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Variety and Temperature Affect the Production of Multiple Stems from Peach Seedling Rootstocks

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Abstract

Seeds of different cultivars of peach rootstocks were assessed to determine the causes of several deleterious traits that affect the quality of the seedlings that result from them. The incidence of triple cotyledons was significantly influenced by variety. It was also found that that the production of multiple stems could be caused by either the production of multiple seeds or by multiple stems originating from cotyledonary axils. The incidence of both traits was heavily influenced by the genotype of the variety. The production of multiple shoots was also affected by temperature with low temperatures favouring their appearance. The incidence of double seeds also varied among cultivars with the average size of seeds from stones that contained double seeds being significantly smaller than for single seeds. The smaller size of double seeds was associated with poorer germination, reduced speed of germination and reduced growth rates. Although there was a strong relationship between seed size and stone (endocarp) size for stones that contained single seeds, no such relationship existed for those stones that contained double seeds. Therefore, selection on the basis of stone size cannot be used to select out stones containing double kernels. The elimination of multiple shoots and double seeds from peach seedlings would reduce the costs associated with rootstock production and could be achieved by removing plants that exhibit these traits from the production system and clonally propagating those trees that are free of such traits.

Zusammenfassung

Genotyp und Temperatur beeinflussen die Mehrstämmigkeit bei Pfirsichunterlagen. In dieser Untersuchung wurde Saatgut von verschiedenen Pfirsichunterlagen-Genotypen beurteilt, um die Ursachen für zahlreiche unerwünschte Merkmale, die die Qualität der aus ihnen hervorgehenden Sämlinge beeinflussen, zu ermitteln. Das Ausbilden von Dreifach-Keimblättern wurde signifikant von der Sorte beeinflusst. Es zeigte sich, dass die Bildung zahlreicher Triebe entweder direkt aus multiplen Samen (im Stein) oder über den Austrieb von Keimblattachselknospen erfolgte. Das Auftreten beider Merkmale wurde sehr stark vom Genotyp beeinflusst. Die multiple Sproßbildung wurde außerdem durch die Temperatur und insbesondere durch tiefe Temperaturen gefördert. Die Häufigkeit von Doppelsamen war bei den Sorten unterschiedlich, wobei die durchschnittliche Größe von Steinen mit zwei Samen signifikant geringer war als bei jenen mit einem Samen. Die geringere Größe der Doppelsamen war gekoppelt mit geringerer Keimrate, geringerer Keimgeschwindigkeit und geringerer Wachstumsrate. Außerdem gab es bei Steinen mit nur einem Samen einen engen Zusammenhang zwischen Samengröße und Stein- (Endokarp-)größe. Dieser Zusammenhang bestand bei Steinen mit zwei Samen nicht. Aus diesem Grund ist eine Selektion auf Basis der Steingröße kein Kriterium für Doppelsamen. Das Ausschalten von multiplen Sprossen und Doppelsamen würde die Kosten für die Produktion von Pfirsichunterlagen senken. Dies könnte durch Aussondern von Pflanzen mit unerwünschten Merkmalen aus dem Vermehrungszyklus in Kombination mit dem Klonen von merkmalsfreien Pflanzen geschehen.

Key words. *Prunus* – multiple shoots – triple cotyledons – seed quality – double seeds

Introduction

The majority of stone fruit rootstocks are produced from seed and the growth and quality of grafted scions is dependant on the quality of the rootstock. The quality of seedlings is determined by a number of factors. In peaches, it has been shown that seed size affects germination percentages, both the speed and the duration of germination as well as seedling growth rates (MALCOLM et al. 2003). As there is a strong correlation between stone (endocarp) size and seed size (MALCOLM et al. 2003), seedling uniformity and growth rates can be improved by nurserymen grading on the basis of either parameter.

In addition to vigour, there is a number of other features of growth that affect seedling quality. Seeds producing multiple shoots are a nuisance to nurserymen who produce rootstocks as additional labour is required to remove them. Sources of multiple shoot production can be due to the presence of multiple seeds within the

one stone (WOODROOF 1978; MONSELISE 1986) or to additional shoots developing in the axils of the cotyledons.

The incidence of multiple shoot production has been poorly studied. Therefore, we have assessed varietal influences on the production of multiple seeds from a single stone; the influence of multiple seeds on average seed size; the germination and growth characteristics of single and double seeds; and, the respective relationships between seed size and stone size in stones containing either single or double seeds. Also examined were the influence of temperature on the production of multiple stems and the production of triple cotyledons.

Materials and Methods

Plant materials

Stones from virus-tested trees of the peach rootstock cultivars, 'Okinawa' (100), 'Floraguard' (300), 'Green Leaf Nemaguard' (600), 'Red Leaf Nemaguard' (825), 'Fay Elberta' (900) and 'Golden Queen' (1100) were obtained from Ausbuds, Victoria. The values in parenthesis refer to the number of chill hours, as defined by WEINBERGER (1950), for flower bud break and were taken from OKIE (1998).

Determination of germination percentage and the incidence of double seeds and triple cotyledons

120 stones from six of the cultivars listed above were cracked, the seeds removed and the proportion of stones containing double seeds recorded. To stratify the seeds, they were placed in moist, autoclaved perlite in an incubator at 5 °C. The duration of stratification for each variety differed depending on their individual requirements and ranged from 1344 hours for 'Okinawa' and 'Floraguard' up to 2352 hours for 'Golden Queen' (M. Ryland, Ausbuds Ltd, Australia, personal communication). Stratification times for each variety were organised so that all seeds were removed from cool treatment and sown at the same time. The seeds were sown in 50 mm diameter pots containing Debco General Growing Mix (Debco Pty. Ltd. Berkshire Park, NSW) and Osmocote slow-release fertiliser and grown in growth cabinets (Thermoline, Sydney, Australia) at 23 °C with a 16 h photoperiod. Seed was assumed to have germinated when the epicotyl was more than 1 mm above the soil mix in each pot. The percentage of seeds that germinated over an eight-week period was recorded from which data for both the mean number of number of seedlings per seed and per stone were obtained. At the end of the experiment, the presence of double or triple cotyledons was recorded after carefully removing the soil from the base of the plants. The experiment was first conducted in 1999 and repeated in 2000.

Effect of seed size of double and single seeds on seedling growth

250 stones from the variety 'Okinawa' were cracked and the seeds removed. The weight of each individual seed and its surrounding endocarp was determined and the proportion of stones containing double seeds recorded. Seeds were separated into two groups on the basis of whether they originated from stones containing a single seed or double seeds. Based on their weight, the seeds from each group were divided into four quartiles with the first quartile containing the lightest seeds and the fourth quartile the heaviest. To stratify the seeds, each quartile was placed in moist, autoclaved perlite in an incubator at 5 °C for 1344 hours. Once stratified, the seeds were sown in 50 mm diameter pots containing Debco General Growing Mix (Debco Pty. Ltd. Berkshire Park, NSW) and Osmocote slow-release fertiliser and grown in growth cabinets (Thermoline, Sydney, Australia) at 23 °C with a 16 h photoperiod. The percentage of seeds germinating over an eight-week period was recorded from which the times for 20, 50 and 80 % of the seeds to germinate were estimated by regression analysis. The height of each seedling was measured at weekly intervals for the eight-week duration of the experiment. The experiment was first conducted in 1999 and repeated in 2000.

Effect of temperature of the development of multiple stems

150 stones from each of the six cultivars were individually cracked and the seeds stratified as above. After stratification, the seeds of each variety were placed in 50 mm pots containing Debco General Growing Mix then randomly divided into 5 aliquots, each totalling 30 seeds. Four of the aliquots from each cultivar were placed in Thermoline growth cabinets at 10, 15, 20 or 25 °C with a 13 h photoperiod. The fifth aliquot of seeds was placed in a shadehouse and the plants grown at ambient conditions. At weekly intervals the number of plants producing multiple stems was recorded. The experiment was repeated three times.

Statistical analysis

Regression analyses and analyses of variance were performed using either TableCurve 2D version 4.0 (Jandel Scientific) or STATISTICA for Windows (StatSoft Inc.). Where data were not homoscedastic according to Bartlett's test, logarithmic transformations were performed. Significant differences between means were determined using Tukey's Honest Significant Difference Test. A contingency chi-square test was performed on germination data to determine homogeneity, with 95 % confidence intervals calculated according to SNEE (1974).

Results and Discussion

There was considerable variation among the rootstock cultivars in the percentage of stones containing double seeds (Table 1). The cultivars, 'Fay Elberta' and 'Green Leaf Nemaguard', only produced stones with single seeds. The cvs., 'Flordaguard', 'Golden Queen' and 'Red Leaf Nemaguard' produced stones, approximately 1–2 % of which had double seeds. In contrast, over 44 % of stones from 'Okinawa' contained double seeds. In this cv., because of the high incidence of double seeds and the large proportion of those double seeds germinating, on a per stone basis, there was a signifi-

Table 1. Percentage of stones containing double seeds and percentage of seedlings producing either multiple shoots or triple cotyledons. Data are combined averages from the 1999 and 2000 seasons with at least 240 seeds or seedlings assessed per cultivar.

Cultivar	Double Seeds (%)	Mean Seedlings per seed	Mean Seedlings per stone	Triple Cotyledons (%)
'Fay Elberta'	0	0.537	0.537	0
'Floraguard'	1.8	0.704	0.708	0
'Golden Queen'	0.8	0.700	0.700	19.9
'Green Leaf Nemaguard'	0	0.840	0.840	0
'Okinawa'	44.3	0.700	1.04	0
'Red Leaf Nemaguard'	2.2	0.385	0.385	0.5
Mean	8.2	0.644	0.701	3.4

Table 2. Size classes and percentage germination of each of the four quartiles of 'Okinawa' seeds (1st = lightest, 4th = heaviest) originating from stones containing single or double seeds. The data from trials held in 1999 and 2000 were combined. At least 22 seeds were assessed per quartile. For both single (S) and double (D) seeds, differences between percentage germination values were determined from 95 % confidence intervals (lower case letters). Differences between average values (capital letters) were determined using Tukey's HSD test.

		1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	Average
Size classes	**S**	0.12–0.19	0.20–0.24	0.25–0.27	0.28–0.34	0.23
	D	0.09–0.14	0.15–0.17	0.18–0.20	0.21–0.26	0.17
% Germination per seed	**S**	22.7^{a}	90.9^{c}	90.9^{c}	100^{c}	76.1^{A}
	D	11.8^{a}	47.1^{b}	97.1^{c}	100^{c}	64.0^{A}
% Germination per stone	**S**	22.7	90.9	90.9	100	76.1
	D	23.6	94.2	194.2	200	128.0
T_{50}	**S**	11.8	6.9	6.2	6.2	7.8^{A}
	D	14.2	9.0	8.5	7.0	9.7^{B}
T_{80}–T_{20}	**S**	15.1	8.7	5.9	5.3	8.8^{A}
	D	5.8	12.6	6.7	6.9	8.1^{A}
Average by quartile						
% Germ per seed		17.3	69.0	94.0	100.0	
% Germ per stone		23.2	92.6	142.6	150.0	
T_{50}		13.0^{A}	8.0^{B}	7.4^{B}	6.6^{B}	
T_{80}–T_{20}		10.4^{A}	10.7^{A}	6.3^{A}	6.1^{A}	

cantly higher number of seedlings per stone compared with the other varieties.

Presumably, the double seeds originated through fertilization of both ovules and the failure of one to abort, as has been found in almonds (DICENTA et al. 1993). There has been only one report of double seeds in peaches and nectarines. PATTEN et al. (1989) found that drought stress increased the number of double ovaries and double fruit in the peach cvs., 'Harvester' and 'Loring'. In almond, the trait is affected by temperature with low temperatures favouring its appearance (GRASSELLY 1972, cited in DICENTA et al. (1993); SPIEGEL-ROY and KOCHBA 1974). Low temperatures may also explain the high incidence of double seeds in the cv. 'Okinawa'. Because of its low chilling requirement (<100 hours; OKIE 1998), under Australian conditions it flowers considerably earlier than other varieties of peach rootstocks, usually in midwinter when temperatures are low.

'Okinawa' seeds from stones containing single seeds were on average 35 % larger than seeds from stones containing double seeds (Table 2). For seeds in the heaviest two quartiles, whether a seed had come from a stone containing single or double seeds had little effect on the germination percentage nor the speed (T_{50}) of germination (Table 2). However, seeds originating from stones containing double seeds in the lightest two quartiles had low percentage germination and took longer to germinate than larger seeds. There were no significant differences between quartiles nor between single and double seeds in the duration ($T_{80–20}$) of germination.

An association between seed size and growth for both single and double seeds was found in the trials held in both 1999 and 2000; the data from the trial held in 1999 are shown in Fig. 2. When plant height at the end of the trial was regressed against the average seed weight of each of the quartiles a quadratic relationship was found that can be described by the equation: height = $397.3 + 192.3 \log_e$ seed weight ($r^2 = 0.97$; $p < 0.001$) (Fig. 3). For the single seeds, analysis of variance showed that the growth of the heaviest (3rd and 4th) quartiles was similar with the plants reaching ~150 mm after eight weeks, whilst growth of the 1st and 2nd quartiles was ~50 and 80 % that of the heaviest quartiles, respectively. For the double seeds, the height of the heaviest quartile was similar to that of the 2nd quartile of the single seeds, whilst the growth of the other three quartiles was close to that of the lowest quartile (lowest seed weight) of the single seeds.

This study has shown that the production of double seeds resulted in their individual seed weights being low and, as found in previous trials (MALCOLM et al. 2003), low seed mass was associated with reduced seed

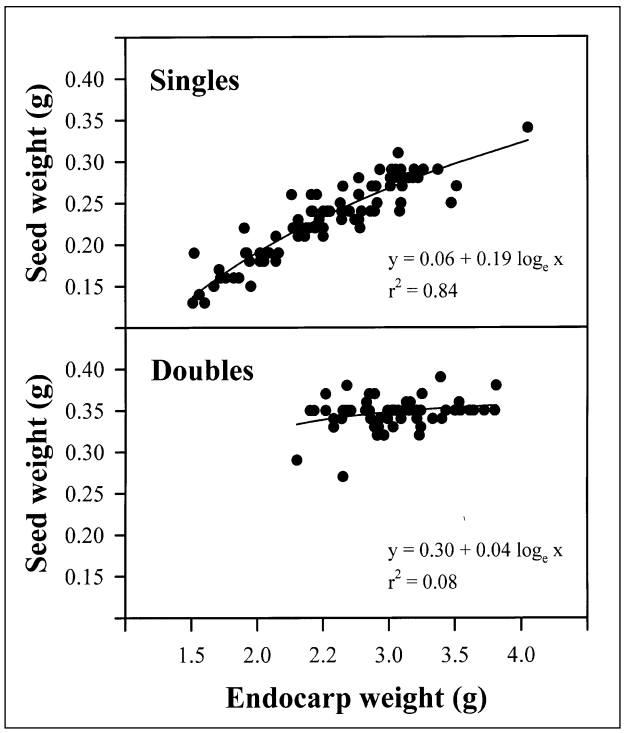


Fig. 1. Relationship between endocarp and seed weight for the cultivar, 'Okinawa', for stones containing single and double seeds. The weights for the double seeds are the combined mass of the two twinned seeds. The data are combined from the 1999 and 2000 trials.

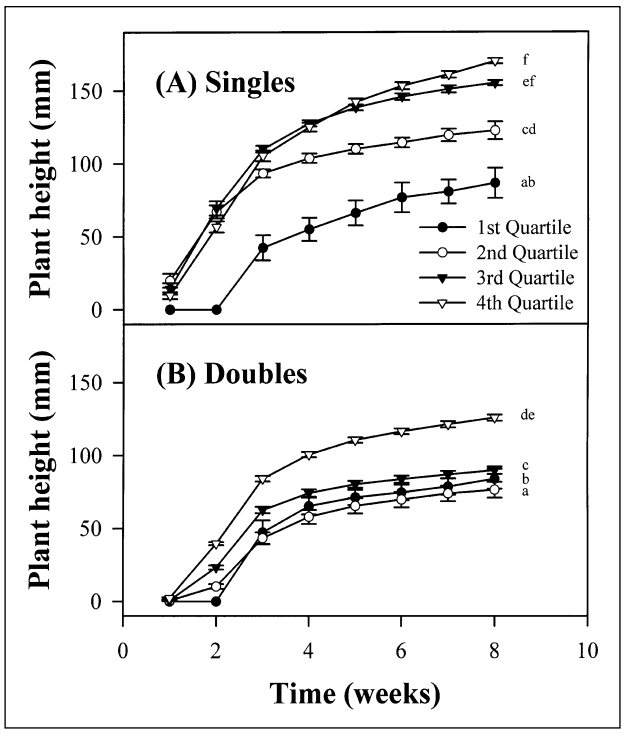


Fig. 2. Weekly height (n≥22) of seedlings from the four quartiles of seeds (1st = lightest, 4th = heaviest) of the rootstock cultivar, 'Okinawa', for seeds taken from stones containing (A) single or (B) double seeds. The data were obtained from the trial held in 1999. Letters indicate significant differences at the 5% level according to Tukey's HSD test between data obtained at eight weeks.

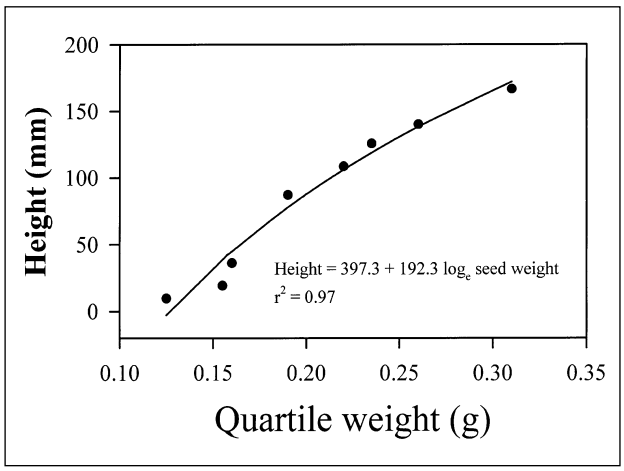


Fig. 3. The relationship between average weight of each quartile of seeds and plant height. At least 22 seeds were assessed per quartile.

quality and seedling growth rates, particularly in the first few weeks following germination. In almonds, the production of double seeds is polygenically inherited, and there appears to be dominance towards double seeds (SPIEGEL-ROY and KOCHBA 1981; DICENTA et al. 1993). The trait also has high heritability (h^2 0.5 ± 0.3) (KESTER et al. 1977). Presumably peach rootstocks are similar to almonds in this regard. As double seeds in peach rootstocks tend to be small and of poor quality, it would be advantageous to breed this trait out of lines used for seed production. Also, the high heritability would explain the variation in this trait seen amongst the varieties used in this study and would assist in the breeding process to eliminate those stones that contain double seeds.

For single seeds of the cv., 'Okinawa', there was a positive correlation between endocarp and seed weight for all the stones used in the 1999 and 2000 trials (Fig. 1A). The relationship between endocarp and seed weight for these seeds can be described by the equation: seed weight = 0.06 + 0.19 $\log_e$ stone weight (r^2 = 0.84; $p < 0.0001$). Thus, there was a steep rise in seed weight as the endocarp (stone) increased. A previous study (MALCOLM et al. 2003) with peach seeds has shown similar results. As concluded in this study, the positive correlation between endocarp and seed weight would allow size selection to be practiced on the basis of either stone or seed weight to improve germination and seedling growth. However in these trials with 'Okinawa', for stones containing double seeds, there was no simple relationship between seed size and the size of the stone (endocarp) and so selection based on stone size could not be used to either improve germination or remove double seeds.

All cultivars produced a number of seedlings that developed multiple shoots with the additional shoots developing from meristems in the axils of the cotyledons (Table 3 and Fig. 4). Both temperature and cultivar had a significant effect on the appearance of multiple shoots. Those seedlings germinated and grown at lower temperatures had more multiple shoots that those pro-

Table 3. Effect of temperature on the number of seedlings of five different peach and nectarine rootstock cultivars developing multiple shoots. Thirty seeds were assessed for each combination of rootstock and temperature and the experiment was repeated three times. Different letters following the means indicate significant differences at the p=0.05 level.

Cultivar	Temperature					Average by cultivar
	10 °C	15 °C	20 °C	25 °C	Ambient	
'Okinawa'	0	3.3	2.2	0	2.2	1.5[a]
'Fay Elberta'	6.7	5.6	2.2	3.3	5.6	4.7[b]
'Red Leaf Nemaguard'	10.0	6.7	5.6	3.3	3.3	5.8[b]
'Green Leaf Nemaguard'	11.1	8.9	5.6	6.7	4.5	7.3[bc]
'Floraguard'	13.4	11.1	10.0	7.8	8.8	10.0[cd]
'Golden Queen'	14.5	17.8	13.4	7.7	6.7	12.0[d]
Average by temperature	9.3[ab]	8.9[a]	6.5[bc]	4.8[c]	5.0[c]	

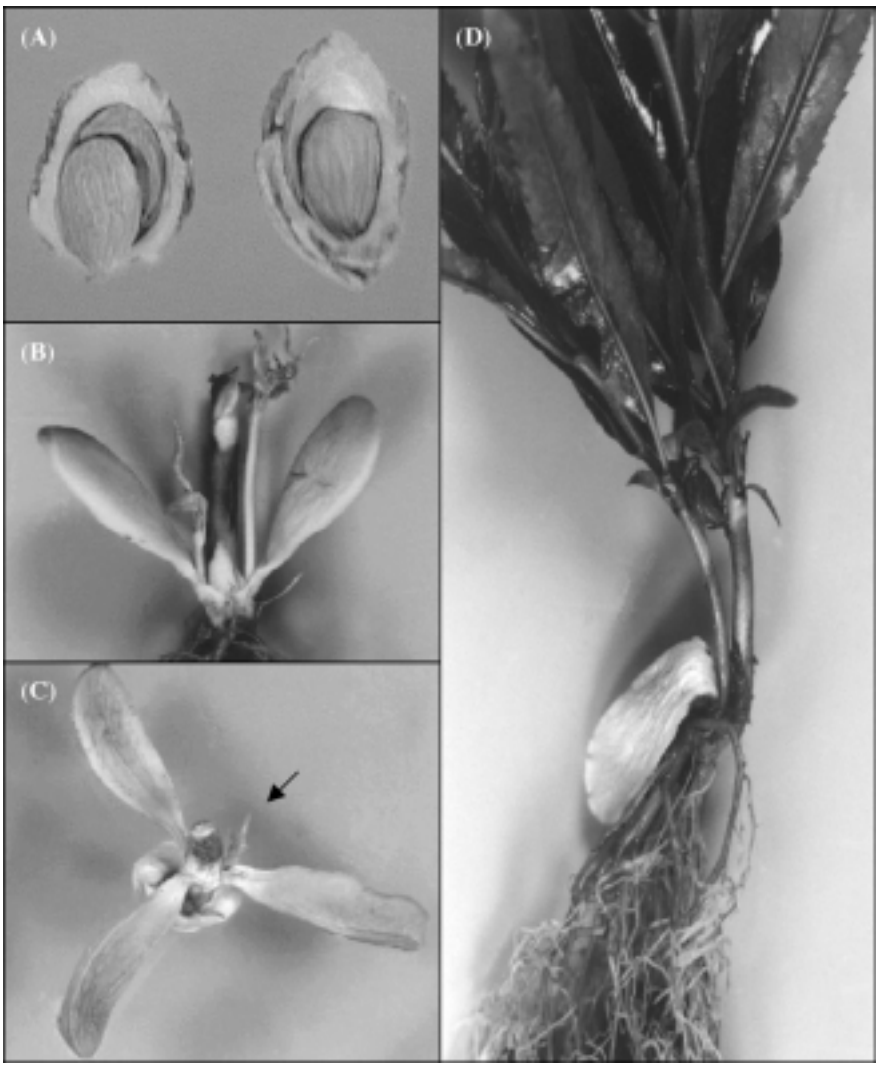


Fig. 4. The development of double (left) and single (right) seeds within the stone (Panel A) and multiple stems from both cotyledonary axes of 'Golden Queen' (main stem removed) (Panel B) and from one cotyledonary axil of 'Red Leaf Nemaguard' (Panel D). Panel C shows the production of both triple cotyledons and an axillary shoot (arrowed) in the cultivar, 'Golden Queen'.

duced at higher temperatures, while the varieties, 'Golden Queen' and 'Floraguard', were more inclined to produce multiple shoots. As 'Golden Queen' (1100 stones) and 'Floraguard' (300 stones) have very different chilling requirements, there appears to be no correlation between chilling requirements and the production of multiple shoots.

The production of multiple shoots may be due to seed damage (ALLARD 1953) possibly induced in susceptible varieties by cold temperatures. Alternatively, it could be due to the relaxation of apical dominance. Many plants in the field have reduced apical dominance at low temperatures (ROSA 1986; MOE 1988; HUCL and BAKER 1990: FAUST and HEINS 1996). The manipulation of temperature to induce multiple shoots could possibly have an application in the mass propagation of clonal rootstocks and could be used to increase shoot production in both field and tissue culture systems. However, *in vitro* studies conducted with honeysuckle (KARHU 1997) and *Hancornia speciosa* (DE PEREIRA-NETTO and MCCOWAN 1999) have shown an increase in shoot production at higher temperatures

and it will be necessary to determine if this contradiction is related to species or is a function of the unusual conditions experienced by plants *in vitro*.

The propensity of the different rootstock cultivars to produce multiple shoots requires further study. Sucker production from the rootstocks of established trees can be a nuisance to both nurserymen and orchardists alike. In the nursery, the production of multiple shoots is a considerable nuisance, as the plants must be desuckered before budding. Often these same plants will continue to produce suckers after budding that also require removal. Certain combinations of rootstocks and scions are prone to producing suckers in the orchard that requires their removal year after year. It would be useful to know if there is any correlation between multiple shoot formation and suckering, as this would help the selection and development of new rootstocks.

In addition to the production of multiple shoots, two varieties also produced seedlings that had triple cotyledons with 19 % of the seedlings from 'Golden Queen' and 0.5 % for 'Red Leaf Nemaguard' showing this trait (Table 1). After stratification, since the seeds were all germinated under the same conditions it is likely that these cvs. may contain a mutation in the genes controlling cotyledon development. The mutation does not seem to have any pleiotropic effect, as the development of the seedlings, including leaf growth, appears to be normal. However, it would be interesting to see if features of adult trees or scions grafted on to them are affected.

This study has shown that several factors affecting the quality of peach seedling rootstocks are controlled by the plant's genotype. The production of triple cotyledons was only observed in the cvs., 'Golden Queen' and 'Red Leaf Nemaguard'. Similarly, the incidence of multiple stems and double seeds varied with cultivar. The elimination of these traits from each of the cultivars would reduce the costs associated with the production of rootstock seedlings. This could be simply achieved by removing plants that exhibit them from the production system and clonally propagating those trees that are free of any deleterious trait.

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