A Tale of Two Farms

To continue the Dickens’ reference, “It was the best of times, it was the worst of times...,” may be an apt description of berry farming in New York today. As I think about the berry growers I see in Western New York, there are plenty of challenges, as well as opportunities. But most of all, I see a huge dichotomy in the scale and skill of today’s berry growers, so much so that it is impossible to describe an “average” New York berry producer, rather, I see at least two very different grower models.

The first is actually growing the fastest, in terms of number of farms. These are small growers, mostly diversified producers for whom berries are one small piece of all of the crops they are growing. According to the 2017 Census of Agriculture, about 268 of the farms reporting berry sales had under 10 acres, and many of these farms were categorized as vegetable farms or other crop farms. This is up from 145 in 2012, a 54% increase. Many of these growers are new to berry production, adding it to diversify their offerings at a farm stand or market, or to improve spring cash flow. We in extension find that these growers are not necessarily connected to the traditional grower community and knowledge pipelines, yet they need a lot of support and information as they become confident berry growers. They are more likely to be members of a plain community (Amish or Mennonite) or female or a minority. These smaller growers need support in identifying and managing the common production challenges (pests, diseases, weeds, fertility), and often want organic or “low-spray” practices to meet the demands of their customers.

The other type of berry farm is what we might think of as the traditional New York berry grower. They have been growing berries for a long time and generally have larger plantings. These growers are familiar with and experienced at managing the normal pests and diseases. They generally know how to do all of the traditional husbandry practices (establishment, renovation, fertility, pruning). These growers have established market outlets, but some of these markets, such as U-pick, are facing cultural and demographic challenges. Extension support for these growers is less about horticultural basics, and more on regulatory issues, labor and marketing. These growers are more likely to utilize commercial scouting services for pest and disease management, although professional scouts and crop advisors are getting harder to find in the fruit industry.

At the same time, a third, entirely novel, category of berry grower is about to start production in our state. News outlets report that Mastronardi Produce and BerryWorld, a British berry growing and marketing company, are building a huge greenhouse in Oneida. The entire project is slated to eventually include four greenhouses, covering 110 acres, three of which will grow tomatoes. In the fourth they plan to grow 32 acres of hydroponic strawberries through the winter with supplemental lights. The company expects to spend almost $120 Million on the project, creating some 200 permanent greenhouse jobs. This is part of a global movement towards growing berries, especially strawberries, close to markets in increasingly intensive climate controlled facilities. Undoubtedly, Mastronardi and BerryWorld were looking at the relatively short distance to major urban markets (in comparison with California) when they decided to build this facility. Perhaps other major berry marketers are looking at similar opportunities.

From the perspective of research and extension, this creates an interesting challenge for outreach and programming. Should we focus on the fundamentals of berry growing to support the increasing numbers of new, small growers? How can we also strengthen established or expanding growers with innovative research and technology solutions suited to larger scale production? There are no easy answers, but the current approach is to try to provide support and resources to all of these growers. Our berry researchers and specialists are working with growers one-on-one, at small regional meetings, and at state-wide meet-

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NEW YORK STATE HORTICULTURAL SOCIETY

President  Ned Morgan, Morgan Farms LLC
3311 Ripple Hill Road, Marion, NY 14505
Cell: 585-752-9771; Fax: (315) 926-7740
email: emonigan11@rochester.rr.com

Vice President  Brett Kast, Kant Farms, Inc.
2911 Dennmore Road, Albion, NY 14411
PH: (585) 589-9557
e-mail: Brett.Kast@kantfarms.com

Treasurer/Sec.  Warden Dobbins, H.H. Dobbins & Son
99 West Ave., PO Box 503, Lyndonville, NY 14098
PH: (585) 765-2271; Fax: (585) 765-9710
Cell: (716) 622-6366
e-mail: wdobbins@wnyapples.com

Executive Director  Paul Baker
6316 Saunders Settlement Rd., Sodus, NY 14132
FAX: (716) 219-4049; Cell: (716) 807-6827
e-mail: pbaker.hort@roadrunner.com

Office Admin.  Karen Wilson
630 W. North St., Geneva, NY 14456
PH: (315) 483-9221; 315-573-4046 (C); FX: 315-483-6408
email: kwill@orcharddale.com

Director  Terrence Robinson, Dept. of Horticulture
630 W. North St. Geneva, NY 14456
PH: (315) 787-2227 (W); Fax: (315) 787-2216
email: TerrRobinson@cornell.edu

Director  Alisha Abden, Hudson River Fruit Distributors
65 Old Indian Road, PO Box 246, Milton, NY 12547
PH: (845) 795-2121; Fax: (845) 795-2618
Cell: (315) 518-3962
email: alisha@hudsonriverfruit.com

Director  Ted Furber, Cherry Lawn Farms
8130 Glover Road, Sodus, NY 14551
PH: (315) 483-9221; 315-573-4046 (C); FX: 315-483-6408
email: atfurber@yahoo.com

Director  Randy Hart, Hart Apple Farms, LLC
230 N. 22, Peru, NY 14532
Cell: (550) 534-5366
e-mail: randy.hart1@gmail.com

Director  Liz Madison, Empire Drip Supply
5812 Middle Rd.
Sodus, NY 14551
315-879-0516
email: emadison1234@gmail.com

Director  Jenn Naab, Northern Orchard
537 Union Rd.
Perry, NY 14572
CELL: 518-645-6500
jenna@northemorchard.com

Director  Joel Creit, Crist Bros. Orchards
65 Crist Lane, WALD, NY 12596
PH: (585) 778-7424 Cell: (650) 629-0761
email: joel@cristapples.com

Director  Richard Breslawski, Charles Breslawski Farm
561 Pines Rd., Hamlin, NY 14464
Cell: (585) 431-0643
e-mail: richb/sc@250@hotmail.com

NEW YORK STATE HORTICULTURAL SOCIETY

APPLE RESEARCH & DEVELOPMENT PROGRAM ADVISORY BOARD

Chairman  Jeff Smith, Ledge Rock Farms LLC
4362 Smith Green Road, Medina, NY 14103
PH: 585-798-3881
jeffsmith24@medina.com

Grower  Kevin Bittner, Singer Farms
Representative
– Western
8760 Coleman Rd., Baker, NY 14012-9697
PH: 716-795-3030 (P); 716-779-7300 (W)
kevins@bittnersingerorchards.com

Walt Blacklcer, Apple Acres
4633 Cherry Valley Sph, Lafayette, NY 13084
PH: 315-677-5144 (W); 315-327-3728 (C)
wbblackler@gmail.com

Ted Furber, Cherry Lawn Farms
8099 Glover Rd., Sodus, NY 14551
PH: 315-483-9221; 315-573-4046 (C); FX: 315-483-6408
atfurber@yahoo.com

Grower  Jennifer Crist Kohn, Crist Brothers Orchard Inc.
Representative
– Eastern
6 Crist Ln., Walden, NY 12586
PH: 651-778-7424 (W); 651-629-2990 (C)

Mason Foreman, Foreman Orchards
2740 Route 23, Peru, NY 12972
PH: 518-643-9527, 518-726-6074 (C); FX: 518-643-9509
gaywagen@yahoo.com

William Shuttsuck, Marketing Order Administrator
NYS Dept. of Ag & Markets
108 Airport Drive, Albany, NY 12223
PH: (518) 485-7306; Fax: (518) 457-2716
william.shuttsuck@agriculture.ny.gov

Peter Ten Eyck, Indian Ladder Farms
342 Altamont-Voorheesville Road
Albany, NY 12203
PH: 518-765-6296; 518-689-6258 (C); FX: 518-765-2700
Peter@indianladderfarms.com

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Duane Smith
Michigan Apple Committee
13701 S. Sedona Parkway, Suite 3
Lansing, MI 48906
PH: 800.456.2753 Fax: 517.669.9506

NYS BERRY GROWERS BOARD MEMBERS

Chair  Liz Madison, Empire Drip Supply
5812 Middle Rd.
Sodus, NY 14551
315-879-0516
email: emadison1234@gmail.com

Treasurer  Chuck Mead, Mead Orchards LLC
15 Scenic Rd., Trum, NY 12583
PH: 845-756-5441 (W); Cell: 845-389-0731
crocketorchards.com

Executive Secretary  Paul Baker
3586 Saunders Settlement Rd., Sodus, NY 14132
CELL: 716-807-4082; FAX: (716) 219-4049
pbaker.hort@roadrunner.com

Bruce Carano, Carano’s Farm
2238 Reed Rd., Bergen, NY 14446
PH: 585-507-2691; pasawappaga@gmail.com

Dave Dudal, Dudal’s Blues Family Farm and Winery
9582 N. Stinson Rd., Machias, NY 14101
PH: 716-353-4301; ddbluessfarm@gmail.com

Tony Emmi, Emmi Farms
1372 S. Ivy Trail, Baldwinsville, NY 13027
PH: 315-374-3777; emmi@emmi.farm

Amy MacFarland, HARD Orchards
17260 Ridge Rd., Holley, NY 14470
PH: 585-638-8838; amy.macfarland@ HARD.com

Andy Underhill, Underhill Farm
4886 Balaton Alba Township Rd.
Batavia, NY 14020
585–813-4488
underhillandrew@gmail.com

Jake Samascott, Samascott Orchards, LLC
5 Sunset Ave.
Kinderhook, NY 12106
518–813-4488
585–813-4488
underhillandrew@gmail.com

Jody Swenson, Sisson’s Apple Farm
9582 N. Sisson Rd., Machias, NY 14101
PH: 315-879-0516
email: jswenson36@gmail.com

Robert Gregory
Hillsdale, MI

Ph.  800.456.2753  Fax 517.669.9506
Lansing, MI  48906

NYS STATE HORTICULTURAL SOCIETY

PH: 315-787-2248; gro2@cornell.edu

NYSAES, Geneva, NY

CALS Communications

Production  Gemma Olidrome
GALS Communications
NYSAES, Geneva, NY
PH: 315-787-2248; gpa@cornell.edu

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Editor  Terrence Robinson
Dept. of Horticulture
New York State Agricultural Experiment Station
Geneva, New York 14456-0462
PH: 315-787-2227
trl@cornell.edu

Subscriptions  Karen Wilson
& Advertising  NYSGA, 6310 W. North St., Geneva, NY 14456
PH: 315-787-2244; Fax: 315-787-2216
wilsonk36@hotmail.com

Design Chris Cooley
Rochester, NY
PH: 315-263-5187; chris@cooleycreative.com

Production Gemma Olidrome
GALS Communications
NYSAES, Geneva, NY
PH: 315-787-2248; gpa@cornell.edu

NEW YORK STATE HORTICULTURAL SOCIETY
Editorial, Continued

ings like the Empire State Growers Expo. Additionally, in an effort to support growers who are exploring innovation in berry production practices, the New York State Berry Growers Association is partnering with Cornell Cooperative Extension and researchers at Cornell to put on a 3-day workshop on managing strawberries in soil-less (table-top) systems. The workshop is planned for mid-February in Ithaca, and will be a combination of classroom presentations and hands-on practice of management techniques, with instruction from Cornell scientists and international technical experts.

This is truly a time of big changes in New York's berry grower community. There is likely a place for many models of berry production and marketing, and we in Extension have a lot to learn to provide relevant support for all of them.

Esther Kibbe
WNY Regional Extension Specialist for Berry Crops


Special to the NYS Fruit Quarterly

Elaine Gotham, 1951-2019: An Eye for the Natural World

It is with heavy hearts that we inform the readers of the New York State Fruit Quarterly that Elaine L. Gotham, age 68, of Naples, NY, died on Friday, November 15, 2019. Elaine was in the midst of laying out this issue, a job that had been her responsibility ever since the quarterly’s inception by the New York State Horticultural Society (NYSHS), with leadership from Robert Becker and Pat Krauss, some 30 years ago. Elaine worked as senior graphic designer for Cornell University at the New York State Agricultural Experiment Station in Geneva, NY, for more than 32 years. She provided her artistic vision and eye for the natural world to the design of agricultural related publications like the Fruit Quarterly, posters, signage, newsletters, cooperative extension circulars and calendars for the departments of horticulture, entomology, food science and plant pathology. Elaine excelled at science communication and clean design. She took great pride in the Fruit Quarterly and the efforts of the NYSHS, working closely with researchers to integrate photographs, tables and illustrations into scientific research to make the articles more accessible and informative for growers and scientists alike. As a fine artist, Elaine bridged the design world from pen and ink to calligraphy to computer-aided design and output. She founded her own freelance business — Gotham City Design — designing logos for local and national businesses from her home studio in the Bristol Hills, as well as photography, illustration, art, jewelry, calendars and spirit stones inspired by her love of Canandaigua Lake and the natural world. Elaine’s art was much sought after for its creativity and heartfelt poeticism. In lieu of flowers, the family suggests a donation to the charity of your choice or the Canandaigua Lake Watershed Association or Saint Mary’s School in Canandaigua. The New York State Fruit Quarterly and NYSHS family will miss Elaine and her creative spirit. Chris Cooley, another member of the Station family, jumped in to help produce this issue. We thank him for his contribution.

Best wishes for a peaceful and happy holiday season, Terence Robinson

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20% savings when you hedge sides, top, and bottom. It takes the guess work out for those factors. You set the box, then they do big cuts and uprights. Also, by hedging you can fill the space better and have a consistent canopy.
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Apple, growers are adopting improved, production systems to remain competitive in the international fruit market (Robinson, 2008). They are doing this in NY State by establishing high-density plantings with smaller trees using new cultivars. These high-density plantings may cost 10 times more to establish than low-density plantings, however, the potential returns of high-density plantings, far exceed those of low-density plantings, particularly during the first 10 years after planting, often returning the grower’s initial investment much sooner than less-costly, low-density plantings. We have recently completed 4 long-term orchard systems field studies which have confirmed that planting highly feathered trees in the Tall Spindle system at 3X11 ft spacing gives the optimum economic result over 20 years (Lordan et al., 2018a,b, c; 2019a, b; Reig et al., 2018; 2019a, b). If the price of fruit is high with a premium variety then the profitability is much higher and the investment can be paid off much quicker but the optimum planting density is the same. If growers grow their own trees and plant lower quality trees then the total lifetime profitability is less and the investment takes longer to pay back. However, if the trees they grow are large and highly feathered then there is no profitability penalty for growing your own trees.

The central component of a high-density production system is the rootstock which provides tree size control to allow for high-density plantings. Our research over the last 20 years has shown that the rootstock influences not only tree size but many other aspects of tree performance including productivity, fruit quality, nutrient uptake efficiency, pest resistance, stress tolerance, and ultimately profitability (Fazio et al., 2019). The evolution from low- to high-density plantings has raised the bar for rootstock performance. A grower’s decision to establish a new orchard with a specific cultivar/rootstock combination has financial implications spanning 20 years. Such a financial risk creates economic issues associated with the adoption of new rootstocks which must be considered when choosing a rootstock.

**Designer Rootstocks**

In the past the primary criteria in choice of rootstock has been will it survive in my climate, is it the right vigor and is it available. Cold damage and fire blight have been the two primary and economically important causes of tree death in North America. In addition *phytophthora* root rot and waterlogging have also caused tree death. Thus, the rootstock decision in the past was usually quite simple with only 1 or 2 choices available to growers. However with the proliferation in apple rootstocks available around the world there is now a dizzying array of choices for apple growers. The Geneva breeding program alone has released 14 apple rootstocks and is poised to release 4 more in the next few years. With so many rootstock choices, we have suggested the term “designer rootstocks” to indicate the possibility of choosing a rootstock suited for the specific climate, soil, cultivar and planting system a grower chooses. There are 4 variables that need to be combined specifically for each orchard before choosing a rootstock. They are:

1. vigor of the variety,
2. vigor imparted by the climate,
3. vigor imparted by the soil and
4. the space allocated to each tree.

Each of these should be considered as pieces of a puzzle specific to each orchard or areas in an orchard in selecting a rootstock. We have further suggested that a rootstock in a modern orchard should be able to grow well enough to fill the space allocated to the tree by the end of the 2nd or 3rd years and begin production in the second year. This requires planting a large tree. If rootstock vigor combined with scion vigor, climate vigor and soil vigor do not result in sufficient growth to fill the space in two or three years then substantial economic penalties in lost yield accrue to the grower. In the past, with limited rootstock choices, growers often planted a given rootstock which was not well matched with scion vigor, climate vigor, soil vigor or tree spacing resulting in trees that took 5-8 years to fill the allotted space or that grew too vigorously for the allotted space and then were difficult to manage in later years. In one our recent papers (Lordan et al., 2019a) has estimated that with high priced varieties, the lost yield when trees fail to fill their space by the end of the 2nd or 3rd year can cost $100,000/acre in lost returns over the first 8 years of an orchard life. This economic reality is often not appreciated by growers who never see the un-realized income from lower than potential yields due to the wrong rootstock choice which is not matched perfectly to scion vigor, soil vigor and spacing.

Most growers are hesitant to plant a novel rootstock that is new to them because of the risk that the rootstock will not do well for them. However it is also true that there are large economic penalties for not adopting truly better rootstocks when planting a new orchard. The introduction of the variety Honeycrisp in the USA in the mid 1990’s has brought new challenges to rootstock
selection. It is a weak growing cultivar that often fails to fill the space allocated to the tree in 2-3 years. However, due to its high market price this variety has been very profitable for growers even though it has often not achieved the goal of filling the space in 2-3 years with dwarfing rootstocks. In addition, its susceptibility to the Ca related disorder, bitter pit, has resulted in the quest for rootstocks which will have the perfect vigor level to fill the space in 2-3 years and will have a specific mineral nutrient profile of higher Ca uptake and a better K/Ca ratio in the fruit to reduce bitter pit. We are working to speed the discovery of such rootstocks which will be ideal for each variety in each location where apples are produced. We will develop in the next 2 years an online decision aid tool to help growers chose the right rootstock for their specific soil, climate, variety and spacing.

No rootstock is perfect and thus growers must consider both the virtues and faults of each rootstock in selecting a rootstock for their new orchard. Through this article we present the rootstock options for NY apple growers from the Geneva® series of stocks and our recommendations.

Geneva® rootstocks

Fourteen Geneva® rootstocks have been released of which 11 are being supported for further commercialization. The 3 three that are no longer supported are G.65 which is too dwarfing in most situations, G.16 which shows severe sensitivity to virus infected bud wood and G.30 which has many spines in the nursery. The remaining stocks are listed below in order of dwarfing.

Geneva 11 is similar in size to B.9 in some trials and similar to M.9T337 in others. It is very precocious, has very high yield efficiency, produces large fruit size and reduces biennial bearing with Honeycrisp however, its vigor is too low for Honeycrisp on poor soils. It is fire blight resistant and has good resistance to Phytophthora root rot, but it is not resistant to woolly apple aphids. It has mild tolerance to apple replant disease. G.11 produces high quality nursery trees. It is proving to be an excellent replacement for M.9 in North America and Europe. It out-yields M.9 by 10-30%. Its stool bed production in the USA in 2018 was ~1,500,000 liners. For Honeycrisp or other weak cultivars like NY1 we recommend this stock be planted at 2-2.5 ft X 10-11 ft (2178-1584 trees/acre). For vigorous scion cultivars such as McIntosh, Fuji, Mutsu, and Jonagold we recommend this stock be planted at 3-3.5 ft X 11-12ft (1584-1037 trees/acre).

Geneva 213 depending on location this rootstock produces a tree that is similar in size to M.9Pajam2 and slightly more vigorous than G.11, thus more similar to G.41. It is the newest Geneva rootstock and therefore has less widespread testing that the other stocks. Nevertheless, it is probably a future star. It out-yields M.9 by 10-30% on virgin soil but by 25-50% on replant soil. Its winter hardiness has not been tested but is likely harder than M.9. Its stoolbed production in the USA is just beginning (<10,000 liners). It should be planted at 2.5-3.0 ft X 11-12 ft (1584-1,210 trees/acre). For vigorous scion cultivars we recommend this stock be planted at 3-3.5 ft X 11-12ft (1320-1037 trees/acre).

Geneva 222 is slightly larger than M.9 and G.41. It has performed well in South Africa and was released in the USA in 2013. It is fire blight resistant and has done well in commercial trials in the USA but it has not generated much interest by the rootstock producers thus it availability on the USA is very limited. If growers want to try this rootstock we recommend it for poor soils or replant soil. For weak growing cultivars we recommend this stock be planted at 2.5-3.0 ft X 11-12 ft (1584-1,037 trees/acre). For vigorous scion cultivars we recommend this stock be planted at 3-3.5 ft X 12-13ft (1210-957 trees/acre).

Geneva 214 is slightly more vigorous than G.41. Field trials in New York State indicate that G.214 is similar in size to M.26 with Golden Delicious and Fuji; however, with Honeycrisp it was similar in size to M.9. It has high yield efficiency, (similar to M.9) but slightly less yield than G.41. However, its graft union is strong compared to G.41. It is resistant to fire blight, Phytophthora root rot, and woolly apple aphid. Nursery trials at Geneva, NY and in Washington State have shown that it is easy to propagate in stoolbeds (much easier than G.41). Field trials in Washington State have shown that G.214 like G.41 has tolerance to apple replant disease. Its stoolbed production in the USA in still low (100,000 liners) but is rapidly increasing. G.214 like G.41 is a good replacement for M.9 on replant sites. For weak growing cultivars we recommend this stock be planted at 3-3.5 ft X 11-12 ft (1584-1037 trees/acre). For vigorous scion cultivars we recommend this stock be planted at 3-3.5 ft X 12-13ft (1210-1037 trees/acre).

Geneva 935 is similar in size to M.26. It is highly precocious has very high yield efficiency. It induces wide branch angles, is highly resistant to fire blight and Phytophthora, and is tolerant of apple replant disease. It is more winter harder than M.9 but not quite as hardy as G.41. It is not resistant to woolly apple aphid. Fruit size is similar to M.9. It is an excellent new rootstock for weak growing cultivars like spur-type ‘Delicious,’ ‘Honeycrisp,’ ‘Sweet Tango’ or ‘Snapdragon’. Its stoolbed production in the USA in 2018 was ~ 2,000,000 liners. We recommend using certified VF (virus free) wood because of some sensitivity to a new, yet to be determined virus combination. We recommend this stock for high density plantings of weak scion cultivars. For weak growing cultivars we recommend this stock be planted at 3-3.5 ft X 11-12 ft (1320-1037 trees/acre). For vigorous scion cultivars we recommend this stock be planted at 3.5-4.0 ft X 12-13ft (1037-839 trees/acre).
**Geneva® 969**  
Geneva® 969 is derived from a cross made in 1976 between Ottawa 3 and Robusta 5 and is one of our newest stocks. Field trials indicate that G.969 is a semi-dwarfing rootstock between the size of M.26 and M.7. It is similar in size to two other Geneva rootstocks, G.935 and G.814. G.969 has very high productivity similar to G.935 but is resistant to woolly apple aphid while G.935 is not. It is resistant to fire blight and *Phytophthora* root rot and has good anchorage in the orchard. It is easy to propagate in stoolbeds. G.969 as well as G.935 and G.814 appear to induce less biennial bearing with Honeycrisp than other stocks and are considered some of the best stocks for Honeycrisp and other weak growing fresh fruit varieties. Its stoolbed production in the USA in 2018 was ~900,000 liners thus it is now readily available. We recommend this stock for high density plantings of weak scion cultivars. For weak growing cultivars we recommend this stock be planted at 3-3.5 ft X 11-12 ft (1320-1037 trees/acre). For vigorous scion cultivars we recommend this stock be planted at 3.5-4.0 ft X 12-13ft (1037-839 trees/acre). G.969 may also be an excellent rootstock for new high density processing orchards. If processing growers are willing to plant at 4-5 ft in row spacings and 14 ft between row spacings then we suggest they use G.969. It is free standing but will require a trellis to support the high early crops it produces.

**Geneva® 814** is also one our newer stocks and is similar in size to G.935, G.969 and M.26. It is highly precocious has very high yield efficiency. It is highly resistant to fire blight and *Phytophthora* and is tolerant of apple replant disease. It is not resistant to woolly apple aphid. It has been shown to produce large fruit size with Gala. It is an excellent new rootstock for weak growing cultivars like Honeycrisp. In one recent trial it had the highest yield of any stock with ‘Honeycrisp.’ Its major weakness is that it is susceptible to common latent viruses and therefore requires virus free scion budwood when budded in the nursery. Its stoolbed production in the USA in 2018 was ~50,000 liners thus its availability is still very limited. We recommend this stock for high density plantings of weak scion cultivars. For weak growing cultivars we recommend this stock be planted at 3-3.5 ft X 11-12 ft (1320-1037 trees/acre). For vigorous scion cultivars we recommend this stock be planted at 3.5-4.0 ft X 12-13ft (1037-839 trees/acre).

**Geneva 202** produces a tree larger than M.26 but less than M.7. It has high yield efficiency and is precocious but not as high yielder as all of the other Geneva® rootstocks. However it is much superior to M.7 and M.26. It is resistant to fire blight, *Phytophthora*, apple replant disease and to woolly apple aphid. It is a useful with weak growing cultivars and as an alternative to M.26 in climates that have problems with woolly apple aphid. It has proven to be quite sturdy and has done well in many climates and many soils. It has become a popular dwarfing rootstock in New Zealand where almost 1 million trees are planted each year. Its stoolbed production in the USA is much less than in NZ and in 2018 was ~300,000 liners. We recommend this stock for high density processing orchards or for Red Delicious plantings. It is superior to other semi-dwarfing rootstocks. Some have used it to induce less biennial bearing with Honeycrisp and it has done very well. However, it was released specifically for use in new processing orchards in NY, MI and PA. We believe this rootstock will be a great step forward for processing orchards and can be planted at medium-high densities. However, up until now it has not been widely available but its stoolbed production is increasing rapidly and in 2018 was ~800,000. It should now be readily available for new processing orchards. We recommend this stock be planted at 5-6 ft X 14-16 ft (622-454 trees/acre) for processing scion cultivars. It is free standing but will require a trellis or a conduit pole/1-wire trellis to support the large early crops this rootstock gives.

**Geneva® 890** is derived from a cross between Ottawa 3 and Robusta 5. It is also one of our newest rootstocks. Field trials in New York State indicate that G.890 is a semi-vigorous rootstock either slightly larger than M.7 or slightly smaller than M.7. It is much more productive than M.7 and is resistant to fire blight, *Phytophthora* root rot, and woolly apple aphid. It is easy to propagate in stoolbeds and is free standing in the orchard. It will grow in many types of soils and climates and it much needed replacement for M.7, MM.106 and MM.111. It is vastly superior to other semi-dwarfing rootstocks. Some have used it in high density plantings for weak cultivars like Honeycrisp and it has done very well. However, it was released specifically for use in new processing orchards in NY, MI and PA. We believe this rootstock will be a great step forward for processing orchards and can be planted at medium-high densities. However, up until now it has not been widely available but its stoolbed production is increasing rapidly and in 2018 was ~800,000. It should now be readily available for new processing orchards. We recommend this stock be planted at 5-6 ft X 14-16 ft (622-454 trees/acre) for processing scion cultivars. It is free standing but will require a trellis or a conduit pole/1-wire trellis to support the large early crops this rootstock gives.

### Recommendations for fresh market apple plantings

For fresh market apple orchards we continue to recommend the tall spindle system with a spacing of 3x11ft at a density of 1320 trees/acre. For optimum economic results, this system requires the use of tall (>6 ft) highly feathered (>10) trees at planting. If this type of tree is used and the proper rootstock vigor is matched to the soil, climate and cultivar vigor, the tree should reach 10 ft tall at the end of the 2nd year. We suggest a final tree height of 12 ft and a narrow canopy profile of 3 ft wide at the base and 1.5 ft wide at the top of the tree. This can be achieved with a combination of dormant limb removal (remove limbs larger than ¾ inch diameter) and summer sidewall hedging. Given these essential parameters of a tall spindle orchard, the choice of rootstock must ensure that the trees achieve the desired height within 2-3 years and begin production in the second year. The rootstock must also be highly productive and not impart excessive tree vigor after the trees fill their allotted space.

To achieve this goal, we primarily recommend Geneva® rootstocks for planting in NY State due to risks of fire blight infection and the risk of winter injury with of M.9 and M.26. In addition
the partial tolerance of apple replant disease of the Geneva® stocks helps ensure that the trees will fill the allotted space by the end of the 2nd or 3rd year. The other popular rootstock in NY is B.9 which is also fire blight tolerant. However, it often does not fill the space allocated to the tree quickly and thus does not achieve high yields in the first 5 years. Another newer Budagovsky stock, B.10 has performed well in our trials and is a viable alternative in some situations.

Among the Geneva® stocks the range of vigor of its allows choosing a unique rootstock for each scion and soil and region within the state. We are working on a smart decision aid system which will allow growers to input their variety, soil type and region of the state and receive recommended rootstocks for that combination. A preliminary and simplified version is presented in Table 2 which has 1st, 2nd, 3rd, and 4th recommended rootstocks for virgin soil, replant soil and various scion vigor categories for different regions of the state. Until the online version of the decision support system is available this table should help growers plan their future fresh fruit orchard.

Recommendations for processing market apple plantings

Our systems trials at Lagoner and Morgan farms from 1994-2004 indicated that even with processing fruit prices higher planting densities were more profitable than low planting densities (Robinson et al., 2001). From those studies we recommend a vertical axis system with an intermediate spacing of 5x14 ft at a density of 622 trees/acre for new processing fruit orchards. For optimum economic results, this system also requires the use of good quality trees at planting. If rootstock vigor is properly matched to the soil, climate and cultivar vigor, the tree should reach 10 ft tall at the end of the 3rd year. We suggest a final tree height of 14 ft and a canopy profile of 6 ft wide at the base and 3 ft at the top of the tree. The tree should have a permanent bottom tier of scaffolds (4-5) and the upper part of the tree should be managed with limb-renewal pruning when limb diameter exceeds 2 inches. Given these essential parameters, the choice of rootstock must ensure that the trees achieve the desired height within 3 years and begins production in the third year. The rootstock must be highly productive and not impart excessive tree vigor after the trees fill their allotted space.

To support this goal we released 2 highly productive semi-dwarfing rootstocks (G.969 and G.890) in 2013. These new rootstocks allow medium high density orchards with moderate initial investment cost that have high early yields and achieve high sustained yields by the 8th year. In table 2 we present our 1st, 2nd, 3rd, and 4th recommended rootstocks for virgin soil, replant soil and various scion vigor categories for different regions of the state. If NY processing growers can adopt these new stocks and the recommended spacing of 5x14 ft, they will achieve much higher early yields with new orchards and higher lifetime cumulative yields.

Conclusions

The greater availability of Geneva® rootstocks now allows all NY apple growers (whether fresh fruit or processing) numerous choices of rootstock with varying vigor levels. If growers can analyze the vigor of the variety to be planted and the vigor of the soil and the vigor imparted by the climate (primarily heat units) and then select a rootstock that will give sufficient vigor to fill the allotted space by the end of the second year or the third year, they will achieve the optimum profitability with the new orchard. The range of vigor offered by the Geneva® rootstocks allow unique scion/rootstock combinations for each situation. This is true for both fresh fruit and processing orchards even though they are planted at different densities. With greater experience each grower can fine-tune his rootstock selection for the soil type on each block planted.

Even after selecting the best rootstock for your situation, that rootstock may not be available at the last minute. Being able to plant the most desirable combination of rootstock and scion requires advance planning and coordination with your nurseryman. There are now 18 licensed rootstock propagators in the USA (Table 3). If you can’t find the rootstock you have selected for your new orchard from your favorite nursery, reach out to the other licensed nurseries and you will most likely find the rootstock you need.

Literature Cited


Terence Robinson is a research and extension professor at Cornell's AgriTech campus in Geneva who leads Cornell's program in high-density orchard systems, rootstocks, irrigation and plant growth regulators. Gennaro Fazio is a plant breeder with the USDA-ARS who leads the Geneva rootstock breeding and development program.
Table 1. Rootstocks which impart beneficial characteristics to 3 common apple varieties.

<table>
<thead>
<tr>
<th>Characteristics that could use improvement</th>
<th>FUJI</th>
<th>GALA</th>
<th>HONEYCRISP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too much vigor Biennial Color</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit Size Productivity Color/Maturity Fire blight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak vigor Biennial Fruit disorders</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rootstocks that have shown to improve Biennial Bearing

- **Western NY and Hudson Valley**
  - Fresh Fruit Orchard (3X11 ft)
    - Weak variety on replant soil: G.935, G.210, G.210, G.210, G.210
    - Very weak varieties (spur types) on replant soil: G.969, G.814, G.814, G.814, G.814

- **Processing Orchard (5X14 ft)**
  - Strong variety on virgin soil: G.11, G.11, G.214, G.935, G.814, G.814
  - Weak variety on virgin soil: G.41, G.213, G.41, G.214, G.935, G.814
  - Weak variety on replant soil: G.935, G.210, G.210, G.210, G.210
  - Very weak varieties (spur types) on replant soil: G.969, G.814, G.814, G.814, G.814

Table 2. Rootstocks recommendations for different regions in NY State based on performance and availability in 2019.

<table>
<thead>
<tr>
<th>Region</th>
<th>Fresh Fruit Orchard (3X11 ft)</th>
<th>Processing Orchard (5X14 ft)</th>
</tr>
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</table>

Table 3. List of licensed Geneva® rootstock producers in the USA.

<table>
<thead>
<tr>
<th>Organization</th>
<th>Contact Person</th>
<th>Email</th>
<th>Address</th>
<th>Phone Number</th>
<th>Website</th>
<th>Licensed Geneva® rootstocks (ranked in order of dwarfing)</th>
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<tbody>
<tr>
<td>Cameron Nursery, LLC</td>
<td>Stacey Gilmore</td>
<td><a href="mailto:cnsales@fastmail.com">cnsales@fastmail.com</a></td>
<td>1261 Ringold Road, PO Box 300, Elliptia,</td>
<td>509-266-4669</td>
<td><a href="http://www.cameron-nursery.com/">www.cameron-nursery.com/</a></td>
<td>G.65, G.11, G.41, G.16, G.222, G.214, G.935, G.969, G.202,</td>
</tr>
<tr>
<td>Carlton Plants LLC</td>
<td>Jason Bizon</td>
<td><a href="mailto:jbizonis@carltonplants.com">jbizonis@carltonplants.com</a></td>
<td>14301 SE Wallace Road, P.O. Box 398,</td>
<td>800-398-8733</td>
<td>carltonplants.com/index.html</td>
<td>G.41, G.222, G.214, G.935, G.969, G.202, G.210, G.890</td>
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<td></td>
<td></td>
<td>14850</td>
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<tr>
<td>Duarte Nursery, Inc.</td>
<td></td>
<td><a href="mailto:sales@duartenuery.com">sales@duartenuery.com</a></td>
<td>1555 Baldwin Drive, Hughston, California,</td>
<td>209-531-0351</td>
<td><a href="http://www.duartenuery.com/contact-duarte/">www.duartenuery.com/contact-duarte/</a></td>
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<td>G.890</td>
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<td>Helios Nursery</td>
<td>Tye Fleming</td>
<td><a href="mailto:tye@heliosnursery.com">tye@heliosnursery.com</a></td>
<td>57 Silvest Road, Orondo, Washington,</td>
<td>509-787-7777</td>
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<td>G.11, G.41, G.222, G.214, G.935, G.969, G.202, G.814,</td>
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<td>-------------------------------------------------------------------------</td>
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<tr>
<td>Plant Quest, LLC</td>
<td>Debbie</td>
<td>10395 SW Old Hwy, 47, P.O. Box 837, Gaston, Oregon, 97119</td>
<td>503-434-9400</td>
<td><a href="mailto:debbie@plantquest@gmail.com">debbie@plantquest@gmail.com</a></td>
<td>N/A</td>
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<tr>
<td>ProTree Nursery, LLC</td>
<td>Sara DeGaff</td>
<td>Brentwood, California, 94513</td>
<td>800-634-1671</td>
<td><a href="mailto:sara@protreenursery.com">sara@protreenursery.com</a></td>
<td>G.41, G.222, G.214, G.935, G.969, G.202, G.814 G.210, G.890</td>
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<tr>
<td>Sierra Gold Nurseries, Inc.</td>
<td></td>
<td>5320 Garden Hwy, Yuba City, California, 95991</td>
<td>800-243-GOLD (4653)</td>
<td><a href="http://www.sierragoldtrees.com/contact-us">www.sierragoldtrees.com/contact-us</a></td>
<td>G.41, G.222, G.214, G.935, G.969, G.202, G.814, G.210, G.890</td>
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<tr>
<td>Van Well Nursery</td>
<td>Pete VanWell</td>
<td>P.O. Box 1339, Wenatchee, Washington, 98807</td>
<td>800-572-1553</td>
<td><a href="http://www.vanwell.net/contact/just-ask-us">www.vanwell.net/contact/just-ask-us</a></td>
<td>G.41, G.214, G.935 G202</td>
<td></td>
</tr>
<tr>
<td>Willamette Nurseries, Inc.</td>
<td>Devon Cooper</td>
<td>25571 South Barlow Road, Canby, Oregon, 97013</td>
<td>800-852-2018</td>
<td><a href="mailto:willametteinfolfo@canby.com">willametteinfolfo@canby.com</a></td>
<td>G.65, G.11, G.41, G.16, G.222, G.214, G.935, G.969, G.202, G.210, G.30, G.890</td>
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Establishment, Persistence and Impact of Native NY Entomopathogenic Nematodes on Plum Curculio In Apples

Arthur Agnello1, Peter Jentsch2, Elson Shields3, Tony Testa4, and Tessa Lessord1

1Department of Entomology, Cornell University, Cornell AgriTech at NYSAES, Geneva | 2Department of Entomology, Cornell University, Hudson Valley Research Lab, Highland | 3Department of Entomology, Cornell University, Ithaca

Keywords: plum curculio, entomopathogenic nematodes, EPNs, biological control, organic production

P lum curculio (Conotrachelus nenuphar) (Fig. 1) is a key pest in eastern US apple orchards, and is considered to be one of the primary pests limiting organic apple production in this region. Unsprayed orchards can sustain more than 60% fruit damage (Fig. 2) and, even in conventionally managed orchards, damage at harvest can average up to 3%. Until about 2002, organic apple orchards could experience almost total fruit loss from plum curculio in New York and other areas in the region where the species is endemic. Researchers and pest managers have observed that organic apple plantings tend to have greater plum curculio infestations because of the availability of fewer effective management options. The cost of plum curculio control and its marginal effectiveness is a major obstacle to profitable organic apple production in New York State. Plum curculio treatment costs in an organic program can range between $150–$450/acre/year, with fruit damage remaining above 5–20%. Adopting a control measure of kaolin clay (Surround) as a physical barrier to the insect’s attack has been one approach in organic apple orchards. However, the economics of control prove difficult, as the product is not only expensive ($25–$50/A per application), but must be repeated weekly (4–5 applications) to maintain suitable coverage for protection during the rain-prone spring period of this pest’s activity. Further control measures needed to be researched to reduce the impact of plum curculio on organic as well as conventional apple production while also reducing costs for the apple producer, which led us to try biological control.

Entomopathogenic (insect-attacking) nematodes (EPNs) are known to be generalist predators, and have had a role in biological control programs against many insect pests, including plum curculio (Shapiro-Ilan et al. 2002; Alston et al. 2005). They are not direct predators of the insect, but rather vectors of an insect pathogenic bacterium that resides in their gut. They enter the insect host through a natural opening or wound, and release the bacteria into the insect’s body, overcoming the immune system and killing the host. In the process, the nematodes reproduce and eventually break out of the cadaver (Fig. 3) as infective juveniles (J1s), which seek out additional hosts. The purpose of this project was to evaluate the potential of NY cold-adapted EPNs to reduce the impact of plum curculio on apple production, reduce the cost of organic apple production, and provide a higher degree of marketable fruit and a higher profit for all apple producers. Beginning in 2012, laboratory and field bioassays were conducted on native NY nematode species, which had been found to persist after a single application (Shields et al. 2009), to determine their potential for use as plum curculio biocontrol agents. In lab trials, we found that exposure of plum curculio larvae (Fig. 4) to the nematodes was more effective than for plum curculio adults, with the highest mortality obtained using a combination of species; up to 75% mortality was seen within 14 days (Agnello et al. 2014). In field trials, we applied nematodes to the soil surface in research apple plantings using an ATV-mounted modified boom sprayer; soil core samples were taken from the treated rows and assessed for evidence of nematode establishment. The establishment of nematode populations in the treated rows was initially less than anticipated, owing to unfavorably dry weather conditions during the first season, but the levels gradually increased. Field bioassays using lab-reared plum curculio larvae placed in small-plot nematode-infected soil arenas resulted in marginal but increasing levels of mortality to the larvae in their development to the adult stage (Agnello et al. 2014). Here we report the further results of these persistence assays and field trials, which continued into 2018.

Methods

A total of eight field sites were established during the project, comprising six new locations in addition to the original Empire and Idared Cornell AgriTech research plantings inoculated in 2012 (Agnello et al. 2014). The new sites were: 1) an organic apple block at the Loomis Farm (Cornell AgriTech), inoculated in 2013; 2) a commercial organic apple orchard south of Geneva (Davies Farm, Red Jacket Orchards), inoculated in 2013; 3) an organic research planting (Eco Block) at the Hudson Valley Research Laboratory, Highland, inoculated in 2014; and three commercial organic apple orchards in the Hudson Valley, all inoculated in 2013: 4) Westwind Orchards (Accord); 5) Prospect Hill Orchards (Milton); and 6) Fishkill Farms (Hopewell Junction).

Inoculation of EPNs. Prior to inoculating NY-native entomopathogenic nematode (EPN) populations at each field location, designated field sites were bioassayed for the presence of native EPNs. GPS waypoints were recorded at each sample block. Soil core samples (300 per site) were taken to Cornell University and bioassayed for EPNs using a standard technique with wax moth (Galleria mellonella) larvae as an indicator for the presence of biocontrol nematodes in the sample. Past research had indicated few to no existing populations of EPNs should be found; however, the Hudson Valley sites had higher natural populations than the Geneva sites. Both nematode species, Steinernema carpocapsae (NY01) and S. feltiae (NY04), were reared at Cornell University.
prior to field inoculation. Soil inoculations were made in the evening using an ATV equipped with a 25-gal tank with 0008 fertilizer stream nozzles. Nematodes were washed and strained through mesh screens to remove debris from rearing cups prior to being added to the ATV tank. EPN infective juveniles (IJs) were then applied to the grassy alleys between the rows and at the base of the trees. EPNs were applied at the equivalent of 450 million IJs per acre. At Westwind Orchards and Fishkill Farms, a truck equipped with a spray rig applied nematodes to areas surrounding established trees outside row blocks at the same rate using similar application techniques and equipment.

**Persistence of EPNs.** Beginning in June 2016, a series of randomly selected soil core samples were taken at each of the trial sites to be assessed at Cornell University for the presence of persisting nematodes in the previously treated areas. The number of samples collected at each site on each occasion ranged from 150-600, depending on the size and layout of the specific site.

**Micro-plot field trials.** To assess the level of nematode establishment in these plots, we conducted larval exposure trials employing laboratory-reared plum curculio larvae, using micro-plot arenas into which mature plum curculio larvae were introduced (Fig. 5). Arenas were set up in orchard rows in which nematodes had been applied by a spray inoculation, in untreated rows, and in untreated rows but receiving individual EPN hand-inoculation treatments and, in untreated rows but receiving individual EPN hand-inoculation treatments, in untreated rows but receiving individual EPN hand-inoculation treatments. Each arena was covered with an emergence trap (Fig. 7), and checked over several weeks for emerged plum curculio adults (Agnello et al. 2014). Impact of nematodes on plum curculio survival was assessed by collecting any plum curculio that completed development and emerged as adults in each of the treatments in the trial sites.

**Fruit damage assessments.** As another method of assessing overall impact of the nematode treatments on plum curculio populations, fruitlet samples were taken from each of the orchards during June of each of the years of the project. In each row of each EPN-treated and untreated plot sampled, 100 fruits from each of 5 trees were assessed for presence of plum curculio oviposition damage (scars).

**Results**

**Multi-year persistence of EPNs.** Orchard surveys using soil assays to evaluate the impact of biocontrol nematodes and spread were conducted once a year in 2016-2018. Soil surveys in the spring at the Geneva sites (2016), and Hudson Valley sites (2016-2018), found that nematodes persisted throughout the orchard sites at which they had been introduced (Fig. 8); levels remained within the expected ranges for each of the sites throughout the project (Table 1). Bioassay results at the Geneva site also showed that the nematodes not only continued to persist in the treated zones of the apple orchard, they also spread throughout the untreated areas of each site. Subsequent soil assays at the Geneva site were therefore discontinued after 2016. This movement of EPNs has been tracked previously in other cropping systems, but this was the first evidence of movement within an orchard environment.

**Micro-plot field trials.** Initial results the first year after nematode inoculations showed slow progress in population establishment and impact on plum curculio larvae; however, by the third year, results in the spray-applied plots for several of the orchard sites approached the levels of plum curculio mortality seen in plots that were directly treated by hand, which was an encouraging sign that the nematode populations would ultimately contribute significantly to the reduction of plum curculio numbers and damage in these test sites (Table 2). Survival and emergence of adult plum curculio would be anticipated to be lower in the hand-inoculated vs. spray-inoculated treatments, as the presence of viable nematodes in those arenas would have been ensured by inoculating them directly in advance of introducing the plum curculio larvae. We observed this trend in two of the trial sites, Loomis and Davies (both in Geneva), while at the remaining sites, results in these two treatments were essentially similar. Survival and emergence as adults would have been expected to be highest in the untreated checks, but this was only the case at one site, the ECO orchard (Highland) in 2016. The growing season was extremely dry that year, classified as extreme drought conditions, which are known to result in greatly reduced nematode activity, as the populations tend to go dormant when there is insufficient soil moisture. It must be assumed that this would have been a major factor affecting the relative activity and efficacy of the nematodes in our orchard sites that year. A similar phenomenon was experienced in 2012, and although...
it appeared that the nematodes were no longer viable after such a dry season, rains during the fall and subsequent spring revealed that the nematode populations were able to rebound with more representative activity seen against the host larvae.

**Fruit damage assessments.** There was initially a uniform trend of lower overall damage levels in treated vs. untreated rows in nearly all plots, with a gradual decrease in the amount of damaged fruit each succeeding year. However, this did not become enough of an established pattern to conclude that the EPN populations were effectively reducing the oviposition damage seen in the fruit at harvest (Table 3). In 2017, results from the plum curculio fruit damage assessments showed that the nematode treatments did not appreciably improve efforts to prevent oviposition damage vs. the untreated checks, and in the case of the Empire block, damage was demonstrably higher in the treated area (similar to 2016). However, overall damage at the Loomis block plots decreased from the previous year (4.6% avg damage in 2017 vs. 8.1% in 2016); considering that the most recent soil core samples showed that the nematodes had spread to all areas of the orchard since their introduction, including previously untreated rows, it could be inferred that their presence was having an impact on plum curculio populations orchard-wide. It was therefore decided not to conduct additional soil bioassays at these sites in 2017.

Plum curculio damage in 2018 was considerably higher across all sites than levels found in 2017. However, in three of the four nematode trial sites at Geneva, the average percent oviposition damage was numerically (although not statistically) lower in the plots treated with nematodes than in the untreated check plots; damage reductions ranged from 29–44% at the Idared, Empire, and Loomis research farm sites. There was no difference between treated vs. untreated plots at the Davies site, similar to the previous year; this was a commercial site being managed under a program using conventional insecticide sprays, so it appears that the nematode treatment did not have an impact on the total fruit damage being caused by this insect.

**Discussion**

Current plum curculio management costs in an organic production program are expensive, and resulting fruit damage can still be unacceptably high. An alternative approach using commercially available nematode strains has been considered; however, results have shown that the nematodes are non-persistent in the soil profile (30-60 days), require high application dosages, and are expensive because producers use them like a biopesticide. Furthermore, since accurate application timing is critical, treating for plum curculio using commercial strains can often be a financial gamble organic producers cannot afford to make. Beginning in 2011, this biocontrol program for management of plum curculio using native cold-adapted entomopathogenic nematodes (EPNs) was initiated at several orchards in NY. Preliminary results indicated that EPNs could have the potential to reduce plum curculio damage in organic orchards by 70-90% (Agnello et al. 2014). Also, the comparative cost of application of biocontrol nematodes was lower ($80/acre, with only a single application needed) than current control measures.

Establishment of EPNs at economically effective levels to date has been slow in both research and commercial plantings. A primary goal of this study was to optimize the effectiveness of native EPNs in reducing the impact of plum curculio on organic and conventional apple production as a management strategy. The advantage of using persistent nematodes in an inoculative approach is that it requires only a single application to the soil surface. However, the impact of orchard ground cover and soil characteristics on the survival, persistence, and effectiveness of EPNs had not been previously recorded. Native nematode strains have been shown to persist using a wide array of soil insect hosts frequented the grass soil habitat. Obtaining realistic predictions of EPN establishment and activity within a set of NY orchard soil and vegetation conditions could help determine how much impact orchard ground cover and soil characteristics are factoring in as a biological control agent for plum curculio.

Abiotic soil characteristics were assessed at each of the Geneva sites during this period (Lessord 2016). The data showed that Loomis is a very unique site overall, having a significantly higher silt content and lower sand content than the Davies, Empire, and Idared sites. Loomis also had higher clay content than Davies and Empire. The Davies, Empire, and Idared sites are all described as loamy soil, and Loomis is a silt loam. The Loomis site also had higher carbon and nitrogen content than the other three sites, as well as a higher carbon:nitrogen ratio. Soil texture has been shown to influence the virulence of entomopathogenic nematodes, but the actual mechanisms underlying the relationship between soil chemistry and entomopathogenic nematode infectivity is unclear. While our findings do not provide direct evidence of a relationship between soil physico-chemical traits and nematode infectivity, they do suggest that further investigation of this aspect of nematode ecology is warranted. For instance, S. carpocapsae can persist in a relatively wide range of soil moisture levels (2-16%) but 2% moisture provides the highest combined survivability and infectivity for this species.

With the results of the trials conducted at these sites in Geneva and the Hudson Valley, it is apparent that the nematode/insect/environment relationship is highly dynamic. Several factors influencing nematode virulence against plum curculio, including both nematode species and soil structure, can impact the virulence of EPNs in the apple system. The following factors should be considered relative to the potential efficacy of EPNs inoculated as a biocontrol tactic (Lessord 2016):

- **Extreme drought conditions can significantly reduce nematode activity and efficacy; populations tend to go dormant when there is insufficient soil moisture.**
- **Nematode populations are able to rebound when**
soil moisture increases, with more representative activity seen against the host larvae.  
- Nematode efficacy may be related to differences in soil texture and mineralogy; soil texture has been shown to influence the virulence of EPNs.  
- Laboratory trials indicate a strong effect of nematode species on the control of plum curculio in given soil conditions; the apparent higher efficacy of *S. feltiae* is related to the depth at which plum curculio burrows in the soil.  
- Bioassays conducted in the laboratory suggest that site characteristics greatly influence nematode infectivity as well as the survivorship of plum curculio; for example, soil macrostructure has been determined as a likely important factor in the agricultural efficacy of EPNs as biological control agents.  

Overall, the nematode treatments have not had the expected impact in reducing total fruit damage caused by plum curculio.  Our results indicate that an application of biocontrol nematodes did not appreciably improve efforts to prevent oviposition damage; however, overall damage at the research sites tended to result in lower plum curculio oviposition damage.  Consequently, the following entry on “Biological & Non-chemical Control” for Plum Curculio was added to the Apple Insect and Mite Notes of the Cornell Tree Fruit Pest Management Guidelines (TFPMG):  

‘Trials to evaluate field applications of entomopathogenic (insect-attacking) nematodes (EPNs) on plum curculio larvae have shown a measurable impact of this tactic against larvae in the soil of orchard plantings during the summer months.  Level of EPN effectiveness is variable and related to soil type, structure, moisture levels, and ground cover, but NY strains of EPNs are generally able to become established and persist in most regions of the state.  Details on this technique, which has been used successfully against alfalfa snout beetle, can be found at: www.alfalfasnoutbeetle.org under “Biological Control”.  Information found at this site includes: Nematode Application Techniques; Video: Rearing Entomopathogenic Nematodes; Video: Generating Nematodes for Field Application; Video: Material & Supply for Rearing Nematodes; Video: Applying Nematodes.’

Additionally, entomopathogenic nematodes have been added as a tactic for the management of plum curculio in the section of the TFPMG on “Arthropod Management Options in Organic Tree Fruit Production”.  

The findings from our trials have made apple growers more aware of the potential of this low-cost program, despite the fact that our overall results were less successful than originally expected.  A number of fruit producers in NY have expressed interest in looking at the possibility of using this biocontrol tactic to manage plum curculio infestations at their orchards, despite the equivocal results we obtained, and some have already made trial applications on their farms.  Our observations have generally supported the expectation of the continued presence and spread of NY-native nematode populations in orchards.  This approach has been shown to have a long-term positive effect against native pests in other cropping systems, although documentation of significantly improved in pest control can take as long as 5–10 years.

### Table 1. Persistence of entomopathogenic nematode (EPN) populations following inoculation, assessed in soil core samples taken in June of each year.  

<table>
<thead>
<tr>
<th>Site/Inoculation Date</th>
<th>% Samples positive for EPN presence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2016</td>
</tr>
<tr>
<td>AgriTech Idared/2012</td>
<td></td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>0–4%</td>
</tr>
<tr>
<td><em>S. feltiae</em></td>
<td>12–36%</td>
</tr>
<tr>
<td>AgriTech Empire/2012</td>
<td></td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>0–4%</td>
</tr>
<tr>
<td><em>S. feltiae</em></td>
<td>18–22%</td>
</tr>
<tr>
<td>Red Jacket Davies Farm/2013</td>
<td></td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>0%</td>
</tr>
<tr>
<td><em>S. feltiae</em></td>
<td>16–24%</td>
</tr>
<tr>
<td>AgriTech Loomis Farm Organic/2013</td>
<td></td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>0%</td>
</tr>
<tr>
<td><em>S. feltiae</em></td>
<td>21–28%</td>
</tr>
<tr>
<td>HVRL ECO Block/2014</td>
<td></td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>1%</td>
</tr>
<tr>
<td><em>S. feltiae</em></td>
<td>22%</td>
</tr>
<tr>
<td>Westwind Orchards Organic/2013</td>
<td></td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>NA</td>
</tr>
<tr>
<td><em>S. feltiae</em></td>
<td>7–17%</td>
</tr>
<tr>
<td>Prospect Hill Organic/2013</td>
<td></td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>0–17%</td>
</tr>
<tr>
<td><em>S. feltiae</em></td>
<td>0–33%</td>
</tr>
<tr>
<td>Fishkill Farms Organic/2013</td>
<td></td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>1–2%</td>
</tr>
<tr>
<td><em>S. feltiae</em></td>
<td>16–22%</td>
</tr>
</tbody>
</table>

* Geneva-area sites not assessed after 2016 because target establishment was reached.  NA, not applicable: Westwind site was not inoculated with *S. carpocapsae* because of soil conditions at this site.

### Acknowledgements

We would like to thank Joe Nicholson (Red Jacket Orchards, Geneva, NY), Steve Clark (Prospect Farm, Milton, NY), Josh Morgenthau (Fishkill Farms, Hopewell Junction, NY), and Fabio Chizzola (Westwind Orchard, Accord, NY), for allowing us to work on their farms.  Invaluable technical lab and field assistance was provided by Dave Kain, Kristin McGregor, Cortni McGregor, Dylan Tussey, Chrissy Dodge, Forest English-Loeb, Emily Pennock, Abagail Davis, Josh Neal, Mikhail Fischer, Amy Sparer, and Danielle Carolie.  We are grateful to Tracy Leskey, Starker Wright, and Torri Hancock (USDA-ARS, Kearneysville, WV) for supplying the plum curculio starter population, and to the NYS Specialty Crops Block Grants Program and the New York Farm Viability Institute for their funding support.

### References

Arthur Agnello is a professor in the Department of Entomology at Cornell AgriTech in Geneva who leads Cornell’s extension program in fruit entomology. Peter Jentsch is an Extension Associate in the Department of Entomology at Cornell’s Hudson Valley Laboratory specializing in arthropod management in tree fruit, grapes and vegetable crops. Elson Shields is a professor in the Department of Entomology in Ithaca specializing in extension and research on insect pests of field crops. Tony Testa is a Research Support Specialist in Elson Shield’s program. Tessa Lessord is a former graduate student in Arthur Agnello’s program; she is currently employed by A.C.D.S. Research, Inc. in North Rose, NY.

Table 2. Percent plum curculio adult emergence in field micro-arena nematode plots, 2012–2017.

<table>
<thead>
<tr>
<th>Year/Site</th>
<th>Spray applied</th>
<th>Hand applied</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idared</td>
<td>70.0 a</td>
<td>31.0 b</td>
<td>41.0 b</td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idared</td>
<td>22.5 a</td>
<td>13.7 a</td>
<td>15.0 a</td>
</tr>
<tr>
<td>Empire</td>
<td>6.2 a</td>
<td>4.3 a</td>
<td>10.0 a</td>
</tr>
<tr>
<td>Prospect</td>
<td>11.3 ab</td>
<td>1.3 a</td>
<td>17.5 b</td>
</tr>
<tr>
<td>Fishkill</td>
<td>8.8 a</td>
<td>8.8 a</td>
<td>12.5 a</td>
</tr>
<tr>
<td>2014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idared</td>
<td>55.0 a</td>
<td>39.0 a</td>
<td>41.0 a</td>
</tr>
<tr>
<td>Empire</td>
<td>44.0 a</td>
<td>14.0 b</td>
<td>32.0 a</td>
</tr>
<tr>
<td>Loomis</td>
<td>25.0 ab</td>
<td>19.0 b</td>
<td>36.0 a</td>
</tr>
<tr>
<td>Davies</td>
<td>24.0 ab</td>
<td>14.0 b</td>
<td>34.0 a</td>
</tr>
<tr>
<td>2015</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idared</td>
<td>27.0 a</td>
<td>8.0 a</td>
<td>14.0 a</td>
</tr>
<tr>
<td>Empire</td>
<td>19.0 a</td>
<td>35.0 a</td>
<td>14.0 a</td>
</tr>
<tr>
<td>Loomis</td>
<td>25.0 a</td>
<td>13.0 b</td>
<td>14.0 a</td>
</tr>
<tr>
<td>Davies</td>
<td>49.0 a</td>
<td>49.0 a</td>
<td>34.0 a</td>
</tr>
<tr>
<td>2016</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idared</td>
<td>18.0 a</td>
<td>19.0 a</td>
<td>13.0 a</td>
</tr>
<tr>
<td>Empire</td>
<td>23.0 a</td>
<td>27.0 a</td>
<td>27.0 a</td>
</tr>
<tr>
<td>Loomis</td>
<td>20.0 a</td>
<td>12.0 a</td>
<td>17.0 a</td>
</tr>
<tr>
<td>Davies</td>
<td>39.0 a</td>
<td>31.0 a</td>
<td>31.0 a</td>
</tr>
<tr>
<td>HVRL-ECO</td>
<td>15.7 a</td>
<td>20.0 ab</td>
<td>32.2 b</td>
</tr>
<tr>
<td>2017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idared</td>
<td>14.0 a</td>
<td>33.0 a</td>
<td>34.0 a</td>
</tr>
<tr>
<td>Empire</td>
<td>18.0 a</td>
<td>21.0 a</td>
<td>10.0 a</td>
</tr>
<tr>
<td>Loomis</td>
<td>31.0 a</td>
<td>33.0 a</td>
<td>23.0 a</td>
</tr>
<tr>
<td>Davies</td>
<td>45.0 a</td>
<td>55.0 a</td>
<td>50.0 a</td>
</tr>
<tr>
<td>Westwind</td>
<td>55.0 a</td>
<td>75.0 b</td>
<td>40.0 a</td>
</tr>
<tr>
<td>Prospect</td>
<td>52.0 a</td>
<td>27.0 b</td>
<td>52.0 a</td>
</tr>
<tr>
<td>Fishkill</td>
<td>20.0 a</td>
<td>83.0 b</td>
<td>85.0 b</td>
</tr>
</tbody>
</table>

Within each site each year, values followed by the same letter are not significantly different (P < 0.05, Student’s t-test).

Table 3. Percentage of fruit in research and commercial EPN trial sites showing plum curculio oviposition damage at harvest, 2012–2017.

<table>
<thead>
<tr>
<th>Site/Inoculation Date</th>
<th>% Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2012</td>
</tr>
<tr>
<td>Idared/2012</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>7.2a</td>
</tr>
<tr>
<td>Nematodes</td>
<td>3.8a</td>
</tr>
<tr>
<td>Empire/2012</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>35.9a</td>
</tr>
<tr>
<td>Nematodes</td>
<td>33.7a</td>
</tr>
<tr>
<td>Davie/s/2013</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>15.1a</td>
</tr>
<tr>
<td>Nematodes</td>
<td>5.7a</td>
</tr>
<tr>
<td>Loomis/2013</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>9.9a</td>
</tr>
<tr>
<td>Nematodes</td>
<td>3.4b</td>
</tr>
<tr>
<td>HVRL ECO/2014</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>7.3a</td>
</tr>
<tr>
<td>Nematodes</td>
<td>10.5a</td>
</tr>
<tr>
<td>Westwind/2013</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>77.5a</td>
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<tr>
<td>Nematodes</td>
<td>94.7a</td>
</tr>
<tr>
<td>Prospect Hill/2013</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>22.3a</td>
</tr>
<tr>
<td>Nematodes</td>
<td>5.1a</td>
</tr>
<tr>
<td>Fishkill/2013</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>91.4a</td>
</tr>
<tr>
<td>Nematodes</td>
<td>92.3a</td>
</tr>
</tbody>
</table>

Within each year, values at each site followed by the same letter are not significantly different (P < 0.05, Student’s t-test).


Nonthermal Production of Value-added Apple Products: Effect of High Pressure Processing on Quality Parameters of Varietal Apple Juice, Apple Puree and Apple Dices in Juice

Huijuan Zheng, Shuang Qiu, Kyle Kriner, Carmen I. Moraru and Olga I. Padilla-Zakour

Department of Food Science, Cornell University, Geneva, NY

Keywords: High Pressure processing, apple juice quality, apple juice, apple puree, apple dices

Consumers are more aware of the importance of the health benefits associated with foods and are seeking high quality, minimally processed fruit products with fresh, natural characteristics, and with no artificial additives. However, minimally processed fruit products undergo faster physiological changes, which may cause faster browning, tissue softening and microbial spoilage compared to the intact fruits (Artés, Gómez, & Artés-Hernández, 2007). This increasing demand from consumers has stimulated the food industry to reformulate products with natural agents and novel processing strategies to produce clean label, high quality products with fresher taste and extended refrigerated shelf-life, without compromising microbiological safety.

To preserve food products, the food industry has relied mostly on thermal treatments to kill the pathogenic and food quality related microorganisms, as well as to inactivate enzymes that can cause undesirable changes. The majority of shelf-stable products are manufactured by the application of heat including canning and drying operations. Heat can negatively affect the organoleptic properties and the nutritional composition of foods. To produce fresh-like, ready-to-eat foods, such as fruit cups, juice and apple puree, thermal processing is not the best option and therefore other technologies are being used and evaluated. (Amit, Uddin, Rahman, Islam, & Khan, 2017).

Nonthermal technologies offer alternatives to thermal processing, including ultraviolet (UV) light treatment, which is currently used successfully to pasteurize apple cider and similar juices. However, the low penetration depth of UV light has prevented its application to opaque liquids or solid foods. Another commercially available technology that is rapidly expanding in the marketplace is High Pressure Processing (HPP), which can be a viable alternative for processing fruit products under the appropriate conditions. This novel nonthermal, environmentally friendly technology, has proven capable of guaranteeing the safety of the products by inactivating vegetative pathogens and some spoilage microorganisms, while retaining the fresh-like attributes of foods (Barba, Esteve, & Frígola, 2012). As spores are not affected, the products need to be refrigerated, but the process renders extended shelf-life of 1 to 6 months depending on the type of food. The product to be processed at high pressure is packaged in a flexible container (plastic bags, PET bottles and trays/cups) and placed in a pressure vessel that is filled with water, which transmits the high pressure (300-600 MPa, typically for 2-3 min) uniformly and instantaneously from all directions throughout the product, independently of the shape or size of the product (Figure 1).

Extensive studies have shown that HPP is effective in killing pathogenic microorganisms and also in inactivating several enzymes at high pressure between 300 – 700 MPa (Chakraborty, Kaushik, Rao, & Mishra, 2014). However, the HPP processing conditions generally used by the food industry only partially inactivate enzymes responsible for quality losses during refrigerated storage.

This research was supported by the New York Apple Research and Development Program

High Pressure Processing (HPP) of food products is a relatively new method of processing which can be an alternative to thermal (high temperature) processing. Our studies with HPP found that apple products showed equal or better retention of quality attributes than thermally processed ones. HPP is a viable technology to produce high quality, refrigerated juice, apple puree and apple fruit cups with extended shelf-life.

Figure 1. Commercial High Pressure Processing Unit (HPP) – Hiperbaric 55. Diagram of the HPP process: the packaged foods are loaded into the unit and subjected to 600 MPa (87,00 psi) for 3 min at 5°C, in a batch process.
storage, such as browning and softening; thus the need to add natural anti-browning agents such as ascorbic acid (Vitamin C) or calcium ascorbate to minimize those changes. The application of HPP to fruit products has been focused on the safety of juices and smoothies, with little information available regarding the effect on texture and overall quality of a mixed solid food/liquid matrix like fruit dices packaged in juice (fruit in a cup). Furthermore, different apple varieties may also behave differently under the high pressure because of different composition and structures.

Apples are among the most consumed fruits due to their flavor, texture, nutritional benefits and appealing red and gold color. New York State is the second apple-producing state in the country and most importantly, grows more apple varieties than any other state. This diversity offers consumers and manufacturers many choices of apples with different applications for fresh and processed, value-added products.

Our research was aimed at evaluating high-pressure processing (HPP) as a nonthermal technology for producing three different types of refrigerated, minimally processed apple products: natural style apple juice, chunky apple puree, and apple dices in juice (apple fruit cup). In our preliminary studies, we identified enzymatic browning as a key factor in limiting the shelf-life of HPP products, thus we selected varieties with low browning potential: Ginger Gold and Autumn Crisp, representing yellow and red apples. In order to offer more nutritive products, we evaluated retaining the skin in the puree and the fruit cup.

Material and Methods

Two apple varieties, Ginger Gold and Autumn Crisp, were obtained from the Cornell Orchards, Geneva, NY, courtesy of the programs of Terence Robinson and Susan Brown. Three product prototypes were produced for each, namely, apple juice, apple puree and apple dices in apple juice.

Apple Processing and Experimental Design Figure 2 shows the flowchart followed to prepare the samples. Apples were divided into four sets to make the different products. One set was used to make peeled apple puree with ascorbic acid (AA), another set to prepare juice without AA, apple puree with AA and peel, and juice with AA. The other two sets were used to make dices with and without peeling. A dicer was set to yield 0.5-inch cubes, which were mixed with 0.2% AA juice at 1:1 ratio.

After processing, half of the samples were packed into HPP compatible containers, the other half into glass jars for thermal processing (TP). The HPP samples were processed under 600 MPa for 3 min at 5 °C in a commercial high pressure processing unit, Hiperbaric 55 (Hiperbaric, Burgos, Spain). The conditions ensure the microbial safety of the samples (>5-log reduction of vegetative cells of pathogens). Water was used as the pressure-transmitting medium. The TP samples were processed in a boiling water bath for 10 min to reach commercial sterility, using a steam kettle.

Physicochemical analysis Viscosity was measured by Brookfield DV II+ Pro rheometer. Soluble solids (as °Brix), total titratable acidity (TA) and pH were measured in triplicate. Color (L∗, a∗, b∗) values were measured using the Minolta Chroma Meter, CR-400 (Minolta Corp., Osaka, Japan).

Table 1 summarizes the results of the evaluation of the samples for color, pH, acidity and perceived sweetness (sugar/acid ratio). Ginger Gold exhibited a higher sugar-acid ratio compared to Autumn Crisp, indicating that it is perceived as sweeter while Autumn Crisp will have a tarter taste profile. The results agree with the taste test which showed that Ginger Gold was rated sweeter for all the products, and might have a broader appeal to consumers. The discussion will be divided by the type of prepared product.

Natural Style Apple Juice: The juice was prepared by grinding and pressing the apples, thus, it was a cloudy juice with the natural pectin present, similar to cider. We selected low browning apple varieties to maintain a light color juice that will appeal to consumers seeking high quality juices. Ascorbic acid was tested as a natural antioxidant to prevent browning of the apple products, at 0.1% concentration based on preliminary trials. The instrumental color data matched very well with the appearance of the samples, shown in Figure 3. Ginger gold juice showed typical light yellow color and Autumn crisp had an attractive pink color. In general, the addition of ascorbic acid resulted in lighter

A numerical total color difference (ΔE) was calculated as:

\[ \Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \]

Texture was determined using the TA.XT Plus Texture Analyzer (Texture Technologies Corp., Stable Micro Systems, Ltd, Surrey, U.K.). To determine the texture of the apple cubes, two types of mechanical texture measurement were conducted.

Compression: A two-cycle compression test (TPA) method with a 25 mm-dia. acrylic flat head cylindrical probe (P/25 L) to compress the 0.5 inch apple cubes to a depth of 20% of the height of the cubes at a rate of 5 mm/s.

Penetration: By measuring the force required for a 2 mm-dia. probe to penetrate apple cubes of 0.5-inch height to a depth of half of the height at a rate of 5 mm/s.

Texture parameters: Hardness (peak force during the first compression cycle, N) and Flesh firmness was the gradient of the linear part of the Penetration curve (Stable Micro Systems Ltd., Surrey, England).

Results and Discussion

Figure 2. Flowchart for the preparation of apple juice (with and without ascorbic acid=AA), apple puree (with and without peel) and apple dices (with and without peel) in juice (apple fruit cup) products. Two apple varieties, Ginger Gold and Autumn Crisp were utilized.
color as expected, with less browning. The thermal processing inactivated the enzymes responsible for browning, thus stopping any subsequent, associated darkening. The HPP process and refrigerated storage will slow down the enzymatic changes, allowing for at least 3 month shelf-life. The difference in color between HPP and TP samples was small, with the largest change seen in the Ginger Gold juice without AA, where the HPP sample was darker. Apple aroma was stronger in the HPP samples, which retained the fresh apple aroma better than the TP samples. Other attributes such as pH, acidity, soluble solids and viscosity (Table 2) were not significantly affected by the processing applied. The results agree with a previous study for cloudy cucumber juice (Zhao, Wang, Liu, Dong, Huang, Xiong, & Liao, 2013).

Chunky apple puree: All the purees were produced with 0.1% AA added to prevent browning, which is accelerated by the grinding effect and exposure to oxygen. The concentration used was effective in maintaining the color, as seen in Table 1 and Figure 3. The color, pH, °Brix and acidity of HPP puree samples were comparable to thermally processed puree as indicated by the small ΔE (Table 1). Figure 3 also showed no significant differences between the HPP and TP puree samples in terms of color and appearance, for the same variety. Comparing between the two varieties, the peeled puree of Autumn Crisp is even lighter than the Ginger Gold one. The puree with peel samples of Autumn Crisp showed pink color contributed by the red anthocyanins present in the peel, which was very attractive. The color was deeper in the HPP sample, indicating some losses of pigment due to the heating in TP samples. Thermal processing had an effect on the viscosity of the purees, significantly increasing the values for the peeled samples, likely due to the gelatinization of the starch and the swelling of the pectin. Similarly to the juice, the HPP puree had a stronger and fresher apple aroma than the TP puree.

Apple dices in juice: This product was prepared by dicing the peeled or unpeeled apples and mixing them at 50% with juice that had 0.2% AA, thus the final concentration of AA was 0.1%, same as the other products. Comparing to the juice and puree samples, we observed and measured more differences with the dices between the HPP and TP samples. The flesh of the dices for all HPP samples were darker (L*), greener (negative a*), and yellower (b*) than the thermally treated dices, showing a large ΔE difference (ΔE >10) between them as seen in Table 1 and Figure 3. The results indicated that the HPP might cause more browning in the flesh than in the thermally treated flesh, which could be due to unwanted enzymatic browning reactions induced by the loss of cell integrity and the incomplete inactivation of the browning enzymes, polyphenol oxidase (PPO) and peroxide oxidase (POD) (Techakanon, Smith, Jernstedt, & Barrett, 2017). Regarding the peel color, HPP peel was greener and darker than the TP peel for Ginger Gold. HPP resulted in redder and darker peel than TP for Autumn Crisp, indicating that HPP had lower extent of pigment degradation than thermal processing. A significant difference in texture was observed due to the processing applied to the dices (see Figure 4). HPP treated apple dices better maintained the firmness and hardness of the flesh and peel compared with thermally treated dices, which were very soft after heating. The mechanical texture results are consistent with the preliminary sensory results, which showed HPP treated dices were crunchier, fresher-like and with better apple aroma than thermally treated ones. The presence of skins in the dices did not seem to affect the texture and acceptability of the product, a positive factor to increase the nutritional value of the apple in a cup.

These results indicated that HPP plus refrigeration offer a viable alternative to provide high quality apple juice, puree and dices, to meet consumer demands for fresher products with clean labels. Other authors have shown that HPP (600 MPa, 5 min) produced fresh-like carrot juice with better sensory attributes compared to thermally treated (80 °C, 7 min) samples (Picouet, Sárraga, Cofán, Belletti, & Dolors Guàrdia, 2015), further validating our findings.

Summary

This study demonstrated the feasibility of using HPP, a novel nonthermal process, to produce high-quality apple products that retain most of the important attributes that characterize fresh apples. As HPP does not inactivate enzymes or spores, the...
Table 1. Instrumental color parameters (L*, a*, b*) and color differences (ΔE), soluble solid content (as °Brix), pH, titratable acidity (TA, g malic acid/100 g sample weight) and sugar - acid ratio (oBrix/TA) of the apple juice, puree and dices in juice treated by thermal processing (TP) or high pressure processing (HPP).

<table>
<thead>
<tr>
<th>Products</th>
<th>Variety</th>
<th>Treat-ment</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE</th>
<th>pH</th>
<th>°Brix</th>
<th>TA (%)</th>
<th>°Brix/TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juice</td>
<td>Ginger Gold</td>
<td>TP</td>
<td>34.5 ± 0.2</td>
<td>2.33 ± 0.06</td>
<td>8.73 ± 0.04</td>
<td>NA</td>
<td>3.59 ± 0.01</td>
<td>13.4 ± 0.6</td>
<td>0.60 ± 0.01</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>31.0 ± 0.5</td>
<td>1.85 ± 0.09</td>
<td>9.63 ± 0.37</td>
<td>3.65</td>
<td>3.59 ± 0.01</td>
<td>13.3 ± 0.1</td>
<td>0.60 ± 0.02</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>TP</td>
<td>31.1 ± 0.2</td>
<td>2.18 ± 0.10</td>
<td>8.12 ± 0.32</td>
<td>NA</td>
<td>3.26 ± 0.01</td>
<td>13.4 ± 0.1</td>
<td>0.63 ± 0.01</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>33.3 ± 0.1</td>
<td>2.89 ± 0.05</td>
<td>9.08 ± 0.10</td>
<td>1.40</td>
<td>3.30 ± 0.01</td>
<td>13.5 ± 0.3</td>
<td>0.62 ± 0.02</td>
<td>14.9</td>
</tr>
<tr>
<td>Juice with 0.1% ascorbic acid</td>
<td>Ginger Gold</td>
<td>TP</td>
<td>32.8 ± 0.4</td>
<td>0.85 ± 0.12</td>
<td>7.75 ± 0.30</td>
<td>NA</td>
<td>3.56 ± 0.01</td>
<td>13.4 ± 0.1</td>
<td>0.64 ± 0.02</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>32.6 ± 0.5</td>
<td>-0.70 ± 0.30</td>
<td>5.65 ± 0.47</td>
<td>2.17</td>
<td>3.58 ± 0.01</td>
<td>13.3 ± 0.0</td>
<td>0.61 ± 0.02</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>TP</td>
<td>30.9 ± 0.3</td>
<td>1.26 ± 0.07</td>
<td>6.06 ± 0.05</td>
<td>NA</td>
<td>3.28 ± 0.01</td>
<td>13.2 ± 0.1</td>
<td>0.86 ± 0.01</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>31.8 ± 0.1</td>
<td>1.79 ± 0.04</td>
<td>8.04 ± 0.49</td>
<td>1.42</td>
<td>3.28 ± 0.01</td>
<td>13.3 ± 0.1</td>
<td>0.85 ± 0.01</td>
<td>15.7</td>
</tr>
<tr>
<td>Peeled Puree</td>
<td>Ginger Gold</td>
<td>TP</td>
<td>46.1 ± 0.7</td>
<td>-4.72 ± 0.13</td>
<td>14.3 ± 0.3</td>
<td>NA</td>
<td>3.59 ± 0.01</td>
<td>13.4 ± 0.1</td>
<td>0.58 ± 0.02</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>48.4 ± 0.8</td>
<td>-5.37 ± 0.06</td>
<td>14.1 ± 0.3</td>
<td>2.39</td>
<td>3.60 ± 0.01</td>
<td>13.2 ± 0.1</td>
<td>0.55 ± 0.02</td>
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</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>TP</td>
<td>45.4 ± 0.2</td>
<td>-4.10 ± 0.06</td>
<td>8.6 ± 0.1</td>
<td>NA</td>
<td>3.27 ± 0.01</td>
<td>13.4 ± 0.1</td>
<td>0.90 ± 0.03</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>48.7 ± 0.8</td>
<td>-5.11 ± 0.09</td>
<td>9.36 ± 0.03</td>
<td>3.54</td>
<td>3.25 ± 0.00</td>
<td>13.2 ± 0.1</td>
<td>0.87 ± 0.04</td>
<td>15.2</td>
</tr>
<tr>
<td>Puree with peel</td>
<td>Ginger Gold</td>
<td>TP</td>
<td>48.4 ± 0.2</td>
<td>-5.36 ± 0.07</td>
<td>15.4 ± 0.2</td>
<td>NA</td>
<td>3.60 ± 0.00</td>
<td>13.4 ± 0.1</td>
<td>0.60 ± 0.03</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>49.7 ± 0.5</td>
<td>-5.86 ± 0.04</td>
<td>15.5 ± 0.1</td>
<td>1.38</td>
<td>3.58 ± 0.01</td>
<td>13.3 ± 0.1</td>
<td>0.59 ± 0.03</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>TP</td>
<td>39.1 ± 0.5</td>
<td>3.87 ± 0.05</td>
<td>8.49 ± 0.03</td>
<td>NA</td>
<td>3.30 ± 0.01</td>
<td>13.4 ± 0.1</td>
<td>0.83 ± 0.02</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>42.3 ± 0.3</td>
<td>2.80 ± 0.03</td>
<td>9.42 ± 0.04</td>
<td>3.57</td>
<td>3.28 ± 0.01</td>
<td>13.2 ± 0.1</td>
<td>0.81 ± 0.02</td>
<td>16.2</td>
</tr>
<tr>
<td>Peeled dices in juice - Flesh</td>
<td>Ginger Gold</td>
<td>TP</td>
<td>74.4 ± 1.8</td>
<td>-4.94 ± 0.33</td>
<td>15.8 ± 0.9</td>
<td>NA</td>
<td>3.64 ± 0.01</td>
<td>13.7 ± 0.06</td>
<td>0.62 ± 0.01</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>59.3 ± 6.3</td>
<td>-7.28 ± 0.30</td>
<td>13.8 ± 1.5</td>
<td>15.30</td>
<td>3.58 ± 0.00</td>
<td>13.8 ± 0.00</td>
<td>0.60 ± 0.01</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>TP</td>
<td>74.7 ± 1.2</td>
<td>-2.88 ± 0.37</td>
<td>11.2 ± 2.1</td>
<td>NA</td>
<td>3.27 ± 0.01</td>
<td>13.8 ± 0.1</td>
<td>0.80 ± 0.01</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>50.5 ± 1.7</td>
<td>-4.64 ± 0.32</td>
<td>13.0 ± 1.7</td>
<td>24.34</td>
<td>3.31 ± 0.01</td>
<td>13.7 ± 0.1</td>
<td>0.92 ± 0.04</td>
<td>14.9</td>
</tr>
<tr>
<td>Whole dices in juice - Peel</td>
<td>Ginger Gold</td>
<td>TP</td>
<td>73.2 ± 1.5</td>
<td>-7.74 ± 0.20</td>
<td>41.7 ± 2.5</td>
<td>NA</td>
<td>3.60 ± 0.01</td>
<td>13.2 ± 0.06</td>
<td>0.59 ± 0.01</td>
<td>22.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>61.8 ± 4.03</td>
<td>-9.64 ± 0.87</td>
<td>32.37 ± 0.77</td>
<td>14.82</td>
<td>3.61 ± 0.01</td>
<td>13.1 ± 0.06</td>
<td>0.58 ± 0.02</td>
<td>22.7</td>
</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>TP</td>
<td>65.22 ± 3.40</td>
<td>5.47 ± 3.15</td>
<td>25.77 ± 3.12</td>
<td>NA</td>
<td>3.26 ± 0.00</td>
<td>13.8 ± 0.2</td>
<td>0.84 ± 0.01</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPP</td>
<td>57.64 ± 3.16</td>
<td>11.68 ± 1.02</td>
<td>22.56 ± 2.20</td>
<td>10.31</td>
<td>3.24 ± 0.01</td>
<td>13.9 ± 0.0</td>
<td>0.88 ± 0.01</td>
<td>15.7</td>
</tr>
</tbody>
</table>

All data were the means ± standard deviation (SD), n = 3. ΔE of each product for each variety was calculated as the difference between TP (control) and HPP samples. NA= not applicable. Puree and dices had 0.1% ascorbic acid added.

Figure 4. Mechanical texture parameters (firmness and hardness) of the flesh and peel of apple dices after HPP or TP treatment. (HPP=high pressure processing; TP=thermal processing).
products need to be refrigerated and protected from oxidation to have extended shelf-life of several months. The addition of ascorbic acid, a natural antioxidant, at 0.1% concentration in apple juice, puree and dices was found to be effective in preventing browning for varieties that have low browning capacity such as Ginger Gold and Autumn Crisp. Future work will establish the shelf-life that can be expected for apple products based on physicochemical and microbiological analyses during refrigerated storage, as well as consumer acceptability through formal sensory panels of optimized products.

Acknowledgements

We would like to thank John Churey, Andy Humiston and LiDestri Foods Inc. for HPP assistance. Funding was provided by the NY Apple Research and Development Program.

Literature Cited


Table 2. Viscosity of apple juice and apple puree samples prepared by thermal processing (TP) or high pressure processing (HPP) from Ginger Gold and Autumn Crisp varieties.

<table>
<thead>
<tr>
<th>Products</th>
<th>Varieties</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP treated</td>
<td>HPP treated</td>
</tr>
<tr>
<td>Juice</td>
<td>Ginger Gold</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>17</td>
</tr>
<tr>
<td>Juice with 0.1% ascorbic acid</td>
<td>Ginger Gold</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>19</td>
</tr>
<tr>
<td>Peeled puree</td>
<td>Ginger Gold</td>
<td>2397</td>
</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>2396</td>
</tr>
<tr>
<td>Puree with peel</td>
<td>Ginger Gold</td>
<td>1334</td>
</tr>
<tr>
<td></td>
<td>Autumn Crisp</td>
<td>1825</td>
</tr>
</tbody>
</table>
Population Dynamics of *Erwinia amylovora* on Apple Flower Stigmas and the Effect of Application Timing, Systemic Activity, and Light Intensity on Antibiotic Efficacy

Suzanne M. Slack,¹ Kellie J. Walters,² Emily A. Pochubay,² Cory A. Outwater,¹ and George W. Sundin¹

¹Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI  |  ²Department of Horticulture, Michigan State University, East Lansing, MI  |  ³Northwest Michigan Horticultural Research Center, Traverse City, MI

Keywords: fire blight, streptomycin, kasugamycin, oxytetracycline, apple flower stigma

FIre blight, caused by the bacterium *Erwinia amylovora*, is a disease of pome fruits that is prone to sporadic epidemics. Many of the epidemics are initiated during the blossom blight stage of the disease, as flower infection accompanied by ooze emergence provides exceedingly large pathogen populations when initial apple shoot growth begins, and susceptibility to shoot blight infection is very high. The flower stigmas are the first location where *E. amylovora* cells can grow to very large numbers, with populations surging to over 1 million cells per flower in as little as a few days depending on environmental conditions. After the pathogen population increases, the availability of moisture through rain or a heavy dew enables the bacteria to move down the style and into the nectary where flower infection occurs through natural openings in the hypanthium (Fig. 1). Once the bacteria are internal within the flower nectary, the disease progresses and the signs and symptoms associated with fire blight become apparent.

Previous work on *E. amylovora* population dynamics on flowers was used to develop disease predictive models such as MaryBlyt and Cougar Blight, as preventing the initial population build up is the major strategy for disease control in commercial orchards. Growers use these predictive models to help guide decisions on which management tactics to implement in their orchards. These tactics generally include the use of agricultural antibiotics to achieve the most effective control. In environmentally-controlled systems, it is known that temperature, relative humidity, and flower age all play a role in pathogen growth on flower stigmas (Pusey and Curry, 2004; Thompson and Gouk, 2003; Dewdney et al., 2007). However, there are still many open questions concerning *E. amylovora* growth on flowers under field conditions, and also how *E. amylovora* populations respond to antibiotic pressure on sprayed flowers. The antibiotics currently registered for blossom blight control are streptomycin, oxytetracycline, and kasugamycin; streptomycin and kasugamycin are bactericidal while oxytetracycline is bacteriostatic. To date, resistance to streptomycin in *E. amylovora* has been reported in the Pacific Northwest of the United States, and in Michigan and New York (Coyier and Covey, 1975; McGhee et al., 2011; Russo et al., 2008; Tancos and Cox, 2016). In Michigan, kasugamycin has typically yielded control comparable to streptomycin, but results with oxytetracycline have been more variable (McGhee and Sundin, 2011).

The goals of this study were to conduct a detailed examination of the population dynamics of *E. amylovora* on flower stigmas under field conditions. By understanding factors affecting the population dynamics of *E. amylovora* on flowers, both with and without antibiotic application, we aim to improve recommendations to better control fire blight outbreaks and prolong the usefulness of the currently available antibiotics.

**Population dynamics of *Erwinia amylovora* on apple flower stigmas**

Flowers in an orchard do not bloom simultaneously, and new flowers continually open until trees reach full bloom. This provides the pathogen *E. amylovora* with the opportunity to continually colonize and grow on freshly opened flowers over the duration of bloom. We were interested in assessing the population dynamics of *E. amylovora* on apple flower stigmas under field conditions to better understand how environmental conditions and flower age impact bacterial growth and to improve our predictive capabilities for fire blight disease development. These field experiments were run at the Michigan State University Plant Pathology Farm in East Lansing and at the Northwest Michigan Horticultural Research Center in Traverse City.

![Figure 1. Diagram of an apple flower with the stigma and nectary organs highlighted (photo courtesy of C. Kent Evans).](image-url)
for E. amylovora growth on flowers (Billing, 1980). Additional examples of data sets exhibiting population surges between day 2 and 3 of sampling are presented in Table 2. Bacteria grow via cell division, as one cell divides into two daughter cells. Each cell doubling event represents one generation of growth. We observed as many as 8.2 cell doublings within one 24-hr sampling period (Table 1). We note that in these examples, the average temperature observed for all except one of these data sets was below 18.3°C (64.9°F), indicating that if flowers are colonized with E. amylovora, significant cell growth can occur at relatively low temperatures. Dr. Eve Billing in England has conducted extensive work on estimating the effects of temperature on growth of E. amylovora (Billing, 1980). In this work, she estimated that no cell doublings would occur on days with average temperatures of 10.5°C (50.9°F) or less, 1-2 doublings would occur on days with average temperatures between 11.0°C (51.8°F) and 13.0°C (55.4°F), and 7 doublings would occur on a day with average temperature of 17.5°C (63.5°F). She noted that potential cell doublings would max out at 12.5 on warm days when the average temperature was between 21.0°C (69.8°F) and 25.0°C (77.0°F) (Billing, 1980). In the data sets shown in Table 1, the number of doublings we observed within one 24-hr period was not absolutely correlated with temperature, which indicates that other environmental factors are also contributing to E. amylovora growth on flower stigmas. However, our results do highlight the capability of the fire blight pathogen to build...

We tagged groups of individual, unopened flowers that were set to open the next day for use in our experiments. This allowed us to perform experiments on freshly opened flowers during the course of bloom. Flowers were inoculated with E. amylovora Ea110, a virulent strain that is marked with rifampicin resistance, to facilitate ease and specificity of tracking. The E. amylovora cell inoculum was applied directly to flower stigmas using a micropipetor at a rate of approximately $10^3$ (1,000) cells per flower. After inoculation, flowers were sampled immediately, and then at 1-day intervals for five days to track populations. Over the course of four years (2016-2019), we generated 35 experimental data sets of E. amylovora population dynamics on 1-day old flowers. Similar experiments were also conducted on 3- and 5-day old flowers to assess the effect of flower age on E. amylovora populations.

We analyzed each of the 35 data sets to determine general trends in population dynamics and general trends in responsiveness of the E. amylovora populations to environmental conditions. All flowers in this study were initially inoculated with $~10^3$ (1,000) colony forming units (CFU) of E. amylovora Ea110 cells per flower; in most data sets, Ea110 populations reached $10^6-7$ (1,000,000 – 10,000,000) CFU per flower within the five day sampling period, with substantial population surges generally occurring within one 24-hr period over the 5-day experiment. After further examination, we noted that most of the population surges on 1-day old flowers occurred either between day 1 and 2 or day 2 and 3 of sampling (example data sets shown in Fig. 2). The population curve from 1-day old flowers shown in Fig. 2 shows a 1,000-fold population increase between day 2 and 3 of sampling. This substantial increase in population size occurred on a day with an average temperature of 7.5°C (45.5°F), which is at least 4.5°C (16.3°F) below the minimum temperature that was previously shown to be conducive...
In four experiments conducted where flowers were sampled at 4-hr intervals overnight, we consistently observed the most rapid growth of *E. amylovora* between 10 pm and 2 am, with as many as 2.5 doublings occurring during this 4-hr period.

**Effect of antibiotic treatments on Erwinia amylovora stigma populations**

While understanding how *E. amylovora* grows naturally and responds to environmental conditions is important, in a commercial orchard there is another variable: the use of antibiotics during bloom for blossom blight management. The main grower strategy for limiting blossom blight infection is to keep populations of *E. amylovora* at low or nonexistent levels during the bloom period to prevent flower infection. Disease prediction models should be used to guide application timing of the three antibiotics, kasugamycin, oxytetracycline, and streptomycin, currently registered for blossom blight control. Although efficacy data are available for these antibiotics, there is relatively little information on the impact of antibiotic application on *E. amylovora* populations. To address this data gap, we conducted a second set of flower stigma population experiments between 2016 and 2018 at the Michigan State University Plant Pathology Farm in East Lansing. In these experiments, conducted on ‘Gala’, approximately 1,000 cells of *E. amylovora* strain Ea110 were inoculated onto flower stigmas either 4 hr. before or 4 hr. after an antibiotic was applied. The antibiotics were applied with an air blast sprayer; in the control treatment, water was applied via air blast. The antibiotics used in this study were kasugamycin (Kasumin; Arysta Corp., used at 64 fl. oz. in 100 gallons), oxytetracycline (FireLine; AgroSource, used at 0.5 lbs. in 100 gallons), and streptomycin (FireWall; AgroSource, used at 0.5 lbs. in 100 gallons). The non-ionic surfactant Regulaid (Kalo Inc.) was added to all antibiotic sprays at a rate of 16 fl. oz. in 100 gallons. Populations of *E. amylovora* strain Ea110 were monitored within 4 hrs. after antibiotic application, and then by daily sampling for 4-5 days after antibiotic application.

**Figure 4. Incidence of infected flowers occurring in each antibiotic treatment per year and for each inoculation timing.** *Erwinia amylovora* was either inoculated prior to antibiotic treatment or post treatment, indicated by “Prior” or “Post”. A total of 20 flowers were rated for disease incidence. Statistical differences between treatments are indicated by letters derived from ANOVA and Tukey’s HSD (*P* < 0.05). Primary axis indicates percent disease incidence and secondary axis indicates the cumulative light integral for the duration of the experiment (four days).

**Figure 5. Results from testing the systemic activity of the three commercially available antibiotics for fire blight control during bloom: oxytetracycline (FireLine), kasugamycin (Kasumin), and streptomycin (FireWall).** The base of the flowers was directly inoculated with *E. amylovora*, and then treated 12 hrs. later with an antibiotic spray at commercial field rates. The flowers were then monitored for disease progression and rated.

populations on flowers even when temperatures are relatively low.

In addition, it is also very important to note that what we are tracking and reporting is *E. amylovora* growth on flower stigmas. Disease prediction models, such as MaryBlyt, provide temperature values that are predictive of infection events. For example, MaryBlyt uses a daily average temperature of 65°F as a threshold for an infection event, i.e., the daily average temperature must be at least 65°F for a blossom blight infection to occur. Our data are indicating that growth on flowers can enable the fire blight pathogen to reach critical population sizes on flowers that would then be capable of causing an infection if the requirements according to MaryBlyt were met [flowers open with petals intact; average daily temperature of 65°F or higher, 198 degree hours < 65°F were accumulated from first bloom open, and a heavy wetting dew or a rain ≥0.01 inch occurred the previous day (Turechek and Biggs, 2015)].

Flowers that were 3 and 5 days old were also inoculated with *E. amylovora* Ea110, and the population was tracked for 5 days. On 3-day old flowers, we typically observed *E. amylovora* growth trends similar to those on 1-day old flowers, however, the highest population achieved was usually at least 10-fold lower than that achieved on 1-day old flowers (Fig. 2). Flowers that were inoculated one day after opening had *E. amylovora* populations increase by 100,000-fold in 5 days, whereas 3- and 5-day old flowers only had a 100-fold increase (Fig. 2).

After we consistently observed that large population surges can occur within a 24-hr time period, we conducted more intensive samplings during these 24-hr periods to narrow the timing window of when the surges were occurring. This intensive sampling revealed that the majority of the population surges were occurring at night, when temperatures are typically at their lowest. In four experiments conducted where flowers
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Disease (percent of flower clusters with blossom blight) was rated in these treatments approximately two weeks after inoculation.

In all three years of the experiment, application of water to trees either before or after inoculation had a positive effect on *E. amylovora* growth, as populations increased by 1,000 to 10,000-fold over the 4-day sampling period after treatment (representative results from 2016 shown in Fig. 3). On trees treated with streptomycin, *E. amylovora* populations were reduced, and remained at very low levels up to 4-5 days after antibiotic application in all three years of the experiment (Fig. 3). Similar results were observed on trees treated with kasugamycin. In 2016 and 2018 on trees treated with oxytetracycline, growth of *E. amylovora* populations was suppressed for 2-3 days and then increased at similar levels to those observed on control trees.

In 2017, the population response to oxytetracycline was more variable, although the net trend was downward over the 4-5 day sampling period (Table 2). Similarly, populations on flowers treated with kasugamycin were more drastically reduced in 2017 compared to the reductions observed in 2016 and 2018. In addition, the disease incidence in the oxytetracycline treatment was markedly reduced in 2017 compared to 2016 and 2018, and the disease incidence in the kasugamycin treatment was lower in 2017 (Table 2; Fig. 4). Thus, the results from 2017 indicated that something was different from the other years in terms of efficacy of both oxytetracycline and kasugamycin, leading us to examine multiple environmental parameters to identify correlations. We measured the incoming solar radiation during the experimental period in each field experiment, and found that solar radiation in 2017 was reduced by 24.9% compared to the 2016 experimental period, and was reduced by 36.0% compared to the 2018 experimental period (Fig. 4).

The reduction in solar radiation intensity during the 2017 bloom period of our experiments seemed to have a large effect on oxytetracycline efficacy and also positively affected the kasugamycin efficacy. In contrast, we did not observe similar effects in the streptomycin and the control treatments. The sensitivity of oxytetracycline to solar radiation has been examined in a previous study on peaches in Georgia where it was shown that 1 full day of exposure reduced the residual amount of the antibiotic by 43.8% (Christiano et al., 2010). Our focus in this study was to examine the light sensitivity of kasugamycin when formulated as Kasumin.

We exposed samples of Kasumin in a growth chamber to a daily light dose that was equivalent to the average observed in Michigan during apple bloom (35 mol m⁻² d⁻¹), and examined the effect of light exposure on reduction in the capability of reducing *E. amylovora* populations in vitro. Light exposure experiments were conducted over three consecutive day periods, and effects were evaluated daily. The results indicate that there was little reduction in efficacy of Kasumin after 1 day of light exposure; however, after two days, the efficacy of Kasumin was reduced by 50% compared to the negative control at the Michigan average daily light integral. After three days, the efficacy of Kasumin was reduced by 75%.

We also wanted to determine if Kasumin had any systemic permeability into apple flowers similar to streptomycin. To study this, *E. amylovora* Ea110 cells were directly inoculated into the base of flowers; the flowers were then treated approximately 12 hrs. later with Kasumin, FireLine, or FireWall by air blast as described above. This time gap between inoculation and the application of the antibiotics allowed the bacterial cells to become internalized and initiate infections within flowers such that disease control would be reduced only if the sprayed antibiotic could penetrate the host tissue. The experiment was repeated twice, and on average 97.5% of control flowers that were treated with water became infected, and we observed 80%, 67.5%, and 15% infection in flowers treated with FireLine, Kasumin, and FireWall, respectively (Fig. 5). The disease incidence observed for Kasumin was similar to that of FireLine, a compound that is known not to be systemic, and much higher than that of FireWall, a known partially systemic compound. Thus, the evidence indicates that Kasumin does not have any systemic properties in apple flowers.

**Conclusions**

Over the course of a four year field experiment monitoring *E. amylovora* populations on apple flower stigmas, we determined that the growth rate of *E. amylovora* was highest on flowers that were open for 1 day prior to inoculation. In general, when tracking *E. amylovora* growth at daily intervals, we always observed a large surge in growth, when populations increased 100-1,000 fold within a 24-hr period. Surprisingly, in many of our replicated data sets, the average daily temperature when large population surges occurred was lower than what we currently think of as optimal or even conducive for *E. amylovora* growth. Results from further intensive sampling experiments also revealed that the *E. amylovora* pathogen is mostly growing at night, specifically between 10 pm and 2 am. Nighttime conditions include falling temperatures that are also accompanied by the occurrence of dew; the added moisture to stigma surfaces along with the lack of solar UV exposure may be key environmental factors favoring *E. amylovora* growth.

Application of the antibiotics streptomycin and kasugamycin to flowers consistently lowered *E. amylovora* populations in our experiments for at least a 3-day period after application. In contrast, oxytetracycline was much less effective in reducing *E. amylovora* populations, and only worked well in 2017, a year in which cloud cover was substantially higher during the bloom period. Since the efficacy of Kasumin was also slightly increased in our 2017 experiment, compared to results from 2016 and 2018, we investigated the light sensitivity of Kasumin, and found that the light intensity of the equivalent of two Michigan sunny days in May (normal apple bloom timing) reduced the ability of Kasumin to lower the growth of *E. amylovora* cell cultures in vitro by 50%. We want to emphasize that the efficacy of Kasumin has been consistently excellent in all field studies we have conducted during the period 2006-2019, so we are not yet sure if the efficacy of Kasumin on flowers in orchards is being lowered to that extent under field conditions. The examination of light sensitivity was conducted only after we observed a slight increase in efficacy during the cloudy 2017 bloom period. Our conclusion from this result is for growers to just be aware of the length of the spray interval when using Kasumin during periods with sunny days.

Because we found that the *E. amylovora* pathogen grows preferentially at night and that light intensity affects the half-life of two antibiotics, oxytetracycline and kasugamycin, we believe that the optimum time to apply antibiotics for blossom blight control is during the early evening hours. It is always easier to reduce bacterial populations that are lower, thus, applying the antibiotics immediately before *E. amylovora* populations are active provides a better opportunity for control. In addition, application
in the early evening will reduce any impact of compound light sensitivity until the following morning and maximize the impact of the antibiotic application.

Acknowledgments

We thank the Michigan Apple Committee, Project GREEEN, a Michigan plant agriculture initiative at Michigan State University, Michigan State University AgBioResearch, and the National Institute of Food and Agriculture for their financial support of our fire blight research. We thank Jeff Schachterle, Emma Sweeney, Leire Bardají, Megan Botti-Marino, Roshi Kharadi, and Jingyu Peng for assistance in conducting flower population dynamics experiments, and we thank Roberto Lopez, MSU Department of Horticulture, for letting us use his controlled light equipment.

Literature Cited


Table 1. Dynamics of Erwinia amylovora populations on apple flower stigmas after 96 hours of exposure to antibiotic treatment. Bacteria was either inoculated prior to antibiotic treatment or post treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth trend of E. amylovora inoculated prior to treatment</th>
<th>Growth trend of E. amylovora inoculated post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>- + +</td>
<td>+ - +</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Kasugamycin</td>
<td>- - -</td>
<td>- - -</td>
</tr>
</tbody>
</table>

Table 2. Log change of E. amylovora strain ‘Ea110’ populations on ‘Gala’ stigmas after 96 hours of exposure to antibiotic treatment. Bacteria was either inoculated prior to antibiotic treatment or post treatment.

<table>
<thead>
<tr>
<th>Environmental variable</th>
<th>Cultivar</th>
<th>CFU Log change</th>
<th>No. of doublings</th>
<th>Doubling time (no. of hours per doubling)</th>
<th>Precip (mm)</th>
<th>Average % RH</th>
<th>Average temp (°C) hourly average</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. Gold</td>
<td>0.2</td>
<td>0.6</td>
<td>38.2</td>
<td>0</td>
<td>53.0%</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>G. Del.</td>
<td>1.1</td>
<td>3.6</td>
<td>6.6</td>
<td>0</td>
<td>61.5%</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Gala</td>
<td>0.8</td>
<td>2.6</td>
<td>9.2</td>
<td>1</td>
<td>92.7%</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>McIntosh</td>
<td>2.0</td>
<td>6.6</td>
<td>3.7</td>
<td>1</td>
<td>92.7%</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>McIntosh</td>
<td>2.5</td>
<td>8.2</td>
<td>2.9</td>
<td>0</td>
<td>61.2%</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Jonathan</td>
<td>1.5</td>
<td>5.1</td>
<td>4.7</td>
<td>0.1</td>
<td>53.1%</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td>Jonathan</td>
<td>1.9</td>
<td>6.3</td>
<td>3.8</td>
<td>0.1</td>
<td>55.7%</td>
<td>16.7</td>
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</tr>
<tr>
<td>McIntosh</td>
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<td>3.3</td>
<td>7.2</td>
<td>0</td>
<td>76.7%</td>
<td>17.2</td>
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<tr>
<td>McIntosh</td>
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<td>2.5</td>
<td>9.6</td>
<td>8.1</td>
<td>78.9%</td>
<td>18.9</td>
<td></td>
</tr>
</tbody>
</table>


Suzanne M. Slack is graduate student at Michigan State University who works with George Sundin, Kellie J. Walters is a graduate student in Horticulture at MSU, Emily A. Pochubay is IPM extension educator in Northwest Michigan, Cory A. Outwater is a research technician who works with George Sundin and George W. Sundin is a research and extension professor of plant pathology who leads MSU’s program in apple disease control.
Investigating the Behavior and Biology of Locally Overwintered Spotted-wing Drosophila

Dara G. Stockton and Gregory M. Loeb

Department of Entomology, Cornell AgriTech, Geneva, NY 14456 | Keywords: SWD, survival, habitat, diet, overwintering, ecology

Since the arrival of spotted-wing drosophila (*Drosophila suzukii* Matsumura) during 2008 (Asplen et al. 2015), larval feeding damage associated with *D. suzukii* infestation in North American berry crops has caused at least $40 million in revenue losses, and the cost of chemical purchases for conventional growers has increased by more than $1000 per hectare annually (Farnsworth et al. 2017). Prior to the arrival of *D. suzukii*, berry growers in the Northeast managed existing pest problems such as blueberry maggot, and cranberry fruitworm, among others, on a limited basis, and spray programs were only occasionally implemented. While insecticide applications are effective at reducing infestation in conventionally grown small fruit crops (Timmeren and Isaacs 2013, Iglesias and Liburd 2017), this is not likely sustainable and New York fruit growers may benefit from a more integrated approach. In recent years ecological concerns have arisen from increased insecticide use including, pollinator and natural enemy declines, secondary pest outbreaks, and insecticide resistance in some west coast populations (Gress and Zalom 2018).

In order to comprehensively manage *D. suzukii* using economically feasible and ecologically sustainable practices, more information is needed regarding the biology and ecology of this invasive pest. For the past several years, we have researched the thermal susceptibility of this species and attempted to define the lower lethal limits of exposure, as it applies to the likelihood of survival in western New York. Our laboratory data suggest that despite early reports of limited cold hardiness, given adequate acclimation, *D. suzukii* may survive at temperatures as low as -7.5 °C for at least three days (Stockton et al., 2018). This indicates that unless the temperature were to drop suddenly below freezing during early fall, when night time temperatures are still warm, the survival likelihood during the winter could be significant. Indeed, mid-winter field trials (Figure 1) in which we monitored survival outdoors in various populations have further indicated that the potential for overwinter survival in this species is similar to other temperate *Drosophila* (Stockton, Wallingford, et al. 2019). After several weeks, small but biologically significant numbers of flies may survive despite moderate durations below freezing. We have recently focused our attention on habitat selection and dietary preferences of *D. suzukii* throughout the year. This is because, in addition to the summer fruiting season, we are interested in the behavior of this pest when preferred fruit are not available. The need to feed appears to be maintained at temperatures above the threshold for chill coma, which we have estimated to occur below -2.5 °C (Stockton, Brown, et al. 2019). Even reproductive diapause is facultative and temperature dependent in this species (Wallingford and Loeb 2016). This means that as soon as it is warm enough the insects may resume foraging. In western New York, ground temperatures under leaf litter remain at or near 0 °C throughout the winter (Stockton, Wallingford, et al. 2019a).

Due to these unique physiological overwintering traits, there is growing interest in potentially targeting off-season bottleneck populations with mass trapping, targeted sprays, or population suppression using biological control agents. However, that requires that we know where to look for *D. suzukii* within a complex environment. We are currently limited in our ability to target off-season populations, because it is difficult to determine where winter and spring populations reside. If the location of *D. suzukii* is detectable during the late fall or early spring and concentrated in a limited number of places, we may be able to use targeted insecticide applications to control already vulnerable population pockets. The goal of our ongoing research is to 1) determine whether *D. suzukii* overwinters locally in western New York state, 2) identify abiotic (e.g. temperature and precipitation levels) and biotic factors (i.e. physiology, diet, behavior) affecting survival probabilities, and 3) develop improved monitoring and pest management tools using that information. Here we discuss our progress understanding the overwintering biology and ecology of *D. suzukii* within the context of insect physiology, off-season refuge use, and acceptance of non-fruit dietary resources.

Figure 1. Sites featuring raspberries are prime candidates for study, even in our overwintering trials, as low numbers of *D. suzukii* are captured up until January near the wooded margins of berry crops (A). We are increasingly interested in the landscape features of those wooded margins, particularly areas of dense brush over, as possible overwintering sites for *D. suzukii* (B). To study these effects, our Cornell AgriTech Loeb Lab team deploys flies in the winter and monitors survival over time, varying habitat type, access to food, and biological acclimation of the insects prior to release (C). This allows us to gather information on the biotic and abiotic factors significant for survival outcomes. We are also interested in fruit waste, such as compost and fruit pomace, as a nutritive resource and/or heat source present on various farms and vineyards along Seneca Lake, NY (D).
results and discussion

off-season refuge use: previous research has shown that during the fruiting season, D. suzukii move readily from the crop to adjacent wooded areas. Infestation is consistently higher in perimeter areas of the crop, compared to interior portions of the planting, and increases further still if a preferred wild host is present along the periphery (Klick et al. 2016, Ballman and Drummond 2017, Leach et al. 2018). Furthermore, ELISA-based marked-recapture studies have shown that movement between the crop and wooded margins occurs with increased frequency as the season progresses with movement up to 120m (Leach et al. 2018). However, in unclear whether decreasing temperatures in the off-season landscape affect these behaviors in winter-morphotype (WM) flies, which are the larger, darker, more cold hardy insects that ride out the winter. Furthermore, any claim of leaf-litter use is speculative at best, with no research to support refuge preferences in the laboratory or in the field. If we understand what refuge features are attractive to WM females we may be able to locate, and therefore manage them more effectively.

To address some of these questions, we developed a series of laboratory assays observing movement and refuge site preferences in the most likely overwintering population, adult female WM flies (Stockton, Brown, et al. 2019). The first question we wanted to address was aggregation, asking if refuge use tends to occur individually or in aggregate pockets. We designed an arena with four identical leaf-like targets and released cohorts of 20 females to monitor their movement in a controlled environment. We found that as temperatures decreased, aggregation occurred among small clusters of females (Figure 1A), more than would be predicted to occur by random (Poisson regression: \( \chi^2 = 245.77, df = 3, P < 0.001 \)). Indeed, when we observed movement at 0 °C, more than half of all insects (55.45%) were clustered under a single target, with the rest forming smaller aggregates (25.5%, 12.6%, and 6.5%, respectively). In a separate assay, we observed the vertical distribution of those aggregations in 3-dimensional space, and placed two targets in a cage. One was resting on the ground, while the other was located approximately 40 cm high. Using this assay, we were able to determine that D. suzukii aggregations tend to occur low to the ground (Poisson regression: \( \chi^2 = 500.22, df = 1 \)).

Figure 2. Aggregation ratios among D. suzukii given four target refuge locations (A). The proportion of D. suzukii found in the top or bottom of a cage and whether or not the insects were hidden under refuge (B). Four food placement treatments varied the position of food within the cage to determine the effect of food placement on movement. We also observed the effect of target color on refuge use (C) and the time of day on refuge use in additional cage trials (D).

Figure 3. Mean (±SE) D. suzukii per sample of fungi, flowers, or green leaves found in Western New York forests during the time of early capture in May 2019 (A). In our second host range survey, we compared development on whole and processed samples including fruit, fungi, and manure (B). Photographs show D. suzukii pupae embedded within the leaves of common milkweed (C), decomposing dandelion petals (D), and eggs laid on unprocessed goose manure (E).
We have also focused our efforts on understanding how geotaxis (orientation towards the ground) may occur independently of access to food. Our data suggest that geotaxis is orientation towards the ground may occur independently of access to food (Poisson regression: $\chi^2 = 0.31$, df = 3, $P = 0.96$; Figure 1B). This is not surprising as we would expect ample opportunities for saprotrophic sponging on leaf-litter and in soil.

Next we wanted to observe the visual characteristics of the refuge site affect selection. We designed a four choice assay in which female WM D. suzukii were released into an arena containing red, black, orange, or green leaf-like targets. When we assessed how the color of the refuge affected use, we found that most female WM D. suzukii orient towards leaf-like targets that are dark in color (Poisson regression: $\chi^2 = 210.26$, df = 4, $P < 0.001$; Figure 1C). Although there was no difference in selection among red and black targets (P = 0.58), it should be noted that among Drosophila spp. discrimination between red and black is limited due to differences in their eye structure (Lebhardt and Desplan 2017). We also found that refuge use was more prevalent during the evening hours, as during the day the insects were more mobile, regardless of the temperature (Figure 1D). In scenario 1, there was no difference in refuge use among flies at 8:00 AM and 3:00 PM and most flies were hidden during both observation periods (Chi-squared test of independence: $\chi^2 = 0.08$, df = 1, P = 0.78). In scenario 2, however, refuge use was much lower at 6:00 PM (Chi-squared test of independence: $\chi^2 = 101.61$, df = 1, $P < 0.001$), suggesting that D. suzukii is most mobile in the evening. All told, these data suggest that certain features within the landscape may be preferred by overwintering female D. suzukii. They likely cluster in small aggregates low to the ground, under leaf-litter or other protective cover dark in color. Although our data indicate that refuge use decreases in the evening, it is unclear whether this behavior extends to temperatures at or near freezing, as colder nighttime temperatures may affect this pattern.

**Non-fruit Dietary Resources:** We have also focused our attention on off-season resource use. This is particularly important given data suggesting that feeding is required in order for D. suzukii to successfully overwinter. Our working model of D. suzukii ecology and behavior suggests that shifts in resource availability may lead to differential dietary preferences at different times of the year (Panel et al. 2018). In the spring, flowering nectaries and other carbohydrate rich resources are documented sources of nutrition for female flies (Mitsui et al. 2010, Tochen et al. 2016). Furthermore, animal manure may be an important nitrogen source for overwintered females preparing for renewed reproduction (Wallington et al. 2018). As summer progresses, D. suzukii shift their attention to fruits and populations grow quickly, but in the fall, as fruit availability declines and the flies enter a state of reproductive dormancy, other resources may be required to survive. During this time, we suspect that feeding habits of D. suzukii largely shift to saprotrophic sources, such fungi in the soil, on bark, or otherwise present in wooded areas, although fallen decaying apples and other pome fruit may also be utilized (Pelton et al. 2016, Bal et al. 2017).

We previously reported that female D. suzukii may use novel, non-fruit resources such as mushrooms and even chicken manure for oviposition and larval development in controlled laboratory assays (Stockton, Brown, et al. 2019). However, little work has been done to comprehensively study the extent of non-fruit host use based on resources available to D. suzukii during the off-season. For that reason, we conducted 2 observational surveys of non-fruit resources during the spring and summer 2019. In the first survey, we collected wild samples of fungi and flowers during May 2019 from the wooded margins of tart cherry orchards near Sodus, New York. In the lab, we transferred gravid females (females ready to lay eggs) onto the samples for 24 hrs. and observed D. suzukii development over the next two weeks. We observed D. suzukii ovipositing (laying eggs) and larvae developing on a number of non-fruit samples (Figure 2A). Among fungal samples, the greatest number of D. suzukii were reared on orange mycena (Mycena leaiana), a saprobic fungus typically associated with deciduous logs. We also positively reared D. suzukii from mossy maze polyope fungus (Cerrena unicolor) and brown cup fungus (Peziza phyllophena). We were unable to identify two of our other fungus samples, but we did observe positive acceptance of their tissues by female D. suzukii.

We report also observing development on flower tissues including white champion (Silene latifolia), Virginia waterleaf (Hydrophyllum virginianum), purple flowering raspberry (Rubus odoratus), and common dandelion flower heads (Taraxacum officinale). Each flower sample was associated with oviposition and subsequent larval development although at lower rates than observed in fungi. In white champion and flowering raspberry, we observed eggs being laid directly into the petal tissue. In all other flower species the eggs were laid on top of the tissue, although the biological significance of this is unclear as eggs are often laid on top of fruit tissue in the lab, as well. However, particularly for dandelion, on which oviposition and development was quite high, mobile larvae were observed foraging throughout the petal tissue and eventually pupated within the decomposing flower (Figure 2B). Among plant tissue we classified as leafy, we also observed...
abundant oviposition and development on milkweed. Although we brought in immature flower buds and leaves, we observed feeding on the leaves and pupation within the leaf surface (Figure 2C). Although the greatest number of pupae were found on milkweed, very few completed pupation (< 5%). In contrast, over 95% of pupae enclosed as adults when reared on dandelion. This is likely associated with high levels of cardiac glycosides found in common milkweed, making it an unlikely host in nature.

In our second survey, we compared maternal host acceptance and larval performance on fruit and non-fruit resources in the lab using two methods (Figure 2D). In the first method, we presented the diets whole and intact, in the manner in which they would be encountered in nature. However, this method fails to standardize surface area and volume of the samples making it difficult to compare among groups. The second method standardized the media by preparing the diets in a puree set in 0.9% agar. Each of the 7 diets tested was sufficient to rear D. suzukii in the lab. The greatest number of flies was reared on raspberry and blueberry, which is consistent with these being preferred hosts (Lee et al. 2011). The first detection of the spotted wing drosophila, Drosophila suzukii, to the North America mainland in California caused great concern, as the fly was found infesting a variety of commercial fruits. Subsequent detections followed in Oregon, Washington, Florida and British Columbia in 2009; in Utah, North Carolina, South Carolina, Michigan, and Louisiana in 2010; and in Virginia, Montana, Wisconsin, Pennsylvania, New Jersey, Maryland and Mexico in 2011. In Europe, it has been detected in Italy and Spain in 2009 and in France in 2010. Economic costs to the grower from D. suzukii include the increased cost of production (increased labor and materials for chemical inputs, monitoring and other management tools. However, D. suzukii also readily oviposited and developed on grape, tomato, mushroom, and goose manure (Figure 2E). Among these last four hosts, more D. suzukii developed on processed samples better than whole samples, suggesting that access to these resources may be limited in nature if undisturbed. We were surprised to note that we did observe complete larval development on intact cow manure, which to our knowledge has been never previously been reported.

In Michigan, there are reports that grape and apple pomace, among other fruit waste, may function as resource reservoirs for D. suzukii in the fall (Bal et al. 2017). However, without large-scale landscape level research, it is unclear whether the shift from frugivorous to saprotrophic resources is widespread. Similarly, we should also note, that despite abundant non-fruit host use in the lab, it is unclear how readily these resources are used in nature. In the lab we regulate humidity and moisture content, exclude predators and competitive species, and otherwise create a hospitable, likely ideal environment in which to develop. These external environmental factors may preclude non-fruit host use in the field and more data are needed to determine how common flower, fungal, or manure use may be, particularly in the spring.

Limitations in Off-season Detection: One of the foundational problems in detecting overwintering pockets of D. suzukii has been devising a method to reliably capture them during the off-season. In New York, we typically cannot detect D. suzukii after December or occasionally January. Trapping for this species currently consists of a standard wet trap filled with some of drowning solution like diluted ethylene glycol to prevent freezing. Commercial olfactory baits like the fermentation lure manufactured by Scentry or Trécé are often included as well (Cha et al. 2012). Although flat panel traps are now available for use in the crop, little data has been collected on their efficacy during the off season (Kirkpatrick et al. 2017).

While these traps work well during the summer months, there may be reason to suspect limited efficacy in winter morphotype populations. Recent data collected this summer by one of our undergraduate summer scholar students indicated that response to presumably attractive targets is differentially limited in winter morphotype flies, even though the winter morphotype is more active at colder temperatures than summer flies (Figure 3A-B). Choice (number captured in traps) increased with temperature and the highest proportion of flies made a choice at 25°C. Below 10°C, few WM flies entered the traps, while more SM flies entered traps than WM flies, even at lower temperatures (Logistic regression: χ² temperature = 1301.94, P < 0.001; χ² morphotype = 35.01, P < 0.001). WM flies were significantly more active than SM flies at temperatures near freezing while at warmer temperatures WM activity was fairly similar leading to a significant interaction between temperature and morphotype (Poisson regression: χ² = 33.41, P < 0.001). At 25°C, activity level decreased as most flies made a choice were actively feeding on diet, possibility leading to misleadingly low activity readings. These data suggest that despite low captures in the field, wild WM D. suzukii may persist in the environment. We just may not be catching them.

Future Research: Future research needs to focus on two main points. First, we need to collect more data on resource use in the wild throughout the year. Molecular techniques now available mean that it is more accessible than ever to test the gut contents of large numbers of flies relatively quickly and with high accuracy. This method is being employed in North Carolina to test for cultivar feeding (Diepenbrock et al. 2018) and in Washington state, large panel, high throughput methods are being used to test landscape level consumption in pear psylla (Cacopsylla pyricola), another economically significant fruit pest (Cooper et al. 2019). Collecting data on the non-crop resource use of D. suzukii during different times of year would provide valuable information regarding seasonal refuge and nutritional requirements and would refine our search for overwintering populations. The second focus of future research should be on trap design. If WM flies do not respond to fermentation odors, we need to identify what stimuli D. suzukii does find attractive. We know that the primary overwintering population is comprised of adult WM females, so focusing on these insects is particularly important. Are these females attracted to the odors of conspecifics? Anecdotal data from Oregon has reported aggregate pockets of D. suzukii clustered on conifer branches (D. Rendon, personal communication). Perhaps the odor of other D. suzukii, which in SM flies appears less important, is more significant when dietary resources are scarce. If this is the case, we may be able to generate a WM specific lure in the lab and extend our ability to address D. suzukii behavior during the winter months, which have previously been inaccessible.

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Methods

Insect colony *Drosophila suzukii* were reared in a 25°C colony room with a 14L: 10D photoperiodic cycle, 55% relative humidity (RH). Winter morphotype (WM) flies were reared similarly, although 5 days after oviposition, bottles designated for the WM colony were moved to a 15°C growth chamber set to 10L: 14D photoperiodic cycle, 55% RH. The colony was composed of a mixture of insects from 2011, 2014, 2016 and 2017 colonies. The colony was maintained on a standard cornmeal diet comprised of 1 liter of water, 40 grams white sugar, 25 grams cornmeal, 9 grams agar and 14 grams torula yeast. The following antifungal agents were also used: 0.6 grams methyl paraben, 6.7 mL ethanol (95%), and 3 mL acetic acid. The diet was replaced every 4-5 days.

Aggregation assay We observed the movement of flies at three different temperatures (10, 5, and 0°C). The number of flies found under four leaf-like targets and the number of flies exposed in a four choice arena was recorded after 24 hours. Each trial was conducted using cohorts of approximately 20 female WM flies. A total of 15-20 replicates were conducted for each temperature treatment. Differences in the number of flies under each of the targets was compared using Poisson regression due to positive data skew. All reported analyses were performed in R (version 3.6.1; the R Foundation for statistical computing (platform x86_64-w64-mingw32/x64); Vienna, Austria).

Movement assay We constructed a vertical height arena containing two leaf-like refuge targets. One was positioned on the bottom of the arena and the other was positioned 40 cm above using a ring stand. Four scenarios were used that simulated food availability at the top and bottom of the cage. This allowed us to see how movement was affected by access to diet. Scenario 1: food on both levels. Scenario 2: Food on top. Scenario 3: Food on bottom. Scenario 4: No food. Food targets used in this experiment consisted of 40 ml cornmeal diet in a standard petri dish. All trials were conducted at 10°C in a walk-in growth chamber mimicking winter conditions (10L: 14D photoperiodic cycle, 55% RH). Each trial was conducted with a cohort of 30 female WM flies. Nine replicate trials were conducted for each of the four scenarios. After 24 hrs., the number of flies located near the bottom and top of the arena cage, as well as whether the insects were hidden or exposed, was recorded. Differences in insect location within the arena (top or bottom of the cage), and refuge use status (hidden or exposed) were compared using Poisson regression.

Visual responses We compared *D. suzukii* movement under four differently colored leaf-like targets in a four choice arena. Targets were black, yellow, green, or red. The number of flies found under each of the targets, as well as the number exposed (not hidden) was recorded after 24 hrs. Each trial was conducted using cohorts of approximately 20 female WM flies. Twenty replicate trials were conducted in total. All trials were conducted in a walk-in growth chamber mimicking winter conditions (10L: 14D photoperiodic cycle, 55% RH). Differences in the mean number of flies found under each target was compared using Poisson regression and posthoc multiple means comparisons using the package *emmeans*.

Time of day assessment The number of flies observed under a leaf-like target or exposed was observed after 24 hrs. Two time scenarios were included. Scenario 1: The flies were checked at 8am and 3pm. Scenario 2: The flies were checked at 8am and 6pm. This allowed us to compare movement as the afternoon progressed. Each trial was conducted using 20 female WM flies. Ten replicate trials were conducted for each time of day scenario. Differences in refuge use at different times of the day were compared using a Chi-squared test of independence for each scenario.

Novel resource assessments In survey 1, we collected various wild flowers, fungi, and leaf material from the woods and property near Sodus, NY where *D. suzukii* infestations are typically recorded earliest in the year (May-June). All samples were collected near tart cherry orchards along Lake Ontario. The samples were brought back to the lab and 10 g were placed in small deli containers along with 30 mL non-nutritive agar to maintain humidity and moisture. Twenty female SM *D. suzukii* were released for 24 hours on each sample. After this time, the females were removed. After two weeks, the number of *D. suzukii* that completed development on each sample was recorded.

In survey 2, we compared the number of *D. suzukii* that developed on various diets prepared whole or processed in agar to standardize volume and surface area. Whole diet preparations were simply 10 g of diet material weighed and plated in 8 oz deli containers. The bottom of the container contained 30 ml plain, non-nutritive agar media (4.5 g agar + 500 mL distilled water) which prevented the samples from desiccating. Processed diet preparations were made using 100 g diet material, pureed with 500 mL distilled water and 4.5 g agar. This mixture was brought to a boil for 1 minute then cooled. An antifungal was added to the diet preparation (see cornmeal diet above). The processed diet (30 ml) was then poured into small deli containers and was set for at least 3 hours prior to use.

Morphotype comparisons WM and SM flies were acclimated to their respective temperatures of 0°C, 5°C, 15°C, and 25°C in growth chambers for 72 hours prior to the study and were 7-10 day old at the time of the assay. Arenas were set up using standard deli cups (16 oz) with a mesh top as to allow airflow. Inside the container was a gated trap (plastic shot glass) filled with 5 ml diet. Three trials were carried out over a period of three weeks and each trial consisted of a total of 4 to 5 temperature treatments with five replicates for each treatment for each morphotype. We visually assessed activity after 24 hours using a 4-point rating scale (0 - least active, 3 - most active). Choice (response) was measured as the number of flies entering the trap out of the total number released. Logistic regression was used to model response to the food target at each temperature among SM and WM flies. Poisson regression was used to model activity level due to positive data skew.

Literature Cited


Continued on page 35
In the Fall 2019 issue of this journal, we wrote about the soil microbiome and some of the suspected causes of apple replant disease. Here we report on a new apple orchard renovation experiment begun in 2017 at Michigan State University (MSU) to test a variety of methods for mitigating apple replant disease.

Since most of the prime orchard ground in Michigan is already occupied by orchards, and old orchards are being replaced with high density orchards on tighter row spacing, new rows are much more likely to overlap with old rows. Replant disease manifests when new trees overlap with old rows and show early decline or failure to thrive. The causes of this decline or failure are numerous and may include some combination of soil chemistry imbalance, plant parasitic nematodes, and/or microbes that cause disease. Possible remedies are also numerous and appear to depend on a number of variables including rootstocks selected at planting and underlying soil characteristics in a given region.

To test and demonstrate some of these remedies under Michigan conditions, we set up a replicated trial at the MSU Clarksville Research Center (CRC) beginning in 2017. The goal of the CRC Apple Replant Field Trial is to test a variety of pre-plant practices and their impact over time on Honeycrisp apple trees budded to either Bud 9 (what we are calling the grower standard) or G-214 (a replant-resistant rootstock developed by Cornell University) in an orchard renovation. This is a report of an on-going project expected to continue through at least the first year of full production.

Accomplishments so far

We set up the orchard renovation experiment in an established (ca. 2007) vertical axe system apple block containing 10 rows of trees on either EMLA 26 (6 rows), Bud 9 (1 row), or M9T337 (3 rows) rootstocks that were on a 15 foot row spacing (Figure 1). We collected soil samples in June of 2017 to assess the suitability of the site for this study (i.e. whether there was any evidence of plant parasitic nematodes on the site) and set up plots that were 22’ wide by 42’ long overlaid such that each set of pre-plant treatments would overlap with old rows and old grass alleys.

The team decided on seven pre-plant treatments: A) fallow for one year, which is the minimum recommendation before planting back to apples; B) planted to soybeans (1 lb./plot) for one year, which is a common rotation practice in Michigan, but that carries some risk of increasing plant parasitic nematodes; C) planted to a non-host cover crop (2 lbs./plot) for one year, in this case black oats which are not known to be a host for plant parasitic nematodes important to apples; D) planted to a biofumigant cover crop (0.6 lbs./plot), in this case oilseed radish, that when incorporated green acts as a biofumigant; E) herbicide to kill old trees before removal to try to reduce the number of live roots before removal followed by fallow; F) herbicide-treated trees before removal followed by the non-host cover crop; and G) what we are calling immediate replant, in which trees were removed in the fall just prior to planting the new orchard in the spring, another relatively common, but not generally recommended practice in the region. Each set of treatments was replicated six times.

For treatments A-D, all of the old trees were removed in the fall of 2017. For treatments E and F, the trees were treated with the herbicide Garlon® 3A (Dow AgroSciences), first applied as a series of trunk injections using a Hypo-hatchet® (Forestry Suppliers, Inc.) in June, then applied to cut stumps 2 July 2018; stumps were pulled in early August (Figure 2). All of the cover crops were sown 6 August 2018, which was when rain was predicted after weeks of dry weather (Figure 3). The oilseed radish was mowed and rototilled 19 September 2018 (Figure 4). The trees in treatment G were removed 16 October 2018.

Two composite soil samples were taken from each split plot corresponding to the old tree rows and old grass alleys 24 October 2018. One sample from each split plot was sent to the MSU Plant Diagnostic Services Lab to obtain counts and identities of the nematodes, olichochaetes (e.g. earth worms), and mycorrhizal fungi. The other sample was split into two portions. One portion was sent to the MSU Soil Testing Lab to obtain information on the physical and chemical aspects of the soil. The other portion was prepared for molecular analysis of the microbial community to identify the proportion of fungi, oomycetes, bacteria and other microbes found in each split plot. We were also looking for specific fungi and oomycetes that have been previously implicated in apple replant disease in other parts of the world.

Honeycrisp budded to either Bud 9 or Geneva’ 214 rootstocks were grown into whips using the paper-sleeve Ellepot system (https://www.ellepot.com/) by a commercial nursery in preparation for transplanting (Figure 5). All plots were planted to the new orchard during the week of 6 May 2019 (Figure 6).

This work was supported in part by the Michigan Apple Committee

In this preliminary report of a multi-year apple orchard renovation experiment, we describe the results so far from the first two years. A replicated trial was set up in 2017 to test a variety of pre-plant practices and their impact over time on Honeycrisp apple trees budded to either a resistant (Geneva 214) or susceptible (B.9) rootstock. We are also evaluating the effects of old tree row vs. old row middle on tree vigor and productivity through the first full year of production.
with posts, trellis wire, and drip irrigation installed soon after. Trees were planted on a 3-foot by 11-foot row spacing in accordance with a tall-spindle style high density orchard. Within each block, half of each new row was planted with seven trees of one rootstock followed by seven trees of the other rootstock, so that each plot contained 28 trees on each of the rootstocks in two rows that either overlapped with the old tree row or the old grass alley. Standard horticultural practices for the tall spindle orchard system have been and will continue to be used throughout the study.

Within one month after trees were transplanted into the new orchard, an initial measurement of tree diameter was taken with a digital caliper at 1 meter above ground (Figure 7). Of the 1,176 trees that were planted, less than 3% failed to survive the transplant operation and were replaced with new trees of the same rootstock. Orchard grass was sown between rows in August 2019.

More about the pre-plant treatments selected

Fallow vs. immediate replant. Leaving an old orchard block fallow for a year in between planting to a new orchard so that the soil can “rest” is the minimum best practice when it comes to orchard renovation. During the fallow period there is no income being generated, but other than tilling or mowing down weeds once or twice during the season, it is one of the least expensive pre-plant options. Establishing a new high density orchard is very expensive and there may practical economic reasons for needing to plant back immediately so that as little time is lost as possible in terms of production. What we hope to determine is whether the 1-year fallow recommendation actually yields better results than immediately replanting following the removal of an old orchard.

Considering cover crops. Of course, leaving a field fallow is not the only thing that can be done while waiting to plant a new orchard. There are a number of good reasons to plant cover crops in a perennial crop rotation. Cover crops can help reduce soil erosion and add to the percent organic matter after they are incorporated. When planted densely like we did, a cover crop can help suppress weed seed germination for weeds that we know act as alternate hosts for some of the same plant parasitic nematodes that carry and can transmit viruses that affect apple. Some cover crops, like the oilseed radish used in our study, are being bred to act as a biofumigant – the plant naturally produces chemicals that as the plant material breaks down in the soil are released with a toxic effect on soil microbes. Cover crops that are also legumes, like our soybean treatment, can help improve nitrogen availability in the soil, though as mentioned earlier, they may also host plant parasitic nematodes that attack apples. Planting a cover crop may also provide a bit of income if it can be harvested and sold for profit. In addition, if the cover crop produces flowers attractive to pollinators, and blooms in mid-summer, it can serve as an important source of pollen and nectar for bees at a time of year when there is a lack of abundant floral resources in natural habitats.

Using herbicide to kill old apple trees. In an old publication, we came across the idea to use herbicide to kill the tree down to the roots before removal. Living apple roots host a diverse microbiome, including both beneficial and detrimental micro-organisms, so the idea is that if you take away or reduce this food source, you could reduce the number of detrimental players in the system. We thought using a forestry tool to administer the herbicide might be a relatively easy way to kill the trees and to be able to clearly see the effects of the herbicide in the tops. What we discovered is that apple trees are really difficult to kill – when you want to kill them. It looked as though the herbicide was being partitioned in the scion – limbs immediately above where the injection had been made were clearly dying, but not the entire top – and
of course we could not see what was happening underground, but we were concerned that this method was not accomplishing the goal. Hence, we resorted to a method of cutting off the tops and painting the stumps with herbicide to finish the job. Given how difficult it was to kill the old trees with herbicide in the E and F treatments, we will be curious to see whether there are any positive effects on the new trees.

**Plans Going Forward**

Going into the fall of 2019, there were no obvious visual differences among the plots in terms of tree vigor, but there does appear to be a general rootstock effect with the Geneva 214 being taller on average than the B.9. Though we did have to replace some trees that failed to survive being transplanted, there was also no pattern to the tree loss in terms of treatments. Soil samples that were taken from each split plot have been processed – results from these tests will be summarized and presented during one of the Apple sessions and in poster form at the Great Lakes Fruit Vegetable and Farm Market EXPO in December 2019.

In the spring of 2020, we plan to assess the trees for winter survival and take another measure of trunk diameter to look at how well the trees grew during the first year. Another set of soil samples will be taken from the established plots to look for changes over time in the microbial community and soil chemistry. We plan to follow these trees into their first full year of production and possibly longer, noting any differences that may arise among the different treatments – in particular whether there are any cumulative effects on production. We also tracked the cost of the different pre-plant practices employed so that we can determine whether they were worth the effort in terms of their effect on fruit production. In other words, stay tuned!

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Julianna Wilson is a tree fruit integrator in the Department of Entomology, Michigan State University; Marisol Quintanilla is an Assistant Professor of Nematology in the Department of Entomology, Michigan State University; Ashley Shade is an Assistant Professor in the Department of Microbiology & Molecular Genetics, Michigan State University; Todd Einhorn is an Associate Professor in the Department of Horticulture, Michigan State University; Amy Irish-Brown is a Tree Fruit Extension educator with Michigan State University; and George Sundin is a Professor in the Department of Plant, Soil and Microbial Science, Michigan State University.

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Dara G. Stockton is a post-doctoral research associate who works with Dr. Loeb and Gregory M. Loeb is a research and extension professor of entomology who specializes in insect control of grapes and berry crops.
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