Detection of Vascular Discontinuity in Bud Unions of 'Jonagold' Apple on Mark Rootstock with Magnetic Resonance Imaging

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Abstract. 'Jonagold'/Mark apple (*Malus domestica* Borkh.) trees that were chip-budded in Washington and Illinois on 31 Aug. or 21 Sept. 1989 were sampled in Apr. 1990 to determine if magnetic resonance imaging (MRI) could be used to nondestructively examine vascular continuity or discontinuity between the rootstock and scion. Images could be placed into three categories based on signal intensity: 1) the rootstock, bud shield, and the bud or new scion growth had a high signal intensity; 2) the rootstock and the bud shield had a high signal intensity, but the scion had a low signal intensity; and 3) the rootstock had a high signal intensity, but the bud shield and scion had a low signal intensity. High signal intensity was associated with bound water in live tissue and the establishment of vascular continuity between the rootstock and scion. Azosulfamide staining and destructive sectioning confirmed that vascular continuity was established when the rootstock, bud shield, and scion had a high signal intensity in images, whereas budding failure occurred when the bud shield and/or the scion had a low signal intensity. Additional trees that had wilted or weak scion growth were collected from Illinois in June 1990. Parenchyma tissue was found in the scion adjacent to the bud shield that interrupted the vascular tissue. Poor scion growth on trees from the 21 Sept. budding in Washington may be attributed to insufficient growth of rootstock and/or scion tissues at the union in the fall.

Mark rootstock induces dwarfing and precocity in apple trees (NC-140, 1987). Cultivars budded on Mark produce a substantial crop in the 3rd year after planting and maintain high productivity for at least 7 years thereafter (NC-140, 1991; Schupp, 1991). While Mark has desirable horticultural characteristics, it is difficult to propagate trees on this rootstock in some areas of the United States (Dick Snyder, personal communication). When problems are observed, trees chip-budded in the fall may fail to grow from the scion bud or the bud begins growth in the spring, but subsequently dies. These types of budding failures have been attributed to poor budding technique, adverse environmental conditions following budding, or incompatibility (Argles, 1937; Howard and Skene, 1973; Simons, 1987). Two major types of incompatibility in fruit trees have been identified by Mosse (1962). Translocated incompatibility is associated with starch accumulation in the scion, phloem degeneration, or phloem compression within the overgrowth of the scion. Localized incompatibility is characterized by cambial or vascular discontinuity in the union.

Although the anatomical aspects of union formation and incompatibility have been studied extensively (Argles, 1937; Herero, 1951; Mosse, 1962), destructive sectioning methods have been necessary to determine union compatibility before scion bud growth in the spring. However, the rapid development of MRI techniques has allowed the nondestructive examination of water status and distribution in plant organs (Brown et al., 1986; Faust et al., 1991; Wang and Wang, 1989). Since MRI is non-

destructive, repeated observations can be made on the same specimen during various stages of growth. In MRI, a sample is positioned in a strong magnetic field and then subjected to a sequence of radio frequency pulses and magnetic field gradients to measure the concentration and relaxation time of protons primarily associated with water molecules. Data are then reconstructed and an image representing the water distribution within the specimen is produced. In the images, variations in signal intensity (brightness) represent differences in water concentration and the binding strength of water associated with fats, lipids, and phenols in living tissues (Brown et al., 1988).

In 1988, some nurseries located in the western United States reported poor results when apple cultivars were budded onto Mark rootstock (Mike Smith, personal communication). However, budding failures on Mark had not been observed in eastern nurseries (Joe Preczewski, personal communication). Thus, a study was designed to investigate these budding problems using MRI and conventional experimental methods. The objectives of this study were: 1) to determine if MRI could be used to detect vascular continuity or disruption in bud unions of 'Jonagold'/Mark and 2) to ascertain if collapse of scion growth was caused by a vascular aberration in the union.

Materials and Methods

'Jonagold' budwood was collected from scion orchards in Louisiana, Mo., on 28 Aug. and 18 Sept. 1989 and shipped by overnight mail to Wenatchee, Wash., or stored at 2C for use in the Illinois nursery. The plantings of Mark rootstock used for this study were located at commercial nurseries in George, Wash., and Atlas, Ill. Eighty scion buds from each collection date were

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Abbreviation: MRI, magnetic resonance imaging.

Fig. 1. Samples of chip-budded trees grown in George, Wash. (A) Magnetic resonance image of a compatible union in which the rootstock (r), bud shield (bs), and scion (s) have a high signal intensity and (B) the same tree after staining with azosulfamide. (C) Magnetic resonance image of a union in which the rootstock and bud shield tissues have a high signal intensity, but the scion has a low signal intensity and (D) the same tree after staining with azosulfamide. (E) Magnetic resonance image of a sample in which the rootstock has a high signal intensity, but the bud shield and scion have a low signal intensity, and (F) the same tree after staining with azosulfamide. Note that the callus tissue (c) has a low signal intensity in magnetic resonance images.

chip-budded onto Mark rootstock at each location 3 days after budwood collection.

Budbreak had not occurred when trees were dug in early Apr. 1990 in Illinois. These trees were selected to determine if a compatible union had formed before budbreak. In contrast, trees sampled from Washington were used to examine tree tissues early in the growing season. Scion growth was present on $\geq 97\%$ of the trees budded in August in Washington. However, only

54% of the scions budded on 21 Sept. had produced growth by April. To ensure the sampling of trees with optimal and poor scion bud growth for MRI studies, four trees each from the 31 Aug. and 21 Sept. budding dates were selected for imaging from both locations. Scion buds were tightly closed when trees were dug in Illinois on 12 Apr. Trees sampled from Washington on 16 Apr. had 2 to 5 cm of scion growth. All trees were shipped by overnight mail to the Univ. of Missouri. The root system of

Fig. 2. Chip-budded trees sampled in Apr. 1990 and stained with azosulfamide. (A) The red dye is present in the rootstock and scion, confirming the establishment of vascular continuity between these tissues. (B) Azosulfamide (arrows) is present in rootstock tissue and absent in the scion tissue, indicating vascular discontinuity between the rootstock and scion. The scion growth broke off above the bud union after staining when the sample was bisected.

each tree was then sealed in a polyethylene bag to prevent desiccation and stored at 2C until MRI experiments were conducted.

Images were obtained at 63.5 Mhz on a 1.5 tesla Magnetom instrument (Siemens Medical Systems, Iselin, N.J.) housed at the Univ. of Missouri Hospital and Clinics. The bud union of the tree was centered in a 3 cm diameter Helmholtz radio frequency transceiver coil mounted on a flexible plexiglass positioning device within the magnet of the instrument.

Two dimensional spin warp imaging was used to acquire seven longitudinal images per bud union. Each image represented a 2 mm thick scan with a 60- or 120-mm field of view and was reconstructed on a 256×256 or 512×512 matrix, respectively, resulting in spatial resolution of $234 \times 234 \times 2000 \mu\text{m}$. A gradient refocused sequence, which employed multiple radio frequency excitations for spatial encoding, was used. The echo time (TE) was 6 ms and the repetition time (TR) was either 50 or 500 ms, with two data acquisition cycles.

After the imaging was completed, rootstock tissue 10 cm below the bud union was discarded. The remaining portion of the tree was then placed in a 1% (w/v) azosulfamide solution for 4 h. Azosulfamide has been used as a vascular tracer in woody tissues (Ashworth, 1982). In our study, the dye was used to verify vascular continuity within the unions of trees. After staining, bud unions were dissected longitudinally and photographed. Images obtained from MRI were then compared to photographs of stained samples.

Additional trees from the 21 Sept. budding date that exhibited wilted or weak (5 to 8 cm) scion growth were collected from Illinois on 15 June 1990 to further examine vascular tissue in the bud union. These trees were bisected longitudinally and sections were stained with toluidine blue 0 (O'Brien et al., 1964).

Vascular tissues in the sections were examined under a dissecting microscope and photographed.

Results and Discussion

Images of the unions could be grouped into three categories: 1) the rootstock, bud shield, and the scion had a high MRI signal intensity (Fig. 1A); 2) the rootstock and bud shield had a high signal intensity, but the scion had a low signal intensity (Fig. 1C); and 3) the rootstock had a high signal intensity, but the bud shield and the scion had a low intensity (Fig. 1E). The high signal intensity was associated with bound water in live tissue (Fig. 1B). The dead cells in the callus tissue that had formed between the rootstock and the bud shield had a low signal intensity in the images (Fig. 1A and B). The low signal intensity probably resulted from either a high concentration of free water in the dead cells or the lack of water in this tissue. After the unions were stained with azosulfamide, it was evident that vascular continuity was established in the union when the rootstock, bud shield, and scion had a high signal intensity in the images (Fig. 2A). Dissection after azosulfamide staining also confirmed vascular discontinuity when the scion or bud shield had a low signal intensity (Fig. 2B). Staining with azosulfamide dye was always more time consuming than the MRI technique. The stained unions also had to be evaluated within 30 min after dissection because the azosulfamide diffused into nonvascular tissues after this time.

Two of the trees budded on 31 Aug. and sampled in April from Washington had compatible unions. In the other two trees, the rootstock and bud shield had established vascular continuity, but the scion appeared dark or absent in images. When these trees were stained and sectioned the following day, necrotic tissue in the scion was observed near the bud shield. Three of

Fig. 3. Section of rootstock and scion tissue of an undamaged tree sampled from Atlas, Ill., on 15 June 1990 and stained with toluidine blue 0. The xylem (x) in the scion growth is not interrupted by parenchyma tissue.

the trees budded on 31 Aug. and sampled in April from Illinois had established vascular continuity between the rootstock and scion, but vascular discontinuity was observed in one tree.

Only one tree budded on 21 Sept. (from Illinois) had a compatible union, and seven of the budded trees had vascular discontinuity. Poor growth of the rootstock and/or scion tissues at the late budding date probably caused the failure of trees to

form a compatible union. Very little callus tissue was observed in the unions of trees budded on 21 Sept. as compared to that in trees budded at earlier dates. By Oct. 1990, only 25% of the 80 original trees budded on 21 Sept. 1989 in Washington had normal scion growth in the field, whereas 79% of the trees budded on 31 Aug. had normal growth.

By June 1990, the scion of many trees grown in Illinois was wilted or broken at the union in the direction of the prevailing wind. Undamaged and wilted trees collected, stained with azosulfamide, and examined at this time had established vascular continuity between the rootstock and the bud shield. However little azosulfamide stain was observed in the scion. Vascular aberrations were absent in sections of tissues from undamaged trees stained with toluidine blue 0 (Fig. 3). In contrast, sections stained with toluidine blue 0 from wilted trees revealed that the xylem in the basal portion of the scion was interrupted by parenchyma tissue (Fig. 4). Water uptake into the scion was apparently restricted beyond the parenchyma. The location of the parenchyma tissue in the scion corresponded with the area where breakage occurred. The reason for the parenchyma tissue formation in the xylem tissue is unknown. Apparently, the tissue aberration in the new scion growth occurred after union formation in the fall. Since 79% of the trees budded on 31 Aug. and grown in Washington produced normal growth in the field, the budding failures could not be attributed to a genetic incompatibility between the 'Jonagold' scion and Mark rootstock.

In conclusion, azosulfamide stained trees verified that MRI can be used to detect vascular continuity or discontinuity in budded trees. However, the reason for discontinuity could not be elucidated nondestructively from images. In trees from Illinois that exhibited scion wilting, parenchyma tissue was found adjacent to the bud shield in the scion. This tissue appeared to limit water uptake in the spring when trees were exposed to warm weather. The failure of trees budded on 21 Sept. to form a compatible union may be attributed to insufficient growth of rootstock and/or scion tissues in the fall.

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Fig. 4. Section of rootstock and scion tissue of a wilted tree sampled from Atlas, Ill., on 15 June 1990. (A) Unstained section. Callus (c) tissue between the rootstock and scion. Note that the xylem (x) is interrupted by parenchyma tissue (arrows). (B) Section from the same tree stained with toluidine blue 0.

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