STUDENT RESEARCH REPORT – REEU PROGRAM 2023

Determining the Feeding Preferences of Rosy Apple Aphids for Six Apple Cultivars

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Abstract

For apple growers, rosy apple aphid (RAA), *Dysaphis plantaginea* (Hemiptera: Aphididae), is one of the most widespread insect pests that cause fruit deformation, leaf curling, and significant crop yield losses when left uncontrolled. To limit pesticide use and input costs, perimeter trap trees could be used to congregate RAA in a few, strategic locations for reduced spray applications. To this end, our study surveyed RAA populations on apple trees grafted with six different cultivars in three blocks at the University of Massachusetts Cold Spring Orchard in Belchertown, Massachusetts. A total of two surveys were conducted in early June to assess which apple cultivar is the most attractive to RAA. Based on prior research on multiple apple insect pests (including RAA), we hypothesized that the Red Astrachan and Ginger Gold cultivars would congregate more RAA compared to the other four cultivars. To test this, ten fruit clusters were randomly sampled per cultivar/tree in each block. Rosy apple aphids were collected and brought back to our lab to quantify aphid incidence, abundance, and parasitism. Additionally, beneficial insects and spiders were identified under a light microscope to record their overall presence in the orchard. We observed statistically significant differences in RAA incidence of injury between apple cultivars only within Empire block at week two (ANOVA; $p = 0.0498$), where RAA congregated on Red Astrachan and Ginger Gold cultivars. Furthermore, there was a marginally-significant trend towards Ginger Gold in the Rock Mountain block during week one (ANOVA; $p = 0.059$). Aphid abundance was only calculated for Rock Mountain and parasitism was averaged across all three blocks for week one due to low sample sizes. These findings indicate that a longer-term study across multiple orchards is needed to better understand the chemical ecology of RAA with regards to grafted apple trees under varying site conditions. Future research will inform growers on the optimal cultivars for RAA aggregation, which could potentially allow farmers to manage multiple apple pests with reduced chemical inputs by focusing sprays on perimeter trap trees.

1 Introduction

1.1 Rosy Apple Aphid Life History

One of the major insect pests for apple growers during the spring and early summer is the rosy apple aphid (RAA) (*Dysaphis plantaginea*). This insect (Fig. 1) causes severe leaf curling, fruit deformation, and significant losses when left uncontrolled (Tougeron et al., 2022). While leaf curling does not harm the tree directly, it acts as a protective barrier from insecticide applications and some natural enemies (Alston, 2010). Prolonged leaf curling may lead to leaf abscission, fruit deformation, and stunting in young trees (Alston, 2010). Furthermore, RAA reaches adulthood in 7-8 days and each female can produce up to 80 offspring in one week (Flint, 2013). Thus, managing RAA populations during the early stages of fruit growth is crucial to ensuring that growers have a marketable crop for the fall season.

Figure 1. A rosy apple aphid (RAA) infestation on an apple leaf. Pictured is a tell-tale example of an RAA infestation. The leaf has begun to curl inwards, providing a protective barrier for the insect. Both winged and wingless forms are present.

RAA is an heteroecious insect (i.e. it goes through multiple hosts throughout their life cycle); their eggs overwinter on the branches and bark of apple trees and hatch in the spring (Fig. 2). First-generation females then produce live young that feed on new vegetative growth, causing the leaves to curl inwards and harden (Bloomers et al., 2004). The second generation adults first appear several weeks after petal fall, with some as winged aphids capable of host migration (Bloomers et al., 2004). Multiple generations are produced in early spring and summer, with each having a successively higher percentage of winged morphs (Beers and Willett, 2007). As the growing season progresses into late June, winged adults migrate to perennial weeds, including narrowleaf and broadleaf plantain (Beers and Willett, 2007). On the plantain, wingless female aphids reproduce asexually through mid-September (Bloomers, 2004). Following the female migration, winged males appear on the plantain, move to apple trees, and mate with winged females (Bloomers et al., 2004). The females lay eggs that overwinter and give rise to next year's generation (Beers and Willett, 2007).

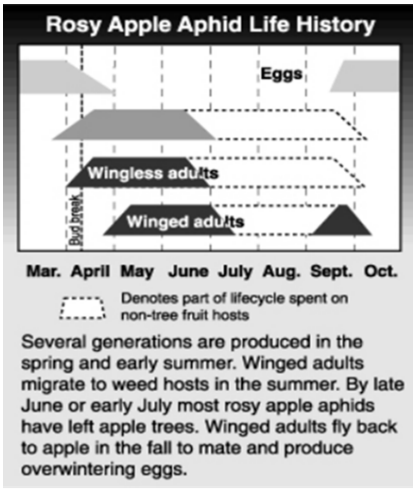

Figure 2. Life history of rosy apple aphids (RAAs) denoting their winged and wingless stages. Monitoring for this insect May-June is important for managing their populations.

1.2 RAA Plant Host Interactions

Once morphed into their winged form, aphids fly without fine directional control (Abbot et al., 2018). Instead, they rely on and use olfactory organs on their antennae to respond to host plant cues (Abbot et al., 2018). At long distances, they use phototactic cues or blind luck for host location (Abbot et al., 2018 as cited in Döring, 2014). To access nutrients from the plant, aphids use a stylet to pierce and consume sap from mesophyll and parenchyma tissues, while simultaneously secreting two types of saliva (Abbot et al., 2018). Secretion of these salivas,

which contain proteins and other molecules, can suppress plant-defense responses (Abbot, et al., 2018). Herbivorous feeding habits and plant interactions are key factors that influence physiological and structural adaptations in coexisting organisms (Mbaluto et al., 2020). Aphids feed on the phloem tissue of plants that contain a range of macromolecules responsible for plant growth, development, and defense (Abbot et al., 2018 as cited in Atkins et al. 2011). To improve nutrient uptake and further decrease plant defenses, aphids can induce galls into the plant's foliage for their own benefit (Kurzfeld-Zexer and Inbar, 2021).

1.3 Pest Resistant Apple Cultivars

In one study, Liberty, a cultivar with the *Vf* gene for scab-resistance, was evaluated in 2003 to test for susceptibility to RAA (Miñarro and Dapena, 2007). Of the 11 cultivars sampled, Liberty was among the least affected cultivars, with physical leaf damage rated at a scale of one out of five (Miñarro and Dapena, 2007). Results from this study suggested that some cultivars with the scab-resistant gene have reduced susceptibility to rosy apple aphid infestations as well (Miñarro and Dapena, 2007).

Another study, published by West Virginia University, tested 23 apple cultivars to determine their susceptibility to several foliar and fruit-feeding pests over the span of four years. Highly susceptible cultivars (40 to 50 RAA colonies per tree) included Fortune, Fuji Red Sport #2, Pristine, Shuzuka, and Ginger Gold (Hogmire and Miller, 2005). Owing to Ginger Gold's high susceptibility to both plum curculio (PC), *Conotrachelus nenuphar* (Coleoptera: Curculionidae) (Hogmire and Miller, 2005) and apple maggot fly (AMF), *Rhagoletis pomonella* (Diptera: Tephritidae) (Hogmire and Miller, 2005), this cultivar may also be attractive to RAA. However, experimental data is needed to confirm such a hypothesis.

In the Piñero Laboratory at UMass Amherst, our group has conducted field-scale studies across 10 apple orchards in Massachusetts to determine 1) if trees grafted with six cultivars are more attractive to PC compared to non-grafted trees and 2) if multi-cultivar grafted trees concentrate higher levels of fruit injury compared to non-grafted trees (Regmi, 2023).). The end-goal of Regmi's experiment was to determine if low-cost, permanent attract-and-kill sites could be developed for monitoring and managing PC. Based on these observations and prior research (e.g., Hogmire et al., 2005) that suggested that Ginger Gold trees were highly susceptible to PC damage, we hypothesized that RAA may prefer Ginger Gold and Red Astrachan cultivars for feeding. Thus, we predicted that these two cultivars would be more attractive to RAA, expressed in terms of RAA incidence and abundance.

2 Materials and Methods

2.1 Study Site

To determine which apple varieties are preferred by RAA s on grafted apple trees, we quantified RAA populations at three different apple tree blocks located at the University of Massachusetts Amherst CSO in Belchertown throughout June. These three blocks are differentiated as Rock Mountain (noted as ***A*** in bar graphs), Empire (noted as ***B***), and X Block (noted as ***C***). Experimental trees were grafted with six apple cultivars (Liberty, Red Astrachan, Ginger Gold, Dabinett, Wickson, and Yellow Transparent) (Fig. 3) in 2018 and 2019.

2.2 Leaf Cluster Collection

RAA sampling ran through the first three weeks of June, sampling twice from CSO (with one week in between sampling dates). Within each block, four grafted, four non-grafted, and four control trees (which were an average of 106 meters away from the grafted block) were sampled (Fig. 4). Additionally, since the majority of the grafted trees contain six apple cultivars and a separate rootstock cultivar, each of these branches were sampled from separately (total of 10 per cultivar). Leaf and fruit clusters were randomly sampled from each of the grafted branches. For the non-grafted and control trees, 20 clusters were randomly sampled. For all tree types, samples were chosen at random by walking around the entire perimeter of the tree and selecting leaves and shoots generally at stomach and chest height.

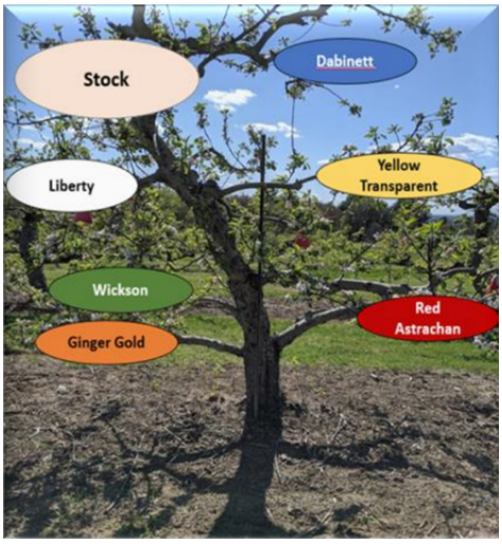

Figure 3. Example of an apple tree with six grafted cultivars. Additionally, a rootstock is present, which may be more attractive to rosy apple aphids.

2.3 Aphid Counting

For each block, the number of fruit clusters with RAA incidence were recorded in a sample of 10 fruit clusters per tree. Leaf samples (one cluster from each cultivar and two clusters from each non-grafted and control tree) were collected and wrapped in a damp paper towel to retain moisture. Afterwards, the samples were taken back to the laboratory at UMass Amherst. Each of the infested clusters was inspected under a light microscope to quantify the severity of the infestation, evaluated as the number of mobile RAAs present per apple cultivar. Since each fruit cluster contained an upwards of five leaves, only the ones with the highest counts of aphids (maximum two per cultivar, per sample) were analyzed under the microscope. Samples that were not observed analyzed the day of were kept inside an ice pack-filled cooler to avoid decomposition until the samples could be analyzed (which occurred within four days of the initial collection date).

2.4 Natural Enemy Estimates

In addition to aphid counting, natural enemies (parasitic and predatory insects and spiders) were recorded and identified at the order level to determine differences in biological control potential across treatments. The National Geographic *Insects of North America Pocket Guide* and the *Natural Enemy Field Guide* from Ohio Statement University were used as references in the field to assist with the identification process. Whenever possible, we also took pictures of the encountered insects to identify later. Parasitized aphid mummies were counted per leaf under the light microscope on all of the grafted cultivars, non-grafted, and control trees to estimate biological control via parasitism rates.

2.5 Data Analysis

While grafted tree rootstocks and non-grafted and control trees were initially observed and sampled from in all three blocks, they were not included in the data analysis for this study as

they did not provide any meaningful results. RAA parasitism by natural enemies was also excluded for similar reasons.

Microsoft Excel (Microsoft Office Suite, v.2307) was used to input the recorded data from each of the apple blocks and create bar graphs, while the statistical analyses were performed using Statistica (TIBCO Software Inc., v.14.0.1). To compare the six apple cultivars, we ran an ANOVA test for Rock Mountain and Empire blocks during week one and week two for RAA incidence. Since sample sizes were too low in X block to analyze RAA incidence, and no statistical tests were conducted for both weeks in this block. For RAA abundance, an ANOVA test was conducted for Rock Mountain only since Empire and X blocks had too few sample sizes. Similarly, RAA parasitism was averaged across all three blocks at CSO due to insufficient sample sizes.

3 Results

3.1 Incidence of RAA Injury

For week one, results from the Rock Mountain block (Fig. 5A) show a trend towards more RAA infestation in Ginger Gold, which was marginally significant (ANOVA; $F_{5,108} = 2.925$; $p = 0.059$) when compared to the other cultivars. However, high variability among samples prevented the analysis from detecting significant differences. Across blocks, the least-infested cultivars were Liberty and Dabinett (Fig. 5A-C). In the case of Yellow Transparent, there was either no grafted branches present on the trees or no RAA infestations in the branches sampled for two blocks. In these cases we did not collect samples for this cultivar. For Empire block, data were highly variable and no differences in RAA infestation were noted (ANOVA; $F_{5,108} = 1.643$; $p = 0.209$) (Fig. 5B). Tree branches grafted with Wickson and Liberty had zero RAA infestation; thus, they are not represented on the graph. For X block (Fig. 1C), no statistical analyses were conducted for RAA infestations because the sample sizes were too low.

Week 1

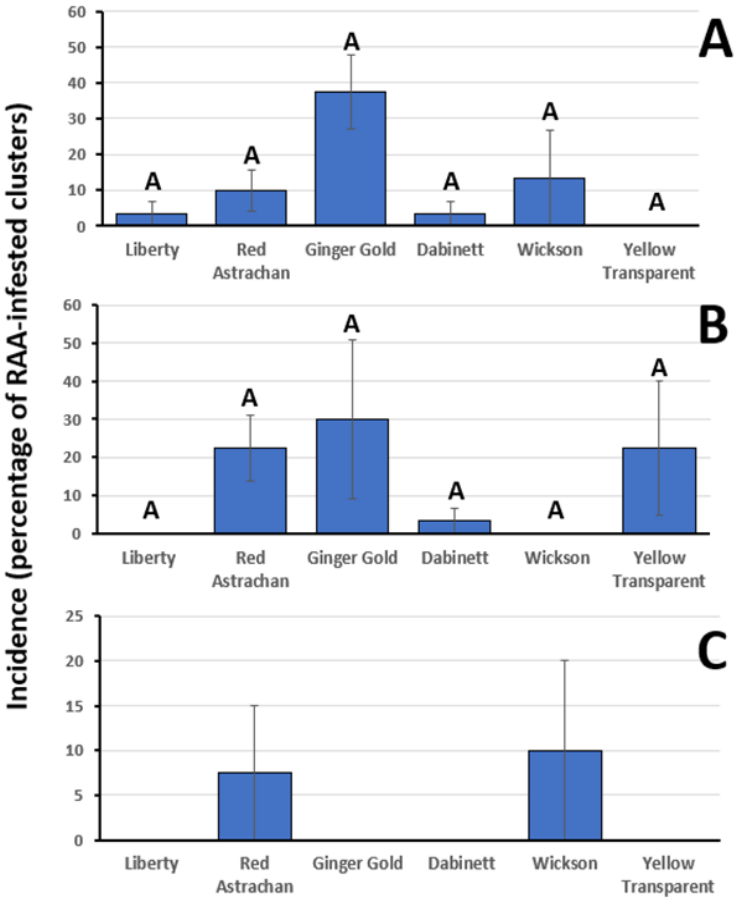

Figure 5. **Rosy apple aphid incidence across all three apple blocks at the UMass Amherst Cold Spring Orchard for week one**. While Ginger Gold concentrated higher counts of injury in Rock Mountain and Empire, no significant differences were observed across all blocks.

Legend
-Rock Mountain Block (Graph **A**)
-Empire Block (Graph **B**)
-X Block (Graph **C**)

For week two, Rock Mountain and X blocks did not show a statistical difference between RAA incidence across apple cultivars (Fig. 6A and C). In Rock Mountain, Liberty and Dabinett did not have any branches infested with RAAs (Fig. 6A). While Ginger Gold reported higher levels of incidence (30%) compared to Red Astrachan, Wickson, and Yellow Transparent, high variability prevented us from detecting any notable differences (ANOVA; $F_{5,108} = 2.302$; $p = 0.090$) (Fig. 6A). Within Empire block, Ginger Gold and Red Astrachan foliage was significantly more infested by RAA (ANOVA; $F_{5,108} = 2.813$; $p = 0.0498$) than Liberty and Dabinett (Fig. 6B). Wickson and Yellow Transparent also received very low levels of RAA infestation when compared to Ginger Gold. In X block (Fig. 6C), no statistical analyses were conducted for RAA infestations because the sample sizes were too low.

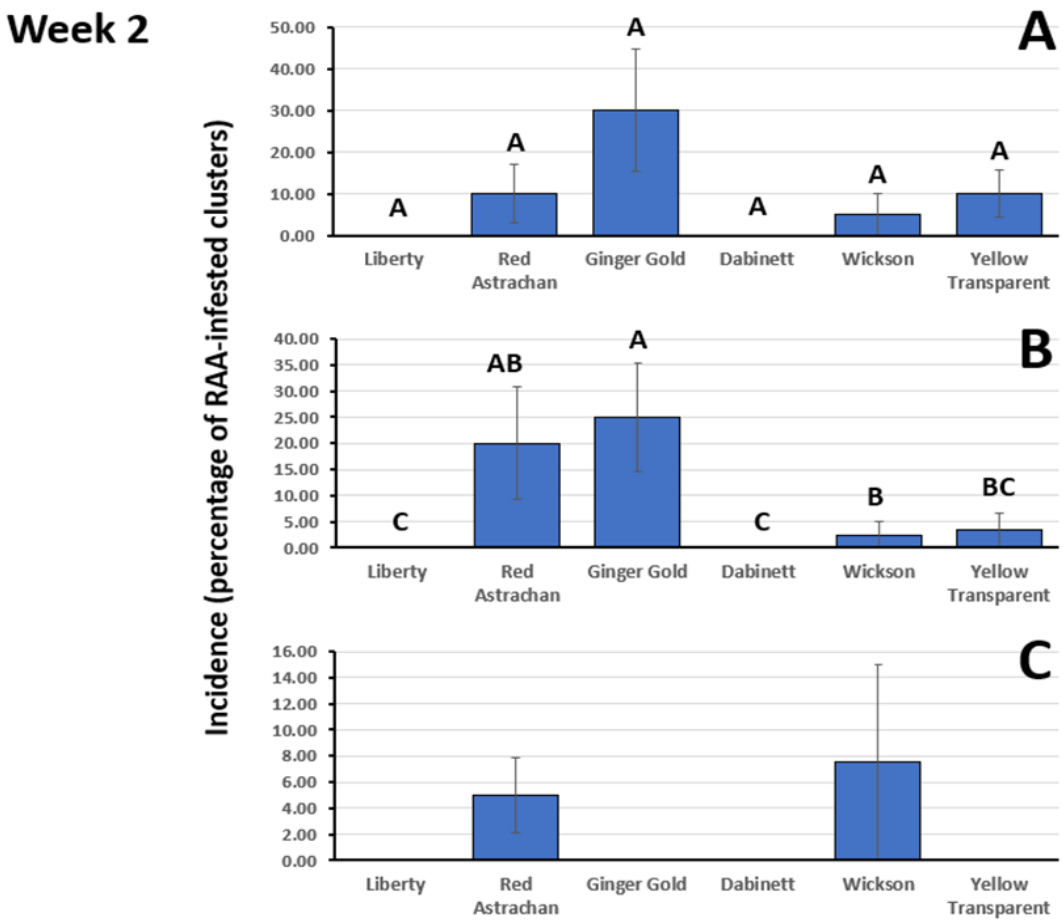

Figure 6. **Rosy apple aphid (RAA) incidence across all three apple blocks at the UMass Amherst Cold Spring Orchard for week two**. In Empire block, Ginger Gold had statistically higher infestations of RAA compared to the other two blocks.

Legend
-Rock Mountain Block (Graph **A**)
-Empire Block (Graph **B**)
-X Block (Graph **C**)

3.2 RAA Abundance

Statistical analyses for abundance were only conducted for the Rock Mountain block during week one due to insufficient sample sizes in Empire and X blocks. In Rock Mountain, there was no statistical difference between RAA abundance and apple cultivars (ANOVA; $F_{5,42} = 0.8361$; $p = 0.546$) (Fig. 7). However, Liberty had lower counts of RAAs present on foliage compared to Red Astrachan, Ginger Gold, Dabinett, Wickson, and Yellow Transparent. By the second week of data collection, rosy apple aphids had migrated from the apple trees onto their perennial hosts. Thus, no statistical analysis for abundance was conducted on any of the blocks from week two.

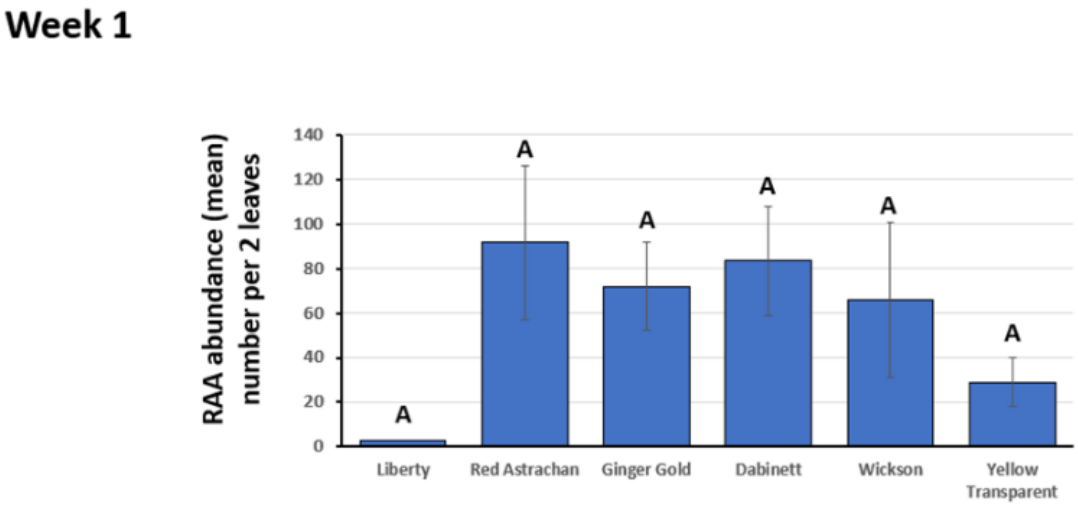

Figure 7. Rosy Apple Aphid abundance in Rock Mountain for week one. Abundance was not analyzed for Empire and X block due to low sample sizes, and no significant differences were observed across all three blocks.

3.3 Parasitism of RAA

Parasitism was averaged across all three blocks at CSO for week one only. Due to high variability in Red Astrachan and Ginger Gold, there are no statistically discernible differences in parasitism levels between apple cultivars (ANOVA; $F_{5,42} = 1.077$; $p = 0.414$) (Fig. 4). Liberty was excluded from Figure 8 due to only one sample being recorded.

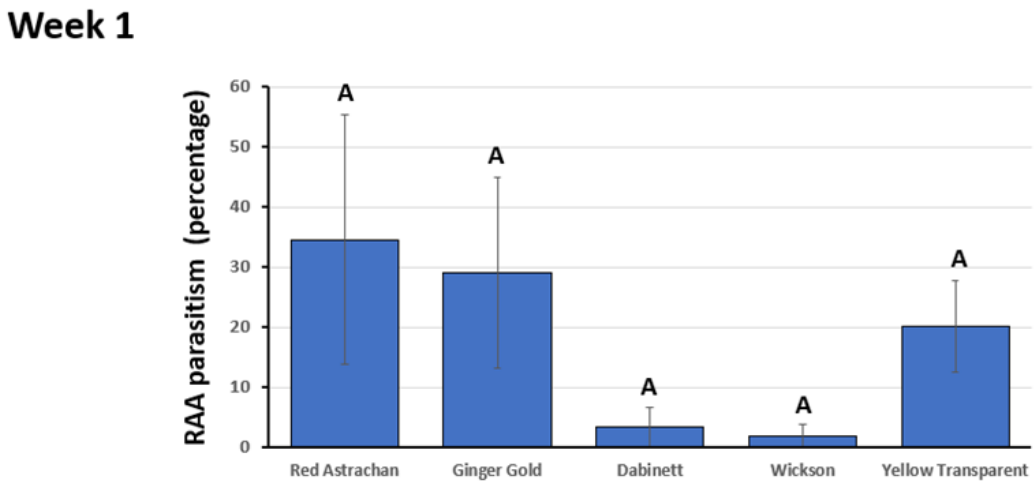

Figure 8. Parasitism of Rosy Apple Aphids for week one across blocks at the UMass Amherst Cold Spring Orchard. While Red Astrachan and Ginger Gold had higher rates of parasitism compared to Yellow Transparent, no significant differences were recorded.

3.4 Presence of Beneficial Arthropods on Apple Trees

As shown in Figure 4, the most abundant beneficial insects and arachnids found on the leaves during week one of the data collection process were aphid predatory midges (15 total), followed by spiders (8 total counts including egg masses). These counts were totaled from all sampled trees, across all three blocks (Rock Mountain, Empire, and X).

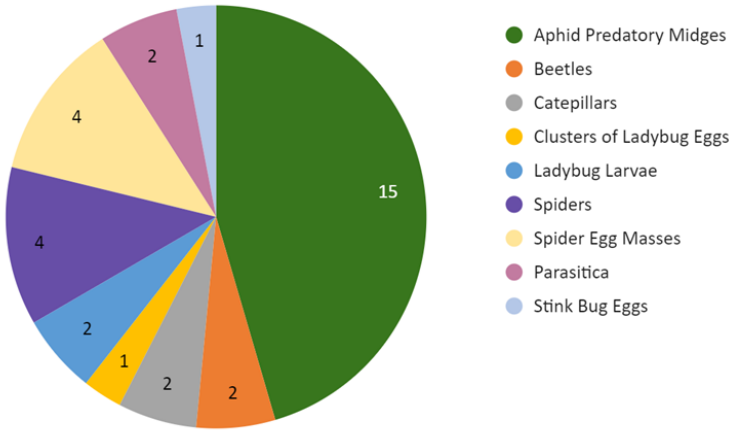

Figure 9. Beneficial insect counts across three apple tree blocks at the UMass Amherst Cold Spring Orchard during week one of data collection. Aphid predatory midges were the most abundant beneficial insect present that feed on rosy apple aphids.

For week two, beneficial counts were substantially lower compared to the first week of data collection, mirroring lower RAA abundance (Fig. 10). One arachnid and five insect groups were present. The most abundant beneficials were spiders (9 total including egg masses) and predatory ear

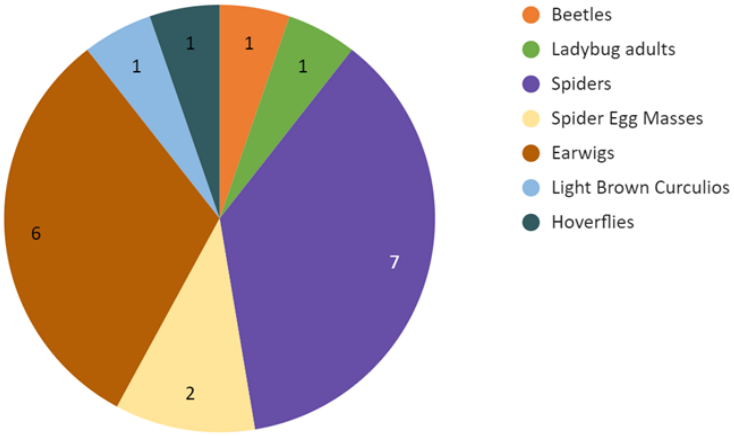

Figure 10. Beneficial insect counts across three apple tree blocks at the UMass Amherst Cold Spring Orchard during week two of data collection. Spiders (including spider egg masses) were the most abundant rosy apple aphid predator, followed by earwigs.

4.0 Discussion

RAA is a major insect pest during the early summer season that negatively impacts apple yields for farmers (Warneys et al., 2018). Prior studies have shown that certain cultivars are more susceptible to RAA damage, including Fortune and Ginger Gold (Hogmire and Miller, 2005). Previously, apple trees grafted with six grafted cultivars (Liberty, Red Astrachan, Ginger Gold, Dabinett, Wickson, and Yellow Transparent) concentrated higher counts of PC damage compared to non-grafted trees in Massachusetts (Regmi, 2023). However, apple trees with multiple cultivars grafted onto a single tree had not been evaluated for RAA incidence and abundance.

During the first week of data collection, while no statistically significant differences among the six cultivars were found across all three apple blocks there was a numerical trend toward greater RAA presence in Ginger Gold. When compared to Ginger Gold, Liberty had a lower percentage of infested clusters, suggesting that it is less attractive than other cultivars to RAA as previously reported by Miñarro and Dapena (2007). Yellow Transparent either did not have any living branches for data collection or no aphids were present on the foliage. In the Empire block, no differences in RAA infestations were noted as the data was highly variable. It is important to note that Empire is considered to be an attractive cultivar to RAA; thus being able to find RAA preferences for particular grafted cultivars may be more difficult than if the stock fruit is relatively less attractive (e.g., McIntosh). Liberty and Wickson did not have any samples with RAA infestations, while Dabinett had only one sample with aphids present. For X block, RAAs were only observed on Red Astrachan and Wickson.

The spring of 2023 was marked with a frost during the month of May, which led to the destruction of the apple crop in many orchards in New England. Such a meteorological phenomenon certainly affected the abundance of apple fruits at the UMass Cold Spring Orchard. Unusual weather patterns may have also influenced aphid counts this year. On May 18th, a historic freeze event caused severe damage to apple blossoms and tree branches across Massachusetts, wiping out a substantial portion of the apple crop for the 2023 growing season (Mize, 2023). Lastly, rainy weather conditions during week two may have also contributed to lower aphids present on the leaves during the data collection process. Once the aphids fall to the ground from rain, they cannot easily climb back onto the leaves before they die (Rettke, 2022).

Data from week two was collected towards the end of June when the majority of the RAA had begun to migrate to their secondary hosts. While Ginger Gold reported higher incidence compared to Red Astrachan, Wickson, and Yellow Transparent, there is high variability within this cultivar in Rock Mountain. Week two data from Empire block supported our hypothesis that RAA tend to prefer Ginger Gold cultivars on grafted apple trees. This corroborates with prior research conducted on PC and AMF that suggested Ginger Gold receives higher levels of injury compared to other cultivars (Hogmire and Miller, 2005). However, the other two blocks did not receive significant differences in RAA incidence for this particular cultivar, suggesting that there may be other environmental factors influencing these results.

In terms of natural enemy abundance, the most prominent arthropods found during week one were predatory aphid midges present on both grafted and non-grafted trees, followed by spiders and spider egg masses. Predatory insects and spiders were substantially lower on week two compared to week one. This may be due to lower RAA counts during the second week of data collection since most of the aphids had migrated to their secondary hosts. Spiders and earwigs were the most prominent organisms during week two. Overall, Rock Mountain contained the greatest number of beneficial insects and arachnids compared to Empire and X blocks.

This study was conducted from early through late June. While there were adequate numbers of aphids present on apple trees during week one of the data collection process (June 5-9), many of the RAAs had migrated to other hosts by week two (June 21-23), making it difficult, for instance, to quantify incidence in X block (Blommers et al., 2004). Furthermore,

RAA abundance could not be calculated for week two due to a very low number of RAA present across the six cultivars. If this study was to be replicated, RAA should be observed in early May, before they have a chance to produce winged females (Warneys et al., 2018). Increasing the sample size to include more trap trees to sample per apple block would also help to reduce variability and produce more robust results.

Unusual weather patterns may have also influenced aphid counts this year. On May 18th, a historic freeze event caused severe damage to apple blossoms and tree branches across Massachusetts, wiping out a substantial portion of the apple crop for the 2023 growing season (Mize, 2023). Lastly, rainy weather conditions during week two may have also contributed to lower aphids present on the leaves during the data collection process. Once the aphids fall to the ground from rain, they cannot easily climb back onto the leaves before they die (Rettke, 2022).

5.0 Conclusion

Results from this study indicate that there were no significant differences between rosy apple aphid incidence and abundance compared to apple cultivar, excluding RAA incidence for Empire block in week two. Ginger Gold was more attractive to RAAs in this particular block compared to the Wickson and Yellow Transparent. Furthermore, during both weeks, there was a biological trend in both the Rock Mountain and Empire blocks towards Ginger Gold. A longer-term study that includes multiple orchards across Massachusetts is needed to confirm my results for a better understanding of the ecology of this pest on grafted apple trees with regards to varying site conditions. Once a (or several) attractive cultivars are identified, apple growers will have a means to control multiple apple pests, namely plum curculio, apple maggot, and rosy apple aphid, by implementing a single, low input and maintenance-cost cultural control with the potential to substantially reduce pesticide use, promoting sustainable food production across the country.

6.0 Acknowledgements

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