Hydathode Anatomy and Adaxial Water Loss in Micropropagated 'Silvan' Blackberry

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Abstract. Foliar anatomical comparisons were made between in vitro-grown plantlets and greenhouse-grown plants of 'Silvan' blackberry (*Rubus* sp.) using scanning and light microscopy. Each apex and marginal serration of in vitroand greenhouse-grown leaves had a terminal hydathode region composed of a scattered, primarily adaxial, group of sunken water pores. Water pores and stomata of plantlet leaves were open, while greenhouse-grown plant leaves had closed water pores and stomata or comparatively small apertures. Internally, the hydathodes of both cultured plantlets and greenhouse-grown plants were delimited by a bundle sheath that flanked the vascular tissues and extended to the epidermis. Between the vascular tissues and the epidermis were loosely arranged epithem cells. The hydathodes of plantlet leaves were simpler than those of greenhouse-grown plants, with fewer water pores and reduced epithem. Water loss from detached leaves of plantlets occurred through both leaf surfaces, although more water was lost from the abaxial surface. In contrast, foliar water loss from severed leaf blades of greenhouse-grown plants was primarily abaxial.

Hydathode function has been debated for more than a hundred years (6). Hydathodes occur in many, but not all, plant families and vary widely in structure among genera within families (4). Hydathodes eliminate water through guttation, from the terminal tracheids of the bundle ends, via water pores. Water pores are incompletely differentiated stomata that, in some plant species, have lost the function to open and close (4). Between the terminal tracheids and the water pores are small, thin-walled, achlorophyllous parenchyma cells—the epithem. Water is apoplastically driven by root pressure through the epithem. Hydathodes are thought to be involved in the regulation of water uptake, maintaining an upward flow of water and minerals, when the transpiration stream is suppressed (6).

Little is known of hydathode anatomy or significance in the Rosaceae. Guttation has been noted in Rosoideae and Spiraeoideae and water pores are known in all four subfamilies (Amygdaloideae, Maloideae, Rosoideae, and Spiraeoideae) (7). Guttation has been reported in *Rubus* (1), and water pores and epithem noted but not illustrated (9). Tissue-cultured 'Silvan' blackberry have hydathodes (3). We have frequently seen guttation drops on leaf apices and serrations of tented ex vitro *Rubus* transplants in the greenhouse, implicating hydathodes in the acclimatization process. *Rubus* hydathode anatomy has never been described and the relation of hydathodes to water loss and the cultural environment is unknown.

The objectives of this study were to compare the anatomy of the hydathode complex in 'Silvan' blackberry leaves, from both greenhouse-grown plants and tissue-cultured plantlets, and to determine whether these structures contribute to evaporative foliar water loss.

Materials and Methods

In vitro 'Silvan' shoots were derived from 1- to 5-mm long shoot tips initiated and micropropagated in basal medium based on Murashige and Skoog (10) salts with thiamine hydrochloride at 1.20 μ M (0.4 mg·liter⁻¹) (8), *mvo*-inositol at 550 μ M (100 mg·liter⁻¹), sucrose at 87.6 mM (30 g·liter⁻¹), *N*-(phenylmethyl)-1*H*-purin-6-amine (BA) at 4.4 μ M (1.0 mg·liter⁻¹) and 1*H*-indole-3-butanoic acid (IBA) at 0.49 μ M (0.1 mg·liter⁻¹). Shoots were rooted in the same medium with BA omitted and IBA increased to 2.46 μ M (0.5 mg·liter⁻¹). Both media were adjusted to pH 5.7. Cultures were incubated at 27° to 28°C and kept under 25 μ mol·s⁻¹·m⁻² cool-white fluorescent light, with a 16-hr photoperiod. 'Silvan' greenhouse control plants were subjected to ambient temperatures (24° ± 5°) and natural light, supplemented with cool-white fluorescent light, to total 100 μ mol·s⁻¹·m⁻² for 16 hr.

For scanning electron microscopy (SEM) and light microscopy (LM), fresh samples were collected from 20 mature leaves of 6-month-old greenhouse-grown plants and an equal number of expanded leaves from rooted shoots (plantlets) after 1 month in rooting medium. Sections, which included the leaf apex and adjoining leaf serrations, were fixed in 4% glutaraldehyde in cacodylate buffer (pH 7.0) for at least 2 hr, washed in distilled water, soaked for 1.5 hr in 1% osmium in 0.1 M cacodylate buffer (pH 7.0), again washed in distilled water, and dehydrated through an ethanol series. Those for SEM were critical-pointdried in CO₂, mounted on aluminum stubs with silver epoxy paste, sputter-coated with gold, and examined and photographed in a Hitachi S500 SEM. Those for LM were embedded in Epon, sectioned (1–2 μ m) on an ultramicrotome, stained with a polychrome stain (11), and examined. Hydathode structure was de-

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termined from photographs of serial and longitudinal sections through the leaf apices.

For determination of water loss from plantlet leaves, 25 cultures were harvested. Plantlet leaves were detached and quickly sorted into groups of five. Leaves were taken at random from greenhouse-grown plants and were treated individually. Petroleum jelly, a nontoxic grease, was used to selectively coat entire leaf surfaces or the adaxial hydathode area only. It was assumed that water loss subsequently would occur from noncoated areas of the leaves. Leaves then were placed on weighing boats and weighed immediately and at 30-min intervals for 3 hr. Between weighing, leaves were kept under ambient conditions of $24^{\circ} \pm 1^{\circ}$ C, 7.5–8.0 µmol·s⁻¹·m⁻² cool-white fluorescent light, and $\approx 10\%$ RH. Each coating treatment was replicated five times for the plantlet leaves and three times for the greenhouse-grown plant leaves. Treatments were compared using two-way factorial analysis of variance ($\alpha = 0.05$).

Results and Discussion

Greenhouse-grown plant leaves were tri- or pentafoliolate with many marginal serrations, while in vitro-grown plantlet leaves were smaller and unifoliolate. Every leaf spex and marginal serration examined with SEM had a terminal hydathode region. The hydathode area was flush with the leaf surface; unlike those reported in Physocarpus (Rosaceae, subfamily Spiraeoideae), which occurred on achlorophyllous, raised pads (7), but resembling those of Potentialla palustris (L.) Scop., subfamily Rosoideae (2). The epidermal cell wall ridges were indistinct and the epidermal surfaces were smooth. The predominant feature was a scattered, terminal, and primarily adaxial group of sunken water pores. Leaves of plantlets had from 1 to 5 water pores on each serration and up to 15 on the leaf apex (Fig. 1A). Greenhouse-grown plant leaves had up to 25 water pores on each leaf serration and up to 50 on the leaf apex (Fig. 2A). Stomata, but not water pores, were present abaxially on the leaf surface, apices, and serrations (Figs. 1B and 2B). Leaf serrations that terminated in a glandular structure resembling a colleter had fewer water pores, which were displaced towards the adaxial leaf surface. There was one gland-tipped hydathode for every 4 or 5 of the aglandular hydathodes. Stomata were distinguishable from water pores by their raised guard cells (a feature exaggerated in cultured plantlet leaves) and conspicuous inner and outer wall thickenings. By contrast, the water pores were localized and adaxial, with guard cells sunk below the level of surrounding epidermal cells, and lacking distinct outer wall thickenings. Water pores (Fig. 1C and D) and stomata (Fig. 1 E and F) of plantlet leaves were open, while greenhouse-grown plant leaves had water pores (Fig. 2 C and D) and stomata (Fig. 2 E and F) that were closed or had relatively small apertures.

Internally, the hydathodes of greenhouse-grown plant leaves (Fig. 3 A and B) were delimited by a bundle sheath of darkstained cells that flanked the tracheary elements of the bundle endings. The bundle sheath extended abaxially almost to the epidermis at the leaf tip, and adaxially reached the epidermis, close to the least terminal water pore at the hydathode border. The epidermal cells of the hydathode region appeared smaller and more cuboidal in cross-section than adjacent epidermal cells. Files of spiral-walled tracheary elements ended varying distances from the leaf tip. Between the terminal tracheary elements and the water pores were sinuous-walled xylem parenchyma cells. Mesophyll cells, some without chloroplasts (epithem), some densely staining and with chloroplasts, filled the area between the xylem and the epidermis.



Fig. 1. SEM photomicrographs of micropropagated 'Silvan' plantlet leaves showing. (A) Adaxial apex with hydathode complex showing scattered water pores, indistinct cell wall ridges, and smooth epidermal surface. (B) Abaxial apex showing absence of water pores. (C) Sunken water pore with guard cells flared but the inner guard cell walls pinched closed. (D) Sunken, open water pore. (E) Open abaxial stomata. (F) Open abaxial stoma with raised guard cells and conspicuous inner and outer wall thickenings. Scale bar = 100 μm (A and B); 5 μm (C, D, and F); 50 μm (E).

The hydathodes of micropropagated 'Silvan' leaves (Fig. 3 C and D) were similar internally to those of greenhouse-grown plant leaves (Fig. 3 A and B). The bundle sheath cells of plantlet leaves had fewer tanniferous materials and stained less distinctly than those of greenhouse-grown leaves. There were fewer epithem cells in plantlet leaves and the tracheary elements tended to terminate closer to the epidermal cells than in greenhouse-grown plant leaves. This difference may tend to make leaves from culture comparatively more vulnerable to evaporative water loss from the leaf vasculature, a situation that may be worsened by the open water pores.

Detached greenhouse-grown plant leaves coated on both surfaces lost the least amount of water (Fig. 4, lower). Plant leaves with only abaxial surfaces coated had similar cumulative water losses (lost through the adaxial surface) to leaves coated on both surfaces. Compared to detached plant leaves coated on both surfaces, leaves coated only on adaxial surfaces lost significantly more water ($F_{1,24}$) = 15.258, $P \le 0.005$). Plant leaves without coating or with adaxial surfaces coated had similar cumulative water losses. Compared to detached plant leaves without coating, those with only the hydathodes coated lost significantly less water ($F_{1,24} = 19.392$, $P \le 0.005$). Of the small amount of adaxial water loss from plant leaves, the hydathodes apparently contribute little. Greenhouse-grown plant leaves lost water primarily through abaxial leaf surfaces.

Compared to detached plantlet leaves coated on both surfaces, cumulative water losses were significantly greater when only



Fig. 2. SEM photomicrographs of greenhouse-grown 'Silvan' leaves showing. (A) Adaxial apex with hydathode complex showing scattered water pores, indistinct cell wall ridges, and smooth epidermal surface. (B) Abaxial apex showing a terminal, but no other water pores, and some abaxial stomata. (C) Sunken water pore. (D) Sunken water pore, small aperture. (E) Abaxial surface stomata. (F) Open abaxial stoma, guard cells with conspicuous inner and outer wall thickenings. Scale bar = 100 μ m (A and B); 10 μ m (C, D, E, and F.



Fig. 3. LM photomicrographs showing longitudinal sections through the hydathodes of the leaf apex of 'Silvan'. (**A** and **B**) Greenhousegrown plant leaf. (**C** and **D**) Micropropagated plantlet leaf. (b, bundle sheath; e, epithem; m, mesophyll; p, palisade; s, stoma; t, tracheary element; w, water pore; x, xylem parenchyma). Scale bar = $50 \mu m$.

one surface was coated, either abaxial ($F_{1,46} = 6.528$, $P \le 0.025$) or adaxial ($F_{1,48} = 25.249$, $P \le 0.0001$) (Fig. 4, upper). However, when detached plantlet leaves were uncoated, coated on the adaxial leaf surface, or only on the hydathode areas,



Fig. 4. Cumulative foliar water loss showing mean and standard error bars (mg) from groups of five detached 'Silvan' plantlet leaves (**upper**) and individual detached greenhouse-grown plant leaves (**lower**) taken at 30-min intervals for 3 hr. Coating treatments included: no treatment; adaxial surface coated; hydathodes coated; abaxial surface coated and both surfaces coated. ²Mean separation within leaf type by Duncan's multiple range test ($P \le 0.05$).

cumulative water losses were similar. Unlike the greenhouse control leaves, abaxial water loss did not account for all the water lost from detached plantlet leaves. Water loss was greatest from the abaxial surface (adaxial surface coated) during the first 1.5-hr after detachment. However, after 1.5 hr, cumulative water loss was the same from either leaf surface. Water is therefore lost from both surfaces of plantlet leaves. In contrast to results found with plant leaves, coating the entire adaxial leaf surface of plantlets was not significantly different from coating only the hydathodes. This clearly implicates the foliar hydathodes of plantlets in ex vitro adaxial water loss.

During the first 30 min, detached plantlet leaves lost significantly more water ($F_{1,30} = 6.397$, $P \le 0.025$) than greenhouse-grown plant leaves, regardless of coating treatment. However, during the five subsequent 30-min time intervals, there were no differences in the rate of water loss between the two groups.

In conclusion, foliar water loss was almost entirely abaxial in severed greenhouse-grown 'Silvan' leaves. It is, therefore, unlikely that water loss is cuticular, and probable that stomata are involved. Abaxial evapotranspiration alone (again, most probably via stomata) did not account for all of the water lost from detached leaves of micropropagated 'Silvan' plantlets during a 3-hr period. A significant water loss occurred from adaxial leaf surfaces [unlike 'Pixy' plum (5)]. The contribution of the hydathodes to adaxial water loss in detached micropropagated 'Silvan' leaves was clearly shown. Reduced epithem and open water pores of cultured plantlet leaves likely promote water loss from the hydathodes.

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Distribution, Storage, and Remobilization of ¹⁴C-labeled Assimilate in Easter Lily

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Additional index words. Lilium longiflorum, bulb, dry weight, daughter scale, mother scale

Abstract. Whole shoots of Easter lily (*Lilium longiflorum* Thunb. cv. Nellie White) were exposed to ${}^{14}CO_2$ at 25, 37, and 51 days after full bloom of the commercial crop. Seven days after each exposure, 20% of the total recovered ${}^{14}C$ remained in the shoot, which included stem roots, 10–25% in stem bulblets, 11–20% in mother scales, and 34–44% in daughter scales. Sink activity increased sharply from the outer mother scales to the inner daughter scales. The fraction of total ${}^{14}C$ in the main bulb decreased, while that in the stem bulblets increased at successive exposures. Another group of plants was labeled repeatedly by dosing with ${}^{14}CO_2$ on the three previous occasions and, also, at 65 days after full bloom. Bulbs were harvested 7 days after the final exposure, stored at 18°C for 14 weeks, and then replanted, At bulb digging, 50%, 30%, and 20% of the total ${}^{14}C$ during storage and were nearly depleted when flower buds were visible the next season. Specific ${}^{14}C$ activity in the emerging flowering shoot was high but decreased dramatically as the leaf number rapidly increased. The shoot and new daughter scales were the principal recipients of mobilized scale reserves, although only 28% of the ${}^{14}C$ lost from mother scales were fall harvest and 3 weeks after anthesis the following year. The daughter bulb contained 64% of the ${}^{14}C$ in the bulb at fall harvest, and lost very little ${}^{14}C$ during regrowth the following year.

Easter lily bulbs are composed of modified leaves, referred to as scales, which are filled with food reserves during the last few months of a growing season (5, 15, 17, 20). A mature lily bulb has two kinds of scales: mother scales from the previous year and daughter scales that develop during the current growing season. Since both mother and daughter scales are initiated and develop over a long period (5), scales at different nodal positions are likely filled at different rates and the filling pattern may change with time (7, 18). Bulblets, which develop along the underground stem, grow rapidly after anthesis (5, 17) and potentially can divert a large fraction of current assimilate away from the bulb and lower its final weight (5). A previous study showed that after anthesis the majority of the ¹⁴C-labeled assimilate translocated out of a single source leaf in 24 hr was in the scales (20). However, the distribution of current assimilate from the entire lily shoot to various sink organs during the active filling period has not been determined.

Following bulb planting, dry weight of a lily plant decreases to a minimum before it increases again. Reserves in the old scales of an Easter lily bulb are depleted gradually during the development of the flowering shoot and formation of a new daughter bulb (17, 18). These scale reserves may be an important carbon source for new growth. Jefford and Edelman (10) reported that much of the dry matter removed from the Jerusalem artichoke tuber was used by the growing daughter shoots. Growth of the daughter bulb in tulip is partially dependent upon the reserves in the mother scales (1). The importance of scale reserves to new growth and the redistribution of reserves among new organs in Easter lily are not known.

The objectives of this study were to determine the long-term

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