

## Fruit Notes

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Cover: Three-year-old tall-spindle apple trees, planted 2.5' x 3'. These trees are part of a Midwest Apple Improvement Association (MAIA) cultivar trial, located in Kingwood Township, NJ. Notice the extensive feathers developed with multiple applications of Exilis\_9.5\_SC annually. Photo Credit: Win Cowgill.

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### Evaluation of a Grower-friendly Attract-and-kill Strategy for Apple Maggot Control in New England Apple Orchards: Research Results for Year Two

Dorna Saadat and Jaime C. Piñero Stockbridge School of Agriculture, University of Massachusetts

Admittedly, most of the damage caused by the apple maggot fly (AMF), *Rhagoletis pomonella*, in commercial orchards originates from adults immigrating from unmanaged hosts. One behaviorally-based approach that was developed for AMF control is an attractand-kill (= AK) system involving either, odor-baited Tangletrap-coated red spheres, or maintenance-free odor-baited attracticidal spheres which have contoured tops that provide sustained release of both insecticide and feeding stimulant under field conditions. However,

while both trapping devices are effective at controlling AMF, grower adoption has not materialized due to concerns involving the amount of labor involved in the case of sticky spheres, costs, and even regulatory hurdles that have largely prevented further research and development of attracticidal spheres.

In the spring 2020 issue of *Fruit Notes* we reported on the effectiveness of a novel AK system, evaluated in 2019, that makes use of synthetic lures deployed in perimeter-row trees in combination with insecticide sprays with 3% sugar (as a feeding stimulant) added to the tank. In that study, we demonstrated that the lures attracted AMF adults to perimeter-row trees where they were presumably killed by the insecticide/sugar sprays, before they could penetrate into the orchard blocks trees. The 2019 study

was conducted in six commercial orchards.

Here, present the results of a 2020 field study that was conducted in 11 commercial orchards located in Massachusetts, New Hampshire, and Maine. Our main goal was to validate the results of the 2019 study.

#### Material & Methods

**Study sites and treatment description.** This research study was conducted in 11 commercial apple

**Table 1**: Area of the attract-and-kill (= AK) and grower control (= GC) blocks and number of AMF lures used in AK blocks in 11 commercial apple orchards located in Massachusetts, New Hampshire, and Maine in 2020. CSO-1= UMass Cold Spring Orchard (CSO) block 1; CSO-2 = UMass CSO block 2.

Orchard	Area AK block / GC block	No AMF lures deployed in AK blocks
A	6.5 ac. / 7.8 ac.	24
В	1.4 ac. / 1.7 ac.	11
С	1.1 ac. / 1.6 ac.	6
D	7.1 ac. / 1.0 ac.	28
E (CSO-1)	3.7 ac. / 1.2 ac.	17
F (CSO-2)	1.9 ac. / 1.2 ac.	12
G	1.2 ac. /1.2 ac.	10
Н	3.3 ac. / 2.6 ac.	16
I	3.3 ac. / 4.0 ac.	16
J	1.1 ac. / 0.5 ac	16
K	2.5 ac. / 2.0 ac.	14

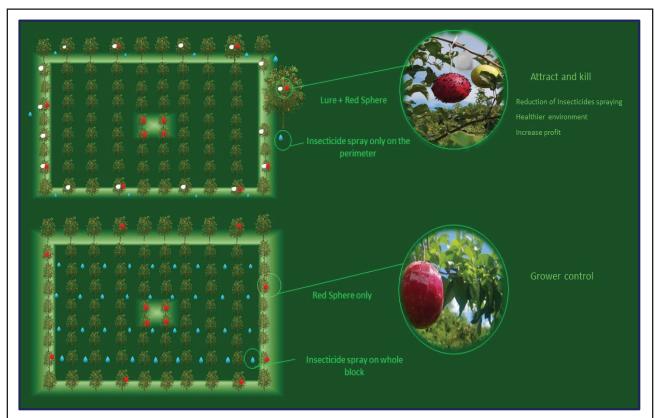
orchards located in Massachusetts (7 orchards), New Hampshire (3 orchards), and Maine (1 orchard). Within each orchard, there were two treatment blocks: (1) Attract and kill (= AK), and (2) Grower control (= GC). The area of each type of block is presented in Table 1.

In the AK block, the "attract" component consisted of AMF lures containing attractive synthetic apple odor (purchased from Great Lakes IPM). The lures were deployed every 30 yards along the entire perimeter of the block. The average lure density was 5 per acre (Table 1). The 'kill' component of this strategy consisted of insecticide sprays mixed with 3% sugar (3 lbs. per 100 gallons of water) applied to the perimeter of the blocks during July and August.

The GC block received no lures and no sugar in the sprays. Insecticides targeting AMF were applied to the entire block, as deemed necessary by the grower. Each participant grower applied the insecticide of their choice, most commonly the organophosphate Imidan (Phosmet), the neonicotinoid Assail Acetamiprid), the anthranilic diamide Exirel (Cyantraniliprole), and the neonicotinoid Belay (Clothianidin).

AMF monitoring and fruit injury assessments. We quantified AMF populations using red sticky spheres (3.5 inches in diameter) on a weekly basis. Both the AK and the GC blocks received 8 sticky spheres on perimeter-row trees (Figure 1). Four unbaited sticky spheres were deployed on the most interior trees of each block to monitor the AMF penetration rate (Figure 1). The number of AMF captured by the red monitoring spheres was recorded every week from trap deployment (in late June) until harvest. Captures by interior spheres were used as an indicator of the relative numbers of AMF adults that penetrated into the block interior.

At harvest, for each block we visually inspected 20 fruits from 16 trees located left and right of a monitoring sphere, and from 8 trees located in the block interior, for a total of 480 fruits per block. Across all 11 orchards and



**Figure 1.** Schematic illustration of the 2020 evaluation of an attract-and-kill (AK) strategy for apple maggot fly control involving (1) use of synthetic lures deployed on perimeter-row trees in AK blocks and (2) sugar (3%) added to the insecticide sprays that were confined to perimeter-row trees only. The efficiency of this management was compared against grower control blocks. The red circles are indicative of the location of red sticky spheres in both types of blocks and the white circles represent lures deployed in AK blocks only.

blocks, 10,560 fruits were visually inspected. All fruits that were suspected of having AMF egg-laying injury upon visual inspection were brought to the laboratory (UMass Amherst) and were kept inside individual plastic containers with moist sand (as a pupation substrate) at 75° F for six weeks. Then, each fruit was dissected for signs of tunneling and/or the presence of AMF pupae in the sand. Here, we are reporting confirmed injury levels, which are lower than the suspected injury levels that were recorded in the harvest surveys.

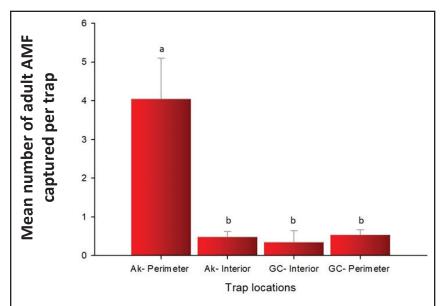
This research was considered effective if (1) AMF numbers on perimeter-row monitoring spheres were significantly greater than the number recorded on interior sticky spheres of AK blocks, and (2) if similar levels of AMF control, as

reflected by infestation rates, occurred in the AK and GC blocks.

#### Results

**AMF trapping:** Results indicated that AMF captures by baited sticky spheres in perimeter-row trees in association with synthetic AMF lures in AK blocks

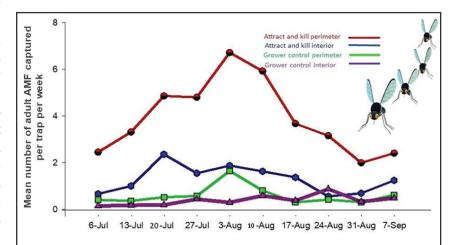
were significantly greater than AMF captures in perimeter rows of GC blocks, which had unbaited spheres (Figure 2). This indicates that the lures were efficient at pulling AMF adults to the perimeter, thus preventing them from penetrating into the block interior. No significant difference was observed between the unbaited monitoring spheres deployed in interior trees of both types of blocks (Figure 2), despite the fact that the interior of AK blocks did not receive insecticides. Since insecticides were used only in the perimeter of AK blocks, the total amount of insecticide used was lower than the amount used in GC



**Figure 2.** Mean number of adult AMF (males and females combined) captured in red sticky monitoring spheres according to treatment. Bars superscribed by the same letter are not significantly different at odds of 19:1.

blocks. As shown in Figure 3, the peak of AMF captures in AK blocks, across all orchards, took place in early August.

**Infestation data:** The confirmed AMF infestation levels were 0.04% across all 11 orchards and, similar to the first-year study (in 2019), the amount of fruit injured (expressed as a percentage) did not differ between AK blocks and GC blocks.



**Figure 3:** Mean weekly captures of wild AMF (males and females combined) in red sticky spheres deployed in perimeter-row trees in attract-and-kill blocks (red line) and in interior trees (blue line). Grower control blocks received unbaited sticky spheres deployed in the perimeter (green line) and in interior trees (purple line).

#### **Conclusions**

Results from this second-year study confirmed that an attract-and-kill approach involving synthetic lures deployed on perimeter-row trees in association with perimeter-row sprays of insecticides containing 3% sugar was efficient in controlling AMF, as determined by trap captures and infestation data, when compared to grower control blocks.

#### Acknowledgments

We thank growers from Massachusetts (Tom and Ben Clark, Keith Arsenault, Al Rose, Joanne DiNardo, Dana Clark, Shawn Mcintire), New Hampshire (Steve Wood, Chuck Souther, and Giff Burnap), and Maine (Harry and Sam Ricker) for allowing us to work on their orchards. We also thank Anna Wallingford, Jeremy Delisle and Sadie McCracken (University of New Hampshire) and Glenn Koehler (University of Maine) for support. Heriberto Godoy-Hernandez, Prabina Regmi, and Ajay Giri supported this research in the Massachusetts orchards. The USDA National Institute of Food and Agriculture, Crop and Pest Management program, funded this work through project 2018-70006-28890.

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### Does the Presence of Trap-crop Plants Enhance the Response of the Invasive Brown Marmorated Stink Bug to Its Synthetic Pheromone?

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In Massachusetts and other New England states, the invasive pest brown marmorated stink bug (BMSB), Halyomorpha halys, is threatening the specialty crops industry. The highest BMSB populations in Massachusetts were recorded in 2019 and 2020. In 2020, we increased monitoring efforts to include farms located in the southwest, northwest, and southeast areas of the state, where no BMSB monitoring had been done before. We found BMSB at every single farm and fruit growers expressed big concerns about future crop damage potentially caused by this pest. Small-scale growers face tough choices about protecting crops from BMSB near harvest, when pest populations are high. Broad-spectrum insecticides are effective but also kill beneficial insects and some materials cannot be applied near harvest. Thus, the threat posed by BMSB to retail and pick-your-own operations is very high.

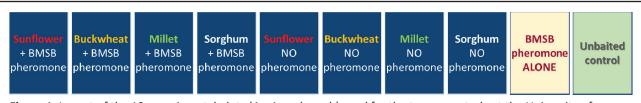
Recent trap-cropping research has revealed that sunflower and sorghum are effective trap crop plants for BMSB in vegetable production systems. In Florida, buckwheat and millet are additional suggested trap crops for leaf-footed bugs and various stink bug species. The BMSB synthetic pheromone is being used by some growers to monitor BMSB populations. Whether the combination of trap crop plants and BMSB pheromone lures attract more BMSB than either, trap crops alone

or the pheromone alone, has to our knowledge not been evaluated in fruit orchards.

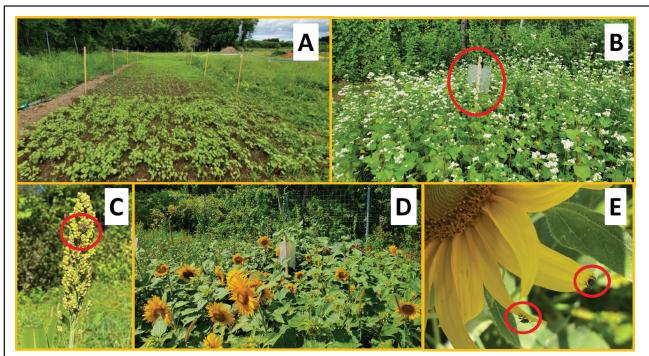
Here, we present the results of a 2020 study that compared the extent to which the presence of trap crop plants (dwarf sunflower, buckwheat, sorghum, and pearl millet) increases BMSB captures in pheromone-baited clear sticky cards when compared to pheromone-baited cards in the absence of trap crops.

#### Materials & Methods

This study was conducted at the University of Massachusetts Cold spring Orchard, in an open area adjacent (about 100 yards apart) to an apple block. In early June, 2020, an area of 5 x 45 yards was plowed and rototilled. This area was then sub-divided into 10 plots of 4 x 4 yards each. The first four plots received one of four trap crop seeds: dwarf sunflower, buckwheat, pearl millet, and sorghum (WGF-type). The four adjacent plots received the same four trap crops. The ninth and the tenth plots were left as bare soil, and they eventually became grassy areas. On July 6<sup>th</sup>, each of the 10 plots received one clear sticky card (8 x 6 inches). All sticky cards were stapled to tomato stakes that were buried into the ground. The first four plots received BMSB pheromone lures (one per plot), which were attached



**Figure 1.** Layout of the 10 experimental plots (4 x 4 yards each) used for the trap crop study at the University of Massachusetts Cold Spring Orchard. Each experimental plot received one baited (plots 1-4 and 9) or one unbaited (plots 5-8 and 10) clear sticky card to quantify BMSB captures.



**Figure 2.** Progression of the trap crop study: (A) View of the experimental plots on June 15, 2020, (B) Buckwheat plants in bloom (picture taken on July 20) with pheromone-baited clear sticky card (encircled), (C) Adult BMSB (encircled) on sorghum panicle (head), (D) Dwarf sunflower plants in bloom (picture taken on August 17), (E) view of two BMSB nymphs (encircled) on sunflower.

to the tomato stake, above the sticky cards. Plots 5-8 did not receive pheromone lures; therefore, these plots only tested the effects of the trap crops alone. Plot 9 received one BMSB pheromone lure, so this treatment represented the pheromone in the absence of trap crop

plants. The 10<sup>th</sup> plot received an unbaited clear sticky card, so this plot served as a negative control (Figure 1).

Once a week thereafter, all sticky cards were inspected and the number of adults and nymphs of BMSB

were recorded (and removed) until September 21, 2020. Figure 2 depicts the experimental plots with trap crop plants at various stages of development.

#### 20 captured per sticky card Mean number of BMSB 18 16 14 12 10 8 6 4 2 Pheromone on trap crop Control Pheromone Trap crop alone (no pheromone on grass

**Figure 3.** Mean number of BMSB captured per clear sticky card, according to treatment. BMSB captures were pulled across the four trap crop species (first and third bars).

#### Results

From July 13 to September 21, 2020, 10 sticky cards captured 84 BMSB (combining adults and nymphs). Similar BMSB captures were recorded among the four trap crop plant species. Figure 3 shows that BMSB captures in pheromone-baited clear sticky cards deployed in association with trap crop plants attracted 60% more BMSBs than pheromone-baited sticky cards de-

ployed in the absence of trap crop plants. Sticky cards deployed in trap crop areas in the absence of BMSB pheromone only captured 2 BMSB (average of 0.25 per card) across the entire period of investigation.

#### **Conclusions**

Based on the results of this single-location, single-season study, it appears that deploying the BMSB pheromone in areas planted with trap crops results in increased numbers of BMSB visiting those areas when compared to the BMSB pheromone deployed alone. Plans are underway to continue with this research at multiple locations throughout Massachusetts to validate the findings of this study.

#### Acknowledgments

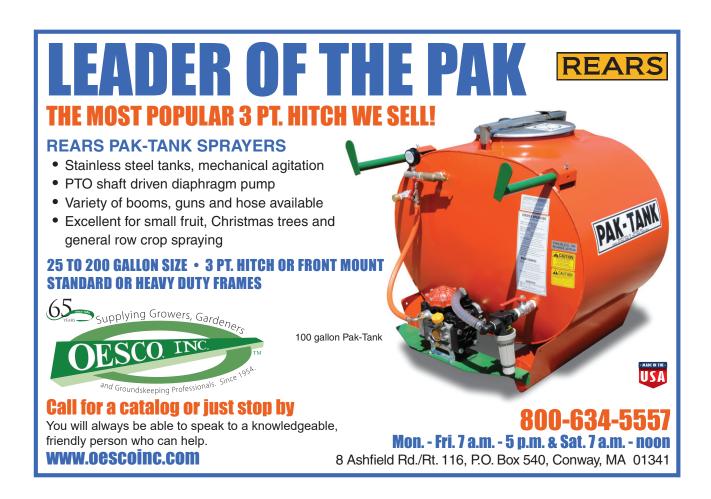
We thank Shawn McIntire and the crew of the Cold

Spring Orchard (Belchertown, MA) for preparing the land for this work. Support for this research was provided by the UMass Stockbridge School of Agriculture.

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# Precision Crop-load Management of Honeycrisp: Flower Bud Identification and Precision Pruning

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#### Win Cowgill

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#### Why precision-prune Honeycrisp?

### • Honeycrisp is very prone to biennial bearing. You can't afford to have "on" and/or "off" years

- Over- or under-cropping Honeycrisp results in biennial bearing and has adverse effect(s) on fruit quality
- Precision crop load management of Honeycrisp, particularly in tall-spindle orchards is recommended, which includes precision pruning, precision thinning, and return bloom sprays
- Understanding what percent of spurs are flowering is an important step in precision pruning, however, with Honeycrisp buds must be dissected and magnified to determine this percent, you cannot simply tell by observation (unlike some other varieties, and note that they can be precision-pruned too)

#### What is precision pruning?

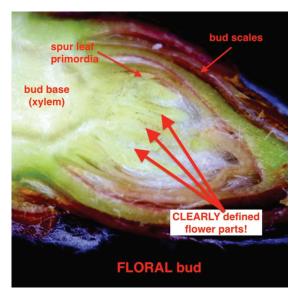
- Precision pruning is determining a desirable crop load (number of apples per tree), determining what percent of spurs (buds) are flowering (or not), and then pruning until a desired bud count is reached
- Note that determining the desired crop load is FIRST determined by the target yield per acre (1,000 bushels for example) then dividing by the number of trees per acre to get apples per tree; alternately you can simply decide how many apples per tree you want based on tree density, age, training system, and fruiting spur branch density

#### Flower buds vs. non-flower buds-why and how?

- Have to be able to identify spur buds as floral or vegetative, remembering, buds are initiated the growing season (in May/June) before dormant pruning
- Factors that affect whether a flower bud is formed (or not formed) the previous season?
  - Honeycrisp induces and then initiates flower buds early, beginning just after bloom and finishing as early as 30-45 days after boom; therefore, fruit thinning must be accomplished early (with blossom and petal fall NAA sprays)
  - Honeycrisp thinned to no more than one fruit per spur will result in more flower bud formation
  - Thinning/return bloom sprays beginning at bloom and petal fall (NAA) and then again (if needed) using NAA in the traditional thinning stage of 8 to 12 mm. fruit size (as necessary) promote flower bud development
  - Keep spurs exposed to sunlight, trees should not be stressed, have excellent nutrition and adequate irrigation, etc.

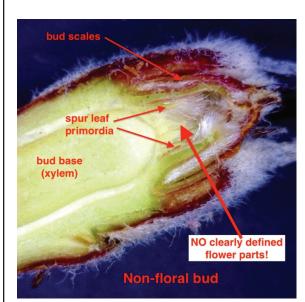
#### Flower buds vs. non-flower buds- identification

- Just before pruning is started, collect a random sample of what appear to be flowering spurs from each block a sample of 30 to 50 buds is recommended
- Make a longitudinal slice right in the center of each bud with single edge razor blade to expose bud insides (make as clean a cut as possible)





These are flower buds!





These are NOT flower buds!

- Magnify with the instrument of your choice -- a binocular-scope or digital microscope -- to identify if flower bud or not, flower parts should be obvious (see pictures)
- Determine, % buds with flower parts vs. no flower parts, for example if a sample of 30 buds is taken, and 18 are seen to have flower parts (12 do not) then it is presumed you would have 60% flowering spurs across the block
- Consider some other factors: large crop last year equals more likely to have non-floral buds (blanks);

bud size, all things being equal, larger buds are more likely floral vs. smaller buds more likely non-floral (but with Honeycrisp it's hard to tell for sure unless you dissect and examine under magnification); cropping history, rootstock, absence of spur buds, etc. Use your experience in addition to procedure described above, but dissection and magnification is the only way to know for sure what percent of buds are floral on Honeycrisp (and perhaps other hard-to-tell apple varieties).

#### Precision-prune and follow-up

- Determine how many buds (floral and non-floral) to leave after precision pruning based on the desired final crop load. For example, if tree starts out with 200 apparent flower buds, and desired crop load is 60 apples, 1.5 times 60 = 90 buds, but if only 60% are floral, then need to leave 150 buds after pruning (remove 50 buds)
- Prune to desired bud count, use thinning cuts, spur bud extinction (particularly those vertical oriented, up or down)
- Adjust bud load by pruning at pink if possible (most growers that is not)
- Apply a bloom thinning spray using NAA
- Apply a petal fall thinning spray using NAA, and then again at 8 to 12 mm. (if necessary) using NAA (and carbaryl if indicated) using the carbohydrate model (to time and adjust rate) and the fruit growth rate model (Malusim app, malusim.org)

- Hand thin if necessary within 35 days of full bloom
- or ethephon at 30 to 35 mm (only if less than 80 degrees F. for ethephon) every seven days, remembering flower bud formation is nearly complete 45 days after bloom. Make sure to cease ethephon sprays 45 days before harvest as to not trigger ripening. Some research has shown that ethephon applied immediately after harvest (@2pints/A) will help promote fruit bud formation the following season.

#### Acknowledgments

Special thanks to Dr. Poliana Francescatto, Valent Biosciences for assistance in ID of floral vs. non-floral buds, and her work while a post-doc at Cornell in identifying early flower bud formation of Honeycrisp apples.



# Evaluating the Efficacy of Multi-cultivar Grafted Apple Trees as Perennial Trap Crops for Multiple Pests: Research Results Year One

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Habitat manipulation through the incorporation of non-crop plants such as trap crops (very attractive plants that lure pests away from the cash crop) into agroecosystems is an ecological approach to pest management. To be effective, trap cropping systems must congregate and retain the pest on trap crop plants, thereby reducing pest populations in the cash crop. The fundamental tenet of this definition involves differential pest preference between plant species, the plants that function as trap crops and those to be protected. It is known that some apple cultivars are more attractive to some insect pests than others. For example, Red Astrachan is a cultivar highly susceptible to apple maggot fly (AMF) attack, and growers have indicated that Yellow Transparent and Dabinett are also favored by AMF over other cultivars. The cultivars Ginger Gold and Liberty are reported to be attractive to plum curculio (PC). While for these two insect pests effective lures are commercially available for monitoring and control (e.g., attract-and-kill systems), their comparatively high cost has prevented growers from adopting monitoring or control systems that are based on synthetic lures. For other insect pests such as tarnished plant bug (TPB) and European apple sawfly (EAS), no lures have been developed. Consequently, we sought to exploit natural sources of plant odor represented by apple cultivars that have the potential to aggregate pests on selected apple trees that are grafted with six cultivars, thereby serving as perennial 'trap crops'.

In the spring of 2018, the lead author (Piñero) sought grower input to gauge the level of interest in research aimed at developing permanent monitoring (and potentially attract-and-kill) sites using selected perimeterrow apple trees grafted with six apple cultivars that are highly attractive to PC and AMF. The growers that were consulted expressed support for the project, and some growers immediately requested scion wood of the

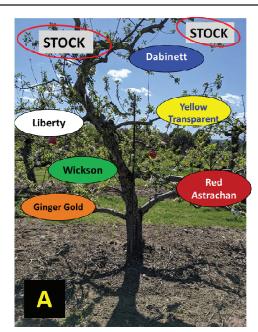
cultivars that were proposed, knowing that it would take at least two years to have experimental trees available for the research.

From the onset, the new concept of multi-cultivar grafting for pest management is considered to be simple (i.e., grower-friendly) and inexpensive. If this new IPM approach proves to be effective, then permanent monitoring sites could be developed and farm inputs might be reduced in support of sustainable agriculture. By mid-May 2018, over 40 trees in Massachusetts, New Hampshire, and Maine had already been grafted. Each grafted tree received 6 cultivars reported to be attractive to PC and AMF. In the spring 2020, the number of trees grafted in 13 commercial orchards (10 in MA, two in NH, one in ME) had exceeded 100.

Here, we present the research results for the first year of insect pest monitoring in grafted and nongrafted trees in 10 Massachusetts orchard blocks. In 2020, the target pests were TPB, EAS, PC, and AMF. The main goal of this long-term study is to establish the attractiveness of perimeter-row trees grafted with multiple cultivars to develop permanent monitoring, and potentially attract-and-kill sites, for multiple pests.

#### Materials & Methods

This study was conducted in 10 commercial apple orchard blocks in Massachusetts. The size of the experimental blocks in Massachusetts ranged from 0.2 to 7.3 acres and most blocks had a density of 3 grafted trees per acre. Four blocks have perimeter-row trees that were grafted in 2018, and six blocks have trees that were grafted in 2019. Each tree was grafted with six cultivars: Liberty, Red Astrachan, Yellow Transparent, Ginger Gold, Dabinett, and Wickson (Fig. 1). For nearly all trees, the grafting was conducted by Jim Krupa (UMass cold Spring Orchard) using the cleft technique.





**Figure 1.** Representative example of one apple tree grafted with six cultivars (A) Early season, (B) Late season. For each grafted tree, non-grafted branches are referred to as 'stock' branches.

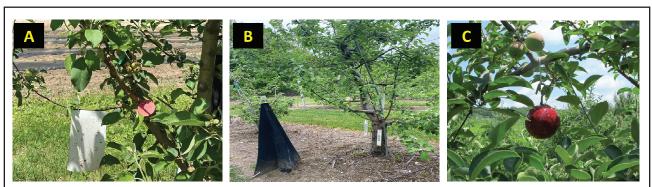
Monitoring insect pest activity: To monitor EAS and TPB, unbaited white sticky cards were deployed on lower branches of grafted and non-grafted tree trees on 30 March, 2020, at the silver tip bud stage (Fig. 2A). PC monitoring was done using unbaited black pyramid traps deployed near grafted and non-grafted trees starting in early May 2020, at the pink tree stage (Fig. 2B). AMF was monitored from 30 June to 18 September, 2020, using unbaited red sticky spheres deployed in optimal position within the tree canopies (Fig. 2C). All insect traps were inspected once a week. Tree phenology was recorded twice a week (data not shown).

**Assessment of fruit injury**. Starting on June 2<sup>nd</sup>, levels of fruit injury by PC, TPB, and EAS were

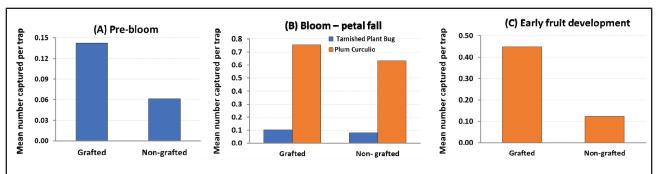
recorded weekly until July 7<sup>th</sup>, 2020. Fresh fruit injury by PC was recorded by marking the scar with sharpie, in order to avoid counting the same fruits. The level of fruit injury by all pests was recorded at harvest (in mid-September, 2020) by sampling 20 fruits from every cultivar of grafted trees and 20 fruits from non- grafted trees.

#### Results

Insect captures in grafted vs. non-grafted trees. During the pre-bloom period, white traps deployed on grafted trees captured 2.3 times more TPB than traps placed on non-grafted trees. However, results are statis-

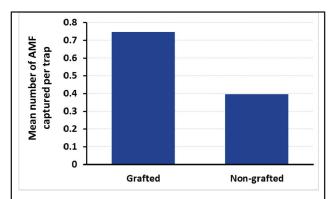


**Figure 2**. Insect monitoring devices: (A) unbaited white sticky card for tarnished plant bug and European apple sawfly, (B) unbaited black pyramid trap for plum curculio, and (C) unbaited red sticky sphere for apple magget fly.



**Figure 3.** Captures of tarnished plant bug (blue bars) and plum curculio (orange bars) on grafted and non-grafted trees at three phenological tree stages: (A) Pre-bloom (B) Petal fall, and (C) Early fruit development.

tically non-significant due to variability among samples (Fig. 3A). From bloom to petal fall, PC and TPB captures were similar in grafted and non-grafted trees (Fig. 3B). During the early fruit development period,



**Figure 4.** Captures of adult apple maggot fly (AMF) on unbaited red sticky spheres deployed in grafted and nongrafted trees.

injury by PC. The least damaged cultivars across the 5-week period were Dabinett, Wickson, Yellow Transparent, and Liberty (Fig. 5). Ginger Gold received the highest levels of fruit injury by TPB (see blue bars in Fig. 6) recorded at harvest.

AMF injury was very low across all blocks. Only a single fruit, sampled from a grafted tree (cultivar: Ginger Gold) was found to be infested by AMF. It is important to note that all trees were subject to standard insecticide applications by the growers so infestation levels were expected to be low.

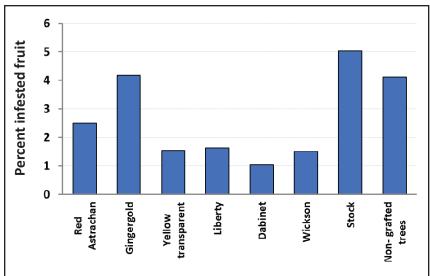
#### **Conclusions**

Based on the first-year results of this long-term study, we recorded evidence supporting our hypothesis that grafted trees may be more attractive to some insect pests than non- grafted trees. Ginger Gold, one of the six cultivars selected for grafting, was highly attractive

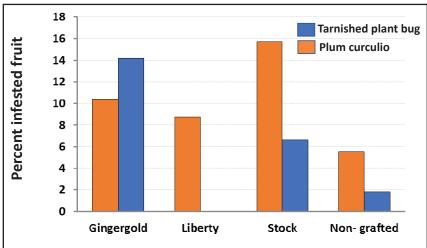
significantly more PCs (over 3 times more) were captured on grafted trees than on non-grafted trees (Fig. 3C).

Across the 10 commercial orchards, unbaited sticky spheres placed on grafted trees captured nearly twice as many AMF as unbaited sticky spheres deployed on non-grafted trees (Fig. 4).

Level of fruit injury caused by insect pests in grafted vs. nongrafted trees. There were significant differences among cultivars in terms of level of fruit injury caused by PC during May and June. Stock fruit (fruit sampled from non-grafted branches in grafted trees), followed by Ginger Gold, received the most



**Figure 5.** Mean percentage of fruit infested by plum curculio in grafted and non-grafted trees across 5 weeks (June 2 to July 7, 2020).



**Figure 6.** Mean percentage of fruit damaged by tarnished plant bug (blue bars) and plum curculio orange bars) in grafted and non- grafted trees at harvest.

to TPB and PC, based on trapping and fruit injury data. Because tree branches were grafted in 2018 and 2019, multiple years of research are therefore needed, under multiple levels of pest pressure, before firm conclusions can be drawn concerning the relative attractiveness of grafted trees to insect pests.

#### Acknowledgments

We thank Joanne Dinardo (Sholan Farms), Keith Arsenault (Ragged Hill Orchard), Tom and Ben Clark (Clarkdale Orchards), Shawn McIntire (Cold Spring Orchard, Belchertown, MA), Kenneth Nicewicz (Nicewicz family farm) Albert Rose (Red Apple Farm, Phillipston, MA), and Mo and Andre Tougas (Tougas family farm) for allowing us to work on their orchards. We also thank Ajay Giri for assistance.

The University acknowledges the support of the Massachusetts Society for Promoting Agriculture

provided in memory of David Chandler, who worked tirelessly to promote the best agricultural practices in the Massachusetts apple industry. The USDA National Institute of Food and Agriculture provided additional financial support for this work through project award number 2019-70006-30445.



# Does the Red Color Enhance Spotted Wing Drosophila Response to Traps Baited with Diluted Concord Grape Juice?

### Lyndsey A. Ware, Elizabeth W. Garofalo, Elsa Petit, and Jaime C. Piñero *University of Massachusetts Amherst*

Spotted wing drosophila (SWD), *Drosophila suzukii*, is an invasive vinegar fly. The female of this species possesses a serrated ovipositor, enabling her to lay eggs in sound, ripening fruit unlike other vinegar flies which only inhabit damaged or rotting fruits and vegetables. This knife-like ovipositor enables the female of this species to penetrate firm fruit surfaces and lay her eggs inside where larvae subsequently hatch and feed, causing damage to fruit and dismay to consumers. To time insecticide sprays effectively and mitigate damage caused by SWD, growers need to monitor SWD populations.

For the past two years we have been evaluating the attractiveness of diluted Concord grape juice, a low-cost and readily available material, to male and female SWD. When diluted at a ratio of 1:3 (= 1 part of grape juice and three parts of water), diluted grape juice showed to be three times more attractive to males and females than one commercial lure under field conditions. In addition, grape juice diluted at the 1:3 ratio

attracted significantly fewer (about three times less) non-targets than one commercial lure, highlighting a potential greater selectivity of diluted Concord grape juice (see Piñero et al. [2019]; *Fruit Notes* summer issue).

Appropriate combinations of visual and olfactory cues could be helpful for earlier detection or in attract-and-kill strategies for SWD control. In general, darker, less reflective colors have been proven to attract SWD. In our previous studies, we have used semitransparent traps. The present study was designed to quantify the extent to which color plays a role in SWD captures in traps baited with diluted Concord grape juice.

#### **Materials & Methods**

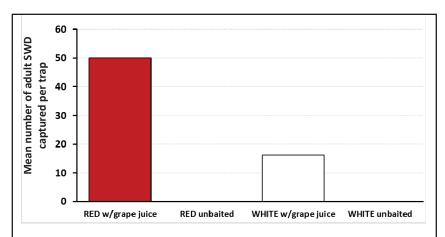
This study was conducted from August 8 to September 15, 2020, in one section of Frontenac wine grapes at the UMass Cold Orchard (Belchertown, MA). Once Covid-19-related restrictions were loosened and appro-

priate safety protocols implemented, team members were able to access the fields and execute this experiment. Traps used were 1 quart in capacity and had 12 pin holes punched around the rim. The holes were big enough to allow SWD adults to enter the trap but small enough to reduce captures of larger insects. Twistable wire extruding from a single hole in the lid allowed for trap deployment on the lower training wires between posts (Figure 1).

We evaluated four treatments, dictated by the combination of two colors: red and white, and two baits: diluted Concord grape juice and wa-



Figure 1. White- and red-painted traps used for the study.



**Figure 2.** Average captures of male and female (data combined) spotted wing drosophila in red- and white-painted traps baited with either, diluted Concord grape juice or unbaited traps. Unbaited traps had 8 ounces of unscented soapy water to kill responding insects.

ter as a control. The traps and the corresponding lids were spray-painted red or white. Diluted grape juice was prepared by mixing two ounces of grape juice and six ounces of tap water. Water-baited traps had one drop of unscented soap to break the surface tension of water thereby making the insects sink.

On August 5, four sets consisting of four traps each (one for each color/bait combination) were deployed along the lower horizontal wire of the trellis. Each set was positioned in a different row, and considered a replicate. The distance between traps was 3 yards. Traps were positioned so that sunlight did not hit traps directly. To minimize fermentation effects, all traps were serviced twice a week. At each inspection ses-

sion, all traps were retrieved and all insects were collected. Traps were then cleaned thoroughly with soapy water, rinsed with deionized water and refilled before being re-hung in the lower canopies. SWD captured were identified according to sex and non-SWD insects were recorded as non-targets.

#### Results

Figure 2 shows that captures of SWD were strongly affected by trap color. Across the entire period of investigation, red-painted traps baited with diluted grape juice captured at least three times more adult

SWD (males and females combined) than similarly-baited white-painted traps. Regardless of color, unbaited traps captured very few insects, and zero SWD.

As for captures of non-target insects, diluted Concord grape juice consistently attracted more SWD than non-target insects (3.6 times more, on average, across all trapping dates) except for the first (August 10) trapping date (Figure 3).

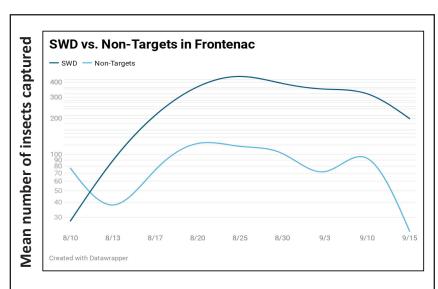
#### **Conclusions**

This study showed that by painting traps red captures of SWD in traps containing diluted grape

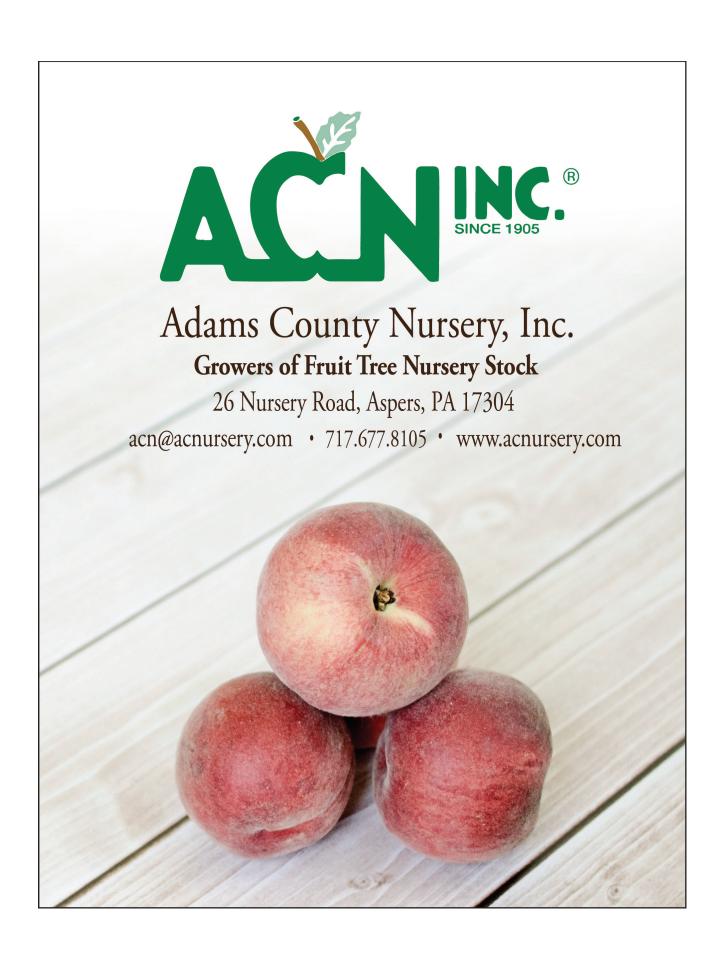
juice are greatly enhanced, thereby demonstrating the importance of vision in this invasive species. This inexpensive trapping system can improve the 'bottom line' by saving on lure costs while maximizing SWD captures.

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Piñero, J.C., Wen, X.J., and Begonis, E. 2019. Using diluted grape juice for early-season monitoring and its potential for Attract-and-kill of Spotted Wing Drosophila, *Drosophila suzukii*. Fruit Notes 84: 12-15.



**Figure 3.** Captures of adult SWD and non-target insects in diluted Concord grape juice-baited traps according to date.



# Evaluation of Novel Kairomone-based Lures for Attracting Male and Female Tortricid Moths in Apple Orchards

Ajay P. Giri and Jaime C. Piñero Stockbridge School of Agriculture, UMass Amherst

Apple orchards are often attacked by several tortricid (Lepidoptera) pests such as Codling moth (CM), Oriental fruit moth (OFM), Obliquebanded leafroller (OBLR), and Redbanded leafroller (RBLR) that are either, key or secondary pests that attack the fruit. Sex pheromone-based lures have been used to (1) monitor male moth populations and (2) control pest species directly via mating disruption. Increased captures of moths of both sexes by the addition of plant volatiles could improve the effectiveness of these systems. The goal of this study was to evaluate the performance of experimental lures with added plant volatiles at captur-

ing males and females of multiple species of tortricids.

#### Materials & Methods

This field study was conducted from May 12 to September 18, 2020, in one commercial apple orchard in Massachusetts. Six olfactory treatments: (1) Pherocon® CML2-P, (2) Pherocon® Megalure CM 4K Dual, (3) Pherocon® Megalure CM 4K Dual + TRE2265, (4) TRE2266, (5) TRE2267 and (6) unbaited traps as control were evaluated in orange Delta-shaped traps (Pherocon® VI, Trécé Inc., Adair, OK). All lures were

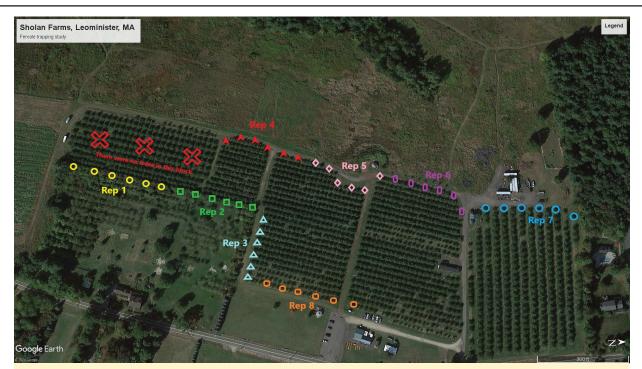
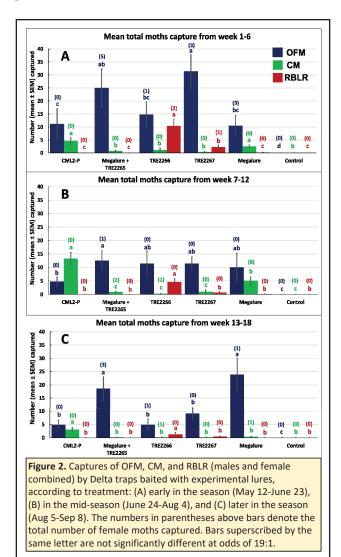


Figure 1. Trap deployment on the perimeter of the orchard. There were 6 treatments and 8 replications.

provided by Trécé Inc. Each treatment was replicated 8 times. Traps were deployed along the perimeter of one apple block (Fig. 1). Each treatment was randomized within a replicate. Traps were 15 yards apart. Traps were examined beginning on May 19 and every 7 days thereafter until September 18. All lures and sticky liners were renewed every four weeks. Once a week, traps were switched one position clockwise within a replication to minimize the effect of position. The orchard received a standard insecticide spray regime.

**Data collection and analysis**: All captured adult moths were identified according to species (i.e., CM, OFM, RBLR) and dissected under stereomicroscope to identify their respective sex. No OBLR were detected in this orchard. Data were analyzed using proper statistical procedures.



Results & Discussion

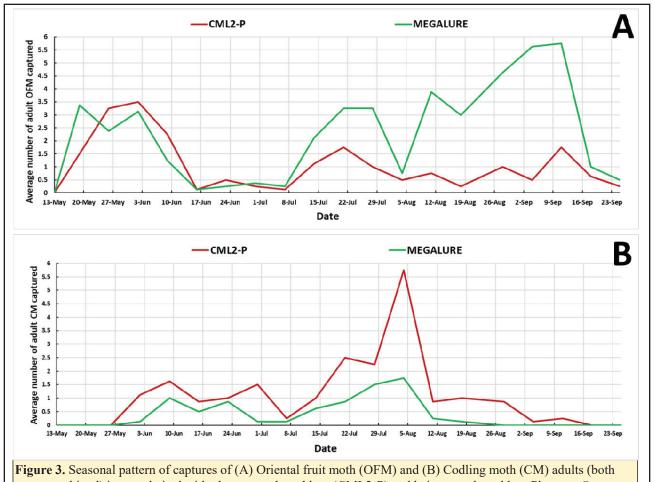
For the first trapping period (May 12 - June 23), TRE2266 and Pherocon® Megalure CM 4K Dual+TRE2265 captured significantly more OFM adults than any other lure (Fig. 2A). More OFM females (9 in all) were captured in traps baited with Pherocon® Megalure CM 4K Dual. Pheromone lure CML2-P and Pherocon® Megalure CM 4K Dual captured significantly more CM than any other lure.

As for RBLR, the lure TRE2266 captured significantly more moths than any other lure in this period.

Results from the second period (June 24 - August 4) revealed statistically similar captures of OFM adults among lures except for unbaited traps, which captured no moths (Fig. 2B). For CM, the lure CML2-P captured significantly more moths than any other lure. The second highest captures were observed in Pherocon® Megalure CM 4K Dual, which also attracted the highest number of CM females (8 in all) during that period. The lure TRE2266 continued to perform best at attracting RBLR adults.

In the third period (August 5 - September 18), traps baited with Pherocon® Megalure CM 4K Dual and with Pherocon® Megalure CM 4K Dual+TRE2265 captured significantly more OFM than any other lure (Fig. 2C). Again, the lure CML2-P captured significantly more CM adults in this period. Likewise, TRE2266 captured significantly more RBLR adults than any other lure. Overall, Pherocon® Megalure CM 4K Dual attracted 50% of all OFM females (n= 18; period 1) and 73% of all CM females (n= 11; period 2).

Temporal pattern of moth captures by Pherocon® Megalure CM 4K Dual and CML2-P lures. Early in the season (May 12 - June 23), captures of OFM (Fig. 3A) and CM (Fig. 3B) by traps baited with the pheromone lure (CML2-P) and the kairomone lure Pherocon® Megalure CM 4K Dual were similar. In the mid-season (June 24 - August 4), traps baited with Pherocon® Megalure CM 4K Dual captured twice as many OFM than CML2-P whereas there was a 3-fold increase in CM captures in traps baited with CML2-P compared to Pherocon® Megalure CM 4K Dual. Late in the season (August 5 - September 23), Pherocon® Megalure CM 4K Dual captured 3 times more OFM than CML2-P. At the beginning of the late season, CM captures were greatest in CML2-P and then captures declined.



**Figure 3.** Seasonal pattern of captures of (A) Oriental fruit moth (OFM) and (B) Codling moth (CM) adults (both sexes combined) in traps baited with pheromone-based lure (CML2-P) and kairomone based lure Pherocon® Megalure CM 4K Dual.

#### Conclusion

Across the entire season, Pherocon® Megalure CM 4K Dual was as attractive or more attractive than the pheromone lure CML2-P to male OFM, revealing a benefit of using plant volatiles for enhanced moth monitoring. In contrast, CML2-P was numerically more attractive to male CM than Pherocon® Megalure CM 4K Dual. This lure attracted the most females of CM and OFM but the addition of plant volatiles (i.e., TRE2265) to Pherocon® Megalure CM 4K Dual did not improve female captures. The lures TRE2266 and TRE2267 performed well at attracting male RBLR and OFM.

#### Acknowledgements

We thank Joanne M. DiNardo for allowing us to work in Sholan Orchards (Leominster, MA), Thanks to Mr. Brent Short (Trécé Inc., Adair, OK) for providing the lures and trapping materials, to Heriberto Godoy-Hernandez, Prabina Regmi and Dorna Saadat for assistance.



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# Evaluation of Hard Cider Apple Varieties to Promote Hard Cider Industry Growth in New Jersey

#### Megan Muehlbauer and Rebecca Magron Rutgers University

Hard cider is one of the most recent alcoholic beverages to gain popularity in the USA. Despite being considered a niche product, sales reached \$428.2 million in 2019. (Jacobsen, J. 2020) These figures indicate there is a significant amount of profit to be realized from growing and pressing apples into hard cider in the United States. In fact, a number of case studies have shown this to be true. Budget spreadsheets developed by Farris, J et al. illustrate the economic feasibility of hard cider production, and despite often requiring significant inputs, profits can still be realized.

Currently New Jersey orchardists already making sweet cider are looking to produce an additional valueadded product. NJ farm wineries seeking to diversity their product line have also considered producing hard

cider and are looking to learn how to grow 'hard cider varieties' to blend with sweet cider. The common question is which variety or varieties are best suited for New Jersey growing conditions.

Apple varieties have historically been chosen for use in hard cider based upon flavor characteristics. Most notably, high sugar, high acid, and complex tannin profiles. Most hard cider producers in New York and Pennsylvania are blending approximately 75% sweet cider with some more traditional hard cider cultivars from England or France to increase the tannin content and acidity and improve flavor profiles. A hard cider variety trial by Duane Green at UMASS showed that some traditional eating apples make very good hard cider as well i.e. Goldrush, Liberty, Golden Russet, Baldwin, Roxbury Russet, Rhode Island Greening and Esopus Spitzenburg, a favorite of Thomas Jefferson's.

Many of the older eating varieties and the European hard cider varieties that are of interest for their use in cider often lack disease and pest resistance, vigor and high yields. In fact, many bloom late and are severely susceptible to fire blight. Field trials, to determine how best to manage these varieties in modern orchards. A number of these trials have been established and are ongoing at Universities including Cornell University, The University of Vermont and Washington State University. Despite these efforts, studies have shown there is a continued need for an increased number of variety trials across diverse climate conditions.

A study by Alexander et al. (2016), illustrated that



Figure 1. Successful cleft grafts of cider apple scions in Aztec Fuji Interstem/M.9 NAKBT337, August 2018.

Table 1. Cultivars included in the trial, and their use in ciders. All varieties included in this study are best used for blending with varieties that have complimentary characteristics.

Variety	Description	
Calville Blanc	Sharp Noteworthy aroma, can be used as a single varietal cider.	
Collaos	Bittersharp Ripens very late in season.	
Dabinett	Bittersweet A well-balanced variety, commonly used for cider.	
Ellis Bitter	Bittersweet Noteworthy for being a tip-bearer.	
Golden Russet	<b>Sharp</b> High acidity, sugar and aroma, a highly recommended cider variety.	
Harrison	Sharp Noted for excellent, well balanced juice quality.	
Kingston Black	Sharp A well-balanced stand-alone variety.	
Margil	Sharp A variety with nuance, rich, intense and aromatic.	
Rein Des Reinettes	Sharp Vigorous with a high sugar content.	
Roxbury Russet	Sharp Notable for holding well in storage.	
Stoke Red	Bittersharp It is used in cider blends and as a single varietal.	
Wickson	<b>Sharp</b> Commonly grown by cider makers, notable for high sugar content.	

four widely utilized hard cider cultivars grown over the course of several years at different locations resulted in significantly different sugar content and tannin profiles in the pressed juice.

As a result, a hard cider variety trial was established in New Jersey, as a means to provide New Jersey apple growers a local resource for yield, vigor and fruit size of locally grown hard cider apples, in addition to other management decisions for establishing a hard cider apple orchard in New Jersey.

#### Experimental Design

This trial was established in 2018 at the Rutgers Snyder Research and Extension Farm in Pittstown, New Jersey. A block of 80, 5-year-old Daybreak Fuji trees on M.9 NAKBT337 top worked to create the trial. Scion wood was obtained from the USDA Germplasm

Repository (Geneva, NY). Twelve hard cider varieties were chosen, which represent the major hard cider apple types (bittersweet, bittersharp, and sharp) Table 1. A total of 2 scions per tree and two trees per variety were grafted (top-worked). Figure 1. In 2019 and 2020 yield and fruit count totals for each tree were tabulated. Tree vigor was recorded by measuring scion wood diameter 12 inches above the graft union of the most vigorous graft per tree, and total height of that same scion was recorded at the end of the growing season.

#### Results

Measurements of diameter illustrated Dabinett had the largest diameter in 2018 (0.57 in), however in 2019 Stoke Red was shown to have the largest diameter (1.5 in). In contrast Margil had the smallest diameter in both 2018 and 2019, 0.22 (in) and 0.55 (in) respectively.

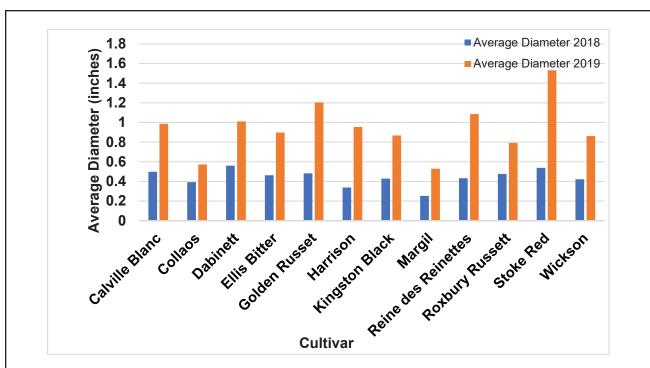


Figure 2. Illustrates the differences in vigor across all varieties as measured by diameters measured 12 inches above the graft union of the tallest scion per tree.

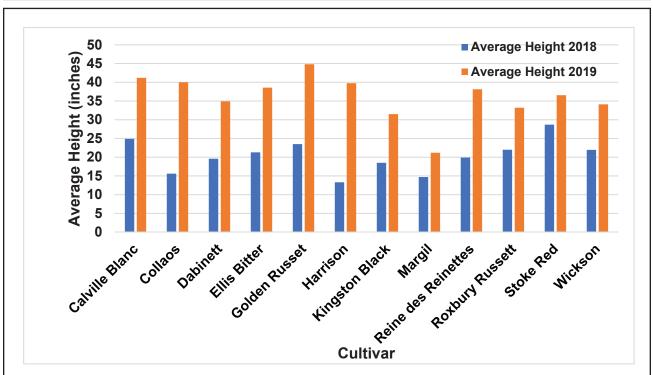


Figure 3. Illustrates the differences in vigor across all varieties as measured by height from the graft union to the top of the leader of the tallest scion per tree at the Rutgers Snyder Research and Extension Farm, Pittstown, NJ.

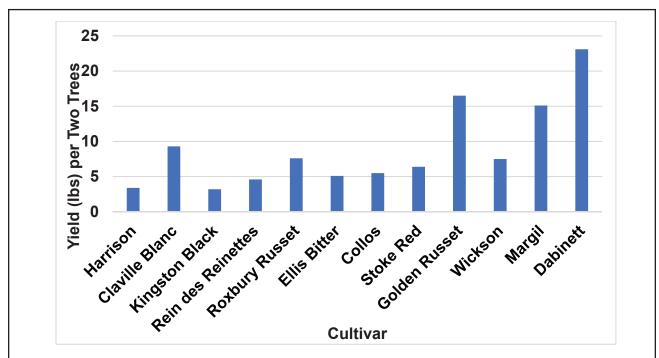


Figure 4. Total yield sampled in 2019 from two trees per variety at the Rutgers Snyder Research and Extension Farm, Pittstown, NJ.

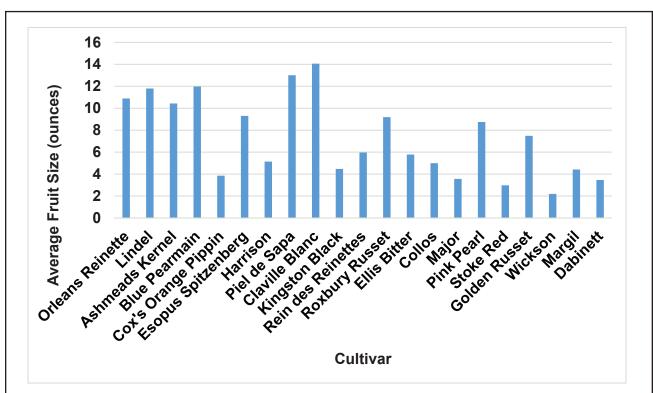


Figure 5. Average fruit size in 2019 was collected by obtaining the total yield per two trees divided by the total number of apples collected at the Rutgers Snyder Research and Extension Farm, Pittstown, NJ.



Figure 6. Mature Stoke Red apples at the Rutgers Snyder Research and Extension Farm, Pittstown, NJ.

Figure 2 The tallest main leader was observed for Stoke Red in 2018 (28 inches) and Golden Russet in 2019 (45 inches). The smallest main leader was observed for Harrison in 2018 (14 inches) and Mivity argil in 2019 (21 inches). Figure 3

Measurements of yield and fruit size showed the highest yielding cultivar was Dabinett (23 lbs./2 trees) and the lowest yielding cultivar was Kingston Black (3 lbs./2 trees). Figure 4 Average fruit size was observed to be the greatest for Claville Blanc (14 ounces) and



Figure 7. Mature Claville Blanc apples at the Rutgers Snyder Research and Extension Farm, Pittstown, NJ.



Figure 8. Mature Golden Russet apples at the Rutgers Snyder Research and Extension Farm, Pittstown, NJ.



Figure 9. Mature Margil apples at the Rutgers Snyder Research and Extension Farm, Pittstown, NJ.



Figure 10. Mature Dabinett apples. Photo Credit: Raintree Nursery.

the smallest for Wickson (2 oz). Figure 5.

#### **Conclusions**

The measurements of tree vigor (Figure 2 and 3) indicated that Stoke Red (Figure 6), Claville Blanc (Figure 7), and Golden Russet (Figure 8) thus far, thrive under New Jersey growing conditions.

Yield data indicated Margil (Figure 9), Dabinett (Figure 10) and Golden Russet (Figure 8) show promising fruit yields (Figure 4).

Fruit size measurements (Figure 5), showed about 75% of the varieties tested would likely be most efficiently harvested if swept off the ground (<150 g), and the other 25% would most likely be best harvested by hand (>150g).

Of the biochemical measurements taken, the pH showed levels comparable with those found in previous studies, while Brix, TA, and Tannins were much more variable (data not shown).

This project will be continued, and data collected

(yield, vigor and biochemical analysis) for several more years to corroborate the project findings.

Best practices for pruning and training of these trees will be investigated, along with PGR's (plant growth regulators) to minimize blind wood and enhance productivity and optimize crop load management.

In addition, this research demonstration plot will also serve as a field lab for showcasing the top working of existing orchard trees, which has garnered interest with NJ growers.

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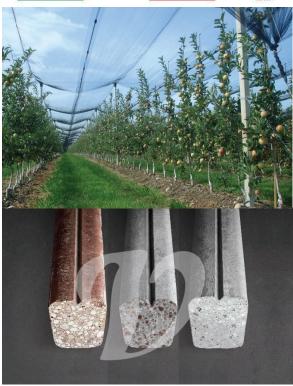
Washington State University. Cider Performance Gallery. www.cider.wsu.edu/ciderweb

Note all photos credit to Megan Muehlbauer unless otherwise noted.

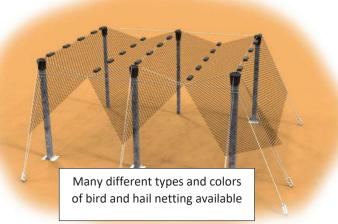
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## Response of Tarnished Plant Bug to Synthetic Aromatic Plant Volatiles

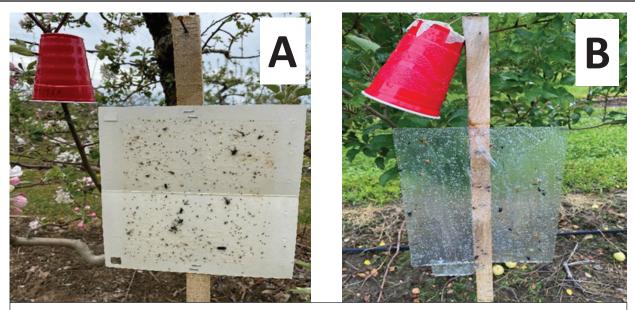
Prabina Regmi and Jaime Piñero Stockbridge School of Agriculture, University of Massachusetts

Many insect species utilize plant volatiles to locate food, oviposition sites, and potential mates. Based on the existing knowledge in plant-insect interactions, commercial lures based on plant volatiles have been developed to monitor some tree fruit pests. Some aromatic plant volatiles (group of related compounds that share some characteristics including strong, pleasant aroma) such as methyl salicylate and benzaldehyde are emitted by trees at various phenological stages and they may be attractive to some insect herbivores. For example, when used in combination with the aggregation pheromone, benzaldehyde has demonstrated to be attractive to plum curculio, Conotrachelus nenuphar. However, no lures have been developed to monitor populations of some insects like tarnished plant bug (TPB), Lygus lineolaris, and European apple sawfly, Hoplocampa testudinea, two early-season apple pests.

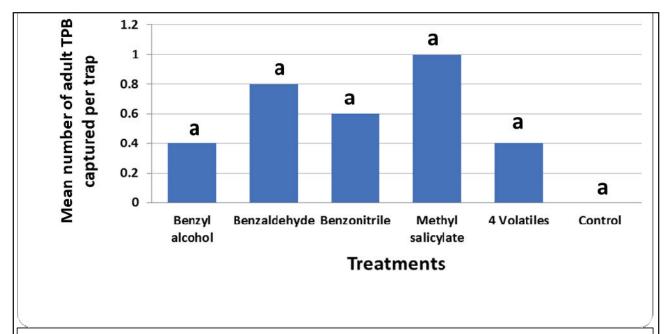
Here, we evaluated the response of adult TPB to four synthetic aromatic plant volatiles (methyl salicylate, benzyl alcohol, benzonitrile, and benzaldehyde) assessed alone and in combination. Additionally, we also evaluated the attractiveness of the commercial lure PredaLure® (methyl salicylate-containing lure) to TPB adults.

#### Materials & Methods

We conducted four different field experiments. The first and second experiments were conducted at the University of Massachusetts Cold Spring Orchard (CSO) in Belchertown. The plant volatiles were formulated in the laboratory using low-density white polyethylene vials (one vial per plant volatile) and were diluted in mineral oil. White sticky cards (Fig. 1A), purchased from Great Lakes IPM, were used for these experiments. Tomato stakes (5 feet tall) were fixed on the ground to place the white sticky cards and the volatile-containing vials. In total, 46 stakes were deployed. The distance between stakes was 2 yards. Each vial was placed inside an inverted red plastic cup to provide additional protection from rainfall and degradation by UV light. Vials were attached to the tomato stakes using wire. The white



**Figure 1.** Traps used for the experiments with tarnished plant bug: (A) white sticky card, (B) clear sticky card. Lures were placed inside the inverted red plastic cups to minimize effects of rainfall and UV light degradation.

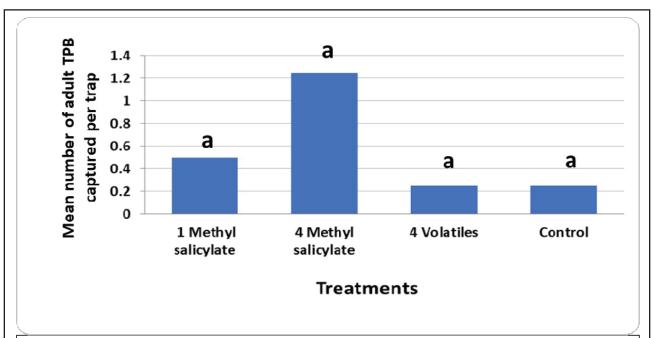


**Figure 2.** Captures of tarnished plant bug on white sticky cards baited with different plant volatiles. Bars superscribed by the same letter are not significantly different at odds of 19:1.

sticky cards were inspected once a week. To minimize the influence of trap location on TPB capture, we rotated the plastic cups attached with lures in clockwise direction within each replication. We replaced all lures every 3 weeks.

For Experiment 1, we evaluated (1) benzaldehyde,

(2) methyl salicylate, (3) benzonitrile, (4) benzyl alcohol, (5) all 4 plant volatiles combined, and (6) unbaited traps (only mineral oil) as control. All treatments were replicated 5 times in a complete randomized block design. For Experiment 2, we tested (1) methyl salicylate single lure, (2) combination of four methyl salicylate



**Figure 3.** Captures (May 1 – June 29, 2020) of tarnished plant bug on white sticky cards baited with different concentrations of methyl salicylate. Bars superscribed by the same letter are not significantly different at odds of 19:1.

dispensers, (3) combination of methyl salicylate, benzaldehyde, benzonitrile and benzyl alcohol, and (4) unbaited traps as control. All the treatments were replicated 4 times and were arranged in complete randomized block design.

The third experiment was conducted at six commercial orchards in Massachusetts from April to June 2020. We compared captures of adult TPB in PredaLure®, which is a commercial lure that contains methyl salicylate (purchased from AgBio, Inc.) versus unbaited cards. Five pairs of cards (one was baited with PredaLure® and the other was unbaited) were deployed in each orchard. Traps were deployed on the lower branches of perimeter-row trees.

The fourth experiment was conducted at the UMass CSO in Belchertown, MA, from July to September 2020 using clear sticky cards baited with PredaLure® and unbaited clear sticky cards. Clear sticky cards (Fig. 1B) were used to quantify the olfactory response of TPB to the lures in the absence of visual cues. The clear sticky cards were prepared in the lab using laminated sheets coated with Tangletrap®. The lure-containing vials and clear sticky cards were placed on the tomato stakes at the height of about 4 feet above ground. Traps in all experiments were checked once a week and all PredaLures® used in experiments 3 and 4 were replaced every 4 weeks.

#### Results

Results from the first experiment showed that, among four aromatic plant volatiles tested singly or the 4-volatiles combined, TPB captures were highest in white sticky cards baited with methyl salicylate, followed by cards baited with benzaldehyde. The 4-

volatile lure seemed to have decreased TPB captures (Fig. 2).

Fig. 3. (experiment 2) shows that white sticky cards baited with the combination of four methyl salicylate dispensers captured about five times more TPB than unbaited control cards, and 2.5 times more than cards baited with one methyl salicylate dispenser. The 4-compound lure seemed to have decreased TPB captures (Fig. 3).

Results from the third experiment revealed that white sticky cards baited with PredaLure® captured 3 times more TPB adults than unbaited sticky cards (Fig. 4A). In the fourth and last field experiment, clear sticky cards baited with PredaLure® captured nearly twice as many TPB than unbaited sticky cards (Fig. 4B).

#### **Conclusion**

Based on our combined results, we gathered evidence suggesting that methyl salicylate is an attractive plant volatile to adult TPB. Yet, further research needs to be done to determine the potentiality of methyl salicylate as a lure to monitor TPB populations.

#### Acknowledgments

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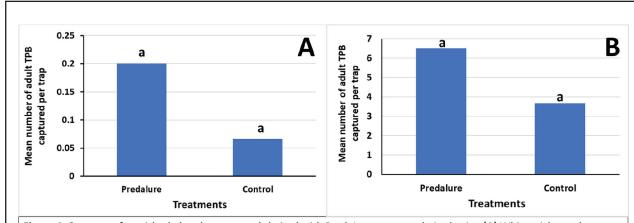
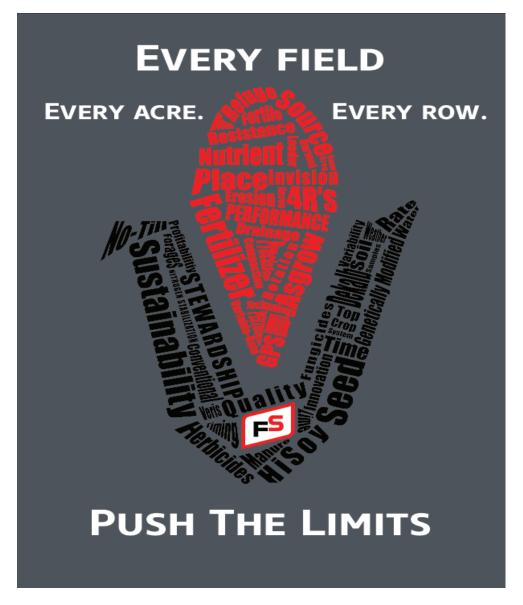


Figure 4. Captures of tarnished plant bugs on cards baited with PredaLure versus unbaited using (A) White sticky cards (experiment 3) and (B) Clear sticky cards (experiment 4). Bars superscribed by the same letter are not significantly different at odds of 19:1.

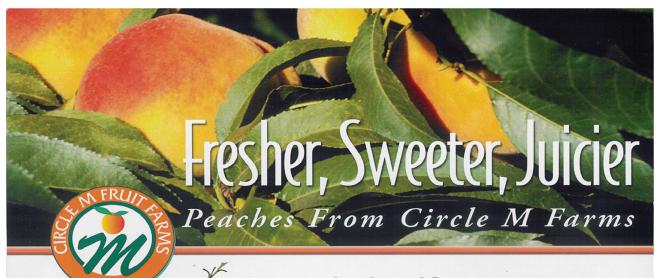


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