stab the apical meristem and surrounding tissues

(Shorthouse et al. 1986). McAlister and Anderson (1932) noted that this does not affect gall formation.

A study was conducted to determine whether the stabbing technique was required for a successful gall and offspring development. J. D. Shorthouse and colleagues hypothesized that success of a gall is directly impacted by this "stabbing" technique (Shorthouse et al. 1986). Lowbush blueberry plants were observed for oviposition, both in the field and in the lab on isolated shoots (Shorthouse et al. 1986). The shoots that oviposition was observed on were slide mounted (Shorthouse et al. 1986) In the field, female *H. nubilipennis* were allowed to oviposition for a maximum of thirty minutes into selected shoots on bushes in the field, once this time elapsed the shoot was covered in mesh cages and examined two weeks later (Shorthouse et al. 1986). Their results rejected their hypothesis, and successful galls were found on shoots with no stabbing (Shorthouse et al. 1986). However, Shorthouse and colleagues could not be certain that they successfully prevented the female from using the stabbing technique in this study (Shorthouse et al. 1986).

Females can distribute eggs into multiple shoots in their short lifetime, including shoots that have been previously attacked by other female wasps (West and Shorthouse 1989). The eggs are described as "silvery white" and "in the shape of an Indian club" (McAlister and Anderson 1932). The cells of the shoot begin to rapidly divide within 48 hours of oviposition (Shorthouse 1986). Scarring on the shoot tissue can be seen above where the eggs were, and no eggs were found in this region (Shorthouse et al. 1986). These shoot tips were found to be dead and no longer growing after two days (Shorthouse et al. 1986). Although some shoots continue to grow, and the gall is not formed at the apex.

After 10 days the egg is surrounded by thick layers of cells which provide protection (Shorthouse 1986; Shorthouse et al. 1986). The gall wasp larvae hatch in approximately 10-14 days (Shorthouse et al. 1986; Hayman et al. 2003a). Twenty days after oviposition the gall is noticeably swelling, and nutritive cells are beginning to form in the cell lining of the chamber (McAlister and Anderson 1932; Shorthouse 1986). The larvae are already hatched at this point and begin feeding on the tissue within the chamber (Shorthouse et al. 1986). Each larva has their own chamber, which was initially the channel in which the egg was deposited, making up a multi-chambered gall (Shorthouse et al. 1990).

As the gall expands the cells continue to divide and the gall increases in size (Shorthouse et al. 1986). Since the eggs were laid on one side of the shoot the shoot swells unevenly causing a "sharp angle" in the shoot (McAlister and Anderson 1932). After 40 days nutritive cells line the chamber (Shorthouse 1986). The nutritive cells are more beneficial to the larvae than other tissues because they contain higher levels of starch, sugars, lipids, and proteins allocated from the blueberry bush via vascular bundles (Dreger-Jauffret and Shorthouse 1992).

Gall development takes 60-90 days; and larvae are growing and feeding during this time (Hayman et al. 2003a). Galls can be round, reniform, and irregular in shape (Shorthouse et al. 1990). Generally, the placement of eggs on the shoot determines the shape of the gall and the number of eggs determines the size of the gall (Shorthouse et al. 1990). When eggs are laid in a concentrated area or few eggs are laid the gall is rounded (Shorthouse et. al 1990). When the eggs are laid in a single row a reniform shape is formed (Shorthouse et. al 1990). Irregular placement of eggs or if more than one female lays into one shoot then an irregular shape gall is formed (Shorthouse et. al 1990). West and

Shorthouse found 30% of the galls surveyed to be spherical or irregular shape, not adhering to the reniform definition (West and Shorthouse 1989). They also found that not all the galls are found at the apex of the shoot (West and Shorthouse 1989). This is most likely due to expanding of the shoot because it was not successfully killed by the stabbing of the female. While the gall is developing, it is mostly green in color but can also have creamy white, pink, and red accents (West and Shorthouse 1989).

Around the time the larvae stop feeding the "chambers become encapsulated by the hard sclerenchyma sheath" and the gall becomes woody in preparation for winter (Shorthouse 1986). The color of the gall turns from green to brown (West and Shorthouse 1989). The larvae become slow moving (McAlister and Anderson 1932; Shorthouse et al. 1986). McAlister and Anderson (1932) report that *H. nubilipennis* overwintering as a mature larva, however Hayman (2003a) refers to this as a state of diapause. Although this is not agreed upon, it is understood that larvae are inactive (McAlister and Anderson 1932; Shorthouse et al. 1986). Once spring arrives larvae exit this phase, pupate, and begin to develop into adults (Hayman et al. 2003a; Shorthouse et al. 1986). A degree day model has been developed by Michigan State University to predict emergence at 160DD with a base of 12.5 degrees Celsius (Michigan State University Enviroweather, n.d.). Adults chew their way out of the gall and begin laying eggs into the new expanding shoots which will form new galls over the growing season (Isaacs et al. 2020).

What is known about the blueberry stem gall wasp life cycle has mostly been studied on lowbush varieties of blueberry. There have been no developmental studies on commercial highbush varieties. Through observations, the gall wasp is believed to behave in a very similar way in the highbush varieties.

Galls as Sinks

Since it is known that tissues of the gall become enriched with nutrients for the gall wasp larvae to feed on, a study looked at galls as sinks for inorganic elements such as copper and nickel (Bagatto and Shorthouse 1991). The sampling sites chosen were north-west of the Inco smelter and were at distances of 6.5 to 74 km from the smelter (Bagatto and Shorthouse 1991). Metal concentration in the galls collected during the winter were higher than metal concentration of the other parts of the lowbush blueberry sampled during the summer at sites close to the smelter (Bagatto and Shorthouse 1991). Roots generally were next highest in metal accumulation, followed by the stems, leaves, and berries (Bagatto and Shorthouse 1991). Nickel and copper concentrations varied when compared against each other, Bagatto and Shorthouse (1991) concluded that this may be because of the degree of mobility in the plant (Bagatto and Shorthouse 1991).

In a later study Bagatto and Shorthouse looked at the mineral nutrient accumulation in galled shoots at different gall stages (Bagatto and Shorthouse 1994). Samples were taken from lowbush blueberry plants, 15 km north of Sudbury, Ontarior, Canada (Bagatto and Shorthouse 1994). They collected 3 phases of the gall development and ungalled shoots for nutrient analysis. (Bagatto and Shorthouse 1994). The 3 phases were initiation (May), growth (late June), and mature (late August) (Bagatto and Shorthouse 1994). Only one sample of the ungalled shoots were collected, and these were collected with the initiation phase gall samples (Bagatto and Shorthouse 1994). All samples were assessed for concentrations of Cu, Ni, Fe, Zn, Mn, Mg, and Ca (Bagatto and Shorthouse 1994). In the initiation phase galls had higher concentrations of Cu, Ni, Fe and Zn than the ungalled shoots (Bagatto and Shorthouse 1994). The growth phases had lower concentrations of Cu,

Ni, Fe, and Zn than the initiation phase (Bagatto and Shorthouse 1994). The reason why the growth phase concentrations were lower is thought to be because of dilution effect where the growth of the plant "dilutes" the weight of the minerals when comparing dry masses (Jarrell and Beverly 1981, as cited in Bagatto and Shorthouse 1994). Mineral concentrations of Mn, Mg, and Ca were higher in the ungalled shoots than any phase of the galled shoots (Bagatto and Shorthouse 1994). In the galled shoots, Mg and Mg concentrations decreased as the gall developed and Ca concentrations remained the same (Bagatto and Shorthouse 1994). When comparing a mature stem mineral concentration, the concentrations varied in greater or lesser concentrations when comparing to the phases or the ungalled shoot (Bagatto and Shorthouse 1994). This may be because mature stems are morphologically, developmentally, and physcologically different (Bagatto and Shorthouse 1994). In all, Bagatto and Shorthouse (1994) could not conclude that there was a pattern in the accumulation of Cu, Ni, Fe, Zn, Mn, Mg, and Ca in mature gall tissues. Rather, the presence of those minerals is for the developing cells and the activity of the gall tissues in response to the larvae (Bagatto and Shorthouse 1994).

Hemadas nubilipennis as a Pest

The blueberry stem gall wasp is a pest in eastern North America (Isaacs et al. 2020). Isaacs and colleagues report that low population levels of the gall wasp is more of a nuisance because bushes with few number of galls can be pruned off (Isaacs et al. 2020). When the population level is high, pruning costs are high and costs outweigh the benefit of managing the field (Isaacs et al. 2020). Two significant impacts that the formation of the

gall from *H. nubilipennis* has on blueberry bushes is the reduction in fruiting surface and contamination of harvested fruit (Hayman et al. 2003a; McAlister and Anderson 1932).

A study conducted in Novia Scotia over two seasons at three different lowbush blueberry fields assessed the galls impact on shoot characteristics and berry production (Hayman et al. 2003a). Placement of the gall on the stem significantly impacted the stem length (Hayman et al. 2003a). When the gall is located terminally, the shoot is found to be shorter with less weight in stems and leaves than shoots with galls basally located (Hayman et al. 2003a). Galls terminally located also had a higher gall weight ratio (Hayman et al. 2003a). Overall, galled shoots had significantly less leaf and stem weight ratios when compared to one or more non-galled shoots (Hayman et al. 2003a). It was also found that two sites had reduction in berry production with shoots that had galls (Hayman et al. 2003a). The reduction in mature berry production was only found to be 3%, which was a conservative estimate; however, this rate may be significantly greater as years go on (Hayman et al. 2003a). McAlister and Anderson (1932) also expressed concern over the reduction in fruiting surface in their study with lowbush blueberry plants. When the stabbing technique is successful, shoot expansion and the fruiting area off that shoot is reduced in the coming years (McAlister and Anderson 1932; Hayman et al. 2003a).

The other potential problem that blueberry growers face is that galls formed by *H. nubilipennis* can contaminate harvested berries (Hayman et al. 2003a). Galls can be removed by the blueberry harvester machine and become mixed in with the harvested fruit (Hayman et al. 2003a). Since galls can be similar in size, it is possible that they may not be sorted out, mistaken as fruit, and sold to the consumer (Hayman et al. 2003a). This can lead to contamination of frozen and fresh berries (Hayman et al. 2003a). Isaacs and colleagues

recommend training personnel or electronic sorters on the processing line to identify and remove galls (Isaacs et al. 2020).

Mobility and Abundance

In the early 1900's reports of *H. nubilipennis* being a pest of lowbush blueberry in eastern North America were noted in Massachusetts (Stebbins 1910), Indiana (Doak 1927), New Jersey (Driggers 1927), and Maine (McAlister and Anderson 1932). A 2020 report suggest that blueberry stem gall wasp has been found most of the northeastern North America region with the addition of Florida and Georgia (Isaacs et al. 2020). Although the blueberry stem gall wasp in native, there are concerns of spreading of large amounts of wasps to areas where it is not a major pest. It is likely that the spread of *H. nubilipennis* will be very slow due to its an inability to fly but rather "hop short distances" (McAlister and Anderson 1932).

In recent years, the abundance of blueberry stem gall wasp in western Michigan has become a real problem for commercial highbush blueberry farmers. Jersey variety was one of the most highly planted varieties in Michigan (USDA 2020), is highly susceptible to gall wasp infestation. In the 2018-2019 Michigan Fruit Inventory reported 27% of the acres of blueberry bushes planted in Michigan were Jersey variety (USDA 2020 as cited in Garcia-Salazar et al. 2020). Many commercial blueberry farmers have begun to feel helpless because every IPM practice they tried would not stop galls from forming and large amounts of wasps emerging. They only way to control them was to complete removal of highly susceptible blueberry varieties from their farm (Isaacs et al. 2020). The most susceptible commercial highbush blueberry varieties are Jersey, Northland, Pemberton, and Bluejay

(Isaacs et al. 2020). This recent shift of the blueberry stem gall wasp becoming an important pest to Michigan blueberry growers is thought to be because of two changes in blueberry pest management (Isaacs et al. 2020). First, the arrival of spotted-wing drosophila (*Drosophila suzukii*, Diptera: Drosophilidae), a major invasive fly pest attacking the fruit, has farmers applying late season insecticide sprays to keep fly larvae out of the fruit. (Isaacs et al. 2020). These late summer insecticide applications occur when natural enemies of the blueberry stem gall wasp's gall are active and may prevent the parasitoid from keeping the gall wasp population in check (Isaacs et al. 2020). Another change in blueberry pest management is the EPA cancelation of the organophosphate insecticide, azinphosmethyl, which was an insecticide used for fruit worm treatment in blueberry and applied during the time adult blueberry stem gall wasp were active (Isaacs et al. 2020). There is evidence that this product may have provided control of blueberry stem gall wasp even when applied after eggs are in the shoot (Fanning and Isaacs 2019a).

Current Blueberry Stem Gall Wasp Management

A common practice for commercial lowbush blueberry field maintenance is burning and/or pruning of the bushes (Hayman et al. 2003b). A study found the greatest reduction of blueberry stem gall wasp emergence in fields that did the combination of a fall mow and a spring burn (Hayman et al. 2003b). Mowing alone or mowing plus a fall burn resulted in a higher emergence rate (Hayman et al. 2003b). The fall burn is thought to be less effective because the leaf litter and gall tissues have a higher moisture rate than those in the spring, allow for a less intense burn (Hayman et al. 2003b). Highbush blueberry growers do not use similar practices, but usually require some pruning of branches in the off-season. The

pruning of branches in fact only promotes more growth for *H. nubilipennis* to attack (Isaacs et al. 2020). Both organic and non-organic farmers of highbush blueberry bushes can hire individuals to hand remove galls from bushes in the off-season. This can become very costly as stated above. Galls should be burned or buried more than 30.5 cm deep (Isaacs et al. 2020). Any galls that are left on the ground, even if they are mowed, will still have adults emerge (Isaacs et al. 2020; Hayman et al. 2003b).

Besides removal of galls, there are other recommendations and natural predators that may help growers. The costliest, but by far the most effective, is removal of susceptible varieties. In regions where gall wasp abundance is high, Isaacs and colleagues recommend replanting susceptible fields, especially Jersey varieties, with a known resistant variety (Isaacs et al. 2020). They recommend a year or more between removal and planting for soil improvements (Isaacs et al. 2020). This will also allow any galls with larvae that may have fallen from the bushes and remain in the field to complete their life cycle without blueberry bushes present.

Two biological control practices that both organic and non-organic farmers can utilize are parasitism and predation though the results may not be significant without the right conditions. Isaacs and colleagues have noticed that small mammals and birds damage galls during late winter into early spring (Isaacs et al. 2020). The impact of this predation can vary based on the field and its surroundings; also, the length and severity of the winter (Isaacs et al. 2020). The studies done in Michigan, U.S. show that damage caused by birds and small mammals reduces the number of wasps emerging but likely does not have a significant impact on population levels (Isaacs et al. 2020). Parasitism of the gall is another biological control option, though this is more successful in fields where insecticides are not

readily used. Isaacs and colleagues found in fields where insecticides are not used, only 50% of the wasps emerging from the gall were *H. nubilipennis* (Isaacs et al. 2020). The other half were parasitoids such as *E. solenozopheriae, O. vacciniicola,* and *Sycophila vacciniicola;* along with other insects (Isaacs et al. 2020). However, in fields treated with conventional insecticides, *H. nubilipennis* was nearly the sole species found in the galls at 99% occupation (Isaacs et al. 2020). The 1% was usually *S. vacciniicola,* which has an active period very similar to *H. nubilipennis* (Isaacs et al. 2020). The other parasitoid species are active during the same but have a second-generation during harvest when many insecticide applications are applied (Isaacs et al. 2020). It is likely that parasitoids will not be beneficial to conventional farmers.

Lastly, chemical control is an option for controlling *H. nubilipennis.* The use of insecticides is limited when targeting adult gall wasp since this time corresponds with bloom when pollinators are present (Isaacs et al. 2020). Since blueberry farmers depend heavily on pollination it is important to avoid using pesticides during this time (Isaacs et al. 2020). Pre-bloom sprays have been found to be ineffective and can have create a risk for pollinators (Isaacs et al. 2020). So, insecticides are recommended to be applied immediately after bloom, some of which target eggs and young larvae within the developing gall (Isaacs et al. 2020).

Azinphosmethyl, the previously registered insecticide, was found to be effective in reducing the total number of galls per bush with approximately 90% reduction in the total number of galls when compared to the untreated control bushes at the end of the season (Fanning and Isaacs 2019a). Finding registered product that is as effective has been a challenge for researchers. In the same study cyclaniliprole (Verdepryn) was found to

approximately reduce the number of galls per bush by 64% (Fanning Isaacs 2019a). Understanding the full impact of this insecticide came when comparing adult emergence. There was a 95% reduction of adults emerged from bushes that were treated with cyclaniliprole compared to untreated bushes (Isaacs and Wise 2020).

Other broad-spectrum foliar insecticides tested and recommended by Isaacs and colleagues include methomyl (Lannate) followed by an application of zeta-cypermethrin (Mustang Maxx) or esfenvalerate (Asana) (Isaacs et al. 2020). Phosmet (Imidan) is also recommended as being effective (Isaacs et al. 2020). The benefit of using these broad-spectrum products is that it may also provide control of other pests such as cherry fruitworm (*Grapholita packardi*) and cranberry fruitworm (*Acrobasis vaccinii*) (Isaacs et al. 2020).

Another study looked at soil applications of systemic insecticides: dinotefuran (Scorpion), flupyradifurone (Sivanto), thiamethoxam (Platinum), imidacloprid (Admire Pro) (Fanning and Isaacs 2019b). These products delivered through soil application had no impact on reducing gall formation (Fanning and Isaacs 2019b). Foliar systemic: spirotetramat (Movento) application reduced the number of galls (28 ± 2.85 mean ± SEM) when compared to untreated plots (43 ± 4.16 mean ± SEM) (Fanning and Isaacs 2019b). Larvae were 99% controlled in galls collected from bushes treated with a foliar application of spirotetramat when compared to untreated control bushes when measuring emergence (Isaacs et al. 2020).

A bioassay involving a dip-method study was used to test the efficacy of insecticides with and without superior oil for reducing blueberry stem galls wasp emergence from mature galls pruned from blueberry bushes (Mason and Isaacs 2011). Azinphosmethyl was

found to reduce emergence by greater than 50% without the addition of oil and around 50% with oil (Mason and Isaacs 2011). Bifenthrin (Brigade) without the addition of oil reduced emergence by about 50%, however with oil emergence was reduced 98% (Mason and Isaacs 2011). Esfenvalerate (Asana) with oil resulted in a 93% reduction in emergence (Mason and Isaacs 2011). Phosmet (Imidan) with oil was also significant in reducing emergence, but only by 38% (Mason and Isaacs 2011). The use of superior oil helped the insecticides penetrate the galls surface and deliver a lethal dose to the mature larvae inside. It is likely that all foliar applications will need a way to penetrate plant tissues to be effect in controlling the blueberry stem gall wasp. Adjuvants such as oils and plant penetrants will aid in delivering the insecticide to the larvae.

Studies by Xu and colleagues (2008) involving the gall wasp *Quadrastichus erythrinae* that forms galls on wiliwili tree leaves tested the efficacy of several systemics, distribution of imidacloprid in the tree, and longevity of soil or injected imidacloprid. Erythrina gall wasp emergence was significantly lower from injected trees with imidacloprid (Imicide/Mauget 4 ml microinjection capsules, 100 g L^{-1}) than the untreated control and all other systemics tested including abamectin (Abacide/Mauget 2ml microinjection capsules, 10 g L^{-1}), drench applied imidacloprid (Merit 2F, 214 g L^{-1}), and drench applied dinotefuran (Safari 20 SG, 200 g kg^{-1}) (Xu et al. 2008). Imidacloprid is found in greatest concentrations in areas nearest to the injection site, though in this study the levels did not meet the minimum residue to provide erythrina gall wasp control (Xu et al. 2008). Xu and colleagues (2008) also found that both galled and ungalled leaves had similar levels of residue and either can be used to determine residue levels. In the imidacloprid longevity study, injected and drench treated trees gradually increased in

residue levels throughout the 15 weeks (Xu et al. 2008). Drenched treated trees died at 15 weeks because of the inability to control the erythrina gall wasp infestation. In the following year, the injected formulation Merit 200 SL and IMA-jet had concentrations high enough to be effective against the erythrina gall wasp (Xu et al. 2008). In all, injection of imidacloprid provided control of erythrina gall wasp of the wiliwili tree for one year and has the potential to provide an additional year (Xu et al. 2008); and may also be useful for control of other gall wasps via injection treatment.

It is apparent that if highbush blueberry farmers do not want to remove susceptible fields of blueberry bushes, chemical control may be their best option. Choosing an insecticide that will deliver a lethal does to the juvenile stages is the biggest challenge. Foliar sprays with adjuvants have proven to be moderately effective. Although, systemic insecticides intriguing since they move within the plant's vascular system. spirotetramat (Movento), a foliar systemic, has shown great lethality of blueberry stem gall wasp larvae. However, the study noted above with soil applied systemics do not appear to have provided great control. More research should be done to test other timings and methods of systemic application on blueberry bushes for control of *H. nubilipennis.*

SYSTEMICS AND SYSTEMIC APPLICATION METHODS

History of Systemics in Plants

Systemic movement of foreign matter (chemical, dye, flavor) within plants have long puzzled and excited scientists. A good systemic chemical or material has the ability to move within a plant from a source to a sink (White 2012). The first recorded injection was a solid

formulation injection (Roach 1939). Hadj de Granade described Ibn-Al-Awarm's work in the 12th century as a solid injection with musk, cloves, saffron and more inserted into the pith cavity of a tree (Roach 1939). Regions of the tree that were used included roots, shoots, or between bark and the tree (Roach 1939). The goal was to "impart perfumes, flavours, and medicinal qualities to fruits, and a yellow or blue colour to roses" (Roach 1939).

Liquid plant injection was recorded to be used by Leonardo da Vinci in the 15th century on peach tree trunks (Roach 1939; U.S. Dept. of Agriculture [USDA] 1960). Leonardo da Vinci found a solution of arsenic and realgar dissolved in boiling water can make the fruit of the tree poisonous (Roach 1939). The peach pest was killed but the fruit was very toxic to consume (Roach 1939; USDA 1960).

In 1602 methods of a solid plant injection was used as recommendation to kill "wormes" in a tree, published in *The Orchard and Garden* (Anon 1602, as cited in Roach 1939). The recommendation was to mix pepper, laurel, incense, and good wine, place it in a hole created downward into a tree which reaches the pith, sealing it with hawthorne (Anon 1602, as cited in Roach 1939). Furthermore, fruits could be made sweeter by injecting honey into a hole about a foot above the roots and seal it with hawthorne branch (Anon 1602, as cited in Roach 1939).

In 1765 mercury was tested as an injection material to kill insects on trees and shrubs (Wilson 1765, as cited in Roach 1939). A drop or two of mercury was dripped into a hole created on a branch with an awl (Wilson 1765, as cited in Roach 1939). It was recorded that the hole was not as deep as previous injections, and never reached the pith (Wilson 1765, as cited in Roach 1939). A wooden plug was used to seal the hole (Wilson

1765, as cited in Roach 1939). There was no harm to the trees and shrubs reported; in fact, they were said to be in "full vigour" for the rest of the season (Wilson 1765, as cited in Roach 1939). The insects inhabiting the branch that was treated had "dropped off" the following day, and after a few more days the insects were off all the other branches, suggesting there was systemic movement of the mercury (Wilson 1765, as cited in Roach 1939).

In the 19th century many scientists were exploring sap accent in trees (Roach 1939). Dissolved dyes, salts, and metals were tested for speed of ascended and/ or diffusion (Roach 1939). Methods that are used in current injection practices begin to be invented. Hartig may have been the first to inject into a hole in a tree from a reservoir, which is a very common way of injection used today (Hartig 1853, as cited in Roach 1939).

Into the twentieth century researchers were using systemics through injection in agricultural and forestry applications with major published finding from Russia, Italy, Germany, America, and England (Roach 1939). Experiments were noted to be on a much larger scale, sometimes several hundred to thousand plants and trees used (Roach 1939). Different concentrations were tested along with seasonal effects on movement of injected materials, and injection pressures (Roach 1939). Dementiey (1914) found that applying pressure to the injection increases uptake rate and this is used in some agricultural applications today (Dementiey 1914, as cited in Roach 1939). Potassium cyanide was injected into apple trees in autumn to target the descending sap which would deliver a lethal does to phylloxera parasites (Perosino 1899, as cited in Roach 1939). Later observed, potassium cyanide injected as a liquid did not persistent in the tree (Dezeani 1913, as cited in Roach 1939). Solid injections of potassium cyanide, potassium chlorate, and iron

sulphate were thought to increase vigor and protect the tree from insects and fungus when applied, but this was not the case (Surface 1914, as cited in Roach 1939). There was no increase in vigor and no treatment of disease was achieved (Surface 1914, as cited in Roach 1939). Later work showed that cyanide disappeared within trees within two days (Elliot 1917, as cited in Roach 1939).

Injection of nutrients was also a part of twentieth century injection research (Roach 1939). Lipman and Gordon discovered pear trees to be lacking in magnesium after an injection of a nutrient solution (Lipman and Gordon 1925, as cited in Roach 1939). The leaves of the injected trees exhibited a deeper green color after a few weeks, suggesting that the tree was lacking magnesium (Lipman and Gordon 1925, as cited in Roach 1939).

Sodium selenate and sodium selenite were tested as systemics applied to soil after USDA scientists found aphids dying on wheat growing in soil containing selenium (USDA 1960). The dose needed to be effect in the USDA experiments on flowers was very close to the maximum tolerance level in the plant so further research of soil applied sodium selenate and sodium selenite was stopped (USDA 1960). In the 1950s and 1960s organophosphates were very popular uses as systemics (USDA 1960). Phorate was being tested as a systemic injected into the root zone on apple trees for spider mite control (USDA 1960). Other organophosphates that were being tested for soil treatment and foliar applications in agriculture and on ornamentals included demeton, mevinphos, schradan, disulfoton, and phosphamidon (USDA 1960). Some of which were effective foliar sprays but the USDA (1960) could not conclude whether it was systemic activity or from direct contact.

In the 1970's Dutch elm disease was not effectively treated by common fungicide application methods so many individuals turned to trunk injection (many articles cited in Doccola and Wild 2012). Wilson (1978) said it best, "Recent enthusiasm for injection procedures in the treatment of Dutch elm disease has heightened interest in the tree injection and infusion in general. It has also made us aware of our lack of basic knowledge of what goes on inside the trees" (Wilson 1978). The lack of basic knowledge that he was referring to was distribution within the tree and how the wounding effected the tree (Wilson 1978). Sodium arsenite, ammonium sulfate, and copper sulfate were used in early eradication of Dutch elm disease (Wilson 1978). Application of these chemicals were done with "ax frills", funnels attached to the tree, and the "Cornell Tree-poisoning Tool" which was an injector (Cope 1931, as cited in Wilson 1978).

Arrival of insects like hemlock wooly adelgid, Asian long horned beetle, and emerald ash borer also increased interest in trunk injection which lead to improved injection technology and formulations (Doccola and Wild 2012). Agricultural pest such as sucking and chewing pests have increased interest in injection for us in agriculture, and lately for integration of integrated pest management (USDA 1960).

How Systemic Insecticides Work

Systemic chemicals are known for their ability to move within the vascular system of a plant (USDA 1960). Systemic pesticides can be absorbed through foliar and root applications or through injection to a region of the plant (USDA 1960). These chemicals also can be used in seed treatment providing protection starting at the earliest stages of

growth (USDA 1960). Depending on the application method, different pathways of the plant are utilized (Chaney 1978). The movement within the plant gives an advantage over non-systemic chemicals that are localized to regions where they were applied (USDA 1960).

The vascular system is made up of the xylem and phloem (USDA 1960; Chaney 1978; White 2012). Xylem and phloem are different from each other in terms of composition and function but may exchange transportation of solutes or water (White 2012). The xylem's role within the plant is to transport water and solutes up into the canopy (Chaney 1978). This is accomplished by root pressure and a negative water potential caused by open stomata on leaves, described as cohesion theory (White 2012; Chaney 1978). Root pressure theory suggests that water is pushed upward from the roots, but this is only seen in some species or during early spring, and is not likely to be the driving force behind the upward movement throughout the growing season (Kozlowski 1961; Chaney 1978). The structure of the xylem is made up of large non-living vessels that have pitted sidewalls and cross walls with openings or perforation for movement across the wall (Chaney 1978; White 2012). Depending on the arrangement and connectivity of the vessels, solutes and water can be delivered to different parts of the canopy (Chaney 1978), which in some cases results in poor distribution of systemics (USDA 1960). Xylem elements in trees have been found in a spiral arrangement and movement within the xylem generally follows this spiral trend up the tree (Kozlowski and Winget 1963). The direction of spiraling can be left or right; and there can also be changing in directions, irregular movement, and vertical movement (Kozlowski and Winget 1963). Majority of chemicals

introduced to the plant through injection or soil application utilize the xylem, moving into the expanding tissues (Chaney 1978; USDA 1960).

In the phloem, organic molecules are usually translocated, meaning this movement is not necessary up or down, rather bidirectional (Chaney 1978; White 2012). The driving force behind the direction of the movement in the phloem is the nutritional needs of the plant (White 2012). Nutrients from stem and leaf sources travel through the phloem and deposit into sinks such as shoot apices, fruits, and roots (White 2012). Phloem makes up a smaller part of the vascular system than the xylem, and the cells of the phloem do not function within the vascular system for much more than a growing season (Evert 1977, as cited in Chaney 1978). The phloem is made of living sieve tubes that have highly perforated cross walls, called sieve plates, and sieve areas on sidewalls (White 2012; Chaney 1978). Foliar application of systemic chemicals can utilize the phloem (Chaney 1978). Injection systemics do not use the phloem because of the positive pressure within the phloem and the quick response of sealing off the wounded area (Chaney 1978).

The cohesion theory, developed by Dixon and Jolly 1894, explains why there is generally an upward movement within the plant (Chaney 1978). The loss of water as vapor through transpiration from the stomata on leaves creates a pull, a decrease in pressure from roots to shoots, for the water and sap for movement from the roots to the leaves (Chaney 1978; Kramer and Boyer 1995). Kramer and Boyer (1995) describe this pull as an energy gradient that controls the ascent of sap and the initial absorption. Since the water is in continuity throughout the plant and water molecules are cohesive this movement is achieved (Chaney 1978). Different plant species can vary in anatomy of the xylem and phloem, along with the rate of transpiration which impact the movement of water and

introduced systemics (Chaney 1978; USDA 1960). Kramer (1937) stated that stomatal closure is a quick response to changes in the environment but the saturation deficit within the plant remains, causing absorption to continue until the deficit is fulfilled (Kramer 1937). This indicates that changes in rate of transpiration have shown a delayed response in absorption (Kramer 1937).

Systemics work well for controlling plant-sucking pests such as aphids, thrips, leafhoppers, spider mites, and few chewing insects (USDA 1960). This is because the insects feed on plant material containing the systemic which was delivered to the area of feeding by the vascular system (Chaney 1978; Campana 1978). Even at the expanding shoot tips systemic residues can be found (Vantimmeren et al. 2011, VanWoerkom et al. 2014, as cited in Wise 2016). Since some sucking insects are vectors for disease there is potential for disease control as well by controlling the insect (USDA 1960).

Foliar Application of Systemic Insecticides

Foliar systemics are applied by sprayers emitting airborne particles onto the surface of the plant (Doccola and Wild 2012). These particles are generally absorbed within a few hours after application (Chaney 1978). The first barrier to pass is the cuticle of the leaf which possess many challenges such as water repellency, permeability, and uneven surfaces (Chaney 1978). Uneven surfaces can be further explained as leaf hairs, trichomes, waxes and resins (Esau 1965 as cited in Chaney 1978). These uneven surfaces increase the contact angle and reduces absorption of water due to its high surface tension feature (Chaney 1978). Young leaves are mostly lacking these uneven surface characteristics and have a thinner cuticle which allows for quick absorption (Chaney 1978). Adjuvants added

to foliar systemics, such as wetting agents and surfactants, helps with penetration because it reduces surface tension, contact angle, and allows for the surface to remain wet for diffusion (Chaney 1978; Bukovac and Petracek 1993).

The process in which the active ingredient diffuses through the cuticle involves leaving the aqueous spray solution to join an aqueous apoplast and crossing the cuticle (which acts as a membrane) in between (Bukovac and Petracek 1993). Penetration of the chemical is thought to be greatest while the spray solution is wet on the leaf surface; spray volume, additives, and environmental factors impact the length of drying time (Wittwer and Teubner 1959; Sargent 1965 as cited by Bukovac and Petracek 1993). The cuticle can also be bypassed in areas around hairs on the leaf (Chaney 1978). After passing the cuticle of the leaf, the systemic either moves through the cell walls, intercellular spaces, or the protoplasm; and enters the phloem which allows transportation of the chemical into other parts of the plants (Kramer 1969 as cited in Chaney 1978). Since it is well understood that there is exchange between the phloem and the xylem, it can be expected that the systemic will be transferred to the xylem and utilize the upward movement (Chaney 1978).

A significant advantage of foliar application of systemics is that translocation movement can be achieved since the phloem is utilized for this application method and is not largely used in any other application method. Uniform spraying is still highly encouraged to ensure good coverage, and increasing the water gallonage can provide better control (Isaacs et al. 2020). The other advantage of foliar application is there is no wounding of the plant, which can create an entry for disease and organisms (Chaney 1978).

The major disadvantages to use of systemics by foliar application is the slow and limited process of being absorbed and moved to other areas of the plant (Chaney 1978).

There are also challenges of applying foliar sprays such as unfavorable weather conditions, UV degradation, accessibility of the foliage to uniform spray, and avoiding off-target drift (Doccola and Wild 2012; Wise 2016).

Soil Application of Systemic Insecticides

Soil applied systemics are absorbed by the roots, transferred into the vascular system, and move upward through the xylem (Doccola and Wild 2012). Though this seems straight forward, there are many barriers to be crossed to achieve upward movement (Chaney 1978).

Root absorption of water and solutes is not restricted to one area of the root (Kramer 1965, as cited in Chaney 1978). The area that exhibits the most rapid absorption is 5 to 10 cm after the tip of the root, known as the zone of rapid absorption (Kramer 1965, as cited in Chaney 1978). The region is before the area of developing suberization and behind the meristematic zone of the root, and usually has root hairs (Chaney 1978). The variation in length of the zone of rapid absorption is dependent on root extension rate (Chaney 1978). Fast growing roots tend to have larger zones of rapid absorption than slow growing roots (McQuilkin 1935, as cited in Chaney 1978). In the zone of rapid absorption, symplastic movement is utilized (Robards 1975, as cited in Chaney 1978). Initially, water and solutes are absorbed by the epidermal cells or root hairs in the zone of rapid absorption (Robards 1975, as cited in Chaney 1978). Then transported through the cortex and endodermis of the root and diffused from root cell to cell in the plasmodesmata, and finally reaching the vascular system (Robards 1975, as cited in Chaney 1978). The

casparian strip, which is a part of the endodermis, does not allow for apoplastic movement between cells in young roots (Esau 1965, as cited in Chaney 1978).

Absorption also occurs in regions of the old, suberized roots, especially seen in trees (Kramer 1969, as cited in Chaney 1978). Suberized regions of roots contain differentiated tissues such a bark cork, and vascular cambia; usually resembling the tissue layout in the plant's stems (Esau 1965, as cited in Chaney 1978). Movement in suberized roots is achieved by apoplastic pathways, regions outside of the cell's walls and in intercellular spaces (Chaney 1978). Since this movement does not directly involve the cells, no metabolic energy is required (Chaney 1978). The transfer to the xylem vessels is not well agreed upon and may not fit a generalized model (Bollard 1960, as cited in Chaney 1978).

Advantages to soil applied systemics are similar to other application methods of systemics. There is no spray drift that may fall upon beneficial insects or other plants (USDA 1960). Soil applications can be applied during windy conditions and in areas where foliar sprays may not be possible due to plant height or surrounding environment.

The disadvantage to soil applied systemics is that there are many beneficial organisms, such as earthworms, macro and micro invertebrates in the soil that may be affected by application into soil (Doccola and Wild 2012; Chagnon et al. 2014). There are also concerns of leaching and movement away from the desired treatment area, and in worst-case scenario towards areas of water (Doccola and Wild 2012). Another disadvantage of soil applied systemics is that they can be slow acting due to the time it takes to be absorbed by the roots, transferred to the vascular system, and then moved upward in the xylem (Doccola and Wild 2012). Some compounds may even bind to the organic matter and never reach the roots (Kramer and Boyer 1995). Also, soil applications

may require greater amounts of the chemical to achieve the same amount of insecticide applied by other methods, potentially causing repeated applications (Doccola and Wild 2012). These disadvantages were observed in a study that compared injected versus drench applied imidacloprid (Xu et al. 2008). Drench treated trees died from gall wasp infestation 15 weeks after treatment, whereas injected treated trees had high enough imidacloprid levels to control the gall wasp and survived (Xu et al. 2008).

Soil characteristics such as soil type and chemical properties are very important to consider when applying a systemic to a soil. It has been found that plants are inefficient in absorbing a systemic applied in sandy/coarse textured or soil containing high organic matter (USDA 1960; Doccola and Wild 2012). Liquids move through sandy soil and coarse textured soils the fastest when compared to other soil types, so it makes sense that roots may not absorb much of the applied systemic before it has leached away (Doccola and Wild 2012). With highly organic soils, the soil has greater ability to hold water and bind with the systemics, making them inaccessible to the roots (Kramer and Boyer 1995). Also, the age of the roots and root extension rate should be considered because absorption should be achieved in a timely matter due to potential of degradation of the systemic by microbes and the soil chemistry, along with binding of the soil and the systemic (Doccola and Wild 2012; Chaney 1978; Campana 1978). Additionally, the amount of precipitation after treatment should be considered because too much precipitation leads to leach (Doccola and Wild 2012). Finally, distribution of the systemic into the soil may affect the distribution into the canopy of plant (Chaney 1978).

Injection of Systemic Insecticides

Systemics can also be introduced to the vascular system by injection (Chaney 1978). Injection directly introduces the systemic to the xylem of the plant by creating a cavity into the trunk, stem, crown, or root of a plant and inserting the chemical (Chaney 1978; Shigo 1978). The phloem is not utilized because it is quick to become sealed off and under positive pressure (Crafts 1961, as cited in Chaney 1978).

Injections of systemics can be pressurized or infused (Chaney 1978). Arborjet Inc (Woburn, MA, USA) and BioForest Technologies Inc (Canada) have created several tools that make trunk injection more efficient and reduce injury (Doccola and Wild 2012). The Arborplug™ (Arborject Inc) is tapped into a drilled cavity and the TREE I.V. is used to injected with a needle into the rubber septum of the Arborplug™ (Doccola and Wild 2012). The Eco-jet Micro-injection System (BioForest Technologies Inc) uses reusable capsules that are inserted into a drilled cavity tree (Doccola and Wild 2012).

There are many advantages to injection of systemics including reduction of pesticide exposure to the environment and workers (Wise 2016). Since the injected chemical is not an airborne spray the potential for off target spray drift is eliminated (Wise 2016). This may also improve public opinion of agricultural practices, specifically use of air blast sprayers in close proximity to the general public (Wise 2016). Also, since the product is not applied to the soil there is little concern for leaching and or reaching off-target soil organisms. A good candidate for injection described by Doccola and Wild (2012) was riparian hemlock trees. The riparian environment contains organisms that may be sensitive to pesticide exposure, including water dwelling organisms (Doccola and Wild 2012). The riparian environment can be protected but the tree will still receive treatment for the pest

it contains. Another example is when foliar application is needed on canopy's of very tall trees, it can be difficult to limit drift and obtain a uniform application (Doccola and Wild 2012). Injection would allow for delivery up into the canopy without putting the environment or workers in danger.

In regard to the agricultural works, since less product is generally used for injection, agricultural workers making the application will come into contact with less pesticide. Workers collecting the fruit or preforming maintenance will not likely encounter any residues on the surface or in the soil of the plant as they would in other treatment methods.

Additionally, UV light degradation and rain wash off, or leaching from rainwater in the soil is not a concern for injected chemicals (Wise 2016). Instead, days of residue activity is lengthened by a couple months compared to a week(s) for a foliar spray of the same product (Wise 2016). A specific example Wise (2016) provides is the longevity of a botanical pesticide, e.g. azadirachtin, was found to have 5 to 7 days of residue activity from a foliar spray applications and 60 to 90 days from trunk injection application. Additionally, azadirachtin used in a study conducted by Wheeler and colleagues (2020) provide two years of control of pear psylla (*Cacopsylla pyricola*) from a single trunk injection and used 75% less product compared to airblast application. Biopesticides are generally more sensitive to environmental conditions and injection into the plant protects and prolongs the activity period (Wise 2016). Injection can generally be done in all weather conditions during the growing season, but there are more favorable conditions associated with leaf stomates open and transpiration occurring.

One significant disadvantage of injection that is unique to the injection process is the formation of a wound on the plant. However, wounding to trees is something that naturally

occurs, and trees has evolved ways to restore the area damaged (Doccola and Wild 2012). Some natural and unnatural wounding examples include insect borers, woodpeckers, and sap collection for maple syrup production (Doccola and Wild 2012).

The healing process begins with parenchyma tissue called callus developing at the base of the wound and enlarge in size (Neely 1978). Those cells divide and this process is repeated until the wound is filled (Neely 1978). The vascular cambium becomes differentiated after three weeks and in continuum with unwounded part of the trunk or stem (Neely 1978). The bark will remain destroyed in that area and will not heal (Neely 1978). Shigo (1978) and Neely (1978) recommend wounding the tree with the smallest, narrowest, shallowest, and roundest wounds possible because this reduces internal damage. Aćimović et al. (2016) found lenticular port formed by double edge blade healed the fastest compared to 9.5 mm and 4.4 mm drilled ports. Injections should be done lower on the trunk and away from previous wounding to cause less injury (Shigo 1978). Additionally, when injecting into roots wounding should not occur on the valleys of root flares because more injury is caused (Shigo 1978). The same author suggests not creating wounds during leaf expansion because this often results in cambial dieback around the wound, and avoiding annual injection wounds. Finally, promoting plant growth with application of water and fertilizer plus pruning and thinning with help support more rapid healing (Shigo 1978). Some trees may respond to wounds with more rigor within the same species (Shigo 1978). Wounds on apple trees from injection have found to “heal” in one to two growing seasons (Aćimović et al. 2014, as cited in Wise 2016).

Another disadvantage is the mobility of an injected systemic chemical can be erratically distributed (Campana 1978). Xu et al. (2008) found that imidacloprid was at

highest concentration nearest to the injection site for wiliwili trees. The larger the tree, the greater the difficulty in achieving an even distribution (Campana 1978).

Importance of Chemical Characteristics

Achieving movement within the plant-by-plant mechanisms is only half of the story for systemics. Richard J. Campana (1978) outlines the functional, non-functional, and practical chemical characteristics that make a chemical a good candidate for systemic applications in *Characteristics of Successful Systemic Chemicals* from *Proceedings of Symposium on Systemic Chemical Treatments in Tree Culture.*

Functional aspects of a systemic include effectiveness, solubility, mobility, stability, absorption, limited toxicity, compatibility, and residual duration; all of which are interconnected (Campana 1978). Effectiveness is measured in distribution of the chemical throughout the plant and the ability of the chemical to target the intended goal (Campana 1978). The goal could be to target a life stage of an organism/ pathogen, provide anti-metabolites, or provide growth regulators and promoters (Dimond 1963, as cited in Campana 1978; Hoffmann et al. 2008). Solubility is the ability of the product to be suspended in solution, without settling out and clogging the vascular system (Campana 1978). Solubility directly affects the mobility of the chemical within the plant and the effectiveness (Campana 1978). Neutral and acid systemic chemicals should be used because they do not interact with the negative charge of the xylem walls, and flow freely (Marsh 1977, as cited in Campana 1978). Particle size also can affect whether a systemic can move within the vascular system (Conversation with Anthony VanWoerkom). Mobility of a system chemical can vary with distance, speed, accumulated/arrival concentration,

which also depended on application method (Metcalf 1966, as cited in Campana 1978). Stability of the chemical within the plant is very important (Campana 1978). The chemical will react with sugars, enzymes, amino acids, proteins, lipids and others in the xylem sap stream (Campana 1978). To have good stability means the chemical designed to interact with the target organism or pathogen remains the same as it moves in the plant (Campana 1978). Absorption is dependent on solubility, mobility, and stability; and determines whether the systemic can pass the various barriers before entering the vascular system (Champana 1978). Limited toxicity to the plant is also very important consideration and aspect of a systemic chemical (Campana 1978). Since systemic chemicals are moving and interacting with tissues and cells of the plant, it is likely to cause some level of phytotoxicity, the key is minimal damage so the plant can recover (Campana 1978). This damage is visible in the form of phytotoxicity, damage to foliage. Internal damage to the xylem can be observed by discoloration in the xylem (Andersen et al. 1978, as cited in Campana 1978). When more than one systemic chemical is applied it is very important to consider the compatibility due to potential synergism, synthetic reactions, and/or deactivation of the chemicals involved (Metcalf 1966, as cited in Campana 1978). A synergism or reaction has the potential to be much more damaging within the plant than being applied through foliar application (Campana 1978). Residual duration can vary based on the ability of the chemical to fix to the surfaces of the vascular wall surfaces and later move upward in the plant, stability of the chemical is important here (Campana 1978). A chemical with a low fixity would move quickly within the plant (Campana 1978). Depending on the targeted organism, required levels of toxicity must be present for an extended amount of time, which can be achieved with a longer release of chemical fixity

(Campana 1978). The example Campana gives is the introduction of a systemic chemical in elm trees before beetle inoculation, and the chemical must persist at toxic level to the pathogen for at least three months (Campana 1978).

The non-functional and practical aspects of systemic chemicals outlined by Campana (1978) coincide with each other. Non-functional aspects are mammalian toxicity and stability in storage (Campana 1978). The practical aspects of systemic chemicals include safety to user, feasibility of application, cost of application, and impact of application (Campana 1978). First and foremost, mammalian toxicity is very important. Systemics and the application methods have many attributes that limit off target exposure to organisms, however user safety and public safety are important for registering the product and should allow for safe use (Campana 1978). Stability in storage is also important because it impacts the effectiveness of the product and costs of keeping the product up to date (Campana 1978). Since systemics can be acidic and designed to be soluble, poor storage stability could also lead to phytotoxicity to the plant (Campana 1978). The feasibility of application and cost of application is making some forms of systemic application more favorable than others as discussed before. All application methods of systemics have advantages and disadvantages when it comes to damage, equipment needed, application time, time it takes to move within the plant, and amount of product needed. Individuals looking to utilize systemics should consider the cost-benefit, their environment, the plant, and take into account how they weigh against other alternatives (Doccola and Wild 2012; Campana 1978).

Risk to Beneficial Insects and Resistance Management

Pesticide applications targeting pests also hold exposure risks to non-target arthropods, including beneficial organisms. Protecting pollinators has been of special interest since colony collapse disorder was named in 2006 (vanEngelsdorp et al. 2007, 2008, as cited in Lu et al. 2020). It is likely that neonicotinoids are not the only cause for this disorder, though many are researching the sub-lethal exposure of neonicotinoids to bees (Lu et al. 2020). In Lu et al.'s (2020) literature review, studies concluded the known sub-lethal exposure of neonicotinoids are changes in foraging and brood development, neurological effects, cognitive effects and as mentioned before colony collapse disorder. Sources of neonicotinoid exposure is more than just from a treated crop; it can be found in the pollen and nectar of plants absorbing the systemic neonicotinoids from treated soil (Lu et al. 2020). Bees may also be exposed by drinking contaminated water from leaf guttation or puddles on the ground (Lu et al. 2020).

Girolami and colleagues found corn to be grown from a seed treated with neonicotinoids to systemically transfer the neonicotinoid up the leaves and out in leaf guttation droplets (Girolami et al. 2009, as cited in Lu et al. 2020). These water sources may also be an exposure route for other beneficial organisms (Girolami et al. 2009, as cited in Lu et al. 2020; Hoffmann and Castle 2012). Hoffmann and Castle (2012) found guttation levels of imidacloprid in soil treated cantaloupe fields to exceed median oral toxicity levels of bumble bees (*Bombus impatiens, Bombus terrestris),* European honeybee (*Apis mellifera*), and beneficial predators (*Orius laevigatus, Podisus maculiventris*) up to a factor of 10 or more for some of these species. Hoffmann and Castle (2012) concluded that guttation from

plants treated with imidacloprid in guttation-prone environments are an important risk to consider for pollinators and beneficial insects and should be further studied.

Preliminary research was conducted on apple tree flowers for systemic insecticide residues from trees injected with one of the following insecticides – imidacloprid, emamectin benzoate, and rynaxypyr (VanWoerkom et al. 2014). Injections were administered at petal fall stage in 2010 and samples were collected in bloom of 2011. Imidacloprid was not detected in the flowers, and the amount of emamectin benzoate and rynaxypyr was below published acute toxicity (ememectin benzoate) and oral LD50 (rynaxypyr) levels (VanWoerkom et al. 2014). VanWoerkom and colleagues (2014) also conclude that since this was a residue analysis on the entire flower, it is likely that the nectar and pollen would have lower residue levels than detected in this study.

In a 2015-2016 study, apple trees were injected with either imidacloprid or emamectin benzoate in the spring or fall of 2015, residues were analyzed in fruit buds (fall) pollen and nectar (spring) (Colsor et al. 2019a). Samples for residue analysis was collected in Fall 2015 and Spring 2016 for both application timings (Colsor et al. 2019a). Emamectin benzoate was found in the nectar and pollen only in Spring 2016 from trees injected in Spring of 2015 (Colsor et al. 2019a). Colsor and colleagues (2019a) concluding that this may have resulted from a reservoir effect where levels of the chemical remain in the woody tissue and were redistributed in the following spring. Imidacloprid responded differently, with higher residue concentrations found in fruit buds in the fall of 2015 following a spring injection and very little amounts found in nectar and pollen from both fall and spring injections (Colsor et al. 2019a). Imidacloprid concentrations in leaves greatly exceeded all detectable residues in fruit buds, nectar, and pollen (Colsor et al. 2019a). Colsor and

colleagues (2019a) concluded that this is due to imidacloprid's inability to load into the phloem, where nutrients are supplied to the nectar and pollen. From this study, Colsor et al. (2019a) recommends using these seasonal treatment findings to protect pollinators from potential insecticides residues and their metabolites.

A study conducted on citrus trees tested whether phloem feeding insects, citrus mealybug (*Planococcus citri*) could expose beneficial insects feeding on the mealybug's honeydew to neonicotinoids applied by soil or a 50% labeled rate foliar applications to the citrus tree (Calvo-Agudoa et al. 2019). Of the mealybugs that survived to produce honeydew, the two neonicotinoids tested (imidacloprid and thiamethoxam) were detected in the honeydew and had some effect on the longevity and survival of the two beneficial insects, a hoverfly (*Sphaerophoria rueppellii*) and parasitic wasp species (*Anagyrus pseudococci*) (Calvo-Agudoa et al. 2019). Thiamethoxam applied by foliar and soil applications had the greatest impact most likely due to the known movement within the phloem, whereas imidacloprid primarily moves within the xylem (Calvo-Agudoa et al. 2019).

Regarding resistance management, Wise (2016) outlines two important characteristics to IPM resistance management - effectiveness and rotation of pesticides with different modes of action. When a pesticide is not effective against majority of the pest population, individuals surviving that treatment will pass their "surviving" genes onto the next generation. The more individuals that survive the treatment, the greater the risk of the that pesticide becoming ineffective against that pest. The rotation of pesticides having different modes of action can be done temporally or spatially (Wise 2016). The temporal strategy refers to the change in pesticide mode of action with the change of generations of

the insect pest during the growing season (Wise 2016). The spatial strategy is commonly used in field crops, where pesticides with different modes of action are applied to the sections or rows of a field (Wise 2016). This strategy can be applied to a field of injected treated plants creating a "mosaic of modes of action" (Wise 2016).

Evaluation of the plants and their environment, choosing the right application method and timing of a systemic can reduce the potential exposure to beneficial invertebrates, such as pollinators, and achieve integrated pest management goals. Doccola and Wild (2012) explained the cost-benefit logic behind determining whether a tree and the environment it is within is a good candidate for injection, but I feel that this can be opened to choosing which systemic application method is best for the plant and pest of interest.

Recent Studies of Systemics for Fruit Pests

In recent years, systemic injection studies for control of orchard insect pests have been conducted on apple (VanWoerkom et al. 2014; Wise 2016; Coslor et al. 2019a; Coslor et al. 2019b), pear (Wheeler et al. 2020), and avocado (Byrne et al. 2020). Soil applied systemics studies have been conducted in citrus (Byrne et al. 2017; Herrick et al. 2019), grape (Van Timmeren et al. 2012 Daane et al. 2020), and blueberry (Isaacs 2019b). Foliar systemic studies involving spirotetramat (Movento) have also been conducted on blueberry (Isaacs et al. 2020), grape (Daane et al. 2020) and many others. For any chemical to be successfully used in an agricultural setting the chemical or its metabolites need to be effective in controlling the pest in the targeted life stage, while meeting residue limits

within the crop (USDA 1960; Hoffmann et al. 2008). Many systemics have been discarded due to excessive persistence (USDA 1960).

In apple, several systemics have been popular in providing one or two, and possibly more years of direct and indirect apple pest feeding on the leaves of injected apple trees (VanWoerkom et al. 2014; Wise 2016; Coslor et al. 2019a; Coslor et al. 2019b). VanWoerkom et al. (2014) found the rate of movement and seasonal trends of the emamectin benzoate, rynaxypyr, and imidacloprid moving from injection sites into the foliage. Residues in leaf samples generally were highest following injection (VanWoerkom et al. 2014). Emamectin benzoate and rynaxypyr were detectable as soon as the same day of injection, and imidacloprid within a week (VanWoerkom et al. 2014). Residue concentration peaked in 14-60 DAT and dropped to a minimum concentration 60-90 DAT, with the expectation of emamectin benzoate reaching its minimum shortly after 30 DAT (VanWoerkom et al. 2014). Residues from trees injected with imidacloprid or emamectin benzoate a year prior showed a similar seasonal trend but at lower concentration levels (VanWoerkom et al. 2014). Rynaxypyr had an irregular residue pattern from trees injected one year prior, and VanWoerkom and colleagues concluded that this is due to possible binding and uneven release from woody tissue (VanWoerkom et al. 2014). All levels of insecticide residues in fruit were below the EPA maximum residue limits (VanWoerkom et al. 2014).

A study conducted in 2013 and 2014 showed similar single year seasonal trend in systemics injected where it peaked in concentration during the mid-season and then decreased; these systemics included imidacloprid, chlorantraniliprole, and dinotefuran (Coslor et al. 2019a). Emamectin benzoate and abamectin showed an increasing residue

level throughout the season (Coslor et al. 2019a). Spinosad was also tested but had very low detection and did not provide protection from the two insect pests (Coslor et al. 2019a). It was concluded that this formulation was a poor candidate for treatment of apple tree pest via trunk injection (Coslor et al. 2019a). Imidacloprid and dinotefuran provided control of potato leaf hopper (*Empoasca fabae*) in field evaluations; other insecticides tested provided control of obliquebanded leafroller (*Choristoneura rosaceana*) in bioassays using leaves of injected trees (Coslor et al. 2019a). All residues in fruit were below the EPA maximum residue limits (Coslor et al. 2019a).

In a study involving nursery apple trees comparing the root injection versus trunk injection at a 1/8 and 1/80 labeled rate, emamectin benzoate injected into the trunk at the high rate moved the fastest into the leaves (Coslor et al. 2019b). However, over time the high rate (1/8) provided similar pest control and residue persistence from both injection areas (Coslor et al. 2019b). It should be noted that Coslor et al. (2019b) also found residues to be poorly distributed into the canopy with root injection, the greater concentrations were found in the roots (1/8 rate) and (1/80 rate) stems (Coslor et al. 2019b). Coslor et al. (2019b) recommends the consideration of the higher rate for trunk injection to provide multiyear control.

In pear, biopesticides abamectin and azadirachtin were applied by foliar spray and injection for control of an insect pest that feeds on the leaves, pear psylla (Wheeler et al. 2020). Injection of these biopesticides provide two years of control (Wheeler et al. 2020). Within the first year, the injected biopesticides preformed as well or better than the foliar application of these products (Wheeler et al. 2020). It was estimated that 75% less product

was used in the injection for two years of control than application by foliar (Wheeler et al. 2020).

In avocado, emamectin benzoate was injected into the trunk to control ambrosia beetles which created galleries with fungi that caused harm to the tree (Byrne et al. 2020). The uptake of emamectin benzoate was sped up by diluting the formulation (Byrne et al. 2020). This diluted formulation always reached the threshold concentration, determined by bioassays, and would provide control of the ambrosia beetles (Byrne et al. 2020). Residues in the trunk persisted at this level for nine months (Byrne et al. 2020).

In grape, Van Timmeren et al. (2012) tested the efficacy of soil applied neonicotinoids (imidacloprid, clothianidin, thiamethoxam, dinotefuran) in grapes for control of early and mid-season insect pests. Results from Van Timmeren et al. (2012) study indicated that neonicotinoids applied through a drip irrigation line and root targeted injection, versus soil banded application, provided control of several insect pests including Japanese beetle (*Popillia japonica*), potato leaf hopper, and grape berry moth (*Eupoecilia ambiguella*) which is a pest of the fruit. Van Timmeren et al. (2012) concluded that this application method has the potential to provide control of grape pest while reducing exposure to workers and the environment, along with increasing efficiency and lowering costs.

Daane et al. (2020), compared foliar applied flupyradifurone (Sivanto), systemic spirotetramat (Movento), and flupyradifurone plus spirotetramat, along with flupyradifurone and imidacloprid (Admire) applied via a drip line for control of mealybug. Differences in insecticides applied were noticeable after harvest, with foliar applied spirotetramat and flupyradifurone plus spirotetramat providing the best control (Daane et

al. 2020). Vines treated with a drip line of admire were no different than the control (Daane et al. 2020).

As stated above in the blueberry stem gall wasp portion, systemics have been tested for control of this gall wasp in blueberry (Fanning and Isaacs 2019b; Isaacs et al. 2020). Common application types are soil applied, and foliar spray of spirotetramat (Movento) (Fanning and Isaacs 2019b; Isaacs et al. 2020).

In citrus, systemics are commonly used for mealybug control (Herrick et al. 2019; Byrne et al. 2017; Calvo-Agudoa et al. 2019). Although there are conflicting results on whether soil applied systemics can successfully be used to control mealybugs in potted citrus, imidacloprid remains to be of popular use (Herrick et al. 2019; Byrne et al. 2017). Thiamethoxam, appears to be a potential effective systemic for mealybugs, in some studies better than imidacloprid (Herrick et al. 2019; Byrne et al. 2017; Calvo-Agudoa et al. 2019).

CHAPTER 2: A SYSTEMIC APPROACH TO CONTROL *HEMADAS NUBILIPENNIS* IN BLUEBERRY COMPARING SOIL DRENCH, CROWN INJECTION, AND FOLIAR APPLIED INSECTICIDES

ABSTRACT

Blueberry stem gall wasp (*Hemadas nubilipennis*) is a pest of highbush blueberry and can pose a challenge to control with foliar sprays due to adult activity during bloom and larval development within the plant tissues. This study tests the efficacy three application methods, soil drench, crown injection and foliar spray replicated over two years (2020, 2021) with the following insecticides: imidacloprid, flupyradifurone, and spirotetramat. We hypothesized that if the insecticide can move systemically within the blueberry vascular system to areas where the blueberry stem gall wasp larvae are developing, then the insecticide will kill the larvae leading to a reduction in gall formation and number of surviving adults per gall. The following insecticides moved successfully from site of entry, roots, crown cavity or foliage, to expanding shoot tissues on potted Jersey blueberry bushes: imidacloprid (crown injection and soil drench), flupyradifurone (crown injection and soil drench), and spirotetramat (foliar spray). Residues of these insecticides were detected at 14DAT and 59/60DAT, which represents the course of time where the gall wasp larva are developing. However, this study found no evidence that the tested insecticides used with these three systemic methods inhibited gall or gall wasp development due to the age of the bushes tested. Future research should evaluate whether these systemics and methods inhibit blueberry stem gall wasp and gall development by evaluating emergence from treated bushes that are older and more vigorous.

INTRODUCTION

The blueberry stem gall wasp, *Hemadas nubilipennis* Ashmead (Hymenoptera: Pteromalidae) is a chalcid wasp measuring two to three millimeters in length (Isaacs et al. 2020) and is native to eastern North America (West and Shorthouse 1989). This gall wasp is known for its ability to form stem galls on expanding shoots of lowbush and highbush blueberry plants (West and Shorthouse 1989). The native host for blueberry stem gall wasp is the lowbush blueberry, *Vaccinium angustifolium* (West and Shorthouse 1989) but it also infests susceptible cultivars of highbush blueberry (Isaacs and Van Timmeren 2016). The most susceptible commercial highbush blueberry varieties are Jersey, Northland, Pemberton, and Bluejay (Isaacs et al. 2020). Jersey was reported to be the most widely planted variety in Michigan at 27% of total blueberry acres in the latest USDA Michigan Fruit Inventory (USDA 2020 as cited in Garcia-Salazar et al. 2020). Isaacs and colleagues (2020) report that low population levels of the gall wasp are a manageable nuisance to growers because bushes with few numbers of galls can be pruned off. However, when the population level is high, pruning costs out weight the benefit of managing the field (Isaacs et al. 2020). This pest has caused many commercial blueberry growers to remove susceptible blueberry varieties from their farms (Isaacs et al. 2020).

Blueberry stem gall wasp adults are only active for a short period of time when blueberry bushes are growing rapidly (Hayman et al. 2003a; Isaacs et al. 2020). This timing corresponds with bloom when most insecticide applications are restricted to protect pollinators (Hayman et al. 2003a). When blueberry bushes are no longer blooming, blueberry stem gall wasps are inaccessible by most foliar applied insecticides because eggs and larvae are developing within the plant in structures called galls. These two issues, adult

activity during bloom and larvae development within the plant, make the blueberry stem gall wasp challenging to control.

The blueberry stem gall wasp life cycle begins with adults emerging from galls formed in the previous year (Hayman et al. 2003a; Isaacs et al. 2020). Females lay eggs into expanding shoots of blueberry bushes and injure the apex of shoots that received oviposition (Hayman et al. 200a3; Shorthouse et al. 1986). Each egg is deposited into a separate "channel" created by the ovipositor (Shorthouse et al. 1986), primarily in the pith of the shoot but may be in contact with vascular tissue and cortex near one end of the egg (West and Shorthouse 1989). Within 48 hours of oviposition the cells around each egg chamber in the shoot rapidly divide forming a gall which provides protection for the larvae that hatch in 10-14 days (Shorthouse et al. 1986; Hayman et al. 2003a). Twenty days after oviposition the gall is noticeably swelling, and nutritive cells are beginning to from in the cell lining of the chamber which the larvae are actively feeding upon (McAlister and Anderson 1932; Shorthouse 1986; Shorthouse et al. 1986). The nutritive cells are beneficial to the larvae because they contain higher levels of starch, sugars, lipids, and proteins allocated from the blueberry bush via vascular bundles (Dreger-Jauffret and Shorthouse 1992).

After 60-90 days the cells in the gall become woody and the overall shape of the gall is globular or reniform shape (Hayman et al. 2003a). Around the time the larvae stop feeding the chambers become "encapsulated by the hard sclerenchyma sheath" (Shorthouse 1986). They remain inactive until spring when they pupate and emerge as adults during another growing season (McAlister and Anderson 1932; Shorthouse et al. 1986; Hayman et al. 2003a).

The formation of the gall impacts blueberry production because it reduces the fruiting area and potentially contaminates fruit pack-out during harvest (McAlister and Anderson 1932; Hayman et al. 2003a). When the gall is located terminally, the shoot is found to be shorter with less weight in stems and leaves (Hayman et al. 2003). Not only does a terminal gall "stunt" the shoots growth, but it also redirects the plant's energy and resources from fruiting and foliar expansion to benefit the insect (Rohfritsch and Shorthouse 1982; Shorthouse et al. 2005). Berry production was reduced 3% in a Novia Scotia study; however, this rate may become significantly greater as years go on (Hayman et al. 2003a). The other problem that blueberry growers face is that galls can contaminate harvested berries (Hayman et al. 2003a). Galls can be removed by the blueberry harvester machine, mistaken as fruit when similar in size during processing, and later be found in frozen and fresh berries (Hayman et al. 2003).

Blueberry stem gall wasp has become an important pest to Michigan blueberry growers because of two changes in blueberry pest management (Isaacs et al. 2020). First, with the arrival of spotted winged drosophila (*Drosophila suzukii*, Diptera: Drosophilidae), a major invasive fly pest attacking the fruit, many late season insecticide sprays are applied to keep fly larvae out of the fruit. (Isaacs et al. 2020). These late summer insecticide applications occur when co-inhabitants (inquiline species) of the blueberry stem gall wasp's gall are active, and these insecticides may kill these natural enemies (Isaacs et al. 2020). Another change in blueberry pest management is the cancelation of an insecticide: azinphosmethyl which was used for treatments for fruitworms in blueberries and applied during the time adult blueberry stem gall wasp are active (Isaacs et al. 2020). There is

evidence that this product may have provided subsequent control of blueberry stem gall wasp (Isaacs et al. 2020).

Efficacy of azinphosmethyl was assessed on Jersey blueberry bushes with confirmed blueberry stem gall wasp oviposition activity (Fanning and Isaacs 2019a). Azinphosmethyl was effective in reducing the total number of galls per bush with approximately 90% reduction in the total number of galls when compared to the untreated control bushes (Fanning and Isaacs 2019a). This study also tested other foliar applied insecticides: the second most effective product was cyclaniliprole with an approximate 64% reduction in the total number of galls per bush (Fanning and Isaacs 2019a). In addition, adult emergence from cyclaniliprole treated bushes resulted in a 95% reduction compared to untreated bushes (Isaacs and Wise 2020).

Soil applications of systemic insecticides: dinotefuran, flupyradifurone, thiamethoxam, imidacloprid no impact on reducing gall formation in Fanning and Isaacs (2019b) study. However, insecticides in this study were not applied at the ideal timing directly following bloom. A foliar application of the systemic spirotetramat reduced the number of galls (28 ± 2.85 mean ± SEM) when compared to untreated plots (43 ± 4.16 mean ± SEM). Larvae were 99% controlled in galls collected from bushes treated with a foliar application of spirotetramat when compared to untreated control bushes (Isaacs et al. 2020). In addition, the following year's blueberry stem gall wasp population was reduced, however, galls were still observed on the bushes.

Systemics insecticides, like spirotetramat, move in the vascular system of plants to reach the juvenile pest stage within the plant tissues (Chaney 1978). Spirotetramat is applied by foliar sprays and utilizes the phloem to move to other parts of the plant both

acropetally and basipetally (Chaney 1978; Nauen et al. 2008). Systemics can also be applied through drip irrigation which are absorbed by the roots, transferred into the vascular system, and move upward through the xylem into expanding tissues (Doccola and Wild 2012; Chaney 1978). Another form of systemic application that is new to blueberry plants is crown injection. This modified version of trunk injection directly introduces the insecticide to the xylem of the plant by creating a cavity into crown of the blueberry bush and injecting insecticides (Chaney 1978). Again, the systemic compound is moved through the xylem to expanding tissues. The overall advantages of these three application methods of systemics is the protection from sources of degradation, like sun and wash-off, and longer residual exposure to insect pests (Wise 2016).

Systemic delivery of insecticides is an ideal approach to controlling blueberry stem gall wasps which spend most of their life feeding within plant tissues. In this study we asked the following questions: (1) can systemic insecticides introduced by crown injection, soil drench, or foliar spray be detected in plant tissues where the gall wasp is active over the course of their larval development and, (2) can systemic insecticides reduce number of galls formed and reduce survivorship of the blueberry stem gall wasp?

We hypothesized that if the insecticide can move systemically within the blueberry bush to areas where the blueberry stem gall wasp larvae are developing, then the insecticide will kill the larvae leading to a reduction in gall formation and number of surviving adults per gall. To test the efficacy of systemic insecticides in blueberries for control of blueberry stem gall wasp, systemic insecticides were applied to potted blueberry bushes by one of the three application methods (crown injection, soil drench, foliar spray). We determined if the systemic insecticides moved to the site of blueberry stem gall wasp

activity by collecting shoots and leaves during the growing season for insecticide residue analysis. We monitored and quantified gall development during and after the growing season, after which galls were collected, and gall wasps were reared to determine survivorship post-growing season. This study was replicated twice over two years.

MATERIALS & METHODS

Source of Potted Blueberry Bushes

Two-year-old potted blueberry bushes (Jersey variety) were obtained from DeGrandchamps Farms Nursery (South Haven, MI) and delivered to Michigan State University's Trevor Nichols Research Center (TNRC) (Fennville, MI; 42.5951°N, 86.1561°W) on March 18, 2020 and March 22, 2021. Bushes were kept outside just as they were at the nursey. Each bush was in a round pot measuring 16.51 cm tall and 15.24 cm in diameter.

Source of Galls and Insects

Since blueberry stem gall wasp is native to Western Michigan (West and Shorthouse 1989), the source of blueberry stem gall wasp used to infest bushes for this study was obtained from commercial farms in Holland, MI, U.S. Blueberry stem gall wasp were immature and dormant in galls pruned off highbush blueberry bushes and stored in cold storage (3.33°C) at TNRC until further processing for infesting potted blueberry bushes.

Infestation of Blueberry Bushes with Blueberry Stem Gall Wasp

In preparation for infestation of the potted blueberry bushes, galls kept in cold storage were sorted. Galls with little to no bird/rodent damage, no emergence holes, and greater than 15mm in length were kept for rearing. In 2020, two containers sealed with fine mesh with approximately 5.56 kg of galls were placed in an environmental growth chamber (16L:8D photoperiod, 27 °C) 5 days apart, on May 13 and May 18, 2020. This corresponded with early pink bud stage on the potted Jersey blueberry plants. For the 2021 study, the same methods were used with two batches of approximately 2.72 kg of galls on May 14 and May 19, 2021.

On May 27, 2020 and May 24, 2021, two sets of 40 bushes were placed approximately a 15.24 cm a part in rows of eight in a mowed grass field at TNRC. Bamboo stakes were sunk into the ground next to each pot and zip ties were used to secure the pot to the bamboo stakes. Each bush received equal amounts of galls from the first batch placed in the environmental chamber (May 13, 2020 for 2020 study; May 14, 2021 for 2021 study). The galls were placed in plastic wine bottle sleeves sealed with zip ties on both ends prior to being attached to the bush. Each mesh sleeve with galls was attached to the middle of the canopy of each bush. A 3.35 m by 3.35 m screened tent (Coleman, Chicago, IL) was placed over each of the two sets of 40 bushes with galls. The bottom of the tent was sealed off by shoveling dirt on the fabric that extended past the ground level. The second batch of galls were added to each bush in the same manner as before, no later than 4 days after the first (June 1, 2020 and May 26, 2021). In 2021, due to early emergence of the wasp, galls were added to the soil surface in each pot on May 24, 2021.

After a majority of the gall wasps had died, the screened tents were removed (June 5, 2020; May 30, 2021). The bushes were sorted and 60 of the healthy bushes with obvious infestation (Figure 2.1a) were selected to be used in this study. The spacing was increased to about 76.2 cm apart and a sprinkler was setup between the two plots to maintain an adequate watering cycle for plant health.

Insecticide Treatment

Gall wasp infested blueberry bushes received an insecticide treatment approximately two weeks following removal of infestation tents (June 15, 2020; June 8, 2021). There were three application methods with varying chemicals and concentrations. The three application methods included injection into the crown, soil drench, and foliar spray. Prior to treatment bushes were randomized and assigned treatments using a complete random block design. There were four replicates per treatment. Amount of chemical applied to each bush was determined by calculating the maximum singular foliar rate per bush for flupyradifurone (Sivanto Prime 200 SL, Bayer CropScience, St. Louis, MO; 0.285ml/bush or 0.057g AI/bush), spirotetramat (Movento SC, Bayer CropScience LP, St. Louis, MO; 0.204 ml/bush or 0.049 g AI/bush) and using the chemigation maximum rate per bush for imidacloprid (Admire Pro SC Flowable, Bayer CropScience LP, Research Triangle Park, NC; 0.285 ml/bush or 0.157 g AI/bush) (Table 2.1). All three insecticides were also applied at a 50% rate (Table 2.1). Untreated control bushes received the same application method as the insecticide treated bushes, but only received distilled water.

Table 2.1. A summary of the insecticides used to treat potted blueberry bushes infested with blueberry gall wasps at an experiment station in Michigan in 2020 and 2021.

Treatment	Form. /bush	AI/bush	Application Type
Untreated Control	-	-	I,F,S
imidacloprid 50%	0.143 ml	0.078 g	I,S
imidacloprid 100%	0.285 ml	0.157 g	I,S
flupyradifurone 50%	0.143 ml	0.029 g	I,S
flupyradifurone 100%	0.285 ml	0.057 g	I,S
spirotetramat 50%	0.102 ml	0.024 g	I,F
spirotetramat 100%	0.204 ml	0.049 g	I,F

Rates (Form./bush) and active ingredient per bush (AI/bush). Application type indicates which insecticides were used for crown injection (I), foliar application (F), and soil drench (S).

Trunk injection methods on nursey apple trees (Coslor et al. 2019b) were adapted for this study by injecting the crown of blueberry bushes in the lab at TNRC. As the crown of the blueberry bush was exposed, a spraying bottle with water was used to clean dirt from the desired drilling surface which was located on the crown of the blueberry bush, within 2.54 cm deep of the soil surface. A power drill was used to create a 6.35 mm cavity, or no deeper than half the diameter of the crown, with a 4.78 mm size drill bit. Treatment solutions totaling 0.5 ml was pipetted into the drilled hole until all the solution was infused into the plant tissue. The injection hole was capped with nylon plug (Widgetco, Houston, Texas), the length of the nylon plug was reduced by half to ensure it fit the cavity. The bush was placed back in the pot and wrapped with 2.54 cm wide Parafilm grafting tape to protect the cavity from infection.

The same amount of imidacloprid and flupyradifurone was used for the soil drench application by adding the insecticides into 75 ml distilled water. The solution was well agitated in a 473 ml deli cup to ensure equal distribution. The treatment solution was applied by pouring evenly into the soil surface of the pot, twice around the bush.

Foliar applications of spirotetramat at two rates were made with a Zep professional spray bottle (Zep Inc, Atlanta, GA). All treatments, including untreated control contained a nonionic surfactant (0.250% v/v, R-11, Wilbur-Ellis, Aurora, CO). Both rates of spirotetramat were added to 50 ml of distilled water and applied to the bush until the leaves were well covered.

In 2020 two and a half hours after treatment administration, bushes that received crown injection and soil drench were lightly watered with a sprinkler, and foliar treatments were watered directly into the pot to dampen soil due to hot temperatures. In 2020 and 2021 approximately eight hours after treatment the bushes were placed back on the regular watering schedule with the sprinkler.

General Maintenance of Potted Bushes

Bushes were watered using a timed sprinkler. Depending on temperatures and rainfall amounts, bushes received 45 – 60 min watering, every 6 or 12 hour during the growing season. Bushes were maintained with a foliar application of Sevin, N-methyl carbamate, (Bayer, Cary, NC; rate: 11.7 ml/L), to protect against Japanese beetles (*Popillia japonica*; Coleoptera: Scarabaeidae). Applications were made in 2020 on July 2, 16, 31, and in 2021 on July 8. A handheld pump spray was used to make these applications.

Insect and Gall Assessments

Fourteen days after treatment (June 29, 2020; June 22, 2021) shoots containing swelling galls were quantified. Small swelling was approximately 5mm or less in length, medium swelling was >5 mm (Figure 2.1b). Number of failed galls was also assessed during

the time that galls were swelling (July 1, 2020; June 22, 2021). Failed galls had no swelling, and the shoot was split open.

At the end of the expansion period of the galls (August 14, 2020; August 24, 2021), each bush was assessed for number of small galls and medium galls. Small galls were less than 15 mm. Medium galls were greater or equal to 15 mm (Figure 2.1c).

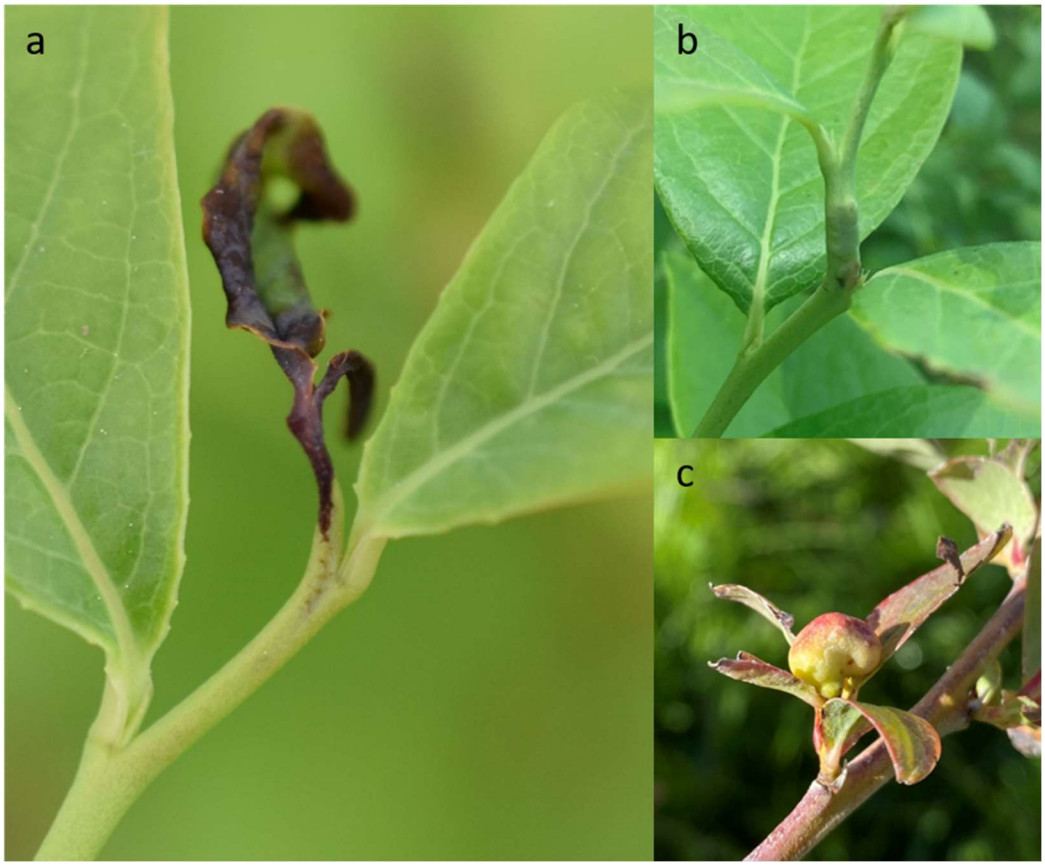

Figure 2.1. Phases of gall development from blueberry stem gall wasp infestation. Oviposition incisions leading up to a dying shoot tip and leaves (a). Swelling gall on young shoot (b). Developed medium gall at shoot apex (c).

A final gall count was conducted post-growing season and galls were removed and stored in cold storage at TNRC (4.4°C). Total gall mass per bush was recorded and galls were placed in perforated souffle cups (118.3 ml) in an environmental chamber (16L:8D photoperiod, 27 °C) for rearing (January 21, 2021).

Collection for Residue Assessment

Residue samples were collected twice corresponding with the swelling gall assessment and the completion of the gall development. The first collection was 14DAT (day after treatment) on June 29 in 2020 and June 22 in 2021. The second collection was 60DAT on August 14, 2020 and 59DAT on August 6, 2021. Thirty undamaged, fully developed leaves were collected per bush. Fifteen shoots with the leaves removed and without any sign of potential gall formation were collected in 2020, ten shoots in 2021. Shoots collected on the 14DAT were specifically chosen to be the same age as the shoots growing when the gall wasp infestation occurred. The shoots collected on 59DAT/60DAT were shoots expanding anytime during the growing season. Samples were stored in sealed, 120 ml graduated glass bottles (Qorpak, Clinton, PA) with 50 ml dichloromethane, 4 grams of magnesium sulfate, and 1 gram of sodium chloride in a walk-in cold room (4 °C) for future preparation for residue analysis.

Residue Sample Preparation and Analysis

The residue sample preparation and analysis methods for this study were adapted from the QuEChERs method (Lehotay, 2011) and used in several other injection studies including VanWoerkom et al. (2014) and Wheeler et al. (2020). All samples were held in cold storage for approximately two months. One day prior to decanting all residue samples, shoot samples were sonicated for five minutes. Samples were decanted by pouring the sample through approximately 12 g of sodium sulfate in 125mm diameter filter paper that was folded into a funnel. Each funnel of sodium sulfate was placed on top of a 120 ml glass bottle that corresponded with the sample being poured. Each solvent was evaporated for

up to 2 days. This left a residue film on the inside of the jar that was dissolved by adding 2 ml of acetonitrile to each jar and swirling for 90 seconds. The sample was filtered with a 0.45 µm PTFE hydrophobic filter attached to a 3 ml disposable syringe and transferred to a 2 ml glass vial. All samples were store in a freezer until processed with analytical equipment. All samples were analyzed using ultra performance liquid chromatography (UPLC) with the limit of detection (LOD) and limit of qualification (LOQ) stated in Table 2.2 for 2020 study and Table 2.3 for 2021. Recovery of insecticide quantified using linear regression and reported as µg of active ingredient per gram of shoot tissue. Standards were obtained from the EPA Standard Repository. The software used was Waters Mass Lynx version 4.2.

Table 2.2. Limit of detection (LOD) and limit of qualification (LOQ) for insecticides used in the 2020 study.

Compound	M+H (m/z)	Qualifier (m/z)	LOD (µg/g)	LOQ (µg/g)
imidacloprid	209	175	0.0025	0.0075
flupyradifurone	125.9	90	0.002	0.006
spirotetramat	216.2	117.1	0.019	0.057

Table 2.3. Limit of detection (LOD) and limit of qualification (LOQ) for insecticides used in the 2021 study.

Compound	M+H (m/z)	Qualifier (m/z)	LOD (µg/g)	LOQ (µg/g)
imidacloprid	209	175	0.001	0.001
flupyradifurone	125.9	90	0.001	0.003
spirotetramat	216.2	117.1	0.0267	0.08

Statistical Analysis of Residue Recovery

Due to incomplete factorial design, a new variable that is a combination of chemical, rate, and method was used for statistical analysis (hereafter referred to "treatment"). The statistical model for the analysis of concentration (µg/g) consisted of treatment, time, and their interaction. The replication and the interaction between treatment and replication

were included as random factors and the latter was used as the error term for time. The normality assumption was tested by examining normal probability plots. Due to the violation of the normality, the dataset was square-root transformed for the analysis. Since residue was recovered generally below the value of 1 in 2021 leaves, a log(X +1) transformation was used. Reported figures and tables show means and standard error of the means. Equal variance assumption was tested by performing Levene's test based on absolute residuals. The optimal model for unequal variances was selected based on AIC and BIC values. When the ANOVA results were significant, a mean comparison using Fisher's LSD was used to present the difference between the two treatments or sample type. 2020 sample of imidacloprid 50% injection shoots at 14DAT was dropped in all active ingredient recovery analysis due to chemical extraction error.

For comparisons of concentration recovered in plant tissue type, each insecticide model consisted of tissue type, treatment, and the interaction of the two within a sampling date. Since this analysis is a split block design, the random variable replicate was within the fixed variable treatment. When the ANOVA results were significant, a mean comparison using Tukey's HSD was used to present the difference between the two treatments or the interaction of treatment and tissue type.

The statistical model for gall categories consisted of treatment, and the random factor was replication. A negative binomial distribution was used for swelling and final gall categories with Laplace method. Reported figures and tables show means and standard error of the means. Mean of untreated control included each method type with replication. When the ANOVA results were significant, a mean comparison using Tukey's HSD was used

to present the difference between the two treatments. All analyses were performed using PROC MIXED procedure in SAS (SAS 9.4, SAS Institute Inc., NC USA).

RESULTS

Residue Detection in Shoot Tissue

The interaction of treatment and time was found to be significant using a two-way ANOVA for concentration of active ingredient recovered in shoot tissue in the 2020 study ($F_{11, 22.2} = 18.41$; $P<0.0001$). In 2020, imidacloprid was recovered in shoots administered at 50% and 100% rates by crown injection at 14 DAT and 60 DAT (Figure 2.2). Replicate 1 was dropped from imidacloprid injection 50% rate at 14 DAT due to issue with residue extraction. There is no significant difference between the two collection dates within 100% rate (14 DAT: 8.06 ppm; 60 DAT: 3.28 ppm), however the mean concentration recovered in shoots from bushes treated with the 50% rate sampled on the 14 DAT significantly greater than in the 60 DAT sample (Fisher's LSD, $P<0.005$) (Figure 2.2). There is no significant difference when comparing the two rates (50% and 100%) within a collection date for imidacloprid recovered in shoots treated with injection method, however, 100% rate resulted numerically greater concentration recovered for both collection timings (Figure 2.2).

In 2020, imidacloprid was recovered from shoots administered by soil drench in shoots collected 60 DAT that received 50% rate, and both 14 DAT and 60 DAT for 100% rate (Figure 2.2). Though there is no significant difference between the collection date within a rate, however the mean concentration recovered in shoots sampled on the 14DAT

from bushes receiving 100% rate (3.93 ppm) are numerically greater than the 60 DAT (2.60 ppm) (Figure 2.2). The 100% soil drench applied imidacloprid resulted in higher mean active ingredient recovered at 14 DAT than 50% soil drench because there was no active ingredient recovered (Fisher's LSD, P=0.0052). There was no significant difference between the two rates for mean active ingredient recovered 60 DAT, however the 100% rate was numerically greater by five times (Fisher's LSD, P=0.0647) (Figure 2.2). When comparing the two methods used to administer imidacloprid in 2020, imidacloprid was recovered in greater concentrations by injection method when compared to soil drench (Figure 2.2). Injection was significantly greater at the 50% rate at 14 DAT (Fisher's LSD, P<0.0001), and greater at the 100% rate (Fisher's LSD, P=0.0976).

In 2020, flupyradifurone was recovered in shoots administered 50% and 100% rates by crown injection at 14 DAT and 60 DAT (Figure 2.2). Mean active ingredient recovered from crown injected bushes at the 50% rate was significantly greater at 14 DAT (0.95 ppm) than at 60DAT (0.01 ppm) (Fisher's LSD, P<0.05). There was no significant difference between the collection date in the 100% rate. Mean recovery of flupyradifurone in shoots was significantly greater at the 100% rate for both 14 DAT (7.77 ppm) and 60 DAT (5.82 ppm) when compared to mean active ingredient recovered from the 50% rate (Fisher's LSD, P<0.05).

In 2020, flupyradifurone was recovered from shoots administered by soil drench in 14DAT that received 50% rate, and both 14 DAT and 60 DAT for 100% rate (Figure 2.2). Though there was no significant difference between DAT shoot collection, the mean concentration recovered in shoots sampled on the 14 DAT (50%: 0.04 ppm; 100%: 1.71 ppm) are numerically higher than the 60 DAT (50%: 0 ppm; 100%: 0.82 ppm) (Figure 2.2).

There was no significant difference between the two rates for mean active ingredient recovered for both collection date, however the 100% rate was numerically greater (Figure 2.2). When comparing the two methods used to administer flupyradifurone in 2020, flupyradifurone was recovered in greater mean concentrations by injection method (Figure 2.2). Injection was significantly higher at the 50% rate at 14 DAT (Fisher's LSD, $P<0.05$), and marginally significantly higher at 100% rate at 14 DAT (Fisher's LSD, $P=0.0668$).

In 2020, spirotetramat was recovered in shoots administered 50% and 100% rates by crown injection at 14 DAT, which is significantly greater than no recovery at 60 DAT (Fisher's LSD, $P<0.05$). Mean recovery of spirotetramat at 14 DAT was significantly greater for the 100% (1.05 ppm) rate compared to the 50% rate (0.29 ppm) (Fisher's LSD, $P<0.05$).

In 2020, spirotetramat was recovered from shoots administered by foliar spray in shoots that received 50% rate at 14 DAT and 60 DAT (Figure 2.2). There was greater mean concentration of spirotetramat recovered at 2.28 ppm for the 50% at 60 DAT than 0.09 ppm for the 50% at 14 DAT (Fisher's LSD, $P=0.0002$). Since there was no detection of spirotetramat from the 100% rate for foliar application, the 50% was significantly greater for 60 DAT (Fisher's LSD, $P<0.001$). There was no significant difference between the two rates for mean active ingredient recovered 14 DAT, however the 50% rate was numerically higher than the no detection of the 100% (Figure 2.2). When comparing the two methods used to administer spirotetramat in 2020, spirotetramat was recovered in a greater mean by injection method at the 50% rate at 14 DAT, 0.29 ppm (Fisher's LSD, $P<0.05$). There was only recovery in the injected spirotetramat 100% rate at 14 DAT which was significantly greater than the no recovery by foliar application (Fisher's LSD, $P=0.0002$). Spirotetramat

was only recovered for 60 DAT in the foliar applied method at 50%, which makes it significantly greater than the no recovery in the injection method (Fisher's LSD, P<0.0001).

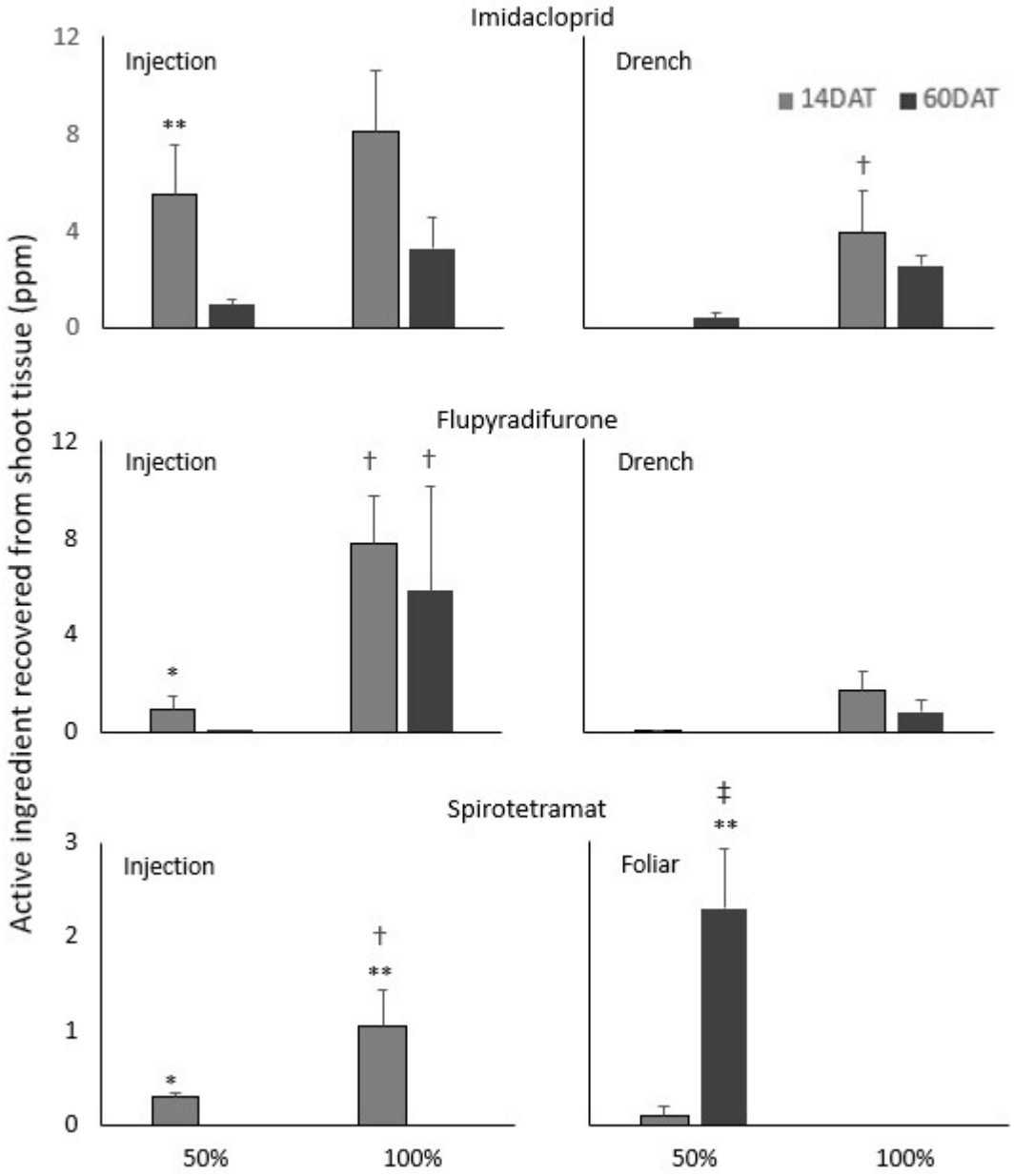

Figure 2.2. Mean active ingredient (µg/g) recovered in shoots at 14 DAT and 60 DAT per 50% and 100% rate in 2020. Treatments include imidacloprid injection and drench, flupyradifurone injection and drench, spirotetramat injection and foliar. Significant difference (*) of mean active ingredient detection between DAT within a rate (Fisher's LSD, * = P<0.05, ** = P <0.005). Significant (†) difference of mean active ingredient detection between rate within a DAT (Fisher's LSD, † = P<0.05, ‡ = P <0.005). Error bars indicate SEM.

The interaction of treatment and time was found to be significant using a two-way ANOVA for concentration of active ingredient recovered in shoot tissue in the 2021 ($F_{11, 84.9} = 10.95$; $P<0.001$).

In 2021 imidacloprid was recovered in shoots administered 50% and 100% rates by crown injection at 14 DAT and 59 DAT (Figure 2.3). The mean concentration of imidacloprid was nearly tenfold greater at 14 DAT (50%: 1.880 ppm; 100%: 2.019 ppm) than 59 DAT samples (50%: 0.203 ppm; 100%: 0.183 ppm) for both rates (Fisher's LSD, $P\leq0.0001$) (Figure 2.3). There is no significant difference when comparing the two rates (50% and 100%) within a collection date for imidacloprid recovered in shoots treated with injection method (Figure 2.3).

In 2021, imidacloprid was recovered from shoots administered by soil drench in shoots on both collection dates (Figure 2.3). Greater concentration of imidacloprid was recovered at 14 DAT than 59 DAT in both rates (Fisher's LSD, $P <0.0001$) (Figure 2.3). The mean concentration of imidacloprid recovered in shoots was as follows, 14 DAT at 50% rate: 0.846 ppm; 14 DAT at 100%: 3.898 ppm; 59 DAT at 50%: 0.015 ppm; and 59 DAT at 100%: 0.065 ppm. The 100% soil drench applied imidacloprid resulted in significantly greater mean active ingredient recovered at 14 DAT than 50% soil drench (Fisher's LSD, $P<0.0001$) (Figure 2.3). When comparing the two methods used to administer imidacloprid in 2021, imidacloprid was recovered in greater concentrations by injection method when compared to soil drench in all comparisons besides the 100% rate at 14 DAT. There was significantly less imidacloprid recovered in crown injection bushes than soil drench at 14 DAT for 100% rate (Fisher's LSD, $P < 0.0001$) and significantly more in recovered in crown

injected bushes than soil drench treated bushes at 59 DAT for the 50% rate (Fisher's LSD, P <0.05) (Figure 2.3).

In 2021, flupyradifurone was recovered in shoots administered 50% and 100% rates by crown injection at 14 DAT and 59 DAT (Figure 2.3). Mean active ingredient recovered from crown injected bushes was significantly greater at 14 DAT than 59 DAT for both rates (Fisher's LSD, P<0.0005). Mean recovery of flupyradifurone in shoots was as follows for the 50% rate, 14 DAT: 0.241 ppm, 59 DAT: 0.005 ppm; and for 100% rate, 14 DAT: 0.648 ppm, 59 DAT: 0.031 ppm. There was greater recovery of flupyradifurone at 14 DAT in the shoots on bushes that received the 100% rate (Fisher's LSD, P<0.005).

In 2021, flupyradifurone was recovered from shoots administered by soil drench in both the 50% and 100% rates for both collection dates, 14 DAT and 59 DAT (Figure 2.3). There was no significant difference between DAT shoot collection, the mean concentration recovered in shoots sampled on the 14 DAT (50%: 0.045 ppm; 100%: 0.074 ppm) are numerically higher than the 59 DAT (50%: 0.006 ppm; 100%: 0.014 ppm) (Figure 2.3). There was no significant difference between the two rates for mean active ingredient recovered for both collection date, however the 100% rate was numerically greater (Figure 2.3). When comparing the two methods used to administer flupyradifurone in 2021, flupyradifurone was recovered in greater mean concentrations by injection method and was significantly greater for both the 50% and 100% rate at 14 DAT (Fisher's LSD, P<0.005).

In 2021, spirotetramat was recovered in shoots administered 50% and 100% rates by crown injection at both collections dates. Mean recovery of spirotetramat at 14 DAT (50%: 10.59 ppm; 100%: 13.37 ppm) was significantly greater than at 59 DAT (50%: 4.92

ppm; 100%: 7.24 ppm) for the 100% rate (Fisher's LSD, $P < 0.05$) and marginally greater for the 50% rate ($P = 0.0603$). Shoots from bushes that received the 100% rate had numerically greater active ingredient recovered than 50% rate for both sample dates (Figure 2.3).

In 2021, spirotetramat was recovered from shoots administered by foliar spray in shoots that received both rates (50% and 100%) at both collection dates (14 DAT and 59 DAT) (Figure 2.3). Numerically, spirotetramat was recovered in greater concentration at 59 DAT for both rates (50%: 15.72 ppm; 100%: 19.26 ppm) than at 14 DAT (50%: 9.62 ppm; 100%: 10.09 ppm); and recovery was numerically higher in the 100% rate than the 50% rate for both sample dates (Figure 2.3). When comparing the two methods used to administer spirotetramat in 2021, spirotetramat was recovered numerically in a greater mean by injection method at 14 DAT and by foliar method at 59 DAT with the 100% rate resulting in significantly greater active ingredient recovered (Fisher's LSD, $P<0.05$).

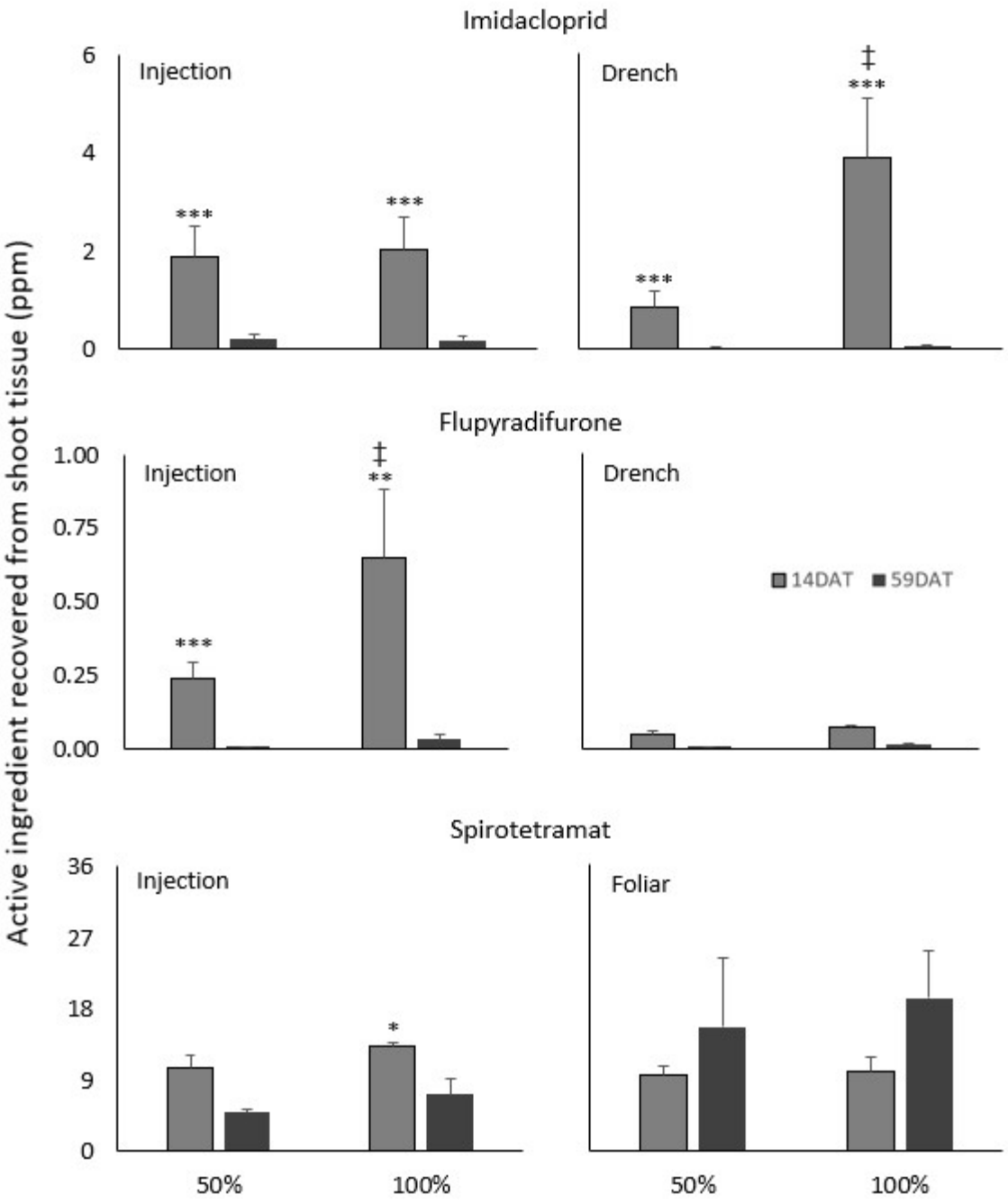

Figure 2.3. Mean chemical residue (µg/g) detected in shoots at 14 DAT and 59 DAT per 50% and 100% rate in 2021. Treatments include imidacloprid injection and drench, flupyradifurone injection and drench, spirotetramat injection and foliar. Error bars indicate SEM. Significant difference (*) of mean active ingredient detection between DAT within a rate (Fisher's LSD, * = P <0.05, ** = P <0.005, *** = P ≤0.001). Significant (†) difference of mean active ingredient detection between rate within a DAT (Fisher's LSD, † = P<0.05, ‡ = P <0.005). Error bars indicate SEM.

Residue Detection in Leaf Tissue

The interaction of treatment and time was found to be significant using a two-way ANOVA for concentration of active ingredient recovered in leaf tissue in the 2020 study ($F_{11, 14.2} = 3.62$; $P = 0.0128$).

In 2020 imidacloprid was recovered in leaf tissue administered 50% and 100% rates by crown injection at 14 DAT and 60 DAT (Figure 2.4). There is no significant difference between the two collection dates within a rate (50% 14 DAT: 0.69 ppm, 60 DAT: 2.22 ppm) (100% 14 DAT: 5.40 ppm, 60 DAT: 4.45 ppm). Imidacloprid recovered from shoot tissue at 14 DAT was significantly higher at the 100% rate when compared to the 50% rate (Fisher's LSD, $P>0.005$).

In 2020 imidacloprid was recovered in leaf tissue administered 50% and 100% rates by soil drench at 14 DAT and 60 DAT (Figure 2.4). Imidacloprid was recovered at a greater concentration at 60 DAT (2.16 ppm) than 14 DAT (0.11 ppm) at 50% (Fisher's LSD, $P<0.05$). When comparing rate within 14 DAT, imidacloprid is recovered in significantly greater concentration at 100% than 50% rate (Fisher's LSD, $P<0.005$). When comparing the two methods used to administer imidacloprid in 2020, imidacloprid was generally recovered in greater concentrations by injection method when compared to soil drench (Figure 2.4).

In 2020, flupyradifurone was recovered in leaf tissue administered 50% and 100% rates by crown injection at 14 DAT and 60 DAT (Figure 2.4). Mean active ingredient recovered from crown injected bushes at the 50% rate was greater at 14 DAT (4.46 ppm) then at 60 DAT (1.26 ppm); and significantly greater in the 100% rate (Fisher's LSD, $P<0.05$). Mean recovery of flupyradifurone in leaf tissue was significantly greater at the

100% rate for 60 DAT sample when compared to mean active ingredient recovered from the 50% rate (Fisher's LSD, $P<0.05$).

In 2020, flupyradifurone was recovered from leaf tissue administered 50% and 100% rates by soil drench at 14 DAT and 60 DAT (Figure 2.4). Though there is no significant difference between DAT leaf collection, the mean concentration recovered in leaf tissue sampled on the 14 DAT (50%: 1.31 ppm; 100%: 2.00 ppm) are numerically higher than the 60 DAT (50%: 0.50 ppm; 100%: 1.78 ppm) (Figure 2.4). Active ingredient recovered from leaf tissue was significantly greater at 60DAT collection for 100% when compared to 50% at 60DAT (Fisher's LSD, $P>0.05$). When comparing the two methods used to administer flupyradifurone in 2020, flupyradifurone was recovered in greater mean concentrations by injection method (Figure 2.4). Injection was significantly higher at the 100% rate at 14 DAT (Fisher's LSD, $P<0.005$) and marginally significant at 60 DAT (Fisher's LSD, $P=0.0565$).

In 2020, spirotetramat was recovered in leaf tissue from bushes administered 50% and 100% rates by crown injection at 14 DAT and 60 DAT (Figure 2.4). There were no significant differences when comparing DAT within rate, and rate within DAT.

In 2020, spirotetramat was recovered from leaf tissue from bushes administered by foliar spray that received 50% and 100% rate at 14 DAT and 60 DAT (Figure 2.4). There was significantly greater mean concentration of spirotetramat recovered for the 50% rate at 14 DAT at 60 DAT (Fisher's LSD, $P>0.005$). There was no significant difference between the two rates for mean active ingredient recovered 14 DAT and 60 DAT, however the 100% rate was numerically higher than the no detection of the 50% (Figure 2.4). When comparing the two methods used to administer spirotetramat in 2020, spirotetramat was

recovered in a greater mean active ingredient by foliar method at the 50% rate at 14 DAT and 60 DAT and 100% rate at 60 DAT (Figure 2.4).

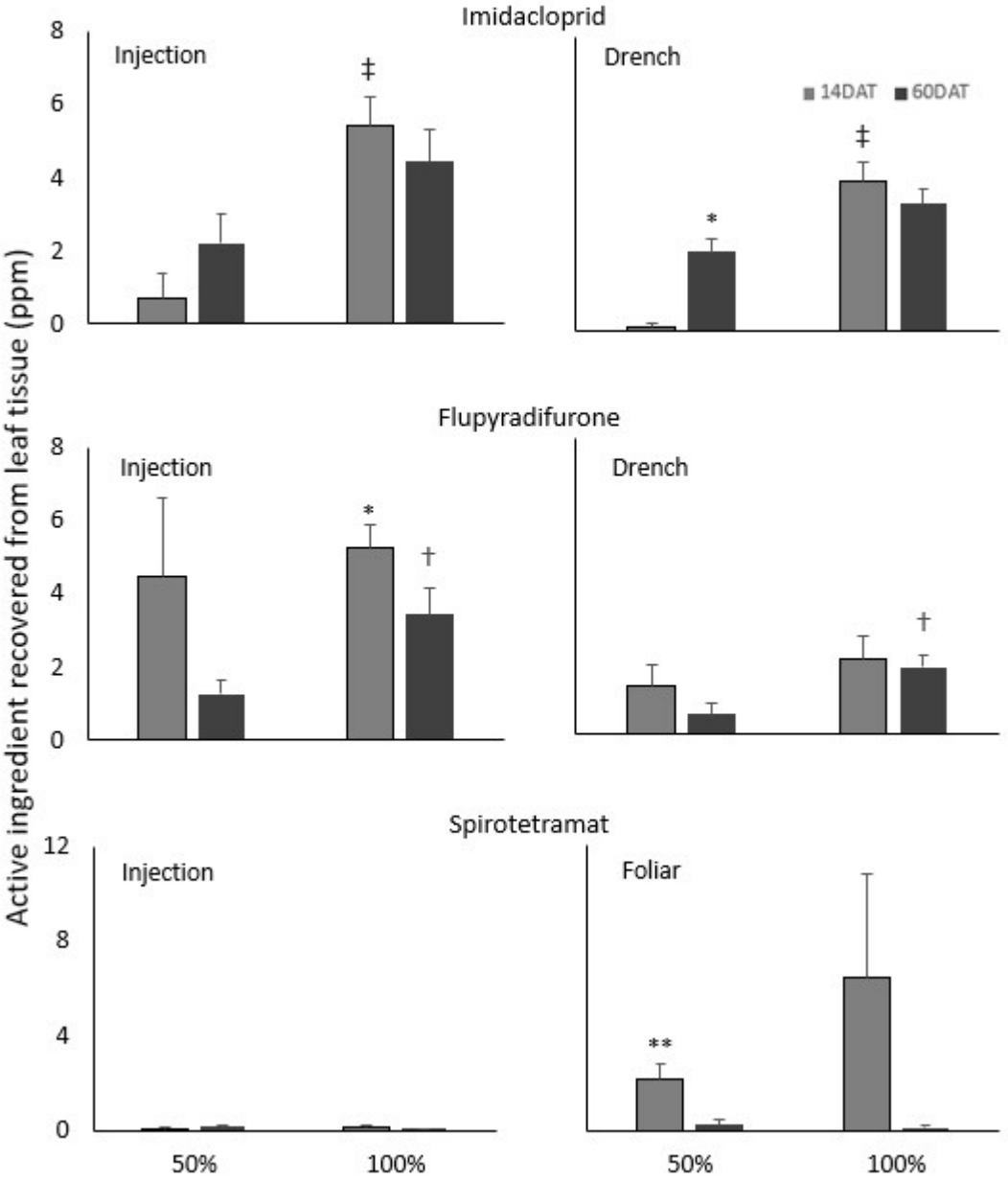

Figure 2.4. Mean chemical residue (µg/g) detected in leaf tissue at 14 DAT and 60 DAT per 50% and 100% rate in 2020. Treatments include imidacloprid injection and drench, flupyradifurone injection and drench, spirotetramat injection and foliar. Error bars indicate SEM. Significant difference (*) of mean active ingredient detection between DAT within a rate (Fisher's LSD, * = P<0.05, ** = P <0.005). Significant (†) difference of mean active ingredient detection between rate within a DAT (Fisher's LSD, † = P<0.05, ‡ = P <0.005). Error bars indicate SEM.

The interaction of treatment and time was found to be significant using a two-way ANOVA for concentration of active ingredient recovered in leaf tissue in the 2021 study ($F_{11, 14.7} = 11.67$; $P < 0.0001$).

In 2021 imidacloprid was recovered in leaf tissue administered 50% and 100% rates by crown injection at 14 DAT and 59 DAT (Figure 2.5). Imidacloprid was recovered from leaves in significantly greater concentration for both rates at 14 DAT (50%: 1.004 ppm; 100%: 0.931 ppm) (Fisher's LSD, $P < 0.05$). There was no significant difference in mean imidacloprid recovered in samples collected from 50% and 100% rates within a sample date.

In 2021 imidacloprid was recovered in leaf tissue administered 50% and 100% rates by soil drench at 14 DAT and 59 DAT (Figure 2.5). Imidacloprid was recovered at a greater concentration at 14 DAT (50%: 0.989 ppm; 100%: 3.856 ppm) than 59 DAT (50%: 0.039 ppm; 100%: 0.185 ppm) (Fisher's LSD, $P < 0.005$ (50%), $P < 0.0001$ (100%)). When comparing rate within 14 DAT, imidacloprid is recovered in significantly greater concentration at 100% than 50% rate (Fisher's LSD, $P<0.05$ (59 DAT), $P = 0.0005$ (14DAT)). When comparing the two methods used to administer imidacloprid in 2020, imidacloprid was generally recovered in greater concentrations by injection method when compared to soil drench and was found significant with the 50% rate at 59 DAT (Fisher's LSD, $P < 0.05$) (Figure 2.5). This trend was different in comparing 100% rate at 14 DAT, the soil drench bushes resulted in greater mean active ingredient recovered (Fisher's LSD, $P < 0.005$).

In 2021, flupyradifurone was recovered in leaf tissue administered 50% and 100% rates by crown injection at 14 DAT and 59 DAT (Figure 2.5). Mean active ingredient

recovered from crown injected bushes at the 50% rate was significantly greater at 14 DAT (0.161 ppm) then at 59 DAT (0.079 ppm) (Fisher's LSD, $P < 0.005$); and marginally greater in the 100% rate (14 DAT: 0.293 ppm; 59 DAT: 0.140 ppm) (Fisher's LSD, $P = 0.0621$). Mean recovery of flupyradifurone in leaf tissue was numerically greater at the 100% rate than for 50% rate for both collection days and was marginally significant for 59 DAT sample (Fisher's LSD, $P = 0.0507$).

In 2021, flupyradifurone was recovered from leaf tissue administered 50% and 100% rates by soil drench at 14 DAT and 59 DAT (Figure 2.5). Though there is no significant difference between DAT shoot collection, the mean concentration recovered in leaf tissue sampled on the 14 DAT (50%: 0.091 ppm; 100%: 0.202 ppm) were numerically higher than the 59 DAT (50%: 0.068 ppm; 100%: 0.098 ppm) (Figure 2.5). When comparing the two methods used to administer flupyradifurone in 2021, flupyradifurone was recovered in numerically greater mean concentrations by injection method (Figure 2.5).

In 2021, spirotetramat was recovered in leaf tissue from bushes administered 50% and 100% rates by crown injection at 14 DAT and 59 DAT (Figure 2.5). There were no significant differences when comparing DAT within rate, and rate within DAT. Active ingredient recovered was numerically greater at 14 DAT (50%: 1.64 ppm; 100%: 2.14 ppm) than 59 DAT (50%: 0.81 ppm; 100%: 1.22 ppm) for both rates. The 100% rate at both dates had numerically greater active ingredient recovered in leaf tissue.

In 2021, spirotetramat was recovered from leaf tissue from bushes administered by foliar spray that received 50% and 100% rate at 14 DAT and 59 DAT (Figure 2.5). The mean concentration of spirotetramat recovered for the 50% rate at 14 DAT, 1.55 ppm, was

significantly less than 59 DAT, 3.92 ppm (Fisher's LSD, P>0.05). Active ingredient recovered from leaf tissue was significantly more in the 100% rate than the 50% rate at 14 DAT (Fisher's LSD, P < 0.05) (Figure 2.5), and numerically different at 59 DAT. When comparing the two methods used to administer spirotetramat in 2020, spirotetramat was recovered in a greater mean active ingredient by foliar method at the 50% rate at 59 DAT, and 100% rate at 14 DAT and 59 DAT which was found to be significant (Fisher's LSD, P < 0.05) (Figure 2.5).

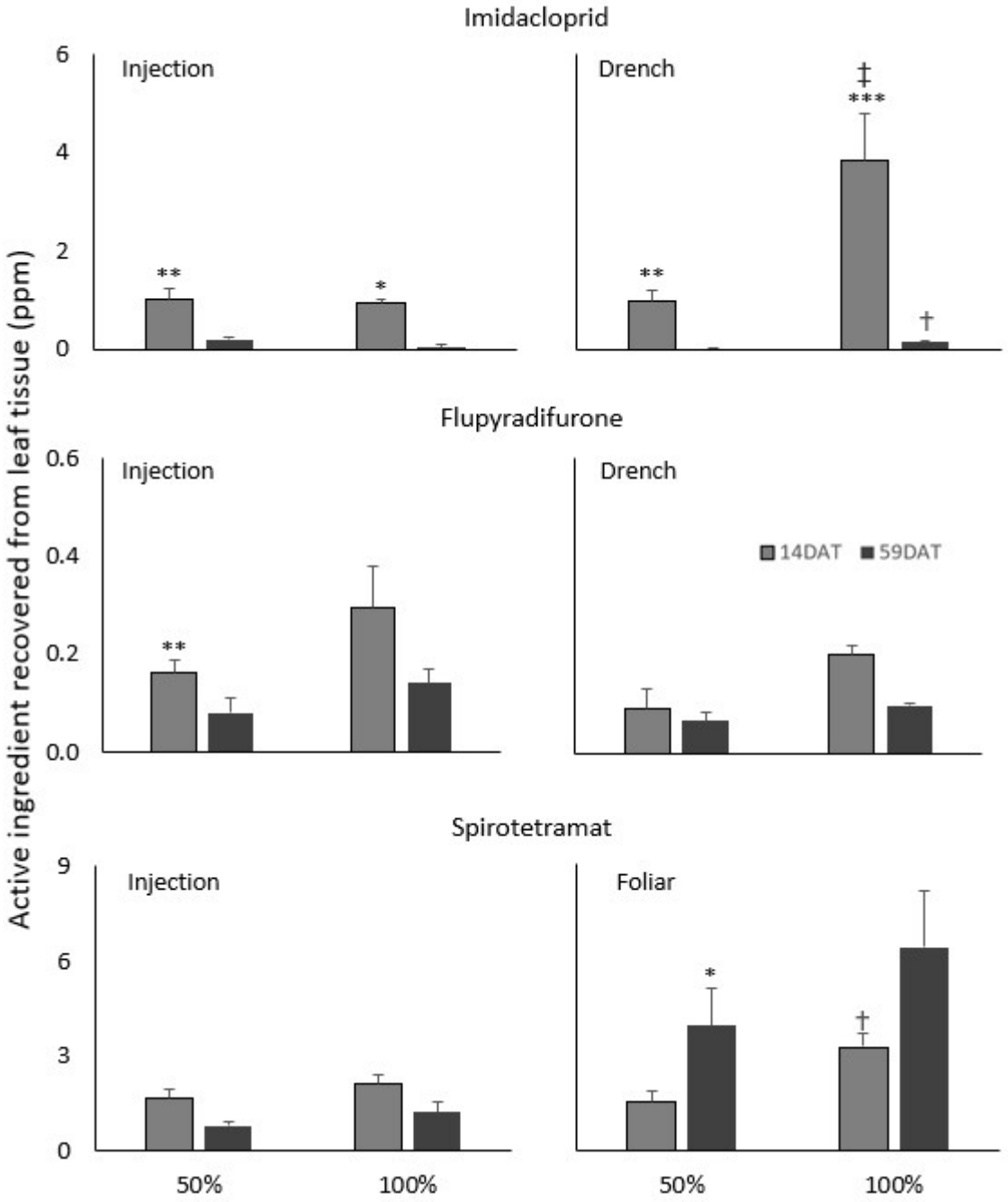

Figure 2.5. Mean chemical residue (µg/g) detected in leaf tissue at 14 DAT and 59 DAT per 50% and 100% rate in 2021. Treatments include imidacloprid injection and drench, flupyradifurone injection and drench, spirotetramat injection and foliar. Error bars indicate SEM. Significant difference (*) of mean active ingredient detection between DAT within a rate (Fisher's LSD, * = P <0.05, ** = P <0.005, *** = P ≤0.001). Significant (†) difference of mean active ingredient detection between rate within a DAT (Fisher's LSD, † = P<0.05, ‡ = P <0.005). Error bars indicate SEM.

Differences in recovery of active ingredient in plant tissue type for treatments within a sample data (DAT) was found to be significant using a two-way ANOVA for samples collected at 14 DAT ($F_{11, 35} = 2.27$; $P < 0.05$) but not at 60 DAT. There was no separation of treatment comparing the two plant tissue types found using Tukey's HSD. Imidacloprid injection treated bushes numerically had greater active ingredient recovered in shoot tissue at 14 DAT and greater active ingredient recovered in leaf tissue at 60 DAT. Imidacloprid 50% injection had 5.49 ppm recovered in shoot tissue in comparison to 0.69 ppm recovered in leaf tissue at 14 DAT. For bushes treated with imidacloprid with soil drench, leaf tissue resulted in generally greater in concentration than shoot tissue for both rates and collection dates. Imidacloprid 50% drench had numerically greater active ingredient recovered in leaf tissue than shoot tissue at 60 DAT. Flupyradifurone 50% injection had numerically greater concentration of active ingredient recovered in leaf tissue than shoot tissue at 14 DAT and 60 DAT. Flupyradifurone 100% at 14 DAT and 60 DAT had the opposite trend, where active ingredient recovered from shoot tissue was generally greater than leaf tissue. Soil drench of flupyradifurone followed the same trend as imidacloprid soil drench, active ingredient recovered from leaf tissue was generally greater in concentration than from shoot tissue for both rates and collection dates; and the 50% rate at the 60DAT had numerically greater active ingredient recovered in leaf tissue. Spirotetramat injection treated bushes followed the trend of having greater active ingredient recovered in shoot tissue at 14 DAT and greater active ingredient recovered in leaf tissue at 60 DAT, though these differences were not as great as the other two injected chemicals. Foliar application of spirotetramat 50% resulted in numerically greater concentration in leaf tissue at 14 DAT and shoot tissue at 60. Leaf tissue had greater

concentration of active ingredient recovered for the spirotetramat 100% treatment due to no recovery in shoot samples at 14 DAT.

Differences in recovery of active ingredient in plant tissue type for treatments within a sample data (DAT) was found to be significant using a two-way ANOVA for samples collected at 14 DAT ($F_{11, 35} = 22.35$; $P < 0.0001$) and 59 DAT ($F_{11, 33} = 2.17$; $P < 0.05$). Mean active ingredient was recovered in greater concentration in shoots than leaves at 14 DAT for spirotetramat for both rates and treatment methods (Tukey's HSD, $P < 0.05$). There was no mean separation for the interaction of treatment and plant tissue sample type using Tukey's HSD, $P < 0.05$. Generally, there was greater active ingredient recovered in shoot tissue samples for both collection dates.

Gall Assessments on Treated Potted Blueberry Bushes

Swelling gall assessment performed 14 DAT (June 29, 2020; June 22, 2021) quantified the number of galls swelling in two size categories (Small ≤ 5 mm, Medium >5 mm) per treated bush.

In 2020, there were no significant differences in mean number of failed, medium or total swelling galls in the different treatments (Table 2.4). However, there was numerically greater mean number of small swelling galls on flupyradifurone 50% administered with injection than imidacloprid 50% administered by soil drench, flupyradifurone 50% administered with soil drench, and spirotetramat 50% and 100% administered by foliar spray (Table 2.4). Mean total swelling galls were numerically less for foliar treatments of spirotetramat when compared to untreated control (Table 2.4).

Table 2.4. Mean ± SEM small swelling galls (≤ 5 mm length), medium swelling galls (>5 mm length) and failed galls per treated blueberry bushes in 2020 study. Total swelling combines small and medium swelling gall categories. Means not followed by the same letter within a column are significantly different base on mean separation using Tukey's HSD, P<0.05.

Treatment	Application Type	Failed	Small Swelling	Medium Swelling	Total Swelling
Untreated Control		1.08 ± 0.51	5.25 ± 1.05 ab	0.67 ± 0.26	5.92 ± 1.08
imidacloprid 50%	Injection	1.5 ± 0.29	5.5 ± 1.5 ab	2 ± 1.68	7.5 ± 2.53
	Soil	1 ± 0.41	3.75 ± 2.06 b	1.25 ± 1.25	5 ± 3.19
imidacloprid 100%	Injection	1.5 ± 0.65	5.25 ± 1.60 ab	0.5 ± 0.29	5.75 ± 1.80
	Soil	0.75 ± 0.48	4.5 ± 2.10 ab	1.25 ± 0.95	5.75 ± 2.95
flupyradifurone 50%	Injection	2.5 ± 0.5	10.75 ± 1.65 a	1.5 ± 1.19	12.25 ± 2.06
	Soil	1 ± 0.71	3.75 ± 1.49 b	1.25 ± 0.25	5 ± 1.58
flupyradifurone 100%	Injection	1.5 ± 0.65	4.75 ± 1.89 ab	0.75 ± 0.48	5.5 ± 2.25
	Soil	2 ± 0.41	6.5 ± 1.26 ab	3.5 ± 1.94	10 ± 2.12
spirotetramat 50%	Injection	2.5 ± 1.5	4.75 ± 2.32 ab	0.75 ± 0.48	5.5 ± 2.60
	Foliar	1.25 ± 0.63	2.75 ± 1.03 b	0 ± 0	2.75 ± 1.03
spirotetramat 100%	Injection	0.25 ± 0.25	4.75 ± 2.43 ab	0.25 ± 0.25	5 ± 2.35
	Foliar	2.25 ± 1.93	2.5 ± 0.65 b	0.75 ± 0.75	3.25 ± 1.31

In 2021, mean number of failed galls was found to be significant for one or more treatments using a one-way ANOVA ($F_{12, 43} = 2.36$; $P = 0.0196$), however there was no mean separation using Tukey HSD. Flupyradifurone 50% administered by soil drench had 3.25 failed galls compared to 9.5 failed galls for the control. There was no difference in mean number small swelling galls per treatment (Table 2.5). For medium swelling galls, the one-way ANOVA was significant ($F_{12, 43} = 3.00$; $P = 0.0040$). There were no significant differences found when comparing the treatments with the untreated control. Imidacloprid 100% soil drench treated bushes and spirotetramat 50% foliar had significantly greater number mean medium swelling galls than flupyradifurone 50% injection using Tukey's HSD. Treatments that had numerically lower mean medium swelling galls than mean number of medium swelling galls on untreated bushes include: imidacloprid 50% injection, flupyradifurone 50% injection, and spirotetramat 50% injection. The one-way ANOVA

found treatment to impact total swelling galls was found to be significant ($F_{12, 43}$ = 2.57; P=0.0116), there was no significant separation found with Tukey's HSD P<0.05. However, the following had total means that were numerically lower than the meal total galls in untreated control (16.67 galls): imidacloprid 50% injection (10.25 galls), flupyradifurone 50% injection (11.5 galls), flupyradifurone 50% soil drench (13.5 galls), flupyradifurone 100% injection (11.67 galls), and spirotetramat 50% injection (11.75 galls).

Table 2.5. Mean ± SEM small swelling galls (≤ 5 mm length), medium swelling galls (>5 mm length) and failed galls per treated blueberry bushes in 2021 study. Total swelling combines small and medium swelling gall categories. Means not followed by the same letter within a column are significantly different base on mean separation using Tukey's HSD, P<0.05.

Treatment	Application Type	Failed	Small Swelling	Medium Swelling	Total Swelling
Untreated Control		9.5 ± 1.37	10.58 ± 0.97	6.08 ± 1.05 ab	16.67 ± 1.76
imidacloprid 50%	Injection	5.5 ± 1.71	6.75 ± 0.63	3.5 ± 0.87 ab	10.25 ± 1.44
	Soil	5.75 ± 0.85	11.75 ± 1.49	7 ± 1.08 ab	18.75 ± 2.53
imidacloprid 100%	Injection	4.25 ± 1.03	12.5 ± 1.32	6.5 ± 2.5 ab	19 ± 1.47
	Soil	7.75 ± 2.25	8.5 ± 1.32	10.75 ± 2.17 a	19.25 ± 1.97
flupyradifurone 50%	Injection	6.25 ± 1.31	10.25 ± 1.93	1.25 ± 0.95 b	11.5 ± 2.66
	Soil	3.25 ± 0.85	9 ± 1.87	4.5 ± 1.94 ab	13.5 ± 1.55
flupyradifurone 100%	Injection	8.33 ± 1.86	7 ± 1.53	4.67 ± 0.88 ab	11.67 ± 1.20
	Soil	7.75 ± 4.09	9.25 ± 2.14	6.75 ± 3.75 ab	16 ± 4.80
spirotetramat 50%	Injection	4.25 ± 0.95	8 ± 1.78	3.75 ± 1.25 ab	11.75 ± 1.70
	Foliar	11.75 ± 4.15	7.5 ± 0.65	8.75 ± 0.95 a	16.25 ± 0.48
spirotetramat 100%	Injection	3.75 ± 1.03	8.5 ± 1.32	6.75 ± 1.89 ab	15.25 ± 2.25
	Foliar	4.5 ± 1.19	7.5 ± 2.02	7 ± 1.08 ab	14.5 ± 0.96

Injection of flupyradifurone 100% only had three replications due to bush dying.

Galls quantified at the end of their expansion (August 14, 2020; August 24, 2021) were categorized into small or medium galls. Small galls were less than 15 mm and medium galls were greater or equal to 15 mm. In 2020, there was no significant differences between untreated control bushes and treatment bushes for mean number of small, medium or total galls (Table 2.6). However, flupyradifurone 50% injection bushes had

numerically greater mean number of small galls than flupyradifurone 50% soil drench and spirotetramat 50% foliar spray treated bushes (Table 2.6). The greatest mean number of total galls were found on flupyradifurone 100% soil drench treated bushes and were numerically greater than flupyradifurone 50% soil drench and spirotetramat 50% foliar spray treated bushes. The mean total galls were fewest on spirotetramat 50% foliar spray treated bushes, and this numerically lower than flupyradifurone 50% injection and 100% soil drench treated bushes. Though there were no significant difference between the untreated control and any of the treatments in mean total gall count, the following had numerically lower mean total galls: imidacloprid 100% injection, flupyradifurone 50% soil drench, and spirotetramat 50% and 100% foliar (Table 2.6).

Table 2.6. Mean ± SEM small galls (< 15 mm length), medium galls (≥ 15 mm length) and total number of galls per treated blueberry bushes in 2020 study.

Treatment	Application Type	Small	Medium	Total
Untreated Control		4 ± 0.66 ab	0.33 ± 0.26	4.33 ± 0.70
imidacloprid 50%	Injection	4.5 ± 1.71 ab	0.75 ± 0.48	5.25 ± 2.10
	Soil	3.5 ± 1.32 ab	0.25 ± 0.25	3.75 ± 1.38
imidacloprid 100%	Injection	3 ± 0.91 ab	0 ± 0	3 ± 0.91
	Soil	4.25 ± 2.02 ab	1.25 ± 1.25	5.5 ± 3.10
flupyradifurone 50%	Injection	7.5 ± 2.72 a	0 ± 0	7.5 ± 2.72
	Soil	1.5 ± 0.65 b	0.25 ± 0.25	1.75 ± 0.75
flupyradifurone 100%	Injection	5 ± 2.68 ab	1.5 ± 1.19	6.5 ± 3.86
	Soil	6.5 ± 2.40 ab	2 ± 1.35	8.5 ± 2.5
spirotetramat 50%	Injection	5 ± 2.35 ab	0 ± 0	5 ± 2.35
	Foliar	1.5 ± 0.65 b	0 ± 0	1.5 ± 0.65
spirotetramat 100%	Injection	3.75 ± 2.06 ab	0.5 ± 0.29	4.25 ± 2.17
	Foliar	2.25 ± 0.95 ab	0.25 ± 0.25	2.5 ± 1.19

In 2021, there were no significant differences between treatments in the two gall categories small and medium, additionally, there were no significant differences in mean total number of galls. Though, the one-way ANOVA for small galls was significant ($F_{12, 41}$ =

2.78; P = 0.0073) and for total number of galls ($F_{12, 41}$ = 2.23; P = 0.0282). Numerically, imidacloprid 50% injection, flupyradifurone 50% soil drench, and flupyradifurone 100% injection had lower mean number of small and total number of galls than the untreated control (Table 2.7).

Table 2.7. Mean ± SEM small galls (< 15 mm length), medium galls (≥ 15 mm length) and total number of galls per treated blueberry bushes in 2021 study.

Treatment	Application Type	Small	Medium	Total
Untreated Control		7.17 ± 0.94	1.92 ± 0.42	9.08 ± 1.05
imidacloprid 50%	Injection	4.5 ± 0.96	1 ± 0.41	5.5 ± 1.04
	Soil	9.25 ± 1.84	2.25 ± 1.31	11.5 ± 3.01
imidacloprid 100%	Injection	9 ± 1.47	3.5 ± 0.87	12.5 ± 1.55
	Soil	11.5 ± 2.90	1 ± 0.58	12.5 ± 3.38
flupyradifurone 50%	Injection	10.67 ± 3.76	0.33 ± 0.33	11 ± 4.04
	Soil	5.5 ± 0.65	2 ± 0.41	7.5 ± 0.65
flupyradifurone 100%	Injection	4.67 ± 1.20	1.67 ± 0.88	6.33 ± 0.67
	Soil	10.5 ± 1.89	1.5 ± 0.65	12 ± 2.48
spirotetramat 50%	Injection	7 ± 2.08	1.67 ± 0.88	8.67 ± 2.96
	Foliar	11.25 ± 1.65	1 ± 0.41	12.25 ± 1.80
spirotetramat 100%	Injection	10.75 ± 2.66	1.5 ± 0.5	12.25 ± 2.43
	Foliar	8 ± 1.96	2.75 ± 0.95	10.75 ± 2.29

The following treatments had 3 replications due to bushes dying: flupyradifurone 50% injection, flupyradifurone 100%, and spirotetramat 50% injection.

Adult Emergence

Due to the poor quality of the gall tissue, no emergence was observed in 2020 and gall masses were not analyzed. In 2021 some emergence was observed but the abundance was too low to perform a statistical analysis.

DISCUSSION

In this study we found that potted blueberry bushes move insecticides systemically from introduced sites of entry like roots (via soil drench), crown xylem (via crown injection) and foliage (foliar sprays) to sites where the blueberry stem gall wasp develops,

shoot tissue. However, this study does not show evidence that the treatments tested inhibit gall and gall wasp development due to the age, size, and vigor of bushes used. Differences in rates, application methods, and time on insecticide recovery discussed below.

Maximal recovery of imidacloprid was dependent on time for crown injection and rate for soil drench. These differences were observed due to the limitations and advantages of the two treatment methods. With crown injection, imidacloprid was put directly into the vascular system and moved rapidly into expanding plant tissue resulting in greatest recovery at 14 DAT. By 59/60 DAT a lesser concentration was recovered likely due to breakdown from plant metabolism and growth dilution from expanding shoots. With imidacloprid rapid movement within the vascular system, the gall wasp larvae are likely feeding on the greatest concentration of imidacloprid at a young larval stage. For soil drench application of imidacloprid, the 100% rate was superior to the 50% rate to deliver maximum amount of imidacloprid residue. Since imidacloprid does have some affinity to soil (Shetlar 2008 as cited in Kurwadkar et al. 2014), the 100% rate overcomes the barrier associated with soil. Additionally, the greater recovery of imidacloprid was observed at the 14 DAT than the 59/60 DAT. Residue recovered in leaf tissue followed similar trends. A similar trend was observed in apple trees treated with trunk injection. Coslor and colleagues' (2019a) observed greater concentrations of imidacloprid in leaf tissue mid-season, where other compounds like spinosad, chlorantraniliprole, abamectin, and emamectin benzoate (high rate) peaked at the end of the growing season.

Delivery of flupyradifurone is influenced by rate in both crown injection and soil drench. A higher rate is required to achieve maximal recovery and therefore deliver greater concentration of flupyradifurone to feeding gall wasp larvae. The low detections of

flupyradifurone in leaf and stem tissues following drench application may be to its high water solubility (3,200 mg l^{-1} at 20°C) and high leachability (GUS leaching potential index = 4.24) (Lewis et al. 2016). Additionally, binding to organic material in the soil may have led to a reduced recovery. Thus, it is likely that flupyradifurone when applied as a drench treatment will not provide maximal residues. Secondarily, flupyradifurone was recovered in greater concentrations regardless of rate, method, and sample type (shoot, leaf) at 14 DAT collection timing in 2020 and 2021. This informs us that flupyradifurone is generally at its highest concentration in shoots during early stages of larval development. Flupyradifurone was recovered in leaf tissue in similar trends.

When comparing delivery of imidacloprid and flupyradifurone, the active ingredient was generally recovered at greater mean concentration in shoot tissue from potted bushes that received the insecticides by injection, when compared to soil drench. A similar scenario was observed with imidacloprid in Xu and colleagues (2008) study on soil drench and injected wiliwili trees. Injected treated trees significantly reduced erythrina gall wasp emergence, whereas drench treated trees died from gall wasp infestation 15 weeks after treatment (Xu et al. 2008). Thus, soil applications generally require greater amounts of the chemical to achieve the same amount of insecticide applied by other methods, which may require repeated applications due to chemical binding to organic matter, leaching, degradation from microbes or soil chemistry, and age of roots (Chaney 1978; Campana 1978; Doccola and Wild 2012; Kramer and Boyer 1995). Our study suggests that injection of imidacloprid and flupyradifurone is the superior way to deliver the greatest amount of active ingredient to shoots rapidly, where the gall wasp develops. Since we know that the vascular system is active in the gall tissue to deliver nutrients to the nutritive larval

chamber lining (Shorthouse 1986; Dreger-Jauffret and Shorthouse 1992), we have reason to believe that the blueberry stem gall wasp larvae will feed upon imidacloprid or flupyradifurone delivered by crown injection.

Without a rapid and automated application system, crown injection may pose a challenge for established blueberry bushes due to the time it will take to expose the crown located below the soil surface and inject the insecticides in each bush. Nevertheless, crown injection on nursery bushes may be feasible due to the ease of mobility of each potted bush. Purchasing treated, potted blueberry bushes from susceptible cultivars may be attractive to growers for preventing the spread of blueberry stem gall wasp and protecting the blueberry bushes in the early years of establishment while they are incorporated into their integrated pest management program.

Secondarily, soil drench is a viable way to deliver imidacloprid and flupyradifurone but with reduced active ingredient delivered, especially observed in flupyradifurone. In a seed-treatment study in soybeans, flupyradifurone was recovered in less concentration than imidacloprid in V2 soybean plants and in greater concentration than imidacloprid under soil moisture stress (Stamm et al. 2015). Though this is a different type of systemic application method, this informs us that uptake of flupyradifurone may requires less water than imidacloprid in certain soil conditions. Additionally, imidacloprid has a greater binding affinity to organic carbon, aka organic matter in soil, than flupyradifurone (Shetlar 2008 as cited in Kurwadkar et al. 2014). In soils with greater amounts of organic matter, there is a greater chance that imidacloprid will remain within the soil increasing longevity of exposure to the roots.

Recovery of spirotetramat is rate and time dependent in 2020. Slower movement was observed in foliar application due to the acropetal and basipetal movement in phloem and potential exchange between phloem and xylem. Rapid movement into expanding plant tissue was observed in injection due to the upward movement in the xylem. The 100% rate delivered greater mean concentration of spirotetramat in crown injection, and this trend may have been observed in foliar if there had not been human error with sample collection/ extraction. In 2021 neither rate nor time influenced recovery in crown injection. The trend of time on spirotetramat recovery observed in 2020 was numerically observed in 2021. The spirotetramat recovered in leaf tissue followed a similar trend to recovery in shoot samples, expect in 2020 foliar applied samples. Greater residue was recovered from leaf tissue at 14DAT than 60DAT in the 2020 foliar applied samples. This suggests that there was movement away from the leaves after 14DAT and we did not see a return to leaves at 60DAT. Movement within the phloem allows spirotetramat to move from the application area to nutrient dependent areas such as expanding tissues of leaves and shoots, which can also include roots (Nauen et al. 2008). In Fanning and Isaacs (2020) study the foliar systemic: spirotetramat (Movento) application reduced the number of galls by nearly a third when compared to untreated plots and reduced adult emergence by 99% (Isaacs et al. 2020). The reduction in adult emergence is likely due to the known phloem transportation of spirotetramat to nutrient dependent areas. We know from research conducted by Shorthouse (1986) and Dreger-Jauffret and Shorthouse (1992) that the cells lining the larval chambers after 40 days from oviposition are made up of nutrient rich cells for the larvae to feed on. It is likely that spirotetramat was delivered along with the starches, sugars, lipids, and proteins (Dreger-Jauffret and Shorthouse 1992) to these

nutritive cells where they were fed upon by the blueberry stem gall wasp larvae. Our study was unable to capture emergence data, but we have gained insight on the movement and accumulation of spirotetramat when applied by crown injection and foliar spray. Spirotetramat performed better as a foliar spray to deliver the greatest amount of active ingredients to the shoots. The accumulation of spirotetramat in the shoot tissue is encouraging and explains why spirotetramat has been effective in the past for controlling blueberry stem gall wasp.

Due to the age of the bushes used in this study we were not able to conclude whether gall abundance or gall wasp emergence was impacted by the methods and systemics tested. This study does however inform us of which combination of application method with which systemic provides the maximal residue recovered. Crown injection of imidacloprid and flupyradifurone, soil drench of imidacloprid and flupyradifurone, and foliar application of spirotetramat have residual activity in shoots over the course of time where gall wasp larvae are actively growing. There are benefits and limitations of each systemic application method, and these should be weighed for treatment feasibility. In future studies, the successful systemics and application methods found in this study should be explored to determine the efficacy on adult emergence in older and more vigorously growing blueberry bushes.

In summary, systemic delivery of insecticides may be useful to blueberry growers in an integrated pest management program and can provide an additional or alternative method to protect blueberry bushes from pests such as blueberry stem gall wasp that feed or live within or on the plant, such as aphids, leafhoppers, planthoppers, beetles, scale, and leafminers.

CHAPTER 3: A BIOASSAY METHOD TO TEST STEM GALL WASP *HEMADAS NUBILIPENNIS* MORTALITY TO SYSTEMICALLY DELIVERED CHEMICALS IN BLUEBERRY BUSH SHOOTS

ABSTRACT

Blueberry stem gall wasp (*Hemadas nubilipennis*) is a pest of highbush blueberry and is a challenge to control with foliar applied insecticides, due to adult activity during bloom and larval development within the plant tissues. Systemic insecticides were evaluated for control of blueberry stem gall wasp using single shoot bioassays and measuring larval mortality. Shoots containing one gall were administered either azadirachtin, imidacloprid, flupyradifurone, or spirotetramat at 10%, 1%, 0.1%, and 0.01% of field rate within a floral pick. Galls were sliced open, and larvae were assessed for mortality after five days of exposure. Additional shoots were used to determine mean active ingredient recovered from galls and leaves. Mean percentage of larval survival correlated negatively with active ingredient recovered. Imidacloprid and spirotetramat were found to have the greatest potential for control of blueberry stem gall wasp and resulted in observed changes in larval body shape and behavior. This bioassay method can be used in the future to inform decisions on use of other systemics by evaluating larval mortality response to percentage of field rate, and active ingredient recovered from gall tissues. The dissection and larval assessment can be used for field evaluations as well.

INTRODUCTION

The blueberry stem gall wasp, *Hemadas nubilipennis* Ashmead (Hymenoptera: Pteromalidae) is localized menace to several varieties of highbush blueberries, *Vaccinium*

corymbosum, grown in Michigan (Isaacs et al. 2020), but also for lowbush blueberry, *Vaccinium angustifolium,* grown throughout eastern North America (West and Shorthouse 1989; Hayman et al. 2003a). The blueberry stem gall measures two to three millimeters in length that feeds and develops in stem galls from egg stage to adult emergence (West and Shorthouse 1989; Isaacs et al. 2020). The formation of galls on the stems of blueberry bushes reduces the fruiting area and potentially contaminates harvested fruit at pack-out (McAlister and Anderson 1932; Hayman et al. 2003a). These galls have also been found to act as sinks for inorganic molecules, such as metal; and nickel and copper has been found in higher concentrations in the gall than any of the other parts of the lowbush blueberry (Bagatto and Shorthouse 1991). Since the blueberry stem gall wasp has the ability to form sinks of plant nutrients and inorganic molecules as part of the plant for their own development, understanding the efficacy of systemic insecticides delivered within a plant on a bioassay level will inform decisions on future control of this gall wasp.

The blueberry stem gall wasp life cycle begins with adults emerging from galls formed in the previous year (Hayman et al. 2003a; Isaacs et al. 2020). Female blueberry stem gall wasp oviposition 5-15 mm below the tip of shoot facing the apex, moving a few millimeters up the shoot each time to lay an egg (Shorthouse et al. 1986, West and Shorthouse 1989). Oviposition on the same shoot happens until 12 to 15 eggs are laid (McAlister and Anderson 1932) and this is repeated on multiple shoots over the wasp's lifetime (West and Shorthouse 1989). When the female gall wasp is done laying eggs, she attempts to prevent the shoot tip from further expansion by using here ovipositor to stab the apical meristem and surrounding tissues (Shorthouse et al. 1986).

The cells in the shoot begin to rapidly divide within 48 hours of oviposition (Shorthouse 1986). After 10 days the egg is surrounded by thick layers of cells which provide protection, and the larvae begin to hatch (Shorthouse 1986; Shorthouse et al. 1986; Hayman et al. 2003a; Shorthouse et al. 1990). Twenty days after oviposition the gall is noticeably swelling, and nutritive cells begin to from in the cell lining of the chamber (McAlister and Anderson 1932; Shorthouse 1986). It should also be noted that there are inquiline wasp species of the gall that become co-inhabitants after the blueberry stem gall wasp initiates the gall (Driggers 1927; McAlister and Anderson 1932).

Gall and larval development take 60-90 days (Hayman et al. 2003). After that period of time the gall turns from green and fleshy to brown and woody (West and Shorthouse 1989; Shorthouse 1986). Galls can be round, reniform, and irregular in shape, depending on the placement of successful eggs (Shorthouse et al. 1990). The larvae stop feeding and the chambers become "encapsulated by the hard sclerenchyma sheath" in preparation for winter (Shorthouse 1986). The larvae become inactive until spring arrives which initiates the larvae to exit this phase, pupate, and chew their way out of the gall as adults (Hayman et al. 2003a; McAlister and Anderson 1932; Shorthouse et al. 1986; Isaacs et al. 2020).

Systemic insecticides move within the vascular system of a plant and target plant-sucking insects and some plant chewing insects (USDA 1960). Systemics can be absorbed through seed, foliar or root applications, or through injection to a region of the plant (USDA 1960). Depending on the application method, different vascular pathways of the plant are utilized, xylem, phloem, or both (Chaney 1978). A majority of chemicals introduced to the plant through injection or chemigation (soil application) utilize the xylem, moving into the expanding tissues (Chaney 1978; USDA 1960). Foliar applied products utilize the phloem

which transports bidirectionally from source to sink (Chaney 1978; White 2012). The overall advantages of systemics are the protection from sources of degradation, like sun and wash-off, and longer residual exposure to insect pests (Wise 2016).

Only recently have systemic insecticides been used to target the blueberry stem gall wasp by foliar sprays and chemigation. In 2019 a study conducted in Michigan, U.S., analyzed the impact of soil applied systemics and a foliar systemic on gall formation. Soil applications of systemic insecticides: dinotefuran (Scorpion), flupyradifurone (Sivanto), thiamethoxam (Platinum), imidacloprid (Admire Pro) were found to have no impact on reducing gall formation in Fanning and Isaacs (2019) study. Foliar application of systemic insecticide spirotetramat (Movento) reduced the total number of galls (28 ± 2.85 mean ± SEM) when compared to untreated plots (43 ± 4.16 mean ± SEM), though this was not statistically significant (Fanning and Isaacs 2019). Isaacs et al. (2020) also found larvae were 99% controlled in galls collected from bushes treated with a foliar application of spirotetramat when compared to untreated control bushes (Isaacs et al. 2020).

In order to understand the potential of using systemic insecticides for controlling gall wasp via vascular delivery, a compound must both demonstrate mobility to the relevant plant tissues, and also show toxicity to the gall wasp life stages present in the galls. The goal of this study was to test the vascular mobility and toxicity to gall wasp larvae of the following systemics: azadirachtin, imidacloprid, flupyradifurone, and spirotetramat. Additionally, to develop a bioassay method for quantifying systemic/gall wasp targeted compounds.

We hypothesized that the gall wasp larvae will incur greater mortality with higher concentrations of the systemic compounds and deliver greater amounts of material into

gall tissue than in leaves. In order to test the mobility and toxicity of the selected compounds two sets of shoot bioassays were setup, one for gall wasp larvae mortality and another for residue analysis. This study was replicated over two years (2020, 2021).

MATERIALS & METHODS

Shoot Collection and Preparation

Young shoots containing medium swelling galls (1.5-2cm length), terminally located, were collected from a privately owned, un-sprayed non-commercial Jersey blueberry field in Fennville, MI (July 9, 2020; July 7, 2021). Shoots were stored in 946.4 ml deli cups with water for transportation to Michigan State's Trevor Nichols Research Center (Fennville, MI). At the research center, these shoots were cut to a standardized length (2020: ~15 cm, 2021: ~11 cm) and all but five mature leaves were removed. When the gall was not terminally located, the shoot above the gall was cut and all other conditions were met.

Bioassay Setup

A bioassay was performed during the 2020 and 2021 growing season. Insecticides tested included azadirachitin (AzaSol SP, Arborjet, Woburn, MA), imidacloprid (Admire Pro SC Flowable, Bayer CropScience LP, Research Triangle Park, NC), flupyradifurone (Sivanto Prime 200 SL, Bayer CropScience, St. Louis, MO), and spirotetramat (Movento SC, Bayer CropScience LP, St. Louis, MO). Amount of each insecticide used was determined by the maximum foliar labeled rate per blueberry bush: azadirachtin (AzaSol SP, 0.117 g/bush or 0.007 g AI/bush), flupyradifurone (Sivanto Prime 200 SL, 0.285ml/bush or 0.057g

AI/bush), spirotetramat (Movento SC, 0.204 ml/bush or 0.049 g AI/bush) and using the chemigation maximum rate per bush for imidacloprid (Admire Pro SC Flowable, 0.285 ml/bush or 0.157 g AI/bush). This amount was the 100% concentration used for serial dilutions to obtain 10%, 1%, 0.1% and 0.01% concentrations (Table 3.1). Each treatment concentration was replicated four times for every bioassay. An untreated control treatment was also included.

Table 3.1. A summary of the insecticides used in 2020 and 2021 bioassays. Amount of insecticide (Form.) and active ingredient (AI) indicates the amount for 100%.

Trade Name	Active Ingredient	Form. (100%)	AI (100%)
Untreated Control	-	-	-
AzaSol	azadirachitin	0.12 g	0.007g
Admire	imidacloprid	0.285 ml	0.157 g
Sivanto	flupyradifurone	0.285 ml	0.057 g
Movento	spirotetramat	0.204 ml	0.049 g

Every insecticide dilution was mixed with water to total 3ml. This insecticide solution was pipetted into 10.16 cm tall, single anchor water pick (Smithers-OASIS, Kent, Ohio). Each water pick received one shoot with a gall. The shoots were given 24 hours under a grow light (16L:8D photoperiod) to uptake the insecticide solution. Once the 24 hours elapsed or solution was absorbed, the shoots were moved into 946.4 ml deli cups with water and placed back under the grow light. This study was duplicated in both 2020 and 2021 for residue analysis.

Mortality Assessment

A mortality assessment was completed five days after the start of the study. Each gall was removed from the shoot. A single edge blade was used to cut thin slices (approx. 2 to 3 mm) of the gall. Due to the placement of the larval chambers, these slices were made

perpendicular to the shoot progression (Figure 3.1). Once a slice was made, larvae that remained untouched by the blade were assessed for mortality by probing the larvae with a blunt dissecting probe up to three times. Based on the response, each larva was assigned to one of the three categories: alive, moribund, and dead. Alive larvae were very active, had full range of motion in their body and were quick to respond when being probed. Moribund larvae either had some range of movement that was slowed or no movement besides with their gut or mouth. Noticeable changes in body shape of an alive larva placed them in the moribund category. Larvae that had no movement in body, gut, or mouth after being poked three times with the dissecting probe and were not punctured by the blade or the probe were recorded as dead.

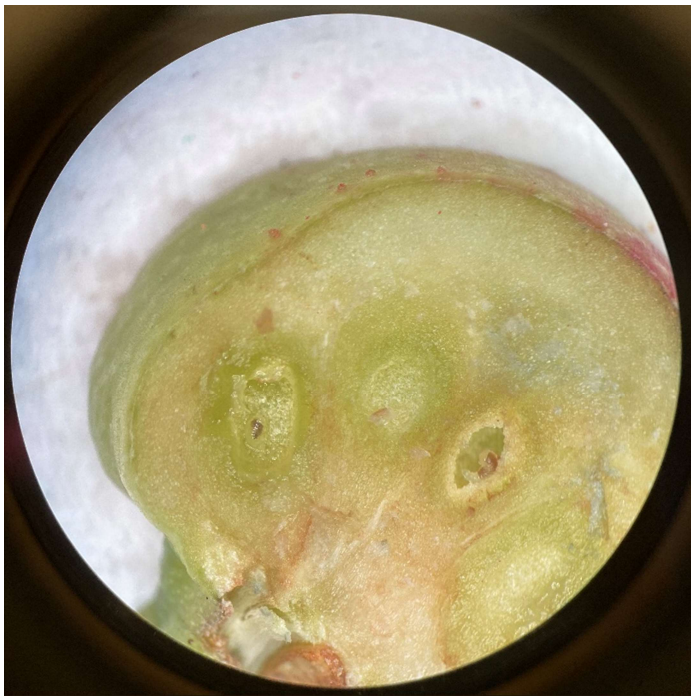

Figure 3.1. Dissected gall showing three larval chambers, each with a larva.

When a part of the gall became woody and it was not obviously caused by the chemical, larvae in that section where not counted (although this was a rare occasion and

generally avoided when selecting shoots). Larvae were not identified to species, but it is likely that inquiline species were present. See appendix for emergence data.

Residue Sample Preparation and Analysis

Residue samples were collected at the same time mortality assessment were completed for the 2020. In 2021, shoots received treatment and were collected a week following mortality assessment. A single gall represented the residue sample (approx. 1 grams) for each treatment concentration replicate. Five leaves were removed from the shoot that was assessed for mortality and five leaves were removed from the shoot that contained the gall for residue purposes. A total of 10 leaves (approx. 3 grams) made up the leaf residue sample.

Methods for residue sample preparation and analysis were adapted from the QuEChERs method (Lehotay, 2011) and used in Wheeler et al. 2020 and Vanwoerkom et al. 2014. All samples were collected into 120 ml graduated glass bottles (Qorpak, Clinton, PA). Fifty milliliters of dichloromethane, 4 grams of magnesium sulfate, and 1 gram of sodium chloride were added to each sample bottle and stored in a in a walk-in cold room (4 °C) for two months.

One day prior to decanting all residue samples, gall samples were sonicated for five minutes. Samples were decanted by pouring the sample through approximately 12 g of sodium sulfate in 125mm diameter filter paper that was folded into a funnel. Each funnel of sodium sulfate was placed on top of a 120 ml glass bottle. Each solvent was evaporated for up to two days. This left a residue film on the inside of the jar that was dissolved by adding 2 ml of acetonitrile to each jar and swirling for 90 seconds. The sample was filtered with a

0.45 µm PTFE hydrophobic filter attached to a 3 ml disposable syringe and transferred to a 2 ml glass vial. All samples were stored in a freezer until processed with analytical equipment. All samples were analyzed using ultra performance liquid chromatography (UPLC) with the limit of detection (LOD) and limit of qualification (LOQ) stated on Table 3.2 for the 2020 study and Table 3.3 for the 2021 study. Recovery of insecticide quantified using linear regression and reported as µg of active ingredient per g of gall or leaf tissue. Standards were obtained from the EPA Standard Repository. The software used was Waters Mass Lynx version 4.2.

Table 3.2. Limit of detection (LOD) and limit of qualification (LOQ) for insecticides used in the 2020 study.

Compound	M+H (m/z)	Qualifier (m/z)	LOD (µg/g)	LOQ (µg/g)
imidacloprid	209	175	0.0025	0.0075
flupyradifurone	125.9	90	0.002	0.006
spirotetramat	216.2	117.1	0.019	0.057
azadirachtin	703.3	585.2	0.00001	0.00003

Table 3.3. Limit of detection (LOD) and limit of qualification (LOQ) for insecticides used in the 2021 study.

Compound	M+H (m/z)	Qualifier (m/z)	LOD (µg/g)	LOQ (µg/g)
imidacloprid	209	175	0.001	0.002
flupyradifurone	125.9	90	0.001	0.003
spirotetramat	216.2	117.1	0.027	0.080
azadirachtin	703.3	585.2	0.007	0.020

Statistical Analysis

An individual model for each insecticide was created when analyzing concentration of active ingredient recovered from plant tissue and larval survival. The estimation method used was an adaptive Gauss-Hermite quadrature. Reported figures and tables show means

and standard error of the means. When the ANOVA results were significant, a mean comparison using Tukey's HSD was used to determine the differences among the different rates.

For comparisons of concentration recovered in plant tissue type, each insecticide model consisted of tissue type, rate, and the interaction of the two. Since this analysis is a split block design, replicate was considered a random variable while rate was the fixed variable. When the ANOVA results were significant for the main factors, a means comparison using Tukey's HSD was used to determine the differences among the treatments.

The statistical models for analysis of percent larval survival included rate as the main variable and replicate as a random variable. Square root transformation was used to correct violation of normal distribution for each larval response variable: percent alive, percent moribund, and percent dead. Reported figures and tables show means and standard error of the means. When the ANOVA results were significant, a mean comparison using Tukey's HSD was used to determine the difference among rates. All analyses were performed using PROC GLIMMIX procedure in SAS (SAS 9.4, SAS Institute Inc., NC USA).

RESULTS

Mortality Bioassays

The bioassay developed for this study was successful for comparing mortality of larvae following exposure to azadirachtin, imidacloprid, flupyradifurone, and spirotetramat treatment dilutions of 10%, 1%, 0.1%, and 0.01%. In 2020 untreated control had 97.2%

alive, 2.8% moribund, and 0% dead mean larval response at 5 DAT. In 2021 untreated control had 100% alive larvae at 5 DAT.

Table 3.4. Mean percentage ± SEM alive, moribund, and dead larval responses in 2020 and 2021 bioassay at 5 DAT for azadirachtin treated shoots. Means not followed by the same letter within a column are significantly different base on mean separation using Tukey's HSD, P<0.05.

	Azadirachtin 2020			Azadirachtin 2021		
Rate	**Alive**	**Moribund**	**Dead**	**Alive**	**Moribund**	**Dead**
UTC	97.2 ± 2.8 a	2.8 ± 2.8 a	0 ± 0 a	100 ± 0	0 ± 0 a	0 ± 0
0.01%	60.8 ± 11.1 ab	6.8 ± 3.6 ab	32.4 ± 12.1 b	93.5 ± 2.9	6.5 ± 2.9 a	0 ± 0
0.10%	90.0 ± 4.1 ab	9.9 ± 4.1 ab	0 ± 0 a	88.7 ± 4.7	11.3 ± 4.7 a	0 ± 0
1%	85.7 ± 10.1 ab	10.7 ± 10.7 a	3.6 ± 3.6 a	98.1 ± 1.9	1.9 ± 1.9 a	0 ± 0
10%	55.0 ± 7.0 b	35.15 ± 9.7 b	9.9 ± 4.2 ab	52.9 ± 18.0	47.1 ± 18.0 b	0 ± 0

In 2020, the one-way ANOVA for rate of azadirachtin on mean percent alive larvae was significant ($F_{4, 12} = 5.26$, P = 0.0111) (Table 3.4). Mean separation using Tukey's HSD (P< 0.05) found a significantly lower mean percentage of larvae lower in the 10% rate compared to the untreated control. The 10% rate did not significantly differ in mean percentage of alive larvae from the other rates tested (Table 3.4). The 10% rate had marginally lesser mean percentage of alive larvae than 0.10% (P = 0.0611). There was a significant one-way ANOVA for mean percentage of moribund larvae ($F_{4, 12} = 4.56$, P = 0.0181) and there was significantly more mean percentage of moribund larvae at the 10% rate than in the untreated control and 1% rate in 2020 (Table 3.4). There was a significant one-way ANOVA for mean percent dead larvae in 2020 ($F_{4, 12} = 9.17$, P = 0.0012). The mean percentage of dead larvae for 0.01% rate was greater by 10-fold than 1% rate and was also significantly greater than the untreated control and 0.10% rate (Tukey's HSD, P < 0.05). The integrity of the gall was compromised in few replications of the 1% and 0.01% rate gall became softened and/ or shriveled.

In 2021 rate of azadirachtin was not significant on the outcome of mean percent alive larvae in galls on shoots administered azadirachtin (Table 3.4). Mean percent alive larvae were numerically lesser in 10% rate compared to all other rates and the untreated control. The one-way ANOVA for rate on mean percent moribund larvae was significant in 2021 ($F_{4, 12} = 11.89$, $P = 0.0004$) (Table 3.4). The 10% rate had significantly more mean percentage of moribund larvae than any other rate and the untreated control (Tukey's HSD, $P < 0.05$). There were no dead larvae found in 2021 bioassay.

Table 3.5. Mean percentage ± SEM alive, moribund, and dead larval responses in 2020 and 2021 bioassay at 5 DAT for imidacloprid treated shoots. Means not followed by the same letter within a column are significantly different base on mean separation using Tukey's HSD, P<0.05.

	Imidacloprid 2020			**Imidacloprid 2021**		
Rate	**Alive**	**Moribund**	**Dead**	**Alive**	**Moribund**	**Dead**
UTC	97.2 ± 2.8 a	2.8 ± 2.8 a	0 ± 0	100 ± 0 a	0 ± 0 a	0 ± 0
0.01%	57.7 ± 11.8 ab	37.7 ± 10.1 b	4.6 ± 3.2	64.2 ± 6.2 ab	34.1 ± 4.6 b	1.7 ± 1.7
0.10%	36.3 ± 11.6 bc	59.5 ± 9.9 b	4.2 ± 4.2	49.6 ± 5.9 b	50.4 ± 5.9 b	0 ± 0
1%	21.1 ± 2.6 c	74.1 ± 2.2 b	4.8 ± 3.4	5.4 ± 3.2 c	71.9 ± 19.2 b	22.6 ± 20.7
10%	27.5 ± 2.8 bc	68.8 ± 3.4 b	3.8 ± 2.4	8.3 ± 3.5 c	62.1 ± 21.1 b	29.6 ± 23.6

In 2020, rate of imidacloprid administered was found to be significant in mean percent alive larvae ($F_{4, 12} = 12.41$, $P = 0.0003$) (Table 3.5). Mean percentage of alive larvae was significantly lower in rates 0.10%, 1%, and 10% when compared to the untreated control (Tukey's HSD; $P < 0.05$). Mean percentage of alive larvae was significantly lower for the 1% rate compared to the 0.01% rate (Tukey's HSD, $P < 0.05$). Rate also had a significant effect on mean percentage of moribund larvae ($F_{4, 12} = 27.02$, $P = <0.0001$) in 2020 (Table 3.5). There were greater mean percentage of moribund larvae found in all rates compared to the untreated control (Tukey's HSD, P<0.05) (Table 3.5). There was marginally lesser mean percentage of moribund larvae found in the 0.01% rate compared to the 1% rate ($P = 0.0641$). The number of dead larvae was not significant and was found to be only about 4%

regardless of rate in 2020. The integrity of the gall was compromised in some replications of the 0.1% rate gall became softened.

In 2021, rate of imidacloprid administered had a significant effect on mean percent alive larvae in 2021 ($F_{4, 12} = 52.14$, $P < 0.0001$) (Table 3.5). The lower rates, 0.01% and 0.10%, were significantly higher in mean percent alive larvae than the 1% and 10% rates (Tukey's HSD, $P<0.005$). There were no significant differences between mean percent alive larvae in 0.01% and 0.1% rates, and no significant differences in 1% and 10% rates. All rates besides 0.01% had significantly lower mean percent of alive larvae than the untreated control (Tukey's HSD, $P < 0.05$). The one-way ANOVA for rate on mean percent moribund larvae was significant ($F_{4, 12} = 6.85$, $P = 0.0041$) and all rates of imidacloprid had significantly more mean percentage of moribund larvae than the untreated control in 2021. There was no significant difference in mean percent dead larvae in 2021. Some replicates had softened and shriveled galls, and phytotoxicity to the leaves at the 10% rate.

Table 3.6. Mean percentage ± SEM alive, moribund, and dead larval responses in 2020 and 2021 bioassay at 5 DAT for imidacloprid treated shoots. Means not followed by the same letter within a column are significantly different base on mean separation using Tukey's HSD, $P<0.05$.

	Flupyradifurone 2020			**Flupyradifurone 2021**		
Rate	**Alive**	**Moribund**	**Dead**	**Alive**	**Moribund**	**Dead**
UTC	97.2 ± 2.8 a	2.8 ± 2.8 a	0 ± 0	100 ± 0 a	0 ± 0 a	0 ± 0
0.01%	40.0 ± 15.2 b	38.7 ± 8.8 b	21.4 ± 18.9	84.4 ± 11.8 a	15.6 ± 11.8 a	0 ± 0
0.10%	71.1 ± 8.5 b	21.9 ± 6.6 b	6.9 ± 4.2	87.1 ± 2.0 a	12.9± 2.0 ab	0 ± 0
1%	57.1 ± 12.1 b	39.3 ± 12.9 b	3.6 ± 2.1	30.4 ± 13.91 b	69.6 ± 13.9 c	0 ± 0
10%	53.9 ± 4.5 b	46.2 ± 4.5 b	0 ± 0	47.3 ± 5.3 ab	52.7 ± 5.3 bc	0 ± 0

There was a significant impact of rate on mean percent larval response for alive for flupyradifurone treated shoots in the 2020 bioassay ($F_{4, 12} = 3.38$, $P = 0.0452$). All rates had significantly lower mean percent alive than the untreated control (Tukey's HSD, $P < 0.05$) (Table 3.6). There were greater mean percentage of moribund larvae in all rates of

flupyradifurone than the untreated control in 2020 ($F_{4, 12}$ = 10.39, P = 0.0007) (Table 3.5). There was no significant impact of rate on mean percent larval response for dead for flupyradifurone treated shoots in the 2020 bioassay. The integrity of the gall was compromised in some replications of the 10% and 0.01% rates. The gall became softened and, in some cases, shriveled.

In 2021 the rate of flupyradifurone was significant for the response of percent alive larvae ($F_{4, 12}$ = 6.82, P = 0.0042). Mean percent alive larvae was significant lower at 1% rate compared to 0.01%, 0.1%, and untreated control (Tukey's HSD, P<0.05). Rate was also significant for mean percent moribund larvae ($F_{4, 12}$ = 14.67, P = 0.0001) (Table 3.6). There were greater mean percent moribund larvae in the 1% and 10% rate than 0.01% rate and untreated control in 2021 (Tukey's HSD, P < 0.05). The 0.10% rate had significantly lesser mean percentage of moribund larvae than the 1% rate (Tukey's HSD, P < 0.05). There were no dead larvae found in the 2021 bioassay. Some galls became softened at the 10% rate in response of phytotoxicity.

Table 3.7. Mean percentage ± SEM alive, moribund, and dead larval responses in 2020 and 2021 bioassay at 5 DAT for imidacloprid treated shoots. Means not followed by the same letter within a column are significantly different base on mean separation using Tukey's HSD, P<0.05.

	Spirotetramat 2020			Spirotetramat 2021		
Rate	**Alive**	**Moribund**	**Dead**	**Alive**	**Moribund**	**Dead**
UTC	97.2 ± 2.8	2.8 ± 2.8	0 ± 0 a	100 ± 0 a	0 ± 0	0 ± 0 a
0.01%	67.3 ± 15.9	21.0 ± 10.7	11.7 ± 9.1 ab	69.2 ± 15.3 a	27.7 ± 12.3	3.1 ± 3.1 a
0.10%	63.5 ± 13.5	20.8 ± 12.5	15.6 ± 15.6 ab	79.7 ± 8.6 a	9.4 ± 6.0	10.9 ± 9.0 ab
1%	64.3 ± 7.5	18.2 ± 12.5	17.5 ± 6.9 ab	59.8 ± 9.9 a	12.9 ± 5.6	27.3 ± 7.4 bc
10%	35.7 ± 17.4	9.5 ± 9.5	54.8 ± 17.0 b	0.1 ± 0.1 b	30.0 ± 12.2	60.0 ± 13.5 c

In 2020 there was no significant impact of spirotetramat rate on mean percent larval response for alive or moribund larvae in 2020. Mean percent alive ranged from 35.7% to 67.2% (Table 3.7). Mean percent moribund ranged from 9.5% to 21.0% (Table

3.7). Some larvae in all rates changed from an elongated body to a compressed, shorter and wider, body. The one-way ANOVA for mean percent dead larvae was significant in 2020 ($F_{4, 12} = 3.87$, $P = 0.0304$). There was significantly greater mean percentage of dead larvae found in the 10% rate compared to the untreated control (Tukey's HSD, $P < 0.05$). The integrity of the gall was compromised in some replications of the 10%, 1%, and 0.01% rates. The gall became softened and, in some cases, shriveled.

In 2021 rates of spirotetramat were significant for the mean percent alive larvae ($F_{4, 12} = 12.44$, $P = 0.0003$). Lesser mean percentage of alive larvae were found at the 10% rate compared to all other rates and untreated control (Tukey's HSD, $P<0.05$). There was no significant interaction of rate of spirotetramat on percent moribund larvae in 2021. There was significant interaction of rate spirotetramat on mean percent dead larvae in 2021 ($F_{4, 12} =12.23$, $P = 0.0003$) (Table 3.7). Less mean percent dead larvae were found at the 10% rate compared to the 0.10% rate, 0.01% rate, and untreated control (Tukey's HSD, $P<0.05$). The 1% rate had significantly lesser mean percent of dead larvae than 0.01% rate and the untreated control (Tukey's HSD, $P < 0.05$). Majority of the galls in the 10% rate became shriveled due to phytotoxicity.

Residue Recovery

Azadirachtin recovered in gall tissue was found to have no significant differences based on rate in 2020 bioassay. Active ingredient recovered in 2020 bioassay ranged from 7.95 ppb to 10.06 ppb (Figure 3.2). Azadirachtin recovered in leaf tissue was found to be significantly different based on rate administered in the 2020 bioassay ($F_{3,12}= 5.96$, $P=0.0099$). The active ingredient recovered for 0.01%, 0.1%, and 1% rates did not

significantly differ and were found to be less than 1 ppb (Figure 3.2). The 10% rate had a mean recovery of 1.13 ppb and was significantly greater than 0.01% and 1% (Tukey's HSD, $P<0.05$) (Figure 3.2). When comparing active ingredient recovered from gall tissue and leaf tissue in the 2020 bioassay, azadirachtin was found to have significantly lower active ingredient recovered in leaf tissue than gall tissue ($F_{1, 12} = 18.33$, $P = 0.0011$). There was no significant interaction using Tukey's HSD between rate and plant tissue type for mean active ingredient recovered for azadirachtin. Few replicates should phytotoxicity in gall and leaves in the 1% and 0.01% rates.

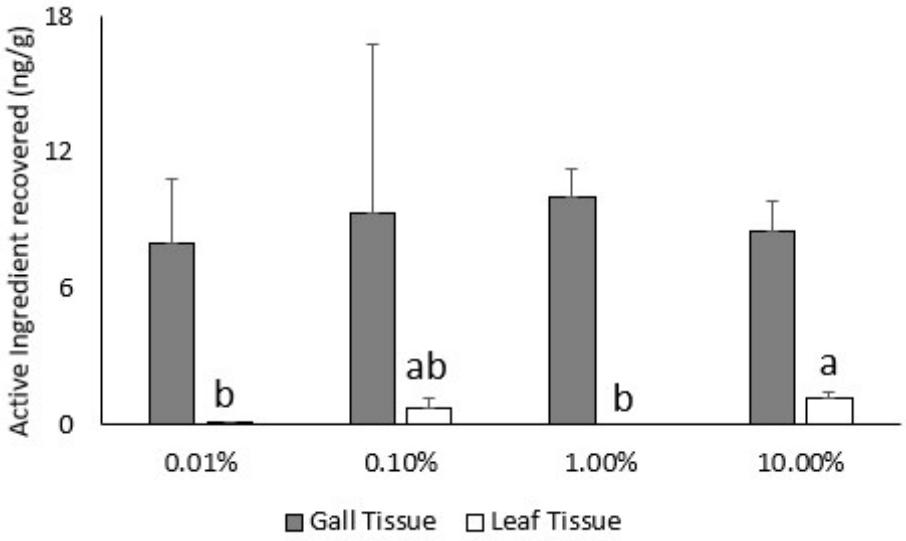

Figure 3.2. The effect of azadirachtin concentration on the mean active ingredient (ng/g) recovered from gall tissue (grey bars) and leaf tissue (white bars) in highbush blueberry shoots in 2020. Bars of the same color with different letters are significantly different using Tukey's HSD, $P < 0.05$. Error bars indicate SEM.

Rate of azadirachtin was found to be significant in the 2021 bioassay for residue recovered in gall tissue ($F_{3, 12} = 6.63$, $P < 0.05$). The mean recovery of azadirachtin in gall tissue at the 10% rate was 2.51 ppm and was significantly greater than any other rate (Tukey's HSD, $P < 0.05$) (Figure 3.3). In the 2021 bioassay, azadirachtin was recovered in leaf tissue was found to be significant based on rate administered ($F_{3, 12} = 159.63$, P

<0.0001). Mean active ingredient recovered at the 10% rate was 11.081 ppm, which is significantly greater than all the other rates tested (Tukey's HSD, P <0.05) (Figure 3.3). As rate decreased so did the mean recovery of azadirachtin. The recovery of azadirachtin did not significantly differ between 1%, 0.1%, and 0.01% rates and all were less than 1 ppm (Figure 3.3). The two-way ANOVA was significant for rate and plant tissue type in the 2021 bioassay ($F_{3, 12} = 33.03$, $P < 0.0001$), and there was significantly greater active ingredient recovered in the leaf tissue than gall tissue on shoots administered 10% rate (Tukey's HSD, $P < 0.05$).

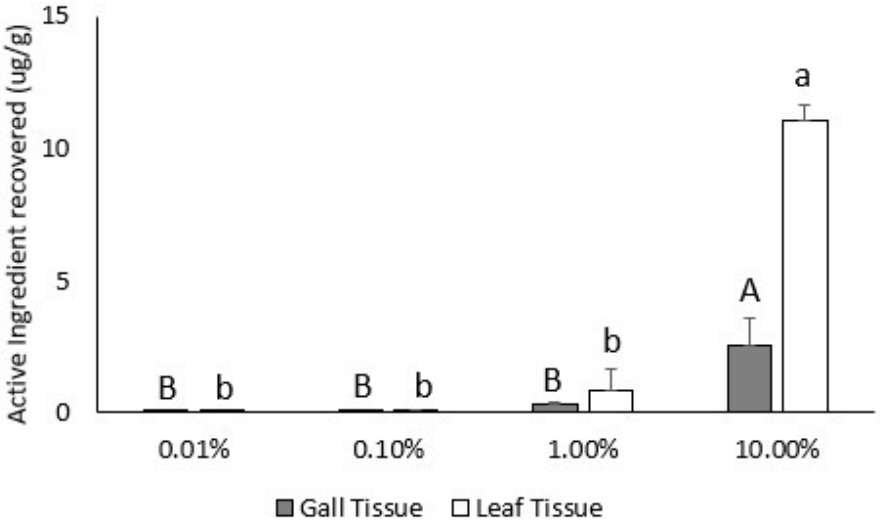

Figure 3.3. The effect of azadirachtin concentration on the mean active ingredient (ng/g) recovered from gall tissue (grey bars) and leaf tissue (white bars) in highbush blueberry shoots in 2021. Bars of the same color with different letters are significantly different using Tukey's HSD, $P < 0.05$. Error bars indicate SEM.

Imidacloprid recovered in gall tissue was found to be significantly different based on rate administered in 2020 ($F_{3,11} = 9.22$, $P = 0.0024$). Residue recovered at the 10% rate was significantly greater than the 0.1% and 0.01% rates in 2020 (Tukey's HSD, P<0.05) (Figure 3.4). This concentration was found to be 16.50 ppm. The residue recovered in gall tissue at 1% was 10.73 ppm and did not significantly differ from any of the other (Figure

3.4). Imidacloprid recovered in leaf tissue was found to be significantly different based on rate administered in 2020 ($F_{3,12}$= 16.71, P=0.0001). Imidacloprid recovered in leaf tissue at 0.01% (0.09 ppm) and 0.10% (0.36 ppm) rate did not significantly differ from each other but were significantly lower than 1% and 10% (Tukey's HSD, P<0.05) (Figure 3.4). Imidacloprid had significantly lesser active ingredient recovered in leaf tissue than gall tissue in 2020 ($F_{1,11}$ = 18.53, P = 0.0012). The mean separation of the interaction of rate and plant tissue type found the mean concentration of active ingredient recovered from leaf tissue at 10% significantly lesser than that of gall tissue (Tukey's HSD, P < 0.05). Few replicates should phytotoxicity in gall and leaves in the 0.1% rate.

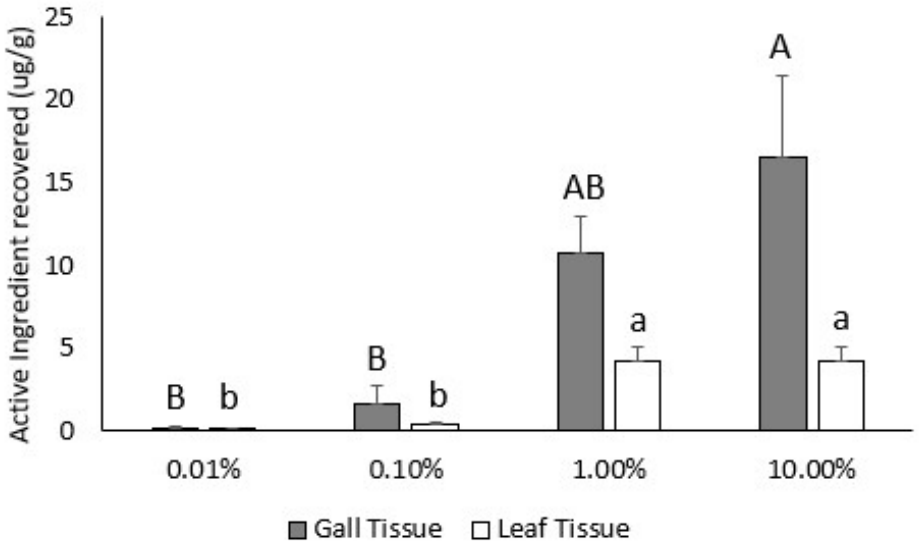

Figure 3.4. The effect of imidacloprid concentration on the mean active ingredient (ng/g) recovered from gall tissue (grey bars) and leaf tissue (white bars) in highbush blueberry shoots in 2020. Bars of the same color with different letters are significantly different using Tukey's HSD, P < 0.05. Error bars indicate SEM.

Imidacloprid recovered in gall tissue was found to be significantly different based on rate administered in 2021 ($F_{3,12}$ = 10.28, P = 0.0012). Residue recovered at the 10% rate was significantly greater than all rates in 2021 (Tukey's HSD, P<0.05) (Figure 5). Residue recovered from gall tissue at the 10% rate in 2021 had a mean recovery concentration of

8.71 ppm. The 1% rate in 2021 had a mean recovery of 3.46 ± 0.65 ppm active ingredient in gall tissue. Rates 0.10% and 0.01% had a mean recovery of less than 1 ppm in gall tissue. Imidacloprid recovered in leaf tissue was found to be significantly different based on rate administered in 2021 ($F_{3,12}$ = 19.62, P<0.0001). In 2021, mean active ingredient recovered in leaf tissue at 0.01% (0.73 ppm) and 0.10% (5.80 ppm) rate did not significantly differ from each other but were significantly lower than 1% and 10% (Tukey's HSD, P<0.05) (Figure 3.5). In 2021, there was no statistical evidence of an interaction of rate and plant tissue type, though leaf tissue had numerically greater concentration at every rate. Few replicates should phytotoxicity in gall and leaves in the 10% rate.

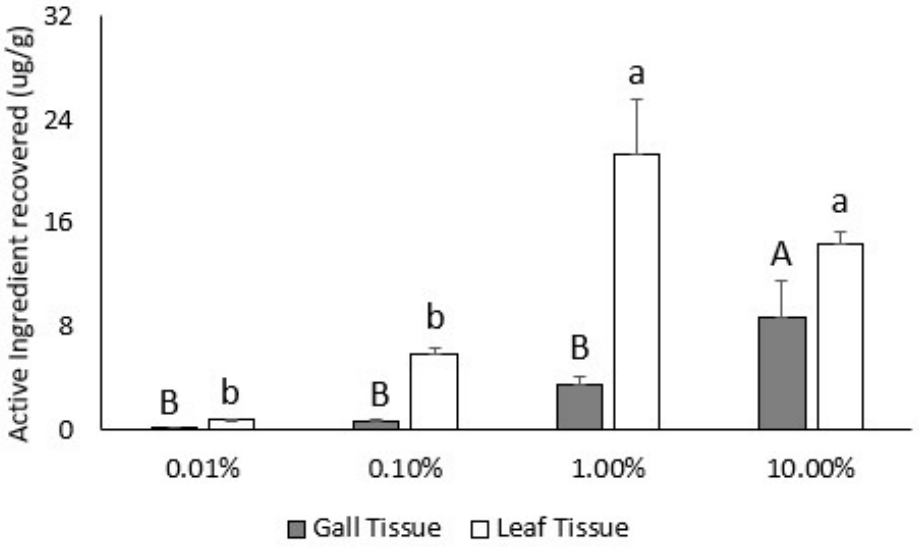

Figure 3.5. The effect of imidacloprid concentration on the mean active ingredient (ng/g) recovered from gall tissue (grey bars) and leaf tissue (white bars) in highbush blueberry shoots in 2021. Bars of the same color with different letters are significantly different using Tukey's HSD, P < 0.05. Error bars indicate SEM.

Flupyradifurone recovered in gall tissue was found to be significantly different based on rate administered in 2020 ($F_{3,12}$ = 4.33, P= 0.0276). Residue recovered at the 10% rate, 9.48 ppm, was significantly higher than the 0.01% rate, 1.11 ppm in 2020 (Tukey's HSD, P<0.05) (Figure 3.6). The 1% and 0.1% rates did not result in significantly different

flupyradifurone recovered in gall tissue from any rate (Figure 3.6). Flupyradifurone recovered in leaf tissue did not significantly differ by rate in 2020. Numerically the active ingredient recovered increased as rate increased, 1.65 ppm to 3.19 ppm (Figure 3.6). Flupyradifurone was recovered in lesser concentration in leaf tissue than gall tissue in 2020 ($F_{1, 12} = 4.86$, $P = 0.0477$). There were no mean separations found to be significant for the interaction of rate and plant tissue type for concentration of active ingredient recovered using Tukey's HSD, $P<0.05$. There was phytotoxicity observed on the leaves and galls in the 10% rate and on galls in the 0.01%.

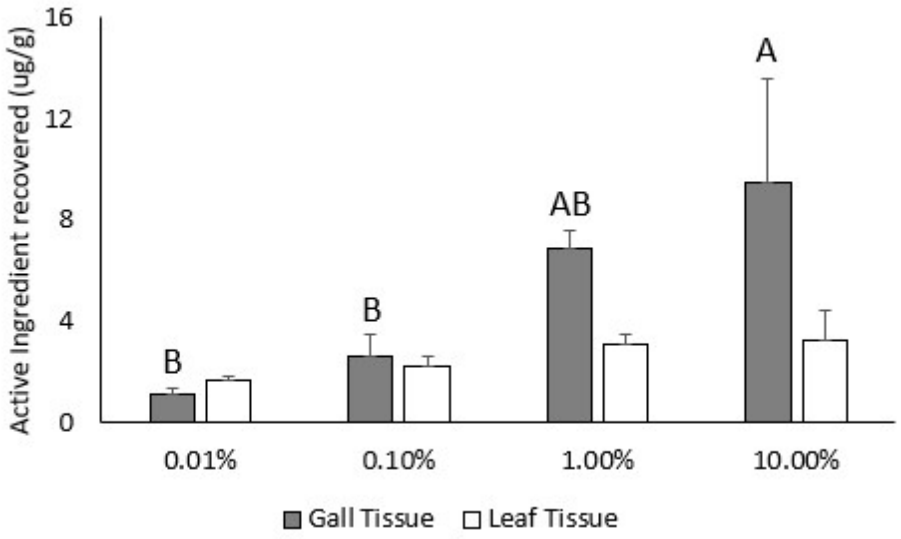

Figure 3.6. The effect of flupyradifurone concentration on the mean active ingredient (ng/g) recovered from gall tissue (grey bars) and leaf tissue (white bars) in highbush blueberry shoots in 2020. Bars of the same color with different letters are significantly different using Tukey's HSD, $P < 0.05$. Error bars indicate SEM.

Flupyradifurone recovered in gall tissue was found to be significantly different based on rate administered in 2021 ($F_{3,12} = 54.59$, $P <0.0001$) (Figure 3.7). Residue recovered at the 10% rate, 18.01 ppm, was significantly greater than recovery from all other rates (Tukey's HSD, $P <0.0001$) (Figure 3.7). The other rates tested did not significantly differ from each other, 1% (5.13 ppm), 0.10% (1.17 ppm), and 0.01% (0.79

ppm) in 2021 (Figure 3.7). In 2021, recovery of flupyradifurone in leaf tissue was found to be significantly different based on rate administered ($F_{3,12}$ = 185.91; P < 0.0001). The 10% and 1% rate had significantly greater mean active ingredient recovered than 0.10% and 0.01% rate (Tukey's HSD; P <0.0001) (Figure 3.7). The mean active ingredient recovered at the 10% rate was 29.93 ppm and was significantly greater than mean active ingredient recovered at the 1% rate, 18.20 ppm (Tukey's HSD; P<0.0001) (Figure 3.7). Rates 0.10% and 0.01% had a mean active ingredient concentration recovered of around 1 ppm. In 2021, flupyradifurone was recovered in greater concentration in leaf tissue than gall tissue ($F_{3,12}$ = 17.32, P = 0.0001). Using Tukey's HSD mean separation, mean active ingredient recovered was greater in leaf tissue than gall tissue in the 10% and 1% rates (P<0.0005). Few replicates should phytotoxicity in gall and leaves in the 10% rate.

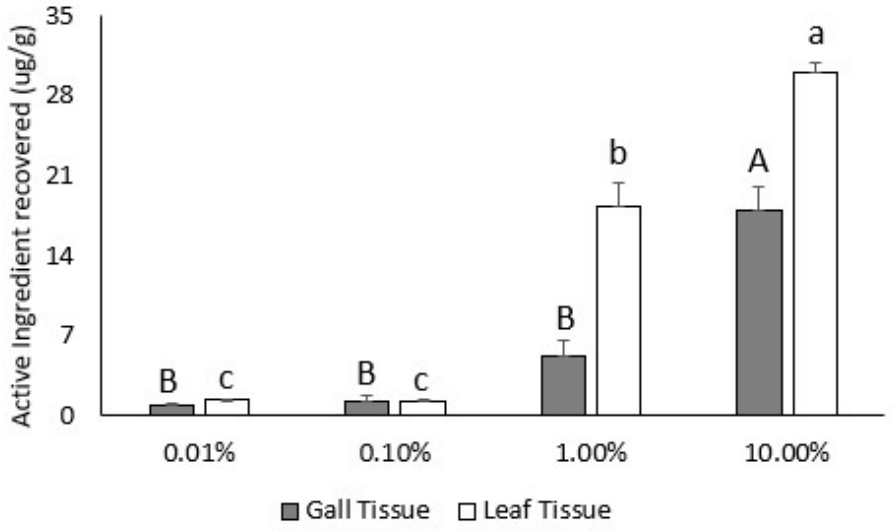

Figure 3.7. The effect of flupyradifurone concentration on the mean active ingredient (ng/g) recovered from gall tissue (grey bars) and leaf tissue (white bars) in highbush blueberry shoots in 2021. Bars of the same color with different letters are significantly different using Tukey's HSD, P < 0.05. Error bars indicate SEM.

Spirotetramat recovered in gall tissue was found to be significantly different based on rate administered in 2020 ($F_{3,12}$ = 9.08, P < 0.005) (Figure 3.8). The 10% rate resulted in

the greatest residue recovered, 74.44 ppm, compared to all other rates (Tukey's HSD, P<0.05) (Figure 3.8). In 2020, spirotetramat recovered in leaf tissue was significant based on rate ($F_{3,12}$ = 5.67, P < 0.05). Mean active ingredient recovered in leaf tissue at rates 0.01% 0.1%, and 1% did not significantly differ from each other (Figure 3.8). The 0.01% and 0.1% rate resulted in significantly lower active ingredient recovered in leaf tissue than 10% rate (Tukey's HSD, P < 0.05) (Figure 3.8). The mean active ingredient recovered from leaf tissue at the 1% rate was not significantly different from any other rate (Figure 3.8). There was no significant difference between overall mean of spirotetramat recovered from leaf tissue and gall tissue using Tukey's HSD, P < 0.05. Few replicates should phytotoxicity in gall and leaves in 10%, 1%, and 0.01% rates.

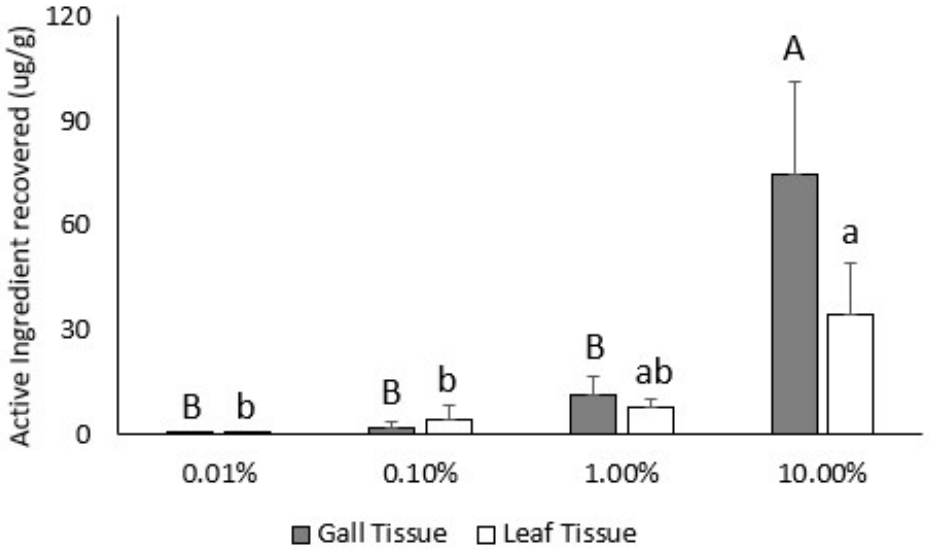

Figure 3.8. The effect of spirotetramat concentration on the mean active ingredient (ng/g) recovered from gall tissue (grey bars) and leaf tissue (white bars) in highbush blueberry shoots in 2020. Bars of the same color with different letters are significantly different using Tukey's HSD, P < 0.05. Error bars indicate SEM.

Spirotetramat recovered in gall tissue was found to be significantly different based on rate administered in 2021 ($F_{3,12}$ = 14.3, P < 0.0005). In 2021, the 10% and 1% rate did not differ from each other for active ingredient recovered but were significantly greater

than active ingredient recovered in shoots that received 0.10% and 0.01% (Tukey's HSD, P < 0.05) (Figure 3.9). In 2021, rate of spirotetramat was found be significant for recovered active ingredient in leaf tissue ($F_{3,12}$ = 33.41, P < 0.0001). After mean separation, the 10% rate was found to have greater concentration of active ingredient than any other rate, 7.28 ppm (Tukey's HSD, P < 0.05) (Figure 3.9). The remainder of rates had a mean recovery of less than 1 ppm (Figure 3.9). The two-way ANOVA for rate and tissue type was found to be significant ($F_{3, 12}$ = 17.05, P = 0.0001). After mean separation using Tukey's HSD (P<0.05), there was greater active ingredient recovered in leaf tissue than gall tissue on shoots administered the 10% rate. There was no further mean separation for interaction of rate and plant tissue type. Few replicates should phytotoxicity in gall and leaves in 10%.

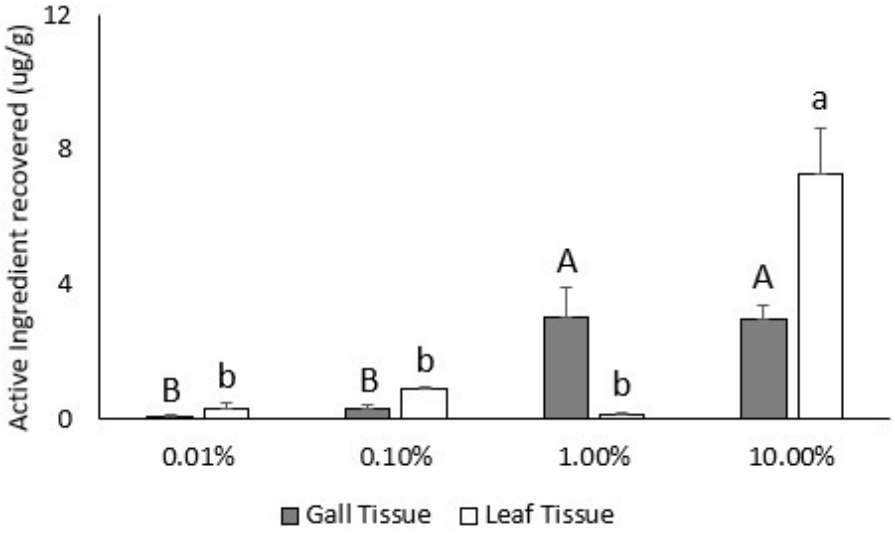

Figure 3.9. The effect of spirotetramat concentration on the mean active ingredient (ng/g) recovered from gall tissue (grey bars) and leaf tissue (white bars) in highbush blueberry shoots in 2021. Bars of the same color with different letters are significantly different using Tukey's HSD, P < 0.05. Error bars indicate SEM.

DISCUSSION

In this study we found all insecticides tested to be mobile in the shoot's vascular system to gall and leaf tissues, however, imidacloprid, flupyradifurone and spirotetramat reached substantially higher levels in plant tissues compared to azadirachtin. The recovery of these insecticides increased with percentage of field rate and the greatest increase in larval moribundity and death was seen at the highest rates, 1% and 10%, indicating a rate-dependent response. However, at these high rates larval survival may not be due solely to direct toxicity, as compound-induced break down of important gall tissues may also make the gall chambers uninhabitable. Variability in mortality from year to year may be due to the greater phytotoxicity observed in 2020. Residue was generally recovered in greater concentration in gall tissue than leaf tissue in 2020 and the opposite was observed in 2021, therefore we cannot conclude if galls act as a sink for chemical residue. The lower residue recovery in leaf tissue in 2020 may be due to the greater amount of phytotoxicity in the leaves at the highest rates.

Azadirachtin moved poorly into the shoots likely due to its chemical properties, thus resulting in limited ability to evaluate its toxicity to gall wasp. We observed when high rates of the solid formulation of azadirachtin were added to small amounts of water, a sticky, thickened product was created. We also observed the need for good agitation. It is likely that these chemical properties inhibited mobility in the shoots resulting in the greatest phytotoxic effects to be observed. In 2020, there were no differences in residue recovered between rates indicating that only small amounts of chemical were able to pass regardless of the rate administered. The larval death and moribund responses in 2020 were likely due to phytotoxicity causing the shoot to die off. In the 2021 bioassay we did

not observe phytotoxicity in as great of magnitude, likely because greater agitation was used, thus leading to more azadirachtin delivered to shoot and leaf tissue in a rate response. Mortality rates still remained low in 2021 likely due to azadirachtin's mode of action. Azadirachtin's mode of action is not well understood, but for some insects it acts as a growth disruptant (Morgan 2009; Thompson et al. 2018) and/or a feeding deterrent (Morgan 2009, Bezzar-Bendjazia et al. 2017; Thompson et al. 2018). It is likely that if azadirachtin had toxic effect on gall wasp larvae these modes of actions were not observable in 5 days. Azadirachtin is an attractive chemical because it is a biopesiticide and could aid organic growers in combating blueberry gall wasp. Further research should be done to assess the long-term effects of azadirachtin on blueberry stem gall wasp larvae.

Larvae exposed to imidacloprid experienced moribund effects such as slowed or no movement of the body besides mouth and/ or gut. Imidacloprid's primary site of action is in the nervous system where it acts as a competitive modulator for the nicotinic acetylcholine receptor (IRAC 2021) resulting in excitatory symptoms (Salgado and Saar 2004) or at low doses an antifeedant response (Nauen 1995 as cited in Salgado and Saar 2004; Nauen et al. 1998 et al. as cited in Salgado and Saar 2004, Wise et al. 2006). In this study we observed larvae to retract into a "c" shape which we believed meant they were not feeding. We are unsure whether the neonicotinoid mode of action or the anti-feeding behavior led to moribund responses. In Fanning and Isaacs study (2019), imidacloprid was applied by soil drench on blueberry stem gall wasp infested blueberry bushes and did not successfully reduce gall formation. Therefore, our bioassay results provide a potential explanation that larval response to imidacloprid does not cause death in time to stop gall formation.

Flupyradifurone is in the butenolide class of insecticides (IRAC 2021) and has a similar mode of action as the neonicotinoids, though was not found to have as great of a toxic effect on the larvae. The moribund behavior observed in this study resulted in slowed to no movement would most likely lead to death with more time. There was more residue recovered in the 2021 bioassay than the 2020 bioassay which resulted in a greater does-dependent response in moribund larvae. Flupyradifurone was also tested in Fanning and Isaacs' (2019) soil drench study and did not successfully reduce gall formation. We can conclude from this bioassay that flupyradifurone may not provide control of blueberry stem gall wasp or may not provide control in a timely matter that would impact gall formation.

Spirotetramat was the only insecticide tested that resulted in proportions of dead larvae in all rates for both years. Spirotetramat's mode of action is inhibitor of acetyl CoA carboxylase, preventing lipid biosynthesis which leads to death (IRAC 2021) which was clearly observed in the compression of some of the larvae bodies. In Fanning and Isaacs' (2019) study, spirotetramat (Movento) was applied by foliar spray and reduced the number of galls by nearly a third when compared to untreated plots. Isaacs et al. (2020) reports reduced adult emergence by 99% compared to untreated control galls. Our findings bridge the gap between treatment and emergence, showing spirotetramat is feed upon in gall tissues leading to growth failure of the larvae.

There are several limitations of a bioassay to consider when making inferences from these results to an application in an infested blueberry field. Systemics can be applied in different ways, and with each way there are biological and chemistry challenges to successful delivery (Campana 1978; Chaney 1978). Beyond the method of application,

there is potential for unequal distribution within the plant and even variance between different plant types (Chaney 1978; USDA 1960). This bioassay looked at the mortality impacts after five days and in a field scenario the larvae would likely have longer exposure to the insecticide residues and beginning at earlier instars. However, we do not know if this will be the case. Future research should aim to look at rates applied in infested fields, and periodic gall samples should be dissected to assess larval mortality.

In conclusion, while this study demonstrates that all four compounds are capable of vascular delivery to blueberry gall tissues; imidacloprid, flupyradifurone, and spirotetramat appear to have the greatest potential as systemics for controlling blueberry stem gall wasp. Azadirachtin was not found to be chemically compatible for delivery within blueberry shoots. Spirotetramat successfully affected the development of the larvae leading to its death. Imidacloprid and flupyradifurone caused slowed movement of larvae, and in some cases imidacloprid caused the larvae to stop feeding. Both responses have not been observable in previous studies that only looked at gall abundance and rearing of adult wasps. Imidacloprid, flupyradifurone and spirotetramat are labeled for use in the state of Michigan for systemic application (imidacloprid & flupyradifurone: soil drench, spirotetramat: foliar) and should be used interchangeably, based on mode of action, in an IPM for resistance management. Further, this bioassay method can be applied to other gall insects and the gall dissection methods can be applied to assess mortality in the field during the growing season.

CHAPTER 4: EFFICACY OF SOIL APPLIED IMIDACLOPRID AND FLUPYRADIFURONE TO CONTROL *HEMADAS NUBILIPENNIS* IN BLUEBERRY, LIBERTY VARIETY

ABSTRACT

Blueberry stem gall wasp (*Hemadas nubilipennis*) is a pest of highbush blueberry and can pose a challenge to control with foliar sprays due to adult activity during bloom and larval development within the plant tissues. This study tests the efficacy of imidacloprid and flupyradifurone applied with a low-pressure drench under commercial field conditions. We hypothesized that if the insecticide can move from roots upward to leaf, shoots, and gall tissues, then the insecticide will kill the internally-feeding larvae, leading to a reduction in gall formation and number of adult wasps. Both imidacloprid and flupyradifurone residues were detected at 14 DAT and 59 DAT, which represents the course of time where the gall wasp larva is developing. However, this study found no evidence that these insecticides inhibited gall development. There was evidence that wasp emergence (*H. nubilipennis* and inquiline species) was reduced in medium size galls. Future research should evaluate imidacloprid and flupyradifurone's success under different water delivery amounts, multiple applications, and soil moisture to improved efficacy.

INTRODUCTION

The blueberry stem gall wasp, *Hemadas nubilipennis* Ashmead (Hymenoptera: Pteromalidae) is a chalcid wasp measuring two to three millimeters in length (Isaacs et al. 2020) and is native to eastern North America (West and Shorthouse 1989). This gall wasp is known for its ability to form stem galls on expanding shoots of lowbush and highbush blueberry plants (West and Shorthouse 1989). The native host for blueberry stem gall

wasp is the lowbush blueberry, *Vaccinium angustifolium* (West and Shorthouse 1989) but it also infests susceptible cultivars of highbush blueberry, *Vaccinium corymbosum* (Isaacs and Van Timmeren 2016) such as Jersey, Northland, Pemberton, and Bluejay (Isaacs et al. 2020). Jersey variety was one of the most highly planted varieties in Michigan (USDA 2020), is highly susceptible to gall wasp infestation. In the 2018-2019 Michigan Fruit Inventory reported 27% of the acres of blueberry bushes planted in Michigan were Jersey variety (USDA 2020 as cited in Garcia-Salazar et al. 2020). Isaacs and colleagues (2020) report that low population levels of the gall wasp are a manageable nuisance to growers because bushes with few numbers of galls can be pruned off. However, when the population level is high, pruning costs out weight the benefit of managing the field (Isaacs et al. 2020). This pest has caused many commercial blueberry growers to remove susceptible blueberry varieties from their farms (Isaacs et al. 2020). Foliar-applied insecticides are generally known to be inadequate for controlling this pest.

Blueberry stem gall wasp adults are only active for a short period of time when blueberry bushes are growing rapidly (Hayman et al. 2003a; Isaacs et al. 2020). This timing corresponds with bloom when most insecticide applications are restricted to protect pollinators (Hayman et al. 2003a). When blueberry bushes are no longer blooming, blueberry stem gall wasps are inaccessible by most foliar applied insecticides because eggs and larvae are developing within the plant in structures called galls. These two issues, adult activity during bloom and larvae development within the plant, make the blueberry stem gall wasp challenging to control.

The blueberry stem gall wasp life cycle begins with adults emerging from galls formed in the previous year (Hayman et al. 2003a; Isaacs et al. 2020). Females lay eggs into

expanding shoots of blueberry bushes and injure the apex of shoots that received oviposition (Hayman et al. 2003a; Shorthouse et al. 1986). Each egg is deposited into a separate "channel" created by the ovipositor (Shorthouse et al. 1986), primarily in the pith of the shoot but may be in contact with vascular tissue and cortex near one end of the egg (West and Shorthouse 1989). Within 48 hours of oviposition the cells around each egg chamber in the shoot rapidly divide forming a gall which provides protection for the larvae that hatch in 10-14 days (Shorthouse et al. 1986; Hayman et al. 2003a). Twenty days after oviposition the gall is noticeably swelling, and nutritive cells are beginning to from in the cell lining of the chamber which the larvae are actively feeding upon (McAlister and Anderson 1932; Shorthouse 1986; Shorthouse et al. 1986). The nutritive cells are beneficial to the larvae because they contain higher levels of starch, sugars, lipids, and proteins allocated from the blueberry bush via vascular bundles (Dreger-Jauffret and Shorthouse 1992).

After 60-90 days the cells in the gall become woody and the overall shape of the gall is globular or reniform shape (Hayman et al. 2003a). Around the time the larvae stop feeding the chambers become "encapsulated by the hard sclerenchyma sheath" (Shorthouse 1986). They remain inactive until spring when they pupate and emerge as adults during another growing season (McAlister and Anderson 1932; Shorthouse et al. 1986; Hayman et al. 2003a).

The formation of the gall impacts blueberry production because it reduces the fruiting area and potentially contaminates fruit pack-out during harvest (McAlister and Anderson 1932; Hayman et al. 2003a). When the gall is located terminally, the shoot is found to be shorter with less weight in stems and leaves (Hayman et al. 2003). Not only

does a terminal gall "stunt" the shoots growth, but it also redirects the plant's energy and resources from fruiting and foliar expansion to benefit the insect (Rohfritsch and Shorthouse 1982; Shorthouse et al. 2005). Berry production was reduced 3% in a Novia Scotia study; however, this rate may become significantly greater as years go on (Hayman et al. 2003a). The other problem that blueberry growers face is that galls can contaminate harvested berries (Hayman et al. 2003a). Galls can be removed by the blueberry harvester machine, mistaken as fruit when similar in size during processing, and later be found in frozen and fresh berries (Hayman et al. 2003a).

Blueberry stem gall wasp is becoming an important pest to Michigan blueberry growers because of two changes in blueberry pest management (Isaacs et al. 2020). First, with the arrival of spotted winged drosophila (*Drosophila suzukii*, Diptera: Drosophilidae), a major invasive fly pest attacking the fruit, many late season insecticide sprays are applied to keep fly larvae out of the fruit. (Isaacs et al. 2020). These late summer insecticide applications occur when co-inhabitants (inquiline species) of the blueberry stem gall wasp's gall are active, and these insecticides may kill these natural enemies (Isaacs et al. 2020). Another change in blueberry pest management is the cancelation of an insecticide: azinphosmethyl which was used for treatments for fruitworms in blueberries and applied during the time adult blueberry stem gall wasp were active (Isaacs et al. 2020). There is evidence that this product may have provided control of blueberry stem gall wasp (Isaacs et al. 2020).

Efficacy of azinphosmethyl was assessed on Jersey blueberry bushes with confirmed blueberry stem gall wasp oviposition activity (Fanning and Isaacs 2019a). Azinphosmethyl was effective in reducing the total number of galls per bush with approximately 90%

reduction in the total number of galls when compared to the untreated control bushes (Fanning and Isaacs 2019a). This study also tested other foliar applied insecticides: the second most effective product was cyclaniliprole (Verdepryn) with an approximate 64% reduction in the total number of galls per bush (Fanning and Isaacs 2019a). In addition, adult emergence from cyclaniliprole (Verdepryn) treated bushes resulted in a 95% reduction compared to untreated bushes (Isaacs and Wise 2020).

Soil applications of systemic insecticides: dinotefuran (Scorpion), flupyradifurone (Sivanto), thiamethoxam (Platinum), imidacloprid (Admire Pro) had no impact on reducing gall formation in Fanning and Isaacs (2019b) study. Though this study was not applied at the ideal timing directly following bloom. A foliar application of the systemic spirotetramat (Movento) reduced the number of galls (28 ± 2.85 mean ± SEM) when compared to untreated plots (43 ± 4.16 mean ± SEM), though this is not statistically significant. Larvae were 99% controlled in galls collected from bushes treated with a foliar application of spirotetramat when compared to untreated control bushes (Isaacs et al. 2020). In addition, the following year's blueberry stem gall wasp population was reduced, however, galls were still observed on the bushes.

Systemics insecticides move in the vascular system of plants to reach the juvenile pest stage within the plant tissues (Chaney 1978). Spirotetramat is applied by foliar sprays and utilizes the phloem to transfer to other parts of the plant (Chaney 1978). Systemics can also be applied through drip irrigation which are absorbed by the roots, transferred into the vascular system, and move upward through the xylem into expanding tissues (Doccola and Wild 2012; Chaney 1978). This process is called chemigation. The overall advantages of these three application methods of systemics is the protection from sources of

degradation, like sun and wash-off, and longer residual exposure to insect pests (Wise 2016).

Chemigation is an ideal approach to controlling blueberry stem gall wasps which spend most of their life feeding within plant tissues. In this study we asked the following questions: (1) can systemic insecticides applied by chemigation reduce galls and/or reduce number of adult wasps emerged (2) does the active ingredient recovered from leaf, shoot, and gall tissue confirm delivery from roots to shoots where galls are formed?

We hypothesized that the insecticides applied by chemigation will move systemically within the blueberry bush to leaf, shoot, and gall tissues leading to a reduction in gall formation and number of surviving adults emerged. In order to test the efficacy of chemigation applied insecticides in blueberries for control of blueberry stem gall wasp, imidacloprid and flupyradifurone were applied to blueberry bushes in a commercial planting using a low-pressure drip system.

MATERIALS & METHODS

Treatment

Four bush plots of "Liberty" variety blueberry bushes on a commercial blueberry farm in North Muskegon, MI were randomized and assigned treatments using a complete random block design. There were four replicates per treatment. Treatments included imidacloprid (Admire Pro SC Flowable, Bayer CropScience LP, Research Triangle Park, NC; 0.285 ml/bush or 0.157 g AI/bush) and flupyradifurone (Sivanto HL, Bayer CropScience, St. Louis, MO; 0.143ml/bush or 0.057g AI/bush). Amount of chemical applied to each bush

was determined by calculating the maximum labeled soil drench rate per bush. Untreated control bush blocks were also included in this study.

Treatments were applied by soil drench post-bloom on June 11, 2021. Drench application equipment included two drip lines, 4.9 meters in length (1.27cm diameter, 60.96cm emitter spacing, DripWorks, Inc., Willits, CA). These drench lines were placed next to the grower's irrigation lines with "U-shaped wire hold downs" (DripWorks, Inc., Willits, CA) to match irrigation emitters (Figure 4.1). The two drip lines connected to a single hose where the treatment solution was contained in a 2-liter bottle (Item# 282-12, R & D Sprayers, Opelousas, LA) and pressurized by a carbon dioxide tank with a regulator. Each treatment plot received 2 liters of water and the assigned chemical. Pressure was approximately 3 PSI. Following treatment, an additional 2 liters of water was used to rinse the lines and to aid in delivering chemicals into the soil.

Figure 4.1. Low-pressure drip setup used to apply insecticides to mature highbush blueberry plants.

Insect and Gall Assessments

Fourteen days after treatment (June 25, 2021) shoots containing swelling galls were quantified. Small swelling was approximately 10 mm or less in length, medium swelling was 10 mm to 20 mm, and large swelling was greater than 20 mm.

On December 23, 2021, each bush was assessed for number of small, medium, and large galls. Small galls were less than 15mm. Medium galls were 15mm to 25mm. Large galls were greater than 25mm. A sample of galls from each size category were removed from the two middle bushes of each plot and stored in cold storage at the Trevor Nichols Research Center [TNRC] (4°C). On January 24, 2022, ten small galls, two medium galls, and one large were selected from each middle bush. Galls of the same size from the same bush

were placed in perforated souffle cups (96ml) in an environmental growth chamber (16L:8D photoperiod, ~23°C, 55% RH) for rearing. The total mass of the galls in each cup was recorded. Emerged wasp adults were counted and identified to species or genera on March 14-15, 2022.

Collection for Residue Assessment

Residue was collected twice corresponding with the swelling gall assessment and the completion of the gall development. The first plant tissue collection was 14DAT (day after treatment) on June 25, 2021. The second plant tissue collection was 59DAT on August 9, 2021. Twenty shoots without any sign of potential gall formation were collected, leaves removed. Shoots collected on the 14DAT were specifically chosen to be the same age as the shoots growing when the gall wasp infestation occurred. The shoots collected on 59DAT were shoots expanding anytime during the growing season, though very young fleshy shoots were avoided. A total of eight galls were also collected at 59 DAT from the middle two bushes of each plot. Samples were stored in sealed, 120 ml graduated glass bottles (Qorpak, Clinton, PA) with 50 ml dichloromethane, 4 grams of magnesium sulfate, and 1 gram of sodium chloride in a walk-in cold room (4 °C) at the MSU Trevor Nichols Research Center in Fennville MI for future preparation for residue analysis.

Residue Sample Preparation and Analysis

The residue sample preparation and analysis methods for this study were adapted from the QuEChERs method (Lehotay, 2011) and used in several other injection studies including VanWoerkom et al. (2014) and Wheeler et al. (2020). All samples were in cold

storage for approximately two months. One day prior to decanting all residue samples, gall and shoot samples were sonicated for five minutes. Samples were decanted by pouring the sample through approximately 12 g of sodium sulfate in 125mm diameter filter paper that was folded into a funnel. Each funnel of sodium sulfate was placed on top of a 120 ml glass bottle that corresponded with the sample being poured. Funnels were removed. Each solvent was evaporated for up to 2 days. This left a residue film on the inside of the jar that was dissolved by adding 2 ml of acetonitrile to each jar and swirling for 90 seconds. The sample was filtered with a 0.45 µm PTFE hydrophobic filter attached to a 3 ml disposable syringe and transferred to a 2 ml glass vial. All samples were store in a freezer until processed with analytical equipment. All samples were analyzed using ultra performance liquid chromatography (UPLC) with the limit of detection (LOD) and limit of qualification (LOQ) stated on Table 4.1. Quantification was done with linear regression. Recovery of imidacloprid reported as µg of active ingredient per gram of plant tissue. Recovery of flupyradifurone reported as ng of active ingredient per gram of plant tissue. When "trace amounts" of insecticide is recovered, the midpoint between LOD and LOQ was used. Standards were obtained from the EPA Standard Repository. The software used was Waters Mass Lynx version 4.2.

Table 4.1. Limit of detection (LOD) and limit of qualification (LOQ) for insecticides.

Compound	M+H (m/z)	Qualifier (m/z)	LOD (µg/g)	LOQ (µg/g)
Imidacloprid	209	175	0.0003	0.001
Flupyradifurone	125.9	90	0.000001	0.0000003

Statistical Analysis

Within each treatment the two statistical models were created for the analysis of concentration (µg/g). The first model consisted of plant tissue type, collection time, and their interaction. Residue collected from galls were excluded since this was only collected at 59 DAT. The replication and the interaction between plant tissue type and replication were included as random factors and the latter was used as the error term for collection time. The second model consisted of plant tissue type sampled at 59 DAT with replication as the random factor. The normality assumption was tested by examining normal probability plots for both models. Due to the violation of the normality, the dataset was square-root transformed for the analysis. Reported figures and tables show means and standard error of the means. When the ANOVA results were significant, a mean comparison using Tukey's HSD was used to present the difference between the two treatments.

The statistical model for gall categories consisted of treatment, and the random factor was replication. A negative binomial distribution was used for swelling and final gall categories with Laplace method. Reported figures show means and standard error of the means. Mean of untreated control included each method type with replication. When the ANOVA results were significant, a mean comparison using Tukey's HSD was used to present the difference between the two treatments.

The statistical model for wasp emergence per gram of gall consisted of treatment, and the random factor was replication. Individual models were created for *H. nubilipennis,* inquiline species, and total wasps within each gall size category. Due to no large galls collected in the untreated plots, and in only 3 replicates of the treated plots, only emergence data was analyzed in imidacloprid and flupyradifurone reps 1-3. The number of

wasps per gram of gall was transformed using log (x +1). Reported figures show means and standard error of the means. When the ANOVA results were significant, a mean comparison using Tukey's HSD was used to present the difference between the two treatments. All analyses were performed using PROC GLIMMIX procedure in SAS (SAS 9.4, SAS Institute Inc., NC USA).

RESULTS

Gall Abundance

There was no significant impact of treatment on mean number of swelling galls within each gall category: small, medium, large, and total. Numerically, there were lesser mean number of small and large swelling galls in flupyradifurone plots compared to the untreated control and imidacloprid plots (Figure 4.2).

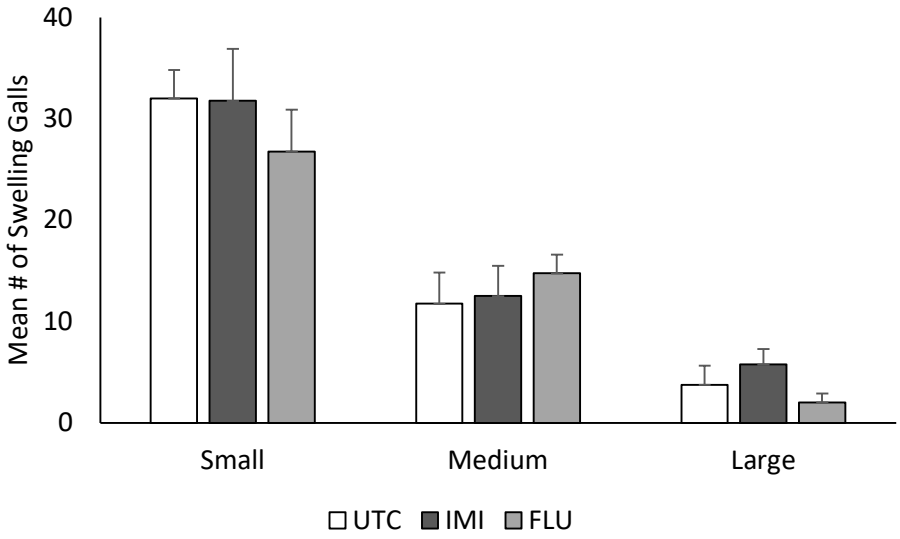

Figure 4.2. Mean ± SEM swelling galls per bush for highbush blueberry treated with different insecticides. Treatments include UTC: untreated control, IMI: imidacloprid, and FLU: flupyradifurone. Assessment was completed 14DAT on June 25, 2021.

There was no significant impact of treatment on mean number of developed galls within each gall category: small, medium, large, and total. Numerically, there were lesser mean number of small developed galls in imidacloprid plots compared to the untreated control and flupyradifurone plots (Figure 4.3). There was numerically lesser mean number of medium and large developed galls in the untreated control compared to the treatments (Figure 4.3).

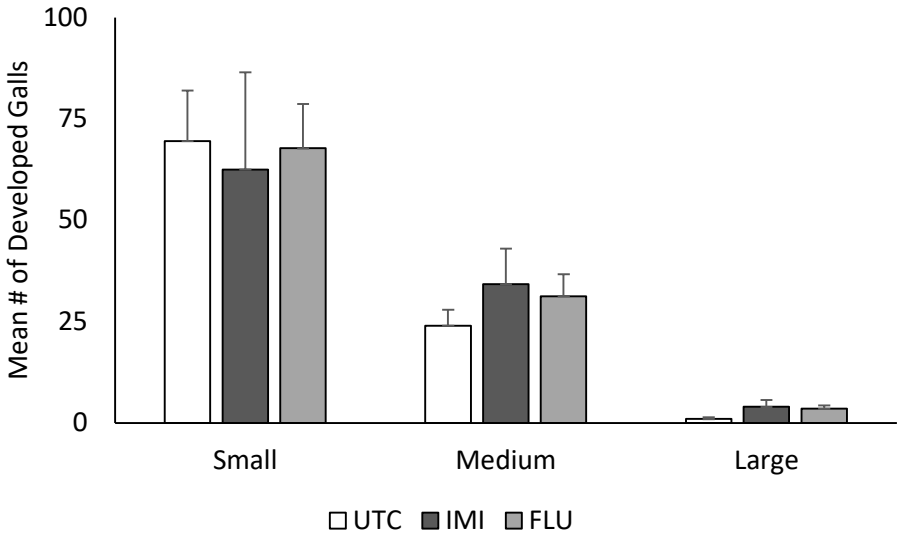

Figure 4.3. Mean ± SEM developed galls per bush for highbush blueberry treated with different insecticides. Treatments include UTC: untreated control, IMI: imidacloprid, and FLU: flupyradifurone. Assessment was completed on December 23, 2021.

Adult Emergence

The mean number of *H. nubilipennis* per gram of galls, inquiline species (Sycophila sp. only found) per gram of galls, and total wasps per gram of galls were quantified within gall size categories: small (Figure 4.4), medium (Figure 4.5), and large (Figure 4.6).

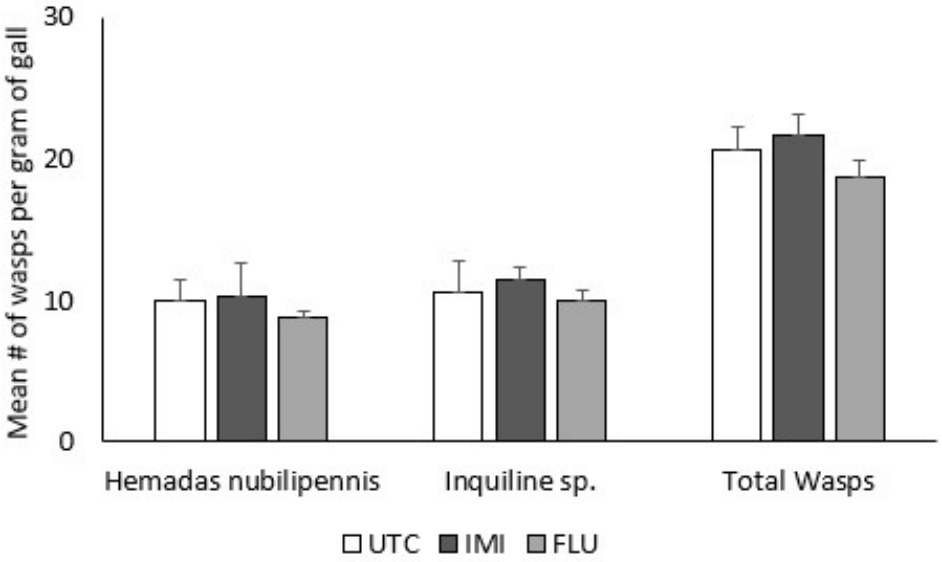

Figure 4.4. Mean ± SEM adult wasps per gram of gall per treatment within the gall size category small. Treatments include UTC: untreated control, IMI: imidacloprid, and FLU: flupyradifurone.

Numerically, there were fewer wasps per gram of small galls emerged from flupyradifurone treated blueberry bush plots. There also appears to be similar abundance of *H. nubilipennis* to inquiline species in small galls.

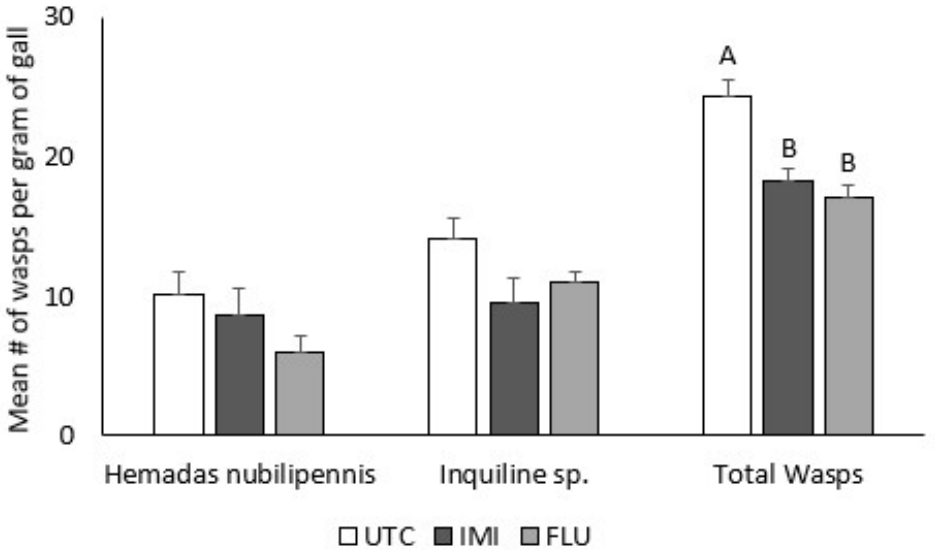

Figure 4.5. Mean ± SEM adult wasps per gram of gall per treatment within the gall size category medium. Treatments include UTC: untreated control, IMI: imidacloprid, and FLU: flupyradifurone. Significant treatment differences within a wasp category (*Hemadas nubilipennis,* inquiline sp., total wasps) are indicated by different letters (Tukey's HSD, $P < 0.05$).

The one-way ANOVA was significant for treatment on mean number of total wasps per gram of medium galls ($F_{2,6} = 15.64$, $P = 0.0042$) (Figure 4.5). Means separation using Tukey's HSD found 25% - 30% greater mean total wasps per gram of medium gall in the untreated control compared to the two treatments ($P < 0.05$) (Figure 4.5). Numerically, there was greater mean *H. nubilipennis* per gram of medium gall and mean inquiline species per gram of medium gall in the untreated control compared to the two treatments (Figure 4.5).

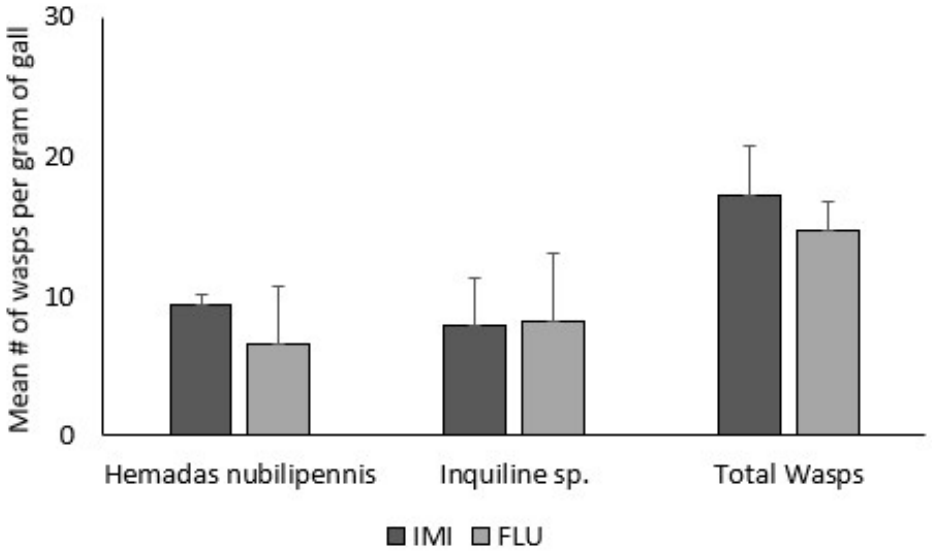

Figure 4.6. Mean ± SEM adult wasps per gram of gall per treatment within the gall size category large. Treatments include UTC: untreated control, IMI: imidacloprid, and FLU: flupyradifurone.

Numerically, there were fewer *H. nubilipennis* per gram of large galls emerged from flupyradifurone treated blueberry bush plots (Figure 4.6). This caused numerically fewer total wasps per gram of large galls emerged from flupyradifurone treated blueberry bush plots (Figure 4.6).

Residue Recovery

There was no recovery of either insecticide in the untreated plot plant tissue samples: leaves, shoots, or galls sampled for both dates sampled. The two-way ANOVA for interaction of plant tissue type (excluding galls) and date was not significant for imidacloprid treated plots, neither was the one-way ANOVA for plant tissue type (excluding galls). However, the one-way ANOVA was significant for the variable date on concentration of imidacloprid recovered ($F_{1,6} = 11.9$, $P = 0.0139$). There was significantly greater imidacloprid recovered at 59 DAT than at 14 DAT (Tukey's HSD, $P < 0.05$). The one-way

ANOVA comparing imidacloprid recovered in plant tissue types: leaves, shoots, and galls at 59 DAT was found to be marginally significant ($F_{2,6} = 4.81$, $P = 0.0566$), and shoot tissue samples were found to have marginally greater imidacloprid recovered compared to gall tissue samples (Tukey's HSD, $P = 0.0685$) (Figure 4.7).

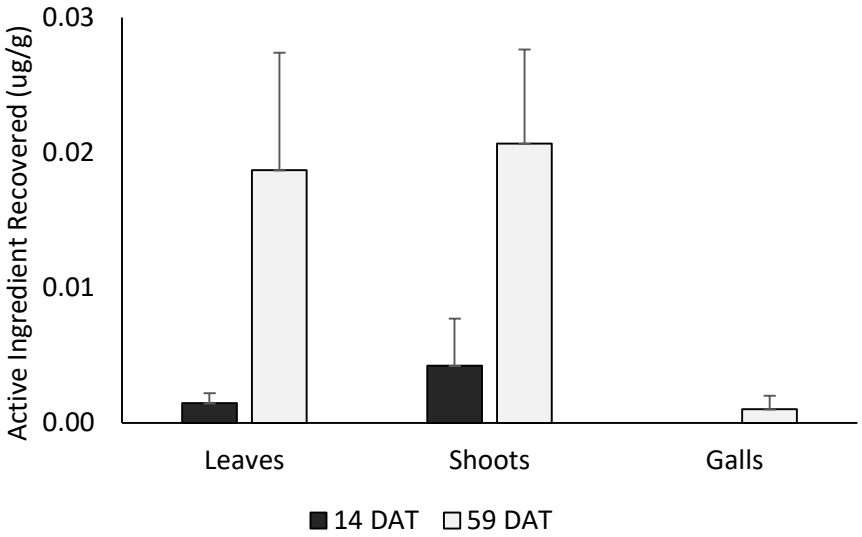

Figure 4.7. Mean ± SEM imidacloprid recovered (µg/g) in highbush blueberry plant tissue type (leaves, shoots, galls) at 14 DAT and 59 DAT. Galls were only collected at 59 DAT.

There was no impact on flupyradifurone recovered from variables date, sample type, and the interaction of date and sample type. The greatest amount of flupyradifurone recovered was at 14 DAT in shoot tissue (Figure 4.8).

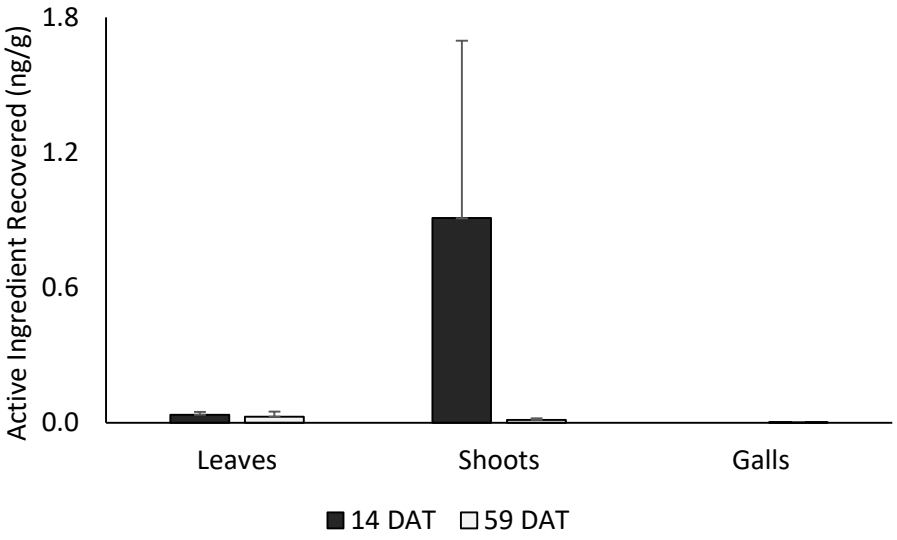

Figure 4.8. Mean ± SEM flupyradifurone recovered (ng/g) in highbush blueberry plant tissue type (leaves, shoots, galls) at 14 DAT and 59 DAT. Galls were only collected at 59 DAT.

DISCUSSION

The results of this study provide new insights into the potential of using drench applications of systemic insecticides for control of blueberry stem gall wasp. We demonstrated successful delivery of imidacloprid and flupyradifurone to blueberry bush leaves, shoots, and galls using drench chemigation methods. For imidacloprid, the residue recovery data suggest that 14 days is not sufficient time to deliver peak residue levels to gall-forming tissues. There was some evidence that both insecticides reduced adult wasp emergence. Since the reduction of adult wasps was 25%-30% and was only seen in medium galls, it is likely that this would not provide a level of control that would be useful to a blueberry grower. The insecticides in this study did not have impact on gall abundances across treatments as observed in Fanning and Isaacs' 2019(b) soil drench study.

Imidacloprid residue was recovered in greater concentration at 59 DAT suggesting that 14 days is not sufficient time to deliver peak residue levels to gall-forming tissues where larvae are early in their development. Overall, residue recovery was lower than expected, due to lack of moisture in soil during application which may have limited imidacloprid uptake into the bushes. In previous research where exposed larvae in galls on shoots were administered imidacloprid in a bioassay, approximately 35% larvae responded with a moribund response after 5 days of exposure at the lowest rate tested with approximately 0.1 ppm residue recovered in gall tissue (Chapter 3). Moribund response nearly doubled at approximately 1 ppm and greater residue recovered in gall tissue (Chapter 3). The residue recovered from shoot and gall tissues in this drench study were too low to cause a moribund or lethal response of the larvae in the galls, according to the bioassay studies in Chapter 3. Imidacloprid has a binding affinity to organic carbon, aka organic matter in soil, therefore, impacting availability to the roots for uptake and increasing the longevity of exposure (Shetlar 2008 as cited in Kurwadkar et al. 2014; Doccola and Wild 2012). Future research should explore different drench application methods to improve imidacloprid uptake into bushes. Additionally, an earlier application may result in peak residue levels at egg hatch and early larval stages. However, the Admire Pro label does not allow for bloom or pre-bloom application in order to protect pollinators (Bayer CropScience 2013).

Flupyradifurone was recovered in nearly 1000-fold less concentration than imidacloprid, at half the active ingredient application rate per bush compared to imidacloprid. This recovery was also much lower than expected due to lack of moisture in soil during application which may have limited flupyradifurone uptake into the bushes.

Flupyradifurone has high water solubility (3,200 mg l^{-1} at 20°C) and high leachability (GUS leaching potential index = 4.24) (Lewis et al. 2016). Flupyradifurone also has lower binding affinity to organic matter in the soil compared to imidacloprid, which may indicate that it does not persist in soil's root zone for uptake throughout the growing season (Shetlar 2008 as cited in Kurwadkar et al. 2014; Doccola and Wild 2012). Therefore, it is possible that flupyradifurone moved through the soil and did not have sufficient exposure to roots for uptake. In a seed-treatment study in soybeans, flupyradifurone was recovered in less concentration than imidacloprid in V2 soybean plants under normal conditions and in greater concentration than imidacloprid under soil moisture stress (Stamm et al. 2015). Though our study was not a seed treatment study, this informs us that successful uptake of flupyradifurone is sensitive to soil moisture, water used for treatment, and soil texture, and would not be "one size fits all" for blueberry growers. In previous research where exposed larvae in galls on shoots were administered flupyradifurone in a bioassay, 13% - 38% of larvae responded with a moribund response after 5 days of exposure for residue recovered at 1 – 2.5 ppm in gall tissue (Chapter 3). Since flupyradifurone was applied at a rate of 7 fl oz/A, which is half of the seasonal maximum, another application may increase insecticide residues in plant tissues, which is allowed by the label (Bayer CropScience LP 2019).

Further research should explore application timing, multiple applications (flupyradifurone), soil moisture, and amount of water used to deliver the insecticide to optimize residue recovery in blueberry bush plant tissues. If greater residue is delivered to the various plant tissues, it may lead to greater reduction in adult wasp emergence. With further research, imidacloprid and flupyradifurone may provide an option for blueberry growers to incorporate into their IPM practices for control of blueberry stem gall wasp.

APPENDICES

APPENDIX A:

Adult Wasp Emergence from Galls in Chapter 3

Ten randomly-selected galls were collected from privately owned, un-sprayed non-commercial Jersey blueberry field in Fennville, MI (December 2021). Each gall was placed in a 96 ml souffle cup with clear lids that had five holes. These galls were placed in growth chambers (~23°C, 55% RH, 16:8 Photoperiod) on January 24, 2022. Emerged wasp adults were assessed March 14-15, 2022. There was greater amount of inquiline species found than *H. nubilipennis.* The rounded average of each species per gall was as follows: 1 *H. nubilipennis,* 30 *Sycophila* sp., 1 *Ormyrus* sp., and 3 *Eurytoma* sp.

APPENDIX B:

Thesis Conclusions

My thesis research address questions associated with systemic insecticide application methods, recovery of active ingredient in shoot and gall tissue, and lethality to blueberry stem gall wasp. From this research, and previously published research, spirotetramat seems to be the best option for reducing blueberry stem gall wasp emergence for growers who plan to keep their highly susceptible highbush blueberry varieties. I observed spirotetramat to perform well as a systemic with detection over the course of a growing season. Spirotetramat disturbed larval development in as quickly as five days, likely leading to high lethality in a field scenario. Additionally, spirotetramat can be easily applied with airblast sprayer, which majority of commercial growers are already equipped with. Imidacloprid and flupyradifurone may be useful for controlling blueberry stem gall wasp with future research using chemigation. Imidacloprid and flupyradifurone have existing labels that include soil delivery in blueberries, and spirotetramat as a foliar application in blueberries.

From my potted study, I successfully captured active ingredient recovered in shoot tissue at two important gall wasp timings, early larval and gall development, and ending of larval and gall development. I found imidacloprid and flupyradifurone to be recovered in greatest concentration in crown injection trees, though soil drench at a 100% rate is a viable option for growers with reduced active ingredient delivered. Spirotetramat preformed equally as well or better as a foliar spray compared to crown injection.

My bioassay results provided new insights of insecticides mode of actions observed on blueberry stem gall wasp larvae leading to moribundity/lethality, previous research has not captured this by counting galls formed or adults emerged. The systemic insecticides ranked from most to least toxic to blueberry stem gall wasp after five days of exposure is imidacloprid, flupyradifurone, spirotetramat, and azadirachtin. The neonicotinoids slowed larva movement and, in some cases, caused the larva to appear retracted and not feeding. Spirotetramat compressed the larval bodies causing complete loss of control of their muscles. Recovery of active ingredient in gall tissue paralleled with larval mortality provides a target for active ingredient recovery in a field study to control blueberry stem gall wasp. My field study however did not reach the target concentrations for imidacloprid and flupyradifurone. Active ingredient recovery was between 100-fold and 1,000-fold difference for imidacloprid, and between 1,000-fold and 10,000-fold difference for flupyradifurone. Improving chemigation application methods and manipulating soil moisture will likely lead to greater active ingredient recovery. With increased delivery of active ingredient, imidacloprid and flupyradifurone may have greater impact on blueberry stem gall wasp emergence in the following year.

Finally, I would like to conclude with advice to individuals who may work with some aspect of this research in the future. The potted bushes used for this study worked very well for understanding the systemic insecticide movement and application of systemic insecticides in a controlled setting but did not work well to show treatment impact on the blueberry stem gall wasp. I found gall abundances on young potted bushes to reflect the ability of the bush to support galls than actual treatment differences. Additionally, there were challenges with keeping potted bushes alive. Some things I learned the hard way

include do not over fertilize, use a sprinkler with a timer to keep the bushes well-watered, and watch for other pests such as tent caterpillars and Japanese beetles that can quickly destroy a bush if not regularly monitored. Lastly, when a study fails to complete one or more of your goals, get creative with a bioassay. Yes, there are limitations with bioassays, yet there is so much that can be discovered on a smaller scale that can largely impact outcomes in field studies.

APPENDIX C:

Record of Deposition of Voucher Specimens

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2022-04

Author and Title of Thesis:

SYSTEMIC DELIVERY OF INSECTICIDES IN BLUEBERRIES FOR CONTROL OF BLUEBERRY STEM GALL WASP, *HEMADAS NUBILIPENNIS*

By

Amber Kay Bosch

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Table C.1. Specimens deposited into Albert J. Cook Arthropod Research Collection at Michigan State University.

Family	Genus-Species	Life Stage	Quantity	Preservation
Pteromalidae	*Hemadas nubilipennis*	Adult	10 (male)	Pinned/Pointed
Pteromalidae	*Hemadas nubilipennis*	Adult	10 (female)	Pinned/Pointed

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