Table 1. Comparison of methods of scheduling irrigation to prevent available soil moisture from falling below 50% in a peach orchard in 1979.

Methods of scheduling irrigation	Date				
	June	July	August		
Soil moisture method (actual dates)	6, 14, 19, 25	13, 17, 23, 30	8, 14, 29		
Climatological method (predicted dates)	3, 11, 15, 19, 25	12, 17, 20, 24, 30	8, 15, 30		

number of hours of bright sunshine per day (n) was obtained from weather data at Harrow, using the Campbell Stokes sunshine recorder.

Effective rainfall (P<sub>e</sub>) was the portion of total rainfall available for plant use. The runoff from irrigated sandy soil is minimal. For light precipitation (P), effective rainfall (P<sub>e</sub>) can be calculated as suggested by Kanemasu et al. (3):

 $P_e = (P/25.4)^{0.75} \times 25.4$ , if  $P \ge 25.4$  mm  $P_e = P$ , if P < 25.4 mm

The maximum daily soil moisture  $(SM_{fc})$  was set to the field capacity. In this study,  $SM_{f.c.}$  and  $SM_{1/2f.c.}$  were 52.5 and 36.0 mm respectively. If daily soil moisture (SM<sub>d</sub>) exceeded field capacity (52.5 mm), then drainage (D) was calculated from  $D = SM_d - SM_{f.c.}$ . If daily soil moisture (SM<sub>d</sub>) was less than 50% ASM (36.0 mm), then irrigation (I) was required. The amount of water required was calculated from  $I = SM_{f.c.} - SM_{d.}$ Climatological data for the 1978 and

1979 growing seasons were obtained from the weather station at the Harrow Research Station and soil moisture data were obtained from an experimental peach orchard 4.8 km southeast of the weather station. The soil type was Fox sand which had the following characteristics: the field capacity in the top 30 cm was 17.5% on a volume basis and the permanent wilting point was 6.53%. Soil moisture was measured with a Nuclear Chicago neutron subsurface probe (no. 4810) and scaler (no. 5920). Routine moisture measurements were made at the 20 cm depth using 12 different sites in the orchard. Irrigation schedules to prevent the available soil moisture from falling below the 50% level, were based on the average of these 12 sites. When the soil moisture content reached the predetermined level equivalent to 50% ASM irrigation was applied to restore the soil to field capacity.

The actual irrigation schedule used in 1978 based on direct soil moisture measurement agreed very closely with the predicted schedule based on the simplified Priestly and Taylor model (Fig. 1E). The parameters used for predicting irrigation requirements in 1978 are shown in Fig. 1 A to D. These comparisons were repeated in 1979 and again very good agreement was obtained (Table 1)

The prediction method for scheduling irrigation described here was preferable to soil moisture method because of its greater simplicity and adaptability for use with computers or electronic calculators.

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# Leaf Anatomy and Water **Stress of Aseptically Cultured 'Pixy' Plum Grown** under Different Environments<sup>1</sup>

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Additional index words. Prunus insititia

Abstract. This study examined the leaf anatomy and water stress of Prunus insititia L. cv. Pixy grown in aseptic culture before and after transfer to the greenhouse and grown in a layerage bed in the field. The depth of palisade cells was significantly less in aseptically cultured plantlets than in greenhouse transfererred plants, and less in greenhouse transferred than in field-grown plants. Percent mesophyll air space was greater in plantlet than in plant leaves. Upper or lower leaf epidermal cell length of plantlets of field grown plants was not significantly different. Stomatal frequency for plantlet leaves was significantly less about 150 stomata per mm<sup>2</sup>) than that of plant leaves (300 stomata per mm<sup>2</sup>). Excised plantlet leaves lost greater than 50% of total leaf water content within 30 min; excised greenhouse leaves lost 50% after 90 minutes.

Plantlets when transferred from aseptic culture to the greenhouse may become water stressed and continued growth or survival may be decreased (9). The purpose of this study was to contrast the leaf anatomy and water stress of 'Pixy' plum plants grown in aseptic culture before and after transfer to the greenhouse with those propagated from a layerage bed in the field.

Leaf anatomy is influenced by light and moisture (2). Leaves developing at high light intensities have more and larger palisade cells than those in shade

(2). Leaves developing under low soil moisture have smaller intercellular spaces and higher stomatal frequencies than those grown with ample moisture (2, 6, 10). Plants grown at high relative humidity have poorly developed epicuticular waxes (9) and higher transpiration rates in dry air than do dry-air plants (8).

In the summer of 1979, 'Pixy' plum plantlets were aseptically cultured by Cheng's (1) method. One half of the rooted plantlets were transferred to peat:perlite (1:1 by volume), fertilized weekly with 473 ppm N, 456 ppm P, and 868 ppm K, and grown in a greenhouse at 27°C and normal day length for 3 weeks. Field plants were grown in layering beds at Oregon Rootstocks, Inc., in Gervais, Oregon.

Leaves were collected from the third to fifth node of aseptically-cultured plantlets. Leaves initiated and matured in the greenhouse were collected from transferred plants. Fully expanded leaves were collected from field grown plants. Samples were fixed in formalin-aceto-al-

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cohol, embedded in paraffin, sectioned and stained in safranin and fast-green (4), and observed with a light microscope.

Palisade, upper epidermal, and lower epidermal cell lengths were measured on 25 cells of each plant leaf. The percent mesophyll air space for each leaf was calculated using an ocular grid. Ten fields of mesophyll cells were examined in 10 sections of each leaf and plant type. To obtain the percent air space, the number of squares  $(25 \times 25 \ \mu m)$  of extracellular space for each field was counted, divided by the total number of squares, and multiplied by 100. The percent air space was analysed for treatment differences using Friedman's test (7) and a multiple comparison based on Friedman's rank sums (3). Stomatal frequency was determined by microscopic examination of siliconrubber, butyl-acetate leaf imprints. Stomatal frequency (the number of stomata per mm<sup>2</sup> of leaf surface) was calculated for 5 randomly chosen microscope fields on each of 20 different plantlet and greenhouse transferred 'Pixy' leaves, and was analysed using a ttest.

For the water stress study, 45 leaves about 20 x 7 mm were excised from 'Pixy' plantlets, and 45 leaf disks 15 mm in diameter were cut from leaves initiated and grown in the greenhouse on transferred plants. Fresh weight of each was determined and samples were placed abaxial side up in an open petri dish. Five samples from each plant type were dried at 21°C for 15, 30, 45, 60, 75, 90, 105, and 120 min and weighed. Oven dry weight was obtained after 48 hr of sample drying at 60°. The percent moisture loss for each sample was calculated by dividing the leaf moisture content after drying at 21° by the original moisture content, and multiplying by 100.

The length of palisade cells was significantly less in plantlets than in leaves of transferred plants (Table 1). Leaf palisade cells from transferred plants were significantly shorter than those of field grown plants. Upper or lower epidermal cell lengths were not significantly different for plantlets or field grown plants (Table 1). However, the upper epidermal cell length of transferred plants was significantly less than those of the other 2 plant types (Table 1). Percent air space in mesophyll tissue was significantly greater in plantlets than in transferred plants (Table 1, Fig. 1A, 1B). Mesophyll air space of transferred plants was not significantly different than that of fully expanded field grown leaves (Table 1, Fig. 1B, 1C). Examination of the most apical leaves indicated that field grown plants had significantly less mesophyll air space than the other plant leaves (data not shown). Stomatal frequency was significantly less for plantlet  $(150 + 60 \text{ stomata per mm}^2)$ than for transferred leaves (300 + 60)stomata per mm<sup>2</sup>).

Excised plantlet leaves lost water

Table 1. Epidermal and palisade cell length and percent leaf mesophyll air space of aseptically cultured, greenhouse, and field grown 'Pixy' plums.

Source of plant	Cell length (µm)			
	Upper epidermis	Lower epidermis	Palisade parenchyma	Air space (%)
Aseptically grown	26.4a <sup>z</sup>	13.8a <sup>z</sup>	20.2a <sup>z</sup>	20.6a <sup>y</sup>
Greenhouse transferred	19.7b	15.8a	31.8b	13.3b
Field grown	26.2a	15.9a	76.9c	9.5b

<sup>2</sup>Mean separation in columns by LSD, 1% level. Each mean was calculated from 25 replicates. <sup>y</sup>Mean separation in the column by a multiple comparison technique based on Friedman's rank sums (4, 8), 1% level. Each mean was calculated from 10 replicates.



Fig. 1. Leaf cross sections from (A) approximately the third node of an aseptically cultured, (B) greenhouse transferred, and (C) field grown 'Pixy' plum. The bar = 100 µm.

more rapidly than transferred plant transferred plant leaves (Fig. 2). Plantlet leaves lost more than 50% leaf moisture 30 min after excision; greenhouse transferred plant leaf disks lost about 50% moisture after 90 min. Analyses by ethvlene and ethane emission and electrolyte leakage indicated that excised 'Pixy' plum leaves were injured at water stress of 50% moisture loss or greater (5). A 50% moisture loss occurred about 1 hr sooner in excised plantlet leaves than in transferred plant leaf disks. We conclude that exised plantlet leaf cells were injured about 1 hr sooner than transferred leaf disks.

Our leaf anatomy data agree with those of similar studies (2, 6, 8, 10). These results indicate that plantlets with smaller palisade cells, larger intercellular spaces, and lower stomatal frequency were injured by water stress more rapidly than transferred plants which were acclimatized to the greenhouse. Further studies will focus on stomatal and cuticular water loss in relation to plantlet transpiration before, during, and after transfer to the greenhouse.



Fig. 2. Percent moisture loss of excised 'Pixy' plantlet leaves and greenhouse transferred plant leaf disks for each drying treatment. Each point represents the mean of 5 samples.

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## Fruit Development in Rabbiteye Blueberry Cultivars<sup>1</sup>

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Additional index words. Vaccinium ashei

Abstract. Fruit growth and development of 6 rabbiteye blueberry (Vaccinium ashei Reade) cultivars were measured from bloom date through harvest season. Fruit development was characterized by 3 divisions of growth: an early period of accelerated size increase, a second period with lesser increase in fruit size, and a third period with a greatly accelerated increase in fruit size which continued until fruit was mature. Fruit size in all cultivars was largest at the first harvest and smallest at the last harvest. Cultivar differences were evident in fruit size, fruit development, titratable acidity, and percent soluble solids.

Measurements on fruit growth and development plus related components have been reported on highbush and lowbush blueberries (2, 3, 4, 6, 7). Little comparable information is available on rabbiteye blueberries (*Vaccinium ashei* Reade), an indigenous fruit with growing economic importance to the southeastern United States.

Environmental conditions greatly influence bloom date and rate of fruit development. Nevertheless, fruit development and ripening, regardless of weather conditions, tend to follow a general growth pattern. Young, working with highbush (V. corymbosum L.) and lowbush (V. angustifolium Ait.) blueberries found that the fruit growth curve could be divided into 3 periods of growth: an early accelerated growth period, a period with little increase in fruit size, and a second accelerated growth period which continued until fruit was mature (7). Although the first and last stages were relatively constant in length, the duration of the middle stage varied from 1 to 42 days on a single plant.

During the period of fruit maturation, changes in chemical and physical characteristics are used as harvesting indicators for various fruit crops and could possibly be utilized for blueberries. Woodruff et al. (6) reported a decrease in acidity and an increase in total sugars of highbush blueberries as fruit growth developed from unripe to ripe stages. They also indicated that titratable acid content decreased as ripening progressed. An increase in the ratio of sugar to acids was associated with ripening in highbush blueberries (4).

The objectives of these studies were to determine for 6 major rabbiteye cultivars the rate of fruit development from bloom through harvest and to ascertain differences in fruit size (volume), soluble solids, and titratable acidity. Comparable plants of 'Briteblue', 'Climax', 'Delite', 'Southland', 'Tifblue', and 'Woodard' rabbiteve cultivars planted in 1974 were chosen for this study. Ripe fruit size was measured through the harvest period for 4 years (1977-1980). Beginning 15 days after flower buds had reached stage 7 (corolla drop) on a floral development scale (5) fruit development, titratable acidity, pH, and percent soluble solids were recorded over a 2-year period. Fruit was randomly selected to represent mean developmental stages of the cultivars. Fruit size was determined by displacement using 25 berries per plant and 8 plants per cultivar on each collection date. Weight per berry data were taken but not reported since weight measurements parallelled volume measurements. Titratable acidity, pH, and soluble solids

were ascertained on 2 plants per cultivar per date using A.O.A.C. methods (1). Titratable acidity was determined by titrating juice samples to pH 8.2 with 0.1 N NaOH. A glass electrode pH meter was used for recording pH and acidity measurements on 25 g of fruit/plant. Percent soluble solids was determined from juice of 10 berries/plant using a refractometer.

Fruit size development measured from 15 to 85 days after corolla drop was more rapid in the early-ripening cultivars such as 'Woodward' and 'Climax' than in the later-ripening cultivars represented by 'Southland' (Fig. 1). Nevertheless, all of the cultivars tested followed the same general fruit size developmental pattern. The growth curve was similar to that for highbush and lowbush blueberries described by Young (7) with 3 divisions of growth. There was an accelerated increase in berry size of 0.0105 cc/day between 15 and 35 days after corolla drop. A slight decrease in the rate of fruit size development occurred between 35 and 65 days, averaging 0.0095 cc per day. However, the second stage of the fruit growth curve was not as distinct from the first stage as that reported for highbush and lowbush blueberries. Between 65 and 85 days after corolla drop, fruit exhibited the greatest increase in size, averaging an increase of 0.029 cc/day. This rate of increase was about 3 times as rapid as the other 2 stages.

Maximum fruit size was reached about 90 days after corolla drop. Fruit was largest in the first harvest for all cultivars. On each successive harvest, fruit size was smaller, averaging a decrease of 0.4 cc/



Fig. 1. Fruit size (cc/berry) development from bloom date until ripe fruit of rabbiteye blueberries.

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