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Apple Rootstocks: History, Physiology, Management, and Breeding

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ABSTRACT

For more than two millennia, superior fruit tree genotypes have been grafted onto rootstocks to maintain the genetic identity of the desirable scions. Until the 20th century most fruit trees were grafted onto seedling rootstocks. Following the classification, evaluation, and propagation of clonal rootstocks during the early 1900s, dwarfing rootstocks became important to the commercial apple industries. Although trees on dwarfing rootstocks are more economical to maintain, and are more precocious and productive than trees on seedling rootstocks, there remains a need for dwarfing rootstocks to be adapted to different growing conditions. During the past 100 years, considerable effort has been made to understand the physiological changes in the scion induced by rootstocks. More recently, molecular techniques have been utilized to identify the genes that control interactions between scion and rootstock. Modern rootstock breeding programs are combining molecular and traditional techniques to develop rootstocks that are dwarfing, productive, and tolerant to biotic and abiotic stresses. In this chapter, the history, development and current use of apple rootstocks, the current understanding of rootstock–scion interactions, and current efforts to develop and evaluate superior rootstocks are discussed.

KEYWORDS: *Malus* × *domestica*, dwarfing, Malling, roots, rootstock–scion interaction

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I. INTRODUCTION

Apple (*Malus* × *domestica*) originated in central Asia and is the most widely grown tree fruit. Apples are heterozygous and do not come true from seed, and apple cuttings are difficult to root. Therefore, to maintain genotypes with desirable characteristics, rootstocks have been used as a

means of propagating fruit trees for more than 2000 years (Roach 1985). Until about 80 years ago, most trees in commercial apple orchards around the world were propagated on seedling rootstocks. Trees on seedling rootstocks were easy to produce, relatively inexpensive, free-standing and performed fairly well on a wide range of soil types and in most climates. Although dwarfing rootstocks have been available for at least 2000 years, attempts to grow trees commercially on these rootstocks usually failed. During the 20th century, the economics of the fruit industry demanded uniform, relatively small, and productive trees. Dwarfing clonal rootstocks offered the potential to satisfy these demands, but these were not widely adopted until researchers developed orchard systems that could take advantage of the beneficial characteristics while minimizing the weaknesses of dwarf rootstocks. The precocity of dwarfing rootstocks caused the leader on young trees to bend and trees to fall over or break. This prevented trees from developing canopies large enough to sustain high yields as the orchard matured. The development of tree support systems, coupled with new tree training techniques, has allowed commercial fruit growers to take advantage of the superior productivity of dwarfing rootstocks, and is responsible for yields per hectare that are more than double those of just 25 years ago. All currently available dwarfing rootstocks have weaknesses, however, such as weak graft unions, propagation difficulties, and susceptibility to biotic and abiotic stresses. The aim of this chapter is to provide an overview of the voluminous work on apple rootstocks that has been conducted and published during the past century, and to identify potential areas for future research.

II. HISTORY

A. Europe

Apple cultivars are difficult to root and do not come true from seed; consequently, for more than two millennia superior apple genotypes have been maintained by grafting or budding onto rootstocks. Until the mid-20th century, seedling rootstocks were most common. It is thought that in the 4th century B.C., Alexander the Great sent to Greece a dwarf self-rooting apple, which later became known as Paradise rootstock (Tukey 1964). For more than 2000 years the use of Paradise was limited to gardens, and it was mentioned in various books as a method for growing small trees that were precocious. Tukey (1964) also described another rootstock called Doucin or English dwarfing stock as only slightly

dwarfing and no more precocious than seedling. During the first half of the 19th century, Dutch Paradise and Doucin were being recommended in England (Loudon 1822; Lindley 1846; Barry 1851) and America (Thomas 1859) for certain cultivars, and especially for espalier training of apple trees to reduce tree size and to induce annual bearing. The rootstock Paradise was described as causing early bearing (Cole 1858), but in his book *American Pomology*, Warder (1867) described the French Paradise dwarfing rootstock and recommended it for gardens only, because it was "…wholly unsuited for orchard planting". By 1914 there was an account of an orchard in eastern Massachusetts, USA with 600 dwarf trees on Doucin stock (Anonymous 1915).

During the late 1800s, San Jose scale (*Quadraspidiotus perniosus*) was a serious pest in North American orchards. Controlling the insect required spraying insecticides or fumigating trees with cyanide gas, and large trees were difficult to treat. Beach (1902) suggested growing dwarf trees on Doucin or Paradise in commercial orchards to facilitate scale control. He noted that these rootstocks were shallow-rooted and required fertile soil, but such trees were more precocious than trees on seedling rootstocks. Beach cited S.T. Wright, of the Royal Horticultural Society, as indicating that commercial production of Paradise was increasing in England, and that the most profitable systems of apple culture utilized dwarf trees because they obtain early returns, orchard work can be performed from the ground, trees are less injured by wind, and interior fruit can be thinned out. It was recommended that dwarf trees be planted 3 m × 3 m (1074 trees per hectare) and such mature orchards could be expected to produce 4700–9500 kg ha^{-1}, whereas standard trees would produce 13 000 kg ha^{-1}. However, lime sulfur sprays soon became available to effectively control scale and American interest in dwarfing rootstocks diminished.

In Europe during the late 1800s and early 1900s, nonuniformity of trees was a problem, with variable tree performance having a negative economic impact on commercial fruit production and also hampering research efforts (Hatton 1917). At that time, seedling rootstocks were referred to as Crab regardless of origin, and those obtained by vegetative propagation (layers) were called Paradise. A second clonal rootstock had been described in France as Doucin, but commercially this became known as English Paradise. An 1869 article in the *Gardeners Chronicle* indicated there was confusion about the identity and nomenclature of various forms of Paradise rootstocks, and new seedling "Paradise" stocks were being grown and tested by several nurserymen (cited by Hatton 1917). In England there were at least five rootstocks with the name Paradise, but with different characteristics. By the late 1800s,

names for these dwarf rootstocks included "Paradise," "French Paradise," 'English Paradise," and "Doucin."

Bunyard (1920) attempted to trace the history of Paradise rootstock and explained the confusion. He felt that there were several dwarfing rootstocks that were collectively known as "Paradise." He divided this collection into two groups: "French Paradise" was the true dwarf and "Broad-leaf Paradise" was not the true dwarf, but was probably a dwarf seedling that rooted easily from cuttings. "Doucin" was one of these dwarfing rootstocks that rooted from cuttings. As rootstock mixtures was suspected to be at least one of the factors contributing to tree variation in the orchard, in 1912 R. Wellington and R.G. Hatton developed a rootstock collection at East Malling, England for evaluation and categorization. Some 71 collections of Paradise were obtained from 35 nurseries in England, Holland, France, and Germany. In most cases, Wellington and Hatton received 12 plants from each nursery. Nursery observations of plant phenology, leaf and bark characteristics, and shoot length during the first year indicated that there was variation within and between nurseries. Over the next few years, additional vegetative characteristics, such as the presence of burrknots and root suckers, date of leaf abscission, tree size, fruiting, fruit characteristics and growth habit allowed segregation of the samples into nine types, designated as Type I to Type IX, later designated as (East Malling) EM I–EM IX. It was suspected that the confusion in the trade had resulted from mislabeling in the nursery and the development of sports from mutations. There was also variation among Crab rootstocks, probably due to different parentage (Hatton 1920a). To evaluate the influence of these rootstocks on scion growth and productivity, the first apple rootstock trial was established at East Malling in 1919, with the scion cultivar 'Lane's Prince Albert' grafted onto 16 clonal rootstocks. In 1920, additional trials were established on four different soil types. Over the next 15 years Hatton (1927, 1935) summarized the results of these trials. First, he noted that the rootstock exhibited a greater influence on the scion than had previously been thought. Trees on EM IX and EM VIII were very small, but the other 14 rootstocks created a continuum of tree sizes, and trees on EM XII were the largest. In addition to tree size, rootstocks also influenced flowering and fruit set, and there was a negative relationship between tree vigor and cropping. However, two rootstocks of similar vigor sometimes differed in cropping. Hatton also noted that trees on EM IX produced larger fruit than trees on EM I. Although the science of statistics was quite young, Hatton used standard deviations to estimate the number of single-tree-replicates that would be required in future experiments to detect differences between two rootstocks.

He estimated that nine replications would be needed to detect a 30% difference in total wood growth, which is similar to recent estimates. Using data from multi-location rootstock trials, Marini (unpublished results) estimated that 10 replications are needed to detect a 23% difference in trunk cross-sectional area at the 5% level of significance.

The identification of a set of rootstocks capable of inducing such a wide range of characters on the scion allowed researchers to use dwarfing rootstocks as a tool to study various aspects of apple tree growth and physiology. In the 1923 East Malling Annual Report there was mention of 17 trial-acres (6.9 ha) of apple trees of 11 cultivars on selected rootstocks. They learned that the rootstock effect was similar with different cultivars on a given soil. Recently, results from a multi-location trial in North America verified the lack of a rootstock × cultivar interaction (Autio *et al.* 2001).

During the 1970s the nomenclature for EM rootstocks changed to M, and Arabic numbers replaced Roman numerals. For example, over the years Type IX changed to EM IX, to M IX, and is currently M.9. During the 1950s to 1970s, the Malling stocks were heat-treated to eliminate known viruses, with the designation East Malling-Long Ashton (EMLA), so the virus-free M.9 is designated M.9 EMLA. The non-treated rootstocks sometimes referred to as "dirty," were usually slightly less vigorous. To eliminate confusion, in this chapter the designations M. (Malling) and MM. (Malling-Merton) will be used while discussing rootstocks released from the first two programs at East Malling.

After classifying the known rootstocks, a joint breeding program was initiated by M.B. Crane of the John Innes Horticultural Institute at Merton and H.M. Tydeman at East Malling (Preston 1956). The resulting Malling-Merton (MM) rootstocks were bred for resistance to woolly apple aphid (WAA; *Eriosoma lanigerum*), which was a common problem in North America and especially in New Zealand. The East Malling clones were crossed with the WAA-resistant 'Northern Spy' and evaluated for WAA-resistance and propagation characteristics (Le Pelley 1927). Crossing 'Northern Spy' with susceptible cultivars produced resistant progeny, and 'Northern Spy' was heterozygous for the trait. This was one of the first truly interdisciplinary fruit research programs, involving both pomologists and entomologists. Of the resulting 3500 seedlings, 15 were selected and numbered Malling-Merton 101–115, but later were renamed MM.101–115. The resulting MM series was described by Preston (1953), and details of their behavior in the nursery, along with photographs of leaves, buds and stems, were published by Tydeman (1952). The clones varied in vigor, but none was as dwarfing as M.9. The clones were then tested abroad (Preston 1955).

A second set of new clones was bred by Tydeman at East Malling to increase the range of vigor and productivity (Tydeman 1933, 1934). The seedlings had M.9 as a common parent and were crossed with other Malling rootstocks. Of the 1000 seedlings, 18 clones were selected for field trial, and an inverse relationship was reported between rootstock vigor and degree of root suckering.

Another group of rootstocks was developed by the English Cider Institute (later the Long Ashton Research Station) as desirable rootstocks for cider apples. Little information is available on these rootstocks, but LA G-6, LA G-7, and LA G-8 were imported by American researchers for evaluation (Ritter and Tukey 1959).

B. North America

By the 1930s there was interest in the United States for smaller trees for economic reasons and because the turnover rate of commercial apple cultivars was becoming more rapid (Tukey and Brase 1939b; Zeiger and Tukey 1960). Tree variability also hindered apple research in North America. During the 1920s, most rootstock trials in North America utilized clonal material imported from the East Malling Research Station (Shaw 1935). In 1920, R.D. Anthony attempted to reduce tree-to-tree variation in his research orchard by importing M.12 for a nutrition study in Pennsylvania (Anthony and Thomas 1923; Anthony 1927). Several years later, additional Malling stocks were imported for orchard trials in Massachusetts, West Virginia, Pennsylvania, Vermont, and New York. In the 1920s, several test orchards on vegetatively propagated roots were established in Pennsylvania (Anthony 1927). In 1927, five apple cultivars on four Malling stocks were planted in trials in Ontario, Canada (Ferree and Carlson 1987). Federal quarantine stopped the importation of rootstocks in 1931, which altered the sources of commercial rootstocks for American nurseries. Seedlings from domestic cultivars replaced French crab rootstocks (Anthony 1946). When the importation of plant material was terminated, there was interest in producing clonal rootstocks in North America, and Tukey and Brase (1935) reported on methods for propagating clonal rootstocks and also described nursery characteristics of M.1–M.16; M.10–M.16 were unnamed (Tukey and Brase 1939a). Based on observations of six-year-old trees, Sudds (1939) concluded that M.2 was worthy of further trial, but M.9 had no commercial potential except for certain special purposes. In 1959, Ritter and Tukey (1959) summarized American experiences with Malling

and Malling Merton rootstocks and with 75 scion–rootstock combinations, including some Long Ashton rootstocks.

Dwarfing rootstocks were not commercially important in North America until the mid-1990s. In fact, pomology text books written before 1960 did not even include the term "rootstock" in their indices. During the 1960s through the 1980s, researchers and commercial growers attempted to grow apples on dwarfing rootstocks with varying degrees of success. Commercial growers had experience with seedling or vigorous clonal rootstocks (mostly M.4, M.7, MM.111, and MM.106) that did not require tree support. They wanted to grow free-standing or non-supported trees because most felt that tree support could not be economically justified. When trees on dwarfing rootstocks were not supported, tree loss was high due to trees falling over, and the short trees caused by leader bending produced low yields. Commercial use of dwarf rootstocks was limited to low-trellis systems, primarily for pick-your-own operations.

By the early 1980s, M.9 had been an important rootstock in Europe for more than 40 years (Vyvyan 1955; Webster 1984), whereas most North American apple growers preferred free-standing central leader trees on vigorous seedling rootstocks or the semi-dwarf M.7, MM.106, and MM.111. Resistance of North American growers to adopt dwarfing rootstocks probably occurred because, unlike in Europe, land and labor were relatively plentiful and inexpensive. Although M.9 was the most popular rootstock in Europe, it had weaknesses, including a lack of winter hardiness, it was difficult to propagate, and the trees required support. In an attempt to overcome some of these problems, European nurserymen and researchers selected clones of M.9, with varying effects on the scion. In North America, dwarfing rootstocks did not become important until the mid-1990s when research from a multi-location trial coordinated by the NC-140 project showed that the vertical axis system (Lespinasse 1980), utilizing dwarf rootstocks, was more profitable than the traditional central leader using semi-dwarf rootstocks (Marini *et al*. 2001c; Marini and Barden 2004). By 2005, the North American apple industry was well on the way to transitioning to intensive orchards utilizing the principles of the vertical axis system with dwarfing rootstocks, such as M.9, B.9, O.3, and M.26.

III. ROOTSTOCK–SCION INTERACTIONS

Interaction between the scion and rootstock genotypes is complex because the root system provides the scion with water, hormones and nutrients, whereas the root requires assimilates, hormones, and other compounds from the scion.

Rootstock can have profound effects on the scion, such as precociousness, flowering intensity and cropping of young trees, as well as tree size, performance in various soils, and tolerance to biotic and abiotic stresses. Recently, grafting has been used to improve productivity in herbaceous plants, and this has stimulated research activity to understand mechanisms underlying the phenotypic variability resulting from rootstock × scion × environment interactions (Albacete *et al.* 2015). During the past decade considerable research has been carried out on the interaction of scion and rootstock in vegetable crops. The physical and biochemical (Marínez-Ballestra *et al.* 2010), hormonal crosstalk between the two plant parts (Aloni *et al.* 2010), and the molecular aspects of these interactions (Kanehira *et al.* 2010; Koepke and Dhingra 2013) have been reviewed for herbaceous plants. At the molecular level, scion–rootstock interactions may be similar for woody and herbaceous plants, but the discussion here will concentrate on research with apple.

A. Influence of Rootstock Roots versus Rootstock Stems

Before rootstock research was stimulated by the classification of the Malling rootstocks, the scion was thought to have a greater influence than the rootstock on the growth and productivity of the tree. Hatton (1920a) was the first to report that the rootstock influenced tree vigor and precocity, and these observations were confirmed by Tydeman (1928). Hatton also found a negative relationship between vegetative vigor and cropping, but rootstocks of a similar vigor sometimes varied in their effects on cropping, flowering, and fruit set. There was disagreement on how much the scion affected the roots, and how much of the scion response was due to the roots versus the rootstock stem piece. Much of this confusion was because American researchers were working with seedling rootstocks and English researchers were working with the newly classified clonal Malling rootstocks. Roberts (1929, 1931a,b) found that the scion greatly influenced the seedling roots when grafted onto pieces of 1-year-old root, but there was little scion effect when budded onto the stem of the seedling rootstock. Roberts concluded that the rootstock influence on the scion came from the stem of the rootstock rather than the roots, and this was verified by Grubb (1939), who reported that the length of a dwarfing interstem increased the dwarfing effect on the scion. However, dwarfing rootstocks influenced scion growth even when the scion was grafted onto a piece of root in the absence of the rootstock stem (Hatton 1931). The scion cultivar had little influence on root depth, and the proportion of fine roots versus coarse roots was affected primarily by rootstock (Hatton *et al.*

1924). Shallow-planted trees on dwarf rootstocks grew slower than deep-planted trees (Hatton *et al.* 1924). Using dwarfing rootstocks as interstems to study dwarfing, Vyvyan (1938) and Knight (1927) found that roots were most important, but the rootstock stem also contributed to dwarfing and the rootstock had a greater effect on the scion when used as a rootstock rather than as an interstem. The interstem did not affect the morphology of the root system. Inarching of vigorous rootstocks to trees on dwarfing rootstocks also enhanced the vigor of the trees (Hearman *et al.* 1937).

As late as the 1950s the relative importance of the scion and rootstock on scion growth characteristics was still being debated. To study the relative influence of the rootstock and scion, Vyvyan (1955) used three rootstock genotypes as the scion and rootstock in reciprocal grafts to obtain nine scion/rootstock combinations. He periodically harvested trees over three years. The ratio of the mean weight of trees with reciprocal "unlike" unions (M.9/M.4 and M.4/M.9) to that of "like" unions (M.9/M.9 and M.4/M.4) was consistently about 0.9, and deviation from unity (1.0) was never significant, indicating that the relative growth rates of composite trees are primarily controlled by the scion genotype. However, rootstock had a greater influence on scion genotype than the scion had on the rootstock, because the size of the composite tree was more similar to the genotype used as the rootstock.

Studying the influence of the scion on the root system is difficult and requires the complete excavations of mature trees (Rogers and Vyvyan 1928; Rogers and Vyvyan 1934; Beakbane and De Wet 1935). In general, when budded to scion cultivars with different growth characteristics, the morphology of the root systems of Malling clones remained constant. Additionally, for rootstocks varying in vigor, the stem:root weight ratio remained constant for a given soil type. After nearly 35 years of work, the general conclusion was that for clonal rootstocks, the rootstock root has the greatest influence on scion growth and precocity, but the rootstock stem enhanced the influence of the root and they can also be slightly modified by the scion. Using dwarfing rootstocks as interstems or bark rings can also influence the scion, but to a lesser degree than when the scion is budded onto the rootstock.

B. Influence of Rootstock on Scion Growth

Before there was much research on rootstocks, dwarfing rootstocks were thought to influence tree vigor by starving the scion of mineral nutrients obtained from the soil, or by partially inhibiting the translocation of photosynthates (Beakbane 1956). Root systems of dwarf rootstocks were

thought to be shallower and to occupy a smaller volume of soil relative to their canopy size. Rogers and Vyvyan (1928, 1934) found that trees on the vigorous M.1 had more upright branches and a root system with greater spread than trees on M.9, while M.9 tended to have a more one-sided root system. However, trees on M.9 had roots that were deeper, with about 70% of the roots below 33 cm, compared to only 27% for M.1. The total root mass was about twice as great for M.1, the fruit:root ratio was twice as high for M.9, but the ratio of stem:root was similar for both rootstocks, as was the proportion of fine roots compared to coarse roots. For a given scion cultivar on a given soil, the stem:root ratio was similar for rootstocks varying in vigor, and the root systems were similar regardless of the scion for trees that were root-grafted onto piece-roots or stem-grafted (Vyvyan 1934; Rogers and Vyvyan 1934).

Later, Rao and Berry (1940) found that carbohydrate concentrations were higher in scions on dwarfing than on vigorous rootstocks. Rootstock can also influence the growth habit of the scion. Seleznyova *et al.* (2003) found that 'Royal Gala' grafted on M.9 had fewer nodes per shoot, and that the initiation of new metamers in scions grafted onto M.9 ceased soon after bud break; the reduced number of nodes resulted in fewer axillary annual shoots during the year, and this growth pattern accumulated.

Although dwarfing rootstocks have been used in commercial apple production for decades, the mechanisms by which they affect various aspects of tree development are still being studied. Until recently, pomologists have used trunk cross-sectional area and branch cross-sectional area (Westwood and Roberts 1970; Moore 1978) to characterize the influence of rootstock on tree growth because they are related to shoot length (Hirst and Ferree 1995) and above-ground tree mass (Barden and Marini 2001b). As trees and branches aged, the number of nodes and length of annual shoots declined, but a rootstock may affect some cultivars more than others (Costes and Garcia 2001). As trees aged over a 5-year period, the number of laterals per annual shoot declined but was similar for trees on M.7 and M.9. However, the number of long laterals and the cross-sectional area of annual shoots were always greater for M.7, and the number of short laterals was always greater for M.9. During the first year after grafting, dwarfing rootstocks suppressed tree size by altering the development of axillary meristems, which affected the type of growth that occurred the following season. Compared to the vigorous M.793 during the first season, M.9 decreased the number, length and node number of sylleptic shoots and/or increased the proportion of floral buds the second season. In another study, trees with many flower buds had fewer and shorter vegetative shoots the following year (Seleznyova *et al.* 2008; Foster *et al.* 2014).

Plant architecture is a relatively new tool for evaluating genetically controlled plant growth (Hallé *et al.* 1978; Kenis and Keulemans 2007). This tool utilizes the metamer as the basic unit of plant structure, consisting of a node, a leaf, an axillary bud, and subtending internodes (White 1979). The addition of metamers results in shoot (axis) extension. A group in New Zealand is using architectural analysis to study the influence of rootstocks and interstems on tree structural development and vigor (Selenznyova *et al.* 2003, 2008). M.9 reduced vigor of vegetative annual shoots and induced an earlier transition to flowering by increasing the number of less vigorous floral axillary annual shoots, resulting in fewer annual shoots with extension growth units along the trunks. The vegetative growth units on the M.9 rootstock had fewer nodes and shorter internodes than trees on MM.106. The number of extension growth units, vegetative spurs and fruiting spurs per annual shoot changed over a 3-year period, but they were not affected by the rootstock (Selenznyova *et al.* 2003). Information from these studies may be useful for rootstock breeders to evaluate the dwarfing potential and precociousness of rootstock selections during the first one or two seasons after grafting. Recent research has demonstrated that effects of rootstock on tree architecture are also influenced by environmental conditions. Shoot length, and the numbers of nodes and sylleptic shoots of 1-year-old trees of a given rootstock–scion combination varied when grown at two locations in New Zealand (Seleznyova *et al.* 2008). In another study, 'Royal Gala' was grafted onto M.27, M.9 and M.793, and grown in containers at three locations, with the experiment being repeated for two years to obtain a range of environmental conditions (Foster *et al.* 2016). Total growth and aspects of the canopy architecture were influenced by rootstock, temperature, and wind, but there was little interaction between rootstock and location. It was concluded that early growth cessation on dwarfing rootstocks was primarily under genetic control, whereas other traits, such as sylleptic shoot growth, flowering and final dry weight, were controlled by the combination of rootstock and growing conditions. The type of scion bud (vegetative versus floral) strongly affected growth; trees developing from vegetative buds had final dry weight 15–45% greater than for trees developing from a floral scion bud. The rootstock-induced effect that was most consistent across locations and years was that dwarfing rootstocks induced an earlier cessation of the primary growth axis.

Data reported by various researchers are often difficult to compare and interpret due to differences in methods. Care must be taken to standardize controls, propagation methods, and rootstock and interstem lengths, planting depth and environmental conditions, because

these factors can influence the growth of the scion (Wertheim 1998). Some researchers compared own-rooted trees to trees on dwarfing rootstocks (Lauri *et al.* 2006), but the lack of a graft union may have altered the growth of the own-rooted trees. Many rootstock experiments aiming to compare rootstock effects on scion architecture, flowering and cropping do not use appropriate controls. The preferred control would be an own-rooted scion genotype grafted with the same genotype as a scion. Since dwarfing rootstocks initially modify scion architecture in either the first (Rao and Berry 1940; van Hooijdonk *et al.* 2010) or the second (Tukey and Brase 1943; Seleznyova *et al.* 2008) year of growth, it would be advantageous for rootstock physiologists and breeders to develop protocols for testing new rootstock selections for size control and precocity.

C. Mechanisms of Stock–Scion Interaction

Over the years, several theories have been suggested to explain the influence of rootstocks on the growth and development of the scion. These theories involve root system size, hormones and other compounds, carbohydrate distribution, hydraulic conductivity and water relations, the anatomy of the graft union, and mineral uptake and allocation. Many of these proposals have been reviewed (Rogers and Beakbane 1957; Tubbs 1973; Lockard and Schneider 1981; Jones 1986), but no hypothesis has totally explained the observed rootstock effects on scion growth and cropping. In a recent review, Koepke and Dhingra (2013) cited a review by Webster (2004), where the influences of rootstock on apple tree growth were discussed along with the potential mechanisms that were essentially the same as proposed 40 years earlier, indicating that little progress had been made towards understanding how rootstocks influence the scion. In many of the studies intended to test these hypotheses, variables such as water relations, assimilates or hormones were measured above and below the graft union, but tree growth was often not measured. In future experiments, some aspects of growth should be measured. One growth variable that is consistently affected by dwarfing rootstocks is early shoot growth cessation; hence, repeated shoot length measurements to determine the date of growth cessation may be a good indicator of dwarfing.

1. Rootstock Anatomy and Morphology. Beakbane (1952) reviewed the literature on anatomic differences of apple rootstocks and roots. In general, the bark:root ratio of young apple trees was related to mature

tree size. The morphology and anatomy of both seedling and Malling clones were not affected by the scion (Vyvyan 1930). Dwarfing rootstocks have higher ratios of bark:wood, but similar ratios of stem:root, have highly parenchymatous xylem and phloem, and contain more living tissue per unit volume of stem and root than vigorous rootstocks (Beakbane 1941; Beakbane and Thompson 1939, 1945). Therefore, the metabolic activity of dwarfing rootstocks per unit volume of tissue is likely greater than for vigorous rootstocks (Rogers and Beakbane 1957). The latter authors hypothesized that a relatively greater proportion of total assimilates would be utilized by the roots of dwarfing rootstocks than vigorous rootstocks, but Hassan (1953, cited by Rogers and Beakbane 1957) found that the rate of respiration per unit of living tissue was higher for vigorous than for dwarfing rootstocks.

The relative proportions of storage to conducting and strengthening tissues in stems and roots were related to the precocity and productivity of the tree. Compared to vigorous rootstocks, dwarfing rootstocks had more living and less conducting and strengthening tissue per unit of root volume. The size of conducting elements was usually positively related to vigor (Beakbane 1941). Therefore, different rootstocks likely vary in their capacity to absorb, store, and utilize nutrients. To study phloem transport in dwarf apple trees, Dickson and Samuels (1956) supplied radioactive phosphorus through a leaf petiole of 3-year-old trees that had been dwarfed by inverting two rings of bark the previous year. The isotope accumulated above the bark inversion, and phloem transport to the roots was reduced. The authors then performed a similar experiment with 5-year-old trees with a M.9 interstem, and the isotope accumulated in the interstem. It was hypothesized that both phloem and auxin transport are retarded in the dwarfing interstem. It is difficult to interpret these results because the authors did not report at what point during the growing season the experiment was conducted. Translocation of the isotope would likely be influenced by the growth stage of the tree (early- versus late-season). In general, vigorous rootstocks have larger xylem vessels, and this supports the concept that dwarf rootstocks may reduce the flow of assimilates and other compounds between the roots and scion. The effect of the xylem on water flow is discussed in the section on water relations (see below). Based on the research with inverted bark rings and phloem-blocking viruses, Rogers and Beakbane (1957) concluded that it seems more likely that phloem transport, rather than xylem transport, would be a limiting factor in rootstocks and interstems.

Simons and Chu (1980) reported that the outer bark of dwarfing rootstocks was thicker than that of semi-dwarfing rootstocks, and

60% of this bark was non-functional phloem. Also, vascular tissues that developed between the rootstock and scion were arranged in a swirling pattern and became necrotic during growth of the tree. More recently, Soumelidou *et al.* (1994) found that the xylem of M.9 linking the bud to the rootstock contained fewer and smaller vessels than for MM.106, and this difference could reduce supplies of water and dissolved nutrients to the scion. These authors hypothesized that hormones, possibly auxin, might be involved in the differentiation of callus cells into vessel elements in the callus between the bud and rootstock. More recent research with herbaceous plants seems to support the role of auxin in graft union formation. At the scion–rootstock interface of a grafted plant, cells expand and divide to form callus, which is undifferentiated stem-cell-like tissue (Sugimoto 2010). Callus tissues surrounding the cut then differentiate to phloem and xylem, and vascular strands connect the scion and rootstock. Eventually, a common cell wall forms between the scion and rootstock and plasmodesmata form across the cell wall. Callus proliferation and cell differentiation continue such that eventually vascular connections are re-established (Melnyk 2016). Yin *et al.* (2012) proposed a model for graft-union developmental stages, and suggested that there is communication between the scion and rootstock resulting in auxin accumulation; the auxin then regulates cell division and differentiation. For apple, Soumelidou *et al.* (1994) reported that the vessels in M.9 in the first year were larger than for MM.106, but smaller the following year. The authors suggested that the difference was due to the level of auxin reaching the bud. Auxin had difficulty crossing the graft interface, and the local accumulation of auxin resulted in highly parenchymatous, abnormal xylem in the rootstock below the bud. The level of auxin was inadequate for true xylogenesis, but the basipetal flow of auxin is unhindered.

2. Assimilate Production and Distribution. During autumn, photoassimilates are translocated out of the leaves to the roots and other woody organs, where they are converted to starch; these reserve carbohydrates are utilized for spring growth (Priestley 1960). Late-season modifications in the carbohydrate status of the tree can affect growth the following spring (Abusrewil *et al.* 1983). Based on experiments with container-grown fruiting and non-fruiting trees, Avery (1970) hypothesized that trees on dwarfing rootstocks are smaller because they produce fewer growing points which continue extension for a shorter period, and are unable to fully utilize photosynthates for growth because of a limiting rate of growth by the root system. The hypothesis

that late-season differences in stored assimilates influence vegetative growth the following season may not be valid, because summer pruning that was severe enough to suppress late-season trunk and root growth did not influence the shoot growth of field-grown or container-grown trees the following season (Marini and Barden 1982a,b).

Research results on the influence of rootstocks on photosynthesis are conflicting. Gregory (1957) reported higher net assimilation rates for vigorous rootstocks over the whole season compared to dwarfing rootstocks, primarily because photosynthetic activity continued later in the season for vigorous stocks. Ruck and Bolas (1956) grew four rootstocks of varying vigor in three types of soilless media with varying pH and found that the vigorous Crab C had higher photosynthetic rates than M.9 at low nitrogen, but at higher nitrogen levels the differences were less pronounced. Ferree and Barden (1971) reported that net photosynthesis (Pn) of container-grown trees on seedling rootstocks was higher than for trees on MM.106. However, in a similar study, Barden and Ferree (1979) found that Pn and dark respiration were unaffected by rootstocks. Schechter *et al.* (1991) found that Pn was higher for field-grown cropping trees on vigorous than on dwarfing rootstocks. Baugher *et al.* (1994) reported that Pn was greater for mature trees on M.7 and MM.111 than on M.9, but Fallahi *et al.* (2002) reported that mature trees on M.9 had higher Pn and transpiration rates than trees on M.7. These results are difficult to interpret, because crop density was not reported and photosynthesis is related to crop load (Palmer *et al.* 1997). Working with 25 rootstocks, Tworkoski and Fazio (2011) reported that photosynthesis and transpiration of 1-year 'Fuji' on dwarfing rootstocks were lower than for trees on semi-vigorous and vigorous rootstocks. Šabajevienė *et al.* (2006) measured leaf pigments of the cultivar 'Auksis' on 12 rootstocks growing in the field. Although the results varied somewhat from year to year, chlorophyll and carotenoid concentrations were higher for trees on M.9 and York 9 than for trees on M.26 and B.9. These results may have varied due to differences in crop load or water relations, but the methods used were reported in too-little detail to interpret the data. Since gas exchange results were so variable, and even when differences were significant they were not very large, at this point there are too few supporting data available to conclude that rootstock vigor can be explained by differences in carbon assimilation. A detailed experiment comparing whole-tree gas exchange measurements for trees on rootstocks of varying vigor is still needed to determine if vegetative vigor may be related to carbon assimilation. Gas exchange measurements for this type of experiment should be made periodically throughout the season to take into account any early cessa-

tion of shoot growth on dwarfing rootstocks. The careful management of crop load and water relations would also be critical for determining the influence of rootstock on gas exchange.

Several studies tested the hypothesis that the greater volume of living tissue in dwarf rootstocks may lead to greater utilization of total metabolites in those tissues. The season following a heavy crop, the vegetative scion growth of 'Lane's Prince Albert' on the vigorous M.4 was 91% that of the previous season compared to only 47% on M.9 (Rogers and Booth 1964). Stutte *et al.* (1994) reported that rootstock affected concentrations of starch, sucrose, and sorbitol at harvest, and concentrations of starch and sorbitol at leaf fall. Roots of trees on MM.111 had greater starch and less sorbitol and sucrose at harvest than roots of trees on M.9. Starch concentrations in young and mature roots of trees on MM.111 were higher than for trees on M.9, and trees on MM.111 had greater shoot and branch starch and less root sorbitol and sucrose at leaf fall. Brown *et al.* (1985) used two scion cultivars on two rootstocks to measure carbohydrates of above- and below-ground (roots plus rootstock shank) tissues of young container-grown apple trees throughout the season. For both rootstocks and cultivars, the dry weight and sorbitol content of above- and below-ground sections of the tree increased until bud set, and declined during the dormant period. For the above-ground portion of the tree, MM.111 had higher starch, sorbitol and soluble sugar concentrations than trees on M.9 from mid-season until budbreak the following spring; however differences in the below-ground portion of the tree occurred only during the early winter. Dwarf rootstocks had higher root sucrose, glucose, and fructose concentrations during dormancy than semi-dwarfing rootstocks under greenhouse conditions, but not when trees were grown in the field (Saavedra 1991). When M.9 was used as an interstock bridge, starch and soluble sugar concentrations increased in the bark above the graft, but declined in the bark below the graft; however, these changes may have been due to the girdling effect of grafting (Samad *et al.* 1999). Taken together, these reports support the concept that carbohydrate concentrations are higher in the above-ground organs of trees on vigorous rootstocks during the late fall and during dormancy, and availability of reserves may influence vegetative growth the following spring. The influence of stored carbohydrates on spring growth is still poorly understood. Most researchers have reported the concentration of various carbohydrates, but the total amount of carbohydrate may be more important than the concentration at any one time. As carbohydrates are converted to sugars and utilized for spring growth, additional starch may be converted to sugars to maintain some critical concentration, above which growth

is not limited. Additional research is needed to determine changes in carbohydrates in various parts of the tree throughout the season.

3. Uptake and Distribution of Nutrients. Since the roots are responsible for absorbing minerals and nutrients for the plant, early rootstock researchers hypothesized that one mechanism by which dwarfing rootstocks exerted their influence on the scion might be by altering the uptake and/or translocation of organic or inorganic materials. It seems likely that the movement of metabolites in the phloem may depend on the size of the sieve tubes, and the relative capacity of the phloem for conducting and storing may be involved in plant vigor (Beakbane 1956). Modifications of the sieve tube size or number occurring at the graft union might hinder the downward flow of organic nutrients and lead to carbohydrate accumulations in the scion. Since dwarfing rootstocks have smaller vessels, movement of water and metabolites in the xylem and phloem would likely be impeded. Work by Rogers and Vyvyan (1934) and Pearse (1940) showed that the size of the root system and uptake of nutrients were not limiting factors in dwarf trees. Trees on M.9 had higher bark:wood ratio and higher Ca, but lower K in bark, wood and leaves compared to more vigorous rootstocks, while P and Mg concentrations were positively correlated with rootstock vigor (Vaidya 1938). Ruck and Bolas (1956) grew non-grafted Crab C and M.9 rootstocks with a range of nitrogen (N) levels, and found that Crab C produced the largest trees. At the lowest N concentration, the mean dry weight of Crab C trees was 31% higher than for M.9, but at the highest N concentration the difference between rootstocks was only 5%. These authors concluded that Crab C absorbed N more efficiently than M.9, especially when N is limited. However, greater amounts of carbohydrates, N, and total amino acids were found in the bark tissue of non-grafted cuttings of M.9 than the more vigorous M.16 (Martin and Williams 1967). Bukovac *et al.* (1958) and Jones (1976) suggested that dwarfing was caused by reduced solute translocation through the rootstock to the scion. Dana *et al.* (1963) also found that trees with dwarfing interstems accumulated N more slowly in scion leaves than vigorous rootstocks, but water loss was not influenced by interstems. Jones (1971) collected sap from the xylem of decapitated mature apple trees, and concluded that nutrient concentrations in the scion alone would not explain the dwarfing effect because the volume of exudate was similar for different rootstocks, and transpiration of the scions was not affected by rootstock (Knight 1925). Concentrations of N, P, and K were higher in the sap of scions on vigorous rootstocks, and there was a greater reduction of these nutrients above the interstem piece for

dwarfing rootstocks. Young container-grown 'Smoothee Golden Delicious' trees on M.9 had higher leaf concentrations of N, Ca, Mg, P, and Zn, but lower concentrations of B and Fe than trees on MM.111 (Zeller *et al.* 1991). Jones (1971) hypothesized that interstocks may simply restrict the movement of nutrients, causing an accumulation near the basal end, or that solutes may be removed from the sap as it moves through the xylem.

Jones (1971, 1974) proposed that M.9 interstems diminished the vigor of scions by reducing nutrient concentrations in the xylem sap stream. The same author later found that sap flowing from above an interstock had lower concentrations of N, P, K, Ca, and Mg than from the rootstock levels, and these differences increased with the dwarfing effect of the interstocks. Nutrient concentrations were higher below the interstocks, but were reduced in the sap flowing from the interstocks into the scion. Analyses of sap from above, mid-way, and below interstems indicated that the changes in concentration were produced in, or close to, the graft union between scion and interstem (Jones 1976). However, the concentration of amino acids was similar above and below the interstem (Jones and Pate 1976). Contradictions in the literature may be due to the fact that some researchers used non-grafted rootstocks, whereas others used grafted trees or interstock trees, and the rootstocks being compared often differed. In addition, experiments with container-grown trees may not reflect conditions in the field. Roots of field-grown trees are not confined, they are exposed to moderate temperatures, and soil-borne communities of microorganisms may interact with the roots and influence the uptake of water and nutrients.

An area of research where there is a dearth of information for fruit trees, but is currently being investigated by agronomists, is the role of root architecture in water and nutrient acquisition. In maize and beans, the depth and spread of roots can affect the uptake of nitrogen, phosphorus and water; cultivar performance in varying soil types appears to be related to the characteristics of the root system (Lynch and Brown 2012). Although root systems of some of the Malling clones were described during the first half of the 20th century, similar information is lacking for newer rootstocks. Detailed characterization of rootstock root systems and the influence of these characteristics on uptake of water and nutrients may be a productive area for future research.

4. Influence on Leaf Nutrient Concentrations. During the 1940s and 1950s, apple orchard nutritional programs were developed for widely spaced trees on vigorous rootstocks. As commercial growers in North America started adopting dwarfing rootstocks, there was a need to

establish nutritional requirements for trees on dwarfing rootstocks and interstems. Tukey *et al.* (1962) was among the first to report differences in leaf nutrient concentrations of apple leaves due to rootstocks and interstems. However, results from different cultivar/orchard combinations were not consistent. Whitfield (1963) reported higher leaf concentrations of Ca and Mg for trees on M.9 than on M.7, and trees on M.2 had higher levels of Ca, Mg, and P than trees on M.16. However, other researchers found no influence of rootstock (Dzamic *et al.* 1980) or interstems (Bould and Campbell 1970) on leaf nutrient levels. In the presence of viruses, trees on MM.106 had higher leaf Mg and Ca than on other rootstocks. High leaf concentrations of Mg for trees on M.26 were reported from several rootstock experiments (Fallahi *et al.* 1984, 1998; Rom *et al.* 1991; Fallahi and Simons 1993; Fallahi and Mohan 2000). One of the challenges associated with determining the influence of rootstock on apple tree mineral nutrition is that rootstocks influence crop load, and crop load can influence leaf nutrient levels (Sadowski *et al.* 1995). In most cases, crop load was not carefully controlled or adequately accounted for in the statistical analyses used in the research outlined above. In future experiments, researchers must carefully control crop load to compare rootstock effects on various aspects of tree growth and physiology.

A number of investigators have reported leaf tissue analyses from rootstock trials, but these results are difficult to interpret and compare because they used different scion cultivars and rootstocks, reported results from varying numbers of seasons (Abdella *et al.* 1982), and crop loads varied or sometimes were not reported. Sometimes, data for two or more seasons were averaged (Poling and Oberly 1970). Over a 4-year period, Fallahi *et al.* (2002) reported that the effect of rootstock (M.7, M.26, and M.9) on 'Fuji' leaf nutrient concentrations varied with season, but trees on M.7 frequently had greater K and trees on M.26 always had greater leaf Mg than trees on other rootstocks. In another study with 'Gala,' results were also inconsistent over years, but trees on M.9 tended to have higher leaf concentrations of Ca than trees on MM.111 (Fallahi and Mohan 2000). Results from a multi-location rootstock trial for 8 years were not very consistent over locations or years within locations, but leaf Ca levels tended to be lower for trees on M.7 than on M.9 or M.26 (Rom *et al.* 1991). In general, it seems that rootstocks have little influence on N and K, and have a greater effect on Ca and Mg, and these two mineral elements have been implicated in bitter pit development. Taken as a whole, results from various experiments support the conclusions of Lockard and Schnieder (1981), that rootstock effects on tree size and precocity are not due to differences in leaf mineral

concentration. Although rootstocks do influence leaf nutrient levels in some years, at present results are too variable to adjust orchard nutritional programs for different rootstocks. Less information is available on fruit nutrient concentrations, which may influence fruit storability. Rootstocks and crop load can affect fruit nutritional levels, fruit quality, and storability. An accurate evaluation of the effect of rootstock on leaf and fruit nutrient levels will require the comparison of rootstocks with a range of crop loads over more than one season, and possibly with more than one cultivar and at multiple locations. Such an experiment would be particularly useful with a cultivar such as 'Honeycrisp,' which is very susceptible to bitter pit.

5. Hormones. Trees tend to maintain a constant top:root ratio. Each scion/rootstock combination has a specific top:root ratio, and attempts to alter this ratio by pruning the top or roots result in the plant changing its growth pattern until the ratio is re-established. Maintenance of the ratio requires interaction between the scion and rootstock. During the 1920s, nurseries had difficulty in producing uniform trees on seedling rootstocks, and research was directed at determining the influence of seed source and scion source on growth of the root system and scion (Maney 1930). The latter author found that not only did the rootstock affect scion growth, but the scion also influenced root growth. There is substantial evidence that rootstock effects on apple tree vigor are mediated by endogenous plant hormones. Some of the first evidence for this concept was reported by Martin and Stahly (1967), who found that the bark of actively growing non-grafted shoots of M.9 had less growth-promoting compounds, and more growth-inhibiting compounds than bark of M.14 shoots.

After reviewing the literature on the dwarfing effects of rootstocks, Lockard and Schneider (1981) proposed that the dwarfing effect of apple rootstocks was due to hormones in both the scion and rootstock. They presented evidence that the primary signal from the shoot to the root was phloem-transported auxin, and the primary signal from the root to the shoot was xylem-translocated cytokinin. Based on a series of experiments involving bark grafts, it was proposed that auxin produced in the shoot tip is translocated down the phloem, and the amount reaching the root influences root metabolism and controls the amount and kind of cytokinins synthesized and translocated to the shoot through the xylem. However, few data were available to support this hypothesis and the suggestion was based primarily on observations of bark grafts and work with callus tissue. This hypothesis now appears to be an oversimplification of the role of hormones in explaining the effect

of rootstocks on scion growth. Kamboj *et al.* (1997) reported higher cytokinin concentrations in shoot xylem sap and root pressure exudate from scions on vigorous rootstocks compared to dwarfing rootstocks, greater [^{3}H]-indole acetic acid (IAA) movement from the scion to roots of vigorous rootstocks, and the ratio of abscisic acid (ABA) to IAA in the rootstock bark was negatively related to rootstock vigor. Unfortunately only the radiolabel, rather than IAA, was measured. Kamboj *et al.* (1999a) found that zeatin was the predominant cytokinin in xylem sap from the dwarfing M.9 and M.27, whereas zeatin riboside was the predominant cytokinin in xylem sap of the semi-dwarfing MM.106. Cytokinin concentrations from sap collected above and below the graft union in composite trees increased with increasing rootstock vigor. Cytokinin concentrations in the shoot sap of non-grafted rootstocks were also positively related to rootstock vigor. Shoot bark of non-grafted M.27 and M.9 had higher concentrations of ABA and higher ratios of ABA:IAA than stems of the more vigorous MM.106 and MM.111 (Kamboj *et al.* 1999b). Since low concentrations of IAA in the cambial region stimulates the differentiation of cambium to phloem (Aloni 1987), the higher ABA:IAA ratios in dwarfing rootstocks may explain the higher bark:wood ratios that have been reported for dwarfing rootstocks.

Researchers in New Zealand studied the role of plant hormones in rootstock-induced scion architecture modification. After grafting 'Royal Gala' onto three rootstocks varying in vigor, van Hooijdonk *et al.* (2006) hypothesized that a reduced root supply of cytokinin to the scion likely controlled bud break, lateral production, and the allocation of growth between plant axes. At the end of the first season, the length and node number of the primary shoot were similar for scions on M.9 and on an own-rooted 'Royal Gala' rootstock control, but trees on M.9 had fewer secondary shoots and fewer grew late in the season. In addition, the dry mass of trees on M.9 was less than on the 'Royal Gala' rootstock control. During the late season, M.9 had greater concentrations of zeatin riboside and lower concentrations of giberillin $(GA)_{19}$ in the xylem sap compared to own-rooted trees. It was concluded that dwarfing apple rootstocks may limit root-produced GA_{19} supplied to shoot apices of the scion, where GA_{19} may be a precursor of bioactive GA_1 required for shoot extension growth (van Hooijdonk *et al.* 2010, 2011). Determining the role of cytokinins and gibberellins in rootstocks is difficult because cytokinin concentrations can change rapidly, especially during the first 15 days after bud break (Tromp and Ovaa 1994). Endogenous cytokinins increase in the spring just before budbreak in shoot xylem sap, and this peak may be responsible for budbreak and branching habit (Cook *et al.*

2001). Gibberellins are difficult to study because there are several bioactive forms and a number of precursors, and they can be converted back and forth; moreover, bioactive forms are often at concentrations that can be difficult to measure (Yamaguchi 2008). Gibberellic acid-insensitive mRNA can move in both upward and downward directions via the graft union within 5 days of grafting (Xu *et al.* 2010). Michalczuk (2002) measured free and conjugated IAA levels in wood, bark and cambial sap of several mature non-grafted rootstocks varying in vigor. Conjugated IAA in bark and wood tissues was higher than the free form and was not affected by rootstock. The level of free IAA in cambial sap was much higher than in the bark and wood tissues, and was lower for M.9 than for M.26 and MM.106, while IAA in cambial sap tended to decline in M.9 later in the season. Using mature trees, Tworkoski and Miller (2007) found that auxin concentrations were a little lower and ABA was a little higher in apical buds of trees on M.9 and M.7 compared to seedling, but the auxin:cytokinin ratio was nearly twice as high in seedling than the other rootstocks. This suggested that the ratio may be a factor regulating sylleptic bud-break and the growth habit in apple scions, and that rootstock modified the hormone concentrations in shoot tips. Tworkoski and Fazio (2011) found that concentrations and fluxes of IAA and cytokinin in xylem exudates of container-grown 'Fuji' were not affected by rootstock when measured after 30 days of growth, but the concentration of ABA per unit volume of exudate per hour (ABA flux) was greater in dwarfing rootstocks than in more vigorous rootstocks. ABA flux was also negatively correlated with vessel cross-sectional area and xylem flow. Scions on dwarfing rootstocks had smaller vessel diameters but greater vessel density, and the resulting total lumen area of vessels in the scion was about 14% greater for vigorous than for dwarfing rootstocks. Hydraulic conductivity was lowest in the most dwarfing rootstocks, suggesting that reduced hydraulic conductivity, caused by an undetermined factor, may lead to water stress and the induction of ABA synthesis. ABA moving up the stems may exert a growth-inhibitory effect, but the role of ABA in size controlling effects of apple rootstocks remains to be elucidated.

The trees used by Tworkoski and Miller (2007) had diverse growth habits, and were budded onto four rootstocks with a wide range of vigor. Although not stated, the treatment structure appeared to be a factorial of six scion genotypes and four rootstocks, and the presence of interaction was not tested. However, there appears to be an interaction between scion genotype and rootstock because the influence of rootstock on trunk diameter was not consistent for all scion genotypes. If tree vigor was controlled solely by rootstock, then one would expect

rootstocks to have a similar effect on tree size, regardless of scion genotype. To elucidate the relative importance of rootstock and scion on tree vigor, additional research is needed, involving reciprocal grafts, and the controls should be rootstocks grafted onto the same rootstock.

Lockard and Schneider (1981) concluded that there was little evidence to support a role for gibberellins in apple dwarfing. However, evidence supporting a role for GA was later presented by Richards *et al.* (1986), who found that M.9 interstems can greatly alter the distribution and metabolism of xylem-applied [^{3}H]GA_4, with an overall effect of reducing the levels of [^{3}H]GA metabolites arriving in the upper shoot and leaves. These authors suggested that endogenous GAs, possibly associated with other hormones, probably contribute to the dwarfing mechanism. ABA-like activity was negatively related to rootstock vigor in tissues of non-grafted rootstocks and levels of GA_{4+7}-like compounds were positively related to rootstock vigor (Yadava and Lockard 1977). Concentrations of GA_3-like compounds were positively related to vigor in above-ground tissues, but negatively related to vigor in the roots. It was hypothesized that rootstock vigor may be related to the ratios of cytokinins and gibberellins in the roots and shoots. Tworkoski and Fazio (2016) used rootstocks of varying vigor as scions on rootstocks of varying vigor. They found ABA and its conjugate, ABA glucose ester, were higher in the root and rootstock shank below the graft union, in the scion above the graft, and in the xylem exudate of M.9 than MM.111 rootstock. Elevated ABA and reduced GA were associated with the more dwarfing rootstocks. Since results for greenhouse- and field-grown trees sometimes differed, it is possible that tree age and environmental stress may interact to affect hormone signals and other size-controlling factors. Additionally, container-grown trees in greenhouses, where roots may be restricted and soil temperatures may be excessive, may not be representative of trees growing in the field. In addition to GA and ABA, other hormones may be involved in size-control. Tworkoski and Fazio (2016) found higher IAA in the rootstock below the graft and in the exudate of MM.111 than for M.9. In reciprocally grafted trees, IAA was higher in the scion above the graft for trees on M.9 than MM.111, but did not differ in rootstock below the graft or the root. It was hypothesized that understanding gene expression associated with hormone metabolism may help explain size-control in rootstocks and assist the selection of rootstocks for size control. Although results are not totally consistent, most of the research supports the concept that rootstock vigor is at least partially mediated by hormones. Since hormones affect tree growth and architecture, it seems likely that rootstock-induced differences in tree growth are related to the interaction of auxin, GA, and cytokinins.

High ratios of auxin and possibly GA to cytokinin may enhance scion vigor directly – or possibly indirectly – by altering cell differentiation, leading to smaller vessels that suppress the movement of water and other growth-promoting compounds through the graft union.

6. Graft Union Failure and Tree Anchorage. Some scion/rootstock combinations have weak unions that break in the nursery or orchard during wind storms. For example, 'Golden Delicious,' 'Gala,' 'Honeycrisp,' and 'Granny Smith' on M.26 sometimes break at the union. Shaw and Southwick (1944a,b) were among the first to report that several apple cultivars performed poorly in the nursery when grafted onto the clonal rootstock 'Spy 227.'

In a review of stock–scion interactions as related to dwarfing, Simons (1987) discussed characteristics associated with incompatibility, which included poor growth and unsatisfactory unions, often resulting in breakage. Graft combinations with mechanically weak unions, which often break, were sometimes associated with mechanical obstruction at the union or with abnormal starch distribution. In a review of graft incompatibility, Andrews and Serrano Marquez (1993) defined incompatibility as "…the failure of the graft combination to form a strong union and to remain healthy due to cellular, physiological intolerance resulting from metabolic, developmental, and/or anatomical differences". They also suggested that incompatibility reduced vascular continuity and the transport of carbohydrates and nutrients across the union, causing these materials to accumulate on either side of the union. Mosse (1962) and Simons (1987) suggested that incompatibility is the primary cause of graft union failure. Some apple cultivars have brittle wood, possibly due to a high proportion of fiber cells that influence flexibility (Simons 1975), and cultivars or rootstocks with brittle wood may be prone to union breakage. Rootstock can also influence the number of fiber cells, the thickness of fiber cell walls, and the proportions of fiber and parenchyma cells (Beakbane and Thompson 1947; Doley 1974). Basedow (2015) compared the xylem tissues of eight scion/rootstock combinations that varied in their tendencies to break, and found that fiber cell wall thickness varied with combination. Weak combinations also had higher percentages of parenchymatous tissue than strong combinations.

In addition to anatomic characteristics, biochemical factors may also contribute to weak graft unions. Compounds, such as peroxidases (Feucht *et al.* 1983), isoperoxidases (Gulen *et al.* 2002), the glycoside prunasin (Gur *et al.* 1968), polyphenols (Dos Santos Pereira *et al.* 2014) and plant hormones, such as auxin and cytokinin (Aloni 1982; Aloni

et al. 2010), may be involved in the division, differentiation, and function of cells in the graft union.

Recent nursery and field observations indicate that unions of some newer cultivars, such as 'Cripps Pink' and 'Scilate' on the new rootstocks G.41 and G.935, are also brittle and trees may break in wind storms and while digging trees in the nursery or planting trees in the orchard. Since tree breakage can have significant economic consequences for nurserymen and orchardists, researchers have evaluated methods for determining union strength and flexibility. Ermel *et al*. (1997, 1999) evaluated histological traits related to different aspects of graft union formation in pear, but there was considerable variation in most traits. Using multivariate analyses, it was possible to discriminate between compatible and incompatible combinations before differences between graft combinations became evident using macroscopic or qualitative microscopic examination. The histological variables responsible for the discrimination were related to bark discontinuity, cambial dysfunction, and starch accumulation in the scion (Ermel *et al*. 1999). Unions with different vascular connectivity could be distinguished using magnetic resonance imaging (Warmund *et al*. 1993) and X-ray computed tomography (Milien *et al*. 2012). Laser ablation tomography (Basedow 2015; Chimungu *et al*. 2015) may be used on newly budded trees to develop three-dimensional models of the unions. Biochemical gene expression and activity may also be used to evaluate graft combinations (Gulen *et al*. 2002; Dos Santos Pereira *et al*. 2014).

The amount of force required to break graft unions has been evaluated for some scion/rootstock combinations. The graft union of 'Gala'/G.30 was more brittle than 'Gala'/M.26, and 'Empire' on G.30 and CG.41 also had some tree breakage (Robinson *et al*. 2003). Rehkugler *et al*. (1979) used a universal testing machine to measure the bending strength of graft unions, and found that 18-year-old 'Golden Delicious' on M.9 and G.30 could withstand only one-third of the force that caused breakage on vigorous rootstocks. Adams *et al*. (2017) applied various plant growth regulators to graft unions in the nursery in an attempt to enhance the flexibility of the union. Using a bench testing machine to measure fracture strength, it was found that foliar applications of prohexadione-calcium and benzyl adenine applied to the union in latex paint increased the flexural strength per scion cross-sectional area, and the flexibility of the union. To avoid tree breakage problems in the future, routine measurement of graft union strength will likely precede the introduction of new rootstocks into commerce. For scion/rootstock combinations with known union weaknesses, many pomologists recommend support

systems with at least three wires so that branches can be tied to wires to prevent trees twisting in the wind.

In addition to union breakage, some rootstocks have long been known to be poorly anchored and tend to lean. The term "anchorage" refers to the resistance of trees to lean or fall over. There is a wide range of anchorage among apple rootstocks. Preston (1955) reported that M.9 and M.4 were weak, M.2 was fair, and M.14 had excellent anchorage. MM.104 was better anchored than either M.6 or MM.111. The cause of poor anchorage for dwarfing rootstocks is likely due to asymmetric root distribution or to brittle root systems, possibly due to short fibers (Rogers and Beakbane 1957). Following wind storms, it became obvious that tree leaning was due to the scion/rootstock combination rather than to the rootstock itself (Marini *et al.* 2001a). In Maine, the angle of leaning from vertical was greater than 30° for 'Starkspur Supreme Delicious' trees on MAC39, B.9, P.1, P.2, P.22, M.26, 16° for M.7, and less than 10° for trees on MAC1 seedling, B.490, and Ant.313. In Massachusetts, no tree leaning was observed for 'Puritan' on six rootstocks, whereas the percentage of leaning trees was 0%, 57%, and 100% for 'Delicious' trees on MM.106, M.2, and M.7, respectively. Following a severe storm in Virginia, less than 10% of 'Starkspur Supreme Delicious' on B.490 and P.22, 'Golden Delicious' on M.26 and 'McIntosh' on M.26 were leaning, whereas more than 80% of the 'Starkspur Supreme Delicious' on M.4 and C.6 were leaning. No 'Golden Delicious' or 'Empire' trees on M.7 exhibited leaning, whereas 88% of the 'Triple Red Delicious' trees on M.7 were leaning. In modern orchards, trees are always supported, so rootstock anchorage will likely be less important (Marini 2001). In Virginia (R.P. Marini, personal observations) and in Michigan (R. Perry, personal communication), vigorous cultivars on M.7 rootstock growing on heavy or clay soils often lean. Investigations in Michigan determined that the loss of anchorage was associated with an asymmetric pattern of roots around the stem shank. Heavy soils appear to exacerbate the situation.

7. Burrknots. Clonal apple rootstocks differ in their tendency to form burrknots (Rom 1973). Burrknots are areas of partially developed adventitious root initials originating in apple tree stem tissue (Rom and Brown 1979). Root primordia form from non-differentiated parenchyma tissue in the bud and leaf gap areas at each node (Swingle 1927). Burrknots form on the above-ground portion of many apple rootstocks, and on some scion cultivars, such as 'Gala' and 'Empire.' These areas can enlarge as the tree grows and cause trunk fluting or partial girdling, which can interfere with vascular transport and stunt the tree (Rom and

Carlson 1987). Burrknots can also be sites for ovipositioning of dogwood borer (*Synthanthedon scitula* Harris), feeding for ambrosia beetles (*Xylosandrus germanus* Blandford), and infection for fire blight (*Erwinia amylovora* Burrill). Since burrknots are undesirable, rootstocks with good rooting characteristics with little burrknot formation would be preferred. Liners of MM.111 produced more large (>20 mm diameter) burrknots than liners of M.26, M.7, and MM.106 (Rom and Brown 1979). In multi-location NC-140 rootstock trials, burrknot severity is usually quantified as the percentage of the rootstock circumference affected. When comparing burrknot data from various rootstock trials, it is obvious that burrknot development varies with site. Soil and climatic conditions, as well as orchard management practices are all confounded in site, so it is impossible to determine which of these factors promote burrknot development. With 'Gala' as the scion, burrknot severity on 5-year old trees was significantly influenced by rootstock at 11 of 20 locations (Marini *et al.* 2000). After 10 years, burrknot development was more severe on B.491, M.27 EMLA, MARK, and M.26 EMLA than on six M.9 clones (Marini *et al.* 2006). In a trial with 'Golden Delicious' as the scion, trees on PiAu 51-4, B.10 and M.26 EMLA had more burrknot development than trees on M.9 NAKBT337, G.11, G.41 and G.935 (Marini *et al.* 2014). In another trial with 'Gala' the severity of burrknots was ranked: M.26 NAKB > M.26 EMLA > B.9 > M.9 NAKBT337 > M.9 RN29 (Autio *et al.* 2013). In addition, there was a poor relationship between burrknot severity after 5 years and that after 10 years (Marini *et al.* 2003, 2014). The number of different-sized burrknots and the burrknot density (burrknots per cm^2 trunk cross-sectional area; TCA) on the 'Gala' scion were recorded for eight dwarf rootstocks in North Carolina and Virginia (Marini *et al.* 2003). For unknown reasons, burrknot development on the scion was greater in North Carolina than in Virginia, but the rankings for the rootstocks were similar except for MARK. At both locations, burrknot density was highest for trees on O.3 and lowest for trees on B.9 and M.27 EMLA, and there was a poor correlation between tree vigor and scion burrknot severity. It is interesting that M.27 EMLA induced few burrknots on the scion, because it usually ranks high for burrknot development on the rootstock shank (Marini *et al.* 2003). When reviewing burrknot data from several NC-140 trials, burrknot development tended to be greatest in British Columbia, North Carolina, and Virginia. Conditions conducive to burrknot development are not known, but may be related to orchard practices, environmental conditions, or soil type. Future experiments aimed at identifying the factors involved in burrknot development are needed.

IV. STRESSES INFLUENCING ROOTSTOCK PERFORMANCE

Unlike the scion cultivar, apple rootstocks are exposed to both above-ground and below-ground stresses. Until fairly recently, rootstocks were not bred to tolerate stresses other than WAA. In general, the adaptation of rootstocks to various stresses was usually identified during field trials, but occasionally rootstocks were subjected to stresses in controlled experiments. More recently, rootstock breeders have been challenging new rootstock genotypes with various stresses, and also studying plant responses to stresses at the whole-plant and the molecular level.

A. Abiotic Stresses

1. Temperature

Cold Stress. Low-temperature injury is one of the most important factors limiting apple production in northern areas, while in other regions high temperatures can adversely affect tree growth. Root cold injury is common in cold apple-growing regions. Increased frequency of temperature extremes associated with climate change may influence tree survival and performance (Quamme *et al.* 2010). Therefore, the industry requires rootstocks that can tolerate extreme temperatures, and it would also be advantageous if the rootstock can impart these characteristics to the scion.

Terminology has long been problematic in the literature related to low-temperature injury in plants. In this chapter, the term "frost" will refer to temperatures below 0 °C during the spring or fall, when trees have leaves or blossoms. "Freeze" will refer to below-freezing temperatures occurring from late fall (after leaf abscission), to early spring before green tissues appear in the buds. The term "hardy" will refer to the plant's ability to tolerate cold stress. Most of the information concerning rootstock cold hardiness comes from field observations following a cold event, and from controlled freezing experiments in the laboratory.

Because it is difficult to evaluate root responses to cold stress, information on apple rootstock hardiness is limited. Sublethal effects of root injury on subsequent tree growth are also difficult to evaluate. The most common methods for evaluating rootstock cold hardiness include: 1) observing tree survival and growth following test winters; 2) exposing young grafted or non-grafted rootstocks to freezing temperatures in the laboratory, followed by the evaluation of specific tissues or plant

survival; and 3) subjecting pieces of rootstock roots or shoots to controlled freezing conditions and evaluating injury. Injury may be evaluated by recording various aspects of growth the following season, or in the case of stem and root pieces by observing tissue oxidative browning, performing differential thermal analysis, or by measuring electrolyte leakage.

Interpreting the literature on cold hardiness from field trials is challenging because the severity of low-temperature injury is influenced by a number of factors such as: 1) time of year when the cold event occurred and when observations were made; 2) temperatures preceding the freezing event; 3) characteristics of the cold event (the rate of temperature drop, the minimum temperature experienced, the length of time at the minimum temperature, and the rate of thaw); 4) the relative health and vigor of the trees before cold stress; 5) tree age; and 6) the amount of insulating snow cover on the ground at the time of the cold event. Although laboratory freezing tests eliminate some of these sources of variation, differences exist in sampling time, storage and preparation of plant material for freezing, and methods used to assess injury. For these reasons, results from different studies sometimes do not agree.

The above-ground parts of woody plants acclimate to low temperatures in autumn in response to short photoperiods and declining temperatures (Weiser 1970). Plants deacclimate in the late winter in response to warm temperatures, and can reacclimate to some extent upon exposure to lower temperatures (Arora and Rowland 2011). Compared to above-ground parts of the tree, apple roots are less cold-hardy, and roots are slower to harden in the fall and slower to deharden in the spring (Chandler 1957; Wildung *et al.* 1973a). The critical mid-winter temperature for apple stem tissue is below –30 °C for hardy cultivars, and the critical temperature for roots is likely between –10 and –18 °C, depending on a number of factors. Trees with root winter injury usually leaf out in the spring, but new shoots grow slowly and often wilt in hot weather. Tissues of shallow roots and the below-ground portion of the rootstock may exhibit oxidative browning. Roots at lower depths, which were not exposed to lethal temperatures, may be uninjured, but may eventually die. Trees often continue to die for at least two years after the cold event (Czynczyk 1979). Trees on sandy or gravelly soils are most prone to winter injury. During a 2-year study, Wildung *et al.* (1973b) reported a root killing temperature below –12 °C in one year and –10 °C the next year, despite a warmer soil temperature the first year. It was suggested that a low soil moisture the second year may have been responsible for the decreased hardiness. It was felt that the

scion and roots developed hardiness independently of each other because root hardiness followed changes in soil temperature. It was also found that 2-year-old roots were hardier than 1-year-old roots.

Several researchers have studied the acclimation and deacclimation of apple rootstocks. Quamme (1990) used 4- or 5-year-old trees budded to two cultivars for controlled freezing tests. The roots of three rootstocks increased in hardiness from late November to mid-January, and then hardiness decreased in March. It was suggested that the capacity to acclimate varies with rootstock. Roots of M.26, MM.106 and M.7 in January were hardy to –14, –10, and –9 °C, respectively, whereas shoots of the same rootstocks were injured at –30 °C. For most of the test period the hardiness rank was M.26 > MM.106 > M.7, and the minimum survival temperature was estimated at –10, –7.2, and –6.7 °C, respectively. Root hardiness responded to changes in soil temperature. Czynczyk and Holubowicz (1984) reported that a winter soil temperature of –11 °C caused root injury and tree death in the orchard, with tree mortality being greater on M.9 than on M.26 or B.9. In another study, roots were exposed to a range of temperatures by sweeping snow and/or covering the soil with mulch to obtain a minimum temperature of –12 °C. Less root injury occurred at –10 °C than at –11.5 °C. To study deacclimation in a 2-year study, Malling and Polish rootstocks were exposed to low temperatures after exposing the trees to 5 or 10 days of temperatures ranging from 1 to 10 °C. In January, 10 days, but not 5 days, of above-freezing temperatures resulted in a loss of hardiness. B.9 lost the most hardiness, followed by M.9 and M.26, whereas P.2 and P.22 deacclimated the least. In mid-February, P.2 and P.22 deacclimated less than B.9 and M.26. In late March, B.9, M.9 and M.26 deacclimated fairly quickly after being exposed to –7 °C. Shoots of Robusta 5 deacclimated earlier in the spring than most apple cultivars (Forsline 1983).

The cold temperature tolerance of the Malling rootstocks has been questioned almost since they were imported to North America. Stuart (1941) was among the first to report on experiments involving controlled freezing of root pieces and stems of stools of Malling rootstocks. Based on electrolyte leakage, the roots of M.3 were most hardy and M.1 and M.9 were the least hardy, whereas the stems of M.3 and M.7 were most hardy and M.8 and M.2 were least hardy. Later, Filinger and Zeigler (1951) reported that all Malling stocks being tested at Kansas were killed during the winter of 1947, and all rootstocks of the K-series of French Crab rootstocks survived. They also exposed rootstock shoots to controlled freezes and found that M.9 was hardier than 16 of the 17 clones of French Crab rootstocks.

A number of researchers have evaluated the cold hardiness of various rootstocks. Lapins (1963) summarized the information in the literature and, of the commercially important rootstocks, he classified M.7, M.2, M.4, M.9 and MM.106 as tender, MM.111, Alnarp 2 (A.2) and Antonovka seedling as moderately hardy, and Beautiful Arcade as hardy. In Poland, Antonovka seedlings suffered more damage than A.2, and Polish 1 (P.1) was more hardy than M.7 or MM.106, but less hardy than MM.111. Of the dwarfing rootstocks, P.2, P.22 and M.26 were most hardy. When M.9 or B.9 were used as interstems on Antonovka seedlings, scions were more hardy when B.9 was the interstem (Czynczyk and Holubowicz 1984). Zurawicz and Lewandowski (2014) tested stool shoots of nine Polish (P), three Malling (M), one Malling-Merton (MM) rootstock and Antonovka seedlings at three temperatures in late winter for 2 years. All plants survived –12 °C and, based on regrowth, P.59, P.60, P.66, P.67, P.68, M.7, and MM.106 recovered best, whereas subsequent growth was poorer for P.2, P.14, P.16, P.22, M.9 and M.26. In British Columbia, P.2 was most hardy, followed by O.3 and then A.2, Jork 9 (J.9), B.9, O.3 and P.2, while M.7 was least hardy (Quamme and Brownlee 1997). When Embree (1988) froze 2-year-old container-grown trees at a range of root temperatures, root hardiness was greater for B.118, B.490, M.26, O.3, A.2 and P.1 than for M.7 or M.2. Privé and Embree (1997a) exposed root tissue to a range of temperatures and, based on electrolyte leakage, reported that results did not agree with regrowth. Kentville Stock Clone 28 (KSC.28) and M.26 had the best regrowth and survival; B.118 and B.490 had fair to good survival and regrowth; O.3, MM.106 and MM.111 had good survival and poor regrowth, whereas M.7 and M.9 had poor survival and regrowth. Roots from 2-year-old rootstocks had less mortality and regrowth than those from 1-year-old rootstocks. Moran *et al.* (2011) froze non-grafted M.26, Geneva 41 (G.41), G.30, B.9, G.11, P.2 and G.935 rootstocks in plastic bags to –8 to –16 °C. Based on regrowth in the greenhouse, G.41, G.11, G.30, B.9, P.2 and M.26 had similar hardiness, whereas G.935 had greater root hardiness than M.26. Wildung *et al.* (1973b) compared non-grafted layers of M.7, M.9, M.26, M.104 and MM.106 for two winters under various mulch treatments in Minnesota, and also used stem and root tissues for controlled freezes. During the first winter, the January soil temperature was –17.8 °C for 5 hours with bare ground, and the same temperature was recorded for only 2 hours with snow cover. In February, the soil temperature was below –12.2 °C on 10 days in bare ground, and –10.6 °C with snow. Trees growing in bare ground had more injury than mulched trees, and M.26 was less injured than the other rootstocks. Survival was 88% for M.26, 50% for M.9, 38% for

MM.104, 12% for MM.106, and 0% for M.7. There was more snow cover the second year, and minimum soil temperatures were –18.3 and –7.8 °C for bare ground and snow-covered ground, respectively. Again, M.7 had most injury, M.26 had the least injury, and the others were intermediate. Freezing tests in November showed no differences in stem hardiness, and slight injury occurred on all clones between –23.3 and –26.1 °C, but all survived –28.9 °C. In November, roots of M.26 and M.7 were killed at –10.6 and –6.7 °C, respectively, and the other rootstocks were intermediate. Freezing in May caused the same trend, with all clones sustaining severe injury near –6.7 °C. Rootstock influenced tree survival following the mid-winter cold event of 2004 in a rootstock trial in the Champlain region of New York State. With 'Honeycrisp' and 'McIntosh' as the scions, O.3, V.1, V.3, G.16, G.30 and Mark had the greatest survival, followed by B.118, M.9 T337, B.9, M.9 Nic 29 and Supporter 4. M.26, MM.111, M.7 and MM.106 had very poor survival (Robinson *et al.* 2006).

Based on his research and reports in the literature, Quamme (1990) classified rootstocks as follows: very tender (M.7), tender (M.2, M.4, M.9, MM.106, and P.16) moderately hardy (M.26, MM.111, MM.104, P.1 and J.9) and hardy (Antonovka seedling, A.2, Beautiful Arcade, O.3, O.8, B.9, P.2, P.22, and P.18).

Freezing trees in pots requires a large freezer and quite a long time because the soil in the pot buffers the temperature. Privé and Embree (1997b) froze non-budded rootstocks at –12 °C in pots containing various types of media. The different media had different insulating properties and provided a range of temperature drop, but root injury and subsequent plant growth were similar for roots in plastic bags, soilless mix, and sawdust. The authors suggested placing the root systems in plastic bags for future whole-plant cold hardiness studies because temperatures throughout the root system were more uniform and less time was required to attain the desired temperature.

There are three aspects of rootstock cold hardiness to consider: 1) the hardiness of the below-ground portion of the rootstock; 2) the hardiness of the above-ground portion of the rootstock; and 3) the influence that the rootstock and scion may have on each other. Stuart (1937) and Quamme (1990) reported that there was no effect of rootstock on scion hardiness. However, Rollins *et al.* (1962) found that when several crabapple cultivars were used as rootstocks they seemed to induce early scion deacclimation. Lapins (1963) also reported that M.7 induced early acclimation of the scion. Quamme and Brownlee (1997) later reported that Robusta 5 imparted about 2–3 °C more hardiness to the scion than other rootstocks. The range in scion hardiness for all rootstocks

was 3.2 °C. Robusta 5 was the earliest rootstock to leaf out in the spring, followed by B.9, M.7, and M.26. Embree and McRae (1991) grafted the hardy 'Wealthy' and the tender 'Gravenstein' on five rootstocks. The trees were grown in pots and the pots were moved to storage in November until freezing in December or February. Injury was assessed by browning of the xylem and cambium of the rootstock and scion as well as the roots, and they also measured regrowth. When exposed to –8 or –11 °C, root survival was not affected by scion cultivar. However, when exposed to –25 °C, trunk tissue survival was consistently lowest for scions on M.7 EMLA, and regrowth was less than for scions on M.26 and MM.111. When Embree (1988) froze the tree tops to a range of temperatures without freezing the roots and trees on M.26, A.2, the Beautiful Arcade seedlings and O.3 had less injury than trees on M.7, MM.106 and MM.111. It was concluded that the rootstock may have an effect on trunk hardiness. Peach flower bud survival was influenced by rootstock in two test winters in New Jersey (Durner 1988), but rootstock did not greatly influence cold hardiness of 1-year-old shoots of peach on several rootstocks in Michigan (Gucci *et al.* 1988).

Ranking of rootstock hardiness is remarkably consistent considering the methods used to obtain the information. Field testing is important because trees varying in age and condition are exposed to low-temperature stress. However, each cold event is fairly unique and injury depends on a combination of factors, such as the minimum temperature, the length of time at the minimum temperature, the rate of temperature drop, time of year, the temperatures proceeding the cold event, snow cover, soil moisture, nutritional status of the tree and cropping history. Although field testing provides "real world" tests, controlled freezing is important for screening new rootstocks and should be incorporated into rootstock breeding programs.

Heat Stress. Some apple-producing regions experience temperatures exceeding 40 °C, and one consequence of global climate change is that these regions will likely experience more frequent high-temperature events that may seriously affect apple tree growth and production. Rootstocks respond differently to root-zone temperatures. Top growth of non-grafted MM.106 and MM.111 was greatest when roots were held at 18 °C and least at 7 °C, whereas top growth of MM.104 and MM.109 responded little to root temperatures. Root growth of MM.104, MM.109 and MM.111 was greatest at 13–18 °C, whereas root growth of seedlings and MM.106 did not respond to temperature (Carlson 1965a).

Plants have evolved physiological and molecular mechanisms to resist heat stress. "Acquired thermotolerance" is the ability to tolerate

otherwise lethal heat stress, and is induced by a short acclimation period at moderately high, but survivable, temperatures. "Basal thermotolerance" is the ability to survive exposure to temperatures above the optimal for growth (Larkindale *et al*. 2005). Stress physiologists are using transcriptomics (signaling components, such as protein kinases and transcription factors) and proteomics (functional genes, such as heat shock protein and catalase) to identify heat stress-responsive genes and proteins in plants. Unfortunately, most of the research on the mechanisms involved in thermotolerance has been conducted with *Arabidopsis* or seedlings (Guo *et al*. 2016).

Heat shock proteins (HSP) are likely the major control proteins during heat stress response, but the functions of these proteins are poorly understood (Qu *et al*. 2013). HSPs are common to many organisms, and tend to increase in response to heat stress in plants, insects (Key *et al*. 1981; cited by Murthy and Ravishankar 2016), and bacteria (Ritossa 1962). Heat acclimation induces the transcription and translation of HSPs, and can be regulated by hormones. HSPs were proposed to act as chaperones to protect cellular proteins against irreversible heat-induced denaturation, to facilitate refolding of heat-damaged proteins, and may facilitate the translocation of unstable proteins for degradation to lysosomes or proteasomes (Boston *et al*. 1996). Larkindale *et al*. (2005) tested *Arabidopsis* mutants that were defective for acquired thermotolerance at five growth stages and found that, in addition to HSP induction, ABA, active oxygen species and salicylic acid pathways were involved in acquired thermotolerance, and that the *uvh6* gene plays an important role in temperature responses. It was concluded that thermotolerance is a complex multigenic process, with different gene sets involved in acquired and basal thermotolerance at different plant growth stages.

Although the apple genome has been fully sequenced, the heat shock transcriptional factor (Hsf) gene family has not been characterized in detail. Jensen *et al*. (2003) reported differences in gene expression due to rootstock in 'Gala' trees that were grafted on M.7 and M.9. Twice as many genes with homology to stress-related genes were identified in 'Gala'/M.7 scions as in 'Gala'/M.9 scions. The same group also identified a clone from 'Gala'/M.7 with homology to *HVA22*, which is a member of a family of stress-regulated genes (ABA, drought, salt, cold) believed to play a role in stress tolerance (Shen *et al*. 2001). Another clone had homology to SP1/POP3, a protein implicated in drought tolerance and *Hsp20*, a heat-shock protein. Using leaves, blossoms and fruit from mature 'Golden Delicious' trees on M.9 rootstock, Giorno *et al*. (2012) identified five *Malus domestica* heat shock families (MdHsfs),

classified in three main groups (class A, B, and C) according to structural characteristics and to phylogenetic comparisons with *Arabidopsis thaliana* and *Populus trichocarpa*. The apple genome comprises 25 full-length Hsf genes, and these are important regulators in the sensing and signaling of different environmental stresses. Rootstock breeders should be able to use this information to facilitate the selection of rootstock candidates with increased heat stress tolerance.

Zhou *et al*. (2016) exposed six non-grafted apple rootstocks to heat stress, and rootstocks of the Shao series (SH series, native to China) showed better heat stress resistance than B.9, CG.24, and M.26. SH1 and SH6 had higher heat stress resistance than SH40. Based on photosynthesis and leaf conductivity, M.26 was least tolerant to heat stress. It was suggested that a high temperature tolerance of the SH series rootstocks may be related to greater osmotic adjustment, because there were smaller reductions in leaf relative water content, higher turgor potentials and higher leaf gas exchange rates compared with the other rootstocks. Following heat-acclimation and exposure to 42 °C, Brestic *et al*. (2011) found that leaves of J-TE-F (from the Czech Republic) had more thermostable photosystem II (PSII) than leaves of MM.106 because ABA stimulated total peroxidases activity and they suggested that J-TE-F may be better adapted in heat-stress regions.

While studying the effect of anthocyanins on heat resistance, Trutneva (2011) found that the scion cultivars, 'Korichnoe Polosatoe' and 'Antonovka Obyknovennaya' grafted onto the red-leaf rootstock Paradizka Budagovskogo had greater resistance to high temperature than the same scions grafted onto a green-leaf mutant Paradizka Budagovskogo. The non-grafted red-leaf type also was more heat-resistant than the non-grafted green-leaf type. It was suggested that the ability to resist heat stress was due to a higher water content of leaf tissues induced by anthocyanins, and also that anthocyanins have antioxidant properties and may act as anti-stress factors.

These studies showed that leaves of rootstocks vary in heat tolerance, and that heat tolerance of the scion can be influenced by rootstock. However, there is a need to include these rootstocks in field trials in regions that are prone to heat stress to verify that rootstock can influence growth and productivity of trees in hot regions.

2. Water Relations. Plants respond to water stress at the physiological, biochemical, and genetic levels. Fruit trees can suffer from too much or too little water. Water relations in a grafted tree may be influenced by the ability of the roots to absorb water, or by the ability to conduct water from the roots through the rootstock stem piece, across

the graft union, and through the scion stem to the leaves. The ability of rootstocks to absorb water at the root surface has not been studied, and information is lacking on the importance of young fine roots. Although recent research suggests that first- and second-order fine roots are most important for water and mineral acquisition, they have typically been ignored in apple root studies.

Excess Water. Many orchards in non-arid regions periodically experience wet soils and flooding. In general, apple trees tolerate wet soils better than many woody species. Apple trees are usually expected to survive flooding for about 6 weeks during the dormant season, but they tolerate flooding less well during the growing season (Rom and Brown 1979). Childers and White (1942) found that 2 to 7 days of apple seedling root submergence reduced transpiration, photosynthesis and leaf respiration. Some roots died after 18 days of submergence, but new roots were observed 8 days after the water was drained. Rom and Brown (1979) submerged the roots of 1-year-old trees on five rootstocks for 6 weeks at different times of the year, and measured regrowth and tree mortality. The order of flooding tolerance was M.26>M.7>MM.106>MM.111, regardless of flooding time from November to April. It was suggested that the fibrous root system of MM.111 may be advantageous under drought, but not under flood conditions. However, these results contradicted field observations where trees on M.26 and MM.106 were sensitive to wet soils (Ferree and Carlson 1987). Makariev (1977) ranked rootstock tolerance to asphyxia as MM.106 (most tolerant), and M.7, MM.111, 'Golden Pearmain' seedling, M.2, M.4, MM.104, A.2, M.26, MM.109 and M.9 (least tolerant). Other researchers reported that MM.106 tolerated flooding better than many other rootstocks, and M.1, M.13 and M.16 had field tolerance to wet soil conditions (Ferree and Carlson 1987). Although MM.106 tolerates wet soils fairly well, tree mortality is often high on wet soils because MM.106 is quite susceptible to *Phytophthora* (Browne and Mircetich 1993). In China, *Malus sieversii* and *M. hupehensis* are commonly used as apple rootstocks. *M. sieversii* is native to the semi-arid region in northwestern China, and *M. hupehensis* is native to a wet climate region in Eastern China. *M. sieversii* is more vigorous and shallow-rooted than *M. sieversii*. Young *M. hupehensis* seedlings tolerated hypoxia better than *M. sieversii*, and the ability to tolerate hypoxia appeared to be related to changes in multiple hormones (Bai *et al.* 2011).

Drought Stress. Many important fruit-producing regions are arid, where rootstocks that perform well under relatively dry conditions are beneficial. In addition, rootstock water relations have been

hypothesized to influence rootstock vigor. Research on drought stress is more voluminous than for root flooding. Drought stress research has been performed with grafted and non-grafted rootstocks, and with container-grown and field-grown trees. Drought tolerance is actually a compromise between plant survival and vegetative growth and cropping. Stomatal closure effectively conserves moisture, but at the cost of reduced carbon assimilation. Water stress can induce physiological and biochemical changes in trees, and rootstock can influence leaf function of the scion. Apple rootstocks influenced leaf size, specific leaf weight and net photosynthesis in the scion (Ferree and Barden 1971) and carbon partitioning to various parts of the tree (Zhou *et al.* 2015). Stomatal closure is related to root physiology, possibly due to chemical signals, such as ABA from roots to the shoot in the transport stream. In grape, the same scion cultivar had different stomatal density and pore sizes when grafted to different rootstocks (Serra *et al.* 2013).

Total plant dry weight gain was usually suppressed by increasing water stress. Based on the dry weight of container-grown non-grafted rootstocks, Sakalauskaite *et al.* (2006) classified MM.106, M.26, B.118, M.9, P.60, P.59, P.2 and B.396 as drought-sensitive, whereas seedling, P.22 and M.9 were drought-tolerant. Based on shoot length and dry weight measurements of drought-stressed, non-grafted rootstocks, Preston and Rogers (1961; cited by Carlson 1967) concluded that MM.106, MM.109 and M.9 were more drought-tolerant than M.2, Robusta 5 and A.2. In another study, based on transpiration and shoot diameter increase, P.22 was more drought-tolerant than P.16 (Klamkowski and Treder 2002). Atkinson *et al.* (1999) exposed container-grown, non-grafted rootstocks to drought stress and after 6 months the dwarfing rootstocks had less root dry matter, but drought affected the production of coarse and fine roots differently depending on the rootstock. For AR295-6, AR360-19 and AR628-2, the production of fine and coarse roots declined with increasing soil water deficit, whereas for AR69-7 and M.26, root production increased slightly. For M.9, coarse root dry matter declined while fine root production increased, and this may enhance the capacity of a root system to extract more water. For M.26, the coarse-to-fine root ratio increased with reduced irrigation. The authors suggested that plants with large root systems may be more drought-tolerant (Higgs and Jones 1990), but the dwarfing rootstocks AR628-3, AR295-6 and AR486-1 produced more root mass than the more vigorous rootstocks, especially M.26, and this response was associated with rootstocks that have O.3 as a parent. Unfortunately, O.3 has not been included in any rootstock experiments involving drought stress. Psarras and Merwin (2000) found a slight shift

towards finer root diameter with water stress of M.9 and MM.111, and total root dry matter production declined whereas root respiration increased with soil moisture.

Hydraulic conductivity through the graft union of apple trees was generally less than that through adjacent scion and rootstock stem tissue (Knight 1926; Warne and Raby 1939). The effect was greater for trees on M.9 than for trees on more vigorous rootstocks, and the differences in conductivity between the union and adjacent stem declined as trees aged. Gur and Blum (1975) found no difference between hydraulic conductivity of compatible unions and adjacent stem tissue for 4- to 10-year-old apple, peach, and plum trees. Olien and Lakso (1984) felt it was unlikely that resistance at the graft union could account for differences in mid-day stem potential. Since root distribution and amounts of roots varied with rootstock (Rogers and Vyvyan 1934), but the shoot:root ratio was similar for all rootstocks in a given soil environment, Olien and Lakso (1984) also felt that the size of root system probably does not affect root resistance among rootstocks, though they postulated that there are differences in root resistance among rootstocks.

Results from experiments using grafted trees may be more relevant to field situations; however, results from such experiments have been inconsistent. Most researchers using grafted trees did not report tree dry weights, but usually reported shoot length, leaf gas exchange, or plant water status responses to drought. Based on field observations, Tukey and Brase (1939c) classified M.1, M.7 and M.16 as tolerant of high soil moisture, M.2, M.4, M.12 were intolerant of low soil moisture, whereas M.7 and M.13 tolerated low soil moisture. Fernandez *et al.* (1997) reported that 1-year-old 'Gala'/Mark trees were most sensitive to drought, MM.111 was intermediate, and M.9 EMLA was least sensitive. Leaf ABA concentrations increased after drought stress and were highest for M.9 EMLA and lowest for Mark. These results were supported by others. 'Granny Smith' on M.9 was most drought-tolerant, followed by seedling and MM.106 (Kaynas *et al.* 1995), and for 'Golden Delicious', M.9 was most drought-tolerant, followed by MM.106 and M.7 (Giulivo *et al.* 1985). However, Chandel and Chauhan (1990) using 'Delicious' as a scion, found that of 11 rootstocks, trees on M.9 grew most poorly when drought-stressed. The same authors later reported that drought tolerance was positively related to levels of proline, ABA, and carbohydrates in the scion leaves (Chandel and Chauhan 1991).

Apple rootstock root systems have low root length per volume of soil, roots are not uniformly distributed under the tree, and rootstocks respond differently to soil structure (Rogers 1939; Fernandez *et al.* 1995). The root system can influence water uptake and transport, and

may also detect soil water deficits and send signals that regulate stomatal functioning. Fernandez *et al.* (1997) reported a positive association between the drought tolerance of three rootstocks and ABA levels in scion leaves. Loveys and During (1984) found that leaf ABA concentrations increased during drought stress, but ABA levels alone could not explain the higher leaf stomatal conductance and photosynthesis measured on grafted scions compared to own-rooted cuttings. Following drought stress, non-grafted M.9, *Gami almasi* (a dwarf apple rootstock native to Iran) and its seedlings had more free proline and soluble sugars, and were more drought-tolerant than MM.106 and trees of the local apple 'Azayesh' (Alizadeh *et al.* 2011a). In *Prunus* rootstocks, drought-induced accumulations of sorbitol, raffinose and proline, conferred drought tolerance (Jimenez *et al.* 2013). Because initial molecular responses were related to the biochemical responses, it was proposed that the accumulation of leaf sorbitol, root raffinose, and root and leaf proline could be used as drought tolerance markers for the early selection of *Prunus* rootstocks. The differential expression of *PSC5* in roots could also be used as a drought tolerance marker. The peach–almond hybrid rootstocks GF 677 and ROOTPAC® R performed better under drought stress than the dwarfing rootstock ROOTPAC 20. Differential rootstock performance could be related to differences in genetic background and vigor. Jimenez *et al.* (2013) also suggested that additional research is needed to determine if these metabolic compounds participate in osmotic adjustments in plants.

Jones (2012) felt there is a need to identify genes that are involved in drought tolerance, and stressed the importance of evaluating fruiting trees on different rootstocks in different hydraulic environments. Marguerit *et al.* (2012) investigated the architecture of the rootstock control of scion transpiration-related traits in grapes by water-stressing the plants. It was concluded that the scion transpiration rate was controlled by the rootstock through different genetic architectures. Genes have recently been identified that may be involved in the response of rootstocks to abiotic stresses. In drought-stressed grapevines, scion transpiration was controlled by a small number of loci, each accounting for less than 10% of the phenotypic variance (Marguerit *et al.* 2012). The genetic control of transpiration rate and water extraction capacity was independent of the genetic control of transpiration rate acclimation. There were eight genomic regions associated with scion transpiration-related traits suggesting hormonal (ABA), and hydraulic signaling between the scion and rootstock plays an important role in responses to water deficit. Scion transpiration rate and its acclimation to water deficit are controlled genetically by the rootstock, through different genetic architectures.

Using 'Gala' as the scion, Jensen (2010) found twice as many genes with homology to stress-related genes in trees on M.7 than trees on M.9, and they suggested that this may account for the observations that tees on M.7 tolerate disease, drought and cold better than trees on M.9 (Carlson 1970; Ferree *et al.* 1995; Wertheim 1998; Cline *et al.* 2001).

Liu *et al.* (2012a) grafted 'Gale Gala' onto seedlings of *M. sieversii* and *M. hupehensis* grown in pots and exposed them to two irrigation treatments. *M. sieversii* was more resistant to drought and had smaller reductions in growth, photosynthesis, leaf area, chlorophyll and relative water content. Following drought stress, leaves and roots from trees grafted onto *M. sieversii* had greater synthesis of ascorbic acid and glutathione, as well as higher activities of super-oxide dismutase, catalase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase. These results suggest that the rootstock can enhance drought resistance by improving the antioxidant system in a plant.

Irrigation systems have become an integral component of apple production in dry regions and are becoming more common in humid regions. However, since periodic drought will likely be more common in many apple-growing regions as the climate changes, breeding for drought tolerance should be considered an important aspect of rootstock breeding and evaluation in the future.

3. Root Characteristics Affecting Water Relations. The effects of soil characteristics, rootstocks, fruiting, and abiotic stresses on the gross structure and growth of apple root systems have been reported, but less is known about the function of apple roots. A better understanding of apple root growth and function is required before the interactive effects of scion and rootstock responses to abiotic stress can be adequately evaluated and to allow rootstock breeders to select for traits that enhance tree performance.

The amount of water available to a tree depends on the volume and distribution of roots under the tree, which may depend on how a rootstock grows in a given soil. Therefore, rootstock response to drought is, in part, likely independent of the physiological characteristics of the rootstock. Because entire root systems of mature trees in the field are difficult to study, there is little information on these aspects of water relations as affected by rootstock. Additionally, during root sampling, the young fine roots – which are most important for the absorption of water and nutrients – are typically not measured. Rootstock interactions with scion growth and cropping (Marini *et al.* 2013a,b) likely also complicate such studies. Studying the effect of rootstocks on water

relations with mature trees is complicated by several factors that may be confounded; vigorous rootstocks have larger root systems that can explore greater soil volume, but also have more leaf area and tend to use greater amounts of water than trees on dwarfing rootstocks (Higgs and Jones 1990). For some, but not all, experiments involving drought stress, the soil and plant water status were measured. Future studies should focus on tree responses to estimates of soil or plant water status, such as pre-dawn water potential or mid-day stem potential (Lakso 2003). In most experiments using non-grafted and grafted trees, M.9 was among the more drought-tolerant and MM.106 among the least drought-tolerant rootstocks. However, comparing data from various experiments is difficult because different rootstocks were compared, and the severity and duration of drought stress varied. Additionally, at least in field experiments, drought stress may have been confounded with ambient air and soil temperatures. Although such research is difficult and expensive to perform, more information is needed concerning the drought tolerance of cropping trees on various rootstocks, not only in the year of the stress but also in the following season.

During the past 30 years considerable progress has been made in understanding how water moves from the soil, into the roots, and eventually to the leaves of grafted apple trees. Under dry conditions, leaf water potential, leaf conductance and water uptake were lower for dwarfing rootstocks (Olien and Lakso 1984, 1986; Higgs and Jones 1990; Hussein and McFarland 1994), which was attributed to hydraulic conductance (Olien and Lakso 1984, 1986; Higgs and Jones 1990). Mature 'Smoothee' trees on M.9 used less water than MM.106 (Cohen and Naor 2002), and differences in water use were attributed to differences in leaf conductance because conductivity of the wood and the maximum sap velocities were similar for the two rootstocks.

The hydraulic conductivity of 1.5 mm-diameter roots from dwarfing rootstocks was lower than similar sized roots from semi-vigorous rootstocks. Water movement into the root from the soil has been considered the major component of root resistance in healthy plants, but the ability of rootstocks to absorb water at the root surface has not been evaluated (Landsberg and Jones 1981). Total root length relative to tree size is more limited in fruit trees than in other species (Atkinson *et al.* 1980). Thus, fruit trees require higher rates of water absorption per unit of root length, increasing the potential for resistance to water absorption and to limit the water transport system. Olien and Lakso (1984) suggested that differences in root resistance among rootstocks may involve water absorption at the root surface and the radii and number of xylem vessels for water transport.

Atkinson *et al.* (1980) described the growth and aging of apple roots, but the function of roots of different diameters and age were not known. Pomologists studying roots have generally classified roots on the basis of root diameter, and usually the smallest classification is for fine roots (<2 mm diameter) (Adkinson 1980; Fernandez *et al.* 1995; Atkinson *et al.* 1999), without considering their function. However, not all roots of <2 mm diameter function equally. The size class approach ignores the position of individual roots on the complex lateral branching system. Pregitzer *et al.* (2002) found that the function of roots of a given size class varied with tree species, and the position of an individual root on the branching system defined the function of the root. Apple roots with small differences in diameter may have very different lifespans (Wells and Eissenstat 2001), and roots of different branching orders can differ in respiration (Huang *et al.* 2005), anatomy (Eissenstat and Achor 1999), lifespan and ability to absorb water and nutrients (Eissenstat *et al.* 2000; Anderson *et al.* 2003). First-order roots (roots terminating in a meristem and devoid of lateral roots), second-order roots (roots with a single set of dependent laterals) and third-order roots, formed at the junction of two second-order roots, are most important for the absorption of water and nutrients. However, first- and second-order roots are usually lost during root excavation, or fall into the same size class. As these young, non-pigmented, white roots age, they exhibit declining hydraulic conductivity (Kramer and Bullock 1966; Nobel *et al.* 1990). Mycorrhizal fungi also colonize the faster growing first-order roots and probably affect the function of the root (Resenes *et al.* 2008). Poplar roots not colonized by arbuscular mycorrhizal fungi survived for a longer period than colonized roots (Hooker *et al.* 1995); however, the methods used were not sufficiently detailed to explain how mycorrhizal colonization of growing roots was observed. It is likely that the fine root architecture and the colonization of first-order roots varies with rootstock and possibly with choice of scion, and these traits may be affected by abiotic stresses. Valuable areas for future research include detailed studies of the fine root architecture and the genetic regulation of root branching and uptake of water and nutrients by first- and second-order roots of different rootstocks.

Rootstock Morphology Affects Water Relations. Differences in morphology and rootstock-induced modifications of graft unions and stem morphology likely affect water relations in the scion, and possibly also the roots. Beakbane and Thompson (1939) and Beakbane (1941) found that scions grafted onto M.9 had fewer wood fibers, more wood parenchyma and wood ray cells per unit area, and more (but smaller) xylem

vessels, and a higher ratio of bark-to-wood than did trees on M.2 or M.12. These observations and others led to the hypothesis that dwarfing might be caused by reduced hydraulic conductance or reduced transport of water, mineral nutrients or solutes from roots to the scion (Beakbane 1956; Bukovac *et al.* 1958; Jones 1976). Subsequent research showed that in both apple and peach rootstocks, the dwarfing effect appears to be related to lowered afternoon water potentials, and the differences were associated with xylem vessel size (Atkinson *et al.* 2003; Basile *et al.* 2003a,b; Tombesi *et al.* 2010b). Moisture stress of young trees affected the sap flow of trees on seedling rootstock less than on Mark, whereas M.7 was intermediate, possibly due to differences in root characteristics, xylem anatomy, or other features related to the bud union (Hussein and McFarland 1994). Pearce (1940) found that trees on vigorous rootstocks absorbed more water per unit of leaf area, but trees on dwarf rootstocks absorbed more water per unit of fresh weight increase of the scion. Atkinson *et al.* (2003) also found that rootstock affected stem hydraulic conductivity and percentage of functional xylem, suggesting that tree vigor may be related to the vascular characteristics of the graft union. However, their experiments were performed with trees grafted just one year earlier and the graft unions may not have healed totally.

Results from a series of experiments with peach and cherry, indicate that the primary physiological mechanism by which rootstocks influence scion vigor appears to be related to the hydraulic conductance of the rootstock (Basile *et al.* 2003a,b; Gonçalves *et al.* 2007; Tombesi *et al.* 2010a,b). Dwarfing peach rootstocks had smaller xylem vessel diameters leading to reduced hydraulic conductance. The same authors also reported that dwarfing rootstocks and dwarfing rootstocks used as interstems had only a slight influence on xylem characteristics of the scion.

Compared to vigorous sweet cherry rootstocks, xylem vessel diameter was smaller and vessel frequency was higher in roots and stems of dwarfing rootstocks (Gonçalves *et al.* 2007; Tombesi *et al.* 2010a), suggesting that dwarf rootstocks restrict water transport (Tyree and Ewers 1991). Olien and Lakso (1984) reported that fruiting 'Empire' trees on M.9 and M.26 had more negative stem potential than trees on M.7, MM.106 and MM.104, and tree size was positively correlated with mid-day stem potential. It was suggested that the difference in mid-day stem potential was due to resistance at the graft union or to root hydraulic resistance because the stem potential gradients in the shoot and transpiration rates of M.9 did not differ from those in MM.104.

The morphology of apple rootstocks varies, but rootstock also can influence the morphology of scion stems. Dwarfing apple rootstocks

tended to have smaller diameter root xylem vessels and a lower percentage of root xylem cross-sectional-area occupied by vessel lumina than vigorous rootstocks (Beakbane 1953). Larger xylem vessels are assumed to have greater hydraulic conductance and increased plant growth under non-stressed conditions (Rodriguez-Gamir *et al.* 2010), but the act of grafting can alter xylem vessel diameters in the scion (Trifilò *et al.* 2007). Bauerle *et al.* (2011), using grafted and non-grafted MM.111 and B.9 trees, reported an interaction between plant growth rate and xylem diameter in response to water stress. Water-stressed trees on MM.111 grew better than trees on B.9, and on MM.111 there was a 25% increase in vessel frequency and a 28% narrower current season xylem ring width compared to B.9. The differential drought responses between plants of differing shoot vigor were a result of root system growth potential. This may identify a link between plastic xylem vessel anatomic responses and hydraulic xylem cavitation control on potential growth rate in both rootstock and scion portions of the plant under limited soil water. Scions on MM.111 had smaller xylem vessel diameters, but produced more xylem vessels, resulting in decreased numbers of emboli during drought.

4. Soil pH and Salinity. In some regions, salinity is an important abiotic stress limiting crop production and, as the climate changes, salinity will likely become an increasingly important problem. As plants remove water from the soil, increasing salt concentrations in the soil reduce water availability to the roots and induce water stress. High salt concentrations in the soil solution are associated with lower stomatal conductance, reduced leaf chlorophyll concentration, decreased leaf water potential and relative water content, and suppressed leaf expansion and overall plant growth, along with increases in leaf concentrations of proline and soluble sugars (Alizadeh and Alizade 2013). In general, apple is more sensitive to chloride than peach (Dilley *et al.* 1957). Tolerance to salinity depends on the uptake and transport of salts by roots (Vitaglino *et al.* 1992). Apple rootstocks varied in tolerance to salt stress (Motosugi *et al.* 1987; Therios and Misopolinos 1989). Leaves of non-grafted MM.106 and 'Azayish' rootstocks growing in varying concentrations of NaCl had higher leaf concentrations of proline and soluble sugars than did leaves of M.9 and 'Gami-Almasi' (Alizadeh *et al.* 2011b). *Malus zumi* is a rootstock used in northeast China which can survive high salinity, and recent research showed an increased expression of genes involved in photosynthesis under salt stress and new mechanisms for the scavenging of reactive oxygen species and osmoprotection (Li *et al.* 2013). However, *M. zumi* was not

compared to other apple rootstocks in that study. In a different study, when container-grown, non-grafted rootstocks were grown in varying concentrations of NaCl, foliar symptoms were most severe on M.4 and M.27, followed by M.11; symptoms were least severe on M.16 and M.26. Leaf concentrations of Cl and Na increased with increasing concentrations in the irrigation water. Concentrations of Cl were lowest in *M. prunifolia*, M.9 and M.26, and concentrations of Na were highest in leaves of M.11 and lowest in leaves of M.16 and M.26 (Motosugi *et al.* 1987). When 'Fuji' was grafted onto nine rootstocks, salt treatments reduced tree growth, while visual foliar symptoms were most severe for M.4 and M.11 and least severe for M.26. In general, M.4, M.11 and M.27 were most sensitive to salinity, M.26 was least sensitive, and M.9 and M.16 were intermediate. 'Fuji' seedlings inoculated with arbuscular mycorrhizal fungi tolerated saline conditions better than non-inoculated seedlings, and Yang *et al.* (2014) suggested that 2% and 4% salt concentrations may be the upper thresholds of salinity tolerance for non-mycorrhizal and mycorrhizal apple trees, respectively.

Some *Malus* species and cultivars, not commercially important in Europe and the Western hemisphere, are adapted to abiotic stresses and may be useful in rootstock breeding programs. When young seedlings of five *Malus* species native to China were grown at a range of pH values in hydroponic solutions, three species grew best at pH 5.5, but *M. sieversii* seedlings grew best at pH 8.5. Growth of *M. sieversii* and *M. robusta* was inhibited at pH 5.5, and growth of *M. prunifolia* and *M. hupehensis* was inhibited at pH 8.5 (Deng *et al.* 2012). The apple cultivars, 'Gami-Almasi' and 'Azayish,' are native to Iran and are used as rootstocks. 'Gami-Almasi' is a dwarfing rootstock (Naseri *et al.* 2011). Since these two rootstocks apparently perform well in arid climates, apple rootstock breeders might consider including them as parents in their breeding programs in an attempt to enhance drought stress and salt tolerance.

B. Biotic Stresses

1. Fire Blight. Fire blight is caused by the bacterium *Eriwinia amylovora* and is a serious bacterial disease on many *Rosaceous* species. The disease was the first bacterium proven to be a plant pathogen. Fire blight is thought to be native to eastern North America, but is present in 47 countries, and is expected eventually to spread to all countries growing pome fruit (van der Zwet *et al.* 2012). Fire blight can infect and kill blossoms (blossom blight), shoots (shoot blight), and woody plant

organs, including the rootstock (rootstock blight) (van der Zwet and Keil 1979). As North American apple growers intensified their orchards during the past 20 years, fire blight became more severe (Momol *et al.* 1999). Closely spaced trees with new fire blight-susceptible cultivars on susceptible dwarfing rootstocks, plus susceptible crab apple pollenizers with extended bloom periods to ensure pollination, provided optimal conditions for fire blight infection and spread. Some young orchards experienced more than 15% tree mortality per year during the first several years after planting. Economic loss associated with one episode of 10% rootstock blight was estimated at $US 8650 per hectare (Norelli *et al.* 2000). Rootstock infection can occur in several ways, including infection of root suckers, internal spread of bacteria from infections of the scion to the rootstock, or direct infection of the rootstock through discontinuities in the bark caused by growth or various types of injuries (Momol *et al.* 1998). Bacteria can move 50 cm down a shoot of a resistant cultivar in just 12 days, and movement was faster following late-season as opposed to early-season inoculations (Norelli *et al.* 2000). This was verified in a rootstock trial in its sixth year in Virginia, where fire blight infected a 'Delicious' leaf after a thunderstorm in July. Within one month the tree on M.26 rootstock died (R. Marini, unpublished results). Apparently, the bacteria moved down the fairly resistant scion, without causing symptoms, and killed the susceptible rootstock. Because fire blight severity is influenced by the combination of cultivar and rootstock (Boyce 1970), in order to avoid rootstock blight growers were encouraged to plant trees on resistant rootstocks whenever possible.

One objective of the Geneva apple rootstock breeding program is to develop rootstocks with fire blight resistance. Based on inoculations of growing shoot tips, Robusta 5 was identified as resistant to fire blight and used as a parent in the program (Gardner *et al.* 1980a). Later inoculation tests showed that Robusta 5 was differentially susceptible to different strains of fire blight, and the strain E4001a infected some rootstocks previously identified as resistant (Paulin *et al.* 1993).

When Norelli *et al.* (2003a) inoculated 49 apple rootstock liners growing in the greenhouse with different strains of fire blight, B.9, O.3, M.9 and M.26 were most susceptible, whereas G.11, G.65, G.16, G.30, Pi Au 51-11, and M.7 were most resistant. Field-grown fruiting 'Royal Gala' trees on G.16 and G.30 were highly resistant to rootstock infection when trees had severe blossom infection compared to susceptible trees on M.9 and M.26. Although shoot-inoculated, container-grown B.9 plants in the greenhouse were susceptible, blossom-inoculated, field-grown trees grafted to B.9 rootstocks were resistant to rootstock

infection. This confirmed unexpected observations from a multi-location rootstock trial where tree survival of 'Gala' on B.9 was higher than for trees on M.9 and M.26 during seasons with severe fire blight infection (Marini *et al.* 2000). In addition, B.9 seemed to transmit resistance to the scion. During a severe fire blight outbreak in Ohio, trees on B.9 experienced some dieback of the scion, but trees on M.9 were killed (Ferree *et al.* 2002). These observations led to a series of experiments to determine why trees on B.9 in the orchard were more resistant than was indicated from greenhouse inoculation of stool shoots.

Russo *et al.* (2008a) found that fire blight could migrate from the infected scion into the rootstock, and that the bacteria survived in both susceptible and resistant rootstocks. Leaf-inoculated non-grafted B.9 plants growing in the greenhouse and the field developed similar symptoms, so environmental conditions did not influence susceptibility. When wounds of 4- or 5-year-old grafted and non-grafted B.9 rootstocks were inoculated, B.9 displayed a level of resistance similar to the resistant G.16. Similarly inoculated M.9 rootstock retained its susceptibility. It was hypothesized that B.9 possessed a novel form of age-related disease resistance. B.9 is highly susceptible to fire blight when leaf-inoculated, but highly resistant when the woody tissue is inoculated and resistance is influenced by shoot tissue maturity. Fire blight-resistant transgenic lines of apple have been developed (Bolar *et al.* 1999), but will likely not become commercialized due to public concerns. Therefore, rootstock resistance to fire blight and other pathogens will depend on breeding programs (Norelli *et al.* 2003b).

2. Viruses. Viral diseases can cause severe economic losses to tree fruit crops. Cembali *et al.* (2003) reported that the virus protection program helped avoid losses to nurseries, producers and consumers, and the combined benefits were estimated at $US 227.4 million a year in the USA. Latent viruses are viruses that usually do not cause symptoms in most commercial cultivars. A number of latent viruses have been identified in apple. The three most common in North America are apple chlorotic leafspot virus (ACLSV), apple stem grooving virus (ASGV), and apple stem pitting virus (ASPV) (Podleckis and Welliver 1995). These viruses often occur in combination (Leone *et al.* 1998; Kundu 2003). Apple mosaic virus (ApMV), tomato ringspot virus (ToRSV) and rubbery wood phytoplasma can also affect apple trees. Some cultivars, rootstocks or specific rootstock/scion combinations may exhibit symptoms ranging from suppressed tree vigor to tree mortality. Antonovka trees infected with apple mosaic virus and rubbery wood were more susceptible to cold injury (Zawadzka 1988). Latent viruses are usually

transmitted by grafting or budding infected scion material onto a susceptible rootstock. Diagnosing virus infection based on symptoms can be misleading because similar symptoms can be caused by nutrient deficiencies, herbicides, insect feeding, and plant growth regulators. In addition, symptoms may be similar to those of other diseases, and may vary with cultivar and rootstock, the combination of cultivar and rootstock, virus strains and environmental conditions (Fuchs 2016). Only laboratory tests can reliably identify viruses in apple trees.

ApMV is related to *Prunus* necrotic ringspot virus. It is graft-transmissible, and may cause variegated foliage on apple trees, such that leaves may abscise prematurely (McCrun *et al.* 1960). Cultivars vary in susceptibility, but 'Golden Delicious' and 'Jonathan' are among the most susceptible, with infection possibly reducing yields by up to 50% (Podleckis and Welliver 1995). ApMV reportedly reduced tree growth and yield in several studies. After 12 years, compared to 'Freyberg' trees on non-infected M.793 rootstock, propagating trees on infected M.793 resulted in a trunk cross-sectional area suppression of 42% and the yield was reduced by 26%, but there was no effect on fruit size and quality (Chamberlain *et al.* 1971a). When 23-year-old trees of four apple cultivars on 18 rootstocks were inoculated with two strains of the virus, yields were reduced by 0% to 40% depending on the cultivar. M.9 and M.25 were the most sensitive rootstocks, whereas M.2 and M.14 were the least sensitive (Ponsette and Cropley 1956).

ACLSV belongs to the Betaflexiviridae family (Carstens 2010), is distributed worldwide, and can infect woody species of the Rosaceae family, such as apple, pear, peach, plum, cherry, and apricot (Desvignes and Boyé 1989). The virus can cause symptoms in some stone fruits, but is symptomless in most apple cultivars. ACLSV was detected in 30% of the trees tested in France (Desvignes *et al.* 1992), and in about 60% of the trees tested in the United States (Waterworth 1993). Apple trees on ACLSV-infected M.9 rootstock or interstem grew poorly and some trees died (Koike *et al.* 1993). Cieślińska and Rutkowski (2008) compared the growth and fruit quality of 'Sampion' and 'Golden Delicious' grafted onto virus-free and ACLSV-infected M.9 rootstock. Trees on virus-free M.9 had higher yields, larger fruit, and fruit with greater red blush and less russetting than infected trees.

ASGV infects apples and pears (Pleśe *et al.* 1975) and produces symptoms on 'Virginia Crab' that include chlorotic leaf spots, stem grooving and pitting, union necrosis, and swelling of the trunk above the graft union. Before modern techniques were developed for virus identification, 'Virginia Crab' was commonly used as an indicator host when indexing for ASGV and ASPV (Waterworth 1972). Although ASGV did

not exhibit symptoms in apple shoot cultures of 'Fuji' compared to non-infected shoot cultures, Chen *et al.* (2014) identified 184 upregulated and 136 downregulated genes that are involved in a wide range of plant functions. Infected shoot cultures had lower rates of photosynthesis at low light levels, but higher stomatal conductance, internal CO_2 concentration and transpiration than non-infected shoot cultures over a range of light levels.

ASPV, also known as pear vein yellows virus, belongs to the Betaflexiviridae family (Nemeth 1986) and infects pome and stone fruit worldwide. ASPV was first reported in apple by Smith (1954) in the United States. Symptoms of ASPV are similar to those of ASGV (Pleśe *et al.* 1975). The virus is symptomless in most apple cultivars, but may cause epinasty and graft incompatibility in 'Northern Spy,' which is used as an indicator host (McCrum *et al.* 1960). Symptoms in 'Virginia Crab' include stem pitting of xylem, usually below the graft union (Podleckis and Welliver 1995).

Apple rubbery wood was first described as a virus in the U.K. (Luckwill and Crowdy 1950), and infects apples and pears around the world. As genetic techniques improved, the disease was later shown to be a phytoplasma belonging to the aster yellows group in apple (Bertaccini *et al.* 1998). Infected trees have flexible limbs and a prostrate appearance due to a lack of normal lignification in patches of the xylem vessels and tracheids in young branches (Beakbane and Thompson 1945). Rubbery wood was found in 75% of the apple trees and 70% of the pear trees tested in New Zealand (Chamberlain *et al.* 1971b). The disease is latent in many cultivars, but produces symptoms in some cultivars, such as 'Gala,' 'Splendour,' 'Golden Delicious,' and 'Red Delicious.' Symptoms are severe in 'Lord Lambourne,' which is used as an indicator host in England. In Poland, apple trees infected with apple rubbery wood or ApMV were more susceptible to winter injury than virus-free trees. In the 1940s, nurseryman noticed that nursery trees sometimes developed rubbery wood symptoms, even when scion wood was taken from the same 'Lord Lambourne' mother trees, suggesting that rootstocks may have been the source of infection. In 1950, Luckwill and Crowdy (cited as personal communication by Ponsette and Cropley 1951) reported that certain rootstocks at Long Ashton were apparently carrying rubbery wood, and in 1949 investigations were initiated to determine if infection also existed at East Malling. Stools of seven rootstocks were obtained from the stool beds at East Malling and budded with non-rubbery wood 'Lord Lambourne.' Some trees on M.1 and M.9 developed rubbery wood symptoms, whereas M.2, M.3, M.4, M.7, M.12, M.14 and Crab C were free of rubbery wood.

However, trees on M.3 and M.8 obtained from a commercial nursery were infected. Later, rubbery wood was found in East Malling stoolbeds for M.1, M.4, M.9 and Crab C, and it was also found in a rootstock trial established in 1920. No viruses were found in the new MM series (Posnette and Cropley 1953). As a result, all Malling rootstocks and cultivars were tested and re-cloned from a single clean plant and distribution of M.1 and M.9 was temporarily stopped until "clean" material was available.

The discovery of latent viruses in some clonal rootstocks and scion cultivars during the 1950s complicated studies intended to shed light on the dwarfing mechanism(s) of rootstocks. Some M.9 and M.1 rootstocks were found to be infected with rubbery wood virus, which can have a dwarfing effect (Posnette and Cropley 1951, 1953). Further studies by Posnette and Cropley (1965) with apple mosaic, cherry, and pear viruses showed dwarfing effects, both with and without leaf symptoms.

Wood (1996) tested many rootstocks being produced by commercial nurseries in New Zealand for virus, and found that most rootstocks were not infected. He reported that rootstocks imported after 1936 (MM.115, MM.102, MM.104, MM.106, M.26 and Merton 793) were not infected, but that older rootstocks (M.1, M.9, M.12, M.13, M.16 and 'Northern Spy') were infected with latent viruses and apple mosaic virus; the newer rootstock, Mark, was infected with stem pitting and chlorotic leaf spot viruses. Only M.12 was infected with rubbery wood.

Several studies were conducted to determine if combinations of latent viruses affected apple tree growth and cropping, and stool production in the nursery. When scions infected with ACLSV, spy decline, platycarpa scaly bark virus, ASPV or rubbery wood were budded onto virus-free M.26, trunk growth and yield for the first two cropping years were reduced by 20% and 30%, respectively, and infected trees had more russetted fruit than virus-free trees (Meljneke *et al.* 1975). Campbell and Bould (1970) performed a three-way factorial experiment involving six levels of soil fertility, three rootstocks, and two levels of virus infection (a combination of four latent viruses versus no virus). MM.104 was found to be most sensitive, followed by MM.111, whereas MM.106 was relatively tolerant. The viruses reduced tree growth, and there were interactions between viruses and soil fertility; when potassium levels were low the trees were small and viruses had little effect on growth. Hickey and Shear (1975) budded 'Delicious' and 'Golden Delicious' infected with ACLSV and SPV onto seedling rootstocks, and grew trees with varying levels of nitrogen. After 5 years there was no effect on growth, leaf nutrient concentration, yield or fruit finish.

For the first 3 years, combinations of some latent viruses had little effect on scion growth (Mundo and Millikan 1963; Posnette and Cropley 1965); however in the fourth year infected trees grew less than non-infected trees (Campbell and Bould 1970). Alleyne *et al.* (1989) reported little difference in water relations of several non-grafted Malling rootstocks that were infected with latent viruses compared to the same virus-free rootstocks. When four cultivars were budded onto MM.106, rootstocks that were infected with a combination of SPV, epinasty and decline virus (EDV), ACLSV and platycarpa scaly bark virus (PSBV), or a combination of SPV, EDV, ACLSV, PSBV and rubbery wood, tree growth was suppressed during the first and second years, indicating that the negative effects of latent viruses may be related to the number of viruses or virus concentration (Campbell 1971).

Apple union necrosis is caused by tomato ringspot virus (TmRSV), is vectored by dagger nematode (*Xiphinema* spp.), and also causes stem pitting in *Prunus*. Many broadleaf plants and weeds are infected with TmRSV and serve as reservoirs for the virus in orchards. Apple cultivars vary in resistance or tolerance to TmRSV. When 'Delicious,' which is tolerant to TmRSV, and some other cultivars are budded onto MM.106, the virus causes a hypersensitive reaction called "brown line necrosis" and trees decline in vigor and die. This disease develops only when the scion cultivar is resistant to TmRSV and the rootstock is tolerant. Rootstocks tolerant to TmRSV include MM.106, M.7, M.26, M.9, MARK, P.2 and B.9, whereas resistant rootstocks include M.4, M.7, O.3 and Novole. Cultivars resistant to TmRSV include 'Delicious,' 'Quinte,' 'Tydeman's Red,' 'Jerseymac,' and 'Jonathan,' whereas 'Golden Delicious,' 'Empire,' and 'York Imperial' are tolerant. Ornamental crab apples and other *Malus* species appear unaffected, as are most apple cultivars on seedling rootstocks (Biggs 2011). This disease was so severe, that the combination of 'Delicious'/MM.106 was not recommended for commercial orchards in Virginia.

Latent viruses can also affect stool bed productivity. The number and weight of M.1 rootstocks produced by infected mother plants over a 5-year period were reduced by 60% and 40%, respectively compared to non-infected mother plants. However, the number and weight of M.7 rootstocks produced by infected mother plants was only slightly reduced (Campbell 1961).

Some rootstocks in the Geneva series, especially G.16, G.30, G.814, G.935 and G.65, are susceptible to one or more of the latent viruses, and have hypersensitive reactions. When these rootstocks are budded with infected material the trees grow well with no symptoms in the nursery, but die during the first year in the orchard. Therefore, virus-free scion material must be used with these rootstocks (Fazio *et al.* 2016).

During the early 1960s, Campbell (1962) reported that no virus-free source existed in England for many commercially grown apple cultivars and rootstocks. Experiments began in 1958 in an attempt to inactivate viruses by heat therapy because the technique was effective for strawberry and raspberry. Posnette and Cropley (1956) reported that apple mosaic virus was inactivated in plants held at 37 °C for 27 days. After exposing young apple trees to 37 °C for up to 20 days, the authors used buds from new shoots to test for viruses on indicator hosts and reported temporary improvement, but system reinfection was rapid. The technique was modified by removing the shoot tip after heat treatment and wedge-grafting the shoot tip onto young apple seedlings. More than one-third of the resulting trees were virus-free. It was felt that heat treatment reduced the virus titer and the shoot tip "grew away" from the virus, so it was important to produce rapid growth under conditions unfavorable to virus multiplication and to isolate the young tip as soon as possible after treatment. More recently, virus immunolocalization in M.9 and M.26 showed that only the few top layers of cells in the apical dome and in the youngest two leaf primordia were free of ASGV, and the upper part of the apical dome and youngest three leaf primordia were free of ASPV (Li *et al*. 2016a). Welsh and Nyland (1965) demonstrated that many apple viruses could be eliminated from apple by exposing container-grown trees to 38 °C for 7 days after 7 days' conditioning at 35 °C.

Sastry and Zitter (2014) explained the development of virus certification in the UK. During the 1960s, the East Malling and Long Ashton (EMLA) research stations developed schemes for virus-free certification and "trueness-to-variety." All of the rootstocks in the EMLA scheme were raised at East Malling, and certified material was supplied to nurseries through the Nuclear Stock Association. In 1969, the first year of the program, over 80 000 stool shoots were issued, and by 1979 the number grew to over 400 000. Initially the rootstocks designated with an "A" (M.9A, M.7A, and M.26A) were free of chat fruit, rubbery wood and apple mosaic, but still carried some other latent viruses. In 1973, the EMLA clones (M.9 EMLA, M.7 EMLA, and M.26 EMLA), that were free of all known viruses, were released. EMLA and non-EMLA trees were compared in seven on-farm trials with varying soil types in the UK using 'Cox's Orange Pippin' as the scion cultivar. For the first four cropping years, trees on M.9 EMLA had yields 40% higher than non-EMLA trees, and trees on MM.106 EMLA were slightly more vigorous than non-EMLA trees, but yields were similar. One benefit of the certification program is that nurseries now produce trees that are more uniform in quality.

3. Soil Microorganisms and Apple Replant Disease. There is a scarcity of orchard sites with characteristics conducive for tree survival and annual cropping, such as frost avoidance and soil water drainage, and not previously planted to apple. In addition, orchards are being replanted more frequently because cultivars or strains rapidly become non-profitable due to changing buyer preferences. Many good orchard sites have been in apples for more than 100 years, and growers are reluctant to take land out of production for the 3–5 years required to properly renovate the soil. Trees planted on replant sites often exhibit poor growth for several years, or for the life of the orchard. In extreme situations tree mortality may be severe.

Apple replant disease is an umbrella term used to describe poor tree performance due to a complex of factors consisting of the buildup of pathogen inoculum and herbicide residue, pH and nutrient imbalance, and soil compaction. Fumigation is often used to eliminate pathogens, insects and weed seeds, but the practice is expensive, provides only temporary disease control, eliminates beneficial soil organisms, and does not alleviate abiotic factors that can negatively affect growth of young trees. Symptoms of the disorder are often obvious within a few months after planting, and may include stunted shoot growth, low root biomass, root tip necrosis, nutrient deficiencies and water stress (Mai *et al*. 1994; Mazzola and Manici 2012). Although abiotic factors may be involved in replant disorder, a number of studies identified biotic factors as the primary cause of "Apple Replant Disease" (ARD). The latter term refers to replant symptoms caused by soil-borne biotrophic pathogens, necrotrophic pathogens, oomycetes and nematodes. Biotrophic pathogens invade and acquire nutrients from living cells without killing the tissue. Many foliar pathogens are biotrophic, but most root pathogens are necrotrophs. Necrotrophic fungal pathogens infect and kill host tissue and extract nutrients from the dead host cells. Oomycetes, such as *Pythium*, are filamentous fungus-like eukaryotic microorganisms that reproduce sexually and asexually. The nematode most often associated with ARD is the root lesion nematode *Pratylenchus penetrans*. Because apple trees are perennial, rootstocks that are tolerant or resistant to ARD may be an important component of ARD-management programs. A number of pathogens have been isolated from diseased apple roots, and the pathogen complex likely varies from orchard to orchard (Mazzola 1998). However, the pathogens most often associated with the disease include *Pythium*, *Phytophthora* spp., *Rhizoctonia solani* and *Cylindrocarpon* spp., as well as the root lesion nematode (*Pratylenchus penetrans*) and bacteria (Jaffee *et al*. 1982a, 1982b; Sewell 1981; Mazzola 1999; Utkhede and Smith 2000; Tewoldemedhin *et al*. 2011; Mazzola and Manici 2012).

Phytophthora species are soil-borne fungi that cause apple root and crown rots. Root and crown rots become more severe when orchard soils are saturated with water for prolonged periods (Browne and Mircetich 1988). One goal of the Cornell-Geneva program was to develop rootstocks with resistance to ARD, particularly to *Phytophthora* (Cummins and Aldwinckle 1974), but rootstock resistance seemed to vary with *Phytophthora* species. Relative susceptibility of rootstocks to *Phytophthora* varied with location, possibly due to different species or even strains of the fungus. Wilcox (1993) compared four container-grown rootstocks with soil inoculated with one of four species of *Phytophthora*, and flooded the pots for varying lengths of time up to 72 hours. Disease incidence varied with species of *Phytophthora*, and was positively related to the length of flooding time. The incidence of disease was greatest for *P. cryptogea*, least for *P. megasperma*, and intermediate for *P. cactorum* and *P. cambivora*. Averaged over all species, crown rot incidence was highest for MM.111, lowest for M.26, and intermediate for O.3 and M.7. Browne and Mircetich (1993) evaluated 13 rootstocks for resistance to three species of *Phytophthora* in artificially infected soil, and rootstocks differed in their susceptibility to different species. Rootstocks that were resistant to *P. cactorum* included M.9, Mark, B.118 and B.9, whereas MM.106, Ant.313 and seedling were highly susceptible. Rootstocks resistant to *P. cambivora* included Mark and B.118; B.9, M.7 and P.18 were intermediate; and the others were highly susceptible. Most of the rootstocks were relatively resistant to *P. cryptogea*, except M.4, MM.111, Ant.313 and P.18.

Pythium spp. have been associated with apple crown and root rots in many countries. Seventeen different *Pythium* species were identified from roots sampled from six orchards in Washington State, and the population of *Pythium* spp. at a given site was dominated by a single species (Mazzola *et al.* 2002). The relative recovery of *Pythium* spp. from apple roots was also consistently lower in organically managed orchards. Precolonization of 'Gala' seedling roots with any one of three nonpathogenic isolates [isolate 584 of *Pythium* MM1, isolate 1-12 of *Pythium* MM3 (*aff. Oedochilum*), and isolate 1-19 of *Pythium* MM5 (*aff. vexans*)] provided biological control of root rot caused by *P. sylvaticum* and *P. ultimum*. Sensitivity to the fungicide, Metalaxyl, varied among species of *Pythium*, and several non-pathogenic species were less sensitive. The sensitivity of 22 apple rootstocks (10 Malling, 9 Malling Merton, Crab apple and apple seedling) to *P. ultimum* was evaluated at different times of the season in the field in India (Sharma and Gupta 1989). Rootstocks were most susceptible to infection in March, and least susceptible in November. Based on lesion size, MM.103 and MM.104 were most susceptible, whereas lesions were smallest for MM.110, MM.115, Crab

apple, M.2 and M.4. The more commercially important M.9, M.7, M.26, MM.111 and MM.106 were intermediate.

Rumberger *et al.* (2004) planted 'Royal Empire' on five rootstocks in the previous tree row or the previous grass lane of an orchard site, and found that trees on M.7, M.26 and G.16 remained smaller when growing in the previous tree rows compared with previous grass lanes, whereas the growth of trees on G.210 and G.30 planted in the two locations was similar. The lack of orchard position on tree growth was considered evidence that Geneva rootstocks were tolerant of soil pathogens. For all rootstocks, the composition of soil bacteria was influenced by orchard position. Leinfelder and Merwin (2006) suggested that using G.30 and G.210 rootstocks and planting in the previous grass lanes instead of the old rows may be a strategy against ARD. Auvil *et al.* (2011) also reported that trees on several Geneva rootstocks outperformed the industry standards (B.9, M.9 and M.26) on replant sites in Washington.

Plant pathogens can begin accumulating in the rhizosphere and apple roots within a year or two after planting (Mazzola 1999). Actively growing apple root tips, especially the tips of emerging lateral roots or primary roots, were highly susceptible to *Pythium ultimum*, which caused tissue necrosis with dark brown coloration throughout the root (Shin *et al.* 2016). The susceptibility of apple roots to pathogens seems to be related to differences in root growth rate, root physiology, and morphology that allow certain types of roots to avoid infection. Tolerance to soil pathogens may also be related to pathogen-induced chemical defenses mediated at the genetic level. Recent research showed that resistance to necrotrophic pathogens involves the production of antimicrobial compounds and cell wall reinforcement to limit pathogen progression and prevent cell death.

As roots grow through the soil they encounter physical, chemical, and biological environments that influence their rhizospheres and in turn plant growth. Root exudates can stimulate or inhibit soil organisms that may release nutrients, colonize the root, or modify plant growth (Watt *et al.* 2006). Root infection may occur at specific locations along the root, and an increased growth rate may limit the time a root is exposed to a pathogen. Wheat root colonization by fungi and bacteria was influenced by root age and type (pioneer versus fibrous or branch roots) (Sivasithamparam *et al.* 1978). Pioneer roots are fast-growing, have relatively large diameters, are relatively long-lived, undergo secondary growth, have limited mycorrhizal colonization, and their primary purpose is to expand the root system (Polverigiani *et al.* 2011). Fibrous roots arise from pioneer roots, are short-lived, do not undergo

secondary growth, are often colonized by mycorrhizal fungi, and are the primary conduits for water and nutrient transport to the stem. In both field and laboratory studies, rates of wheat root growth interacted with bacteria. Slow-growing roots in cool or hard soil had more *Pseudomonas* bacteria around their tips and were more heavily colonized by *Rhizoctonia* than were faster-growing roots in warmer or loose soil (Neate 1987; Watt *et al.* 2003). A grape rootstock with high rates of root growth and rapid root initiation was more tolerant of phylloxera than a rootstock with a lower root growth rate (Bauerle *et al.* 2007). To test the hypothesis that tolerance to ARD is related to root growth rates, Atucha *et al.* (2014) grew M.26 (susceptible to ARD) and CG.6210 (later designated as G.210 and tolerant to ARD) liners in pasteurized or non-pasteurized soil from a replant site. First- and second-order roots of M.26 were larger in diameter and had higher nitrogen concentrations than roots of CG.6210. Regardless of rootstock, roots lived longer in pasteurized soil. In non-pasteurized soil, but not in pasteurized soil, M.26 roots lived longer than CG.6210 roots. It was hypothesized that rootstocks with thinner, faster-growing roots that can be shed more easily and with higher root turnover may tolerate ARD infection by investing fewer resources in individual root construction. Emmett *et al.* (2014) also found that ARD organisms did not colonize the entire root system. First- and second-order roots were more heavily colonized by ARD pathogens and exhibited cortical tissue senescence compared to third-order roots. CG.210 had a finer branching structure with smaller diameter and thinner cortex than M.26. Pathogen colonization was lower in pioneer roots than in first-order fine-feeder roots. Defense compounds, such as the phenolic, phloridzin, increased with root order, with highest concentrations in third-order roots. Phenolic concentrations were also higher in roots growing in non-pasteurized compared to pasteurized soil. Atucha *et al.* (2014) suggested that pioneer roots receive differential investments of defenses than first-order fine-feeder roots. Pioneer and first-order roots of CG.6210 had lower populations of pathogens compared to M.26; therefore, rootstock tolerance to replant pathogens may be related primarily to characteristics of first-order and pioneer roots.

Young roots are colonized by beneficial mycorrhizal fungi as well as pathogenic fungi, and the two types of fungi may compete with each other. Arbuscular mycorrhizal fungi (AMF) colonization of M.26 roots was associated with faster-growing roots and roots that grew for a longer duration, leading to longer roots. Fast-growing roots were colonized by AMF within 3 days of emergence, but colonization usually occurred within 7–15 days, whereas non-AMF colonization occurred more than

25 days after root emergence (Resenes *et al*. 2008). Therefore, colonization by AMF fungi may restrict infection by pathogenic organisms. Current research at the Pennsylvania State University indicates that there is an interaction of AMF and non-mycorrhizal fungi with different soil-applied nitrogen sources (E. Lavely, personal communication). Since fast-growing roots were colonized by AMF and not by ARD pathogens, future work should concentrate on the potential interaction of these fungi to determine if AMF colonization can suppress or prevent colonization by pathogenic fungi, and if rootstocks can influence this relationship.

Plants respond to pathogen infections by activating defense genes that produce reactive oxygen species (ROS), synthesize pathogenesis-related proteins, localize cell-wall reinforcement, and produce antimicrobial compounds. Low-molecular-mass secondary metabolites with antimicrobial activity that are induced by stress are called *phytoalexins*, and are considered molecular markers of disease resistance (Ahuja *et al*. 2012). Resistance to pathogens with a broad host range is complex, and components of host resistance are being identified (Mengiste 2012). Salicylic acid, jasmonic acid and ethylene are hormones that regulate genes which are induced and/or repressed by the action of transcriptional regulators involved in pathogen immunity (Moore *et al*. 2011). Molecular-level responses to pathogens have been studied in much more detail in above-ground plant organs than in roots. Researchers at Cornell University and the United States Department of Agriculture tested the hypothesis that the molecular defense responses to necroptrophic pathogens are similar in foliar and root tissues. Jasmonic acid and ethylene signaling pathways are involved in the defense of foliar pathogens, and Shin *et al*. (2016) reported that these pathways are also functional in apple root systems induced by *Pythium* inoculation. The same group also found that, following *Pythium* inoculation, the ethylene/jasmonic acid biosynthesis gene and an apple chitinase gene were upregulated. In a later study, the induction was identified of genes regulating ROS and antioxidant metabolism, hormone biosynthesis and signaling (ethylene, jasmonate and cytokinin), cell wall fortification, and antimicrobial secondary metabolism (Shin *et al*. 2016). It was concluded that there is a high degree of conservation regarding the molecular framework of defense responses compared with those observed with foliar tissues. Now that breeders know that pathogen-induced chemical defenses are similar in above-ground and below-ground organs, marker-assisted and genomics-assisted rootstock breeding technologies can be incorporated into rootstock breeding programs (Kumpatla *et al*. 2012).

Results from rootstock trials and observations by commercial growers have indicated that some rootstocks, especially some of the Geneva rootstocks, perform better in replant situations than the widely grown Malling rootstocks. Isutsu and Merwin (2000) compared the growth of 941 rootstock genotypes in soil collected from replant sites that was fumigated or not fumigated, and found that G.65, CG.6210 and G.30 were tolerant to ARD. Based on field trials, Robinson *et al.* (2006) concluded that G.935 and G.202 had good tolerance to ARD. In a replant study in Washington State, G.11 and G.30 were more tolerant to lesion nematode than M.7, M.9, M.26, MM.106, and MM.111. Trees on M.26, MM.106 and MM.111 were more susceptible to *Pythium* spp. than trees on B.9 and rootstocks in the Geneva series (Mazzola *et al.* 2009). In replant trials in North Carolina, trees on G.30 and G.210 performed better in replant soils than trees on M.26 and M.7 (Parker *et al.* 2014).

To aid rootstock breeders in selecting genotypes with tolerance to ARD, researchers recently began to study the genetic mechanisms involved in ARD tolerance. Zhu *et al.* (2014) discussed the current status of breeding tools and possible approaches which may be employed to develop apple rootstock genotypes with resistance to ARD pathogens. The mechanisms of resistance to ARD pathogens have not been well studied, but molecular characterization of root responses to infection may enhance future marker-assisted and genomics-assisted breeding for resistance. Specific genes associated with ARD resistance have yet to be identified, but rootstock breeders are beginning to use genomic tools to connect desirable traits and tightly linked allelic forms of genes that are known to impart superior traits (Zhu *et al.* 2014).

Apple trees form symbiotic relationships with naturally occurring vescular-arbuscular mycorrhizal fungi (AMF; sometimes referred to as VAM), which penetrate the cortical cells of the roots and help plants absorb nutrients (especially phosphorus) from the soil and often improve plant growth (Gerdemann 1968; Mosse 1973). Sometimes, AMF colonization enhances tolerance to biotic and abiotic stresses (Menge *et al.* 1978; Guillemin *et al.* 1994). Mosse (1957) was the first to report the benefits of AMF on apple tree growth, and showed that inoculated apple seedlings accumulated higher K, Fe, and Cu, and less Mn in leaves and roots. However, the influence of mycorrhizal fungi on apple tree growth depends on soil conditions. Mycorrhizae-inoculation enhanced apple seedling growth in fumigated orchard soils without supplemental P more often than when P was applied (Hoepfner *et al.* 1983). Mycorrhizal fungi can also enhance the water status and drought tolerance of apple trees by improving the absorption and translocation of water by external hyphae (Runjin 1989). Sumorok *et al.* (2011)

identified three species of AMF from under non-mulched apple trees, and up to five species under trees mulched with manure, and showed colonization to be greater under several types of organic mulches compared to non-mulched trees.

Some studies have indicated that AMF inoculation may enhance tree growth in the nursery, especially the establishment of micro-propagated rootstocks (Cavallazzi *et al*. 2007). During acclimation, micro-propagated Marubakaido and M.9 rootstocks were inoculated with a mixture of *Scutellospora pellucida*, two isolates of *Glomus etunicatum*, and *Glomus* sp. After 81 days, inoculated Marubakaido were larger than non-inoculated plants, but the opposite was true for M.9 plants (Locatelli and Lovato 2002). In a study comparing sources and doses of Fe, roots of MM.106 treated with a high rate of Fe-EDDHA on a calcareous soil had more AMF than roots of M.9 and M.26 (Coskan *et al*. 2009). AMF inoculation of micro-propagated MM.106 plants stimulated shoot growth through improved P nutrition; subsequently, Fortuna *et al*. (1996) suggested that mycorrhizal fungal inoculation in nursery production may enhance the growth of plantlets during the acclimation phase of micro-propagated trees. Morin *et al*. (1994) inoculated four micro-propagated apple rootstocks with one of four AMF, and grew the plants in containers with nursery soil with high levels of P. Although M.26 tended to respond better than P.16, inoculation enhanced the growth of all rootstocks. Regardless of the mycorrhizal fungal species, inoculated plants were larger and had larger leaves and higher leaf P concentrations than non-inoculated plants. Despite high soil P, enhanced growth was related to improved P nutrition.

In a millet field trial, AMF colonization was strongly influenced by the interaction of genotype by location; the growth responses differed between the genotypes and they also differed in their response to P uptake and AMF inoculation (Krishna *et al*. 1985). Therefore, it is likely that AMF colonization and response to colonization may vary with rootstock (Locatelli and Lovato 2002). Miller *et al*. (1985) examined roots from a multi-location rootstock trial with nine rootstocks at 18 locations in the USA, and identified three to eight species of AMF depending on location, with the level of colonization varying with location. The proportion and intensity of colonization were negatively correlated with soil Zn and, at most locations, P levels. Mycorrhizal colonization was not influenced by rootstock. AMF have been evaluated for their potential involvement in ARD. AMF can inhibit plant root diseases through mechanisms that are not well understood (Taubebaab and Baltruschat 1993; Azcón-Aguilar and Barea 1996). The potential mechanisms include the stimulation of host plant resistance pathways,

interactions between AMF and pathogen mycelium, enhanced nutrient uptake (especially P), and modification of the rhizosphere microflora. Ridgway *et al.* (2008) inoculated M.26 stool shoots with one of four mycorrhizal fungi and grew them in soil from a replant site or non-replant soil. Although no AMF fungi reduced the disease symptoms on the main and feeder roots, plants inoculated with *S. calospora* and *A. laevis* had the most colonized roots and the greatest growth. Inoculating the roots of 'Gala' on M.26 with the bacteria *G. intraradices*, *E. aerogenes* (strain BS), and *B. subtilis* (strain EBW-4) increased yield and tree growth and reduced root infection by *P. cactorum* and *P. ultimum* (Utkhede and Smith 2000). It was suggested that some of these AMF might be applied as a post-plant drench as an alternative to soil fumigation. Gąstoł and Domagała-Świątkiewicz (2015) applied several pre-plant treatments to 'Topaz' trees on M.26, including root inoculation with the AMF liquid and granular inocula (MicroPlant E, MicroPlant M, and MicroPlant S) that contained combinations of *Glomus intraradices*, *G. mossae*, *G. gregatum*, *G. etunicatum*, *G. desertícola*, *G. monosporus*, *G. brasilianum*, *Gigaspora margarita*, *Rhizopogon* sp., *Scleroderma* sp., *Suillus* sp., *Laccaria* sp., as well as bacteria strains *Bacillus* sp. and *Azotobacter* sp. before planting on a replant site. After 3 years, trees treated with liquid formulations of AMF were larger and had higher yields than non-treated trees. Treated trees tended to have higher leaf levels of N, P, K, Ca, S, and Cu. Few studies have been conducted to identify the potential interactions of AMF inoculation of different rootstocks under long-term field conditions, and it would be interesting to know if new rootstocks from active breeding programs differ in their ability to support different mycorrhizae, and if the different rootstocks respond similarly to AMF colonization.

A rapidly expanding area of research in the area of host plant–herbivore interaction is chemical ecology. Plants being attacked by herbivores release various chemicals, such as alkaloids, terpenes, and phenolic compounds, to defend themselves (Baldwin *et al.* 2006). Injured plants can also defend themselves by releasing volatile organic compounds that attract predators and/or parasites (Whitfield 2001). Abraham *et al.* (2015) found that larval feeding activity of the beetle *Melolontha melolontha* on M.9 roots induced the production of camphor in the roots, as well as methyl salicylate and (E,E)-α-farnasene and nine other volatiles from leafy shoots. Grape root borer larvae responded differently to root extracts (Bergh *et al.* 2011) and root pieces (Rijal *et al.* 2013) collected from different grape rootstocks. The identification of behaviorally active root volatiles may spur research into differences among commercially important rootstocks in the composition of root

volatiles, the concentration of behaviorally active compounds, and their potential effects on host suitability to – and preference by – insects and nematodes that feed on apple roots.

4. Apple Rootstocks for Organic Management. Organic horticulture is one of the fastest-growing agricultural sectors. Organic farming refers to an integrated system that combines conservation practices with modern technologies, but excludes common conventional inputs such as synthetic pesticides and fertilizers. In general, trees on dwarfing rootstocks are easier to spray for controlling pests, and may be preferable for orchards managed with organic practices. Compared to conventional orchards, there is usually more weed pressure in organic orchards. Therefore, dwarfing rootstocks with vigor similar to M.26 may perform better than less-vigorous rootstocks used in conventional orchards. The Malling rootstocks were not selected for resistance to pests, but the Malling-Merton series was selected for resistance to woolly apple aphid. The Geneva rootstocks were selected for resistance to woolly apple aphid and some pathogens, but field testing has not been completed and few reports exist of rootstock comparisons using organic production practices.

Most of the research involving organic apple production has focused on various orchard floor management systems. In a Swedish study, five orchard floor systems were compared with M.9 as the rootstock and trees in the Swiss sandwich system had high yield and yield efficiency and the largest fruit (Tahir *et al.* 2015). In the sandwich system, annual or perennial crops are grown in a narrow strip within the tree row and the soil to each side of the strip is tilled. A Michigan study used 'Pacific Gala' to compare three orchard floor management systems with M.9 RN29, M.9 NAKBT337 and Supporter 4 as rootstocks (Stefanelli *et al.* 2009). The interaction of rootstock by floor management system was significant for most response variables. After 6 years, trees on M.9 RN29 grown in the Swiss sandwich system were most productive. In a Danish study, 'Retina,' a cultivar with resistance to apple scab, was grafted onto M.9, J.9 and M.26 rootstock and grown with six ground cover management treatments (Pedersen and Pedersen 2004). After 5 years, trees on M.26 had the largest trunks and M.9 had the smallest trunks. Rootstock had no effect on diseases, but trees on M.9 had a higher percentage of fruit with red apple aphid injury than trees on M.26. Trees on J.9 had more marketable fruit than trees on M.26. In 2008, an organic trial was established in Germany with 'GoldRush' grafted onto G.16, CG.11 and M.9 (Pfeiffer 2014). After 5 years, the cumulative yield was highest for trees on CG.11 and lowest for trees on M.9, while trees on

G.16 had the highest percentage of fruit with russetting. All fruit on all rootstocks had some apple scab in 2013. Results were similar after 7 years, and the author suggested that G.11 should be considered for organic orchards due to high yield potential (Pfeiffer 2016).

Three new rootstock selections from the East Malling program were evaluated in conventional and organic systems at East Malling (Saunders 2012). After 8 years under organic management the new selections did not have significantly different yields or yield efficiency from M.9. However, AR295-6 was promising because it had vigor similar to M.9 and yield efficiency was equal to or greater than M.9.

Because several Geneva rootstocks often performed better than rootstocks from other programs on replant sites, it seems that Geneva rootstocks may be well suited for organic orchards. In 2015, a multi-location organic apple rootstock trial was established at 15 North American locations with the scion cultivar 'Modi.' The trial includes 10 Geneva rootstocks plus M.9 NAKBT337 as a standard. After the first season, the largest trees were on G.890 and G.41, whereas trees on G.222 and G.16 were the smallest (Autio 2016). This study will provide valuable information on rootstock performance in one organic orchard system. However, some reports have indicated that rootstock performance may depend on the orchard floor management system that is used. Therefore, future rootstock trials should compare several rootstocks in several orchard floor management systems to better understand potential interactions between rootstock and orchard floor systems. Additional research is also needed to determine if some rootstocks are more competitive with vegetation in the tree row, and if some rootstocks can better utilize fertilizers from organic sources.

V. INTERSTEMS

One of the major reasons that dwarf rootstocks were not widely planted in North America until recently was because their root systems are one-sided or brittle, and non-supported trees are poorly anchored, break easily, or fall over, and the central leaders lean or bend under the weight of a crop (Brase 1953). American growers felt that the high establishment costs associated with supported systems required for dwarfing rootstocks could not be economically justified. Additionally, Malling rootstocks were thought to lack adequate cold hardiness for many North American apple-growing regions. A potential solution to this problem was to use a hardy dwarf interstem with a vigorous rootstock to produce a free-standing, cold-hardy, semi-dwarf tree. The practice of

double-grafting to produce interstem trees had been used to produce dwarf trees for many years, but interstem trees were more expensive and the system is more complex because it involves two graft unions. Virginia Crab is winter-hardy, tolerates collar rot, and was commonly used as a rootstock and as an interstem during the 1930s and 1940s in North America. Stark Brothers Nursery in Missouri sold a four-part tree with Virginia Crab root, 10 cm-long Clark Dwarf stem piece, Hibernal hardy stem and the cultivar, and some commercial orchards planted these trees with little research data to support their use. The rootstock known as Clark Dwarf was later identified as M.8 (Brase 1953). The plantings were generally not very profitable because the trees were too small for their spacing and tended to lean, produced excessive root suckers, and had virus problems (Carlson 1992). By the 1950s there were reports of poor tree growth due to incompatibility or "uncongeniality" with some scion cultivars, possibly caused by a virus (Tukey *et al*. 1954; Miller 1954). Later, it was learned that Virginia Crab was sensitive to stem pitting (McCrum *et al*. 1960), caused by apple stem grooving virus, which is latent in most apple scion/rootstock combinations.

At the turn of the 20th century, European nurserymen budded apple cultivars onto vegetatively propagated layers, and felt that the scion cultivar had no effect on the root system. American nurserymen propagated trees by bench grafting onto seedling rootstocks or seedling root pieces, and they felt that the scion cultivar influenced the root system to such an extent that they could identify some cultivars by observing the roots upon digging in the nursery (Hatton 1931). In an attempt to determine the effects of the scion and rootstocks on each other, researchers used interstem trees as a tool for studying rootstocks, and learned that the scion had little influence on the roots of Malling rootstocks, but the scion influenced the morphology of seedling roots (Knight 1927; Amos *et al*. 1930; Vyvyan 1930).

Following World War II, increasing labor costs again sparked commercial interest in smaller trees and the use of interstems to produce hardy, precocious, semi-dwarf trees in America. In general, trees with dwarf interstems were smaller and more precocious than trees on seedling roots, but to a lesser extent than trees on dwarfing rootstocks (Tukey and Brase 1943; Carlson 1965b; Carlson and Oh 1975), and the degree of dwarfing was related to the length of the interstem (Grubb 1939). When various clonal rootstocks were used as interstems on different Crab seedling rootstocks, the inherent vigor of the rootstock had less effect on tree size than did the interstem, and the vigor ranking of rootstock clones was similar whether used as an interstem or rootstock. The size of 12-year-old 'Delicious' and 'Jonathan' trees decreased at a declining

rate as the length of M.8 interstem on A.2 rootstock increased from 10 to 30 cm. The yield per tree declined linearly with increasing interstem length, but 10 and 20 cm-long interstem trees had higher yields than the A.2 control. Yield estimates, based on tree spacing adjusted for canopy diameter of the 12-year-old trees, showed that yield per hectare increased at a decreasing rate as the interstem length increased (Carlson and Oh 1975). Roberts and Blaney (1967) placed interstems of varying lengths at 8 cm or 30 cm above ground, and found that the lower placement had a greater effect on scion growth; however, they found that the increased flowering was not closely related to growth suppression. The desire for free-standing semi-dwarf trees during the 1970s resulted in a number of rootstock trials on American research stations and in commercial orchards. In most trials, the rootstocks were seedling, MM.111 and MM.106, and M.26 was used as the interstem. Early observations of young trees indicated that interstems often developed burrknots and had excessive suckering, but the latter could be reduced by deep planting (Costante and Lord 1980; Simons 1980). Root suckering was a problem in other trials, and the number of suckers per tree was positively associated with tree vigor (Ferree 1982). Sometimes, tree mortality was unacceptable because fire blight infected the susceptible interstem. Grower experiences with interstem trees were variable, sometimes because the potential size of the trees was unknown and trees did not fill their allotted space because trees were spaced too widely and young trees were allowed to crop too heavily (Brown 1983). In general, interstem trees and those on dwarfing rootstocks performed better when supported by a trellis or a tree stake (Brown 1983). Freestanding 'Mutsu' trees on M.9/MM.106 or M.9/MM.111 were larger but more productive than trees on MAC.9 (later named Mark) rootstock (Ferree and Schmid 1997). Results from 18-year experiments with 'Delicious' and 'Golden Delicious' showed that M.9/MM.106 interstem trees were similar in size or smaller than trees on M.9, whereas M.9/MM.111 trees were similar in size to M.26 (Barden and Marini 1997, 1999). Survival of trees on M.9/MM.106 was lower than for trees on M.9, M.26 or M.9/MM.111. The cumulative yield efficiency for both interstem trees was similar to M.9. Lord *et al.* (1985) compared M.9 and M.27 interstems on MM.111 and MM.106 rootstocks with trees on M.26, M.9 and M.27 (where trees on M.9 and M.27 were supported with stakes). After 8 years all interstem trees were similar in size, and had cumulative yield and yield efficiency similar to trees on M.26. Experiences acquired by researchers and commercial growers showed that interstem trees required support for maximum productivity. Non-supported leaders of interstem trees tended to bend under the weight of a crop, and trees usually did not

obtain the canopy volume required for high yields. By the late 1980s, most researchers and commercial growers felt that interstem trees offered few advantages over dwarfing rootstocks.

VI. INFLUENCE OF ROOTSTOCK ON FRUIT CHARACTERISTICS

A. Fruit Mineral Content

Several preharvest and postharvest apple fruit disorders are associated with the mineral content of the fruit, and rootstock researchers have evaluated the nutrient levels of leaves and fruit from rootstock trials. In a pear rootstock trial, rootstock did not affect fruit weight, but flesh firmness and leaf mineral element accumulation were influenced by rootstock (Ikinci *et al.* 2014). When comparing the mineral concentration of apples from four cultivars used as scions and interstocks on M.9 rootstock, Eaton and Robinson (1977) found that leaf and fruit mineral concentration was affected by scion cultivar, but not by interstocks and there was no scion–interstock interaction. 'Delicious' fruit on Mark generally had relatively high Ca concentrations, and fruit from trees on OAR1 had relatively low Ca concentrations (Autio 1991). Early results from a rootstock trial with 'Honeycrisp' indicated that rootstock may influence mineral element concentrations of leaves and fruit, but results were not consistent from year to year (unpublished data, NC-140). There is also recent evidence that rootstock can influence nutrient absorbance and transport in the scion (Fazio 2016). As research reveals the genetic control of nutrient uptake and transport, orchard nutritional programs may take into consideration specific rootstock–scion combinations.

B. Fruit Quality and Maturity

Hewetson (1944) was among the first to report the effect of interstems on apple fruit quality. He reported that an M.9 interstem advanced fruit maturity because fruit were redder and abscised earlier than fruit on trees with more vigorous interstems. Therefore, growers were sometimes advised to harvest fruit from such trees one week earlier than for other rootstocks (Perry and Dilley 1984). This led to additional research on the influence of rootstocks on fruit quality. In a 2-year study with 'Empire' grafted to MM.111 and two lengths of M.9/MM.111 interstem sections, Perry and Dilley (1984) compared various aspects of fruit quality and maturity. They reported that fruit on interstem trees became climacteric earliest, but flesh firmness and starch disappearance were

not influenced by rootstock. Lord *et al.* (1985) evaluated 'Empire' fruit quality from eight rootstocks or interstock combinations over 3 years, and found that the effect on fruit weight was inconsistent; however, fruit from trees on M.27/MM.111 became climacteric later than those from trees on M.26 and M.27 for two of the three years, but the delay was small. Flesh firmness was not influenced by rootstock, but the soluble solids concentration (SSC) was higher for fruit from trees on M.27 than on M.26, M.9/MM.111 and M.27/MM.111. Senescent breakdown was greater in fruit from trees on M.26 than on M.9, M.27 or the two interstems. In a 2-year study comparing six rootstocks, fruit on OAR1 rootstock had greatest SSC, yellow color and firmness, but fruit were also smaller than fruit on trees on other rootstocks (Fallahi *et al.* 1985). In a 1-year study, Drake *et al.* (1988) evaluated the influence of three rootstocks on maturity and storage of 'Goldspur' apples. Fruit from trees on seedlings entered the climacteric earliest, followed by M.26 and MM.111. Fruit from trees on M.26 developed the reddest color, and the highest acids, SSC and Ca concentrations. Over a 2-year period, 'Granny Smith' and 'Greenspur' fruit from trees on M.26 had higher SSC and Ca concentrations, but poorer red blush and a higher percentage of sun scald than fruit from trees on seedling or MM.111 rootstocks; moreover, fruit from trees on M.26 appeared to mature earlier than those on MM.111 (Drake *et al.* 1991). Autio (1991) and Barden and Marini (1992) evaluated the effect of rootstock on 'Delicious' fruit quality harvested from a multi-location rootstock trial. Autio (1991) reported that fruit size was consistently largest on trees on M.9, and smallest from trees on OAR1. When the effects of crop density (CD) was accounted for by using an analysis of covariance, M.27 advanced fruit ripening and M.7 delayed fruit ripening. Autio also suggested that the effects of rootstock on fruit storability were likely related to their effects on maturity and Ca levels. Over a 3-year period, SSC – but not flesh firmness, starch rating and red color – was consistently high for fruit from trees on Mark and O.3, but other maturity and quality indices were not consistent over the years. Data for SSC, starch rating and ground color were ranked and summed to calculate a maturity index, which was highest for fruit from trees on O.3, Mark, and M.26 than for fruit from trees on M.27, OAR1, MAC24, M.9 and M.7 (Barden and Marini 1992). When averaged over 2 years, 'Auksis' apples harvested from trees on Bulboga rootstock had the highest maturity index of 12 rootstocks, and were firmer and had a lower starch rating than fruit from trees on P.2 (Kviklienė and Kviklys 2006). From these published studies, some rootstocks appeared to influence fruit maturity and quality, but fruit quality may also be influenced by crop load, and in most

cases crop load was not controlled or accounted for. Differences in red color development may be related to canopy size and shading. Of the rootstocks tested, fruit from trees on M.26 seem to have a high SSC. A multi-location rootstock trial involving the most commonly planted rootstocks (M.9, B.9, M.26, G.935, G.41, G.30 and MM.111), and carefully managed to control crop load for several years, would allow researchers to evaluate the effect of rootstock on nutrient uptake, fruit quality, and fruit storability.

C. Fruit Size

Fruit size is an important economic factor, and rootstocks that produce large fruit would be advantageous. Average fruit weight (FW) is usually reported for rootstock trials and the effect of rootstock on FW has been inconsistent, especially where crop load was not controlled. Hatton (1935) was the first to report that fruit from trees on M.9 were larger than fruit from trees on other Malling rootstocks. 'Gala' seasonal fruit growth and FW were not affected by rootstock in Wisconsin (Al-Hinai and Roper 2004). These results confirmed previous results from rootstock trials where FW was not consistently affected by rootstock (NC-140 1996; Barden and Marini 1997, 1999, 2001a,b; Fallahi and Mohan 2000). Jackson and Blasco (1975) reported that 'Cox's Orange Pippin' fruit from trees on M.9 were larger than those from trees on M.26, M.7, and MM.106. This report was confirmed by Preston *et al*. (1981), where 'Bramley's Seedling' fruit from trees on M.9A were larger than those on M.27, M.26, and MM.106. Compared to trees on M.7, M.26 and M.9/MM.111 interstem, 'McIntosh' trees on M.9 produced the largest fruit, and interstem trees the smallest fruit (Autio and Southwick 1993). As part of the first NC-140 multi-location trial, Autio *et al*. (1991) compared nine rootstocks in Massachusetts, and found that 'Delicious' FW was consistently higher for trees on M.9 EMLA and lowest for trees on OAR1. However, Elfving and Schechter (1993), using trees from the same trial in Ontario, reported that fruit weight was not influenced by rootstock, but was negatively and linearly related to the number of fruit per tree.

Similar to various aspects of fruit quality and maturity, an important factor that often complicates evaluation of the influence of rootstock on FW is the crop density (CD; fruit per cm^2 trunk cross-sectional area), which may vary with rootstock and year. Vigorous rootstocks often have high numbers of fruit per tree, but relatively low CD. For trees of similar size, FW is negatively related to the number of fruit per tree and CD

(Forshey and Elfving 1977; Elfving and Schechter 1993). More recently, some researchers have used analysis of covariance (ANCOVA) to adjust FW estimates for variation in CD (Autio 1991; Barritt *et al.* 1995, 1996, 1997; Marini *et al.* 2001b). An ANCOVA model contains both continuous variables (CD) and group indicator variables (rootstock). Means that are adjusted for the covariate (least squares means) can then be compared with a multiple comparison. These are actually means estimated at the average CD of all trees in the experiment. However, if the range of the CD is not similar for all rootstocks, or if CD interacts with rootstock, a normal ANCOVA is not appropriate (Milliken and Johnson 2002). A significant rootstock–CD interaction indicates that the influence of CD on FW (slope) depends on the rootstock. Rootstock researchers often use ANCOVA, but rarely test the interaction of CD × rootstock, and simply compare rootstock means at the average value of CD. Marini *et al.* (2002, 2008) found that for rootstock trials the assumptions required for a normal ANCOVA were usually not satisfied because the ranges of CD were not always similar for all rootstocks, and the slopes were usually not homogenous for all rootstocks and for combinations of year and rootstock. A strategy for analyzing these types of data sets, using an unequal slopes model was recently suggested by Marini and Ward (2012). In two multi-location rootstock trials where data were analyzed with an unequal slopes model, there was a three-way interaction (location × rootstock × CD) (Marini *et al.* 2002, 2008). At some locations, normal ANCOVA was appropriate, so data were analyzed by location. For most combinations of year, location and rootstock, trees on M.9 produced larger fruit than trees on M.26. In an attempt to verify these results, a multi-location trial was established specifically to evaluate the influence of rootstock on the relationship between 'Golden Delicious' FW and CD (Marini *et al.* 2012). Over five cropping years, trees were hand-thinned to create a range of CDs and the four-way interaction of location × rootstock × year × CD was significant, so data were analyzed by location. At eight of the 12 locations CD interacted with year and/or rootstock, and an unequal slopes model indicated that slopes were most negative for trees on M.26 and least negative for trees on M.9, indicating that FW was most affected by CD for trees on M.26. Regardless of CD, FW was generally lowest on G.16 and highest for trees on M.9, with M.26 being intermediate. This experiment also provided an opportunity to evaluate the influence of rootstock on return bloom after adjusting for previous season CD, and flower density was not consistently influenced by rootstock (Marini *et al.* 2013b). Ferenc (2009) also found that alternate bearing was not greatly influenced by rootstocks varying in vigor from M.9 NAKBT337 to MM.111.

Although multi-location trials – where there is an attempt to control crop load – is the best way to evaluate rootstock effects on fruit size and fruit quality, adjusting crop loads to predetermined target levels is challenging due to frost, biennial bearing, and difficulties in counting fruit in the early season. Failure to control or account for these factors may be responsible for conflicting results in the literature.

VII. GENETICS AND BREEDING

A. Genetic Factors Controlling Important Rootstocks Traits

The study of the inheritance of important apple rootstock traits can be a rather difficult endeavor, as some of the traits investigated are measured on a different genotype (the scion) and are collected over many years of the life of an orchard. Despite these difficulties, there have been some major advances in understanding the complexity and inheritance of inherent (belonging to the rootstock genotype) and induced (measured in a grafted scion) rootstock traits in recent years. The advent of genome sequences (Velasco *et al.* 2010; Li *et al.* 2016b), molecular genetic maps (Liebhard *et al.* 2003; Segura *et al.* 2009; Antanaviciute *et al.* 2012; Moriya *et al.* 2012; Jensen *et al.* 2014), quantitative trait mapping (Fazio *et al.* 2014), root-specific Expressed Sequence Tags (Gasic *et al.* 2009), and microarrays (Jensen *et al.* 2010; Chagne *et al.* 2012; Bianco *et al.* 2014) have facilitated research on traits which were difficult to discern in rootstock breeding populations. Individual traits are discussed and grouped as induced or inherent below.

1. Induced Traits

Dwarfing and Early Bearing. The length of time required to measure these traits in the field after producing a seedling population makes them important targets for marker-assisted breeding (Fazio and Mazzola 2004). The first report of research into the understanding of how M.9 and close relatives might induce dwarfing on grafted scions came from New Zealand as part of a major effort on genome mapping of apple traits (Gardiner *et al.* 1994; Rusholme-Pilcher *et al.* 2004; Pilcher *et al.* 2008) where the group, using bulked segregant analysis data from a cross between M.9 and 'Robusta 5' (R.5), reported that a major gene *Dw1* on the top of chromosome 5 of apple was responsible for dwarfing in M.9-derived material. The effect of *Dw1* was confirmed and the effect of a new dwarfing locus *Dw2* in the middle of chromosome 11 was

identified when two independent populations (O.3 × R.5 and G.935 × B.9) were analyzed with quantitative trait mapping (Fazio *et al.* 2014). This effort also was the first to reveal the relationship between dwarfing and early-bearing induction by identifying an allele modeling two genetic factors *Eb1* and *Eb2*, which summarily co-located with *Dw1* and *Dw2*. The interaction between *Dw1-Eb1* and *Dw2-Eb2* was confirmed by two independent studies (Foster *et al.* 2015; Harrison *et al.* 2016a), where the addition of a third locus on chromosome 13 affecting bark morphology might play a role in dwarfing scions. Two flowering loci, MdFT1 and MdFT2, were identified in apple (Kotoda *et al.* 2010). MdFT1 was expressed in apical buds in the adult phase, but not in the juvenile phase, whereas MdFT2 was expressed in flower buds and young fruit. An interesting area of research would be to determine if rootstock can influence the expression of these genes.

Nutrient Concentration in Scion Leaves and Fruit. Apple rootstocks perform the essential function of foraging for mineral nutrients contained in soil substrates. These nutrients are transported through rootstock vascular tissues to scion tissues and used in various essential plant functions, including photosynthesis. Fazio *et al.* (2013) were first to report in all fruit tree rootstock species the inheritance of genetic factors affecting nutrient concentration in grafted scions. They used quantitative trait analysis of measurements of leaf mineral concentrations for K, Na, P, Ca, Cu, S, Zn, Mg, Ni and Mo in 'Golden Delicious,' 'Red Delicious,' and 'Gala' scions grafted onto a segregating population of apple rootstocks to map loci that affected these individual traits (Fazio *et al.* 2013, 2015a). This analysis also revealed a complex landscape for rootstock-induced nutrient concentrations, as several of these traits are correlated (share similar physiological pathways), and at the same time identified major loci for K and P concentration on chromosomes 5 and 11.

Rootstock-Induced Resistance and Gene Expression Changes. A major development in the understanding of how rootstocks influence the development, productivity and resistance in a grafted scion came from a series of studies that showed that rootstocks influenced differential expression of genes related to tree architecture (Jensen *et al.* 2003, 2010) and disease resistance (Jensen *et al.* 2011, 2012). The researchers found that rootstocks had significant effects on the fire blight susceptibility of 'Gala' scions, and rootstock-regulated gene expression patterns could be correlated with differences in susceptibility. In addition, they found that each rootstock triggered a distinct, reproducible scion gene expression pattern with 116

gene transcripts whose expression levels were correlated with tree size and encoded sorbitol dehydrogenase, homeobox-leucine zipper, and hevein-like proteins associated with larger trees and a transcript predicted to encode an extensin-like protein associated with smaller trees. Similar methods were used to identify genes and gene expression quantitative trait loci (eQTL) associated with disease resistance and the concentrations of phenolic compounds in leaves (Jensen *et al.* 2014). Several known and unknown flavonoid compounds clustered with the expression profile of APPLE00R00024377 (At3g55120), a chalcone-flavanone isomerase located on chromosome 12, whose expression segregated 1:1 in the O3R5 population. There was also a significant correlation between the expression of APPLE00R00024377 on the expression of the next pathway step APPLE0F000003679 annotated as a flavonoid 3′ hydroxylase IIb [*Malus* × *domestica*] found on chromosome 6, then APPLE0F000019828 (eQTL on LG07 at 36.958 cM with LOD of 9.13 physically located on chromosome 13 annotated as a putative UDP-galactose-flavonoid 3-O-galactosyltransferase [*Malus* × *domestica*]), and APPLE0F000021492 (eQTL located on LG07 at 36.958 cM with LOD of 14.04 physically located on chromosome 13 annotated as a UDP-glucose:flavonoid 7-O-glucosyltransferase [*Malus* × *domestica*]). The methods of eQTL discovery and analysis have been useful to discover gene networks and major switches of gene networks. Furthermore, they determine the complexity of a trait for rootstock breeding purposes, and underscore the importance of the influence of the "genetic background" when transferring major factors during breeding.

2. Inherent Traits

Resistance to Fire Blight. The rootstock phase of fire blight is caused by the anaerobic, Gram-negative bacterium *Erwinia amylovora*, which causes visible symptoms as the bark oozes yellow brown liquid and kills the whole tree by girdling it below the graft union. Whilst spraying antibiotics such as 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 an apple rootstock: 1) a multi-genic type similar to that found in *Malus robusta* cv. 'Robusta 5' where green tissues and flowers are not affected by the bacterium (Aldwinckle *et al.* 1974; Cummins and Aldwinckle 1974); and 2) an ontogenic type of resistance found in Budagovsky 9 (B.9) rootstock, where the green tissues are severely

affected, but 2-year-old and older wood seems not to react to the bacterium (Russo *et al.* 2008c). 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' × '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). Transformation approaches using 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). The inheritance of the B.9 type of resistance is currently not known.

Resistance to Woolly Apple Aphids. The development of apple rootstocks resistant to woolly apple aphids (WAA) was one of the first breeding objectives developed in the Malling-Merton (MM) apple rootstock improvement program, as the disease pressure of these aphids made the cultivation of apples very difficult in the southern hemisphere (Cummins *et al.* 1983). The donor parent of resistance to WAA was 'Northern Spy' which, when crossed with several Malling selections, resulted in the WAA-resistant vigorous rootstocks MM.106 (Northern Spy × M.1) and MM.111 (Northern Spy × Merton 793). The 'Northern Spy' type of resistance seems to be monogenic (the *Er1* locus) and has been mapped to chromosome 8 of apple. Monogenic resistance to WAA derived from 'Robusta 5' (Bartish *et al.* 1999) has been mapped to chromosome 17 (Er2 locus), and has been utilized extensively in the Geneva, NY, 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, 2005; Sandanayaka and Backus 2008). Marker-assisted selection is being used to select parents and cull progeny that do not possess the resistance locus (Fazio *et al.* 2011, 2015b; 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.

Resistance to Crown Gall. This work is being conducted to identify markers for marker-assisted breeding at the Morioka, Japan research station (Moriya *et al.* 2010). The researchers used *Malus sieboldii*

'Sanashi 63' as the resistance source to map the crown gall resistance gene *Cg*. They applied a genome scanning approach on the mapping population JM7 (*cgcg*) × *Malus sieboldii* Sanashi 63 (Cgcg) to map the resistance to linkage group (LG) 2, where only flanking markers CH03b01 and NZmsPal92 showed good allelic association with Cg.

Resistance to Powdery Mildew. One of the most important parents for several Geneva® rootstocks is *Malus robusta* cv. 'Robusta 5'. It has been used as a source of resistance to powdery mildew caused by *Podosphaera leucotricha* in apple breeding programs (Hemmat *et al*. 1994; Bartish *et al*. 1999). The resistance has been mapped to a single locus, Pl1, located on chromosome 12 of the apple genome (Markussen *et al*. 1995; Evans and James 2003; Dunemann *et al*. 2007; Bus *et al*. 2010), and is thought to be associated with a *NBS-LRR* type gene. Jensen *et al*. (2014) identified several other expressed genes on chromosome 12 that might be needed in synchrony to exhibit full resistance.

B. Apple Rootstock Breeding Programs

Prior to the efforts of scientists at the East Malling Research Station near Kent in England, there were no formal breeding, characterization and selection programs for apple rootstocks (Hatton 1917, 1919, 1920a,b; Hatton and Rogers 1945). Traces of Jaune de Metz, Paradise and French Paradise apples that are thought to be synonymous with the dwarfing and precocious apple rootstocks known as M.9 and M.8 and other related landraces in central Europe can be found in several pomology books prior to 1900 (Monceau 1768; Loudon 1822; Lindley 1828; Rivers 1866), and may very well have been initially selected because, on their own roots, the trees are dwarf, precocious, and produce early season edible fruit: apple trees that could be grown in small quarters and gardens (Tukey 1964). While dwarfing, precocity and productivity were the first traits to be evaluated in East Malling and John Innes Institute in Merton, England, the attention of the British breeding programs shifted to breeding for resistance to woolly apple aphid (*Eriosoma lanigerum*) resulting in the release in the 1950s and 1960s of the Malling (M.25, M.26, M.27) and Malling-Merton (MM.101–MM.115) series. The East Malling breeding program, after a hiatus due to programmatic and budgetary reasons, has been revived and is in the process of evaluating material from crosses made during the late 1900s and making new crosses, producing genetic maps and other rootstock breeding support data (Fernandez-Fernandez *et al*. 2008; Antanaviciute *et al*. 2013; Harrison *et al*. 2016b). Additional breeding programs have developed

in other countries with common goals of dwarfing, precocity, and productivity and specific goals depending on country needs: cold tolerance, disease and insect resistance, and propagation propensity. Such programs are described in the next few paragraphs according to world regions where they have operated (Europe, North America, and Asia). Tree fruit breeding programs are long term, and the time required to propagate and evaluate rootstocks is even longer than for scion cultivars. Several of the breeding programs that began during the period 1930–1970 have been terminated because of the intensive and long-term input requirements.

1. European Apple Rootstock Breeding Programs

Czech Republic. The main goals of this breeding program, which started in 1957, in Techobuzice (Prague) were winter hardiness, dwarfing and propagation ease (Cummins *et al.* 1983). The program produced the JTE (J = Jablon = Apple; TE = Techobuzice) series of rootstocks of which some were dwarfing similar to M.9 (JTE-E, JTE-F, JTE-H), one super-dwarfing (JTE-G) similar to M.27, and the rest semi-dwarfing similar to M.7 (JTE-B, JTE-D) (Wertheim 1998). Several of these rootstocks have been tested worldwide and have yield efficiencies similar to or better than M.9 (Bonany *et al.* 2004), but sensitivity to fire blight has slowed acceptance by industry. This breeding program has been terminated.

Germany. Efforts of breeding and selection of apple rootstocks in Germany date back to the East Malling era at Proskau, Berlin-Dahlem and Pillnitz (Cummins *et al.* 1983), but only the Dresden-Pillnitz program survived World War II, continuing to produce new crosses and the Supporter® series of rootstocks (Fischer 1994a,b, 1997a,b; Fischer *et al.* 2000). The breeding program aimed to produce rootstocks that exhibited good propagation properties, nursery tree growth, yield and resistance to *Erwinia amylovora* (fire blight), *Venturia inaequalis* (apple scab) and *Podosphaera leucotricha* (powdery mildew). The program produced several apple rootstocks, including Pillnitzer Supporter® 1 (M.9 × *M. baccata*), Pillnitzer Supporter® 2 and Pillnitzer Supporter® 3 (M.9 × *M. micromalus*). The rootstock Pi-80, also known as Supporter® 4, has been tested worldwide with some acceptance by apple industries. It was selected from a cross between M.9 × M.4, is a semi-dwarfing rootstock suitable for intensive or semi-intensive planting systems, with good cold tolerance and performance similar to M.26. This rootstock, which is susceptible to *Erwinia amylovora* and similar to M.26, has shown some susceptibility in replant soils. In NC-140 testing this

rootstock suffered the highest mortality after 10 years (Autio *et al.* 2013). While the program has essentially terminated breeding efforts, an additional set of semi-dwarfing rootstocks (Pi-AU 36-2, Pi-AU 51-4, Pi-AU 51-11 and Pi-AU 56-83) is being tested in apple-producing countries.

Italy. A relatively recent breeding program, operated by a consortium of nurseries in northern Italy, has been operating since the 1990s. This consortium produced a new rootstock named CIVP 21. According to the nursery, this rootstock was derived from an open pollinated population of PAJAM®2 Cepiland. It produces trees with vigor similar to M.26, with flat or open branching and with deep roots. This rootstock is still being tested in various locations in Europe to gauge tolerance to specific and general replant disease, weak soils and productivity compared to other industry standards.

Poland. Abiotic stresses linked to local growing conditions, such as cold temperatures and heavy soils, motivated the establishment of an apple rootstock breeding program at the Skiernievice Research Institute of Pomology in 1954. The P (Polish) series of apple rootstocks derived from crosses made in the 1950s include five clonal rootstocks P.1, P.2, P.14, P.16 and P.22 which were released during the 1970s and 1980s. Some rootstocks in this series (P.1, P.2, P.16, P.18 and P.22) have been field tested by the NC-140 project (Marini *et al.* 2000, 2006; Crassweller *et al.* 2001; Hirst *et al.* 2001; NC-140 1996) and have shown little improvement over the standard M.9 and similar susceptibility to fire blight while maintaining resistance to crown rot. Additional breeding efforts in the 1960s and 1970s produced some 40 families of hybrids, from which 6420 seedlings were raised of which 60 clones were selected for stoolbed and orchard performance tests (Jakubowski *et al.* 2000). The resulting material was released for testing in the 1990s, including the rootstock P.60, which has properties similar to M.9, and additional clones derived by mutagenesis (Zagaja *et al.* 1991; Jakubowski *et al.* 1993, 2000). Replant disease tolerance and productivity of this new set of rootstocks is being evaluated and is showing some promise for P.66 (susceptible to fire blight) and P.67 (mildly susceptible to fire blight), with tree size and productivity similar to M.26 (Zurawicz *et al.* 2011, 2013). This breeding program is active, making new crosses and selections oriented towards more disease-resistant properties.

Romania. Rootstock research in Geoagiu produced a series of rootstocks from supposedly open pollinated M.9 and named G.21/963,

G.22/975 (dwarfing) and G.7/963 (semi-dwarfing) rootstocks (Casavela 1977, 1983; Romania 1979). These rootstocks were selected for improved rooting and local adaptation to apple-growing regions in Romania (Movileanu 1989), and have not been tested widely elsewhere in the world. This breeding program is not active. An additional Romanian program was active at the Fruit Research Institute in Voinesti which produced apple rootstocks Voinesti 1 (M.4 × 'Cretesc') and Voinesti 2 (M.9 × 'Cretesc') that produces a tree similar to M.27 (Wertheim 1998; Mazilu and Viscol 1999). This program is no longer conducting active breeding.

Russia. The Budagovsky breeding program started in 1938 at the Minchurin College of Horticulture in Minchurisk, with productivity and cold hardiness as the main goals. The most widely grown apple rootstock from this program is Budagovsky 9 (B.9 or Bud.9), obtained from a cross between M.8 and 'Red Standard' (Pieniazek 1971; Barritt *et al.* 1990; Webster and Tobutt 1994). This rootstock was initially thought to be susceptible to fire blight, and intrinsically it is when new shoots are challenged with live *Erwinia amylovora* cultures. However, as a finished tree it develops a form of ontogenic resistance which has held true in plantings in north east of USA (Ferree *et al.* 2002; Russo *et al.*, 2008a,b). While B.9 was productive and yield-efficient in several NC-140 trials (Hirst *et al.* 2001; Marini and Barden 2004; Marini *et al.* 2006), it lacks replant tolerance (Auvil *et al.* 2011). Two strains of B.9, a European clone and a clone propagated in the American Pacific northwest, exhibited different growth characteristics in the nursery and raised concerns that trees propagated on the two clones might perform differently. There were no differences in DNA or susceptibility of the rootstock liners or grafted trees to fire blight (LoGiudice *et al.* 2006). The two clones were compared at six locations with 'Gala' as the scion, and after 10 years few differences existed between the two clones. Other widely known rootstocks from this program are B.491 (similar to M.27) and B.118 (semi-vigorous similar to MM.111). The breeding program in Minchurisk continues to breed and produce new apple rootstocks that are being tested in Europe and in the USA. Among these are the super-dwarfing rootstock B.7-17-22 (similar to M.27), the dwarfing rootstock B.10 (also known as B.62-396), semi-dwarfing (B.67-5-32), and semi-vigorous (B.7-20-21, B.7-3-150, B.70-6-8 and B.64-194). Rootstock B.10 is gaining interest in the industry given the initial data on productivity, notwithstanding susceptibility to replant disease, fire blight and *Eriosoma lanigerum* (WAA).

Sweden. The Alnarp Fruit Tree Station breeding program had goals of cold hardiness and propagation ease, and in 1944 released 'Alnarp 2' (A.2), a vigorous rootstock susceptible to collar rot (Wertheim 1998). An additional breeding program in Balsgard made crosses with Malling rootstocks, generating the BM (Balsgard Malus) series of rootstocks of which only BM.342 ('Mank's Codlin' × M.4) was widely tested and found to be unproductive and unsuited for high-density systems (Callesen *et al.* 1997; Ystaas *et al.* 1997).

2. North American Apple Rootstock Breeding Programs

Canada. Three breeding programs operated by Agriculture and Agri-Food Canada (AAFC) developed the Ottawa series, the Kentville series, and the Vineland series of rootstocks were started in 1959. The aim of these programs was to produce winter-hardy rootstocks, resistant to crown rot and adapted to local environments. The Ottawa program's most famous selection is 'Ottawa 3' (O.3; 'Robin' × M.9), a rootstock with productivity and yield efficiency similar to M.9, with high winter hardiness, but it is difficult to propagate and susceptible to fire blight and some latent viruses. At the Kentville, Nova Scotia cold-hardy clones were developed from 'Beautiful Arcade' seedlings, and 'Antonovka' was the primary pollen source. The hardiness selection was made in the nursery after a severe winter killed all but 30 of the 9000 seedlings. The 30 Kentville Stock Clones (KSC) were evaluated by Embree and Crowe (1986) with 'McIntosh' and 'Red Delicious' as the scions. After 13 years, only KSC 25 and KSC 28 had TCSA similar to or smaller than M.7, and the clones have not been widely tested.

Several Vineland rootstocks are still being tested in various research programs, including the NC-140, where they have demonstrated some resistance to fire blight and acceptable yield efficiency compared to M.9 and M.26 standards (Hutchinson 1977; Cline *et al.* 2001; Marini *et al.* 2006; Hampson 2012; Hampson *et al.* 2012). Another program that operated a breeding program started in 1970 at the Agriculture and Agri-Food Canada (AAFC), Horticultural Research and Development Center (HRDC), Quebec. This produced the SJM series of rootstocks developed by crossing 'Nertchinsk' × M.9, 'Osman' × 'Heyer' 12, *M. robusta* Robusta 5 × M.27 and 'Nertchinsk' × M.26 (Khanizadeh *et al.* 2005, 2011; Carisse and Khanizadeh 2006). Additional recent testing indicates that SJM-15, SJM-150, SJM-189, SJM-5198, and SJM-5128 might be suited for high-density production systems in British Columbia (Hampson 2016). No new breeding efforts have been evident in Canada since these series were produced.

United States of America. Two main breeding programs have operated in the U.S. One started in 1959 at Michigan State University that made selections from open pollinated seedlings of M.9, M.16, A.2, and R.5. This program produced a very yield efficient rootstock tested as MAC-9 and later named 'Mark' (Carlson 1981; Carlson and Perry 1986; Ferree and Schmid 1994), which is still being employed by certain segments of the U.S. industry. The second 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 would complement 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). While initial releases of G.11 and G.65 were tarnished by mixtures in the propagation material, these were eventually resolved. The true G.65 produced trees similar to M.27 with small fruit; therefore it was not adopted by the industry. However G.11 (M.26 × R.5), which produces trees similar to M.9 in performance and resistance to fire blight, is slated to reach production of close to 4 million rootstocks planted per year in the U.S. Apple rootstocks G.41 and G.202 (M.27 × R.5) are similar or better in productivity than M.9 and M.26, respectively, showing tolerance to replant disease and resistance to fire blight and woolly apple aphids; these are gaining traction in the U.S. with a production close to 4 million per year. Other rootstocks released by the program include dwarfing (G.935, G.214, G.814, G.213) and semi-dwarfing to semi-vigorous (G.890, G.969, G.210) derived from O.3 × R.5 crosses. All exhibit high yield efficiency, productivity, resistance to fire blight, tolerance to replant disease, and some have resistance to WAA. All these rootstocks have performed well in regional and national (NC-140) testing, and are being implemented by industries in South Africa, New Zealand, Brazil, Europe (Czynczyk and Bielicki 2012), and U.S. Several novel traits have been identified in the Geneva® germplasm, including the 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; Tworkoski and Fazio 2016; Tworkoski *et al.* 2016).

3. Asian and Pacific Islands Apple Rootstock Breeding Programs

China. The search for improved, locally adapted apple rootstocks and the search for cold-hardy rootstocks able to thrive in non-irrigated land spurred the initiation of several apple rootstock improvement programs during the late 1970s through the early 2000s (Mong 1991; Xiang *et al.* 1995). Most of the apple industry in China relies on seedling *Malus hupehensis*, *Malus prunifolia*, and *Malus baccata* rootstocks, where some are apomictic (Lei *et al.* 1998). Their breeding strategies have included the development of apomictic populations and clonal rootstock types (Liu *et al.* 1989; Wu *et al.* 1990; Sha *et al.* 2011; Ma *et al.* 2012). The earlier breeding programs were conducted at the Institute of Pomology, Shanxi Academy of Agricultural Science, Taigu, Shanxi Province, at the Liaoning Research Institute of Pomology, Xiongyue, Liaoning Province, at the Zhengzhou Fruit Research Institute, CAAS, Zhengzhou, Henan Province, and at the Qingdao Agricultural Experiment Station in the Shandong Province. The later breeding programs are at Northwest Agriculture and Forestry University in Yongling, Shaanxi Province and at China Agricultural University. The Shanxi program produced the SH series, which includes extremely dwarfing and semi-dwarfing lines that are easy to propagate vegetatively and show precocity, drought resistance, and good compatibility with scions (Shao *et al.* 1988). The Qingdao station produced the Qhingzhen series 'Qingzhen 1' and 'Qingzhen 2,' two apple rootstocks with high apomictic characteristic and tree size between M.26 and *Malus hupehensis* seedlings (Sha *et al.* 2011). The Zhengzhou Fruit Research Institute produced U8, which was derived from the cross M.8 × 'Balenghaitang' (*Malus micromalus*) made in 1974, and is supposedly similar in dwarfing as a vigorous M.9. Research on apple rootstock physiology, germplasm and genetics are common (Li *et al.* 2007; Yang *et al.* 2008; Zhu *et al.* 2009; Fan and Yang 2011; Gao *et al.* 2011; Han *et al.* 2011; Wan *et al.* 2011; Jin *et al.* 2012; Liu *et al.* 2012; Xiao *et al.* 2015; Duan *et al.* 2016; Zhou *et al.* 2016), but there seems to be a paucity in the literature regarding the comparative performance of all these apple rootstocks in coordinated trials in China and outside of China. There appears to be a consultative collaboration between the New Zealand breeding program and several of the Chinese breeding programs to improve the process of breeding and selection of new apple rootstocks (Zhang *et al.* 2016).

Japan. Apple rootstock breeding at the Fruit Tree Research Station in Morioka, Inwate, Japan produced *Malus prunifolia* Marubakaido and the JM series of apple rootstocks JM1, JM2, JM5, JM7 and JM8 (Tsuchiya 1979, 1988) in a search for disease resistance to local problems (Bessho and Soejima 1992; Moriya *et al.* 2008). All were derived from the cross M.9 × 'Marubakaido' made in 1972. Rootstock JM5 is super-dwarfing (M.27 type), and JM7 is similar to M.9 in productivity and is considered the best of the series (Tamai *et al.* 2003; Autio *et al.* 2013). These rootstocks were selected for hardwood propagation unique to that country (Yoshida and Muramatsu 1998). It seems that the program is still making crosses and developing new germplasm to this date.

New Zealand. The Plant and Food Research rootstock breeding program has produced, and is testing, a series of rootstocks (White and Tustin 2000; Tustin *et al.* 2014) derived from populations used in research on the inheritance of dwarfing (Rusholme-Pilcher *et al.* 2004, 2008; Foster *et al.* 2015) and pest resistance traits (Bus *et al.* 2008; Gardiner *et al.* 2012; Zhang *et al.* 2016). The current advanced selections are derived from Robusta 5 and 'Aotea' to combine their WAA, fire blight and/or crown rot resistance with the dwarfing trait of M.9 from original families made by Stuart Tustin in 1986 and 1987 (S. Tustin, personal communication). The program is one of the very first to implement Marker Assisted Breeding for three of these traits (Bassett *et al.* 2015; Bus *et al.* 2017). This includes the selection for WAA resistance gene pyramids to achieve more durable resistance, since there is a significant risk of biotypes developing that can overcome single-gene resistances (Sandanayaka *et al.* 2003). These rootstocks are slated to be evaluated in North America in a NC-140 uniform rootstock trial in 2018.

VIII. ROOTSTOCK EVALUATION

Until the 1980s, rootstock evaluation required many years because results from different locations often conflicted. Variable results were due to differences in tree management, environmental and soil conditions, choice of scion cultivar, and differences in experimental designs, numbers of replications, response variables measured, and statistical analyses of data. Tree management is critical because in multi-location trials, yield per tree sometimes changed by up to 30% when a researcher retired and trees were managed by a different

person. During the late 1970s, pomologists from across North America established uniform rootstock trials, under the auspices of the North Central 140 (NC-140) project, to quickly expose rootstocks to a wide range of soil and climatic conditions while holding most other factors constant (Cowgill *et al*. 2017). Over the past 35 years, 19 apple rootstock trials have been conducted. In 1998, a similar international multi-location rootstock project was established in the Baltic States and Byelorussia under the name "Baltic Fruit Rootstock Studies" (Bite *et al*. 2004; Kviklys *et al*. 2012). A multi-location rootstock trial was also established in the Netherlands in 1987 (Mass and Wertheim 2004). To facilitate collaborative research in Europe, 52 scientists from 21 fruit research institutions from 16 European countries joined the informal voluntary European Fruit Research Institutes Network (EUFRIN) Rootstock Working Group. Results from a survey of 32 European horticultural institutions in 2007–2008 indicated that most institutions conduct rootstock research and four have rootstock breeding programs (Kviklys 2011). The largest apple rootstock trials are in Poland, Lithuania, and Latvia, whereas the most extensive rootstock collections are at the Research Institute of Pomology and Floriculture, Poland and the Pure Horticulture Research Centre, Latvia. Northern and Eastern Europe focus on rootstock cold hardiness, whereas central and Western Europe work on soil-borne pathogens and fire blight resistance. Western Europeans are interested in rootstocks in the M.9 and M.27 vigor class, whereas northern countries consider more vigorous rootstocks to be more cold-tolerant. Kviklys (2011) stated that rootstock research is declining due to its long-term applied nature and lack of government funding. A similar trend is occurring in the U.S. for the same reasons. Kviklys recommended that international multi-state uniform rootstock trials may be an efficient way to evaluate rootstocks and study location × rootstock interactions, but international projects are more difficult to establish and manage than the large NC-140 project that is funded primarily by one country and supported by a combination of federal, state, and industry funds. Słowiński (2001) summarized in tabular form rootstock evaluations from 148 publications from 1981 to 2000.

The NC-140 project has allowed rapid evaluation of new rootstocks because trees are exposed to widely varying biotic and abiotic stresses, and results from the project have made it possible to develop rootstock recommendations for different regions of North America. Based on previous experiences and observations, experimental designs, statistical analyses and management protocols have evolved to enhance the

efficiency of the trials. Below are some of the changes that were adopted:

1. For the first several trials, rootstocks with a wide range of vigor (M.27 to MAC.24) were compared. Planting trees with large variation in vigor next to each other created management problems, resulted in highly variable data sets, and vigorous trees may have influenced the performance of adjacent trees. The current NC-140 protocols attempt to group rootstocks according to vigor, and new trials include rootstocks classified as only dwarf or semi-dwarf. However, some rootstocks, such as P.1, Pi Au 56-83 and Pi Au 36-2, were more vigorous than expected.
2. Early trials utilized 10 single-tree replications in a randomized complete block design (RCBD) at each location, because it was assumed that the soil variation within blocks was less than the variation between blocks. However, blocks explained little variation at most locations and a formal test of the relative efficiency of blocking showed that it was not very effective (R.P. Marini, unpublished results), so newer trials are utilizing completely randomized designs. Using data from one of the trials, it was learned that eight single-tree replicates were nearly as effective as 10 single-tree replicates. However, as trees often die during the study and open cells complicate the analyses, a generalized randomized complete block design, with two trees per rootstock randomized within four or five blocks, may be more effective. In general, five blocks with two trees per block could be expected to detect differences of about 23%, 26%, and 30%, respectively, for trunk cross-sectional area, yield per tree and yield efficiency (YE, kg per cm^2 TCA) (R.P. Marini, unpublished results).
3. Differences in soil fertility and length of growing season affect the ultimate tree size. Requiring a standard planting distance was not very satisfactory because trees may not fill their space at a low-vigor site, and trees may crowd each other at a high-vigor site. To accommodate for differences in site vigor, cooperators are now able to choose from one of two spacings.
4. Choice of cultivar is a challenge when rootstocks are evaluated over a wide range of growing conditions. Cultivars that are hardy enough to survive in cold climates often perform poorly in warmer climates. Therefore, cooperators must be willing to compromise for the good of the project. Growers sometimes complain that rootstock trials are relevant to local conditions only when scion cultivars commonly grown in the region are used. A trial was

established at 11 locations with four cultivars on five rootstocks to determine how much variation was explained by the cultivar × rootstock interaction, and the interaction was minimal (Autio *et al.* 2001). Therefore, the relative performance of rootstocks is usually not influenced by cultivar.

5. Over the years, statistical software packages have greatly improved, and can now be used to analyze generalized linear mixed models with unbalanced data sets, non-normal data, and random effects. Multiple comparisons with slicing techniques can be performed to compare rootstocks within each location. As software packages evolve, learning these new, more appropriate analyses will continue to be a challenge for pomologists.
6. Including one or more commonly used rootstocks as standards in every trial is important for comparative purposes, and to evaluate the interaction of rootstock × location. NC-140 now includes M.9 NAKBT337, M.26, and sometimes B.9, and M.9 Pajam 2 in dwarf trials, and M.26 in semi-dwarf trials as standards. The relative difference between M.9 and M.26 for TCA is often quite large between trials, and even between locations within multi-location trials. For example, within the same multi-location trial, trees on M.9 NAKBT337 had TCAs from 30% to 130% the size of trees on M.26 (Marini *et al.* 2006, 2014). Depending on the trial and location, trees on B.9 had TCAs 40% to 100% the size of trees on M.26.

By exposing rootstocks to widely varying conditions, multi-location rootstock trials sometimes provided unexpected results. Below are some of the unexpected discoveries by NC-140:

1. When the crop load is adjusted for TCA, trees on M.9 often produce larger fruit, and trees on G.16 often produce smaller fruit than trees on other rootstocks.
2. B.9 provided some fire blight resistance to the scion.
3. Trees on G.30 broke at the graft union in wind storms, even when trunks were supported with conduit.
4. Although the Vineland series was selected for cold hardiness, trees on Vineland rootstocks survived better than trees on Malling rootstocks in the southeastern USA.
5. Rootstock can influence burrknot development above the graft union on ‘Gala’ trees.

6. G.41 and G.935 are the only rootstocks in the M.9 to M.26 size class, respectively, to have YEs similar to or greater than M.9 and M.26.
7. There is a wide range of vigor among the M.9 clones (Warmund 2001).
8. Most dwarfing rootstocks perform poorly in hot dry climates.
9. Root suckers and burrknot severity are greatly affected by location.
10. Clones of B.9, with different growth habits, influenced tree growth and cropping similarly (Autio *et al.* 2013).
11. Geneva rootstocks propagated from normal stool beds and from stool beds established with tissue culture plants generally perform similarly (Autio *et al.* 2011).

During the 1970s, there were 11 apple rootstock breeding programs in the world. Today, only seven programs are currently active, although rootstock selections are still being evaluated from some of the non-active programs. Since rootstocks can have an important impact on the economic viability of an orchard, continued rootstock breeding and evaluation is important. However, as federal and university funding for agricultural research declines there is a greater emphasis on obtaining external funds to support research and graduate students. It is difficult to obtain funding for long-term applied research such as rootstock breeding and evaluation, and therefore industry support will become increasingly important. In the future, it is likely that rootstock research will exist only in regions with industries large enough to support the work. Protection of intellectual property is another obstacle to rootstock and cultivar testing. New rootstocks are often licensed to nurseries before being tested widely, and pomologists interested in evaluating rootstocks may not be able to obtain new rootstocks before they are commercially available. Comprehensive rootstock testing depends on individuals with good international relationships with rootstock breeders and nurserymen, as well as a long-term commitment to rootstock breeding and evaluating. Pomologists with those relationships, especially at the international level, are retiring and are not being replaced, and consequently breeders may have to make greater efforts to have their selections tested widely. During the next 20 years, new rootstocks will likely replace the currently available Malling and Geneva rootstocks. As breeders identify genes that control tree vigor, flowering, pest resistance and nutrient uptake, and new breeding methods are incorporated into the breeding programs, genetic improvement of apple rootstocks will likely advance at an accelerated rate.

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