J. AMER. SOC. HORT. SCI. 115(3):364-368. 1990.

Growth of Transplants and in Vitro-cultured Clones of Asparagus in Response to CO_2 Enrichment and Supplemental Lighting

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Additional index words. Asparagus officinalis, seedlings, hardening, micropropagation, acclimatization, greenhouse

Abstract. **Asparagus *(Asparagus officinalis* L.) transplants and in vitro-cultured clones were grown and acclimatized under two photosynthetic photon flux (PPF) conditions (ambient and ambient + 80 µmol·s^{-1}·m^{-2}) and three atmospheric CO_2 concentrations (330, 900, and 1500 ppm). Short- and long-term effects were measured in the greenhouse and after two seasons of growth in the field, respectively. In the greenhouse, CO_2 enrichment (CE) and supplemental lighting (SL) increased root and fern dry weight by 196% and 336%, respectively, for transplants and by 335% and 229%, respectively, for clones. For these characteristics, a significant interaction was observed between SL and CE with tissue-cultured plantlets. In the absence of SL, CE did not significantly increase root or shoot dry weight. No interaction was observed between CE and SL for transplants, although these factors significantly improved growth. It was possible to reduce the nursery period by as much as 3 weeks with CE and SL and still obtain a plant size comparable to that of the control at the end of the experiment. Long-term effects of SL were observed after two seasons of growth in the field. Supplemental lighting improved survival of transplants and was particularly beneficial to in vitro plants. Clones grown under SL were of similar size as transplants after 2 years in the field.**

The use of transplants is becoming increasingly popular for the establishment of asparagus plantings. Its advantages over conventional planting of l-year-old crowns include the prevention of root diseases, superior stand establishment, and reduced production costs associated with high seed prices and digging-replanting operations (Benson, 1979; Ombrello and Garrison, 1978; Williams, 1979). Many papers have dealt with the optimization of growing conditions, such as container size and media (Dufault and Waters, 1984), fertilizer type (Precheur and Maynard, 1983) and ratio (Adler et al., 1984; Fisher and Benson, 1983), growth regulators (Adler et al., 1985), and inoculation with VA mycorrhizae (Powell et al., 1985). However, these papers have not addressed two often limiting factors for the growth of transplants: CO_2 and light. Despite an abundant literature on the beneficial effects of carbon dioxide enrichment (CR) and supplemental lighting (SL) for the production of vegetable transplants (Hurd, 1968; Porter and Grodzinski, 1985), no data are available, to the best of our knowledge, on the influence of these factors on the growth of asparagus.

Recent advances in asparagus tissue culture (Chin, 1982; Desjardins et al., 1987a; Khunachak et al., 1987) offer the possibility for large-scale cloning of superior plants. However, in many instances, clonal material has a poorer performance than expected, which may be attributed to persisting effects of tissue culture or to a poor adaptation of clones to external conditions. Recent reports by Desjardins et al. (1987b) and Hayashi and Kozai (1987) have shown that CE and SL improve growth and reduce the acclimatization time of tissue-cultured strawberry ex vitro. A similar effect of these treatments on asparagus clones would be very desirable.

The objectives of the experiment were to determine the effect of supplemental lighting and CO_2 enrichment on the growth of asparagus transplants and ex vitro asparagus clones in a greenhouse. The residual effects of these pretreatments to plants in the field were also determined.

Materials and Methods

Asparagus seeds ('Lucullus mid-early' and 'Viking-2G') were sown in an artificial medium (Pro-mix BX, Premier Peatmoss, Rivière-du-Loup, Que., Canada) in 7.6-cm-diameter × 12.7-cm-deep (220 cm^3 volume) compressed peat pots (Jiffy Products of America, West Chicago, Ill.). Two seeds were placed in each pot. Plants were transferred to treatments when germination was ≈ 50% (2 weeks after sowing). After germination, plantlets were thinned to one per pot.

Asparagus clone G-171 was obtained from the Univ. of Guelph, Guelph, Ont., and originated from an outstanding selection of the cultivar Viking-2G. It was multiplied and rooted according to Desjardins et al. (1987b). Rooted plantlets were washed free of agar, leaf area was halved by cutting ferns to reduce the evapotranspirative area, and plantlets were transferred to a Pro-Mix BX artificial substrate. Plants were then placed in a greenhouse under humidity tents (>95% RH) for 10 days, until the appearance of one ex vitro spear, after which they were transferred to the treatments. At the time treatments were imposed, plant size was comparable to that of seedlings of the two other cultivars.

Plants were randomly distributed in treatments and were arranged in a split-plot design. The main plots were unreplicated and consisted of three concentrations of atmospheric CO, (330, 900, and 1500 ppm). The sub-plots were two light treatments [natural light and natural light supplemented with an 80 µmol·s^{-1}·m^{-2} photosynthetic photon flux (PPF)] replicated twice. Cultivars and clones were placed in sub-sub-plots. Carbon dioxide concentrations were controlled and monitored (± 50 ppm) in each of the 24-m^2 compartments with an infrared gas analyzer

Received for publication 31 Mar. 1989. We would like to express gratitude to the Canadian Horticultural Council, the Canadian Food Processors Association, Agriculture Canada, and Québec's Ministry of Agriculture. Fisheries, and Food for their financial support. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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(APBA 2.50E; Priva Computers, Vineland Station, Ont., Canada). Carbon dioxide was added during the photoperiod from a compressed CO, supply (Liquid Air Canada). Natural radiant energy was measured with a LI-COR radiometer with a quantum sensor (LI-185; LI-COR, Lincoln, Neb.). Lamps were turned on when natural light was lower than 250 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Supplemental lighting, supplied by 1000-W HPS lamps, provided 80 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at plant level for a photoperiod of 16 hr. Artificial lighting supplied between 15% and 25% of the total radiant energy received by the plants. Ambient temperature was maintained at 24C/18C day/night; when air temperatures were higher than 25C, forced-air ventilation was used. Plants were fertilized daily by irrigation (50 ml) with the nutrient solution recommended by Fisher and Benson (1983) and adapted to the specifications of Precheur and Maynard (1983).

At weekly intervals, plants were sampled and washed to remove soil particles. Leaf and root fresh and dry weights, leaf area, and total number of ferns and roots were obtained from 10 plants per experimental unit. Leaf area was measured with a LI-3000 portable area meter (LI-COR).

After 8 weeks of growth in the greenhouse, plants from the 18 previously described treatments were transferred to the field to observe the residual effects. The experimental plots were located at Agriculture Canada, Lavaltrie Experimental Farm, 50 km northeast of Montreal. Transplants and clones were planted on an upland sand of pH 6.1 and with an organic content of 2.5%. The experimental design was a randomized complete block with 20 plants per treatment replicated four times. Transplants and clones were compared to crowns of 'Lucullus mid-early'. Fertilization practices were as recommended by Agriculture Québec (Conseil des Productions Végétales du Québec, 1980). The number and height of the spears and survival of plants were measured after two seasons of growth in the field.

An analysis of variance was performed using a general linear model on SAS (SAS Institute, Inc., 1982) on the means of each experimental unit. Homogeneity of variances was previously verified using a Bartlett test (Anderson and McLean, 1974). Single degree-of-freedom contrasts were performed to determine the response of growth characteristics to CO_2 enrichment and supplemental light. Contrasts were declared nonsignificant if the probability of the relationship occurring by chance alone exceeded 5.0%.

Results and Discussion

Greenhouse experiments

Large differences in growth were observed between clones and transplants at all sampling dales, and the variances were heterogeneous, even if provision was made to place clones and transplants under treatment conditions when their size was comparable. Therefore, the effect of treatments on transplants and clones was compared separately.

After 52 days of growth, no interaction was found between cultivars, CE, and SL for fern and root dry weights and for leaf area. Moreover, there was no significant difference between cultivars for these characteristics. Thus, results for the two cultivars were pooled. Supplemental lighting at 80 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (PPF) significantly increased fern dry weight and root and crown dry weight accumulation (Table 1). Significant differences were observed as early as 22 days after the beginning of the treatments. These differences were maintained throughout the experiment. Supplemental lighting increased fern and root dry weight by 66% and 84%, respectively, at the end of the experiment. Enrichment with CO_2 at 1500 ppm gave the highest dry weight accumulation with increases relative to 330 ppm of 114% and 104% for fern and root dry weight, respectively, after 52 days of treatment. Increases in leaf area followed a similar trend. Since there was no replication on the main plots, no statistics were computed for this factor. After 52 days of treatment, the interaction between SL and CE was significant at $P = 0.07$ and a synergistic effect was observed (Fig. 1A). At that time, CE at 1500 ppm and SL resulted in the largest increase in fern and root dry weight (196% and 336%, respectively) over the control without CE and SL. For these treatments, a plant dry weight comparable to that of the control at the end of the experiment was obtained 3 weeks earlier. Asparagus transplants responded similarly as other C_3 plants (Porter and Grodzinski, 1985). Cladophylls of this species possess a C_3 metabolism (Downton and Törökfalvy, 1975) and elevated concentrations of CO_2 may increase net assimilation by reducing photorespiration. The presence of elevated partial pressures of CO_2 probably reduced the oxygenase activity of ribulose biphosphate carboxylase/oxygenase and increased proportionally the carboxylase activity, leading to increased carbon fixation.

Table 1. Growth of 'Lucullus mid-early' and 'Viking-2G' asparagus transplants maintained under three atmospheric CO_2 concentrations and two levels of light.[z]

	Dry wt(g)																	
	Ferns						Roots and crown						Leaf area (cm^2)					
	Days of treatment						Days of treatment						Days of treatment					
Treatments	14	22	31	39	46	52	14	22	31	39	46	52	14	22	31	39	46	52
Supplemental lighting ($\mu mol \cdot s^{-1} \cdot m^{-2}$)																		
None (ambient)	0.028	0.083	0.139	0.204	0.323	0.518	0.010	0.022	0.052	0.101	0.170	0.270	1.9	3.2	7.5	12.2	22.9	42.5
80	0.058	0.135	0.272	0.382	0.509	0.860	0.019	0.038	0.113	0.231	0.294	0.512	3.4	6.4	15.7	19.2	40.1	57.3
Significance																		
t test	NS	**	**	**	**	**	NS	**	**	**	**	**	NS	**	**	**	**	**
CO_2 concn (ppm)																		
330	0.026	0.074	0.145	0.196	0.271	0.449	0.012	0.030	0.055	0.106	0.158	0.281	1.7	3.1	6.7	9.3	19.1	31.9
900	0.033	0.099	0.172	0.276	0.356	0.654	0.011	0.022	0.066	0.137	0.181	0.330	2.0	3.8	11.8	12.7	30.7	53.1
1500	0.063	0.154	0.299	0.405	0.621	0.965	0.022	0.038	0.126	0.224	0.359	0.574	4.2	7.5	16.3	25.0	44.8	64.8
SE mean (±)	0.008	0.013	0.007	0.034	0.044	0.035	0.002	0.004	0.026	0.017	0.024	0.033	0.31	0.54	1.9	2.2	3.0	5.2

[z]Supplemental light was supplied with 1000-W HPS lamps for a photoperiod of 16 hr at a PPF of 80 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (PAR).
[NS,**]Nonsignificant or significant at $P = 0.01$ using a *t* test.

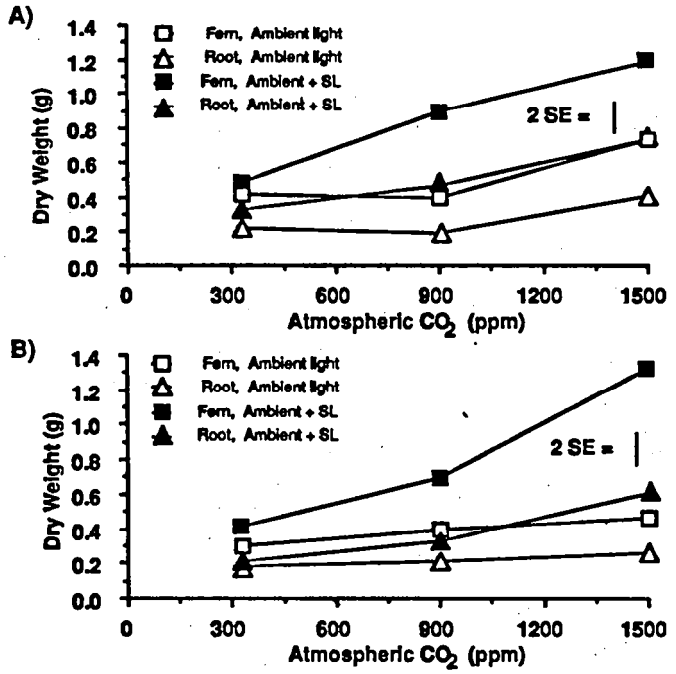

Fig. 1. Fern and root dry weight accumulation of asparagus **(A)** transplants and **(B)** clone G-171 after 52 and 42 days of growth, respectively, under CO_2 enrichment (330, 900, and 1500 ppm) and supplemental lighting [ambient, and ambient + 80 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (PAR)].

Clone G-171 also benefited from CE, and SL. After 22 days of treatment, SL significantly increased dry weight accumulation (Table 2). After 22 days, fern, root, and crown dry weight were increased by 44% and 18%, respectively. No interaction was observed between CO_2 and SL for these criteria. CE also increased most aspects of growth, with the 1500-ppm treatment being more effective than 330 and 900 ppm. After 42 days of treatment, a significant interaction was observed between CE and SL for fern *(P* = 0.017) and root and crown dry weight *(P* = 0.029) (Table 2). A distinct synergistic effect between CE and SL was observed at that time (Pig. 1B), since the increase in dry weight was higher when CE and SL were used in combination than the summation of the individual effects of CE and SL. Plant growth and quality were highest with CE at 1500 ppm and SL, increases in fern and root dry weights were 335% and 229%, respectively. Plants of in vitro origin responded as well to CE and SL; however, clones showed an even more intense synergetic response to CE and SL than did transplants (Fig. 1). A similar phenomenon was observed with tissuecultured strawberry (Desjardins et al., 1987) and tomato transplants (Hurd, 1968). In contrast to the effects observed for transplants, CE had very little effect on dry weights of nonlighted clones at the end of the experiment. It has been shown that in vitro-cultured sweetgum leaves have undifferentiated chloroplasts with thylakoid membranes not assembled in distinct grana (Wetzstein and Sommer, 1982). The absence of structured chloroplast mem-, branes explains the low photosynthetic activity observed for leaves in vitro. Light intensity is one of the most important environmental factors influencing leaf anatomy of leaves in vitro (Donnelly and Vidaver, 1984). These authors have shown that in vitro culture conditioned the anatomy of raspberry leaves and that epigenetic changes were carried over to the leaves developing ex vitro. Supplemental lighting of asparagus clones may hasten the development of adapted leaf structure and thus would provide a proper environment for CE to improve growth. Transplant leaves develop under greenhouse conditions that lead to a normal anatomy. Therefore, the leaves can respond more favorably to CE even in the absence of SL.

Field experiment

After 2 years of growth in the field, no interaction between CE and SL was observed for any of the measured characteristics: CE had no significant effect on plant survival, whatever their origin was; however, SL significantly improved transplant survival (Table 3). The population stand was higher for transplants than for clones with all treatments, but was slightly inferior to 1-year-old crowns. There was no difference in percent survival between cultivars of transplants.

CE did not improve the number of spears per plant for transplants or clones (Table 3). Moreover, SL had no effect on transplants but increased the number of spears of clones, by 31%, compared to the control. A similar response was observed for the plant height, where a significant increase of 49% was mea-

Table 2. Growth of asparagus clone G-171 maintained under three atmospheric CO_2 concentrations and two light levels.[z]

	Dry wt (g)								Leaf area (cm^2)			
	Ferns				Roots and crown							
	Days of treatment				Days of treatment				Days of treatment			
Treatments	15	22	29	42	15	22	29	42	15	22	29	42
Supplemental lighting ($\mu mol \cdot s^{-1} \cdot m^{-2}$)												
None (ambient)	**0.082**	**0.091**	**0.278**	**0.384**	**0.069**	**0.117**	**0.156**	**0.217**	**4.3**	**6.6**	**15.7**	**17**
80	**0.082**	**0.122**	**0.402**	**0.812**	**0.071**	**0.180**	**0.179**	**0.382**	**4.7**	**9.5**	**21.5**	**36**
Significance												
t test	NS	**	**	**	NS	**	NS	**	NS	**	NS	NS
Interaction CE × SL	NS	NS	NS	0.017	NS	NS	NS	0.029	NS	NS	NS	NS
CO_2 concn (ppm)												
330	**0.067**	**0.089**	**0.217**	**0.359**	**0.069**	**0.118**	**0.119**	**0.199**	**4.9**	**6.7**	**12.1**	**16.5**
900	**0.072**	**0.088**	**0.372**	**0.544**	**0.058**	**0.143**	**0.186**	**0.270**	**3.6**	**8.8**	**23.4**	**24.2**
1500	**0.107**	0.141	0.439	0.893	0.084	0.183	0.201	0.431	5.0	**8.6**	**21.6**	**40.3**
SE mean (±)	0.013	0.018	0.067	0.060	0.009	0.016	0.022	0.028	0.7	**0.9**	**4.7**	**3.6**

[z]Supplemental light was supplied with 1000-W HPS lamps for a photoperiod of 16 hr at a PPF of 80 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (PAR).
[NS,**]Nonsignificant or significant at *P* = 0.01 using a *t* test.

Table 3. Long-t& effects of CO_2 enrichment and supplemental lighting during transplant raising and clone acclimatization after two seasons of growth in the field.

Light ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	CO_2 (ppm)	No. of shoots/plant		Ht (cm)		Percent survival	
		Transplants	Clone	Transplants	Clone	Transplants	Clone
Ambient	330	8.18	4.43	1.13	0.58	80.5	60
	900	1.43	6.73	1.00	0.75	85.5	80
	1500	8.03	5.43	1.17	0.65	87.7	70
Ambient + 80 SL	330	7.4	6.36	1.16	0.93	94.4	73
	900	7.56	7.03	1.08	0.96	92.2	66
	1500	6.11	8.46	1.07	1.04	95	83
1-year-old crown Lucullus		9.13		1.57		100 ± 0	
Significance							
CE		NS	NS	NS	NS	NS	NS
SL		NS	0.02	NS	0.005	0.005	NS
CE × SL		NS	NS	NS	NS	NS	NS
Cultivars		0.03		0.0001		0.0001	
Lucullus vs. Viking		NS		NS		NS	
Transplant vs. clone		0.01		0.0001		0.0001	

sured with SL. On average, transplants produced more spears per plant and were taller than clones.

As a result of SL treatments, clones reached a size comparable to that of transplants. In comparison, in the absence of CE and SL, clones were much smaller and less vigorous. It appears that the increases in dry weight observed in the greenhouse led to improved growth in the field. Such a correlation between dry weight accumulation and yield has been reported by Ellison et al. (1960). Moreover, the adaptation of the leaves brought about by SL, as discussed earlier in the greenhouse experiment, may have led to improved photosynthetic ability in the field, thus explaining their improved response. Gas exchange and anatomical studies are needed to verify these hypotheses.

The optimization of growth conditions and, more specifically, the use of SL in the greenhouse appear to be essential to provide proper growing conditions during acclimatization of asparagus clones if one is to properly evaluate the potential for production of plant material originating from tissue culture.

In summary, CE and SL applied during raising of transplants and clone acclimatization in the greenhouse improved plant quality and reduced the nursery period. For transplants, increases in dry weight were observed with CE and SL. These effects did not contribute to improved yield components, such as number of shoots per plant or height of the plant. It seems that SL contributed significantly to an improvement in plant survival. The results presented here demonstrate the importance of SL for the acclimatization of asparagus clones. A significant synergism between CE and SL was observed and improvement in growth of the plantlets was obtained after 2 years of growth in the field. The treatments used during greenhouse acclimatization should be among the factors closely examined when comparing the productivity of plant material of in vitro or seed origin.

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J. AMER. SOC. HORT. SCI. 115:(3)368-374. 1990.

Mechanical Harvestability of Y-shaped and Pyramid-shaped 'Empire' and 'Delicious' Apple Trees

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Additional index words. *Malus domestica,* trunk recoil-impact shaking, fruit bruising, stem pulling, storage rot

Abstract. **Mature 'Empire' and 'Redchief Delicious' apple *trees (Malus domestica* Borkh.) trained to a Y-shaped trellis (Y/M.26) or trained as pyramid-shaped central leaders (CL/M.7) were mechanically harvested with the Cornell trunk recoil-impact shaker during 4 years. With 'Empire', fruit removal from the Y/M.26 trees (85% to 90%) was significantly less than from the CL/M.7 trees (95% to 97%). With 'Delicious' there were no differences in fruit removal (90% to 95%) between the two' tree forms in any year. When the catching pad was on the ground, fruit grade based on damage was only slightly better for the Y/M.26 trees than for the CL/M.7 trees. When the catching pad was raised up near the Y/M.26 canopy, fruit grade was significantly improved for the Y/M.26 trees and was better than the CL/M.7 trees. Fruit grade for both cultivars ranged from 83% to 94% Extra Fancy with 5% to 16% culls for the Y/M.26 trees and from 74% to 88% Extra Fancy and 11% to 21% culls for the CL/M.7 trees. Skin punctures, skin breaks, and number of large and small bruises were lower and the percentage of nondamaged fruit was higher with the Y/M.26 trees when the pads were close to the canopy than when the pads were on the ground. The CL/M.7 trees had higher levels of ail types of fruit damage than did the Y/M.26 trees. Damaged fruit from the CL/M.7 trees was mainly frm the top half of the tree, while fruit from lower-tier scaffold branches had low levels of damage. Mechanically harvested fruit from the Y/M.26 trees had lower incidences of fruit rot and flesh breakdown after a 6-month storage period than did fruit from the CL/M.7 trees. Stem pulling was high with both systems and averaged 60% for 'Delicious and 30% for 'Empire'. The advantage of the single plane Y-trellis system for mechanical harvesting appears to be that the catching pads can be placed close to the fruit, thereby reducing fruit damage.**

Considerable effort has been made in the study of mechanical harvesting of fresh fruits (Brown, 1980; Brown et al., 1983; O'Brien et al., 1983). In some fruit crops, especially those used in processing, harvest mechanization has become common, but with fresh-market fruits problems remain. The major obstacle to mechanical harvesting of apples is excessive fruit damage, particularly when fruit are intended for the fresh market. Most of the work with mechanical harvest of apples has focused on adapting the machines to existing tree forms, but the problem of fruit damage is too great with existing large trees. To reduce fruit damage, Tukey (1971) suggested the tree be molded to fit the machines.

Studies of the sources of bruising during the shake-catch mechanical harvest of apples have shown that about one-half of the damage occurs near the site of detachment and one-half arises when fruit falls through the tree and is caught (Millier et al., 1980, 1984; Pellerin et al.,. 1977). Damage from detachment and from fruit falling through the tree was reduced by using a recoil-impact shaker instead of the common inertial shakers (Millier et al., 1980, 1984; Pellerin et al., 1979, 1982). The recoil-impact shaking concept has recently been added to a complete over-the-row harvester (Miller and Peterson, 1989; Peterson et al., 1985). These authors have also shown a reduction in damage levels from impact shaking compared to inertial shaking.

Damage resulting from fruit falling through tree canopies has been shown to increase logarithmically with increasing distance above the catching surface in standard tree forms (Lakso et al., 1978). This damage could be reduced by improved tree canopy designs that limit the distance of fall or distribute the fruit more horizontally. Lakso et al. (1978) removed the central leader of standard-sized 'McIntosh' trees, leaving short, relatively flat open-center trees, and found a substantial reduction in mechanical damage during shake-catch operations. However, such trees had lower total production and excessive vegetative growth (Lasko et al., 1978). Miller and Peterson (1989) have developed modified central leader trees for mechanical harvest with an over-the-row machine that have had lower fruit damage levels than larger central leader trees.

Further modifications of tree canopy form that place the fruiting zone predictably could offer further reductions in fruit damage levels (O'Brien et al., 1983). The Tatura Trellis, which is an inclined planar canopy in a V form, was developed for me-

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