Xylem Differentiation and Boron Accumulation in ‘Italian’ Prune Flower Buds

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Abstract. Since B levels in prune (Prunus domestica L.) flowers may affect fruit set, the accumulation of B in buds was studied for 2 months prior to bloom to determine when B enters buds and the importance of the xylem as the supply route. Bud anatomy and the distribution of azosulfamide dye in excised prune twigs indicated that xylem in the axes of flower buds differentiated and became functional only when buds begin to swell, 5 weeks before bloom. Discontinuous xylem connections prior to bloom may impede B movement into dormant buds. In intact trees, B accumulated in buds slowly before bud swelling, but rapidly as buds accumulated dry matter from swelling until bloom. The concentration of B in xylem exudate was unchanged from 19 Jan. until bloom on 1 Apr., whereas marked increases in the concentrations of P, K, Ca, and S began 5 weeks before bloom. These changes suggest that the remobilization of B in the xylem of branches prior to bloom is more limited than that of other elements. Transpiration from buds was calculated from the rate of Ca accumulation in buds and Ca concentrations in xylem exudate, by assuming all Ca entered buds via the xylem. Using calculated daily transpiration rates and concentrations in xylem exudate, it was estimated that only 26% of the B entering buds was supplied by the xylem.

Boron sprays applied to ‘Italian’ prune (Prunus domestica L.) trees with sufficient leaf B levels (35 ppm) increased flower B levels from 78 to 165 ppm and enhanced fruit set (7). Boron applications have increased set on ‘Italian’ trees with sufficient leaf B levels in other studies as well (9, 12). Chaplin et al. (9) suggested that the B requirement for optimum fruit set in prune may not normally be met by the general B nutrition of the tree.

Factors affecting the movement of B to prune flowers and possible reasons for an inadequate B supply have not been studied. Prune flowers have contained 6 times as much B as dormant flower buds, and nearly all of the B which accumulated in flowers was derived from reserves in nearby branch tissue (12). Flowers of pear, apple, and sweet cherry also contained much more B than was stored in dormant flower buds (25). Boron accumulated slowly in these buds as they began to swell and rapidly as bloom approached.

The extent to which the xylem and phloem systems transport B to flower buds is unknown. Boron moves passively within the transpiration stream to transpiring organs such as leaves (17). Remobilization of B in the phloem typically is limited (11), although evidence of phloem mobility has been seen in cotton and turnip (18), broccoli (4), subterranean clover and peanut (8), and apple (24). The movement of B in the phloem may depend on its concentration in tissue. In grape, evidence of B transport out of mature leaves was seen only when leaf concentrations were above a certain level (21). In apricot and prune trees exposed to excessive soil B levels, large quantities of B accumulate in fruit and bark, suggesting that B may move out of leaves and into fruit and bark via the phloem when the tissue concentrations are high (10).

An understanding of how and when B enters prune flowers may explain why flowers normally receive insufficient B for optimum fruit set. This work was conducted to determine the importance of the xylem system in supplying B to prune flower buds prior to bloom. Since xylem differentiates in the axes of many Prunus flower buds when they begin to swell in the spring (1, 2), we also wanted to know when xylem differentiates in prune flower buds and whether a discontinuous vascular connection may impede B movement into flower buds.

Materials and Methods
Xylem differentiation and B movement into dormant buds. Xylem connections in axes of ‘Italian’ prune flower buds were studied anatomically by collecting flower buds from January until bloom in April. Buds were vacuum infiltrated with CRAF fixative (chromic acid-acetate-acid-formalin), dehydrated in an ethanol-tertiary butyl alcohol series, and imbedded in paraffin for sectioning with a rotary microtome (15). Sections were stained with safranin-fast green.

Vascular development in prune flower buds was studied by following the movement of dye through 15 to 30 cm long twigs collected every week from mid-January until the green tip stage in mid-March. The base of each twig was immersed in a 1% solution of azosulfamide and kept in the dark at 20°C for 3 to 4 days. Excised buds were bisected longitudinally and examined under a dissecting microscope to determine the extent of dye movement.

Boron uptake by prune twigs and movement into dormant buds was studied by removing 8 twigs, 15 to 20 cm long, from each of 3 ‘Italian’ prune trees on 19 Jan. Twigs also were removed from 3 common chokecherry (Prunus virginiana L.) trees for comparison. The base of each twig was placed in either distilled water or 200 ppm B solution and kept in the dark at 20°C for 4 days. Sections from the twig bases just above the level of the solution, spurs and dormant flower buds then were collected and analyzed for B.

Nutrient concentrations in xylem exudate and developing buds. Boron accumulation in ‘Italian’ prune flower buds and concentration in xylem exudate were studied in a 10-year-old western Oregon orchard for 2 months prior to bloom. Levels of other nutrients in buds and xylem exudate also were measured. Samples of 50 to 100 flower buds were collected and analyzed for nutrient concentrations from each of 8 trees at 10 to 15 day intervals from 28 Jan. until bloom in early April. Xylem exudate was sampled from 6 adjacent trees at 10 to 20 day intervals from 19 Jan. until bloom. Exudate was extracted under suction.
(5) from 4 branches (20 cm long) per tree, yielding 8 to 12 ml of extract per tree. Exudate samples were evaporated to dryness in crucibles. Both exudate and tissue samples were ashed at 450°C, dissolved in 5% nitric acid and analyzed for nutrient elements with an inductively coupled plasma emission spectrometer. Samples were standardized against National Bureau of Standards leaf tissue. Boron-containing glassware was avoided.

Flower bud transpiration rates were measured on a single 'Italian' prune tree with a LI-COR LI-1600 steady state porometer at 3 stages of bud development: 1) green tip; 2) first white; 3) full bloom. A section of twig containing flower buds was inserted into the porometer chamber. Bark surface within the chamber was covered with parafilm to prevent transpiration through the bark. At each growth stage, transpiration was measured on 20 twigs, each with 1 to 3 buds. The same twigs and buds were used for measurements during each growth stage to reduce variability.

Results

Xylem differentiation and B movement into dormant buds. Xylem vessel elements were seen first in the axes of flower buds when they began to swell, 5 weeks before bloom. Elongated cells observed in the axes of buds prior to swelling did not appear to be functional xylem elements (Fig. 1), since they lacked secondary wall thickening and stained dark green, indicating the presence of cytoplasm. Xylem vessels were observed in the axes of buds collected from early bud swelling (2 Mar.) until bloom (1 Apr.). Secondary wall thickening was clearly visible, and cells no longer stained dark green, indicating cytoplasm was absent (Fig. 2).

The distribution of azosulfamide dye in cut twigs also indicated that the xylem in the axes of buds became functional when buds began to swell. Dye moved into the cut ends of prune twigs and to the top of twigs within 3 days. When twigs were collected prior to bud swelling, dye moved to the base of buds within 3 days, but did not enter flower primordia, even after 5 days in dye solutions. When twigs were collected after buds began to swell, dye moved into flower primordia within 3 days.

Boron solutions moved readily through the cut ends of dormant prune but not chokecherry twigs (Table 1). Placing the cut ends of prune twigs in B solutions increased B concentrations in sections from the base of twigs and in spurs, but did not change the concentrations in dormant buds. Boron concentrations in base sections, spurs, and dormant buds of chokecherry were unaffected by B solutions.

The Ca content of buds changed little in January and February, but increased in March as bloom approached (Fig. 4). Calcium content of buds increased 40% from January until bloom. The concentration in xylem exudate began to increase in February, and reached a peak 10 days before bloom. Bud dry weight began to increase 5 weeks before bloom, with the most rapid

Table 1. Boron distribution in excised twigs of 'Italian' prune (Prunus domestica L.) and chokecherry (P. virginiana L.) placed in distilled water and B solutions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>'Italian prune' B (ppm dry wt)</th>
<th>Chokecherry B (ppm dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>Spur</td>
</tr>
<tr>
<td>Distilled water</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>200 ppm B</td>
<td>34*</td>
<td>36*</td>
</tr>
</tbody>
</table>

*F-test significantly different at 5% level.
accumulation of dry matter occurring just before bloom (Fig. 4).

Flower buds at green tip, first white and full bloom stages transpired an average of 0.5, 1.1, and 1.3 μg water/bud/sec, respectively.

Discussion

Xylem in the axes of prune flower buds differentiated and became functional when buds began to swell 5 weeks before bloom. A similar pattern was observed in the flower buds of other Prunus species (1, 2). To test if an earlier bloom date may change the time of xylem differentiation relative to bloom, prune branches were forced to bloom indoors on 7 and 27 Feb. Observations of bud anatomy and dye distribution within branches again indicated that xylem in the axes of buds becomes functional when buds begin to swell, regardless of the date of bloom (data not shown). It was not possible through anatomical observations to determine if phloem was present at this time.

Boron and dye solutions moved readily through the xylem systems of cut prune twigs, suggesting that a significant amount of transpiration may occur through the bark of twigs. The fact that B solutions moved readily into spurs, but not dormant buds (Table 1), suggests that either the discontinuous xylem connection in the axes of dormant buds impedes the flow of water and B, or the tight bud scales minimize bud transpiration and water movement into buds prior to swelling. Either may explain why very little B was observed to move into prune buds prior to bud swelling. Chokecherry twigs were included in this study for comparison, because dormant chokecherry buds appear to have a continuous xylem connection (2). Boron solutions had no effect on B levels in chokecherry twigs (Table 1), indicating solutions did not move into twigs of this species. Although the base of all twigs was recut under water to preserve a continuous water column in the twig, a broken water column could impede water and B movement. It is not clear why B solutions moved into prune but not chokecherry twigs. Neither prune nor chokecherry twigs appeared to desiccate or shrivel during the experiment.

Mean B concentration in xylem exudate (0.26 ppm) was lower than concentrations reported for annual plants. Boron concentrations in the sap exuding from the base of detopped tomato plants ranged from 1.0-5.2 ppm (6). Sap collected in a similar manner from sunflower plants contained 0.68-0.82 ppm B (14). Raven (20), using reported values of B concentration per unit dry weight of plant tops and volume of water transpired per unit increase in dry weight, calculated the expected B concentration in the xylem stream to be 0.01-0.6 ppm. The range in Ca concentrations in xylem exudate observed in this study (30-80 ppm) compares well with concentrations reported in the xylem exudate of Lupinus albus L. (60-90 ppm) (13), Quercus rubra L. (17-18 ppm) (23), apple (110-120 ppm), and pear (120-180 ppm) (16).

Whereas the concentration of B in the xylem exudate did not change significantly over the sampling period (Fig. 3), a 170% increase in the concentration of CA was seen, beginning 5 weeks before bloom (Fig. 4), and similar increases were seen in the concentrations of S (200%), K (320%), and P (570%) beginning at the same time (data not shown). Marked increases in the concentrations of N, P, and Mg in the xylem exudate of apple trees occur during bloom (5), and substantial increases in the concentrations of Cu and Fe were reported in pear trees just prior to bloom (3). As suggested earlier (3, 5), increases in the concentrations of mineral elements in the xylem as growth resumes in the spring may reflect nutrients which were absorbed the previous season and released to the xylem from storage tissue. The fact that B concentrations in the xylem changed little
prior to bloom suggests that B is not as easily remobilized as P, K, Ca, and S. Skok and McIlrath (22) have shown that the soluble fraction of tissue B declines to near zero as plants become increasingly B deficient, suggesting that plants with high tissue B concentrations may have a greater proportion in soluble form than those with low B. If branches contain high B concentrations in the spring, an increased proportion of B may be in a form available for release to the xylem stream or other transport systems.

An understanding of how much B enters buds in the transpiration stream may help to identify factors that limit B supply to buds. Although B and Ca began to accumulate in flower buds as the xylem differentiated 5 weeks before bloom, the proportion of B in buds increased at a much greater rate. Boron content increased 600% (Fig. 3) and Ca content 40% (Fig. 4) from the end of January until bloom (1 Apr.). The amount of S, P, and K in buds increased from 7 to 14 times over the same period (data not shown). Although there was a close correlation between the time of xylem differentiation in the axes of buds and nutrient accumulation, buds also began to accumulate dry matter at this time, suggesting that the phloem was also functional although we could not determine if sieve elements were present in bud sections.

The amount of B entering buds in the transpiration stream can be determined from the B concentrations in xylem exudate and the transpiration rates of buds. An estimate of the water transpired/bud/day can be made by assuming that all Ca enters buds via the xylem. There is general agreement that long duration of B transpired/bud/day can be made by assuming that all Ca enters buds. Although we could not determine if sieve elements were present in bud sections.

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The transpiration rates of buds estimated in this manner increased sharply 5 weeks before bloom, and continued to rise until bloom (Fig. 4). Measured transpiration rates also increased as buds opened and approached bloom, but they were considerably higher than calculated values. At full bloom, the measured transpiration rate was 1.3 μg H₂O/bud/sec or 0.11 ml H₂O/bud/day, compared to a calculated rate of 0.034 ml H₂O/bud/day. Transpiration measurements were made in the early afternoon when the air temperature was close to its daily maximum. The vapor pressure gradient between the bud and air and the transpiration rate were likely maximal at this time, and the average transpiration rate over a 24 hr period was probably considerably lower. The surface of buds was kept dry during transpiration measurements, which also could have resulted in an overestimation of transpiration rates. Measurable precipitation fell on 19 days during the 5 weeks prior to bloom (total precipitation, 3.4 cm), and flower buds were often wet during this time. A film of water on the surface of buds could reduce transpiration rates substantially.

The calculated daily transpiration values (Fig. 4) can be used to estimate the amount of B delivered to buds via the xylem. Multiplying the daily transpiration rates (ml H₂O/bud/day) by the B concentration in xylem exudate (0.26 ppm) (Fig. 3) gives the amount of B supplied to buds each day via the xylem. Over the period from 29 Jan. until bloom, B supplied to buds by the xylem totaled 0.28 μg, which was only 26% of the 1.07 μg B accumulating in buds over this time. Similar calculations indicated that the xylem supplied 17% of P, 25% of K, 28% of Mg, and 71% of S accumulating in buds over the same period (data not given). In Lupinus angustifolius L., the xylem contributes 5% of P, 21% of K, and 55% of Mg accumulating in fruit (13). The proportion of B delivered to prune flowers which enters via the xylem may be lower than the 26% calculated in this study. Calculated transpiration values were based on the assumption that 100% of Ca entered buds by the xylem. If a small percentage of Ca entered buds via the phloem, the actual transpiration rates would be reduced, and the percentage of the total B content of flowers supplied by the xylem would be even smaller. An estimated 12% of the Ca accumulating in Lupinus angustifolius L. fruit is supplied by the phloem (13).

Reducing the transpiration rate of forced flowers reduced the Ca supply to flowers more severely than B supply (12). Flowers blooming in low relative humidity (29%), where transpiration was high, accumulated 13% more B and 25% more Ca than flowers blooming in 86% RH. The fact that B supply to flowers is less affected than Ca by transpiration rate is further evidence that the primary route of B supply to flowers is not the xylem.

This work indicates that very little B moves into buds prior to bud swelling. Passive movement of water and B into dormant buds may be limited by the discontinuous xylem connection in the axes of buds or the low rate of transpiration and water demand by dormant buds. The limited dry matter accumulation in buds indicates that they are weak carbon sinks at this time, and suggests that symplastic flow to buds also would be limited. Boron began to accumulate in buds as they increased in dry weight. Nearly 85% of the B content of prune flowers arrived in 5 weeks between bud swelling and bloom, and an estimated one-fourth of the B was supplied by the xylem stream. The proportion of B entering buds via the xylem may be lower than the 26% calculated in this study. Calculated transpiration values were based on the assumption that 100% of Ca entered buds by the xylem. If a small percentage of Ca entered buds via the phloem, the actual transpiration rates would be reduced, and the percentage of the total B content of flowers supplied by the xylem would be even smaller. An estimated 12% of the Ca accumulating in Lupinus angustifolius L. fruit is supplied by the phloem (13).

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Use of Early Flowering Genes to Reduce Generation Time in Backcrossing, with Specific Application to Lettuce Breeding

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Abstract. A partially dominant allele for early flowering in lettuce (Lactuca sativa L.) reduces flowering time by about one-half. This property is useful in backcross breeding procedure to accelerate the transfer of useful alleles to a desired recurrent parent. Application of the technique to transfer resistance to lettuce mosaic is described through 4 backcrosses. Generalization of the technique for other species is discussed.

Plant breeding is a slow, long-term process for the improvement of agricultural crop production and quality. Any technique that can accelerate the process without adversely affecting the results is likely to be adopted by plant breeders. Techniques vary in their degree of usefulness or in their generality. Certain techniques are specifically applicable to one or a few crops. Bud pollination, for example, is a technique that permits self fertilization in cruciferous crops, thus accelerating the process of inbreeding.

There are a number of techniques that are broadly applicable in reducing the time required to complete a breeding project. One can grow successive crops in each of 2 geographical locations, taking advantage of day length and temperature changes to produce 2 or more seed crops per year. One can accelerate generation time by treatment with plant hormones, especially gibberellins, to reduce the time to flowering.

Within crop species, there is usually significant variation in flowering time. This variation may be temperature and/or photoperiod related (2) and it may be inherited in a quantitative or qualitative manner (10). Earliness may be a favorable production character in crops for which the fruit or the seed is the harvested product. Earliness of flowering, or premature bolting, is not a favorable trait in root and foliar crops, nor in fruit or seed crops if there is a correlation between early flowering and reduced yield.

Genes for earliness, however, may be useful in backcross breeding, as a tool to accelerate the transfer of useful alleles to an adapted cultivar, an inbred line, or a population. In order to be useful, earliness must have the following characteristics:

1) inheritance must be qualitative to permit isolation of single genes;
2) there must be a substantial reduction in flowering time;
3) early flowering must be dominant or partially dominant, or at least the heterozygote must be distinguishable from the late homozygote.

Review of Literature

Discrete early flowering genes have been identified in all species that have been studied in detail (10, 16). In peas, recessive early flowering alleles have been reported by Gottschalk (4) and Wellensiek (17). Murfet (9) described a 2-gene epistatic combination in peas with one dominant and one recessive for...