

## LEAF CHARACTERISTICS AND FRUIT GROWTH AS INFLUENCED BY SHADE IN 'BRAEBURN' APPLE TREES<sup>1</sup>

Patricia I. Garriz<sup>2</sup>  
Graciela M. Colavita<sup>2</sup>  
Hugo L. Alvarez<sup>2</sup>

### ABSTRACT

The effects of shading branches to 20 % of available light from 40 days after full bloom until harvest time on leaf characteristics and the seasonal fruit growth pattern of 'Braeburn'/MM.111 apple (*Malus xdomestica* Borkh.) trees were evaluated in the High Valley region of Argentina. Sunlight reduction yielded significantly, contrasting anatomical features, but it did not influence stomatal density. Chlorophyll meter readings were higher in the shaded than in the exposed leaves (44.6 and 35.9 SPAD units, respectively). Shading reduced the biomass of spur and shoot leaves by 35 and 37 %, respectively; shoots attained significantly greater leaf area of individual leaves than spurs, and surface area of both vegetative components was not affected by light stress. Shaded leaves had significantly lower specific leaf weight per fruiting spur leaf as compared to the control (4.8 vs. 7.1 mg dry weight cm<sup>-2</sup>, respectively). Low irradiance also resulted in smaller fruit diameter ( $\approx$  70 % of control), lower soluble solids content ( $\approx$  44 % of control) and poor coloring, whereas length/diameter ratio was unchanged.

Key words: *Malus xdomestica*, fruit growth, shade.

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<sup>2</sup> Departamento de Biología Aplicada, Facultad de Ciencias Agrarias, Universidad Nacional del Comahue, c.c. 85, 8303 Cinco Saltos, R. N., Argentina. E-mail: pigarriz@uncoma.edu.ar

## RESUMO

### INFLUÊNCIA DO SOMBREAMENTO NAS CARACTERÍSTICAS DAS FOLHAS E CRESCIMENTO DOS FRUTOS EM MACIEIRAS 'BRAEBURN'

Na região do Alto Valle (Río Negro, Argentina), avaliaram-se os efeitos do sombreamento (20% da luz disponível) a partir dos 40 dias depois da floração completa até o momento da colheita, em ramos de macieiras (*Malus xdomestica* Borkh.) 'Braeburn'/MM.111, nas características das folhas e no crescimento estacional dos frutos. A redução da luz solar produziu mudanças anatômicas significativas, mas não influenciou na densidade de estomas. As leituras da medição da clorofila foram maiores nas folhas sombreadas que nas expostas (44,6 e 35,9 unidades de SPAD, respectivamente). O sombreamento reduziu a biomassa das folhas dos dardos e dos brotos em 35 e 37%, respectivamente; a área foliar das folhas individuais foi significativamente maior nos brotos que nos dardos e a área superficial de ambos os componentes vegetativos não foi afetada pelo estresse luminoso. O peso foliar específico das folhas sombreadas nas esporas frutíferas foi significativamente menor que no controle (4,8 vs. 7,1 mg de peso seco cm<sup>-2</sup>, respectivamente). A baixa irradiação também resultou num menor diâmetro do fruto ( $\approx$  70% do controle), mais baixo conteúdo de sólidos solúveis ( $\approx$  44% do controle) e uma pobre coloração, enquanto a relação comprimento-diâmetro não foi modificada.

Palavras-chaves: *Malus xdomestica*, crescimento do fruto, sombreamento.

## INTRODUCTION

Shading frequently occurs in tree canopies and it has become increasingly important to study its effects on leaf and fruit growth. According to Lakso (9), of all environmental factors, solar radiation should be considered first, due to its primary role as the source of energy that drives the biological production of dry matter, which ultimately limits fruit yield. Presently, it is well known that in pome fruits, young fruits are not strong sinks (6). Fruit set and/or fruit size can be decreased by early competition with vegetative shoots or by low irradiance (3) and it has been shown that shoot growth has priority over fruit growth for partitioning in light-limiting conditions (1). However, fewer data are available on the influence of shade on later stages of leaf and fruit development and a better understanding of the basic mechanisms underlying the response of apple trees to light stress may contribute to achieving higher fruit quality.

'Braeburn' is a chance seedling originated in New Zealand in the early 1950s and it is a spurry, late harvest apple cultivar, as described previously by Greene (8). We have found no reports of experiments designed to determine which resources or processes have a limiting performance in 'Braeburn' growing under the environmental conditions of Argentina.

The objective of this study was to compare changes in leaf and fruit characteristics on branches of apple trees cv. Braeburn that were a) shaded and nonshaded late in the season, and b) girdled to prevent carbon transport to or from other sites.

## MATERIALS AND METHODS

Five-year-old 'Braeburn'/MM.111 apple (*Malus domestica* Borkh.) trees were studied growing in sandy loam and trained to palmette leader at Comahue National University experimental farm (38°56'S, 67°59'W), on a sandy loam soil. The experimental site was located in an arid region, with mean annual rainfall of  $\approx 250$  mm. During the growing season, daily maximum irradiance was  $1586 \text{ W} \cdot \text{m}^{-2}$  (23 November 1998). The trees were spaced  $4.0 \times 2.3$  m and row orientation was north-south. Surface-flood irrigation was applied.

Treatments were (i), sun-exposed branches (control); and (ii), shaded branches. Artificial shading was started on 4 November 1998, 40 days after full bloom (DAFB) and ended 173 DAFB. Fruits were hand-thinned to leave a maximum of two fruits per spur. Two comparable branches on each of three uniform trees were selected for good exposure and similarity of size and one branch of each pair was chosen at random and then shaded. Branches were girdled at the base by removing a 5 mm ring of bark to isolate them from any carbohydrate transfer from the rest of the tree. On each branch, eleven fruits were tagged and equatorial fruit diameter was measured at two-weekly intervals ( $n = 33$  per treatment and date) with a Vernier caliper (model 30-410-5, General Supply Corporation, Jackson, Miss., USA). Shading was achieved by covering branches with 80 % polypropylene shade material mounted on frames.

At harvest, the fully expanded leaf closest to the fruit was collected from twenty-one fruiting spurs of each irradiance environment. Discs were excised between the midrib and leaf margin, using a cork borer of 9 mm diameter and the SPAD chlorophyll meter (model 502, Minolta Co., Ltd., Osaka, Japan) was used to measure the green color of apple leaves. Samples were subsequently oven-dried at 80 °C for 48 h; dry weight was determined to obtain specific leaf weight (SLW, dry weight per unit area). Tissue subsamples for microscopic examination were taken ( $n = 8$  per treatment), fixed in formalin-acetic acid-alcohol (FAA) and dehydrated in ethyl alcohol. Tissue was then embedded in paraffin blocks and sectioned at 15  $\mu\text{m}$  with a microtome (model 1207, Leitz G.M.B.H., Wetzlar, Germany). Tissue sections were affixed to glass slides, stained with safranin and fast green and analyzed with a Carl Zeiss light microscope (model Standard RA 34, Oberkochen, Germany).

Fruit were harvested when ripe. Six-fruit samples were weighed with an electronic scale (model Mettler P1210, Mettler Instruments AG, Zurich, Switzerland) and length/diameter ratio was recorded. Soluble solids concentration (SSC, %) was measured on the expressed juice with a hand-held refractometer (Brix 0 - 32 %, Erma, Tokio, Japan). Starch pattern index was estimated for the equatorial region of each fruit by staining with an iodine-potassium iodide solution by the procedure of Lau (10), and rating 0 - 6, where 0 = all stained and 6 = no staining. External color was estimated visually, as described by Quamme, Macdonald and Hampson (12).

Spurs and shoots were removed over the entire branches; for each vegetative component, leaf dry weight (LW) was determined. Thirty leaves per treatment were randomly subsampled; the relationship between leaf area (LA) determined with a LA meter (Cid, Inc., Vancouver, Wash., USA) and LW was evaluated by fitting regression models.

Data were analyzed using the Statistical Analysis System GLM (SAS Institute, Cary, N.C., USA). Differentiation among the mean values was done by the Student's *t*-test.

## RESULTS AND DISCUSSION

### *Leaf morphology*

Sun leaves were thicker (220.2  $\mu\text{m}$ ) than shade leaves (179.6  $\mu\text{m}$ ), because of differences in the dimension of the palisade cell layer and the spongy parenchyma, indicating that the leaves acclimatized through changes in leaf structure (Table 1). Contrasting anatomical characteristics were also found in leaves of pears (6) that had been exposed to different irradiance regimes during 62 days, but sun leaves were thicker than shade leaves because of differences in the dimension of the palisade cell layer and the epidermis with cuticle.

TABLE 1 – Structure of 'Braeburn' spur leaves on girdled limbs as affected by shade (80 %) from 40 days after full bloom until harvest time

Treatment*	Thickness ( $\mu\text{m}$ )				Leaf	Stomatal density (Number of stomata $\cdot$ $\text{mm}^{-2}$ )
	Palisade parenchyma	Spongy parenchyma	Upper epidermis	Lower epidermis		
Control	73.7 a	114.9 a	17.5 a	14.0 a	220.1 a	206.5 a
Shade	62.4 b	87.6 b	15.9 a	13.8 a	179.6 b	215.4 a

\* Means with the same letter in each column did not differ significantly by the *t*-test at  $P \leq 0.05$ .

Controlling of differentiation and activity of stomata is important because they regulate gas exchanges involved in the photosynthetic process (13) and water loss in plant tissues (14). In this study, it was found that shading had no significant effect on stomatal density (Table 1). Different results were obtained by Loreti et al. (11) in trials on 'Stark Red Gold' nectarine leaves, where stomata were less numerous with low available irradiance, and by Streitberg (15), who reported that stomatal density was somewhat reduced in various apple cultivars under shaded conditions. Furthermore, stomatal frequency is characteristic of the cultivar (2); an average of 206.5 stomata · mm<sup>-2</sup> at 173 DAFB on control leaves was determined in this study. SPAD meter readings were substantially higher for shaded (44.6) than for exposed (35.9) leaves, which indicated higher chlorophyll accumulation in regions of lower light intensity.

### *Leaf growth*

LA was linearly correlated with LW; analysis of covariance indicated that the models to estimate LA for exposed and shaded leaves differed significantly; therefore, a specific equation was used for each light condition:

$$\text{Control: } Y = 6.4809 + 0.0584X, R^2 = 0.87 (P < 0.0001)$$

$$\text{Shade: } Y = 4.7917 + 0.1155X, R^2 = 0.93 (P < 0.0001)$$

Where  $Y = \text{LA (cm}^2\text{)}$  and  $X = \text{LW (mg)}$ . Shading reduced the biomass of spur and shoot leaves by 35 and 37 %, respectively (Table 2); this emphasizes the importance of irradiance as a determining factor in leaf activity. Shoots attained larger LA of individual leaves than spurs (Table 2), whereas shaded leaves had lower SLW per fruiting spur leaf as compared to the control (4.8 vs. 7.1 mg dry weight · cm<sup>-2</sup>, respectively).

**TABLE 2** – Final mean leaf dry weight and leaf area of different 'Braeburn' canopy components, as influenced by shade (80 %) from 40 days after full bloom until harvest time, when sampled from girdled limbs.

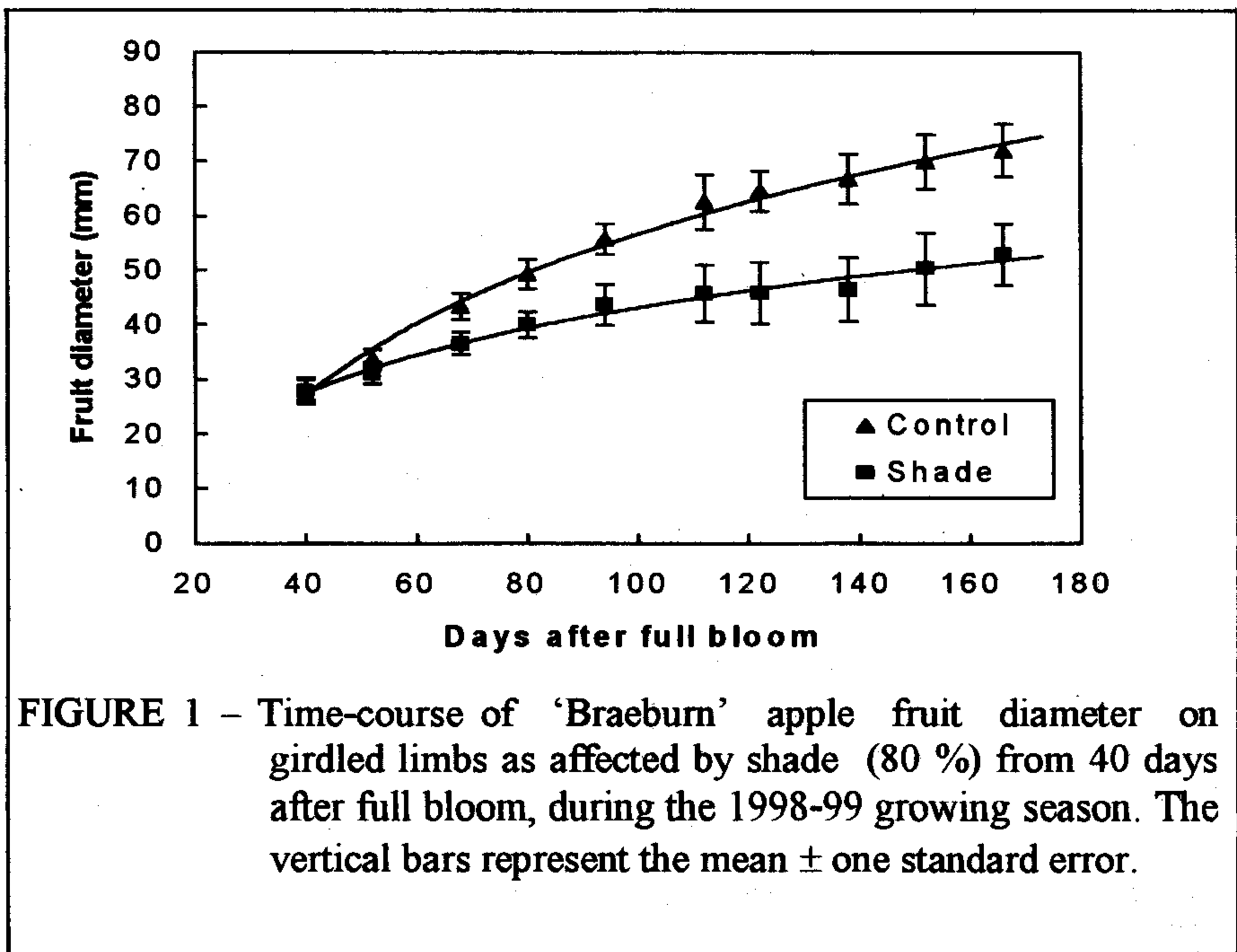
Treatment*	Spurs		Shoots	
	Dry weight (mg · leaf <sup>-1</sup> )	Leaf area (cm <sup>2</sup> · leaf <sup>-1</sup> )	Dry weight (mg · leaf <sup>-1</sup> )	Leaf area (cm <sup>2</sup> · leaf <sup>-1</sup> )
Control	146.3 a	15.0 a	243.8 a	20.7 a
Shade	95.1 b	15.8 a	154.5 b	22.6 a

\* Means with the same letter in each column did not differ significantly by the *t*-test at  $P \leq 0.05$ .

In general, as shade increases, vegetative morphological characteristics of the tree change to facilitate light harvesting, and leaves are larger (4). However, according to our results, surface area of both 'Braeburn' spur and shoot leaves was not affected by shading (Table 2).

### *Fruit growth*

Shading was applied when fruit diameter was  $28.2 \pm 2.0$  mm. The seasonal growth patterns were different for fruits from exposed and shaded branches (Fig. 1); maximum growth rates were  $0.61$  and  $0.33$  mm · d<sup>-1</sup>, respectively. Significant decreases in the fruit growth rates of the treated fruits were detected from 52 DAFB through harvest time. Since shading reduced the biomass of spur and shoot leaves and the branches were isolated from other sources by girdling, the carbon supply was probably limiting for the growth of the shaded leaves and fruits and at the end of the season the latter accumulated 66 % less fresh weight, as compared with control (57.3 vs. 167.0 g, respectively). Fruit sinks have been described by their ability to attract assimilates, denoted by sink strength (7). Additionally, the direct light exposure of fruits has been shown to increase fruit temperatures and would be expected to induce a higher fruit transpiration flux as well; this may stimulate the translocation of nutrients and hormones to the fruit sink, thus increasing sink strength (9).



*Fruit quality*

A basic indicator of internal fruit quality is the total SSC, mostly in the form of sugars. From our results, the quality of the shaded fruits declined, since SSC was reduced by 56 % as compared to the control (Table 3). Unlike these results, previous work performed in the same location showed that exposed pear fruits from nongirdled branches had similar levels of SSC, compared with shade treatments imposed during extended periods. This might be due to compensation by assimilates import from other branches (5).

**TABLE 3 – Effects of shade (80 %) from 40 to 173 days after full bloom on fruit characteristics in 'Braeburn' apples harvested from girdled limbs**

Treatment*	Soluble solids Conc. (%)	Starch index (1-6)	Red over color (%)	Ground color (%)	Length/diameter ratio
Control	15.1 a	5.5 a	63.3 a	Yellow	0.88 a
Shade	6.6 b	6.0 a	0.3 b	Green	0.87 a

\* Means with the same letter in each column did not differ significantly by the *t*-test at  $P \leq 0.05$ .

Light-limiting conditions did not influence fruit shape, reflected in the length/diameter ratio. Full sun exposure resulted in fruit with a red solid over color ranging from 40 to 90 % of the surface and a uniform yellow ground color, whereas shading was strongly detrimental to fruit appearance, suggesting that it affected anthocyanin biosynthesis (Table 3).

### CONCLUSIONS

The main conclusions from this study were as follows: 1) Light stress involved leaf morphology changes and increased chlorophyll content, whereas stomatal density was unchanged. 2) Shading significantly reduced the biomass of shoot and spur leaves and specific leaf weight; shoots attained greater LA of individual leaves than spurs, and surface area of both vegetative components was unaffected by sunlight reduction. 3) Fruit showed a marked sensitivity to low irradiance, which inhibited accumulation of soluble solids, and diminished size and coloration;

however, irradiance-limiting conditions did not influence the length/diameter ratio.

This study was severe in affecting light exposure; while shading decreased light to 20 % of the incident radiation, reductions of irradiance to only 5 % of ambient levels have been measured in the interior of the tree at noon on clear days in February. Further work is needed to elucidate the mechanisms of physiological responses of 'Braeburn' trees to different stress conditions.

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