Differences in Drought Tolerance among Gisela® Cherry Rootstocks Determined Using Automated Weighing Lysimeters

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Abstract. The Gisela® series of dwarfing rootstock are widely used because they enable high-density production, but they may be sensitive to drought. Drought tolerance may be associated with root-zone distribution and depth or with physiological adaptation to low water potential. Here we describe a novel technique for determining physiological tolerance to drought when root distribution is held constant. In two matching studies, we continuously measured transpiration of two groups of eight trees using a 16-container automated weighing lysimeter system in a greenhouse. With this system, Gisela 3, 5, and 12 (G.3, G.5, and G.12) rootstocks were subjected to multiple, controlled drought cycles based on reductions in whole-tree transpiration. To provide an equivalent amount of stress for each tree, water was withheld until the daily transpiration rate had decreased to less than 250 g of water transpired per tree per day. Each tree was then drip-irrigated to bring the root-zone back to about field capacity. G.3 and G.12 rootstocks also had greater leaf area and trunk diameter. Both transpiration data and harvest data indicate physiological differences among rootstocks. Because root-zone volume was constant, these differences are not associated with changes in root distribution or depth. These data indicate that G.5 is less adapted for regulated deficit irrigation strategies that include long irrigation intervals.

Irrigation can use well over half of all diverted water (Fereres et al., 2003; Goldhamer et al., 2003), and there is tremendous incentive to improve irrigation efficiency (Costa et al., 2007). Maximizing water productivity requires scheduling irrigation based on crop water status rather than on a fixed schedule (Fereres and Evans, 2006). In annual crops, water status is typically inferred from measurements of soil water potential—but this approach is uniquely challenging with trees because of the extensive depth and spread of their root systems.

There are more than 1.6 million hectares of orchards in the United States with a production value in excess of $13 billion (U.S. Department of Agriculture, 2015). These high-value tree crops require precision irrigation management to conserve water, and there are many irrigation management strategies. Regulated deficit irrigation (RDI) is a technique that induces a level of water stress during periods where the fruits are less affected by drought such as the lag (DWII) phase of stone fruit development (Chalmers et al., 1981). Partial root-zone drying (PRD) provides irrigation to only half of the root system while withholding it from the other half, inducing an ABA-mediated drought response without reducing turgor (Dry and Loveys, 1998). Water may also be conserved by replacing only the amount of water transpired the previous day using a high-frequence dripper irrigation system (Neilsen et al., 2004). Despite increased use of micro-sprinkler and drip irrigation systems, many orchard managers in the western United States continue to use long-interval (7 to 10 d) irrigation cycles. This type of irrigation with intermittent water stress may be particularly damaging to rootstocks that explore limited volumes of soil. Accordingly, rootstocks for orchards irrigated at long intervals must be adapted to periodic water stress. Whether from reduced amount or frequency of irrigation, precision water stress has the potential to reduce water consumption, improve fruit quality, and minimize nutrient leaching and runoff.

Some fruit crops are well-suited to deficit irrigation because water stress can improve economic return (Costa et al., 2007). Multiple studies indicate that precision water stress has a greater effect on vegetative growth than on reproductive growth in fruit trees (Boland et al., 2000a, 2000b; Mitchell et al., 1989). Reduced irrigation thus reduces pruning costs. Increases in water productivity resulting from appropriately timed water stress have been reported for many orchard crops including tart cherries (Papenfuss, 2010; Papenfuss and Black, 2010), peaches (Girona, 1989; Girona et al., 1993) and apples (Einhorn and Caspari, 2004; Fallahi et al., 2010; Leib et al., 2006).

An improved understanding of the response of tree water status to soil water potential is needed to improve irrigation strategies. Increased depth and distribution of rooting can improve tolerance to water stress (Black et al., 2010), and rootstocks with deeper roots may be more efficient at soil water extraction (Pérez-Pérez et al., 2008, 2010; Romero et al., 2006). Although dwarfing rootstocks can explore large volumes of soil, they may be more sensitive to stress because of less extensive root systems (Beckman and Lang, 2003; Black et al., 2010). A rootstock may also be able to recover more quickly and completely following a drought-stress event, which would be desirable in the case of a production orchard.

The Gisela® Series rootstocks were developed in Giessen, Germany (Callesen, 1998) and are clonal rootstocks that produce dwarf trees. Gisela 5 (G.5) is a triploid hybrid of Prunus cerasus ‘Schattenmorelle’ and Prunus canescens (Franken-Bembenek, 1998). Gisela 3 (G.3) is a sibling to G.5 (Franken-Bembenek, 2004). Gisela 12 (G.12) is a hybrid of P. canescens and P. cerasus (Lang, 2000). G.5 produces a tree that is 50% to 65% of trees grown on a Mazzard rootstock, while G.12 produces a tree that is 65% to 80% of a Mazzard (Lang, 2000). G.3 produces a tree slightly smaller than a G.5 (Franken-Bembenek, 2004; Kappel and Lang, 2008; Roper et al., 2019). These rootstocks are well-suited for high-density cherry production for both sweet and tart cherries, are resistant to several pathogens, and induce precocious bloom (Andersen et al., 1999; Callesen, 1998).

G.5 has been widely recommended for use in high-density plantings, despite anecdotal evidence that it may be more susceptible to drought. The literature contains conflicting reports on the drought sensitivity of G.5 rootstocks. Santos and Gonçalves (2000) reports that G.5 had greater drought resistance than P. avium, Maxma 14, Edabriz, and Cab 11E; but Gonçalves et al. (2003) reports that G.5 rootstocks were more sensitive to water stress than more vigorous rootstocks such as Maxma 14 and P. avium. Lang (2000) reports that G.5 was “fairly” drought sensitive. Vercammen (2002) observed that in dry environments, G.5 had low vigor and small fruit, unless it was carefully irrigated; but that it was preferable to Colt and Limburgse Boskriek because of its higher production efficiency (yield efficiency).

Comparing the drought response of rootstocks in containers facilitates making genetic selections. Tworkoski et al. (2016)
withheld water from containerized apple trees with multiple rootstock and scion combinations and found that rootstock selection influenced drought resistance as measured by changes in ABA levels and leaf water potential. Jiménez et al. (2013) found differences in drought tolerance among four peach rootstocks as evidenced both by physiological measures and by reductions in growth. These containerized studies showed that physiological adaptations influenced drought responses.

Trees can osmotically adjust to adapt to drought stress, or they may avoid drought with deep roots. Both adaptations impact the overall drought tolerance of a rootstock. Separating physiological adaptation from root depth and water acquisition is important to developing precision irrigation strategies for cherries and other tree crops because it would allow for the combination of these traits in rootstock breeding programs. Many studies have measured single-leaf stomatal conductance and photosynthesis, but this causes significant difficulties in extrapolating from single leaves to whole plants (Jones, 2004). The variable effects of root depth and distribution can be minimized when rootstocks are grown in containers. Water availability in containers is reproducible and can be controlled, allowing for analysis of whole-tree adaptation to drought. Transpiration of the whole tree can be measured from changes in mass, which in a containerized system with no leaching is due to the uptake and transpiration of water from the root zone. Weighing lysimeters, thus, provide a way to determine whole-tree transpiration rates over hours, days, and weeks (Ben-Gal et al., 2010). The precision offered by these systems allows for reproducible, controlled, dry-down and rewetting cycles. Additionally, because water can be slowly added using drip emitters, colloids are preserved, and compaction of the soil in the container can be minimized. To ensure good aeration of the root-zone, manyi microprune by pinching off the apical and lateral meristems to reduce the differences in plant size. After 30 d, eight uniform trees of each rootstock were selected and transplanted into 22 L containers in a mixture of 30% peat and 70% sandy loam topsoil. Rootstocks were arranged in a complete block design. To ensure steady nutrient availability, this mixture was amended with 5 g per L of slow-release fertilizer (Polyon 15–6–11, 1- to 2-month release; Koch Turf & Ornamental, Wichita, KS). Greenhouse temperatures were 25 °C day, 20 °C night, and 50% humidity.

Each container was placed on a weighing lysimeter with a load cell (Transducer Techniques ESP-35, Temecula, CA). Chard et al. (2004) describe the principles of the lysimeter system and provide a detailed list of its components and operation (Fig. 1). Immediately after transplanting, the media was irrigated using two drip emitters per pot. To minimize puddling and preserve soil colloids, water was applied for only 15 s out of every minute. Complete irrigation of the media took about 8 hours.

Field capacity is defined by the amount of water left in a soil after the gravimetric water has drained from it. Even though our containers had drainage holes, containers inhibit water drainage because there is no deep soil column below to create a pull on the water column. The point at which water stops dripping from the containers is called container capacity. To achieve field capacity, we used a vacuum extraction (0.8 atmospheres; 81 kpa) to remove excess water. This strategy allowed us to establish a well-watered baseline approximating field capacity to which we could re-wet the soil in each container after each dry-down cycle. This method helped remove excess water (about 200 mL per container) that might lead to root hypoxia and helped maintain adequate air-filled porosity throughout the container.

After vacuum extraction, the mass of each container was recorded and used as a well-watered baseline that closely approximated field capacity, to which we could re-wet the soil in each container following each water-stress episode. All subsequent irrigations were based on this well-watered baseline, and vacuum was not applied after any other irrigations. To minimize evaporation from the soil surface, a 2-cm thick layer of perlite was added to the top of each container. Each container was irrigated independently. Transpiration rates were calculated every 30 min based on changes in mass using a data-logger–based controller. Transpiration and growth in plants are highly correlated (Adams et al., 2018; Brêda and Granier, 1996; Obojes et al., 2018; Welander and Ottosson, 2000). Accordingly, cumulative daily transpiration provided an indication of growth and water stress. Irrigation was withheld until daily transpiration decreased from about 700 g to less than 250 g per day per tree. After daily transpiration decreased to 250 g per day, containers were drip-irrigated the following night to restore them to the well-watered baseline mass approximating field capacity in the root-zone. Based on the results of the first study, the set point for irrigation was increased (less water stress) in the second study. Trees were watered when the transpiration rates reached 30% of the peak rate in the first study and 50% of the peak rate in the second study.

Each container was subjected to at least six dry-down and irrigation cycles over 81 d. Beginning on the 25th day of dry-down cycles, the trunk diameter of each rootstock was measured at 3 cm above soil level using a digital micrometer. Trees by this time were <0.5 m tall. This location was marked, and repeated measurements of trunk diameter were taken at the same location. Frequent micropruning was used throughout the experiment to control plant height and shape.

A common problem with lysimeter studies is that larger plants more rapidly extract water from the finite volume of a container.
Table 1. Growth metrics at final sampling for Gisela rootstocks. In both studies, the trunk cross-sectional area (TCSA) of the trees were matched initially, but G.12 and G.3 rootstocks had greater TCSA at final sampling (grew faster) than G.5 rootstocks. G.3 also had greater leaf and stem dry mass than G.5 in the second study.

<table>
<thead>
<tr>
<th></th>
<th>Leaf mass dry (g)</th>
<th>Stem mass dry (g)</th>
<th>Leaf:stem ratio (g:g)</th>
<th>Total leaf area (cm²)</th>
<th>Specific leaf mass (g·m⁻²)</th>
<th>Trunk cross-sectional area</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.5</td>
<td>42.4</td>
<td>71</td>
<td>0.60</td>
<td>3450</td>
<td>123</td>
<td>0.99</td>
</tr>
<tr>
<td>G.12</td>
<td>48</td>
<td>75</td>
<td>0.64</td>
<td>4110</td>
<td>117</td>
<td>ns</td>
</tr>
<tr>
<td>P value</td>
<td>0.06</td>
<td>ns</td>
<td>ns</td>
<td>0.02</td>
<td>0.04</td>
<td>ns</td>
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</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Day 25 (cm²)</th>
<th>Day 82 (cm²)</th>
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</thead>
<tbody>
<tr>
<td>G.5</td>
<td>0.99</td>
<td>1.43</td>
</tr>
<tr>
<td>G.12</td>
<td>1.00</td>
<td>1.59</td>
</tr>
<tr>
<td>G.3</td>
<td>0.24</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>&lt;0.01</td>
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ns = nonsignificant.

We minimized this problem by watering when the trees reached a similar reduction in daily transpiration.

Data were normalized to the irrigations by assigning the day before irrigation to be the reference (Day 0). Transpiration and trunk diameter measurements for each consecutive day followed as days after the irrigation (Day 1, Day 2, and so on). When the next irrigation occurred, it was used as the new reference. The maximum length of a dry-down cycle was 11 d. Once days were organized by dry-down cycle, the data were combined to return the data to a time-series format, which is indicated as normalized days after initializing water stress.

The study was ended by a destructive final sampling on Day 82. Leaf fresh and dry mass, total leaf area, and stem fresh and dry mass were determined. The root-ball was removed from each container, shaken to remove media, and visually evaluated for root distribution.

G.5 and G.3. The weighing lysimeter system we used has capacity for only 16 plants. After completing the comparison of G.5 and G.12, a similar procedure was followed to compare G.5 and G.3 rootstocks. Peat was not added to the soil in this comparison because aeration was adequate in the first comparison, so the containers were filled entirely with a sandy loam soil. The mass of moist soil in each container was equalized by wetting the media before planting the rootstocks in the containers. To avoid compacting the wet soil, a section of PVC pipe just larger than the root-ball of each living tree was added to each container to equalize the mass of moist soil in each container. The PVC pipe was then removed and the rootstock planted in the hole that the pipe had reserved. The starting mass of each container was equalized to provide similar water availability for each tree. Despite equal initial mass, transpiration rates differed, and each container was monitored and irrigated independently after the first irrigation. As in the first trial, each container was irrigated when the daily total transpiration was less than 250 g per tree.

Destructive growth analysis was on Day 109. Final sampling methods were identical to the first study. Pairwise t-tests were used to analyze differences between rootstocks.

Fig. 2. (A) G.5 and G.12 rootstock transpiration recovery following irrigations. Beginning at the second irrigation, G.12 transpiration rates recovered more quickly from drought stress than those of G.5, reaching pre-stress transpiration rates within 2 or 3 d. G.5 trees never resumed pre-stress transpiration rates, but G.5 transpiration rates decreased more quickly as dry-down cycles progressed.

(B) G.3 and G.5 rootstock transpiration recovery following irrigations. G.3 rootstocks had higher transpiration rates than G.5 rootstocks for several days after irrigation. Both G.3 and G.5 recovered to pre-stress transpiration rates, but G.5 transpiration rates decreased more quickly as dry-down cycles progressed.

Results

Final sampling data. G.12 trees were larger at final sampling than G.5 trees (Fig. 1). Leaf dry mass was not significantly different between G.5 and G.12 (P = 0.06) (Table 1). There was no difference in stem dry mass or in the ratio of leaf-to-stem dry mass between G.5 and G.12. Leaf area was significantly greater for G.12 than for G.5 rootstocks (P < 0.02) as was specific leaf mass (P < 0.04). There was no significant difference in trunk cross-sectional area (TCSA) at the beginning of the study (G.5 = 0.99 cm²; G.12 = 1.00 cm²); but, at final sampling, G.12 TCSA averaged 1.59 cm² and was significantly greater than G.5 TCSA (1.43 cm²) (P = 0.01). The growth rate (increase in TCSA) was also significantly greater for G.12 than for G.5 (58.7 µm² per day vs. 44.1 µm² per day) (P < 0.01) (data not shown).

At final sampling, leaf dry mass and stem dry mass were significantly greater for G.3 than for G.5 (P = 0.01 for both) (Table 1). There was no significant difference in the ratio of leaf-to-stem dry mass between G.3 and G.5. In spite of micropruning, the leaf area of G.3 averaged 1800 cm², which was significantly greater than the average of 1310 cm² for G.5 (P < 0.01). Specific leaf mass of G.3 was also significantly greater than that for G.5 (P < 0.01). There was no significant difference in TCSA between rootstocks at the beginning of the study; G.3 averaged 0.24 cm², while G.5 averaged 0.27 cm². At final sampling, G.3 TCSA averaged 1.02 cm² and was significantly greater than G.5 TCSA (0.68 cm²) (P < 0.01). The growth rate was also significantly greater for G.3 than for G.5 (58.8 µm² per day vs. 33.2 µm² per day) (P < 0.01) (data not shown).

Root system comparison. All of the trees explored the entire volume of soil; and, upon removal from the container, all root balls were held together by the tree root systems. There were no visible differences in the depth or distribution of roots at harvest. Root mass was not compared because washing the roots also removes many of the fine roots, which have a large impact on water uptake in plant
root systems (Rewald et al., 2011). Washing also does not typically remove all the soil particles from the root systems. All rootstocks appeared very similar in both studies.

Recovery of transpiration following drought. There was no significant difference in the recovery of transpiration rate for G.5 and G.12 rootstocks after the first irrigation (Fig. 2A). However, beginning with the second irrigation, G.5 rootstocks recovered more slowly than G.12 during the 3 or 4 d immediately following irrigation. G.12 trees resumed pre-stress transpiration levels within 2 or 3 d, while G.5 trees never fully regained their pre-stress transpiration levels. Five days after irrigation, the transpiration rate of the rootstocks converged when G.5 trees reached their new maximum transpiration rate, and 6.12 trees were beginning to experience drought stress. (Fig. 2A).

There was no significant difference in the recovery of transpiration for G.5 and G.3 rootstocks after the first irrigation (Fig. 2B). However, G.3 transpiration rates were significantly greater on the second and third days after irrigation (P = 0.01 and P = 0.03, respectively). Five days after irrigation, transpiration rates converged (Fig. 2B).

When the last four dry-down cycles were pooled, G.5 and G.12 transpiration rates were not significantly different the day before irrigation, but G.12 trees had significantly greater transpiration for five days after irrigation, but they were not significantly different the day before irrigation or the 2 d after irrigation, but G.3 trees had significantly greater transpiration for the third through the seventh day after irrigation (Fig. 3B).

Discussion

The G.5 trees in the first study did not regain their initial pre-stress daily transpiration rate of 800 g per day (Fig. 2A), but they were able to recover to their initial rate in the second study (Fig. 2B). This result is likely associated with the higher level of imposed stress in the first study where irrigation occurred at a 70% decrease in transpiration as compared with a 50% decrease in transpiration rate in the second study (Fig. 2A and B). In the first study, the first stress cycle may have sufficiently stressed the G.5 trees so that they never completely recovered. While there was no leaf abscission, there was some leaf scorching. Because the stress was less extreme in the second study, the trees more fully recovered.

The threshold of 250 g per day daily transpiration was selected by careful observation of the trees in the initial study as they became more water stressed. In the first water-stress cycle, incipient wilting started to occur at a daily transpiration rate of about 250 g per day. The trees appear to have adjusted osmotically because successive dry-down cycles did not result in any visible wilting, although leaf turgor could have been reduced by the imposed stress.

It is unlikely that differences in growth were the result of waterlogging and hypoxia in the root-zone. Field soils are not commonly used in containers because rapid irrigation causes ponding of water, which causes compaction and hypoxia. The air-filled porosity in these studies was preserved with slow, controlled irrigation; and even then, the maximum volumetric water content occurred only after each irrigation at 7- to 10-d intervals.

In both studies, G.5 rootstocks grew more slowly than the other rootstocks and had reduced leaf area per tree. When whole tree transpiration rate immediately following the last irrigation before harvest was divided by total leaf area, G.5 rootstocks had a higher transpiration rate per unit leaf area than G.12 rootstocks (P < 0.01) or G.3 rootstocks (P = 0.04). However, the volume of the tree canopies was similar, so G.12 and G.3 rootstocks would have had greater self-shading of leaves. These relatively more-shaded leaves would have had a lower transpiration rate per unit leaf area; and, thus, the average transpiration rate per unit leaf area would be expected to be less. The trees may have osmotically adjusted.

Differences among rootstocks could be caused by rate of regeneration of fine roots in response to the repeated dry-down cycles (Rewald et al., 2011). Fine roots are important to water uptake but quickly desiccate during drought and must be regenerated (Atkinson et al., 1999; Jacobs et al., 2009).

Because G.12 and G.3 trees grew larger than G.5 trees, they more rapidly depleted the water in their containers with each successive dry-down cycle and, thus, experienced slightly more water stress as the experiment progressed.

Fig. 3. Gisela rootstock mean transpiration for seven days after irrigation. (A) G.12 transpiration recovered more quickly and completely for the first 5 d after irrigation. Beyond 5 d, the rates did not differ. (B) G.3 transpiration rates weren’t different from G.5 rates on the day immediately after irrigation, but they were higher than G.5 from 3 to 7 d after irrigation. + indicates P ≤ 0.10; * indicates P ≤ 0.05; and ** indicates P ≤ 0.01.
Despite increasing stress levels, G.3 and G.12 grew faster, as evidenced by daily transpiration rate and harvest data. This effect emphasizes the differences in tolerance among rootstocks. These data provide clear evidence that there are physiological differences among rootstocks that are in addition to any differences in root-zone distribution.

The fact that G.3 and G.12 continued to grow despite repeated dry-down and rewetting cycles suggests that they may be better able to adapt to water stress and may be better-suited to a precision irrigation system where schedules create long intervals between irrigations. However, G.5 also has the desired precocity and dwarfing characteristics needed for high-density cherry production systems and has been used successfully in such systems, where only water used during the previous day is replaced (Neilsen et al., 2004). This rootstock seems to be well-suited to high-frequency drip irrigation systems designed to deliver irrigation daily based on evapotranspiration demand (Neilsen et al., 2005, 2010, 2014). Understanding the physiological adaptability of rootstocks to water stress provides a basis for selecting a rootstock adapted to maintaining acceptable yield and quality while still reducing season-long irrigation volume—regardless of the irrigation strategy.

Whether the irrigation volume reductions are a result of longer intervals as with RDI or of precise replacement of daily consumption as with high-frequency drip irrigation, irrigation is based on need rather than on timing as Fereres and Evans (2006) suggest. Evaluation of physiological adaptability to water stress would enable the selection of rootstocks adapted to the various approaches to precision water stress. Combining physiological adaptability and root vigor may lead to rootstocks with an increased overall drought resistance as Tworkoski et al. (2016) suggest in apples. Such optimized orchards could take advantage of the precocity of dwarfing rootstocks like the Gisela® series, while customizing the rootstock selection to the irrigation system. This rootstock characterization would enable rootstock selection to be based more on adaptability to water-reducing irrigation strategies on a per-orchard basis than about finding the best general overall rootstock.

Interactions between the scion and the rootstock contribute to the overall potential drought resistance of a tree (Adams et al., 2018; Tworkoski et al., 2016). Grafting Gisela® rootstocks with common scion would provide a way to further evaluate drought tolerance of whole trees because potential interactions between rootstocks and scions and the contribution of any graft incompatibilities to drought tolerance could then be studied. We are preparing to examine these interactions in future studies.

While containerized studies in greenhouses may not perfectly translate to field conditions, controlled environment studies are frequently used before large-scale, long-term field studies because they allow for the control of variables that would not be controllable in the field. In these comparisons, the weighing lysimeter system allows for equalization of the rooting volume; controlled, gradual, dry-down cycles; and precise measurements of transpiration, all of which are extremely difficult in the field. Using field soils with controlled drought stress and precision analysis of transpiration allows for the separation of the influence of rooting depth and distribution from other physiological characteristics that also affect the ability of a rootstock to tolerate drought stress. Without this degree of control, such an analysis would be extremely difficult.

Tolerance of drought is an important selection criterion in breeding programs. Historically, selection appears to have emphasized rootstock vigor, precocity, and pest resistance; however, these data suggest that differences in rate of recovery from drought may also be an important selection criterion—particularly when selecting rootstocks for use in precision water-stress systems and in arid climates. The weighing lysimeter method we used provides a way to evaluate physiological adaptability of not only rootstocks, but also of rootstock-scion combinations. Adding a physiological compatibility component to the rootstock selection process has the potential to reduce water use in orchards, while maintaining the utility and profitability of high-density orchard systems.

**Literature Cited**


