Insect resistance in cranberry

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Insects and pathogens are considered a major problem in cranberry production; insects are estimated to reduce yield by 1-2% and without spraying, cranberry false blossom, a phytoplasma vectored by a leafhopper, would eliminate commercial cranberry production (Dan Schiffhauer, personal communication). Spraying of chemical insecticides is the most common practice to combat pathogenic microbes and herbivorous insects, but beneficial insects, such as honeybees, important pollinators of cranberry, and natural enemies of herbivores, such as predators and parasitoids, could be affected as well. Therefore, we studied the defensive mechanisms that cranberry plants themselves use to combat insect pests. We focused on the most important insect pests of cranberry in New Jersey, the third largest cranberry producing area in the United States: gypsy moth [*Lymantria dispar* L. (Lepidoptera: Erebidae)], Sparganothis fruitworm [*Sparganothis sulfureana* Clemens (Lepidoptera: Tortricidae)], spotted fireworm [*Choristoneura parallela* Robinson (Lepidoptera: Tortricidae)] and blunt-nosed leafhopper [*Limotettix vaccinii* Van Duzee (Hemiptera: Cicadellidae)].

1. Cranberry resistance to gypsy moth

Gypsy moth is an invasive pest that is especially destructive to oaks in the northeast of the United States. The insect occasionally does extensive damage to cranberry (REF). In a greenhouse setting, we evaluated the resistance of seven cranberry varieties to this pest insect. We measured direct, as well as indirect defenses – direct defenses include the production of toxic compounds that have a direct negative impact on the feeding insects (REF), and indirect defenses include the emission of volatile organic compounds in response to herbivory that can attract the natural enemies of the herbivores (REF). There were wild varieties, cultivated varieties that were developed around 1940 (early) and cultivated varieties that were developed in the past few years (recently). The early cultivated varieties are nowadays widely used. For many crops, artificial selection for increased yield and quality has negatively affected other traits, such as resistance to herbivores (REF). For cranberry, however, we expect recent cultivars to display higher resistance qualities, as they were bred not only for high yield, but also for high insect and pathogen resistance.

To study direct defenses, we evaluated larval performance on the different cranberry plants. Plants were wrapped in polyester sleeves in which neonates were introduced, which were weighted after 7 days of feeding. We counted the number of damaged leaves as an additional measure of resistance (REF). Plants were treated with jasmonic acid (JA), a plant hormone that plays an important role in the regulation of defenses (REF), to study possible differences in induced plant defenses. Non-treated (control) plants were used to study constitutive defenses. We collected leaf material of JA- and gypsy moth-treated cranberry plants and stored it at -20°C for analysis of toxins. To study indirect defenses, plants were treated with JA, subjected to gypsy moth feeding, or non-treated (control), after which volatiles were collected for 3h according to (REF).

Preliminary results show that there were no differences between the resistance of control and JA-treated plants (Two-way ANOVA, Ptreatment = 0.157, Pvariety < 0.001, Pinteraction = 0.938; Figure 1A). These results imply that JA-induced defenses do not affect gypsy moth performance, although previous research did show a negative effect of JA-treatment on insect growth (REF). Overall, there were differences in resistance between the varieties (Figure 1A), but there was no clear correlation between resistance and cultivation status. It is remarkable, though, that the wild variety Howes is most susceptible to insect feeding, and both recent cultivars (Crimson Queen and Demoranville) are most resistant.

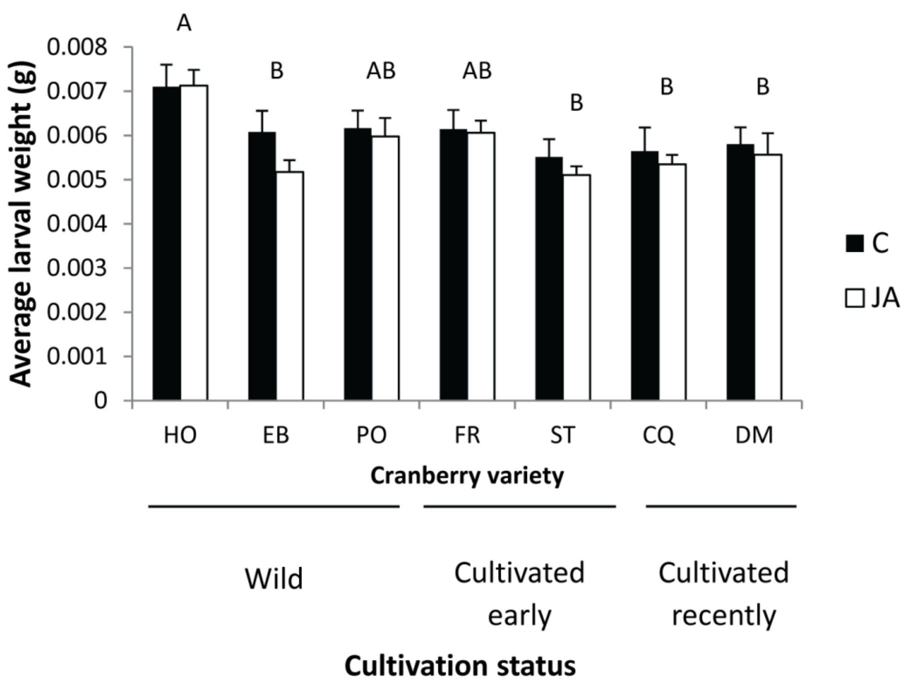

Figure 1. (A) Performance of gypsy moth neonates on seven cranberry varieties with (JA) or without (Control) previous induction of defenses through treatment with the defense-related plant hormone jasmonic acid. Neonate weight was assessed after 7 days of feeding. Each plant received 5 neonates – the average weight of all larvae that could be retrieved from each plant was taken as a measure of gypsy moth performance. (B) Average number of cranberry leaves per plant that was damaged by gypsy moth. HO, Howes; EB, Early Black; PO, Potter; FR, Franklin; ST, Stevens; CQ, Crimson Queen; DM, Demoranville. N=14-15. Different letters indicate significant differences (Two-way ANOVA, P < 0.05).

Damage data…

We are currently analyzing the toxins that are produced in the leaves upon insect feeding, as well as the emission of volatiles of the different cranberry varieties. Elucidating mechanisms of resistance against insect pests in cranberry will help improve the development of more sustainable pest management practices in existing varieties, as well as development of novel varieties with improved resistance qualities.

2. Cranberry resistance against Sparganothis fruitworm and spotted fireworm

Sparganothis fruitworm and spotted fireworm are native pests of cranberry in New Jersey. Larvae feed on foliage and developing fruits and extensive damage can significantly reduce yield (REF). In an experimental cranberry bog, we evaluated the resistance of ten cranberry varieties to these pest insects. There were wild varieties, cultivated varieties that were developed around 1940 (early) and cultivated varieties that were developed in the past few years (recently). We studied mainly direct defenses and evaluated performance of the larvae on the plants, directly in the bogs or in the laboratory on uprights and berries that were collected from the bogs. We used control, non-treated plants and plants that received a treatment with methyl jasmonate (MeJA), a JA-derivative that also functions as an elicitor of defenses (REF). Plants were treated pre-bloom (MeJA early), during bloom (MeJA late), or received a treatment at both timepoints (MeJA both).

When evaluating the effects of the treatments in the widely-used cranberry variety Stevens, we did not observe differences in larval growth on detached uprights of control or MeJA-treated plants, neither for Sparganothis fruitworm (Kruskal-Wallis one-way ANOVA on ranks, $P = 0.026$, no significant differences in post-hoc test; Figure 2A) nor for spotted fireworm (One-way ANOVA, $P = 0.496$; Figure 2B).

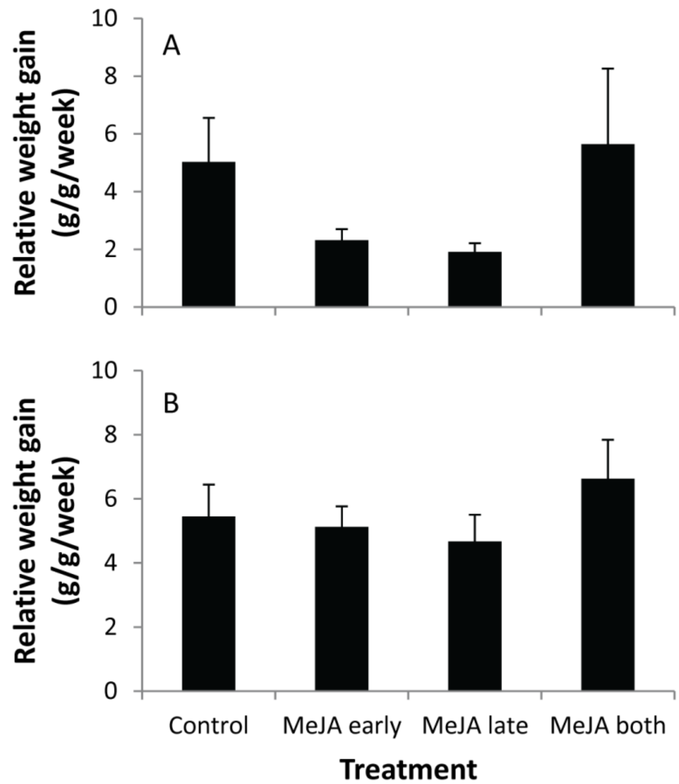

Figure 2. Performance of Sparganothis fruitworm (A) and spotted fireworm (B) on detached uprights of cranberry plants (variety Stevens) from bogs treated with methyl jasmonate pre-bloom (MeJA early), during bloom (MeJA late), on both timepoints (MeJA both), or non-treated (Control). N = 16-20. There were no significant differences.

There were no significant differences in larval growth on detached berries from the MeJA-treated plots either (Sparganothis fruitworm, One-way ANOVA, P = 0.067; Spotted fireworm, One-way ANOVA, P = 0.331), nor where there differences in the amount of berries that were damaged (each larva was supplied with 10 berries; Sparganothis fruitworm, One-way ANOVA, P = 0.846; Spotted fireworm, Kruskal-Wallis one-way ANOVA, P = 0.709). These results indicate the treatments with MeJA did not have measurable ecological effects on larval performance, although effects of MeJA-treatments have previously been found at the level of adult moth attraction (REF).

When assessing larval growth on the different cranberry varieties subjected to the different treatments in the field – larval weight was assessed after 7 days of feeding in a field cage – we did not observe differences for Sparganothis fruitworm (GLM, Ptreatment = 0.462, Pvariety = 0.805; Figure 3A) or spotted fireworm (GLM, Ptreatment = 0.815, Pvariety = 0.606; Figure 3B).

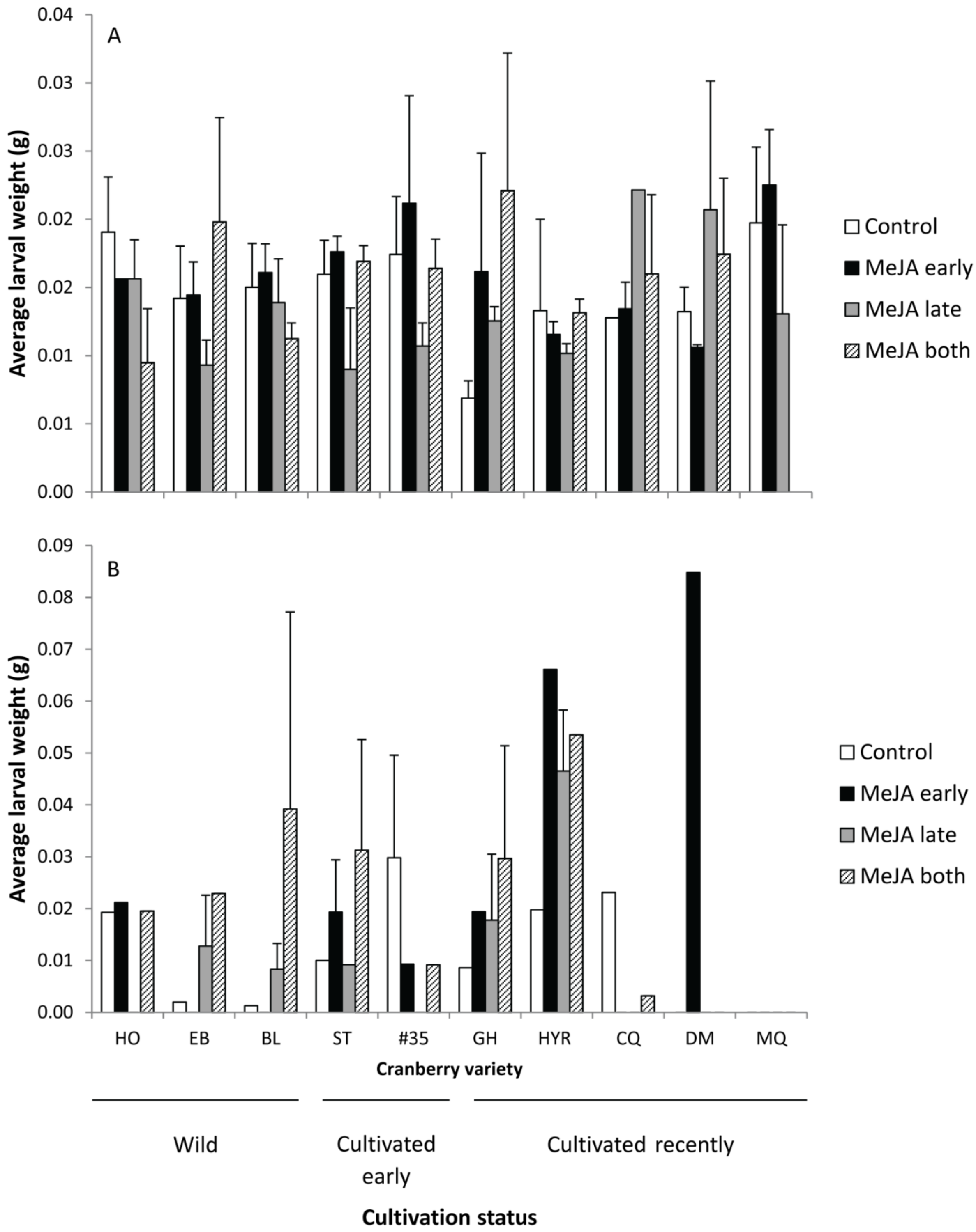

Figure 3. Performance of Sparganothis fruitworm (A) and spotted fireworm (B) in field cages on different cranberry varieties treated with methyl jasmonate pre-bloom (MeJA early), during bloom (MeJA late), on both timepoints (MeJA both), or non-treated (Control). N = 1-4. There were no significant

differences. HO, Howes; EB, Early Black; BL, Ben Lear; ST, Stevens; #35, Number 35; GH, Grygleski; HYR, HyRed; CQ, Crimson Queen; DM, Demoranville; MQ, Mullica Queen.

At the end of the growth season, we selected an area of one square feet in each plot where we harvested all berries. We did not observe any differences in yield between treatments, but we did observe differences between varieties (Two-way ANOVA, Ptreatment = 0.649, Pvariety < 0.001, Pinteraction = 0.991; Figure 4). The recent cultivars HyRed, Crimson Queen and Mullica Queen, as well as the wild variety Ben Lear, produced the highest quantities of berries, while the wild variety Early Black produced the lowest berry quantity.

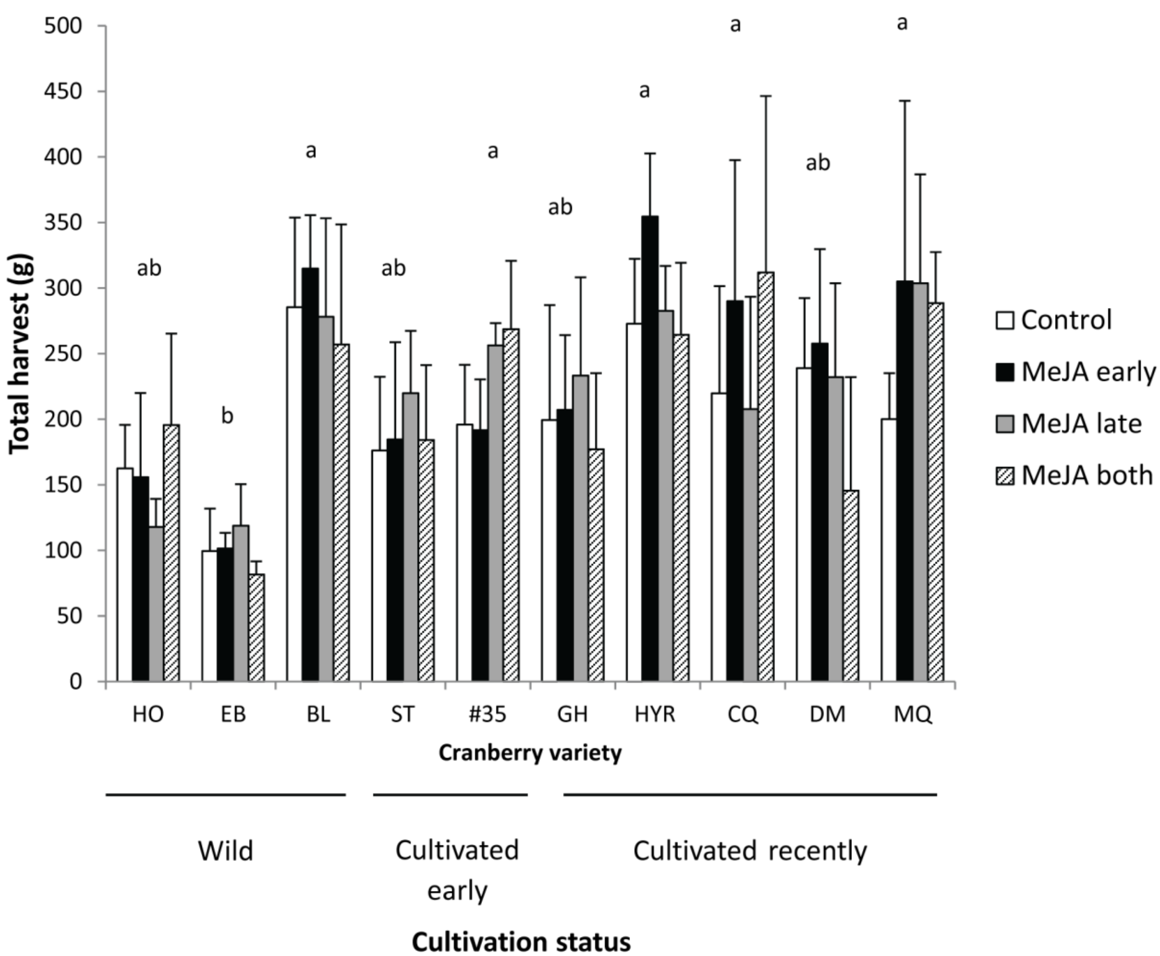

Figure 4. Yield of different cranberry varieties subjected to treatment with methyl jasmonate pre-bloom (MeJA early), during bloom (MeJA late), on both timepoints (MeJA both), or non-treated (Control). N = 1-4. Different letters indicate significant differences (Two-way ANOVA, P < 0.05). HO, Howes; EB, Early Black; BL, Ben Lear; ST, Stevens; #35, Number 35; GH, Grygleski; HYR, HyRed; CQ, Crimson Queen; DM, Demoranville; MQ, Mullica Queen.

Quite a few berries subsided to cranberry fruit rot, an important pest of ripened berries (REF). The treatments did not influence fruit rot, but there were significant differences between the varieties. For instance, wild variety Howes was most resistant to rot, while wild variety Ben Lear, as well as most recently cultivated varieties, were highly susceptible (Two-way ANOVA, $P_{treatment} = 0.603$, $P_{variety} < 0.001$, $P_{interaction} = 0.997$). We found few insect-damaged berries, and there were no differences in the amount of insect damaged berries between varieties or treatments (Two-way ANOVA, $P_{treatment} = 0.390$, $P_{variety} = 0.082$, $P_{interaction} = 0.734$). When considering only the sound berries, the wild varieties Howes and Early Black had the lowest weight per berry, while the recent cultivar Crimson Queen had the highest weight per berry (Two-way ANOVA, $P_{treatment} = 0.529$, $P_{variety} < 0.001$, $P_{interaction} = 0.746$).

Our results indicate that MeJA treatments did not impact plant defense to insects or pathogens to the level that it impacted yield. Various cranberry varieties differed in their susceptibility to fruit rot, but we did not observe differences in their levels of insect resistance. Ecological interactions are intricate and consist of much more than just the plants and the insect herbivores. Since greenhouse experiments showed differences in resistance to gypsy moth between plant varieties, we aim to study the different varieties in more detail under field conditions. Because of the high number of varieties and treatments that we analyzed, the sample size for each treatment was rather low. We aim to decrease the number of treatments and varieties and increase the sample size for future experiments, in order to obtain a more robust dataset concerning the effects of jasmonates on insect performance. Treating plants with jasmonates had led to decreased amounts of insect damage and increased attraction of natural enemies in other agricultural settings (REF), so JA and JA-derivatives have high potential for the development of novel, more sustainable methods of crop protection.

3. Blunt-nosed leafhopper

Blunt-nosed leafhopper vectors a phytoplasma that causes cranberry false-blossom, an important crop disease. False blossom causes defects in the development of the flowers, reducing fruit set and therefore seriously reducing yield (REF). It has been reported that plant viruses and phytopathogenic bacteria can manipulate the volatile emission of their host plants, thereby influencing the behavior of potential vectors (REF). We considered the possibility that our phytoplasma influences cranberry volatile emissions.

We first evaluated the performance of the leafhoppers on healthy and false-blossom infested cranberry plants (recently cultivated variety Crimson Queen). There was no difference in weight between leafhoppers that developed on healthy vs. diseased plants for 30 days, indicating that the disease does not influence plant direct defenses or nutrition for the leafhopper (Two-way ANOVA, $P_{status\ (healty\ vs\ false\ blossom)} = 0.788$, $P_{developmental\ stage\ (nymph\ vs\ adult)} = 0.271$, $P_{interaction} = 0.339$; Figure 5A). There was also no difference in developmental stage in terms of the numbers of leafhoppers that were still in nymphal stage and that became adults (Chi-square test, P = 0.272, Figure 5B).

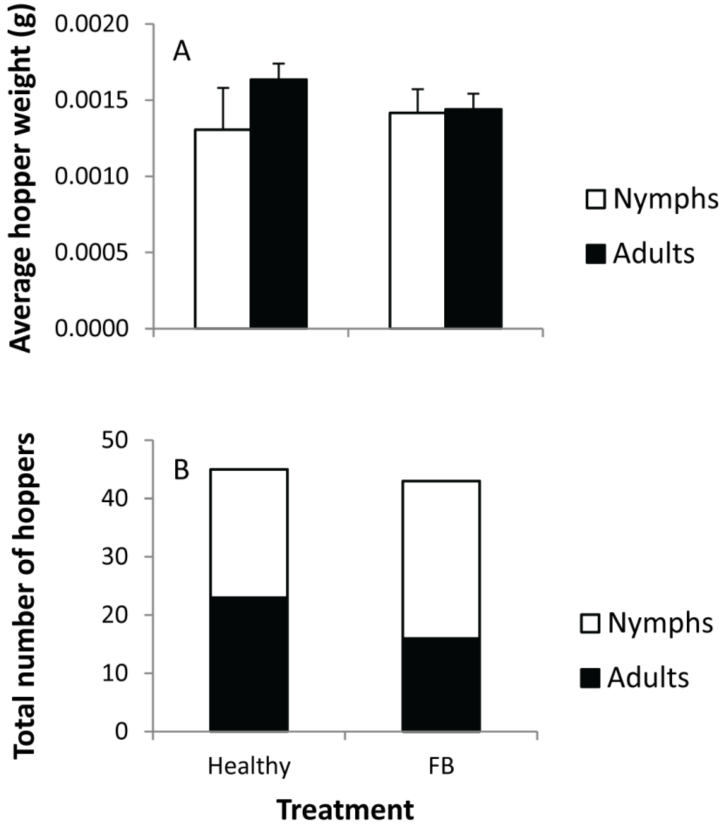

Figure 5. Blunt-nosed leafhopper weight (A) and developmental stage (B) after 30 days of feeding on healthy and false-blossom (FB) diseased cranberry plants, variety Crimson Queen. First-instar nymphs were introduced in small cages on cranberry uprights and allowed to feed for 30 days. Then, the amount of leafhoppers that was in nymph or adult stage was assessed, and leafhopper weight was measured. There are no significant differences (A, N=5-7, B, N=9).

We are currently analyzing the volatiles that healthy and diseased plants emit. If differences are found, next field season, we will study attraction of leafhoppers to these volatiles in a y-tube olfactometer, to assess whether false blossom influences the physiology of cranberry plants and thereby attraction of an important vector for false blossom. More knowledge about the interactions between the disease and the cranberry plants will help to develop practices to reduce the disease.

Conclusions and outlook

We evaluated the resistance of various cranberry varieties to important chewing and sucking insect pests. In a greenhouse setting, we observed a difference in cranberry resistance to gypsy moth, but against our expectations, this difference was not directly correlated to domestication status. We did not observe differences in cranberry resistance to Sparganothis fruitworm and spotted fireworm in a field setting, possibly because of the myriad of intricate ecological interactions. We did not observe a difference in performance of blunt-nosed leafhopper on healthy and false-blossom diseased cranberry plants. Blunt-nosed leafhopper is a vector of the phytoplasma that causes false-blossom, so these results indicate that false blossom does not “improve” the plant for the performance of its vector. Overall, these results have given us more information on the interactions between cranberry and important pest insects, and may help to contribute to the development of novel, more sustainable crop protection mechanisms.