

Rootstock Effects on the Flowering of 'Delicious' Apple. II. Nutritional Effects with Specific Reference to Phosphorus

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Abstract. In each of 3 years, vegetative spurs were sampled from 1-year-old wood of 'Starkspur Supreme Delicious' apple trees (*Malus domestica* Borkh.) growing on B.9, M.26 EMLA, M.7 EMLA, P.18, and seedling rootstocks. Mineral concentrations of spur leaves and bud apical meristems were determined, and related to spur bud development. The spur leaf P concentration decreased during the growing season each year, but was unaffected by rootstock. Spur leaves of trees on B.9 rootstock had 30% higher Ca concentrations than trees on M.26 EMLA or seedling rootstocks. In each year, trees growing on M.26 EMLA rootstocks had the highest leaf Mg concentrations. Mineral concentrations were generally unrelated to spur leaf number, leaf area, leaf dry weight, or specific leaf weight. Phosphorus concentrations in spur bud apical meristems declined during two of the three growing seasons of the study and were unaffected by rootstock. Bud P concentration was weakly negatively related to bud diameter and bud appendage number in one year of the study. More vigorous spurs (as indicated by higher spur leaf number, leaf area, and leaf dry weight) had higher bud K levels during each year. No relationships between bud development and either spur leaf mineral concentration or bud apical meristem mineral levels were evident, suggesting that a direct role of mineral nutrition influenced by rootstock at the site of flower formation was unlikely.

Rootstocks have profound effects on the flowering and fruiting of apple trees (Ferree and Carlson, 1987; NC-140, 1990), however the mechanisms involved remain obscure. In studying the effects of rootstocks on the morphology of apple shoots, previous work (Hirst and Ferree, 1995a) has shown that flower density (the number of flower clusters on a given length of branch) could not be attributed to changes in spur density, but was closely related to the proportion of spurs that formed flowers. Further studies demonstrated that the timing of flower formation and the degree of bud complexity required before flower formation (the critical appendage number) were unaffected by rootstock (Hirst and Ferree, 1995b). Therefore, the effects of rootstock on flowering appears to be due to internal control mechanisms which affect the proportion of spurs that become floral.

Phosphorus applications can increase flowering the following year (Bould and Parfitt, 1973; Neilsen et al., 1990; Taylor and Goubran, 1975; Taylor and Nichols, 1990; Williams and Thompson, 1979). In these studies, only a small portion of the increased flowering in response to added P was due to increased growth. Flowering responses were primarily due to flower production in a higher proportion of spurs. Relationships between the P concentration of mid-shoot leaves and the number of flower clusters have been demonstrated (Bould and Parfitt, 1973; Neilsen et al., 1990; Taylor and Goubran, 1975). In their review of nutritional ranges of fruit trees, Shear and Faust (1980) reported the normal range of P for apple leaves to be 0.15% to 0.30%, but Bould and Parfitt (1973) concluded that adverse effects on growth and flowering may occur at sub-clinical or symptomless deficiency levels. They determined critical P levels of 0.25% (Bould and Parfitt 1973), which is close to the

critical level of 0.32% reported by Taylor and Goubran (1975). Similar trends were also evident in the data of Neilsen et al., (1990). These levels are substantially higher than those commonly observed in apple leaves (Awad and Kenworthy, 1963; Poling and Oberly, 1979; Rom et al., 1991; Warner and Heeney, 1985).

Rootstock effects on flowering may be due to differences among rootstocks in phosphorus uptake from the soil or differences in distribution within the tree. Uptake of P by roots is influenced by rootstock, and is positively related to rootstock vigor (Bukovac et al., 1958). Further work revealed the increased P uptake by more vigorous rootstocks was not entirely due to the presence of a larger root system, since uptake of ³²P per gram of root was also higher in more vigorous rootstocks (Atkinson, 1973). In addition to affecting the uptake of P, rootstocks also affect the amount of P transported across the graft union in the xylem, with a greater degree of depletion in more dwarfing rootstocks and interstocks (Jones, 1971, 1976). Several dwarfing rootstocks may exhibit decreases in both phosphorus uptake and transport to the scion.

Rootstock has been shown to influence leaf mineral nutrition, including P, in a number of studies, but results have been inconsistent. For example, trees of 'Delicious' and 'Golden Delicious' growing on MM.106 rootstocks had higher P levels than similar trees on MM.111 rootstock (Schneider et al., 1978) but West and Young (1988) found similar P concentrations in 'Delicious' trees growing on these rootstocks but lower concentrations in 'Golden Delicious' trees on MM.106 compared with those on MM.111. Trees on seedling rootstock have been reported as having relatively high (Schneider et al., 1978) moderate (Fallahi, et al., 1984a) or low (West and Young, 1988) phosphorus levels. In a study of 'McIntosh' trees on 16 rootstocks, no rootstock effect was found (Poling and Oberly, 1979), while Rom et al., (1991) found no consistent differences among 9 rootstocks studied over 8 years. Such contradictory reports may be due to scion cultivar, soil fertility, location, cultural practices, and year-to-year variation, each of which may affect mineral element uptake (Wutscher, 1989).

The lack of clear rootstock influence on foliar P levels, when obvious differences in uptake and transport across the graft union are evident, is puzzling. In studies with applied P, higher P resulted in increased flowering, but dwarfing rootstocks (which generally

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promote heavy flowering) have lower uptake and transport characteristics. If the mechanism by which P affects flowering is a direct one on the buds, then the measurement of P using conventional foliar sampling, where the leaves sampled are some distance from the potential flower buds, may be inappropriate to assess rootstock influences on P levels as related to flowering. Compared with shoot leaves, spur leaves are in close proximity to the site of flower formation and their analysis may be more closely related to P levels within the bud. For a measure of P within the apical meristem itself, electron microprobe analysis may be useful, where a relative amount of the mineral elements present in a very small area (within a diameter of a few μm) can be obtained (Postek et al., 1980, R.E. Whitmoyer, personal communication). Although energy dispersive X-ray spectrophotometry is not quantitative, it is nonetheless a useful tool to determine the relative amounts of minerals in a small area (Postek et al., 1980).

Mineral nutrients other than P have also been shown to influence apple flowering. Increased flowering of apple occurred in response to nitrogen application, especially when supplied in the ammonium form (Edwards, 1986; Gao et al., 1992). Similarly, potassium has been associated with flower and fruit production (Campbell and Bould, 1970).

This study was conducted to measure rootstock effects on the levels of mineral nutrients, especially phosphorus, in spur leaves and in the apical meristem of buds of a common scion, and to examine the relationship of these levels with the degree of flower formation.

Materials and Methods

Spur sampling. Vegetative spurs were sampled from 'Starkspur Supreme Delicious' trees growing on B.9, M.26 EMLA, M.7 EMLA, P.18, and seedling rootstocks as previously described (Hirst and Ferree, 1995b). These trees formed part of a rootstock evaluation program (NC-140, 1990) and were planted in 1984 as randomized complete blocks with 10 single tree replications. Sampling of spurs from the previous years' growth was conducted from 1991-1993 from mid-July, about 70 days after full bloom, (DAFB) until October 138-172 DAFB. Samples were also collected at 22 DAFB in 1993 to study changes at an earlier stage of development. Either three (1991) or two (1992, 1993) buds were sampled from well illuminated branches on each tree on each sampling date. Terminal bud diameter, spur leaf number, spur leaf area, and spur leaf dry weight were determined for sampled spurs, and dried leaves were stored for subsequent analysis. Spur buds were stored in a formalin : ethanol : acetic acid solution (McLaughlin and Greene, 1991) until dissection and electron microprobe analysis. Spectra collected from fresh buds appeared broadly similar to those obtained from buds stored in FAA solution (data not presented).

Foliar analysis. Foliar analyses were performed on spur leaves collected over the period 70-115 DAFB during each year (mid-July to late August). In 1991, leaves from six sampling dates over this period were analyzed, whereas in 1992 and 1993, leaves from three dates were analyzed, corresponding to periods before, during, and subsequent to flower formation as previously determined (Hirst and Ferree, 1995b). Dried leaves were pooled from the two (1992, 1993) or three (1991) spurs sampled from each tree on each date, and 500 mg of finely ground tissue was ashed for 4 h at 500C. The ash was then dissolved in 25 ml of 2.4 N HNO₃ and analyzed using an inductively coupled plasma (ICP) spectrophotometer (Watson and Isaac, 1990) at the Research Extension Analytical Laboratory (REAL) in Wooster, Ohio.

Electron microprobe analysis. Microprobe analysis of one bud from each of five trees of each rootstock was carried out for buds

collected before the onset of apical doming. Apical doming is the first visible sign that flower formation has occurred (Fulford, 1966; Luckwill and Silva, 1979) and in these trees was first observed at the end of July (Hirst and Ferree, 1995b). In 1991, buds were sampled on three dates before apical doming, whereas buds were collected on two sampling dates in 1992 and 1993 before flower formation. Bud scales, transition leaves, and outer leaves of terminal buds were removed to expose the apical meristem. Buds were removed from the preservative solution and air dried for about 10 min. The apical 2 mm of buds was removed by a transverse cut perpendicular to the longitudinal axis of the bud, and the uncoated bud apical section mounted on a carbon planchette using silver conducting paint. Bud apical meristems were observed with an IS1 scanning electron microscope at a magnification of 200-500 \times with a 30-kv accelerating voltage and an electron beam directed at an angle of 45 $^{\circ}$ to the samples. Using an energy dispersive X-ray spectrophotometer (9900; Edax), spectra were collected from three positions (<10 μm diameter) near the center of the apical meristem. Preliminary investigations determined that the principal minerals present in the apical meristems of 'Starkspur Supreme Delicious' buds were P, S, K, Fe, and Ca, and that the relative levels of P were higher in the center of the meristem than around the periphery ($P \leq 0.05$). Measurements were restricted to these five elements and all measurements were made near the center of meristems. Data obtained from EDAX analysis are relative rather than quantitative, therefore the levels of the various minerals were expressed as a proportion of the total of the five elements studied rather than as absolute concentrations. The three readings from each bud were similar and averaged before statistical analysis using analysis of variance.

Results

In each of the three years of this study, the phosphorus concentration of spur leaves declined over the course of the growing season (Tables 1-3). The decline in P levels was similar for trees on all rootstocks with no interaction between rootstock and time of growing season evident in any years of the study. In 1991, spur leaves from trees on M.26 EMLA rootstock had higher concentrations of P than trees on other rootstocks (Table 1). No rootstock effects on the level of P were observed during 1992 or 1993. In all years, most of the treatment effect on P levels was due to time of the growing season (Tables 1-3). Leaf phosphorus concentration increased each year from 1991-1993, with means of 0.137%, 0.144%, and 0.147% respectively ($P \leq 0.05$).

The relative effects of rootstock and time of the growing season on the levels of other minerals were reasonably stable from year to year (Tables 1-3). Variation in the levels of foliar Ca, Mg, Mn, and Zn were most affected by rootstock, whereas the levels of K, Fe, B, Al, and Na were primarily determined by time when the leaves were sampled. The trends in mineral levels over the course of the growing season were similar among rootstocks for most of the elements studied, but significant interactions between rootstock and time of sampling were evident for B, Cu, and Na.

In all years, leaves sampled from trees on B.9 rootstocks contained higher calcium concentrations than trees on the other rootstocks, and B.9 trees generally had 30% higher Ca concentrations than trees on seedling rootstock (Tables 1-3). There was a trend of higher Ca levels in P.18 rootstock than in M.7 EMLA, M.26 EMLA, and seedling rootstocks, although this was only significant in 1992 (Tables 1-3). In all years, increases in Ca concentration over the course of the growing season were evident, but these changes were of minor importance compared with the rootstock effect. Calcium concentra-

Table 1. Effect of rootstock and time of growing season during 1991 on mineral concentrations of spur leaves of 'Starkspur Supreme Delicious'

	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Al (ppm)	Na (ppm)
Days after full bloom											
67	0.143	1.25	1.72	0.498	183	127	28.1	9.93	34.5	310	131
74	0.150	1.18	1.80	0.503	189	134	27.8	8.70	33.3	417	153
85	0.138	1.01	1.68	0.485	172	107	33.9	7.85	31.6	359	104
99	0.137	1.00	1.77	0.507	181	127	27.7	8.11	31.9	374	111
109	0.123	0.83	1.88	0.489	182	97	34.0	5.96	29.6	346	111
117	0.125	0.83	1.83	0.476	176	93	26.3	7.14	30.5	319	73
LSD (<i>P</i> = 0.05)	0.004	0.09	0.09	0.021	30	10	1.0	0.96	1.8	26	12
Rootstock (RS)											
B.9	0.132	1.06	2.03	0.431	192	113	30.5	8.53	33.6	366	106
M.26 EMLA	0.143	0.87	1.64	0.561	198	114	29.6	7.47	32.9	363	108
M.7 EMLA	0.136	1.02	1.80	0.530	169	115	29.4	8.93	33.5	350	110
P.18	0.134	1.09	1.81	0.478	125	109	28.0	6.90	28.5	355	123
Seedling	0.134	1.05	1.62	0.464	219	119	30.7	7.92	31.0	335	123
LSD (<i>P</i> = 0.05)	0.004	0.08	0.08	0.019	27	9	0.9	0.87	1.7	23	11
Source of variation (% SS)											
Date	82.9***	76.8***	16.1***	4.7	2.3	89.3***	84.4***	53.6***	32.9***	77.7***	63.1***
RS	12.7***	18.8***	77.7***	91.0***	77.8***	3.8	7.8***	17.6***	44.5***	7.3	5.5***
Date × RS	4.4	4.4	6.2	4.3	19.8	6.8	7.8**	28.7**	22.5	15.0	31.3***

*, **, ***Significant at *P* = 0.05, 0.01 or 0.001, respectively.

Table 2. Effect of rootstock and time of growing season during 1992 on mineral concentrations of spur leaves of 'Starkspur Supreme Delicious'.

	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Al (ppm)	Na (ppm)
Days after full bloom											
71	0.158	1.35	1.75	0.494	193	124	26.2	10.06	25.6	289	129.1
89	0.140	1.26	1.75	0.462	191	102	27.2	9.23	24.3	280	80.0
107	0.133	1.20	1.86	0.467	182	107	30.9	7.81	26.2	313	63.1
LSD (<i>P</i> = 0.05)	0.004	0.07	0.08	0.018	26	12	0.9	0.97	1.6	26	18.2
Rootstock (RS)											
B.9	0.144	1.25	2.17	0.433	210	115	27.1	9.76	28.5	333	122.4
M.26 EMLA	0.140	1.28	1.59	0.532	192	116	26.1	8.34	27.1	291	121.2
M.7 EMLA	0.145	1.22	1.66	0.469	162	108	29.0	10.80	23.5	281	72.3
P.18	0.145	1.31	1.90	0.464	154	112	28.7	8.96	23.7	283	81.5
Seedling	0.144	1.27	1.63	0.474	224	105	29.6	7.28	24.0	281	56.3
LSD (<i>P</i> = 0.05)	0.005	0.09	0.11	0.024	33	16	1.1	1.26	2.1	33	23.5
Source of variation											
Date	70.4***	65.6***	5.3*	13.9**	3.0	66.8**	60.9**	22.3***	11.1	23.8*	32.1***
RS	3.2	15.7	92.9***	76.6***	91.2***	13.9	25.9***	36.5***	74.8***	50.3*	29.5***
Date × RS	6.5	18.7	1.8	9.6	5.9	19.3	13.2***	41.2***	14.1	25.8	38.4***

*, **, ***Significant at *P* = 0.05, 0.01, or 0.001, respectively.

tions were similar among years. Magnesium concentration was always highest in M.26 EMLA and lowest in trees on B.9 rootstock, with the levels in other rootstocks being intermediate (Tables 1-3). B.9 and M.26 EMLA trees had the highest Zn concentrations and P. 18 generally had the lowest levels.

Potassium levels declined during the growing season in each year. Rootstock had a relatively small effect whereas trees on P. 18 had higher K than those on M.26 in 1991, and in 1993 leaves from trees on P. 18 and seedling rootstocks contained a higher concentration of K than those on M.7 EMLA (Tables 1-3). Declines in Fe concentration were observed during the 1991 and 1992 growing seasons, but a slight increase was evident in 1993. The foliar concentration of Al varied within the 1991 and 1992 growing seasons, and no overall trend was established, but a marked increase occurred during the 1993 growing season. Sodium generally decreased over the course of each growing season.

The levels of B were determined by time of season, rootstock, and the interaction between them (Tables 1-3). In 1991, peaks in B levels for all rootstocks occurred at 85 and 109 DAFB, and the differences among rootstocks was greater at the dates when peak levels were observed. The nature of the interaction in 1992 was such that B.9 had relatively higher levels of B during the middle of the growing season, compared with other rootstocks. Over all years, leaves from trees on seedling rootstock had the highest concentration of B with lower levels present in trees of M.26 EMLA and P. 18 rootstocks.

Spur leaf mineral concentrations were generally not well correlated with spur leaf characteristics such as leaf number, spur leaf area, leaf dry weight, and specific leaf weight (data not presented). Although some significant relationships were evident, these were weak, variable from year to year, and each accounted for less than 20% of the variation as determined by regression analyses.

Table 3. Effect of rootstock and time of growing season during 1993 on mineral concentrations of spur leaves of 'Starkspur Supreme Delicious'.

	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Al (ppm)	Na (ppm)
Days after full bloom											
71	0.168	1.21	1.68	0.412	210	102	25.7	11.22	32.1	192	117.3
95	0.141	1.05	1.83	0.416	200	112	25.6	8.13	31.3	318	64.3
110	0.132	0.87	1.88	0.430	210	121	27.5	8.17	31.6	336	73.1
LSD (<i>P</i> = 0.05)	0.004	0.05	0.08	0.019	24	7	0.8	2.18	1.5	22	9.5
Rootstock (RS)											
B.9	0.146	1.03	2.08	0.365	217	109	26.1	8.38	35.2	293	75.1
M.26 EMLA	0.147	1.00	1.67	0.516	226	116	25.6	7.98	33.5	284	95.1
M.7 EMLA	0.148	0.97	1.75	0.414	181	109	26.3	9.73	30.1	280	86.5
P.18	0.144	1.11	1.82	0.402	174	103	25.1	11.14	29.5	268	72.8
Seedling	0.148	1.09	1.66	0.399	234	119	28.3	8.65	30.0	286	95.2
LSD (<i>P</i> = 0.05)	0.005	0.07	0.11	0.024	31	9	1.0	2.81	2.0	28	12.3
Source of variation											
Date	97.4***	87.7***	20.9***	2.0	3.4	59.3***	33.4***	26.6***	2.3	95.2***	60.9***
RS	1.0	11.7***	68.9***	96.8***	90.8***	30.9*	53.1***	15.8***	94.5***	1.5	10.2***
Date × RS	1.6	1.2	10.2	1.1	5.8	9.7	13.5	57.6***	3.2	3.3	29.0***

*, **, *** Significant at *P* = 0.05, 0.01 or 0.001, respectively.

Table 4. Effect of rootstock, time of growing season [days after full bloom (DAFB)] and their interaction on the relative levels of P, K, Ca, Fe, and S in bud apical meristems of 'Starkspur Supreme Delicious' during 1991.

	Relative mineral nutrient level (%) ²				
	P	K	Ca	Fe	S
DAFB					
67	37.4	6.1	29.9	8.5	18.1
74	31.1	5.1	26.8	17.5	18.8
85	27.1	5.2	31.7	16.8	19.0
LSD (<i>P</i> = 0.05)	5.2	1.3	4.6	3.3	1.9
Rootstock (RS)					
B.9	33.2	4.8	30.3	13.7	18.2
M.26 EMLA	31.4	6.9	30.1	13.8	17.4
M.7 EMLA	27.6	5.3	33.3	14.6	19.0
P.18	34.8	4.8	28.1	12.1	20.1
Seedling	33.4	5.5	25.5	17.1	18.4
LSD (<i>P</i> = 0.05)	6.7	1.6	5.9	4.3	2.5
Source of variation					
Date	53.5***	11.7	24.3	61.2***	5.9
RS	18.5	33.7	40.4	10.2	28.2
Date × RS	28.0	54.6	35.2	28.6	65.9

²EDAX analysis expressed as a proportion of the total of the elements studied.

*, **, *** Significant at *P* ≥ 0.05, 0.01, or 0.001, respectively.

The relative amount of P in bud apices was not affected by rootstock during any year, but declined during the 1991 and 1993 seasons (Tables 4-6). The interaction between time of season and rootstock for P in 1993 (Table 6) was due to the P level in buds from B.9 and P.18 trees remaining constant between sampling dates, while a decline between the first and second sampling dates was evident for trees on other rootstocks. Trees on M.26 EMLA had a higher relative S content in 1992 than trees on B.9 or seedling rootstocks (Table 5). The effect was small and not observed in either of the other years. The Ca content of bud meristems increased over the course of the 1993 season (Table 6), but was not similarly affected in other years (Tables 4-5). Bud Fe levels increased over the early part of the 1991 and 1993 seasons (Tables 4-5), and remained constant after 74 DAFB in 1991.

The relative levels of all minerals in the bud apex were affected

Table 5. Effect of rootstock, time of growing season [days after full bloom (DAFB)] and their interaction on the relative levels of P, K, Ca, Fe, and S in bud apical meristems of 'Starkspur Supreme Delicious' in 1992.

	Relative mineral nutrient level (%) ²				
	P	K	Ca	Fe	S
DAFB					
71	36.5	8.5	23.1	12.1	19.7
81	37.7	7.5	20.9	12.1	20.9
LSD 0.05	3.6	1.9	4.0	2.5	1.5
Rootstock (RS)					
B.9	35.9	6.1	24.0	15.0	18.7
M.26 EMLA	36.9	6.7	22.8	10.6	22.7
M.7 EMLA	38.4	9.7	20.0	11.2	20.6
P.18	38.7	9.0	19.9	11.7	38.7
Seedling	35.6	8.4	23.3	12.1	19.0
LSD 0.05	5.7	3.0	6.3	3.9	2.4
Source of variation					
Date	6.9	10.6	11.5	0	11.8
RS	34.3	77.6	29.5	67.2	72.8*
Date × RS	58.7	11.8	59.0	32.8	15.4

²EDAX analysis expressed as a proportion of the total of the elements studied.

*, **, *** Significant at *P* ≥ 0.05, 0.01, or 0.001, respectively.

by year, but over all years, rootstock had no effect (Table 7). Bud P levels increased each year and the same trend was evident for all rootstocks, while other minerals were variable from year to year. The year × rootstock interaction for S was primarily due to high levels for M.26 EMLA in 1992, followed by lower levels in 1993.

No relationships between the relative levels of minerals in the bud apex and the levels determined for spurs leaves were evident (data not presented). However, spur leaf data were derived from the two (1992-1993) or three (1991) spurs sampled from each tree whereas the mineral levels in the bud apex were determined in only one of these buds.

The bud appendage number was related to the relative level of P in the apex of the bud only in 1993, when the relationship was negative and rather weak (Table 8). In 1993, more vigorous spurs, (as indicated by higher leaf area, dry weight, SLW, and terminal bud

Table 6. Effect of rootstock, time of growing season [days after full bloom (DAFB)] and their interaction on the relative levels of P, K, Ca, Fe, and S in bud apical meristems of 'Starkspur Supreme Delicious' in 1993.

	Relative mineral nutrient level (%) ^z				
	P	K	Ca	Fe	S
DAFB					
22	43.7	6.6	14.9	14.0	20.8
71	36.6	6.8	19.8	16.9	19.8
LSD (<i>P</i> = 0.05)	3.6	1.3	2.8	2.6	1.3
Rootstock (RS)					
B.9	43.5	6.3	16.9	11.4	21.7
M.26EMLA	37.7	7.1	20.0	16.6	18.6
M.7EMLA	40.6	6.3	17.3	15.4	20.4
P.18	38.2	7.6	16.3	18.1	19.9
Seedling	40.9	6.2	16.2	15.6	20.7
LSD (<i>P</i> = 0.05)	5.8	1.3	4.4	4.2	2.1
Source of variation					
Date	44.8***	1.6	53.3**	14.5*	9.4
RS	16.1	26.3	20.4	36.5*	48.7
Date × RS	39.1*	72.2	26.3	48.9**	42.0

^zEDAX analysis expressed as a proportion of the total of the elements studied.

*,**,***Significant at *P* ≤ 0.05, 0.01, 0.001, respectively.

Table 7. Effect of year and rootstock on the relative level of minerals in bud apices of 'Starkspur Supreme Delicious'.

	Relative mineral nutrient level (%) ^z				
	P	K	Ca	Fe	S
Year					
1991	32.3	5.5	29.4	14.2	18.6
1992	37.1	8.0	22.0	12.1	20.3
1993	40.1	6.8	17.4	15.3	20.3
LSD (<i>P</i> = 0.05)	3.2	1.0	2.7	2.3	1.2
Rootstock (RS)					
B.9	37.6	5.9	23.8	13.2	19.5
M.26 EMLA	35.5	6.8	24.2	13.6	19.6
M.7 EMLA	35.8	7.1	23.4	13.6	20.0
P.18	37.2	7.1	21.4	14.0	20.2
Seedling	36.3	6.7	21.9	15.0	19.3
LSD (<i>P</i> = 0.05)	4.0	1.3	3.4	2.8	1.5
Source of variation					
Year	73.3***	58.4***	86.7***	30.8*	36.8***
Rootstock	4.0	10.1	3.9	7.3	6.1
Year × RS	22.6	31.5	9.4	61.9	57.1**

^zEDAX analysis expressed as a proportion of the total of the elements studied.

*,**,***Significant at *P* ≤ 0.05, 0.01, 0.001, respectively.

diameter) had lower P levels in bud apices, but again the relationships were weak with each explaining less than 30% of the variation for each of these spur characteristics. The relative level of K in the bud apex was positively related to leaf area and leaf dry weight from 1991-1993 and with leaf number per spur in 1991 and 1992.

Spurs with more leaves had higher concentrations of foliar P in each year of the study (Table 9). These relationships were weak and explained < 20% of the observed variation. Leaves from spurs with higher concentrations of B had lower specific leaf weight in 2 of the 3 years of the study, but the relationships explained a small proportion of the variation. Relationships between other spur characteristics and leaf mineral concentrations were weak and variable from year to year.

Table 8. Correlation coefficients between the relative levels of minerals in bud apices of 'Starkspur Supreme Delicious' and spur characteristics.

	Relative mineral nutrient level (%) ^z				
	P	K	Ca	Fe	S
	1991				
Leaf number	0.26	0.47 ^z	-0.29	-0.28	-0.12
Leaf area	0.22	0.40*	-0.22	-0.28	-0.08
Dry weight	0.22	0.50*	-0.24	-0.22	-0.22
Bud diameter	---	---	---	---	---
SLW	-0.02	0.37	-0.07	0.23	-0.44*
Bud appendages	0.10	0.28	-0.12	0.09	0.003
TCSA	-0.03	0.00	-0.05	0.06	0.07
Yield efficiency	0.05	0.02	0.07	-0.11	-0.08
	1992				
Leaf number	0.25	0.53**	-0.47*	-0.02	0.13
Leaf area	0.27	0.75***	-0.41*	-0.36	-0.004
Dry weight	0.26	0.75***	-0.40*	-0.35	-0.02
Bud diameter	---	---	---	---	---
SLW	0.00	0.34	-0.18	0.07	-0.07
Bud appendages	0.33	0.34	-0.29	-0.23	0.08
TCSA	0.05	0.18	-0.03	-0.18	-0.02
Yield efficiency	-0.24	-0.14	0.26	0.11	-0.09
	1993				
Leaf number	-0.06	0.19	0.14	-0.16	-0.03
Leaf area	-0.35*	0.45**	0.25	0.12	-0.21
Dry weight	-0.44**	0.33*	0.34	0.18	-0.20
Bud diameter	-0.53***	0.19	0.43**	0.28	-0.21
SLW	-0.45**	0.09	0.41**	0.21	-0.45**
Bud appendages	-0.47**	0.06	0.41**	0.26	-0.13
TCSA	-0.19	0.04	-0.08	0.38*	-0.15
Yield efficiency	0.10	-0.12	0.14	0.22	-0.004

^zEDAX analysis expressed as a proportion of the total of the elements studied.

*,**,*** Significant at *P* ≤ 0.05, 0.01, or 0.001, respectively.

Discussion

Rootstock effects on spur leaf P concentration were small in magnitude and inconsistent from year to year. In a similar study (Rom et al., 1991), the year of sampling had the largest effect on shoot leaf P, with rootstock accounting for a only a small amount of the variation. Other workers (Dzamic et al., 1980; Poling and Oberly, 1979) have also found leaf P concentrations to be unaffected by rootstock. In the present study, other minerals were affected by rootstock with stable relationships across years (for example Ca, Mg, Mn, and B) however, no correlations of mineral status with rootstock vigor and productivity were apparent. Similarly foliar mineral concentrations were generally unrelated to spur characteristics (Table 9), which have previously been associated with productivity (Rom and Ferree, 1984).

Relationships between the spur leaf P concentration and the bud appendage number were evident in each year (1991, *r*² = 0.12, *P* = 0.001; 1992, *r*² = 0.37, *P* = 0.001; 1993, *r*² = 0.03, *P* = 0.05), and in all cases the slopes were negative. Although these relationships are statistically significant, they probably have little physiological meaning, and may be the result of two coincident events rather than indicating a causal relationship. Within sampling date, there was also no relationship between foliar P concentration and bud appendage number (data not presented).

Decreases in the levels of P and K over the course of a growing season were found in this study and have been reported previously

Table 9. Correlation coefficients between characteristics of 1-year-old vegetative spurs growing on 2-year-old branches with spur leaf mineral concentrations.

Mineral	Spur leaf number			Spur leaf area			Spur leaf dry wt			Specific leaf wt		
	1991	1992	1993	1991	1992	1993	1991	1992	1993	1991	1992	1993
P	0.40***	0.33***	0.36***	0.11	0.18*	0.03	0.28	0.08	0.03	0.44**	-0.40***	0.03
K	-0.40**	0.06	0.32***	-0.24	-0.01	0.09	-0.32*	-0.07	0.05	-0.21	-0.16*	-0.16
Ca	-0.17	-0.07	-0.09	-0.12	-0.06	-0.02	-0.14	-0.07	0.01	-0.01	-0.05	0.13
Mg	0.32	0.05	0.03	-0.35*	0.05	0.03	0.31*	-0.03	0.01	-0.08	-0.29***	-0.09
Mn	-0.19	0.18*	0.07	-0.32*	0.09	-0.19*	-0.20	0.05	-0.14	0.29*	-0.14	0.22**
Fe	0.17	-0.09	-0.10	0.15	-0.11	-0.09	0.16	-0.17*	-0.07	0.11	-0.23**	0.05
B	0.14	0.08	-0.05	0.19	0.24**	0.09	0.07	0.17*	0.02	-0.32	-0.26**	-0.33***
Cu	0.28	0	0.06	0.09	-0.10	0.07	0.13	-0.11	0.05	0.07	-0.03	-0.09
Zn	-0.01	-0.28***	0	-0.11	-0.19*	-0.21*	-0.08	-0.21**	-0.20*	0.10	-0.13	0.03
Al	0.43**	-0.20*	-0.31***	0.31*	-0.24**	-0.15	0.33*	-0.28	-0.11	0.02	-0.10	

*, **, *** Significant at $P \leq 0.05$, 0.01, 0.001, respectively.

(Awad and Kenworthy, 1963; Fallahi et al., 1984a; Mason, 1958; Rogers and Batjer, 1953; Westwood, 1978). The levels of mineral nutrients in spur leaves determined in this study relate well with the levels in shoot leaves (Poling and Oberly, 1979; Rom et al., 1991) suggesting little difference in mineral accumulation between spur and shoot leaves.

Calcium levels were generally highest in leaves from trees on B.9 and lowest from those on M.26 EMLA and seedling rootstocks. This agrees with a recent finding that 'Fuji' apple leaves on M.26 had significantly lower Ca than those on M.9 or M.7 (Fallahi, personal communication). In a study comparing mineral nutrition of 'Starkspur Golden Delicious' trees on six rootstocks, leaf Ca concentrations from trees on M.26 EMLA were found to be intermediate among the other rootstocks (Fallahi et al., 1984a) but fruit from these trees had the lowest Ca concentrations (Fallahi et al., 1984b), and tended to have more bitter pit (Fallahi et al., 1985) than fruit from trees on other rootstocks. Lower fruit Ca levels from M.26 EMLA rootstock were also found by Autio (1991) but these were not related to the incidence of bitter pit.

Magnesium levels were consistently highest in leaves from M.26 EMLA and lowest in leaves from trees growing on B.9 rootstock. These trends are the opposite of those observed for Ca and would suggest a negative relationship, similar to the pattern in apple fruit (Ferguson and Watkins, 1989), but overall there was no relationship between Mg and Ca concentrations in spur leaves.

Manganese concentrations were highest in trees on seedling rootstocks and lowest on P. 18 rootstocks. Therefore trees in a similar vigor class displayed both high and low extremes of Mn. The disorder, internal bark necrosis (also known as measles), is related to high Mn levels, but the highest levels recorded in this study fall short of those reported to be associated with necrotic symptoms (Ferree and Thompson, 1970).

Trees on seedling rootstock generally had higher concentrations of B in their spur leaves than trees on other rootstocks whose concentrations were variable among years. A number of studies have found shoot leaf B to be unaffected by rootstock (Awad and Kenworthy, 1963; Fallahi et al., 1984a; Poling and Oberly, 1979).

Previously, crop load effects on apple fruit mineral levels have been demonstrated (Fallahi et al., 1984b; Volz et al., 1993). Although some variability in crop load was apparent in the trees used in this study (Hirst and Ferree, 1995a), these fluctuations were relatively small. Deviations of crop load (normalized for tree size) from the average crop load for each rootstock over the 3 years of this study averaged 20%, with a maximum of 30% for trees on

seedling rootstock in 1991. It seems unlikely that variations in crop load had a marked effect on leaf nutrient levels.

Foliar analyses were determined from bulk samples comprised of the two (1992 and 1993) or three (1991) spurs sampled per tree on each sampling date to provide sufficient tissue for analysis. In contrast, levels of mineral nutrients in the bud apical meristem were measured in one bud from each tree for each sampling date. Analysis of bud apical meristems and foliar samples differed not only in the type of tissue analyzed, but in the sampling procedures and measurement method (qualitative vs. quantitative). The lack of relationship between foliar and bud data is, therefore, not surprising.

Over the 3 years of this study, most of the variation in bud mineral nutrient levels was accounted for by year, and rootstock accounted for 4% to 10% of the variation depending on element (Table 7). Year also accounted for most of the variation in shoot leaf mineral levels (Rom, et al., 1991) whereas only minor rootstock effects were evident.

The only consistent relationships between bud mineral levels and spur characteristics were for K, which was generally higher in more vigorous spurs as indicated by spur leaf number, leaf area, and leaf dry weight (Table 8). In a previous study, these characteristics were related with flowering, where spurs forming flowers had more leaves with higher total spur leaf area (Hirst and Ferree, 1995b). If the relative level of K in the bud was related to flowering, it would be expected to vary with the proportion of buds in which flowers formed. Yet low numbers of flowers formed in 1993, and high and intermediate numbers formed in 1992, and 1991, respectively (Hirst and Ferree, 1995b). Over these years, however, the relative K level of bud apices was fairly constant, suggesting that the level of K in bud apical meristems was unrelated to flowering. Of the elements in the bud meristem studied, none exhibited trends similar to those demonstrated for flower formation.

Although previous studies have shown that rootstocks differ in their ability to absorb and translocate P (Atkinson, 1973; Jones, 1971, 1976), results from the present study suggest that rootstocks do not affect the amount of P at the site of flower formation, the bud apical meristem. A role for phosphorus in the mechanism of rootstock control of flowering cannot be ruled out, but a direct role of phosphorus at the site of flower formation induced by rootstock seems unlikely. Soil phosphorus levels have been shown to be associated with root cytokinin biosynthesis in *Plantago* sp. (Baas and Kuiper, 1989) and tomato (Menary and van Staden, 1976). A similar mechanism may be operating in apple, where phosphorus influences on flowering is mediated by changes in endogenous cytokinin levels.

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