4. Johnson, L. A. and D. E. Carroll, Jr. 1973. Organic acid and sugar contents of Scuppernong grapes during ripening. *J. Food Sci.* 38: 21-29.
5. Kushman, L. J., and W. E. Ballinger, 1963. Influence of season and harvest interval upon quality of 'Wolcott' blueberries in eastern North Carolina. *Proc. Amer. Soc. Hort. Sci.* 83:395-405.
6. _____ and _____. 1975. Relation of quality indices of individual blueberries to photoelectric measurement of anthocyanin content. *J. Amer. Soc. Hort. Sci.* 100:561-564.
7. _____, and _____. 1975. Effect of season, location, cultivar, and fruit size upon quality of light-sorted blueberries. *J. Amer. Soc. Hort. Sci.* 100:564-569.
8. McClure, W. F., R. P. Rohrbach, L. J. Kushman, and W. E. Ballinger. 1975. Design of a high-speed fibre-optic sorter. *Trans. Amer. Soc. Agr. Engr.* 18:487-490.
9. Norris, K. H., and W. L. Butler. July 1961. Techniques for obtaining absorption spectra on intact biological samples. *I.R.E. Trans. Bio-Med. Electronics BME* 8:153-157.
10. McClure, W. F., R. P. Rohrbach, W. E. Ballinger, and L. J. Kushman. 1977. Rapid measurement of the quality and composition of fresh foods. *Proc. 1st Intern. Congress on Engineering & Food.* Boston, Mass. August 9-13, 1976 (in press)

J. Amer. Soc. Hort. Sci. 103(5):634–638. 1978.

Growth Controlling Properties of Apple Stem Callus, *in Vitro*[1]

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Additional index words. Malus domestica, rootstocks, tree dwarfing

Abstract. **Stem calli of various clones of apple (*Malus domestica* Borkh.) separated by filter paper, were grown on defined media to assess effects of one clone on growth of another *in vitro*. Certain clones affected the growth of the other when grown in the stock (callus in contact with media) or scion (on filter paper on stock callus) positions, or when placed on the media adjacent to each other (separated by filter paper). Both findings indicate the presence of mobile compounds from one clone capable of affecting growth of another clone.**

No generally accepted theory adequately explains the mechanism by which stocks dwarf scions that accounts for most, or all, of the known effects, especially those of interstocks. Beakbane (3), suggested competition for food and differential transport of water or ions were important as dwarfing mechanisms but they are no longer considered satisfactory explanations for dwarfing. The fact that dwarfing rootstocks have a higher proportion of bark than vigorous rootstocks (2, 4, 18) may be important. However, the dwarf rootstock or interstock only increases the bark percentage of the scion adjacent to the dwarfing stock tissue (12).

Both growth promoters and inhibitors have been found in apple tissues (8, 13, 16). They include gibberellins (9, 11, 22), cytokinins (10), auxins (8), abscisic acid (21, 22), p-coumaric acid and phloridzin (13, 14). Thus, the kinds and levels of growth substances found in apple tissue are adequate for growth control by stocks and interstocks to involve promoters, inhibitors or both.

Messer and Lavee (15) have shown that callus of the rather vigorous rootstock Malling Merton (MM) 111 grew more rapidly *in vitro* than the callus of the more dwarfing stock Malling (M) 9, indicating that tissue culture has potential for studying growth and its control in apple. Fuji and Nito (6) used tissue culture to study specific and interspecific graft compatibility. They did not use the technique to study the mechanism of dwarfing even though their illustrations show marked effect of one kind of callus on growth of another.

This report is on the use of tissue culture to study the effect of apple stem callus on growth of callus of other apple cultivars. The test calli were grown in stock-scion positions or adjacent to each other and on the complete nutrient medium *in vitro*. Relationship of these findings to the mechanism of stock-scion growth control is discussed.

Materials and Methods

Callus was derived from portions of shoots less than 1-year-old from apple trees free of known virus. Callus of the following commercial cultivars and rootstocks was used: 'Golden Delicious,' 'Jonathan,' 'Starkrimson,' 'Antonovka,' M 7, M 9, M 26, MM 106 and MM 111. The callus was grown on Fox's medium (5) in a growth room maintained at 26-28°C under 16 hr of light from daylight fluorescent lamps at an average of 50 μeinsteins m^{-2} sec^{-1}. In each experiment, a uniform volume of callus was used to inoculate Petri dishes which were placed in plastic bags to reduce moisture loss from the medium during the growth period of approximately 7 weeks. Fresh wt of tissue was recorded at the end of each experiment and is reported herein.

Two types of experiments were conducted; the first consisted of placing callus of one cultivar on the medium (stock position) with callus of a second cultivar placed on the first (scion position) with sterile filter paper placed between them (Fig. 1). In the other type experiment, the 2 inoculation calli were placed side-by-side on the medium separated by filter paper (Fig. 1).

In most experiments there were 3 different callus pairs per dish with each dish being a replicate. All experiments had sufficient replications for at least 10 observations per treatment. However, in the experiment reported in Fig. 2 there was one side-by-side grouping per Petri dish to compare the growth of callus of 5 different clones beside each other in all combinations. Each of the 5 replicates, which were in a randomized complete block design, consisted of 25 Petri dishes. They consisted of 5 dishes in which the 2 callus tissues were from the

[1]Received for publication March 20, 1978. The investigation reported in this paper (78-10-32) is in connection with a project of Kentucky Agriculture Experiment Station and is published by permission of the Director.
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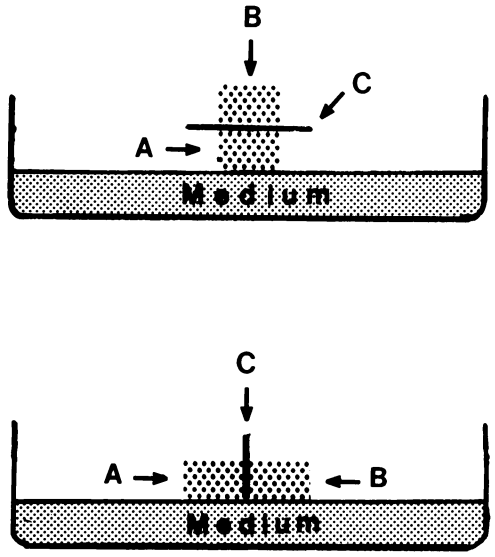

Fig. 1. Orientation of calli in experiments, (top) stock-scion position experiments; A = stock-position callus, B = scion-position callus, C = filter paper. (bottom) adjacent position experiments A, B = apple calli, C = filter paper.

same clone (one for each of the 5 clones) and 2 dishes containing each of 10 possible pairs of clones. Since this experiment had a nested error structure with 2 error variance components, i.e., among dishes and within dishes, the method of Fuller and Battese (7) was used in the statistical analysis of the data. Two models were tested. One consisted of treatment means; the other a 2-factor model of differences in average growth of clones (direct effect), differences in average effects on growth of adjacent clones (indirect effect) and interaction of direct and indirect effects. Since in this experiment the variance of a difference between treatment means was not the same for all treatment comparisons, Student's t-test rather than Duncan's multiple range test was used to test significance of pairwise comparisons.

Results

Studies with calli in the stock-scion positions. Apple scions on MM 111 rootstocks usually grow more rapidly than those on M 9. 'Golden Delicious' callus wt was greater when grown in the scion position over MM 111 callus than over M 9 (Table 1). The same general effect was evident in comparisons of growth of M 9, MM 106 and MM 111 callus as the scion over M 9 or MM 111 (Table 1). Thus, in these tests, apple callus wt in the scion position *in vitro* was as would be expected on the basis of field performance of the same cultivar combinations. It is noteworthy that 'Golden Delicious' callus wt was less when grown on itself than when on MM 111, and M 9 grew less on 'Golden Delicious' than on either itself or MM 111 (Table 1). In 2 of 3 experiments, M 9 weighed more on MM 111 than when grown on itself, but the stimulatory effect of MM 111 was not present when MM 106 was the scion callus though that cultivar did weigh more over MM 111 than over M 9. Stimulation of scion growth by vigorous interstocks and rootstocks under field conditions has been reported (17). Some scion calli grew equally well on 2 stock calli (Table 1). The data are not particularly surprising, except when one considers that all calli in the scion position was undifferentiated and had no vascular system or cellular connection or immediate contact with the stock callus.

'Golden Delicious' callus wt grown as the stock was equal under 'Golden Delicious' and M 9 callus, but M 9 callus in the stock position weighed less when grown under itself than when grown under 'Golden Delicious' or MM 111 (Table 2). MM 111 grown in the stock position weighed more under M 9 callus than under MM 111 or MM 106. It also weighed more under M 9 than under 'Golden Delicious' in one experiment with a similar trend in a second experiment (Table 2). MM 106 grew slightly more under itself than under MM 111. Thus, the data in Table 2 show that in at least some combinations, callus in the scion position affected growth of callus in the stock position as has been reported under field conditions (17).

Table 3 gives data on callus wt of different cultivars when grown on callus of a given clone in the stock position. Callus of 2 sources of 'Golden Delicious' and 'Starkspur' in the scion position on M 9 or MM 106 show no significant differences in fresh wt but MM 106 scion callus grew more than M 9 which, in turn, grew more than MM 111 when MM 111 callus was in the stock position (Table 3).

Studies with calli adjacent to each other on the media. The following data are from adjacent calli of 2 cultivars each grown on the media to eliminate nutrient availability as a factor in observed response. MM 106 grew the same amount when positioned beside either 'Starkrimson' or MM 106. In a second experiment when grown beside 'Golden Delicious', callus wt was more than when grown beside MM 106 which in turn was more than when grown beside 'Starkspur' (Table 4). In these tests, weights of M 9 grown beside M 9, 'Golden Delicious' and 'Starkspur' were similar as were weights of MM 111 when grown beside M 9, MM 106, MM 111, 'Golden Delicious' and 'Starkspur' (Table 4).

MM 106 callus wt was greater when grown beside MM 106 than was 'Golden Delicious', 'Starkspur', or 'Starkrimson' (Table 5). 'Golden Delicious', M 9 and 'Starkspur' grew similarly beside M 9 as did 'Golden Delicious', MM 111 and 'Starkspur' beside MM 111 (Table 5). However, MM 106 callus wt grown beside MM 111 was more than M 9 or MM 111. Thus, in these tests of effect of one kind of callus on growth of callus adjacent to it, a particular clone may or may not affect growth of callus of the other clone *in vitro*.

Callus wt of 5 cultivars (M 7, M 26, 'Jonathan,' 'Antonovka,' and 'Golden Delicious') was obtained using callus grown adjacent to each other in all combinations. Fig. 2-I is a comparison of callus wt of each clone grown beside a common clone and 2-II is a comparison of callus wt of a given clone grown beside each of the clones. When grown beside M 26 'Jonathan' grew more than any clone other than M 7, and M 7 also grew more than M 26 or 'Antonovka' (Fig. 2-I). 'Golden Delicious' callus grown beside 'Jonathan' weighed more than did M 26, with growth of the other 3 clones beside 'Jonathan' being intermediate. M 7 grew more beside 'Antonovka' than did any of the other clones when grown beside 'Antonovka'. There was no significant difference in callus wt of the 5 clones when grown beside M 7. 'Jonathan' and M 7 grew more beside 'Golden Delicious' than did the other clones (Fig. 2-I).

A given clone's callus wt grown beside each of the 5 clones (Fig. 2-II) show that M 26 and 'Antonovka' grew about the same beside each of the 5 cultivars in the test. 'Jonathan' callus wt grown beside M 26 and 'Golden Delicious' was greater than when grown beside the other clones (Fig. 2-II). M 7 grew more beside 'Golden Delicious' than beside 'Jonathan' or itself and an intermediate amount beside M 26 and 'Antonovka'. 'Golden Delicious' callus weighed more when grown beside 'Jonathan' than when grown beside 'Antonovka' or M 7 grew more beside 'Antonovka' than did any of the other clones when grown beside 'Antonovka'. There was no significant difference in callus wt of the 5 clones when grown vars, (Fig. 2-II) but the other clones callus wt was among the lowest when grown beside M 7 (Fig. 2-I). Average weight of all clones grown beside 'Golden Delicious' was greater than their average beside any other clone except M 26 (Fig. 2-I),

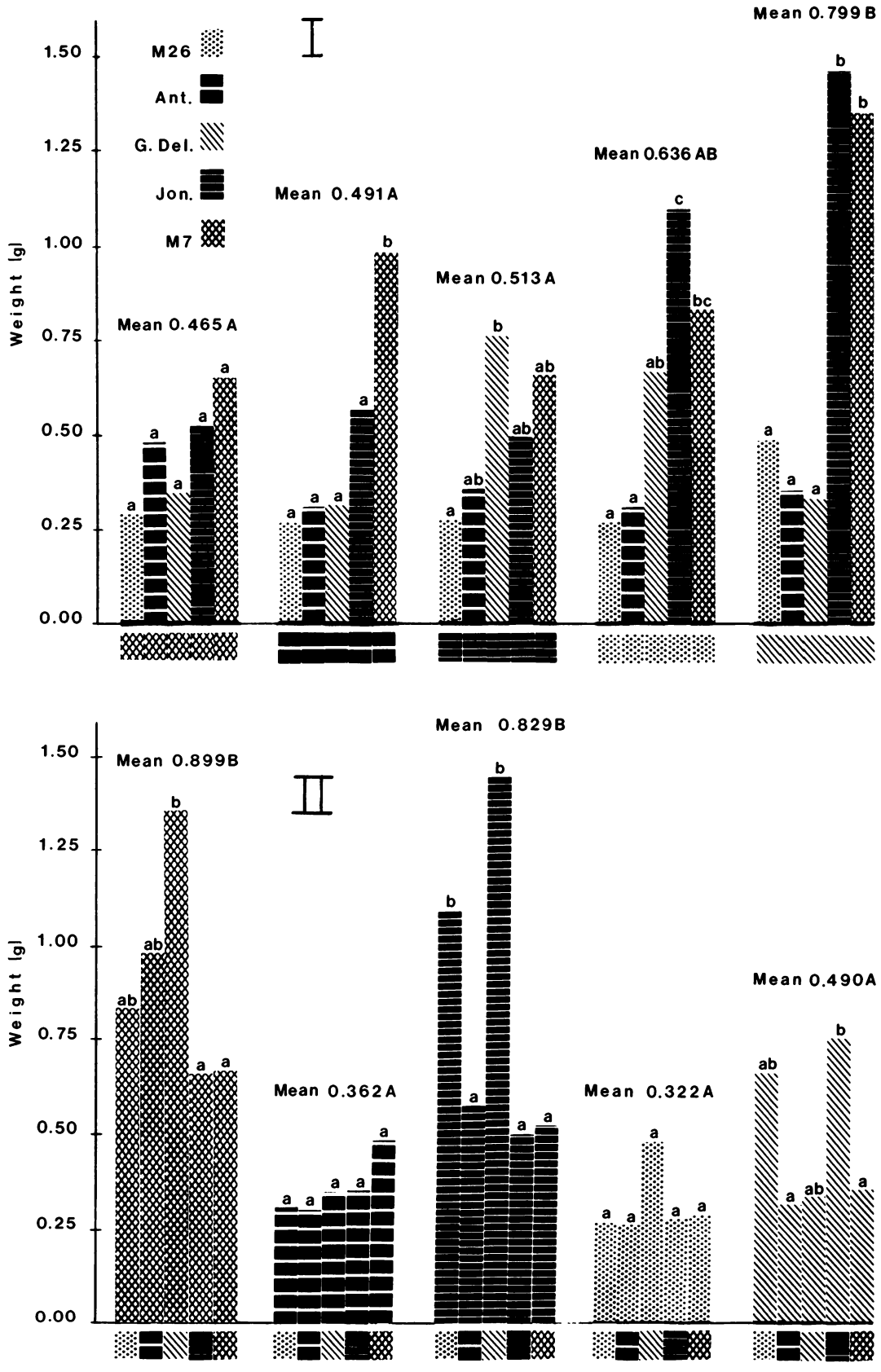

Fig. 2. Callus wt of various apple clones grown beside each other in all combinations. (I) Weight of each of 5 clones (group of verticle bars in graph) when grown beside a given clone (see symbol bars below graph). (II) Weight of a given clone (group of vertical bars in graph) when grown beside each of the 5 clones (see symbol below graph under each bar). Lower case letters (a, b) denote significant differences in wt within each bar group in the graph (Student t-test). Upper case (A, B) denote significant differences in mean wt of clones grown beside clone identified below part I, and of bar groups in part II (Student t-test).

Table 1. Weight of callus tissue of various apple clones grown in the scion position over callus of various clones in the stock position, *in vitro*.

	Scion wt (mg)				
	Stock clone				No. of
Scion clone	M 9	MM 111	Golden Del.	MM 106	expt.
Golden Del.	88 a[z]	106 b	83 a		2
Golden Del.	76 a	99 b			3
M 9	82 b	103 b	52 a		1
M 9	105 a	183 b			2
MM 106		239 a		253 a	2
MM 106	160 a	226 b		263 c	1
MM 111	81 a	107 b			1

[z]Mean separation within rows by Duncan's multiple range test, 5% level.

yet 'Golden Delicious' grew less beside the other clones than did 'Jonathan' and M 7 (Fig. 2-II).

Weight of one clone adjacent to another was significantly different from the adjacent clone when the following were grown together: 'Golden Delicious' beside 'Jonathan,' 'Golden Delicious' beside M 7, 'Jonathan' beside M 26, and M 7 beside M 26. Growth of the 2 clones was not significantly different in the 6 other possible combinations (Fig. 2).

Discussion

These data indicate that stock-scion growth effects in the field could occur even when physical aspects of the graft union are not a factor. Apparently stock-scion growth effects can also occur in the presence of an adequate mineral supply for callus of 1 clone affected growth of another when each was in contact with a common nutrient source. The data also indicate organized structures (root, stem, leaf) are not essential for field stock-scion growth effects which occurred in their absence *in vitro*.

These data (especially Table 1 and Fig. 2) indicate stimulation of growth of callus of one clone by callus of another clone (M 9 grew more on MM 111 than on M 9). Similar growth stimulation has been observed under field conditions (17). Growth responses of a scion cultivar in the orchard may be specific for particular stock-scion combinations (19, 20). This is also true for callus growth (M 7 and 'Jonathan' grew comparably beside 'Golden Delicious' but M 7 grew better than 'Jonathan' beside Antonovka' as shown in Fig. 2-I). This indicates the possibility that one or more specific compound(s) from one clone may promote the growth of another clone. These data do not demonstrate it, but it is reasonable to expect similar phenomena with growth inhibitors. Specific compounds produced by one clone that affect growth of other clones could be: (a) inhibitory compounds, (b) compounds that either enhance breakdown or change endogenous growth substances which influence growth of adjacent tissue or (c) a compound which promotes growth of only those clones that do not have a mechanism to inhibit its action. This concept is supportive of Tubbs' (19) comment on the need to understand the effects of mutual interactions of rootstock and scion cultivars.

Another important aspect of these findings, in relation to the mechanism of stock dwarfing, is the mobility of the compound(s) involved. They apparently passively diffuse through filter paper or agar. This is not in accord with Ball's (1) suggestion that substances translocated from one callus clump to

Table 2. Weight of callus tissue of various apple clones grown in the stock position under callus of various clones in the scion position, *in vitro*.

	Stock wt (mg)				
	Scion clone				
Stock clone	M 9	MM 106	MM 111	Golden Del.	Starkspur
Golden Del.	796 a[z]			794 a	
M 9	150 a			271 b	
M 9	223 a		523 b	592 b	
M 9				199 a	218 a
MM 111				230 a	273 a
MM 111	647 b			401 a	
MM 111	266 c	250 b	219 a		
MM 111	145 b		111 a	124 ab	
MM 106				330 a	280 a
MM 106	642 a	911 a	894 a		
MM 106		280 b	261 a		

[z]Mean separation within rows by Duncan's multiple range test, 5% level.

Table 3. Weight of various apple clones grown in the scion position over callus of selected rootstock clones in the stock position, *in vitro*.

	Scion wt (mg)		
	Stock clone		
Scion clone	MM 106	M 9	MM 111
Golden Del.[z]	241 a[x]	252 a	
Golden Del.[y]	263 a	302 a	
Starkspur	255 a	276 a	
M 9			269 b
MM 111			198 a
MM 106			298 c

[z], [y]Golden Delicious, callus of shoots secured from 2 different sources.
[x]Mean separation within columns by Duncan's multiple range test, 5% level.

Table 4. Weight of apple callus when grown beside callus of apple clones, *in vitro*.

	Callus wt (mg)					
	When grown beside					
Clone	Starkrimson	MM 106	M 9	Golden Del.	MM 111	Starkspur
MM 106		461 b[z]		605 c		259 a
MM 106	1119 a	1298 a				
M 9			254 a	213 a		187 a
MM 111		277 a	266 a		317 a	
MM 111				347 a	347 a	257 a

[z]Mean separation within rows by Duncan's multiple range test, 5% level.

Table 5. Weight of apple callus when grown beside callus of apple clones, *in vitro*.

	Callus wt (mg)				
	When grown beside				
Clone	MM 106	MM 106	M 9	MM 111	MM 111
M 9			279 a[z]	322 a	
Golden Delicious	374 a		284 a		273 a
MM 111				368 a	289 a
Starkspur	314 a		265 a		282 a
Starkrimson		422 a			
MM 106	599 b	1298 b		637 b	

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

another are actively absorbed by callus cells in the scion position from those in the stock position.

These data are interpreted as indicating that mobile compounds synthesized or altered in living cells of stock or scion may be responsible for stock-scion growth effects observed in the field. Many growth substances or their precursors are synthesized in growing leaves or shoots and translocated basipetally through the phloem. These compounds may be activated, deactivated or otherwise altered when they pass through the living cells of the stock. The altered compounds may then be the growth regulating compound(s) translocated to growing points where they influence growth.

Our study indicates that tissue culture techniques may be of value to study other aspects of stock-scion relationships to: a) isolate and identify compound(s) produced from clonal callus grown on defined media; and b) to screen new stock-scion combinations.

Literature Cited

1. Ball, E. A. 1971. Growth of plant tissues upon a substrate of another kind of tissue. *Z. Pflanzenphysiol.* 65:146-158.
2. Beakbane, A. B. 1952. Anatomical structure in relation to rootstock behavior. Proc. XIII Int. Hort. Congr. I p. 152-158.
3. _____ . 1956. Possible mechanism of rootstock effect. *Ann. Appl. Biol.* 44:517-521.
4. _____ and E. C. Thompson. 1939. Anatomical studies of stems and roots of hardy fruit trees II. The internal structure of the roots of some vigorous and some dwarfing apple rootstocks and the correlation of structure with vigour. *J. Pomol.* 17:141-149.
5. Fox, J. E. 1963. Growth factor requirements and chromosome number in tobacco tissue culture. *Physiol. Plant.* 16:793-803.
6. Fuji, T. and N. Nito. 1972. Studies on the compatibility of grafting of fruit trees. *J. Japan Soc. Hort. Sci.* 41:1-10.
7. Fuller, W. A. and G. E. Battese. 1973. Transformations for estimation of linear models with nested-error structure. *J. Amer. Stat. Assn.* 68:626-632.
8. Gur, A. and R. M. Samish. 1968. The role of auxins and auxin destruction in the vigor effect induced by various apple rootstocks. *Beitr. Biol. Pflanzen* 45:91-111.
9. Ibrahim, I. M. and M. N. Dana. 1971. Gibberellin-like activity in apple rootstocks. *HortScience* 6:541-542.
10. Jones, O. P. 1965. Observations on the growth effects of xylem sap from apple trees. Rpt. E. Malling Res. Sta. for 1964. p. 119-123.
11. _____ and H. J. Lacy. 1968. Gibberellin-like substances in the transpiration stream of apple and pear trees. *J. Expt. Bot.* 19:526-531.
12. Lockard, R. G. 1976. The effect of apple dwarfing rootstocks and interstocks on the proportion of bark on the tree. *Hort. Res.* 15: 83-94.
13. Martin, G. C. and E. A. Stahly. 1967. Endogenous growth regulating factors in bark of EM IX and XVI apple trees. *Proc. Amer. Soc. Hort. Sci.* 91:31-38.
14. _____ and M. W. Williams. 1967. Comparison of some biochemical constituents of EM IX and XVI bark. *HortScience* 2:154.
15. Messer, G. and S. Lavee. 1969. Studies in vigor and dwarfism of apple trees in an *in vitro* tissue culture system. *J. Hort. Sci.* 44: 219-233.
16. Miller, S. R. 1965. Growth inhibition produced by leaf extracts from size controlling apple rootstocks. *Can. J. Plant Sci.* 45:519-524.
17. Rogers, W. S. and A. B. Beakbane. 1957. Stock and scion relations. *Annu. Rev. Plant Physiol.* 8:217-236.
18. Thompson, E. C. 1952. Anatomical studies of stems and roots of hardy fruits. V. The histology of the roots of the apple Malling Crab C when budded with certain scion and rootstock varieties. Rpt. E. Malling Res. Sta. for 1951. p. 76-81.
19. Tubbs, F. R. 1973. Research fields in the interaction of rootstocks and scions in woody perennials. Part I. *Hort. Abstr.* 43:247-258.
20. _____ . 1974. Scion-rootstock influences on growth and flowering. *Proc. XIX Int. Hort. Congr.* III. p. 281-291.
21. Yadava, U. L. and D. F. Dayton. 1972. The relation of endogenous abscisic acid to the dwarfing capability of East Malling apple rootstocks. *J. Amer. Soc. Hort. Sci.* 97:701-705.
22. _____ and R. G. Lockard. 1977. Abscisic acid and gibberellin in the ungrafted apple (*Malus sylvestris*) rootstock clones. *Physiol. Plant.* 40:225-229.

J. Amer. Soc. Hort. Sci. 103(5):638–640. 1978.

Calcium and Magnesium Levels in the Annual Rings of 'McIntosh' Apple Wood[1]

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Additional index words. Malus domestica, acid rain

Abstract. **Analyses were made of Ca and Mg in a consecution of annual rings of 3 mature 'McIntosh' apple (*Malus domestica* Borkh.) orchard blocks in New York (acid rain region) and also 3 mature 'McIntosh' blocks in British Columbia (arid-irrigated region) in an attempt to assess the long range effects of acid rain on Ca levels in apple trees. Differences in patterns of Ca and Mg deposition in the wood did not appear to be caused by acid rain.**

An historical study of Ca in 'McIntosh' apple leaf samples sent by orchardists to Ithaca for analysis indicated Ca levels have recently declined precipitously in the 3 New York state apple growing regions (Fig. 1). The decline in leaf Ca was not accompanied by a decline in soil pH or available soil Ca (Table 1). Levels of leaf P, B, Mn, Zn and soil Mg and K did not change significantly during the period of Ca decline in the leaves (data not shown). Finally, it did not seem likely that the decline in leaf Ca was caused by the use of dolomitic limestone to correct soil pH because leaf Mg levels remained constant. For example, during the period 1964-1977 in the Champlain Valley, where leaf Ca decline was steepest, leaf Mg levels remained between 0.25 and 0.30% dry wt (r_{yr}, mg = –0.39 NS). We concluded from these observations that the decline in 'McIntosh' leaf Ca was not a normal nutritional disorder. When an expansion of the historical study indicated the decline in leaf Ca was not confined to 'McIntosh' apple (Table 2), we began to look for possible causes outside the arena of cultural practices.

Meteorological data indicated the average pH of annual precipitation (ppt) in Northeastern U.S. declined from pH 4.72 in 1955-56 to pH 4.39 in 1973-73 (2, 5). Recent ppt pH

[1]Received for publication February 22, 1978.
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