Understanding How Rootstocks Dwarf Fruit Trees

Chris Atkinson and Mark Else

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The manipulation of plant roots to reduce excessive shoot growth and promote flowering and cropping has been practiced for centuries. For example, pots have been used for 2,000 years to grow figs because the restriction of root growth by the container makes the fig fruit more profusely. Rootstocks also have been used for many centuries as a means of propagating recalcitrant-rooting clonal scions. Throughout the world, selective breeding and development programs have provided many apple rootstocks that are able to withstand pests and diseases, poor soils and unfavorable climatic conditions. The attributes required of a rootstock have become more sophisticated over the years, but limiting excessive growth, enhancing the cropping efficiency (yield per tree size) and reducing the time required for a tree to come into cropping (precocity) remain the primary targets. The achievement of some of these goals has enabled much of the world's temperate fruit production (in particular, apple production) to be intensified through the use of trees which crop early, have high yields and can be planted at high numbers per unit land area.

If growers wish to select the most appropriate rootstock to maximize cropping and fruit quality under their orchard conditions, they have to rely on information from field trials. These trials are designed to collect a range of data, which is used to describe how a rootstock performs under the "local" conditions. From planting, perennial fruit trees take time to develop into effective cropping units and their yield changes as they age. Factors such as these, combined with the influence that local climate can have on the cropping cycle, mean that orchard-based rootstock trials must run for several years. Pre-orchard evaluation trials, carried out as part of the breeding and development assessments, are also lengthy. Therefore, it takes many years before a novel rootstock can be released to the nursery for propagation and evaluation by growers.

The information collected from these evaluation trials may not always provide sufficient insight to predict rootstock performance at sites with different climatic or soil conditions. In part, this is because we have a rather poor understanding of the mechanisms by which rootstocks influence scion growth and development (Beakbane, 1956). This lack of knowledge dictates that breeding programs will always be lengthy and expensive endeavors. An understanding of the key physiological mechanisms which determine the benefits of using a rootstock will enable a more predictive approach to be developed to evaluate rootstocks and will also speed breeding programs by identifying molecular markers. Molecular markers linked to desirable rootstock traits must first be identified. For example, a DNA fragment (gene) may be associated with the production of an enzyme that enhances fruit set. Once the location of this fragment of genetic material (marker) has been established and its benefits confirmed, DNA from other rootstocks can be screened quickly for the gene of interest. This approach would enable the selection of desirable rootstocks at the seedling stage, thereby reducing the need for time-consuming and expensive field trials. However, before such marker-assisted selection is feasible, the processes and, ultimately, the genes that endow a rootstock with a desired characteristic must be identified.

> *. . . the yielding efficiency of the tree increases with the extent of scion dwarfing.*

Our understanding of how rootstocks influence scion vigor, flower induction and cropping must, therefore, be improved. Attempts to explain the rootstock influence in terms of a graft union effect on the translocation of minerals or water, or those due to differences in root anatomy, have not proved satisfactory. Recent studies have suggested that rootstocks may influence scion growth and cropping by direct or indirect effects on plant hormones, either via synthesis, metabolism or transport from root to shoot and from shoot to root.

This article will review briefly what rootstocks do and how they are believed to influence scion growth and development. Work at HRI-East Malling will be described showing how our understanding of the ways in which rootstocks influence the scion has been advanced.

WHAT DO ROOTSTOCKS DO TO SCIONS?
Rootstock Effects on Scion Growth and Vigor

Rootstocks have a profound influence on the growth of worked scions (Tubbs,

1973a,b). Dwarfing rootstocks, in simplistic terms, reduce the amount of scion dry weight. This effect is achieved by a reduction in both the rate at which vegetative shoots grow (extend) and the time period over which they grow. Compared to an invigorating rootstock, a dwarfing rootstock directs a greater proportion of dry weight into fruit production rather than into vegetative growth. We do not know how this is achieved, but if dwarfed shoots grow more slowly and do not grow for as long during the season, then dwarfed scions might be expected to have less leaf area than those on vigorous rootstocks. The effects of a reduction in leaf area means that scion shoots on dwarfing rootstocks may produce less photoassimilate relative to scions on vigorous rootstocks. Differences in the way leaves are orientated on the tree may also influence their ability to intercept solar radiation and convert this into fruit production via photosynthesis. Factors such as these may contribute to the observed reduction in tree size associated with dwarfing rootstocks, but they do not necessarily explain differences in the way dry weight is partitioned between tree growth and cropping.

Rootstock Effects on Flowering

One of the great advantages of using rootstocks is that they induce precocious scion flowering. Not only are flowers produced earlier in the tree's life cycle, but also large numbers of flowers are produced per unit tree size. This effect is particularly evident with rootstocks that dwarf scions, but as Webster (1994) points out, these two factors just may be coincidental, rather than biologically linked. However, the ability of dwarfing rootstocks to induce changes in dry weight distribution in favor of reproductive development and, therefore, flower and fruit production suggests a causal link.

Rootstocks may influence the number of flowers on a tree through changes in scion architecture, particularly with respect to branch angle (orientation) and shoot development. The production of new shoots may be modified such that flower bud induction is favored rather than vegetative shoots. Developmental changes of this type can be seen on 1-year-old wood as an increased production of short shoots and flower buds. Rootstocks may also induce increases in the number and size of flowering spurs on older wood.

There have been many cases where rootstocks have been reported to enhance the ability of flowers to set fruit. "Flower quality" is often difficult to characterize

accurately; suggestions are frequently made that an increase in size and the general appearance of the flower may influence its ability to set fruit. The number of spur leaves associated with the flowers can also modify fruit set. Different types of flowers often have different fruit setting and retaining abilities. For apple, flowers on 2-year-old spurs set more fruit than older spurs or axillary flowers. Some differences in flower quality are due to changes in the ability of the ovule (female part of the flower) to remain alive sufficiently long enough for the male pollen grains to grow down into the flower and fertilize the ovule. Any impact that the rootstock can have, for example, on the length of ovule life, or to increase the speed at which the pollen tube grows could increase the flower's ability to set fruit.

The time at which flowering occurs and the duration over which flowers can be successfully fertilized can contribute to a tree's fruit-setting ability. Some rootstocks alter flowering time and this may be due to changes in the chilling requirement of different rootstocks. The rootstock effect on flowering date may also have a bearing on low temperature injury; flowers opening later in the spring are more likely to avoid damage induced by frosts or low temperature. There is also more direct evidence that rootstocks can influence the ability of flowers to withstand low temperature injury, but it is unclear how this is achieved.

Rootstock Effects on Cropping and Fruit Quality

By reducing tree size, dwarfing rootstocks produce less vegetative growth and yields, on a per-tree basis, are lower than those for a tree on a vigorous rootstock (Tubbs, 1973a,b). However, the smaller tree stature permits trees to be planted at higher densities, thereby enabling much larger yields to be achieved per unit of planted area. Furthermore, the yielding efficiency of the tree increases with the extent of scion dwarfing. Measurements that account for differences in tree weight, canopy volume, light interception and trunk cross-sectional area all show an increased yield efficiency associated with dwarfing rootstocks.

Fruit on dwarfing rootstocks also tends to be larger. This appears to be true even when differences in total yield per tree (crop load) are taken into account. There is little experimental data to suggest how these changes come about but they do not appear to be due to a rootstock-induced extension of the growing season. Flowers on the rootstock M.9 often develop later than those on more vigorous rootstocks. Fruit on dwarfing rootstocks may grow at faster rates but, as

yet, this has not been confirmed, nor has the impact of such a possibility on fruit cell size, cellular structure and texture of the fruit.

There is indirect evidence, gleaned from leaf mineral analyses, in which invigorating and dwarfing rootstocks may differ in their abilities to take up mineral nutrients from the soil. Unfortunately, the ways in which these differences impact on the fruit mineral content are less well documented. The evidence available suggests that some rootstocks have a much greater potential for taking up minerals that are important with respect to fruit quality, i.e., calcium. Unfortunately, little is known about the mechanisms that underlie the relative abilities of rootstocks to acquire different minerals. This attribute does not appear to be explicable in terms of basic differences in root architecture.

HOW DO ROOTSTOCKS WORK?

Work on apple at HRI-East Malling has examined how rootstocks differ in their inherent capacity to produce hormones and the ways in which these biologically active substances are transported within the rootstock and/or scion. To date, this work has been directed toward improving our understanding of the mechanisms by which rootstocks influence vegetative growth. To achieve this, we have focused on the coordinating processes by which the movement of key hormones or "signaling molecules," primarily in xylem sap, influences various shoot and root developmental processes. We believe that many previous studies of root-to-shoot signaling in fruit trees failed to recognize, and account for, the influence that sap flow rates can have on the concentrations of hormones and other solutes in the sap. To determine accurately the passage of hormones from roots to shoots in the transpiration stream, it is necessary to take samples of xylem sap flowing at rates similar to those present in the intact tree. Information on both the concentration and the delivery rate (concentration multiplied by sap flow rate) of key signal molecules is needed to establish whether changes in the movement of hormones between root and shoot underlie the dwarfing effect.

To determine the roles that transport of physiologically active substances play in the "rootstock effect," we have considered several aspects of rootstock function, two of which are reviewed below. The first of these is root-derived substances. We have quantified the amount and rate at which root-derived substances (mineral ions and hormones) are exported in the xylem from the root, through rootstock and scion

stems and into the canopy, and evaluated their importance in determining shoot behavior. Secondly, we have quantified the development and functional capacity of the hormone transport systems through observations of anatomy and quantitative measurements of hydraulic function.

Root-Derived Substances

Recent studies of hormone transport from roots to shoots have emphasized the need to collect samples of xylem sap for hormone analysis at rates similar to those in intact, transpiring trees. This need arises because of the confounding influences of sap flow rates on the concentrations of solutes in the xylem sap. We have, therefore, designed and built two unique split-top whole plant pressure chambers that enable experiments to be conducted on intact young fruiting trees (with root volumes up to 25 dm^3). By applying a positive pressure to the roots, we can replicate the sap flow rates that occur in the intact tree. The concentrations of hormones and other solutes in these sap samples will then reflect accurately those in the transpiration stream prior to sampling. However, when trying to determine whether dwarfing and invigorating rootstocks produce and export different amounts of hormones, concentrations alone may mislead because they can be dependent on sap flow rates.

To account for these interactions, hormone delivery rates must be calculated (concentration multiplied by the sap flow rate). By these means, the delivery rates of different hormones or minerals can be calculated and compared between dwarfing and invigorating rootstocks. Accounting for the variable effects of sap flow rates on xylem sap composition is especially important in studies looking at the rootstock effect where trees of different size and, therefore, transpiration rates are being compared. In the past, researchers have obtained sap from trees by simply removing the entire scion and collecting sap that exudes under osmotic pressure from the roots (Jones, 1974, 1986). However, flows of such sap are very slow and highly variable within and between rootstocks. Inevitably, the composition of this xylem sap often has very little resemblance to that flowing previously through intact, transpiring trees. Hormone and ion concentrations in osmotically exuding sap cannot reliably reflect conditions in the intact tree.

Using our pressure-chamber approach, we have shown that the rate of sap flow had a large impact on the xylem sap composition. Slow flowing sap concentrated solutes and faster flowing sap diluted solutes. This dilution of solutes was not proportional to the increase in sap flow rates, a doubling of sap flow did not halve the concentrations. An inevitable consequence of this was that solute delivery rates increased with faster flow rates. The slow flowing osmotically exuding sap concentrated solutes through a lessening of dilution; however, solute delivery rates were still only 4% of those estimated to be passing to the scions in intact trees. Our data imply that the use of this method of sap collection when trying to assign causal status to xylem-borne signaling molecules is risky and may lead to erroneous conclusions.

The delivery of potassium, calcium, magnesium, sodium and hydrogen ions was similar from MM.106 and M.9 rootstocks. However, when the effects of leaf area and root mass were taken into account, the delivery rates of all ions and total solutes were greater from M.9 rootstocks than from MM.106 rootstocks. These findings imply that the ability of the rootstocks to take up and export ions in sufficient quantities to the scions is not an integral part of the dwarfing mechanism.

Our findings are contrary to some previous reports but are supported by numerous observations that mineral concentrations in leaves of scions grafted onto dwarfing and invigorating rootstocks are similar. We also demonstrated convincingly that the graft union did not alter the ionic composition or the solute content of the xylem sap as it passed through the tissues in the graft union.

We have also used our pressure chamber method to validate the classical method as used by Bollard (1953). Bollard's method was used to collect sap samples from *orchard-grown* trees to estimate reliably the concentrations and deliveries of abscissic (ABA) and auxin (IAA) moving in the transpiration stream of MM.106 and M.9 rootstocks subjected to root manipulation treatments. When the effects of the irrigation and root restriction treatments on leaf development were accounted for, the treatments did not influence the transpiration rates of the scions at any time during the season either in trees worked on M.9 or MM.106 rootstocks.

Delivery of ABA per unit leaf area in xylem sap from root-restricted and nonirrigated MM.106 rootstocks was high in September, yet the transpiration rates through branches in these trees was not lowered. Since ABA is a potent regulator of water loss from leaves, these results imply that either the ABA was being directed (sequestered) away from its site of action (the stomatal complex) or that the stomatal guard cells themselves were desensitized to ABA and, therefore, unresponsive. The delivery of ABA was not different from non-root-restricted, irrigated M.9 and MM.106 rootstocks during June and July but was perhaps greater from M.9 than from MM.106 rootstocks during September.

Generally, the delivery of IAA per unit leaf area in xylem sap extracted from scions worked on MM.106 and M.9 rootstocks decreased throughout the season. The source of this IAA may have been the developing apple seeds whose concentrations of IAA are initially high, then gradually decrease to very low levels in ripening fruit. Deliveries of IAA were greater from MM.106 than from M.9 rootstocks during June but were similar thereafter. Shoot extension growth of scions worked on M.9 rootstock ceases at the end of June while that on scions worked on MM.106 rootstocks is maintained throughout July into August. Since IAA is generally considered to have stimulatory effect on shoot growth, the continued shoot extension of scions on MM.106 rootstocks may be attributable to the relatively high export of IAA early in the season.

Overall, these preliminary data suggest the mechanistic basis of dwarfing may not be readily explained in terms of changes in individual signaling molecules. The "dwarfing effect" is complex and most likely regulated by a number of signaling pathways acting in tandem rather than in isolation. However, having developed robust methods to detect accurately the transport of these putative signals, we are now in a position to capitalize on our founding efforts and further extend our understanding of the dwarfing effect.

Functional Capacity

Attempts to quantify the amount of functional xylem area in stem sections by staining with aqueous safranin solution proved to be very informative. The staining and apparent movement of the aqueous safranin solution in the rootstock shank was confined to tissue, which was actively connected to functional xylem (Atkinson et al., submitted). This suggests that in the rootstock shank little radial xylem flow was taking place and areas stained are likely to reflect actual functional xylem. More important, however, was the reduced amount of area stained with aqueous safranin in the scion on top of the dwarfing M.27 rootstock. This suggests a reduction in functional (sap conducting) xylem area. This effect might be explained by a decline in the hydraulic conductance across the graft union.

Direct measurements of stem hydraulic conductance revealed several important points. There was evidence to suggest that the vigor of the rootstock onto which a scion was grafted influenced the scion hydraulic capacity (Atkinson, unpublished). This occurred independently of conductance changing simply in relation to rootstock-induced changes in stem diameter or supported leaf area. A measurement of stem hydraulics across a combined series of resistances, from the rootstock shank, through the graft union, to the scion, showed that conductance was related to rootstock vigor. This was is in agreement with the studies that quantified the movement of aqueous safranin solution across the graft union and those of Warne and Raby (1938) and Simons (1986).

Vigorous rootstocks had the highest total conductance. When the graft union resistances were calculated, the largest factor contributing to this variation between rootstocks was the graft union itself. These results show that the hydraulic resistance of an M.27 dwarfing rootstock graft union was much greater than that of the vigorous rootstock MM.106. The semi-dwarfing rootstock M.9 was of an intermediate value between these two rootstocks. The measurements used to make these calculations were normalized to account for rootstock differences in stem cross-sectional area, but differences in conductance existed between rootstocks on a unit stem cross-sectional area basis. Differences in whole intact stem conductance and, therefore, sap flow in the xylem between rootstocks may be less evident as the graft union cross-sectional area increases with dwarfing rootstocks. Such an increase in the graft union morphology may be a mechanism by which dwarfing rootstocks attempt to overcome the hydraulic limitation imposed by the graft union tissue and its abnormal xylem anatomy (Soumelidou et al., 1994).

CONCLUDING REMARKS

The results of this work show that xylem-borne substances are not delivered in proportion to sap flow, so differences in tree transpiration or leaf area have considerable influence on signal molecule concentration and delivery. Delivery rates of a range of ions were greater for the vigorous rootstock MM.106 compared to the semi-dwarfing M.9. However, when effects of flow rate, leaf area and root mass were taken into account, delivery rates of all the ions examined and total solutes were greater from M.9

rootstocks compared to MM.106. Therefore, the ability of the root system to take up ions and export them to scions in sufficient quantities may not play an integral role in the dwarfing mechanism.

Evidence from anatomical studies shows that various features of both the phloem (sugars, some ions and some hormones) and xylem (water and some hormones) transport systems differ markedly between rootstocks of different vigor. Previously, it had not been possible to determine if these differences actually contributed to the dwarfing effect by inducing differences in the delivery of a potential signal molecule. Equally there was speculation that the anatomical changes associated with the graft union between the scion and rootstock played a role in the rootstock effect. We have shown from direct measurements of stem tissue hydraulics (water flow per unit time) on young fruiting apple trees that scion sap flow was determined by leaf area, but this can be directly influenced by the type of rootstock on which they are grafted. We have also shown that the hydraulic capacity of the rootstock shank itself was very different and is related to rootstock vigor. But perhaps more important than either of these two factors was the observation that the graft union between a dwarfing rootstock and a scion offered considerably higher resistance to water flow than that evident with an invigorating rootstock. This result shows that water flow per unit of stem tissue was lower for the dwarfing rootstock compared to the invigorating rootstock. It also explains, perhaps, why stem tissues swell with some graft unions to compensate for this restrictional influence on water transport. This restrictional influence on stem sap flow, combined with the frequently observed smaller amounts of total root dry weight per plant evident with dwarfing compared to invigorating rootstocks, implies that water transport to the shoot is highly likely to influence shoot growth. The implications are that the early development of the graft union and the establishment of shoot extension growth will be limited by the transport of water from soil to shoot.

We also have evidence that the graft union is unlikely to influence the transport of ions within xylem sap. Samples of sap, collected using the spilt-top pressure chambers, above and below graft unions of M.9 and MM.106 did not differ markedly in the ionic concentrations irrespective of sap flow rate.

There is little doubt that hormones such as the auxins, cytokinins and gibberellins, along with abscissic acid, have major roles in determining shoot and root growth and development. These chemicals are synthesized in one part of the plant in response to a stimulus and then transported to another region of the plant to induce a response. These chemical signals influence the coordination of root and shoot growth and development. How these hormone signals influence crop development and fruit quality is unclear. Through analyses of hormonal physiology it will be possible to determine their influence on gene expression relative to plant development. Used in conjunction with modification of hormone biosynthesis or signal transduction, a more detailed knowledge of the mechanisms that affect rootstock size control will emerge. Eventually the need for grafted rootstocks may even be negated by altered root development in own-rooted scion varieties. Opportunities will also exist to modify the roots of crops for which no dwarfing rootstocks exist presently.

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