

Project Summary

The U.S. apple industry features 244,000 acres of orchards which produce 240M bushels each year with a farm gate worth of almost 4 billion dollars. Apple rootstocks are the foundation of a healthy and productive apple orchard. They are the interface between the scion and the soil, providing anchorage, water, nutrients, and disease protection that ultimately affect the productivity and sustainability of the orchard. Dwarfing and early bearing apple rootstocks provide unique advantages in fruit growing as they increase the efficiency of fruit production by making the orchard amenable to high density and automated or mechanically assisted operations. Most commercial dwarfing apple rootstocks being used by the U.S. industry are susceptible to devastating diseases (fire blight, apple replant disease, viruses), can be intolerant to other abiotic stresses (cold, drought, nutrient deficiencies, poor water quality) and may not be physiologically compatible with existing grafted scion varieties. This research project concerns breeding and evaluation of improved apple rootstocks and developing an understanding of the genetic and physiological components of apple rootstock traits. In cooperation with other USDA units, universities, and private concerns, the project aims to develop and release improved apple rootstocks and apply genomic, phenomic and bio-informatic tools for marker assisted breeding of apple rootstocks while leveraging discoveries in plant nutrition and root morphology. Research work in greenhouse, laboratory, nursery, and field plots, whether located at the PGRU or at cooperators' facilities, will be used to evaluate the characteristics of interest and examine new rootstock selections for commercial adaptation. The project utilizes cost efficient state of the art technologies to understand how rootstocks can make the orchard more productive and apply such knowledge to develop improved rootstocks. This research impacts all U.S. apple producing regions, with the potential to improve productivity, safety and survivability of apple orchards by 10% to 20% when new rootstock technologies are implemented, and increasing labor efficiency by enabling mechanization of cultural practices.

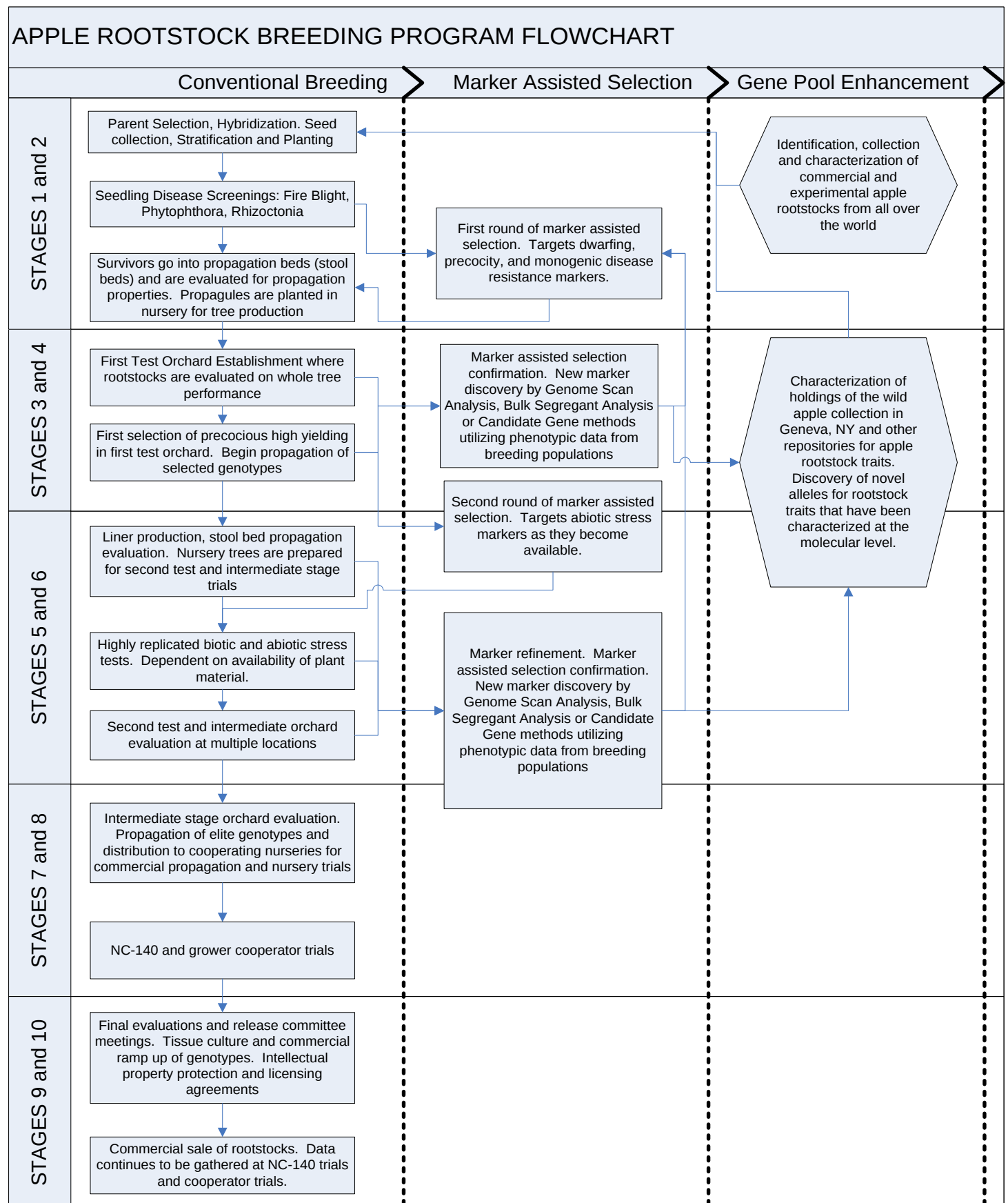


Figure 1. The breeding program has plants in all stages of breeding

Objectives

The Geneva® Apple Rootstock Breeding Program has a good track record of providing superior apple rootstock genotypes that are more productive and disease resistant than currently available rootstocks for the U.S. and world apple industry. The program will pursue this by means of best affordable technology available, including novel methods of high throughput phenotyping, genomic and bioinformatic tools.

- **Objective 1: Develop and release improved apple rootstocks by leveraging advances in marker assisted breeding, including construction of genetic maps, establishing trait associations, gene discovery for important rootstock traits (dwarfing, early bearing, yield efficient, fire blight resistant), and screening for novel alleles for important rootstock traits. [NP301, C1, PS1A, PS1B]**
 - o Sub-objective 1A *Perform all breeding and evaluation stages involved in the 15-30 year process (timeline depending on intensity of phenotyping and need to fast-track) of developing new rootstocks with the assistance of recently developed breeding tools, such as high throughput phenotyping and marker-assisted breeding.*
 - o Sub-objective 1B *Identify and characterize novel germplasm, genes, alleles and trait loci through quantitative trait analyses leveraging new genetic-physical maps.*
- **Objective 2: Identify and dissect important rootstock traits that modify gene activity in the scion, toward enhancing drought tolerance, tree architecture, propagation by nurseries, root growth and physiology, nutrient use efficiency, and disease resistance; incorporate this knowledge into breeding and selection protocols. [NP301, C3, PS3A; C1, PS1A]**
 - o Sub-objective 2A *Identify components of rootstock induced traits that modify gene expression and metabolic/physiological profiles of grafted scions to increase tolerance to abiotic stresses, improve fruit quality and storability, increase tree productivity, disease resistance and nutrient use efficiency.*
 - o Sub-objective 2B *Validate relationships between trait components and overall apple tree performance in different rootstock-scion combinations and incorporate new knowledge into breeding and selection protocols.*

Need for Research

Description of problem to be solved:

The United States has 7,500 apple producers who, collectively, grow 240 million bushels of apples on average each year on 322 thousand total acres of land. The farm-gate revenue, or wholesale value, of the U.S. apple crop annually is close to \$4 billion, with a predicted additional \$14 billion related downstream economic activity each year (U.S. Apple Statistics). Members of the U.S. apple industry and industry groups (Washington Tree Fruit Research Commission – WTFRC, New York Apple Research and Development Board - NYARDP, U.S. Apple, etc.) have prioritized national and localized research needs that address problem areas identified by stakeholders. For example research needs in the “critical” and “high priority” for 2017 by the WTFRC included “soil health” and “improved rootstock and scion genetics”, highlighting the importance of new rootstock technologies to promote sustainability, efficiency and increased productivity for their industry

(http://www.treefruitresearch.com/images/2017_apple_hort_postharvest_priority_list.pdf). Secondary to the choice of a scion variety, the choice of rootstock is perhaps the most important orchard establishment decision growers make because rootstocks affect productivity, fruit quality, orchard longevity, mechanization and many other aspects of apple production. Another aspect that was not evident until recent experiments is that there is a scion by rootstock interaction that can be leveraged to make the system more (or less) efficient i.e. the scion variety Honeycrisp produces more good quality apples with G.890 as a rootstock than G.210. Understanding the underpinnings of that interaction and designing new rootstocks that can be classified or localized to a specific environment and scion variety is very important to apple growers. The scion-rootstock-environment interaction begins at the soil-root interface where water, soil properties, nutrients and rhizospheric biota mingle with rootstock genetics to affect whole tree traits like drought tolerance, nutrient uptake efficiency, anchorage, and replant disease disorder etc. The

interaction continues as the rootstock transcriptome, metabolome, phytohormone apparatus sends and receive signals from the scion – with the graft union as the interface between the two genotypes. More research is needed to understand those interactions and produce improved rootstocks that increase the profitability of the apple industry.

Relevance to ARS National Program Action Plan:

Apple growers require improved, economically and environmentally sustainable production systems to compete in the international fruit market. They are doing this by establishing high-density orchards of high-value cultivars. The apple rootstock determines many key aspects of tree performance, including tree size, productivity, fruit quality, nutrient uptake efficiency, pest resistance, stress tolerance, and ultimately profitability. New, improved rootstocks that incorporate improvements in biotic and abiotic stress tolerance/resistance traits are essential to grower profitability because in modern orchards, rootstocks are subjected to numerous biotic and abiotic stresses – rhizospheric pathogens, temperature, water availability, soil pH, and fertility. These stresses end up affecting not only tree productivity, but also the quality of fruit being harvested. The returns from high-density plantings far exceed those of low-density plantings. However, the initial investment may cost 10 times more for high-density plantings than low-density plantings, thus greatly enhancing economic risk. A key component of high-density apple production is the rootstock. The rootstock can induce early cropping, thus allowing close plant spacings. It is critical to develop more rapid means of screening potential rootstock candidates for susceptibility to stresses, to understand how different rootstocks respond to biotic and abiotic stresses, and to develop recommendations for the use of particular rootstocks under changing orchard conditions and production practices. Understanding factors contributing to apple root physiology – stress tolerance, nutrition, and growth related gene networks is vital. Knowledge of the physiological mechanisms that underlie these responses will allow for the development of genetic maps, molecular markers for target traits, new marker assisted breeding strategies, cultural practices, and ultimately practical means for mitigating various stresses for industry.

The proposed research is relevant to the NP 301 Action Plan, Component 1 – Crop Genetic Improvement; Problem Statement 1A: Trait discovery, analysis, and superior breeding methods; Problem Statement 1B: New crops, new varieties, and enhanced germplasm with superior traits; and to Component 3 – Crop Biological and Molecular Processes; Problem Statement 3A: Fundamental knowledge of plant biological and molecular processes.

Potential benefits expected from attaining objectives –

NP 301 Action Plan Anticipated Products to which the project will contribute -

- 1. Higher yielding plants.
- 2. Plants with resistance or tolerance to diseases and pests.
- 3. Plants tolerant to environmental changes or extremes.
- 4. Plants optimized for production efficiency.

Specific project products and/or outcomes:

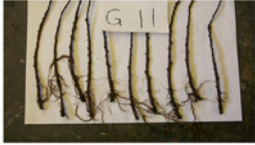
- New apple rootstock varieties with superior performance with regards to dwarfing, productivity and disease resistance.
- New understanding about genetic effects of apple rootstock on several whole tree health traits including tolerance to replant disease, nutrient absorption and translocation.
- New understanding about the application of tree architecture modifying rootstocks to make the orchard/nursery more amenable to mechanized operations.
- Incorporation of new alleles/traits in the apple rootstock breeding pool. Identification and characterization of new gene pools for apple rootstock traits.

- Genetic maps, robust, haplotype-specific molecular markers linked to important traits, and new marker assisted breeding protocols.
- Generation of mass-gene expression profiles of rootstocks in breeding populations and integration of expression profiles with marker assisted breeding.

Customers of the research and their involvement

Our customers are all apple growers, especially those in the regions affected by fire blight (Northeast, Midwest, Northwest U.S.) and growers who plant in soil that is symptomatic for Apple Replant Disease. These customers include small, medium and large conventional and organic fruit growing companies – growers like Jennifer Crist and Jim Bittner in New York and Mike Wittenbach in Michigan who have planted field trials of Geneva® rootstocks because of their need to find better stocks that are resistant to fire blight and that will perform well under organic management (Singer Farms -Bittner); growers near the tri-city area in Washington where they witnessed increased incidence of fire blight in the last five years; and growers in the Yakima (WA) who will need to replant a quarter of older orchards in the next 10 years while virgin land, optimal for apple orchards, is becoming rare in the same area, leaving no alternative to replanting on previous orchard sites needing fumigation with harmful chemicals. Another customer group is made up of organic apple growers and growers participating in integrated pest management like Stemilt Growers Inc., the largest multiple apple variety shipper in the US and the largest organic apple grower in the state of Washington. Two other important customer groups are apple growers planting high density orchard systems and nurseries supplying North America that specialize in the production of apple rootstock liners and finished apple trees, like Richard and Brett Adams of Willow Drive Nurseries (Ephrata, WA), who are propagating test rootstocks for research trials in the US, Devin Cooper, owner of Willamette Nurseries (Canby, OR), Brett Smith of Treco Nursery (Woodburn, OR), Cliff Beumel of Sierra Gold Nurseries (Yuba City, CA), and Todd Cameron of Cameron Nurseries (Quincy, WA) who are among the several nursery operators that are propagating Geneva® rootstocks and selling liners or finished trees to growers everywhere in the US. Additional customers include international apple nurseries and growers that have found value in adopting superior apple rootstock varieties produced by this program.

Commercial apple trees are a combination of two different genetic types: the **rootstock** (root system) and the **scion** (aerial system) which bears fruit.



1. The rootstock mother plants are layered with saw-dust in a stoolbed to generate rooted rootstock shoots

2. The rooted rootstock shoots are harvested from the mother plant and planted in a nursery

3. A bud from a scion variety like Gala or Granny Smith is grafted on the rootstock apple tree.

4. The scion bud grows into a shoot and then into a mature apple tree. The rootstock will influence the productivity, size and precocity of the apple tree.

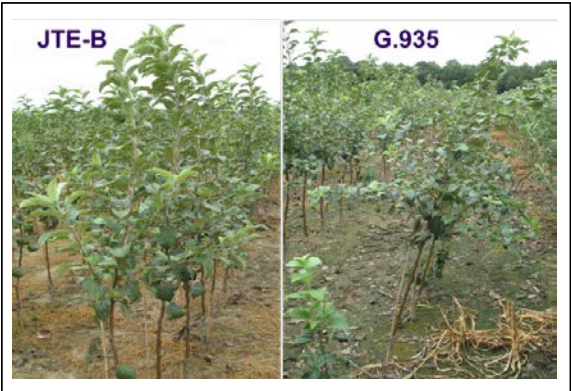


Figure 3. Comparison of nursery tree architecture featuring a flat branching rootstock, G.935 (right), versus an upright branching rootstock (JTE-B).

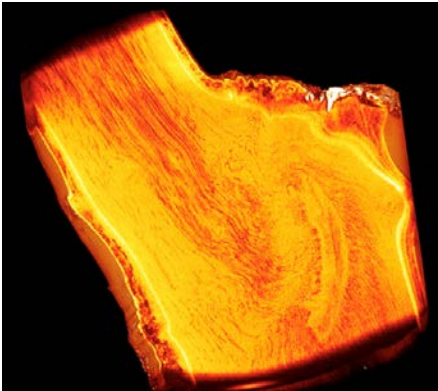


Figure 4. Micro CT scan of a graft union of Honeycrisp on G.41 showing the region where the bud was placed and the unorganized formation of some vessels that may contribute to graft weakness.

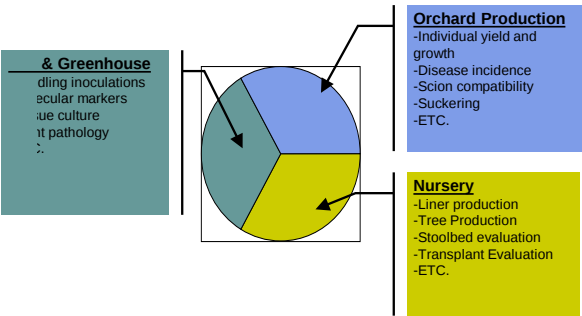


Figure 5. Activities of the apple rootstock breeding program. Laboratory, orchard and nursery components are essential for the success of the program.

Table 1. Selection traits for breeding apple rootstocks. These traits are sometimes evaluated at different locations by the many collaborators of the apple rootstock program.

	YEARS	
Fire Blight resistance	1 or 7	Greenhouse/Field
Phytophthora resistance	1	Greenhouse
Replant Disease Complex	1 or 7	Greenhouse/field
Wholly apple aphid res.	1-5	Greenhouse/field
Juvenility nursery - Spines	3-4	Field/Stoolbed
Stoolbed rooting	3-4	Field/Stoolbed
Growth habit - Brittleness	3-4	Field/Stoolbed
Dwarfing	8-12	Orchard
Precocity	8	Orchard
Suckering	8	Orchard
Yield – Biennial bearing	12	Orchard
Cold hardiness	15	Orchard
Drought tolerance	4	Orchard
Graft union compatibility	5	Orchard

Scientific Background

History and foundation germplasm - The foundations of a productive and healthy orchard are the rootstocks that provide anchorage, water and nutrients essential to the above- ground portions of the trees. The utilization of composite trees has increased the efficiency of breeding productive apple trees by dividing the selection of scion traits and rootstock traits into two genetically (and functionally) different specimens, which are then brought together through grafting. The art and science of grafting scions onto rootstocks spans several millennia; it is thought that it was used initially to aid in the clonal propagation of desirable scion varieties for fruit and nut production (Janick, 2005). In these millennia, it is likely that very little attention was dedicated to the selection of a particular rootstock chosen for its properties (ease of propagation) and the properties it imparted to the scion (Tukey, 1964; Rom and Carlson, 1987; Webster, 2003; Webster and Wertheim, 2003). Clonal selection and the beginning of the science of rootstocks seems to have originated in the latter half of the last millennia, where at least for apple, certain rootstock clonal selections were identified to impart unique productivity and architectural properties (early bearing and dwarfing) onto the grafted scion variety (Monceau, 1768). It is very likely that these properties existed or were selected directly on own-rooted trees first as these trees were early bearing, inherently dwarfed and production of fruit from these curious apple plants was early and abundant compared to seedling trees (Loudon, 1822). The combination of small architecture and productivity is optimal for cultivation in fruiting gardens typical of monasteries, aristocratic and wealthy middle-class dwellings (Rivers, 1866), where the ‘Paradise’ apple, ‘Jaune de Metz’ (Lindley, 1828) otherwise known as Malling 9 (and relatives) could make grafted scions dwarfed, becoming popular in central Europe for making composite dwarfed trees (Hatton, 1917). Scientists at the East Malling Research Station in the United Kingdom, collected many clonal rootstocks from around Europe and painstakingly characterized each of them eliminated duplicates and established foundation material of rootstocks named ‘Malling 1-16’ (Hatton, 1919; 1920). Rootstock “Malling 9” (M.9) and its sport mutations became the primary rootstock that fueled the green revolution of dwarfed apple orchards that occurred in the twentieth century in many apple production regions of the world. Narrow crosses among the Malling rootstocks resulted in two widely used rootstocks: Malling 26 (M.26) and Malling 27 (M.27) that have improved propagability, and different forms of the early bearing and dwarfing effects. Most of the dwarfing founding germplasm was interrelated and had a narrow genetic base (Oraguzie et al., 2005; Gharghani et al., 2009), suggesting the need to introduce new forms of disease resistance and improvement on other horticultural characteristics through wide crosses with germplasm that exhibited the desired phenotypes (Aldwinckle et al., 1999; Momol et al., 1999; Forsline et al., 2002). The results of these wide crosses have produced a series of rootstocks that combine disease and insect resistance with productivity, and represent the second generation of rootstock technologies applied worldwide (Fischer, 1991; Wertheim, 1998; Fischer et al., 2000). The apple rootstock breeding program operated in Geneva, NY is the only one that has commercial deployment of results from these wide crosses. This breeding program has operated in Geneva, NY since 1968 by Cornell University Geneva Campus and joint with the U.S. Department of Agriculture Agricultural Research Service since 1998 (Johnson et al. 2001; Fazio et al. 2015b). This program produced the Geneva® series of apple rootstocks by crossing germplasm that complemented the weaknesses of the Malling germplasm (susceptibility to fire blight, woolly apple aphids, crown rots) and systematically crossed such germplasm with all available dwarfing, precocious rootstock germplasm available to the program (Gardner et al. 1980a, b). The parent Robusta 5 became the source of resistance to fire blight and woolly apple aphids (Aldwinckle et al. 1976; Aldwinckle and Lamb 1978; Cummins et al. 1983). Several novel traits have been identified in the Geneva® germplasm including induction of flat branching (or open tree architecture), increased nutrient concentration, and induction of bud-break in low chilling environments (Fazio and Robinson 2008; Fazio et al. 2012, 2013; Jensen et al. 2012). The Geneva® breeding program continues to make new crosses to improve tolerance to drought and other biotic and abiotic stresses that can be ameliorated in apple rootstocks. (Fazio et al. 2015b; Shin et al. 2016; Tworowski and Fazio 2016; Tworowski et al. 2016). As our understanding of physiology of apple trees, both at the whole tree level and at the cellular level, has increased, so has the understanding of how and what scion properties are modulated by rootstocks, thus increasing the target traits that may be

selected to improve whole tree performance by improving rootstock performance (Fazio and Mazzola, 2004). Improving rootstock performance involves two sets of very different types of traits: the inherent apple rootstock traits (rooting for propagation, lack of spines and burr knots, resistance to root pathogens, cold hardiness, etc.) which deal with the interaction between rootstocks and the environment, and scion traits that are modulated by rootstocks (tree architecture, productivity, etc.) that represent the interaction between rootstocks and scions.

Breeding methods -Breeding apple rootstocks can be a very lengthy process (Johnson et al., 2001a); there are two ways to accelerate the process: the application of marker- assisted breeding (MAB) in the pipeline and/or the intensification of later stages of field testing (Fazio et al., 2015b). The first aims to eliminate substandard germplasm (non-precocious, non-dwarfing, susceptible to diseases, etc.) from the parental and progeny pools via the development and application of robust diagnostic markers. The second is to increase the number of clonal plants tested for each elite genotype and subject them to multiple phenotyping tests and environments that represent production regions. The theoretical benefits from the application of marker technologies to breeding have been reported in publications (Bus et al., 2000; Fazio et al., 2003; Antanaviciute et al., 2012; Bassett et al., 2015). In 2011 the USDA- ARS apple rootstock breeding program located conducted an internal analysis of the economic impact of applying molecular markers in the breeding program by itemizing the cost per genotype for each stage of selection. A detailed description of the rootstock breeding program stages can be found in Fazio et al. (2015b). Briefly: stages 1 and 2 deal with parent selection, crossing, culling seedlings with disease inoculations and propagation of survivors; stages 3 and 4 deal with the establishment of plants as rootstocks in field orchards and propagation beds (see figure Fig. 1); stage 5 includes the evaluation of propagation beds and replicated tests on biotic and abiotic resistance. Stages 6—7 are secondary highly replicated tests and stages 8—10 deal with pre-commercial testing with multiple varieties and multiple locations. The Geneva breeding program elected to conduct the first round of MAB before stage 3, which involves the initial propagation of plants surviving *Phytophthora* root rot and fire blight screens. The cost of genotyping with two markers including DNA extraction and labor was about \$10 per seedling. The cost to phenotype each seedling for dwarfing and precocity during stage 3 (9 years of evaluation) in 2010 dollars was \$15.40/year for 9 years = \$138. The cost savings by culling non-dwarfing individuals was significant, and in 2012 we were able to plant 2 orchard rows of well- replicated, high- density first- test orchard instead of the 12 previously planted.

Scion traits affected by rootstocks - Until recently, the number of traits that were recognized to be modulated by apple rootstocks was pretty small: tree vigor, early bearing, and water use. This list has been expanded to new architecture components such as canopy shape and bud break (syllaptic branching), and effects on fruit size and quality, on disease resistance and on nutrient availability in the scion. Perhaps the biggest breakthrough in our understanding of rootstock effects on scions is the study that monitored gene expression changes in scion tissue by different apple rootstocks (Jensen et al., 2003; Jensen et al., 2010; Jensen et al., 2011; Jensen et al., 2012). At the cellular level, signals sent from the root system of different genotypes to the scion can change the expression levels of genes, which in turn change the composition of proteins and related metabolic processes and compounds in the scion. While there are no experiments in apple that have described the opposite interaction, it is safe to assume that this dramatic change likely occurs as signaling from the scion affects the way roots behave and grow. The science behind understanding the issue of communication and affinity between scion and rootstock seems to be in its infancy and has a lot of potential as the concept of “designer rootstocks” gets more traction in the industry.

Tree vigor -The reduction in tree vigor (Figure 2) is perhaps the most important trait imparted by apple rootstocks to the grafted scion (Tukey, 1964). It is imparted to the scion as an early termination of overall season growth (Seleznyova et al., 2008). The benefits due to this trait in modern orchards are enormous and range from increased efficiency in picking and tree management operations, including mechanization, to the decrease of pesticide inputs, ladder accidents, and other ergonomic issues (Groot, 1997a; Groot, 1997b; Masseron and Roche, 1999; Robinson et al., 2007; James and Middleton, 2011). At the physiological level, the dwarfing trait has increased the effective light interception and partition to fruit

production in the orchard and increased the production per unit area by at least 30% when compared to non-dwarfing rootstocks (Brown et al., 1985; Strong and Miller Azarenko, 1991; Atkinson et al., 1998). This means that for an industry worth \$4 billion like the U.S. industry at least \$ 900 million are a result of the efficiency gained through dwarfing rootstocks. While the genetic components to this trait have been described to be the interaction of two main loci (Fazio et al., 2014b) and perhaps additional modifying loci (Harrison et al., 2016, Foster et al. 2015) it is important to mention that this is a complex trait that has fairly big interaction components and that the total effect of these components results in the overall vigor of the tree. Therefore for any scion 'S', the vigor 'V' is equal to the inherent growth dynamic genetics of the scion 'Sg', plus the dwarfing genetic components of the rootstock 'Rg', plus their interaction, plus the interaction of the whole composite tree with environmental effects 'E' such as fertility, water availability, diseases, soil type, soil pH, and soil type, or orchard management, weed competition, and the like, so that when scion vigor is measured, the genetic components of dwarfing rootstocks are only a part of the equation. This is exemplified by observing the effect of stunting caused by soil-borne replant disease, which has a similar effect to the dwarfing loci in apple and sometimes confuses the estimation of vigor potential of a rootstock (Auvil et al. 2011).

While several architectural dwarfs have been identified in domesticated and wild apple populations (Fazio et al., 2009a; Fazio et al., 2014a), this material has not produced commercially viable rootstocks or has not been tested for similarity to the dwarfing characters offered by the alleles contained in M.9, M.8, M.13 and other Malling rootstocks belonging to the initial set selected in East Malling. The dwarfing trait has been shown to be highly heritable, modulated mainly by the combination of alleles of locus Dw1 found on chromosome 5 (Rusholme et al., 2004; Pilcher et al., 2008) and locus Dw2 found on chromosome 11 (Fazio et al., 2014b). Models that take into account some or all combinatorial allelic effects of these two loci have been able to explain upwards of 80% of the genotypic variation for dwarfing (Foster et al., 2015). The two loci interact with each other and do not necessarily seem to be additive, meaning that the lack of one dwarfing locus effect in the model negates the effect of the other. Several physiological models based on phenotypic observation have hypothesized the involvement of hormone signaling (Zhang et al., 2015; Tworowski and Fazio, 2016), graft union anatomy (Tworowski and Miller, 2007; Tworowski and Fazio, 2011), hydraulic conductivity (Atkinson et al., 2003; Cohen et al., 2007), dry matter partitioning to fruit production, or a combination of these (van Hooijdonk et al., 2011), while the underlying causative genes are still largely unknown. Evaluation of this trait for breeding still requires a lengthy period of 7–10 years for the first observation and perhaps another 10–12 years for multi-location trials with multiple scions. As mentioned in the beginning of this section, multiple field trials are needed to evaluate rootstock interactions with different scions and environmental factors.

Induction of early bearing in scions - Apple seedlings planted on their own roots experience a juvenile period anywhere from 4–7 years before they reach sexual maturity and bloom and fruit (Visser, 1967). Some dwarfing rootstocks have the ability to induce early bearing or reduce the juvenile period to 2 years in extreme cases (Visser and Schaap, 1967; Visser, 1973). Early bearing is a major selection criterion for improved apple rootstocks because the intensive types of cultivation of apple require a quick return on investment (early production of apples) to offset the installation and infrastructure costs to build the orchard (Cummins et al., 1995; Robinson et al., 2007; Robinson et al., 2011). The genetic loci underlying the rootstock-induced trait 'early bearing' were first described by Fazio et al. (2014), who identified two loci, Eb1 and Eb2, that roughly co-located with Dw1 and Dw2, perhaps indicating that the two traits may be physiologically and genetically interconnected. Several studies have described the rootstock-induced partitioning effect of photosynthate into sexual (fruit) and vegetative portions of the tree (Seleznova et al., 2008), comparing the effects of different rootstocks (Marini et al., 2006a; Autio et al., 2011b; Autio et al., 2011c), crop loads on tree growth (Marini et al., 2012), productivity, and bienniality (Marini et al., 2013). However, there is paucity in the literature about the causative elements for these rootstock effects. Breeding for this trait requires field evaluation for four years for the first observation in a replicated experimental orchard and then an additional 5 years in multi-location, multi-scion trials. Visser (1967) showed that scions with reduced juvenility also seemed to be more productive when grafted on M.9

dwarfing rootstocks, indicating the possibility of an inherent scion effect on early bearing and the need to test this scion—rootstock interaction in replicated trials.

Induction of sylleptic branching in the nursery and other architectural changes - Early and abundant fruit production is related to the number of flowering buds produced in the nursery phase and early establishment of the tree in the orchard (Ferree and Rhodus, 1987; Robinson et al., 1991a; Robinson et al., 1991b; Theron et al., 2000). This number can be influenced not only by the early bearing effects of the rootstocks discussed previously, but also by the ability of the rootstocks to produce prolific sylleptic branching (feathers on a nursery tree) in the nursery and later in the orchard. Early yield has been associated with nursery tree caliper, tree height, and number of feathers. Rootstocks with wider genetic diversity than M.9 and Budagovsky 9 (B.9) have been shown to influence the production of sylleptic branches and the formation of crotch angles, that produce trees with a more open (flatter branches) structure (Fazio and Robinson, 2008a; Fazio and Robinson, 2008b). This characteristic is mostly observed in rootstocks developed by the Geneva, New York, breeding program, especially with rootstocks G.935, G.213 G.41 and G.214 (Figure 3). The strength of these effects vary with different scions and continue through the life of the tree in the orchard, as observed in the millions of trees planted on G.935 rootstock throughout the world. One additional characteristic that may be related to sylleptic branching is the ability of some rootstocks in the Geneva breeding program to induce bud break and flowering in low chill environments. This effect was observed in a highly replicated trial in Southern Brazil with ‘Gala’ grafted on three rootstocks (G.213, M.9 and, Marubakaido with M.9 interstem (an interstem is a section of the trunk grafted in between the rootstock and the scion usually made from a third rootstock variety to bridge incompatibility or leverage the qualities of the interstem to increase precocity of the whole tree) where it was observed that in Spring time flowering and bud break were 35% higher in trees with G.213 rootstocks, resulting in higher productivity of the trees (Francescato, pers. comm.). Breeding for these characteristics requires a lot of time and effort as the effects are confounded by the interaction with the dwarfing potential of the rootstocks and the difficulty of measuring crotch angle and branch length of thousands of replicated nursery trees. Our understanding of the genetic effects underlying these traits is in its infancy as the trait was first described in 2007. It is likely that research and breeding efforts aimed at uncovering the genetic factors for these traits will lead to more productive apple trees.

Propagation traits - Apple rootstocks can be clonally propagated by sterile in vitro methods, soft and hard wood cuttings (Bassuk and Howard, 1980), and by layer or stool cuttings (Adams, 2010). While efforts to breed rootstocks amenable to in vitro culture are virtually impossible due to the complexity of media and growing conditions, efforts to improve rooting ability in layering beds and cuttings although difficult may result in superior rooting genotypes. Breeding for nursery performance can be quite complicated as many factors influence apple rootstock performance in the different nursery phases and at times may conflict with field performance. A prime example of this is the fast and easy adventitious rooting trait, highly desired in the propagation phase but correlated with the development of burr knots in the orchard – a harmful trait in certain orchard environments especially where dogwood borers and other insect borers may be present (Bergh and Leskey, 2003). These difficulties can be overcome with improved nursery management practices developed for the establishment of new layer beds, that which include utilization of different propagation techniques like cuttings (Hansen, 1989; Deering, 1991) or micropropagation (Castillo et al., 2015; Geng et al., 2015), and the treatment with plant growth regulators such as prohexadione calcium in the nursery (Adams, 2010) to increase production of primary adventitious roots. The genetics of adventitious root formation have been investigated in the Geneva breeding program revealing a complex trait with low heritability. Therefore, while it may be possible to breed for rooting traits, the importance of these traits is dwarfed by the importance of low suckering and lack of burr knots in the orchard.

Another characteristic affected by the rootstock is graft compatibility. Historically, most problems that were blamed on compatibility turned out to be virus related (Cummins and Aldwinckle, 1983; Lana et al., 1983), however, certain rootstock/scion combinations under unspecified grafting and nursery management conditions have shown a tendency for weak graft unions in very young trees (Robinson et

al., 2003). Graft incompatibility can arise because of the disruption of normal healing between grafted tissues and can result in anatomical and physiological symptoms, biochemical and mechanical issues that lead to graft failure or tree death (Simons and Chu, 1983, 1985; Skene et al., 1983; Simons, 1985; Simons and Chu, 1985). While it is likely that the method of grafting (chip budding, whip -and- tongue grafting, and machine V grafting) (Hartmann et al., 1997) has an effect on healing and union strength at various stages in the nursery cycle, there may be plant- growth- regulator-related and metabolic- compound - related signals that prevent the formation of a strong graft union. Efforts to understand the ability of the rootstock/scion combination to generate enough connective tissue where they meet is underway in the Geneva breeding program through the use of X-Ray tomography (CAT Scans) shown in figure Fig. 4. Some nurseries report that large caliper stocks may not form as strong a graft union as small caliper stocks; therefore, a rootstock genotype that produces smaller caliper liners from the stool bed may be more suitable for nursery tree production.

Drought tolerance - It is difficult to define drought tolerance without an objective reference or phenotype to measure and it is even more difficult to define in a rootstock independent of the scion- specific tolerance (Higgs and Jones, 1991; Virlet et al., 2015). The economic definition of drought tolerance (little or no loss of marketable fruit production) is different from some of the physiological definitions, which range from loss of photosynthetic activity (Massacci and Jones, 1990), to shoot and root growth under stress (Atkinson et al., 2000), to water use efficiency. Perhaps drought- tolerant rootstocks are of little value where irrigation water is available and relevant only in regions that utilize rainwater and may experience long stretches of drought. However, as climate changes and fresh water availability is threatened in traditional apple growing regions, the search for rootstocks that can thrive with less water is becoming more and more important (Ebel et al., 2001). It has long been recognized that there are differences in apple rootstock reaction to drought (Preston et al., 1972; Cummins and Aldwinckle, 1974; Ferree and Schmid, 1990), but those observations mostly dealt with spurious drought events and compared vigorous and non-vigorous types (Chandel and Chauhan, 1993; Fernandez et al., 1994). Decreased sensitivity to drought was attributed to ‘Malling 9’ rootstock when compared to ‘Mark’ (Fernandez et al., 1997) in a potted tree study. A comparison of hormonal drought response between M.9 and MM.111 rootstocks indicated that both rootstocks provided drought resistance but by mechanisms which appear to differ — M.9 produces higher levels of abscisic acid (ABA) that may regulate stomatal opening while MM.111 possesses a more extensive root system (increased soil exploration index) (Tworkoski et al., 2016). Water use efficiency, defined as the ratio of biomass produced to the rate of transpiration, and decreased sensitivity to drought (Xiang et al., 1995; Bassett et al., 2011) has been described in wild apple populations indicating the possibility of using this descriptor as a selection method. Breeding for such a complex trait may be possible only at latter stages of selection as discernment of field-meaningful data requires experiments with high replication, special equipment to control water delivery and use, and very- high- density morphological and physiological measurements. Perhaps gain can be made by selection of components of the trait such as improved root morphology, plant growth regulator signals, and nutrient uptake once their effect is identified in breeding populations possessing all the other ‘important’ traits.

Cold tolerance - Several rootstocks seem to be tolerant to the different types of cold events that can cause injury of cambial and root tissues (Embree, 1988). Damaging cold events can be quite different in their mode of action as mid-winter, events can have very different modes of action than late fall or spring cold events (Cline et al., 2012). Therefore, the methods used to evaluate sensitivity to differing cold injuring events need to address the physiological conditions specific to each event (Quamme et al., 1997; Moran et al., 2011a; Moran et al., 2011b). Fluctuating temperatures in late fall, early winter and early spring are associated with hardening and de-hardening of tissues. This hardening and de-hardening process may have a strong genetic component (Forsline and Cummins, 1978), where a group of *Malus* rootstocks seem to have improved ability to be insensitive to such temperature fluctuations and remain dormant and cold-acclimated. Harvesting rootstock liners during these periods and subjecting them to increasingly low temperatures to show cambial damage is perhaps the most meaningful way to select cold hardy apple rootstocks. Observation of black-heart damage can also aid in the discernment of rootstock/scion

combinations that are susceptible to mid-winter injury (Warmund and Slater, 1988; Warmund et al., 1996). Genes associated with cold response have been described for ‘Gala’ scions (Wisniewski et al., 2008), and similar genes may be found in apple rootstocks. However, the understanding of segregating factors that influence the different types of cold stress adaptation is virtually non-existent, making genetic or genomic-informed breeding impossible, and therefore selection relies entirely on highly replicated phenotyping.

Root morphology and architecture - Phenotypic variation in the morphology of roots has been associated with increases in yield and tolerance to abiotic stresses in several crops (Sousa et al., 2012; Chimungu et al., 2014; Lynch et al., 2014; Burton et al., 2015; Zhan et al., 2015). Harnessing genetic and phenotypic variation in root morphology traits in apple rootstocks may improve productivity, tree size, drought tolerance, nutrient uptake, anchorage and other related whole tree functions (Eissenstat et al., 2001). Ample phenotypic variation has been characterized in wild *Malus sieversii* populations and within the Geneva apple rootstock breeding program where genetic factors for fine root formation (highly branched fine roots) have been mapped to chromosome 4 and 11 of the apple genome (Fazio et al., 2009b). Other traits that may be important to characterize may be the volume explored by the roots, the angle of the roots, the longevity of the roots and so on, etc., which are all traits that are difficult to phenotype and for which robust genetic markers may be extremely useful. In Geneva, New York, the apple rootstock breeding program measured several scion and root morphology characteristics of nursery trees of related (half-sibs) *Malus sieversii* seedlings which showed correlation between canopy volume/tree size and number of thick roots (0.38, $P < 0.001$), and a less pronounced correlation between tree size and root mass (0.25, $P < 0.001$), indicating a feedback loop between scion and root growth: the ability of the canopy to support the growth and expansion of a larger primary root system increased the vigor of young trees by their ability to produce root systems with strong primary hierarchy (Fazio et al., 2014a). Apple root systems vary in seasonal growth patterns (Eissenstat et al., 2006), which may affect their ability to forage for nutrients and water, and even colonization with beneficial mycorrhizae (Resendes et al., 2008). Root turnover rates may also play a significant role in tree nutrition and productivity as well as disease resistance or evasion as demonstrated by experiments that utilized replant-tolerant rootstocks from the Geneva breeding program (Atucha et al., 2013; Emmett et al., 2014). While these root traits can be targeted for marker assisted breeding (MAB), the understanding of genes, gene expression patterns and physiological attributes associated with these traits in rootstocks is limited compared to our understating of scion traits; therefore, more research is needed to understand these traits before they become the subject of selection based on genetic markers. The program is currently leveraging aeroponic systems (Appendix B figures 1-3) and mini-rhizotrons to phenotype apple root systems.

Nutrient uptake - Another set of root-related traits deals with the genetic variation and inheritance of absorption and translocation of macro- and micronutrients by the rootstock to the scion (Tukey et al., 1962). Rootstocks have been shown to vary significantly with regards to their intrinsic ability to forage for nutrients as well as transfer them up to various sinks in the scion, including fruit, perhaps affecting organoleptic, post-harvest qualities of the fruit and disease resistance (Lockard, 1976; Westwood and Bjornstad, 1980; Om and Pathak, 1983; West and Young, 1988; Chandel and Chauhan, 1990; Rom et al., 1991; Sloan et al., 1996; Chun and Chun, 2004; Kim et al., 2004). Transgenic, cisgenic, or conventional breeding approaches have been suggested to increase nutrient uptake of minerals such as zinc to improve productivity of the orchard (Swietlik et al., 2007). Most research on nutrient uptake by apple rootstocks has focused on developing the best management practices for nutrient application on a genetically restricted set of rootstocks, and it was not until a large set of genetically diverse rootstocks were observed in different soils and pH treatments that the physiological diversity of apple roots was revealed (Fazio et al., 2012). The analysis of scion nutrient concentration in leaves and fruit in several rootstock field trials in New York State have indicated the possibility that specific rootstocks may affect fruit quality of Honeycrisp apples showing that certain rootstocks are able to transfer higher calcium levels to the fruit and that the calcium-linked disorders typical of Honeycrisp are a result of scion-specific intrinsic challenges in the movement of calcium into the fruit (Fazio et al., 2015a). Investigation of the inheritance of nutrient uptake and translocation in a full-sib population of apple rootstocks revealed

quantitative trait loci (QTL) influencing scion leaf mineral concentrations of potassium (K), sodium (Na), phosphorus (P), calcium (Ca), zinc (Zn), magnesium (Mg) and molybdenum (Mo), with the most significant ones on chromosome 5 for potassium, chromosome 17 for sodium and lower significance QTLs for calcium, copper, zinc, and phosphorous (Fazio et al., 2013). Concentrations of some nutrients were highly correlated (K and P, S and P), indicating common nodes in the networked pathway that takes nutrients from the soil through the rootstocks to diverse sinks in the scion (Neilsen and Havipson, 2014). The very different mechanisms (interaction with soil biota, active and passive transport, vessel composition and size, etc.) that impact absorption and transport and the fact that crop load and irrigation can also influence mineral concentrations (Neilsen et al., 2015) makes these traits difficult to improve without the aid of a robust understanding of molecular genetic factors involved. Modeling those factors to achieve a particular balance of nutrients in selected scions is therefore very complicated.

Disease and pest resistance - Commercial application of improved disease and insect resistance can be observed in the Geneva, New York, breeding program. Since its inception, the program focused on developing apple rootstocks resistant to fire blight, a North American disease caused by *Erwinia amylovora* while maintaining the resistance to crown and root rot caused by *Phytophthora cactorum* (Aldwinckle et al., 1972; Gardner, 1977; Gardner et al., 1980). This effort over three decades produced rootstocks that are not only resistant to fire blight and crown rot, but that are tolerant to the replant disease complex, and are also resistant to woolly apple aphids WAA (*Eriosoma lanigerum*).

Resistance to fire blight- Fire blight is a devastating disease caused by the anaerobic, gram-negative bacterium *Erwinia amylovora*, which causes visible symptoms in blossoms, green tissues, fruit and some woody tissues of apple scions and rootstocks. While this disease seems to have originated in the Eastern part of North America, it has now spread to most of the apple growing regions of the world. Rootstock blight on susceptible rootstocks (M.9, M.27 and M.26) can be devastating as the infection results in girdling and death of the rootstock shank eventually killing the whole tree – entire orchards and millions of trees have been destroyed because of rootstock blight. While spraying antibiotics like Streptomycin can alleviate the onset of rootstock blight, genetic resistance of the rootstock is the best preventive treatment. Rootstock resistance to *E. amylovora* is found in several wild apple species and these have been utilized to breed a new series of fire blight -resistant rootstocks. There seem to be two main types of resistance in apple rootstock: a multi-genic type similar to that is found in *Malus robusta* ‘Robusta 5’ where green tissues and flowers are not affected by the bacterium (Aldwinckle et al., 1974b; Cummins and Aldwinckle, 1974) and an ontogenic type of resistance found in Budagovsky 9 (B.9) rootstock where the green tissues are severely affected, but two- year- old and older wood seems not to react to the bacteria (Russo et al., 2008). Genetic inheritance of the ‘Robusta 5’ type of resistance has been described as having a strain- specific component on chromosome 3 identified as a gene belonging to the NBS-LRR class of resistance genes (Fahrentrapp et al., 2013; Broggini et al., 2014; Kost et al., 2015) and other minor QTLs on linkage groups 5, 7, 11, and 14, which do not seem to be strain- specific detected in a non-rootstock population (‘Idared’ x ‘Robusta 5’) (Wohner et al., 2014). Another locus that is non -strain specific was discovered on linkage group 7 in a rootstock population derived from a cross between ‘Ottawa 3’ and ‘Robusta 5’ (Gardiner et al., 2012). Cis-genic approaches with the LG03 gene proved only partially successful, suggesting a more complex pathway of resistance than just one gene recognition of the pathogen (Kost et al., 2015).

Replant disease complex - The specific apple replant disease complex is a syndrome observed as stunting and poor growth of young apple trees planted in soil that was previously planted with an apple or pear orchard. This complex disease causes major production losses throughout the life of the orchard. The main causative agents implicated in this syndrome are *Cylindrocarpon destructans*, *Phytophthora cactorum*, *Pythium spp.*, *Rhizoctonia solani* and various pathogenic nematodes (Mazzola, 1998). The occurrence of one or more of these agents will affect the severity of the syndrome and may explain some of the site -to- site variation observed in replant land. This is one of the major problems faced by orchardists as virgin land becomes more rare, major infrastructure investments (hail nets, irrigation, etc.) become more prevalent and require a ‘replant -in- place’ type of renewal of the orchard and as fumigation

chemistries are restricted by environmental laws (Auvil et al., 2011). The removal of the old orchard leaves a major pathogen load in the soil, which overwhelms the young root system of nursery trees. Fumigation treatments (Methyl Bromide, Chloropicrin, and Nematicides) seem to be effective for less than a year as the pathogens implicated in this disease quickly recolonize the sterile soil, and fallow treatments (undesirable because they leave the land in an unproductive state) have shown mixed results, with replant symptoms sometimes appearing even after 4 years of fallow (Leinfelder and Merwin, 2006). Alternative treatments like seed meal amendments, fertilizers, compost teas, and solarization have been proposed and are in various phases of research and development (Utkhede, 1999; Utkhede and Smith, 2000; Mazzola and Mullinix, 2005; Mazzola and Manici, 2012). In addition to the combination of pathogens involved in each orchard, factors like soil type, climate and other edaphic conditions seem to affect the severity of the complex, making it difficult to diagnose (Fazio et al., 2012). The effects of the disease complex are usually measured by comparing the growth of the same rootstock in sterile soil (pasteurization or chemical treatment) to a biologically active soil collected from the rhizosphere of the old orchard (Leinfelder et al., 2004; Rumberger et al., 2004; Yao et al., 2006a). A comprehensive study of multiple rootstock accessions and *Malus* species indicated that there was sufficient phenotypic diversity to enable growth in non-pasteurized soil (Isutsa and Merwin, 2000); however, the only reported commercially applicable genetic tolerance to the replant disease complex seems to be derived from progeny of 'Robusta 5' and other wild apple species. Certain root genotypes have been reported to promote unique types of microbial communities, indicating a specificity or perhaps a pseudo- symbiotic effect of specific root systems that defeat the presence of pathogenic microbes (Yao et al., 2006b; Rumberger et al., 2007; St. Laurent et al., 2010). Breeding and selection for *Phytophthora* resistance is performed by inoculating young seedlings (Aldwinckle et al., 1974a). New studies leveraging Next-Generation sequencing of *Pythium* challenged rootstock seedlings show upregulation of disease resistance- related pathways in resistant plant material indicating the possibility to select for specific resistance to *Pythium* components of replant disease (Shin et al., 2016). The placement of several apple rootstocks and breeding populations in sterile culture (micropropagation) has enabled identification of separate genetic effects of resistance to the individual replant components, as these rootstocks were inoculated with cultures of *Rhizoctonia* species and *Pythium* species independently. While this set of experiments is still ongoing (Zhu, personal communication), preliminary reports indicate segregation of QTLs affecting this trait and the possibility of developing molecular markers to select superior genotypes.

Resistance to wooly apple aphids - Woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera: Aphididae) has become a more severe pest in apple production areas in the past few years. The retirement of powerful organophosphate pesticides has also increased pressure on orchards. Orchards with resistant rootstocks have been shown to eliminate need for spraying for this pest because the insects cannot overwinter in the rhizosphere. Monogenic resistance to WAA derived from 'Robusta 5' has been mapped to chromosome 17 (Er2 locus) and has been utilized extensively in the Geneva, New York, and New Zealand breeding programs (Bus et al., 2008). Another resistance locus (Er3) from Aotea rootstock has also been mapped on chromosome 8, although it is not as effective as Er1 and Er2 (Sandanayaka et al., 2003; Sandanayaka et al., 2005; Sandanayaka and Backus, 2008). Phenotypic evaluation of this trait consists of rearing insects on susceptible germplasm and then transferring a specific number of insects on actively growing shoots of seedlings or replicated clones in a confined space (usually a netted greenhouse), then observing feeding and proliferation of WAA during a 2 month period after transfer (Beers et al., 2006). The monogenic nature of this type of resistance makes it amenable to utilization of cis-haplotype- specific markers to select parents and cull progeny that do not possess the resistance locus (Bassett et al., 2015). Other sources of WAA resistance are known in the *Malus* germplasm but very little is known about the genetic inheritance of these sources.

Rootstock tolerance to phytoplasma and viruses - Apple viruses and phytoplasmas can cause losses in productivity by interdicting basic plant functions, deforming branches and roots, and by making fruit unmarketable. To date, these pathogens are known to be spread by grafting, where infected clonal rootstocks or scions are the media for transmission (Wood, 1996; James et al., 1997; Silva et al., 2008).

While the goal of apple industries throughout the world should be to work only with material that has been certified tested for viruses, phytoplasmas and other graft-transmissible agents, the eradication of these agents has been elusive due to propagation practices of some growers and homeowners that use infected sources of budwood. It is recommended that apple rootstock improvement programs pay some attention to phenotyping apple rootstocks for susceptibility to some or all of the possible graft-transmissible viruses or phytoplasmas (Lankes and Baab, 2011). Efforts have been made in Germany and Italy to produce rootstocks resistant to the proliferation phytoplasmas (*Candidatus Phytoplasma mali*) found in certain accessions of *M. sieboldii* (Seemuller et al., 2007; Seemuller et al., 2008) and *M. sargentii* (Bisognin et al., 2008, 2009; Jarausch et al., 2008; Bisognin et al., 2009). Susceptibility to Apple Stem Grooving Virus has been observed in ‘Ottawa 3’ rootstocks and some of its derivatives (G.16 and G.814) which exhibited stunting or death upon being grafted with an infected scion. The slow decline caused by graft union necrosis among certain rootstock/scion combinations in the presence of Tomato Ring Spot Virus (ToRSV) (Tuttle and Gotlieb, 1985a; Tuttle and Gotlieb, 1985,b) observed in MM.106 rootstock grafted with ‘Delicious’ scion is also of concern when breeding apple rootstocks. A large trial is underway in collaboration with Cornell University and Virginia Tech to evaluate 50 genotypes for this sensitivity (Robinson, personal communication). Furthermore, there is paucity of genetic studies that describe the inheritance of susceptibility of *Malus* germplasm to viruses and phytoplasmas, making genetically informed breeding impossible. In the Geneva breeding program, virus- sensitive parents like G.16 have been utilized for crosses, and efforts to map susceptibility loci are underway in collaboration with Cornell University virologists as a prerequisite to marker development to be utilized for culling susceptible seedlings before resources are wasted on growing them in larger field trials.

Coordinated testing and evaluation programs in the world

The varied environments where apples are grown suggest that no one rootstock will be well adapted to all environments and that coordinated, independent evaluation of new material from breeding program be performed by local pomologists. There are some organizations in certain apple growing regions in the world that aim to independently test rootstocks in a regimented way covering multiple environments and scions (Elfving and McKibbin, 1990; Schechter et al., 1991; Usa, 1991; Kviklys, 2011). A considerable program of tree fruit rootstock evaluation in the United States, Canada and Mexico is conducted by a group of 35+ researchers, extension specialists and industry collaborators within the CREES (cooperative research and extension services of the USDA) multi-regional project NC-140 (www.nc140.org) and in Europe through EUFRIN (www.eufrin.org). As a group the NC-140 researchers have made significant contributions to tree fruit rootstock research over the last 3 three decades and have conducted highly coordinated impactful research for the tree fruit industry (Rom and Rom, 1991; Fernandez et al., 1995; Perry, 1996; Autio et al., 1997, 2011a,b; Barritt et al., 1997; Marini et al., 2002; Marini et al., 2006b; Autio et al., 2011a; Autio et al., 2011b). Other organizations featuring coordinated international research on apple are RosBREED (www.rosbreed.org) (Iezzoni et al., 2010), FruitBreedomics (www.fruitbreedomics.com) and, the Genome Database for the Rosaceae (www.rosaceae.org) are advancing the development of new knowledge about physiology, phenomics, genetics, and genomics of Rosaceous crops and providing useful infrastructure to the development and evaluation of new apple rootstocks (Evans et al., 2012; Evans, 2013a; Evans, 2013,b; Peace et al., 2014; Chagne et al., 2015; Guan et al., 2015; Liverani et al., 2015; Mauroux et al., 2015; Fresnedo-Ramirez et al., 2016). The ultimate goal for all these organizations is to make apple growing more efficient, more environmentally friendly, more profitable for those that grow apples and more nutritious for the customers that eat apples, and the development of new apple rootstocks is an important cog in this intricate effort.

Relationship to Other Projects Search -

This research is closely tied to the evaluation and utilization of the apple germplasm collection 8060-21000-025-00-D, “Management of Apple, Cold-Hardy Grape, and Tart Cherry Genetic Resources and Associated Information” in Geneva NY with G.Y. Zhong and C.T. Chao as principal investigators – this project is the main source of novel breeding material for our program. CRIS PROJ NO: NYC-625410 “Identification and validation of novel genetic loci linked to fire blight resistance in apples” managed by Dr. Khan at Cornell University is closely associated to our research program in the development of

resistance to fire blight in apple rootstocks. CRIS PROJ NO: CALW-2016-04616 “Characterizing genotype-specific apple root biochemistry and its implications for rhizosphere microbial ecology in apple replant disease (ARD)” is also closely associated with the program as we provide much of the germplasm and some of the root samples to investigate genotype specific associations with rhizospheric biota. Dr. Fazio was one of the inceptors and Co-PI of NIFA SCRI CRIS PROJ NO: NYC-145543 “Accelerating the development, evaluation, and adoption of new apple rootstock technologies to improve apple growers’ profitability and sustainability” led by Dr. Cheng at Cornell University which aims to study many aspects of apple rootstock influence on fruit production that are multidisciplinary and related to the breeding program. The program also works closely with Dr. Zhu of the USDA ARS Tree Fruit Research Laboratory in PROJ NO: 2094-21220-002-10T “Phenotyping resistance traits of apple rootstock germplasm to replant pathogens” where germplasm from the breeding program is being used to discover QTLs and genes related to apple replant disease resistance. We collaborate with Dr. Mazzola also in Wenatchee in PROJ NO: 2094-21220-002-08T “Managing rhizosphere/soil microbiology via apple rootstock chemistry” to study aspects of soil biology related to apple rootstocks. We also collaborate with several scientists at the USDA Appalachian Tree Fruit Research Station in Kearneysville, WV, with PROJ NO: 8080-21000-023-00D “Genetic improvement of fruit crops through functional genomics and breeding” where we are investigating the effect of specific genes on tree architecture and PROJ NO: 8080-21000-024-34S “Three-dimensional modeling system for fruit trees” to see how rootstocks influence the architecture of apple trees. PROJ NO: 8080-21000-024-00D “Integrated orchard management and automation for deciduous tree fruit crops” with Dr. Tabb to investigate how apple rootstocks can aid in developing optimal canopies for orchard automation. The program also collaborates closely with ~35 scientists from all apple growing regions in North-America participating in the NC-140 multi state project CRIS PROJ NO: MO-MSPS0006 “Improving economic and environmental sustainability in tree-fruit production through changes in rootstock use”. PROJ NO: 8060-21000-026-02N “Development of apple rootstock technologies for U.S. and Brazilian apple growers” is one of the many international research projects aimed at studying apple rootstock performance. Our project is the result of a close collaboration between Cornell University and USDA ARS. This collaboration has been ratified with a Cooperative Research and Development Agreement (CRADA No. 58-3K95-4-1668-M)

APPROACH AND RESEARCH PROCEDURES

Objective 1: Develop and release improved apple rootstocks by leveraging advances in marker assisted breeding, including construction of genetic maps, establishing trait associations, gene discovery for important rootstock traits (dwarfing, early bearing, yield efficient, fire blight resistant), and screening for novel alleles for important rootstock traits.

Sub-objective 1A Perform all breeding and evaluation stages involved in the 15-30 year process of developing new rootstocks with the assistance of recently developed breeding tools, such as marker-assisted selection.

Non Hypothesis Goal Driven 1A Perform all breeding and evaluation stages involved in the 15-30 year process of developing new rootstocks with the assistance of recently developed breeding tools, such as high throughput phenotyping and marker-assisted breeding.

Experimental design 1A: We will select new parents based on their genetic potential and field performance. We will generate new genotypes by crossing these parents and we will continue data collection and subsequent selection on approximately 4,000 genotypes that are at different evaluation and selection stages of the breeding pipeline. This includes performing multi-state and international advanced orchard trials, advanced cooperator trials with commercial stool-bed nurseries and first test orchards on location.

A Crossing Block composed of elite germplasm and commercial varieties is in place in Geneva, NY and will be used to generate new populations segregating for rootstock quality traits (propagation, dwarfing,

and precocity) and disease resistance. We will follow the ten-stage selection and evaluation protocol outlined in Johnson (2001) with some modifications regarding the utilization of molecular markers to assist selection and the incorporation of newly identified traits. This process is also outlined in a flow chart diagram attached to this document describing the breeding program (Page 6). We currently have plants in all of these stages and expect to perform all operations within these stages during the next five year period. Due to space and resource constraints we begin a new breeding cycle (lasting 15-30 years) every three years. We expect to initiate two breeding cycles during the five year period of this project.

Stage 1. Parental Selection, hybridization, disease screenings, stool plant establishment, Years 1-2 / 2,000-10,000 seedlings. Parental combinations that have complementary characteristics are chosen for hybridization; for example, an easily propagated dwarfing parent might be crossed with a disease resistant parent. Seeds are collected from the fruit of these crosses, and the seed are stratified (cold treated to break dormancy) and germinated. We then inoculate the seedlings with fire blight bacteria (*Erwinia amylovora*) (Gardner *et al.* 1980) and crown rot fungus (*Phytophthora* spp.) (Cummins and Aldwinckle 1974). Based on the results of previous selection cycles we expect to eliminate 50-80% of the seedlings and establish the rest as single plant stool tree populations. DNA is extracted from all surviving seedlings and tested for dwarfing loci and other markers associated with important traits using high throughput PCR markers (SCARs) that have been developed in our laboratory (Appendix B Figure 4). Depending on the parents used, markers generally eliminate 75-95% of surviving seedlings. *Contingency:* If parents selected for breeding do not have the necessary horticultural and resistance traits then we will select from a pool of novel accessions. If a new virulent pathovar overcomes known resistance then we will search the accessions for resistance to the new pathovar. If PCR markers don't perform as expected for selection, then we will search for alternative methods of high throughput screening.

Stage 2. Stool plant selection, nursery liner establishment, nursery tree growth, Years 3-4 / 25-100 stool trees. Genotypes are propagated as single tree stool-bed plants which are then used to propagate rootstock liners harvested from genotypes that show adequate rooting (at least three adventitious roots per shank) and do not have brittle wood. Liners are moved to a nursery (McCarthy farm, Cornell University – Geneva, NY) for years 5 and 6, where finished trees are produced (it takes at least 2 years to make a finished tree: harvest rootstock liner from mother plant in Fall of year 1; plant in field nursery in Spring of year 2; graft scion bud in Summer of year 2; cut tops of rootstock liners in Spring of year 3 to allow grafted bud to push; allow grafted bud to grow a full finished tree in Summer of year 3; harvest finished tree in Fall of year 3; plant finished tree in the field experiment in Spring of year 4 – the procedure can be cut one year if bench-grafts are used instead of August budding). In years 5 and 6 stool trees are again evaluated for resistance to fire blight and for infestation levels with woolly apple aphids (Johnson 2000), and susceptible genotypes are discarded from the nursery and from the stool tree fields. *Contingency:* If stool bed tests for rooting don't work on a particular year then we will repeat them on a subsequent year. If field fire blight and woolly apple aphid tests are inconclusive we will repeat them in a more controlled environment (greenhouse) in successive years.

Stage 3. First test orchard establishment, precocity evaluation and selection, Years 5-6 / 25-50 rootstock genotypes. Because marker assisted selection for dwarfing has been implemented, we expect the vast majority of rootstocks to be dwarfing and we will plant four to six finished trees on each rootstock genotype in a medium density first test orchard in two locations in the U.S. In addition to the test genotypes, size standard varieties are included (M.27, M.9, M.26, MM.106). Trees are trained to develop an open branching pattern, but pruning is allowed to pattern a slender spindle system. Data is collected and analyzed annually for yield, yield efficiency, tree vigor, suckering, nutrient uptake efficiency and response to any unique stress events. *Contingency:* If dwarfing phenotype is not recovered efficiently by markers then we will cull larger trees in the orchard.

Stage 4. First test orchard evaluation and selection, elite stool bed establishment, Years 7-12 / 10-15 rootstock genotypes. Rootstock genotypes that exhibit precocity and adequate yield efficiency by the fourth leaf (year 10 of breeding cycle) are propagated to increase plant material for an elite stool bed in two locations (Geneva, NY and at cooperating nurseries in the North West). Stool beds are developed from liners retained from stage 2 or from root cuttings of older orchard trees. *Contingency:* If we are not

able to identify precocious genotypes in the test orchard then we will test individuals from different crosses and populations. If we are not able to propagate the selections using root cuttings we will consider the utilization of other methods to establish new elite stool beds.

Stage 5. Liner production, stool bed evaluation, nursery tree growth, Years 10-15 / 5-10 rootstock

genotypes. The important characteristic of this stage is that by this time we have enough stool bed material (liners) to be able to run replicated tests and produce a reliable estimate of how resistant or tolerant a selection is to the different biotic and abiotic stresses that the genotypes will be faced within the life of an orchard. This critical number of plants per genotype is between 100-1000 and is very difficult to achieve with conventional propagation methods and may be best achieved through micropropagation (Fazio et al. 2015b). At this stage we produce trees with several scion/rootstock combinations to test graft union compatibility and strength. Liners in the nursery are budded with selected scions to produce 30 high quality finished trees. First test orchards are removed after harvest in year 15 (after 9th leaf). After 30 trees are produced in the nursery, liners are collected from elite stool beds and subjected to evaluations of disease resistance and stress tolerance, extreme temperature soil tests (trees are grown in heated pots), replant soil tests (Isutsa and Merwin 2000), fire blight tests, crown rot tests, virus resistance / hypersensitivity tests, graft union strength tests (3-4 year old finished trees with several scion/rootstock combinations are subjected to mechanical stress at the graft union) while in stages 5-7 (Fazio, 2015). Protocols and methods for these techniques are published (J. Cummins and H. Aldwinckle 1974).

Contingency: In the event that the stress tolerance tests are not adequate then we will investigate novel ways to test for cold tolerance, soil heat tolerance, etc. If second battery of disease resistance screens reveals susceptible individuals then we will investigate to correct initial screens.

Stage 6. Intermediate stage orchard establishment and early evaluation, Years 16-18 / 10 rootstock

genotypes. Intermediate stage orchards are planted beginning in year 16 at three sites representing a cross-section of domestic apple production environments. Each year's planting includes commercial standard dwarfing genotypes (M.9, B.9, M.26) and 5 to 10 elite rootstock genotypes that have shown promise in elite stool bed liner production, initial test orchard performance, and biotic and abiotic stress resistance screens. These orchard trees are evaluated for precocity in their early years.

Stage 7. Intermediate stage orchard evaluation, commercial stool bed trials, Years 19-21 / 5

rootstock genotypes. Intermediate stage orchard trial data collection continues (Russo et al., 2007). Biotic and abiotic stress screenings of rootstock liner trees is completed. The most promising rootstock genotypes (a maximum of 5 per year) from the Cornell/USDA program are distributed to cooperating nurseries for commercial stool bed trials (50 liners to each of 2 cooperating rootstock nurseries) beginning in year 19. The most promising Geneva rootstock genotypes are submitted for phytosanitary certification (NRSP5, Prosser WA) to enable international distribution. It is possible to start the technology transfer to cooperating nurseries at stage 5 or 6 because these nurseries may be the location where the finished trees for intermediate trials are prepared. In that contingency we will transfer plant material (rootstock liners) to cooperating nurseries under contract to generate finished trees. Data on nursery tree performance will be collected at this stage (Fazio et al., 2008a). This will also allow cooperating nurseries to take a first look at these selections and learn to the best cultural practices adapted to the new genotypes.

Stage 8. NC-140 and on-farm trials, distribution to all cooperators, Years 22-24 / 2 rootstock

genotypes. Intermediate stage orchard trial data collection continues (Robinson et al., 2006). For outstanding rootstock genotypes from the intermediate stage orchard trials and commercial nursery stool bed trials, liner production from cooperating nurseries is used to propagate trees for NC-140 and/or on-farm trials. Each multi-state NC-140 trial and on-farm grower cooperator trial is unique and follows methods and protocols that are established by the cooperators participating in the trial. Generally, data on yield efficiency, productivity, precociousness, hardiness, incidence of disease, tree size is collected for each rootstock for a period of 8-12 years. Best rootstock genotypes, as determined by each unique individual trial's protocol, are distributed to domestic cooperating nurseries for propagation, and to international cooperating nurseries and institutions for propagation and local evaluation trials. Internationally commercially successful genotypes join the USDA/Cornell rootstock evaluation program

as stage 8 materials following evaluation in biotic and abiotic stress screenings – this is done to provide a benchmark for all commercial rootstocks.

Stage 9. Final evaluations and selections, commercial ramp-up, patent applications, Years 25-27 / <1 rootstock genotype. Plant material for rootstock genotypes demonstrating marked improvement over commercially available varieties based on results from cooperator and NC-140 trials is increased in commercial stool beds and micropropagation facilities. Intermediate stage evaluation orchards are removed after 11th leaf. Plant Patent and UPOV protection applications are filed on commercially viable rootstock genotypes given that the IP protection is necessary for successful deployment and implementation of the new genotypes.

Stage 10. First commercial sale of Geneva rootstocks, elimination of all unreleased genotypes from trials, Years 28-30. Data collection continues for NC-140 and on-farm grower cooperator trials. Unreleased genotypes that showed promise but were not demonstrably superior to commercially available rootstocks are eliminated from the program or selected for release in alternative markets (ornamental etc.).

Contingencies: There are several contingency points in this part of the project as there are many stages. Amendments to the breeding protocol will be made to include innovations in propagation, molecular markers.

Collaborations: Domestic collaborators: Awais Khan, Cornell University, collaborates on fire blight resistance characterization and inoculation, phytophthora root rot inoculations, tissue culture and genetic engineering. T. L. Robinson, Cornell University, provides second test and intermediate orchard evaluation, national and international evaluation of Geneva rootstocks, and commercialization. John Norelli (fire blight resistance), Chris Dardick (modification of plant architecture by rootstocks), all from ARS Kearneysville, West Virginia; C.T. Chao (novel gene pools for rootstock traits) ARS, Geneva, New York; Mark Mazzola and Yanmin Zhu (replant tolerant rootstock genotypes) ARS Wenatchee, Washington. Willow Drive Nursery provides advanced testing locations for stool bed evaluation, nursery tree development and is one of our stakeholders. Dr. Stefano Musacchi (Wenatchee, WA) is testing advanced selections in Washington State. The Washington Tree Fruit Research Commission performs advanced apple rootstock testing including intermediate and commercial trials in organic and replant situations. The NC-140 network of collaborators (list available at www.nc140.org) is essential for proper testing of local adaptations of these new genotypes.

International collaborators: A network of international test sites in which we are actively involved was established in the past 10 years. These test sites are in Germany, France, Italy, Poland, New Zealand, Australia, South Africa, South Korea, Brazil, Uruguay, and Chile and include representatives from major research institutions (University of Bologna, Italy, IRTA, INRA, EMBRAPA) and representatives from international nursery organizations.

Sub-objective 1B Identify and characterize novel germplasm, genes, alleles and trait loci through quantitative trait analyses leveraging new genetic-physical maps.

Hypothesis 1B Novel genotyping and phenotyping techniques will allow the discovery of novel sources of germplasm and alleles to be used as new parents in the breeding program.

Experimental Design 1B. The program has utilized Single Nucleotide Polymorphism (SNPs), Insertion-Deletion (InDel), microsatellite or simple sequence repeats (SSR) markers to generate DNA fingerprints of apple rootstock populations and potential parents. While these genotyping methods have positive aspects, they mostly lack information about functionality (direct connection to genes) and are not set up to generate haplotype data based on combinations of polymorphisms in longer stretches of DNA. Because of advances in sequencing technologies, in the next five years the program will transition to genotyping by AmpSeq (Yang et al., 2016) which allows the multiplexed (380 individuals x 300 amplicons – Cadle Davison pers. comm.) genotyping of stretches of DNA amplified by PCR. Amplicons are targeted to

genes or specific regions of the genome and the assembly of the sequences onto the apple genome (Velasco et al. 2010; Fazio et al., internal resource), differentiating and grouping sequences based on similarity and haplogroups. Segregation will allow the distinction between homologous and homeologous genes (*Malus* is an ancient tetraploid). We will target a set of genes whose expression is segregating in rootstock breeding populations that have been characterized by gene expression QTL (eQTLs) of published microarray experiments (Jensen et al., 2011; Jensen et al., 2012, Jensen et al., 2014) and eQTLs (internal data, unpublished) derived from RNAseq analyses of areal and root tissues of apple rootstock breeding populations. We will choose germplasm that possesses the best combinatorial arrangement of desirable loci from within the breeding program and novel germplasm from the *Malus* collection based on feedback from past and future phenotyping experiments (Fazio et al. 2014a; Bassett et al., 2011). As clonally propagated material becomes available, we will design a series of replicated pot experiments (based on statistical power analysis for key parameters) where we will treat rootstocks and finished trees with different water regimens and aeroponically delivered pH treatments. Means and effects will be calculated and relevant multivariate analyses will be conducted for measured parameters including tree growth, photosynthesis, nutrient concentration in the leaves or fruit, tree architecture parameters (bud break, rooting, root morphology, branching, growth, flower induction). We are currently running phenotyping experiments involving root growth imaging and sensing with CI-600 root imagers and root analysis software (CID Bio-Science, RootSnap, Giaroots, etc.) and pH-nutrient treatments in connected aeroponic systems (Appendix B Figures 1-3). We see potential for these measurements to help us and apple growers make more informed decisions regarding the type of rootstock that matches their pedo-climatic conditions. Once the methodology is standardized and the results are interpreted it will be incorporated into the breeding process, possibly at stage 5-8 where availability of enough clonal replicates (rootstock liners) is assured. We will seek collaborations with expert physiologists, plant pathologists, etc. to help in the design and interpretation of these experiments.

Contingencies If AmpSeq is difficult to implement, we will seek similar, high throughput genotyping systems as they become available or continue using *Malus* Consortium Illumina SNP Chips. Expert labor, cost, instrument, greenhouse, plant and land availability are all limiting factors to the success of these experiments. We will tailor each experiment based on resources available each year.

Collaborations Dr. Cadle-Davidson (USDA ARS, Geneva) on AmpSeq matters. Dr. Terence Robinson program (Cornell University) and other visiting scientists in his laboratory on evaluation of advanced breeding lines in different orchard systems. Drs. Dardick and Tabb (USDA ARS, Kearneysville, WV) in matters related to tree and root architecture and imaging. Dr. Michael Grusak (USDA ARS, Fargo, ND) in matters regarding nutrient content, impact on tree health and possibly human nutrition. Dr. Moran (University of Maine) to investigate low temperature stress on apple rootstocks. Plant pathologists at Cornell University and Dr. Mark Mazzola (USDA ARS, Wenatchee, WA) to devise and interpret experiments involving soil pathogens associated with replant disease, and Dr. Lee Kalcits (Plant Physiologist, Washington State) on matters of nutrient partitioning by apple rootstocks.

Objective 2: Identify and dissect important rootstock traits that modify gene activity in the scion, toward enhancing drought tolerance, tree architecture, propagation by nurseries, root growth and physiology, nutrient use efficiency, and disease resistance; incorporate this knowledge into breeding and selection protocols.

Sub-objective 2A Identify components of rootstock induced traits that modify gene expression and metabolic/physiological profiles of grafted scions to increase tolerance to abiotic stresses such as tolerance to drought, improve fruit quality and storability, increase tree productivity, disease resistance and nutrient use efficiency.

Hypothesis 2A Genetic maps, QTL analyses, RNAseq, gene expression QTLs, whole genome sequences can be used to deconstruct traits to their segregating components.

Experimental Design 2A The Geneva breeding program has successfully used genetic maps in combination with phenotypic data to identify Quantitative Trait Loci (QTLs) associated with several important traits in apple rootstocks **leveraging very diverse interspecific crosses**. We know from Jensen et al., 2011 and 2012, that gene expression in the scion is modified by apple rootstocks, what we do not know is how genetically complex these scion expression modulations are in segregating rootstocks. Similarly to what has been done within our program with eQTLs, we will plant 1-2 apple rootstock replicated segregating populations, graft them with the same scion (Honeycrisp for example) and measure gene expression by 3' RNAseq (www.lexogen.com), tree growth, photosynthesis, metabolites, nutrient concentration in the leaves or fruit, tree architecture parameters (bud break, branching, growth, flower induction) on the scion to detect rootstock induced gene expression QTLs and possibly associate them with physical traits measured on the same trees. **For example, we have now very strong evidence of rootstock mediated nutrient absorption and translocation (our group was the first in the world to publish on the genetics of such traits) as demonstrated by this dual clustering diagram of boron concentration in grafted Honeycrisp scion from a field experiment with four years of data and multiple.** We would take contrasting rootstocks and progeny to see what genetic elements are fostering such differences. **In this case we have G.935 that consistently confers higher levels of boron and B.9 that has lower levels consistently.** We also have a segregating population that is derived from the cross between G.935 and B.9. **Depending on resources (funds and scientific effort) we plan to hold maximum of two experiments per year.** The next iteration is to apply abiotic stresses on the same populations (drought, pH, lack of nutrients) and follow with performing similar measurements. The next phase is to connect known gene networks (based on functional annotations – MapMan and KEGG analysis software) segregating in the rootstocks with gene networks modulated in the scion by segregating rootstocks using multivariate analyses such as Ward's dual clustering analyses on correlation coefficients, principal component analyses and gene neural network analyses. The program has acquired next generation sequences (Illumina platform) of several founding parents of the apple rootstock breeding program (G.41, M.27, O.3, R.5, M.9, Dolgo), a complete genome assembly of R.5 (obtained by combination 100X Sequel – Pacific Biosciences, BioNano and Phase Genomics data) and has developed an internal database of aligned haplotypes for small genomic regions of interest such as genes with drastically changed expression (expressed or not expressed) identified in the eQTL discovery process. All of the above processes are very data intensive. To accomplish these tasks we will use a combination of off the shelf genomic analysis software, like SAS JMP Pro, Geneious (www.biomatters.com), JoinMap/MapQTL (www.kyazma.com), CLC Genomics Workbench (www.clcbio.com), we will also utilize genomic and breeder's tool box resources available through the Genome Database for Rosaceae (www.rosaceae.org), and other bioinformatic tools specifically designed by collaborators.

Contingencies If methodology described above is not cost effective or fails to produce high quality data, we will seek different methods to genotype and phenotype plants in the populations. We will also continue to explore and adopt the best available, cost effective methodology to harness DNA information for breeding improved apple rootstocks. Current bioinformatic and statistical tools may not be well suited for our dataset types – we will seek advice from the NEA Computational Biologist and/or Statisticians and, when necessary, devise new tools to enable the analysis and correct interpretation of the results.

Collaborations We will collaborate with Dr. Udall and his team (Brigham Young University) with regards to the R.5 genome assembly to be used in this study. Dr. Dardick (USDA ARS, Kearneysville) will aid with mapping and statistical analyses.

Sub-objective 2B Validate relationships between trait components and overall apple tree performance in different rootstock-scion combinations and incorporate new knowledge into breeding and selection protocols.

Hypothesis 2B *Scion gene expression and phenotype modulation by segregating rootstocks transfers to other apple scions.*

Experimental Design 2B Based on the results of experiments in sub-objective 2A, we will validate the strongest rootstock induced scion trait associations on other apple scion varieties like Gala, Golden Delicious, SnapDragon, Autumn Crisp. As an example, if Gene A segregating in rootstock population R had an effect of increasing or decreasing phenotype Y (including gene expression) in Honeycrisp, does it have the same effect on SnapDragon scion? An experiment to validate such effect would only require a subset of rootstock germplasm segregating for such locus (15 with and 15 without) to test the mean separation between phenotypes of the groups (Jensen et al. 2015). We just started experiments in containers and in aeroponics deal with pH: three different levels (5.5, 7 and 8) to measure growth, nutrient uptake and root gene expression of grafted trees. Exploratory experiments with aeroponics: drought response where nutrient mist can be turned off for a number of hours (TBD) or drastically reduced. Items to be measured: physiological traits (photosynthesis, transpiration and hormonal activity), root and scion gene expression. Validated rootstock loci will be transitioned to working DNA markers and published.

Contingencies In the event that we are not able validate effects of selected rootstock loci on phenotypes (including gene expression) of other scion varieties, we will select a new set of loci to validate.

Collaborations We can perform this type of analysis in house, but will seek expertise and collaborations as needed.

Physical and Human Resources

The program enjoys cooperation with Cornell University under a Cooperative Research and Development Agreement (CRADA) 58-3K95-4-1668-M specifically with the Cornell University New York State Agricultural Experiment Station in Geneva (NYSAES). Approximately 25 acres of land belonging to NYSAES are dedicated to the apple rootstock breeding program, including land set aside for stool beds, stool nursery, tree nursery, first test orchards, and replicated trials. NYSAES provides rented greenhouse space for controlled experiments (inoculation, propagation), an aeroponic system able to accommodate 350 plantlets and 2000 sq. ft. cold storage space for storage and processing of finished trees and liners during winter months. The Field Research Unit of NYSAES provides (at a subsidized cost to the USDA) equipment and personnel necessary to conduct large field operations such as pruning, pesticide and herbicide spraying of orchards, etc. A Kubota and a John Deere tractors owned by the program are used for small field operations. Our laboratory is located in the Horticulture Section of the Cornell University Campus of NYSAES. It possesses modern molecular genetic laboratory equipment (water baths, freezers, pipettors, centrifuges etc.). This lab is equipped with a sequencing apparatus ABI3130 genetic analyzer for determining SSR parental allele sizes and a capillary sequencer to perform genotyping and DNA fragment sequencing. Seven 96 well thermal cyclers with high throughput agarose gel apparatuses (96-192 samples) are available to genotype with markers having allele size polymorphisms greater than 10bp. A ROCHE real time PCR machine capable of High Resolution Melting is also available to the lab. The program also has access to the Cornell Institute of Biotechnology Resource Center for services like Next Generation Sequencing, Microarrays, etc. Computer resources include a dedicated Dell and HP computers that are able to work with bioinformatics software like CLC Genomics workbench (www.qiagen.com) and Geneious (www.biomatters.com). Stable human resources for this project are Lead Scientist Geneticist Gennaro Fazio, Research Leader and Geneticist Gan-Yuan Zhong (5%), Field/Lab technician Todd Holleran, Lab Technician Sarah Bauer, and temporary labor pool during the summer months.

Project Management and Evaluation

This project is the result of collaboration between USDA ARS and Cornell University that is ratified with a CRADA. While the USDA has a lead role in the project, project co-directors (Dr. Robinson or his staff and Dr. Khan or his staff) meet regularly to evaluate progress, make critical management decisions about testing and release of apple rootstocks. We hold annual meetings and semi-annual phone conferences with Nursery and Industry cooperators to evaluate the progress of the breeding program. We hold regular (bi-weekly) staff meetings to plan and discuss progress on the project's milestones and regular meetings with collaborators in PGRU to coordinate efforts on germplasm collection and evaluation. We utilize tools like AT&T Connect to routinely share data, presentations and interact with collaborative scientists worldwide. Our ARS Location (Geneva) holds regular monthly meetings that include the Research Leader and all location scientists to coordinate research projects and discuss advancements in research.

We also routinely communicate with Area Director and members of the Office of National Programs staff regarding general direction of project, interaction with industry and progress with milestones.

Data Management

This project produces several types of data assembled in different sizes from small to very large. The smaller sets are phenotypic evaluations of several traits collected during the process of evaluation of genotypes in the breeding program – these sets also include the statistical analyses associated with the traits. Larger data sets are generated as a result of next generation sequencing of samples in the breeding program. All data is backed up and stored on an ARS secure server. Because we work under a CRADA (Cooperative Research and

Development Agreement) with Cornell University, the datasets germane to breeding program genotypes can only be released to the public upon agreement of both parties. This release has been accomplished in many cases through consensual publication. Sequencing datasets are usually published and deposited in related databases like www.rosaceae.org or NCBI when they are processed for publication.

Milestones

Project Title	Development of Biotic and Abiotic Stress Tolerance in Apple Rootstocks			Project No.	8060-21000-026-00D
National Program	301, Plant Genetic Resources, Genomics and Genetic Improvement				
Objective	1. Develop and release improved apple rootstocks by leveraging advances in marker assisted breeding, including construction of genetic maps, establishing trait associations, gene discovery for important rootstock traits (dwarfing, early bearing, yield efficient, fire blight resistant), and screening for novel alleles for important rootstock traits.				
Subobjective	1A. Perform all breeding and evaluation stages involved in the 15-30 year process of developing new rootstocks with the assistance of recently developed breeding tools, such as marker-assisted selection.				
NP Action Plan Component	1– Crop Genetic Improvement				
NP Action Plan Problem Statement	Problem Statement 1B: New crops, new varieties, and enhanced germplasm with superior traits				
Hypothesis	SY Team	Months	Milestones	Progress/ Changes	Products
Perform all breeding and evaluation stages involved in the 15-30 year process of developing new rootstocks with the assistance of recently developed breeding tools, such as high throughput phenotyping and marker-assisted breeding.	Fazio	12	Nearly all the procedures in this objective are cyclic in nature – almost every year we select parents, make crosses, select genotypes from first test orchards, inoculate seedlings, send test selections to cooperators, plant nursery, plant orchards, collect yield data, conduct Marker Assisted Breeding for dwarfing, WAA resistance, etc.		Seed from crosses, selections, trees for testing on cooperators farms. Selections distributed to nurseries for advanced testing. Selections distributed to cooperators for comparative testing in multiple U.S. environments prior to release.
	Fazio	24	Select and establish 5 new female parent trees in crossing block		
	Fazio	36	Implement new selection protocols for nutrition and root architecture		Rootstock Release – this will be a rootstock that has probably been in the pipeline for 20 years.
	Fazio	48	Select and establish 5 new female parent trees in crossing block		

	Fazio	60	Implement new protocols for replant disease selection.		Rootstock Release – this will be a rootstock that has probably been in the pipeline for 20 years.
Project Title	Development of Biotic and Abiotic Stress Tolerance in Apple Rootstocks		Project No.	8060-21000-026-00D	
National Program	301, Plant Genetic Resources, Genomics and Genetic Improvement				
Objective	1. Develop and release improved apple rootstocks by leveraging advances in marker assisted breeding, including construction of genetic maps, establishing trait associations, gene discovery for important rootstock traits (dwarfing, early bearing, yield efficient, fire blight resistant), and screening for novel alleles for important rootstock traits.				
Subobjective		1B. Identify and characterize novel germplasm, genes, alleles and trait loci through quantitative trait analyses leveraging new genetic-physical maps.			
NP Action Plan Component		1– Crop Genetic Improvement			
NP Action Plan Problem Statement		Problem Statement 1A: Trait discovery, analysis, and superior breeding methods			
Hypothesis	SY Team	Months	Milestones	Progress/ Changes	Products
Novel genotyping and phenotyping techniques will allow the discovery novel sources of germplasm and alleles to be used as new parents in the breeding program.	Fazio	12	Identification of target genes (1500) and generation of PCR primers in preparation for AmpSeq. Amplicon optimization and validation.		New set of apple primers for multiplexed AmpSeq – deposit to Rosaceae database.
	Fazio	24	Identification of parents and populations for screening with AmpSeq. Run AmpSeq on selected individuals. Design phenotypic experiments on selected individuals (pH, water use efficiency, nutrient uptake, root morphology, etc.)		
	Fazio	36	Analysis of AmpSeq data, identification of unique alleles and haplotypes. Connection to legacy genotypic and phenotypic datasets.		Candidate loci for incorporation into breeding program. If needed, transition to gel based PCR marker types.

	Fazio	48	Selection and crossing of new individuals based on AmpSeq.		Unique parents with desirable combinations of desired trait loci.
	Fazio	60	Second set of phenotypic experiments – to be determined upon need.		
Project Title	Development of Biotic and Abiotic Stress Tolerance in Apple Rootstocks			Project No.	8060-21000-026-00D
National Program	301, Plant Genetic Resources, Genomics and Genetic Improvement				
Objective	2. Identify and dissect important rootstock traits that modify gene activity in the scion, toward enhancing drought tolerance, tree architecture, propagation by nurseries, root growth and physiology, nutrient use efficiency, and disease resistance; incorporate this knowledge into breeding and selection protocols.				
Subobjective	2A Identify components of rootstock induced traits that modify gene expression and metabolic/physiological profiles of grafted scions to increase tolerance to abiotic stresses such as tolerance, improve fruit quality and storability, increase tree productivity, disease resistance and nutrient use efficiency.				
NP Action Plan Component	Component 3 – Crop Biological and Molecular Processes				
NP Action Plan Problem Statement	Problem Statement 3A: Fundamental knowledge of plant biological and molecular processes.				
Hypothesis	SY Team	Months	Milestones	Progress/ Changes	Products
Genetic maps, QTL analyses, RNAseq, gene expression QTLs, whole genome sequences can be used to deconstruct traits to their segregating components.	Fazio	12	Selection of segregating populations and generation of clonally replicated materials for experiments.		
	Fazio	24	Grafting of same scion, planting in pots or aeroponic systems.		
	Fazio	36	Growth, phenotype measurements and sampling of RNA for 3'RNAseq. Subjection of experimental units to mild drought stress.		Database of rootstock induced phenotype modulation (including gene expression) in non-stressed vs. stressed plants.
	Fazio	48	Analysis of first year data, collection of second year data.		First gene network analysis and first set of candidate loci to test in sub-objective 2B

	Fazio	60	Analysis of second year of data		Publication of research findings
Project Title	Development of Biotic and Abiotic Stress Tolerance in Apple Rootstocks			Project No.	8060-21000-026-00D
National Program	301, Plant Genetic Resources, Genomics and Genetic Improvement				
Objective	2. Identify and dissect important rootstock traits that modify gene activity in the scion, toward enhancing drought tolerance, tree architecture, propagation by nurseries, root growth and physiology, nutrient use efficiency, and disease resistance; incorporate this knowledge into breeding and selection protocols.				
Subobjective		2B Validate relationships between trait components and overall apple tree performance in different rootstock-scion combinations and incorporate new knowledge into breeding and selection protocols.			
NP Action Plan Component		1– Crop Genetic Improvement			
NP Action Plan Problem Statement		Problem Statement 1A: Trait discovery, analysis, and superior breeding methods			
Hypothesis	SY Team	Months	Milestones	Progress/ Changes	Products
Scion gene expression and phenotype modulation by segregating rootstocks transfers to other apple scions.	Fazio	12			
	Fazio	24			
	Fazio	36	Preparation of comparative rootstock subsets (presence/absence of target locus) and grafting of different scions.		
	Fazio	48	Growth and collection of first year data in stressed and non-stressed comparisons.		First validation of locus effects. Validation of gene networks associated with target loci. Identification of new genes associated with target pathways.
	Fazio	60	Measurement of second year of data. Transition of validated loci into haplotype specific markers to be used in breeding.		

Prior Research Accomplishments**Terminating Project Number:** 8060-21000-026-00D**Title:** Development of Pest, Disease Resistance, and Stress Tolerance in Apple Rootstocks**Project Period (beginning and ending dates- Month/Day/Year):** March 28 2013-March 27 2018**SY Time – Investigators:** Gennaro Fazio (100%) Gan-Yuan Zhong (5%)**FTE 1.85:** Todd Holleran (100%) Sarah Bauer (85%)**Project Accomplishments and Impact:****Objective 1 Develop superior apple rootstocks, applying advances in marker-assisted breeding for important traits such as dwarfing, precocity, wooly apple aphid resistance, and tree architecture.**

Technology Transfer - The major impact of the project relates to the adoption by the U.S. industry of new varieties released in the last cycle where, production of Geneva rootstocks increased from less than 1 million rootstocks/year in 2013 to roughly 10 million rootstocks/year in 2017. This was accomplished by numerous on farm field trials that proved the worth of these new disease resistant and productive rootstocks to the U.S. industry. These technology transfer efforts in the project earned the “Excellence in Technology Transfer” award from the Federal Labs Consortium (competed against NASA, DOE, NIH, and other federal agencies) in April, 2015 and discoveries on apple rootstock dwarfing loci published by the project earned the Outstanding Fruit Research Paper Award by the Journal of the American Society for Horticultural Science in August 2015.

Release of New Apple Rootstock G.814. The project provided new solutions and opportunities to the apple industry by releasing this rootstock that addressed issues like replant disease and fruit quality and size. This clonally propagated apple rootstock G.814 is a dwarfing, productive, early bearing, highly yield efficient, resistant to fire blight. Although it is susceptible to wooly apple aphids and to Apple Stem Grooving Virus (ASGV), G.814 has shown tolerance to the replant disease complex. G.814 produces scions that when fully developed are about 40% the size of a standard seedling apple tree. G.814 has the potential to increase productivity of larger, high quality fruit in marginal replanted orchard land.

Release of new apple rootstock G.213. Some apple scions like Gala have a tough time blooming in low winter chill subtropical regions. Apple growers needed yield efficient, disease resistant apple rootstocks when growing apples in low chill environments or when current environments are affected by climate change. The project is releasing a new productive, disease resistant apple rootstock which improves bud break and productivity in low chill environments such as Southern U.S., California and Brazil. This rootstock was developed over a 35 year process where it survived a series of inoculations with apple rootstock pests and pathogens (fire blight, crown rot and wooly apple aphid) and was tested over 25 years with multiple grafted scion varieties in multiple environments increasing productivity and producing scions that when fully developed are about 30-40% the size of a standard seedling apple tree. When clonally propagated in the stool bed the mother plants produce rootstock liners that are 30 to 50 cm tall with few spines. This new rootstock seems to be the first in the world that possesses the characteristic of increasing bud break in low chill environments of scion cultivars like Gala, therefore it will increase production of high quality fruit in apple growing regions in the U.S. and worldwide that are affected by low winter chilling hours.

Characterization of rootstock nutrient uptake. Apple rootstocks can affect the nutritional status of Honeycrisp and Fuji apples. Mineral nutrient status (calcium, potassium, nitrogen, magnesium and phosphorous) affects the eating quality, health, storability and appearance of apples like Honeycrisp causing many apples to be discarded or apple trees to produce poorly. Recently published in a grower oriented journal (New York Fruit Quarterly) is a first of its kind description of how apple rootstocks affect the mineral nutrient and nitrogen concentration of Honeycrisp and Fuji fruit grafted on more than 40 rootstocks in replicated field trials in New York apple growing regions. As the availability and knowledge of diverse rootstocks increases, it will increase the potential to impact fruit productivity, quality and ultimately profitability of our apple orchards. The choice of the best rootstock for the site, scion and orchard system is going to become more important than ever. The

ability to match the nutritional requirements of a scion cultivar to a specially tuned rootstock will enhance orchard management in the future by allowing healthier trees and more efficient use of fertilizers. This study, first of its kind, lays the foundation for this line of research and will provide better choices to our apple growers in terms of rootstock technologies.

The results of a decade long field trial of 48 apple rootstocks grafted with the apple variety Fuji were published. Rootstocks had significant influence on fruit yield and fruit nutrient concentration. Several Geneva® rootstocks evaluated showed considerable promise as alternatives to M.9. CG.6006, CG.8189, CG.4004, CG.5087, CG.4011, G.969, G.935, and G.890 had good performance on 'Fuji'. The rootstock induces changes in the concentrations of leaf and fruit nutrients. Cumulative yield efficiency had a moderate positive correlation with leaf Ca concentration. G.214, JM.10, CG.4003, M.9, G.935, CG.4088, CG.2406, G.969, and G.210 had low alternative bearing which means stable production of flowers and fruit year over year. All these data help apple growers determine the best rootstock for their local growing condition and variety and provide apple consumers with consistent high quality apples.

Graft Union Strength of Geneva Apple Rootstocks. In collaboration with Utah State University we published research on graft union flexural strength which won the 2016 U.P. Hedrick award of the Journal of the American Pomological Society. Apple rootstock 'Geneva® 41' ('G.41') and other rootstocks form weak graft unions with multiple scions, this is a problem in the nursery stage of tree development especially under high wind conditions which may cause losses upwards to 80% of trees in some rootstock/scion combinations – one grower reported the loss of 60,000 trees worth more than \$600,000. Exogenous plant growth regulators (PGR) can influence vascular differentiation and wood formation, and thus may improve graft union strength. A series of commercial and experimental PGR formulations were applied to trees on 'G.41' rootstock over two seasons, and graft union strength and flexibility were measured. Benzyl adenine (BA) applied in paint solution to the graft union significantly increased the flexural strength per scion cross-sectional area and the flexibility of the union. In addition, foliar applications of Prohexadione-Ca also increased graft union flexural strength and flexibility, but temporarily limited scion extension growth. Applying PGRs in the nursery to more brittle rootstock-scion combinations may be an option for improving graft union strength and preventing tree losses. However, more efficient methods of application are needed for this approach to be commercially viable.

We are continuing work to further develop accomplishments in Objective 1: a. we will continue to release new apple rootstocks; b. we continue to work on nutrient uptake genetic and breeding; c. we continue to work on the analysis of micro-CAT scans of graft unions to establish morphometric parameters of comparison between strong and weak unions.

Objective 2: Devise and apply genomic and bioinformatic tools for marker-assisted breeding of apple rootstocks including identification of the genes underlying resistance to the replant disease complex and to nutrient uptake efficiency.

The bioinformatic portion of this objective was accomplished before the separation of Dr. Baldo (20%) from the project early during the course of this reporting period.

Tool for identification of gene variants for rootstock breeding. Once a genomic region is identified as being important for the modulation of a specific important trait it is difficult to identify other apple plants that may contain the trait associated genomic region. To solve this we have analyzed raw Genotyping-by-sequencing data from 1995 accessions of apples (31 species) from the Geneva apple germplasm repository and selected additional breeding material. The SNPs identified were made viewable on the published genome assembly, while diversity is also viewable in chromosome order in Tassel software. This makes it possible to identify SNPs among wild and breeding material near and in candidate genes of interest. This tool has the potential to speed up the breeding process, and identify new sources of important apple rootstock traits. (Baldo, Zhong)

Validation of location and effect of dwarfing genes in the apple genome and relationship to early bearing induction in apple trees. One trait that makes apple rootstocks very special in the realm of fruit production is the ability to dwarf trees and make them more productive earlier in the life of the orchard. It is estimated that the implementation of these traits in U.S. apple production has increased productivity by more than \$0.75B in the past 30 years. Being able to track the origin and effect of these traits will enable the breeding and selection of new disease resistant improved apple rootstocks. In FY 2013 we completed a genetic map that utilized markers developed by the RosBREED consortium to examine the genetics of dwarfing and early bearing in two apple rootstocks breeding populations. The results of this genetic quest confirmed earlier findings by our lab and made possible modeling of dwarfing gene interactions. This accomplishment made quicker the development of new early bearing apple rootstocks and improved prediction of dwarfing potential of such apple rootstocks. (Fazio)

We made a first report about the genetics of nutrient absorption by rootstocks in tree fruits.

We utilized quantitative trait analysis in a breeding population to uncover gene locations for leaf mineral concentrations of leaves for important plant nutrients like potassium (K), sodium (Na), phosphorous (P), calcium (Ca), zinc (Zn), magnesium (Mg) and molybdenum (Mo). We also noticed that several nutrient concentrations were correlated indicating the co-absorption or common transport mechanisms for some nutrients. We found significant positive linear correlations between Ca and Cu, Mg, P, and S. A significant correlation was also detected between Cu and K, Cu and P, also between K and P and between S and P. Segregation of a major gene for leaf K concentration in certain rootstocks had strong effects on the concentrations of other nutrients in the leaves, suggesting that it might be a good target for selection in the breeding program. As this is a first report, we are attempting to understand the physiological influence of these genes on other measurable traits in apple rootstocks and scions. It is possible that even subtle changes in plant nutrients caused by variable gene combinations in the rootstocks can affect productivity and disease resistance of apple trees. (Fazio)

Genes activated during infection with components of replant disease. RNA-seq technology was applied to identify the transcriptomic changes associated with apple root defense response to *Pythium ultimum* infection. Genes encoding homolog proteins with functions of pathogen detection such as chitin elicitor receptor kinase (CERK) and wall-associated receptor kinase (WAK) were among the differentially expressed apple genes. The biosynthesis and signaling of several plant hormones including ethylene, jasmonate and cytokinin were specifically induced in response to *P. ultimum* inoculation. Genes encoding enzymes of secondary metabolisms, cell wall fortification and pathogenesis related (PR) protein, laccase, mandelonitrile lyase and cyanogenic beta-glucosidase were consistently up-regulated in the later stages of infection.

We are continuing work to further develop accomplishments in Objective 2: we are using data generated in this objective to clone and identify genes and gene networks associated with root and rootstock traits which is related to Objective 2 of the new project plan.

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Past Accomplishments of Investigators

GENNARO FAZIO

EDUCATION

Ph.D. University of Wisconsin-Madison Department of Plant Breeding and Plant Genetics. Area of study: Application of molecular markers for breeding, quantitative genetics, plant variety identification. November 2001.

M.S. Agronomy-Molecular Biology, Brigham Young University, Provo, Utah, April 1997. Area of study: Identification of molecular markers linked to crown rot resistance in tomato.

B.S. Molecular Biology and Agronomy, Chemistry minor, BYU, Provo, Utah, 1995.

University of Bari, Italy, Agronomy Department, attended 1986-1987.

PROFESSIONAL EXPERIENCE

Research Geneticist, Plant Genetic Resources Unit, USDA-ARS, Geneva NY (2001-present).

Adjunct Professor, Dept. of Horticulture, Cornell University, Geneva NY (2001-present).

Research Assistant, University of Wisconsin-Madison (1997-2001).

ACCOMPLISHMENTS

1. Patented and released 13 apple rootstocks (G.41, G.935, G.202, G.210, G.214, G.890, G.969, G.814, G.213, G.778, G.228, G.189 and G.222) in the U.S. and internationally. These new rootstocks provide protection from fire blight, tolerance to replant disease, and increased productivity in the orchard. Production of these new Geneva® rootstocks in 2017 approached 10 million plants in the U.S. and is increasing.
2. Discovered inheritance of dwarfing, precocity, fire blight resistance, root morphology, scion branch angle modification in apple rootstocks.
3. Developed marker assisted breeding scheme for selection of dwarfing, precocious, disease resistant rootstocks.
4. Developed concept and initial experiments for the use of prohexadione (Apogee) in apple rootstock liner production. Several apple rootstock nurseries worldwide have adopted this method to increase quality and yield of propagation beds.
5. Discovered quantitative trait loci associated with nutrient absorption and translocation into the scion for important plant nutrients like potassium (K), sodium (Na), phosphorous (P), calcium (Ca), zinc (Zn), magnesium (Mg) and molybdenum (Mo). This study is the first one of its kind performed on apple rootstocks.
6. Characterized in a collaborative study the influence of apple rootstocks on the expression of scion genes.

SELECTED HONORS

Co-awardee of the 2016 U.P. Hedrick award of the Journal of the American Pomological Society.

Journal of the American Society for Horticultural Science Outstanding Fruit Research Paper Award for the year 2014, awarded August 2015.

Federal Laboratory Consortium for Technology Transfer “Excellence in Technology Transfer” award winner in 2015 for “New Productive, Disease Resistant Apple Trees”.

North Atlantic Area Technology Transfer Award Winner in 2014 for development and commercialization of apple rootstocks critical for the U.S. industry.

SELECTED RECENT INVITED PRESENTATIONS in reporting period 2014-2018:

Utah State Horticulture Association Annual meetings Jan. 2017 Keynote “Overview of current rootstock technologies” and “Breeding methods for development of new apple rootstocks”

Kazakh National Agrarian University Dec. 2016 “Review current research on apple breeding” and “Apple rootstock technologies to aid Kazakh apple growers”.

International Fruit Tree Association Annual meetings Feb. 2016 “Rootstocks Matched to Varieties”.

Europa Fruit Tree Rootstock Consortium Aug. 2015 Angers, France. “Breeding and selection of Geneva® apple rootstocks”

University of Guelph, Department of Plant Agriculture Winter 2015 Seminar Series. “Leveraging genomic resources to breed a difficult perennial crop: apple rootstocks”

Ohio Produce Growers and Marketers (OPGMA) 2015 Congress. January, 2015, Sandusky, OH.
 “Updates from the National Apple Rootstock Breeding Program”

Washington State Horticulture Society Annual Meetings, Special Rhizosphere Symposium. Kennewick, WA. December 2014. “Optimal tree nutrition and fruit production begins underground – the apple rootstock story”

The Arsenal, Central Park, NYC Parks, New York, NY. October 2014. Title: “Importance of germplasm conservation in context with the loss of wild apple forests in Kazakhstan”

SELECTED PUBLICATIONS in reporting period 2014-2018

Fazio, G., 2017. Evaluating and improving rootstocks for apple cultivation.

<http://dx.doi.org/10.19103/AS.2016.0017.08> © Burleigh Dodds Science Publishing Limited, 2017.

Stuart Adams, Brent L. Black, Gennaro Fazio and Nicholas A. Roberts 2017. The Effect of Plant Growth Regulators on Apple Graft Union Flexural Strength and Flexibility. *J Am Pom Soc. (APS)* 71:8-18

Norelli JL, Wisniewski M, Fazio G, Burchard E, Gutierrez B, et al. (2017) Genotyping-by-sequencing markers facilitate the identification of quantitative trait loci controlling resistance to *Penicillium expansum* in *Malus sieversii*. *PLOS ONE* 12(3): e0172949.

<https://doi.org/10.1371/journal.pone.0172949>

T. Twirkoski, G. Fazio and D.M. Glenn 2016. Apple rootstock resistance to drought. *Scientia Horticulturae* 204:70-78.

T. Twirkoski and G. Fazio 2016. Hormone and growth interactions of scions and size-controlling rootstocks of young apple trees. *Plant Growth Regulation* 78:105-119.

S. Shin, P. Zheng, G. Fazio, M. Mazzola, D. Main and Y. Zhu 2016. Transcriptome changes specifically associated with apple (*Malus domestica*) root defense response during *Pythium ultimum* infection. *Physiological and Molecular Plant Pathology* 94:16-26.

Z. Migicovsky, K.M. Gardner, D. Money, J. Sawler, J.S. Bloom, P. Moffett, C.T. Chao, H. Schwaninger, G. Fazio, G.-Y. Zhong and S. Myles 2016. Genome to Phenome Mapping in Apple Using Historical Data. *The Plant Genome*.

Fazio G., T.L. Robinson and H.S. Aldwinckle 2015. The Geneva apple rootstock breeding program. *Plant Breeding Reviews* 39:379-424.

Twirkoski, T., Fazio, G. 2015. Effects of Size-Controlling Apple Rootstocks on Growth, Absciscic Acid, and Hydraulic Conductivity of Scion of Different Vigor, *International Journal of Fruit Science*, DOI: 10.1080/15538362.2015.1009973

Volk, G., Chao, C.T., Norelli, J., Brown, S., Fazio, G., Peace, C., McFerson, J., Zhong, G.-Y., and Bretting, P., 2015. The vulnerability of US apple (*Malus*) genetic resources. *Genetic Resources and Crop Evolution*:1-30

Zhu, Y., Fazio, G., Mazzola, M. 2014. Elucidating the molecular responses of apple rootstock resistant to ARD pathogens: Challenges and opportunities for development of genomics-assisted breeding tools. *Nature Horticulture Research*. doi:10.1038..

Fazio, G., Chao, C. T., Forsline, P. L., Richards, C., and Volk, G. 2014 Tree and root architecture of *Malus Sieversii* seedlings for rootstock breeding. *Acta Hort.* 1058:585-594

Fazio, G. 2014. Breeding apple rootstocks in the 21st century – what can we expect them to do to increase productivity in the orchard?. *Acta Horticulturae*. 1058:421-428.

Robinson, T., Fazio, G., Aldwinckle, H. 2014. Characteristics and performance of four new apple rootstock from the Cornell-USDA apple rootstock breeding program. *Acta Hort.* 1058:651-656.

Jensen P.J., Fazio, G., Altman, N., Praul, C., McNellis, T.W. 2014. Mapping in an apple (*Malus x domestica*) F1 segregating population based on physical clustering of differentially expressed genes. *BMC Genomics*. 15: 261

Fazio, G., Y. Wan, D. Kviklys, L. Romero, R.R. Adams, D. Strickland, and T.L. Robinson, 2014. Dw2, a New Dwarfing Locus in Apple Rootstocks and Its Relationship to Induction of Early Bearing in Apple Scions. *J Am Soc Hortic Sci* 139:1-12.

Gan-Yuan Zhong, Ph.D.

Research Leader/Supervisory Plant Geneticist, USDA-ARS, Grape Genetics Research Unit, Plant Genetic Resources Unit

Education:

- 1991 Ph.D. Genetics, University of California, Davis.
- 1985 M.S. Crop Genetics and Breeding, Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, Beijing, China
- 1982 B.S. Agronomy, Jiangsu Agricultural College, China

Professional Experience and Research Accomplishments:**January 2010 – Present, Research Leader, USDA-ARS, Plant Genetic Resources Unit**

- Characterized fruit composition and content of polyphenolic compounds in the USDA-ARS *Vitis* germplasm
- Co-led an effort in genotyping USDA-ARS apple and grape collections and mapping populations using the SNP chips and genotyping-by-sequencing techniques.
- Contributed to various USDA-ARS NPGS missions and activities, particularly those relevant to the USDA-ARS Geneva genetic resources.

May 2007 - Present, Research Leader, USDA-ARS, Grape Genetics Research Unit

- Demonstrated genome-scale mRNA transmission between scions and rootstocks in grapevine
- Determined the likely genetic and molecular basis for several fruit quality traits of grapes
- Investigated genetic factors controlling resistance to root-knot nematodes and evaluated the feasibility of developing a transgenic solution to root-knot nematodes in grapevines
- Investigated the genetic and molecular mechanisms controlling grapevine plant architecture

2004 - 2007, Senior Research Scientist, Pioneer Hi-Bred International, Inc. A DuPont Business

- Developed and implemented molecular markers and other molecular breeding technologies in maize research and product development
- Conducted genetic dissection of complex traits in maize
 - Developed and implemented molecular characterization tools for maize transgenic trait and technology development

1995 – 2004, Research Manager/Scientist, Pioneer Hi-Bred International, Inc.

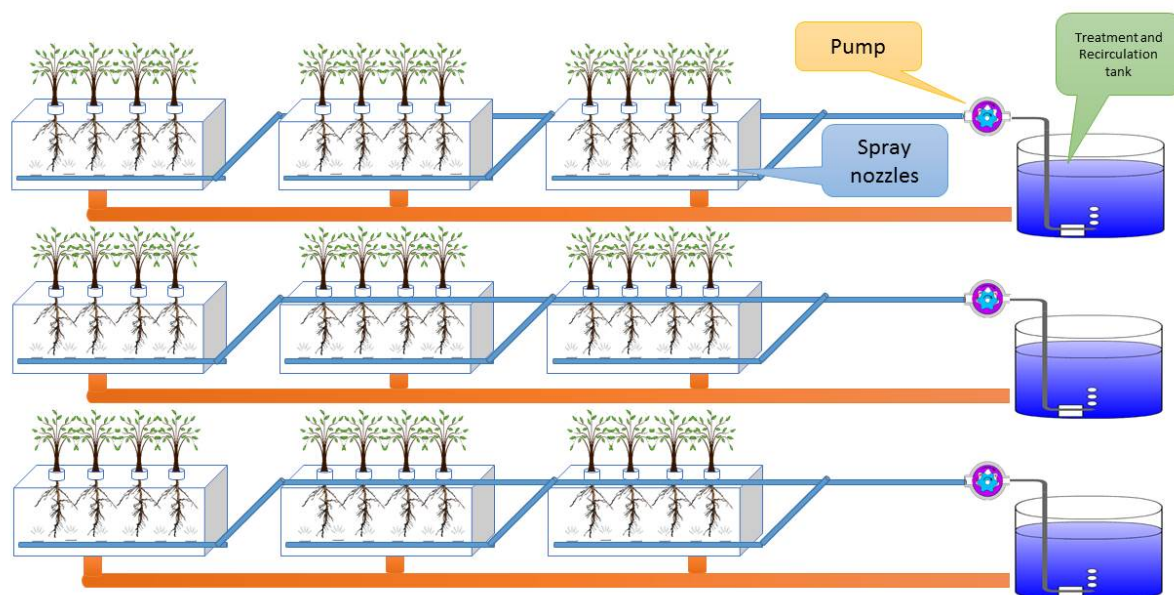
- Led a Transgene Genetics Project for research on transgene silencing and position effect of transgenes in maize
- Carried out QTL mapping of seed quality traits in maize
- Managed product development process, regulatory compliance, and field operations for transgenic maize product development

Recent publications

- Fresnedo-Ramírez, J., Sun, Q., Hwang, C. F., Ledbetter, C. A., Ramming, D. W., Fennell, A. Y., M. Andrew Walker, James J. Luby, Matthew D. Clark, Jason P. Londo, Lance Cadle-Davidson, Gan Yuan Zhong, Reisch, B. I. 2016. Toward the elucidation of cytoplasmic diversity in North American grape breeding programs. *Molecular Breeding*, 36(8), [116]. DOI: 10.1007/s11032-016-0538-z
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- Volk, G.M., Chao, C.T., Norelli, J.L., Brown, S.K., Fazio, G., Peace, C., McFerson, J., Zhong, G., Bretting, P.K. 2015. The vulnerability of US apple (*Malus*) genetic resources. *Genetic Resources and Crop Evolution*. 62(5):765-794. DOI: 10.1007/s10722-014-0194-2.
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- Cousins, P., Zhong, G. 2015. Hybrid and selfed seedling progenies of *Vitis* hybrid 'Roger's Red' grape segregate for tendril distribution. *Acta Horticulturae Proceedings (ISHS)* 1082:373-378.
- Yang, Y., Labate, J.A., Liang, Z., Cousins, P., Prins, B.H., Preece, J.E., Aradhya, M.K., Zhong, G. 2014. Multiple loss-of-function 5-O-Glucosyltransferase alleles revealed in *Vitis vinifera*, but not in other *Vitis* species. *Theoretical and Applied Genetics*. 127(11):2433-2451.
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- Wan, Y., Schwaninger, H.R., Baldo, A.M., Labate, J.A., Zhong, G. Simon, C 2013. A phylogenomic analysis of the grape genus (*Vitis*) reveals broad reticulation and concurrent diversification during quaternary climate change. *BMC Evolutionary Biology*. 13:141.
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Appendix B – Additional Figures.



Appendix Figure 1. Schematics of aeroponics system built in Geneva NY in 2017. We plan to expand capabilities of these systems or build new ones with additional sensors.



Appendix Figure 2. Ability to study root systems without the encumbrance of soil is one of the advantages of growing apple roots in air.

Treatments and Diagnostics Possible with Aeroponics

Integration with robotics will allow multiple automated diagnostics and treatments

- Mineral nutrients
- pH
- Temperature (cold or heat shock) with the addition of a transducer
- Plant growth regulators
- Root pathogens
- Salinity
- Drought
- Easy access roots – RNA, gene expression
- Root growth
- Architecture
- Genotype specific root metabolites
- Disease resistance
- Nutrient induced architecture
- Root gas exchange and respiration

Appendix Figure 3. Examples of treatments and diagnostic measurements possible with aeroponic systems.

Lower Chromosome 12 (Mbp)

LG12R5

15.8 CH01g12
17.7 GD5
22.6 CH01R02
23.2 C18022
23.4 A12305
23.5 A4850
23.6 A02331
23.7 A10720
23.8 A21525
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Validate outcome on existing phenotyped populations and older datasets using good statistical methods

301 Fazio 8060-21000-026-00D Post Plan