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Abaxial Transpiration and Water Loss in Aseptically Cultured Plum[1]

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Abstract. **Scanning electron microscopy was used to investigate leaf epicuticular wax of *Prunus instititia* L. 'Pixie' from aseptically cultured plants before and after acclimatization to the greenhouse. Leaves from plants acclimatized for 2 weeks in the greenhouse had more adaxial wax than those from non-acclimatized (culture flask-grown) plants. Acclimatized plants had more adaxial than abaxial wax. No abaxial wax was observed on leaves of non-acclimatized plants. Stomata were present on the abaxial leaf surface only of both acclimatized and non-acclimatized plants. Epicuticular wax layers surrounded guard cells of acclimatized plant leaves but were not present on non-acclimatized plant leaves. Weight changes in non-acclimatized plant leaves coated with silicon rubber on adaxial, abaxial, and both surfaces indicated that excised leaf water loss occurred only through the abaxial surface. Water loss from plants during the acclimatization process thus may be due to abaxial cuticular and stomatal transpiration.**

Poor survival after transfer to greenhouse conditions limits commercial *in vitro* production of many plants. Only 50% of carnation (5), 60% of thornless blackberry (3), and 50–81% of rose (7, 11) aseptically cultured plants survived when transferred to the greenhouse. Boxus and Quoirin (1) reported poor survival of aseptically cultured plum plants during transplanting, but Jones and Hopgood (8) and Rosati et al. (10) reported good survival by maintaining high humidity after transplanting.

Desiccation and wilting are major causes of low survival when plants are transplanted (12). In cauliflower (4) and carnation (12) plants, lack of epicuticular wax was believed responsible for excessive transpiration. This study sought to determine whether epicuticular wax differed in aseptically cultured plum leaves before and after transfer to the greenhouse and whether water loss occurred from the abaxial or adaxial leaf surface.

Materials and Methods

'Pixy' plum rootstocks were cultured as described by Cheng (4). Three-week-old aseptically cultured plants, about 6 pairs of leaves and 8 roots, each about 1 cm long, were selected for uniformity.

For scanning electron microscopy (SEM), 20 non-acclimated plants were divided into 2 groups. The first remained in the original culture medium at 100% relative humidity (RH) with 16 hr of light at 150 $\mu Em^{-2}s^{-1}$ and at 21°C. The second was transplanted into 6.25-cm plastic pots contining 1: sphagnum peat moss: 1 perlite, acclimatized in a 21° ± 5° greenhouse at about 50% RH during July, 1979, and watered twice daily.

After 2 weeks, leaf sections 5 × 8 mm from the unexpanded upper and center leaves from both groups were mounted on SEM stubs and frozen immediately in liquid nitrogen –40°C and coated with 200°Å gold. Samples were viewed in an ISI MSM-2 scanning electron microscope at a 30° angle and 15 KV.

For measurement of transpirational water loss, non-acclimated leaves 6–8 mm long were collected from the 3rd or 4th node. Five excised leaves were weighed immediately and every 30 min thereafter for 3 hr. Fifteen leaves were coated with Dow Corning III Compound (Dow Corning Corp., Midland, MI 48640; formerly called Molykote III compound), a non-phytotoxic silicon material, on the adaxial, or both surfaces, and leaves were then weighed in separate Petri dishes. Each treatment was replicated 5 times, placed on a laboratory bench with 75 $\mu Em^{-2}s^{-1}$ of light and 23°C ± 2°.

Results and Discussion

Qualitative observations of micrographs of acclimatized and non-acclimatized leaves revealed that epicuticular waxes were deposited primarily on adaxial surfaces of both treatments (Fig. 1, 2). Epicuticular wax occurred on adaxial leaf surfaces of 2-week acclimatized plants than on those of non-acclimated plants. This difference was particularly apparent on the youngest, uppermost leaves (Fig. 1B, 2B). Waxes on acclimated leaves were randomly arranged and appeared crustier than those on leaves of non-acclimated plants (Fig. 1A, 2A).

These results agree with those on cauliflower (6) and carnations (12), and suggest that desiccation after transfer from *in vitro* conditions may result from poor epicuticular wax formation. In other studies, non-acclimatized leaves lost twice as much water within 30 min after excision as did leaves of acclimatized plums (2, 9). Desiccation in aseptically cultured cauliflower plants resulted from excessive transpiration through a cuticle lacking epicuticular waxes (5). Sutter and Langhans (12) reported that only 10% of non-glaucous carnation derived from tissue culture survived during acclimatization process. Absence of epicuticular waxes was the probable cause of poor survival of non-glaucous plantlets.

Silicone rubber applied to leaf surfaces of aseptically cultured plants demonstrated that water loss was entirely abaxial. Abaxial leaf surfaces transpired about 20 mg of water after 1 hr. Adaxial leaf surfaces transpired less than 2 mg after 1 hr. This test did not identify the cause of water loss. Absence of wax deposition on abaxial leaf surfaces may contribute to abaxial water loss from acclimatization. However, little abaxial wax was observed on the 2-week-old greenhouse plants, and these showed no water loss.

We conclude that water loss from excised plum leaves during aseptic culture occurs primarily abaxially and may be either cuticular, resulting from lack of epicuticular wax, or stomatal.

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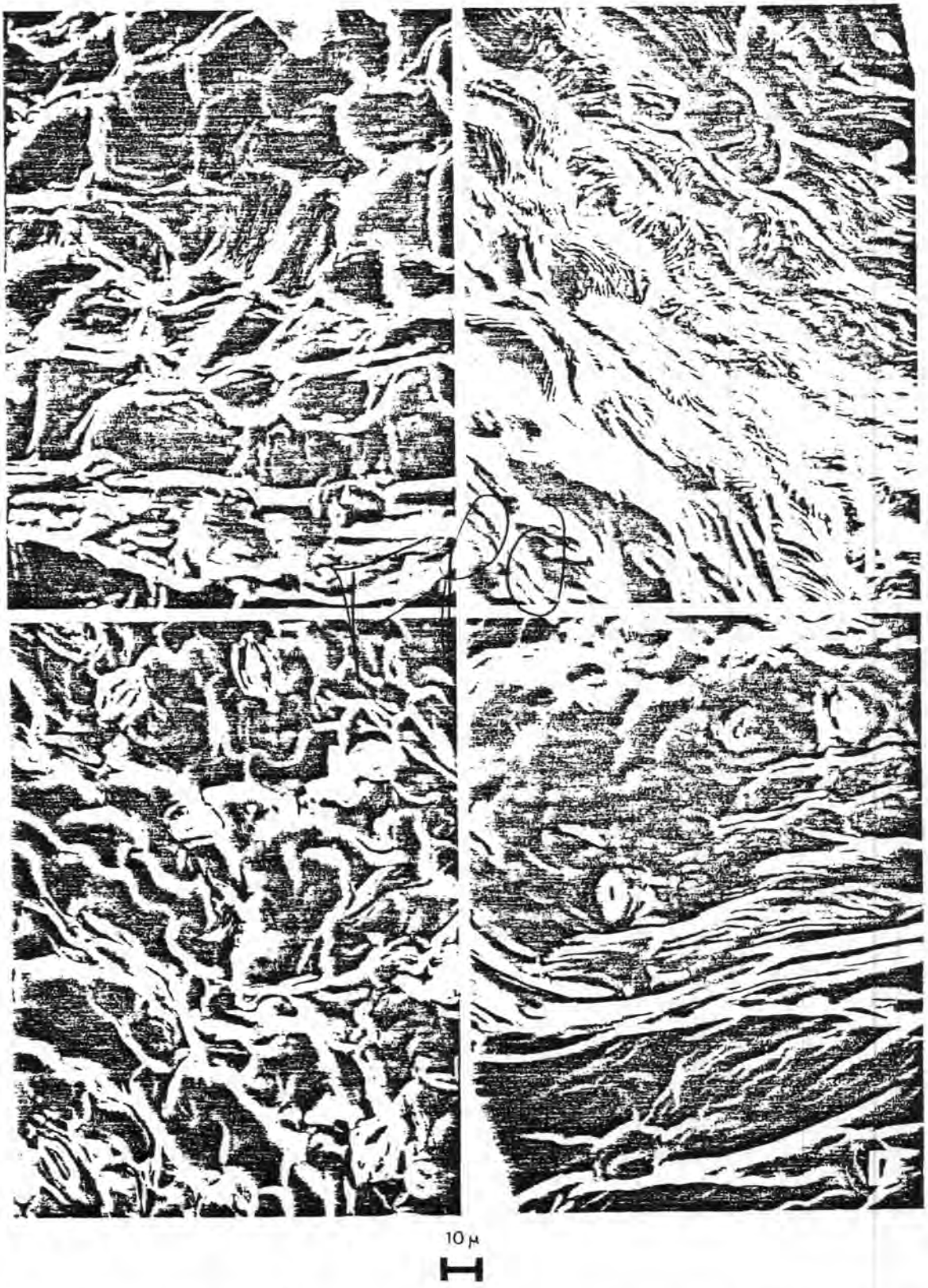

Fig. 1. Scanning electron micrographs of adaxial and abaxial surfaces of leaves of greenhouse-grown 'Pixie' plum plants 2 weeks after transplanting from tissue culture. A) Adaxial surface on mid leaf. B) Adaxial surface of uppermost leaf. C) Abaxial surface of mid leaf. D) Abaxial surface of uppermost leaf. (700 ×).

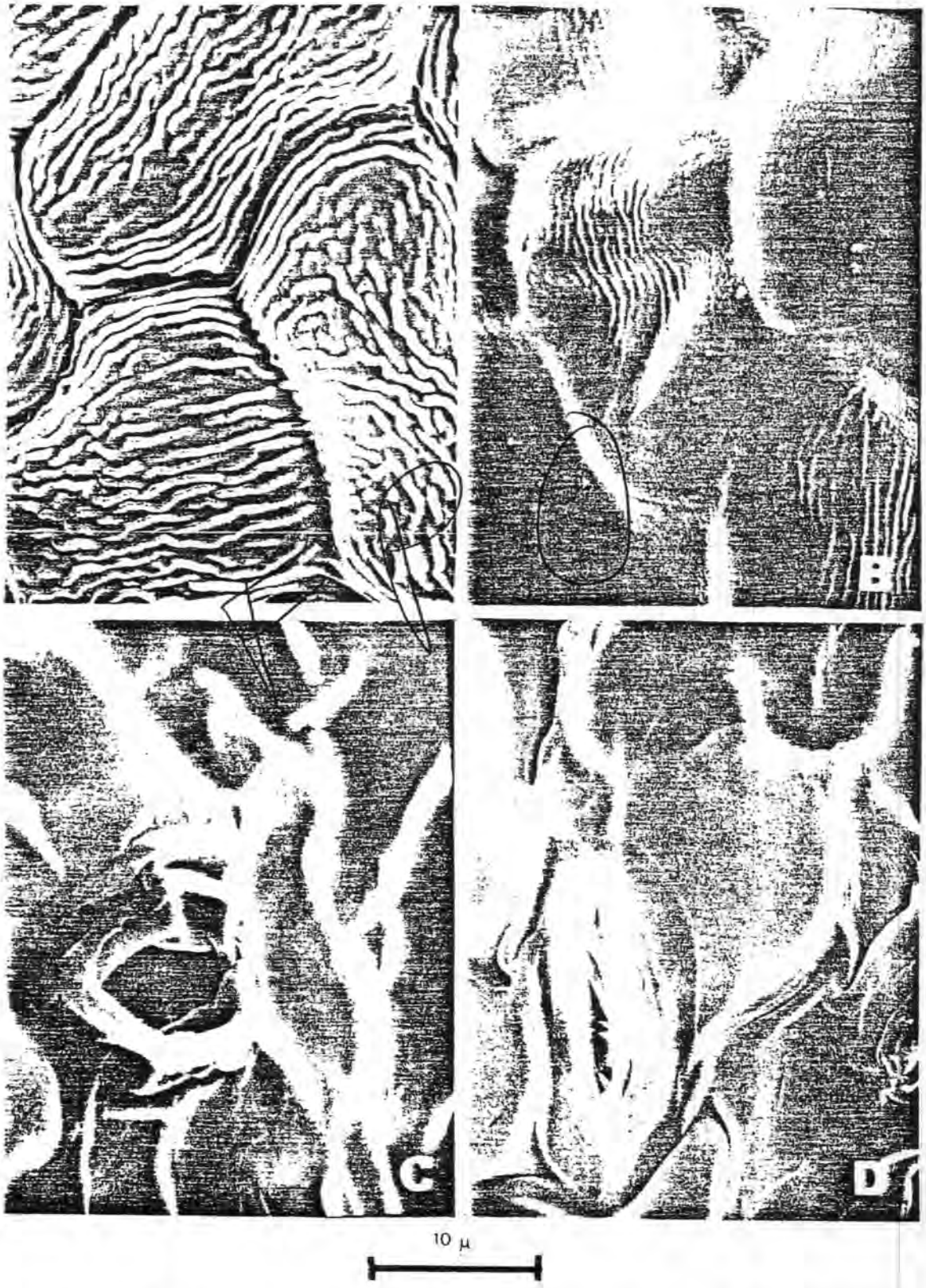

Fig. 2. Scanning electron micrographs of adaxial and abaxial surfaces of leaves of tissue culture-grown 'Pixie' plum plants. A) Adaxial surface of mid leaf. B) Adaxial surface of uppermost leaf. C) Abaxial surface of mid leaf. D) Abaxial surfce of uppermost leaf. (3000 ×).

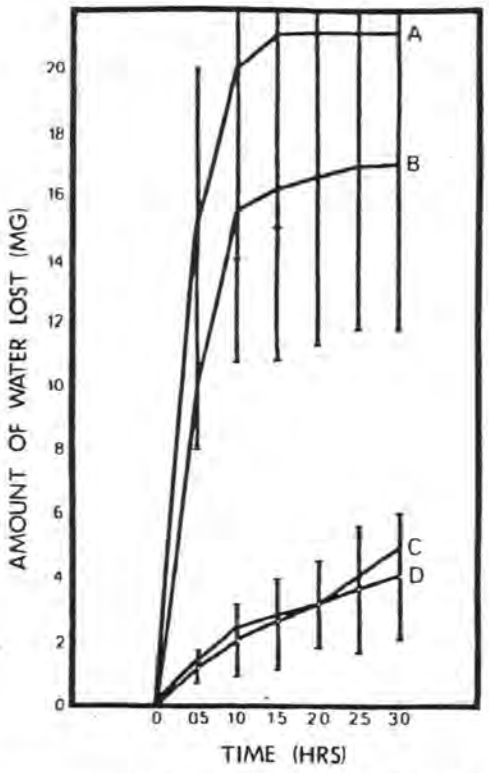

Fig. 3. Relative amounts of water loss from leaves of 'Pixie' plum plants. A) Adaxial surface of leaves covered with Dow Corning III Compound. B) Control. C) Adaxial surface of leaves covered. D) Both surfaces of leaves covered. Vertical lines denote SD.

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Chromosomal Behavior in Octoploid Strawberry Progenies and their Parental Clones during Meiosis[1]

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Abstract. **Chromosomal behaviro during diakinesis and metaphase I was studied in 4 octoploid cultivated strawberry progenies and their parental clones (*Fragaria x ananassa* Duch.). In all genotypes studied, most of the chromosomes were paired as bivalents. Loose bivalents and/or univalents were observed in some pollen mother cells (PMCs) during diakinesis. Two nucleoli were observed in a few PMCs. Twenty-eight bivalents in diakinesis indicated that the progenies and their parents were octoploid, with $2n = 8\ x = 56$ chromosomes. Some bivalents associated together were observed for all genotypes; however, the configurations of these associations were only end-to-end, end-to-side, or side-to-side, but not ring or chain configurations. The appearance of these configurations, therefore, indicated that they are probably formed by the aggregation of bivalents and are pseudomultivalents, not genuine multivalents.**

All large-fruit cultivated strawberries are derived from hybrids of the American species *Fragaria chiloensis* (L.) Duch. and *F. virginiana* Duch., and are referred to as *F.* x *ananassa* (15, 18). Through the study of the chromosomal behavior during meiosis of *Fragaria* polyploids, secondary association between bivalents has attracted the attention of several investigators for assessing the relationships between the different genomes. However, the assesment of genome homology based on chromosome pairing is difficult and subjective (14). Sebastiampillai and Jones (19) wrote: "Cytogenetical studies in *Fragaria* polyploids have been made by several workers with a view to assessing phylogenetic relationships. But interpretations and conclusions of these workers with regard to the nature of polyploids are not always in agreement." Secondary association between bivalents during

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