282

ever, even with this line, levels of suckering and burrknotting remained low following improved conventional propagation.

Overall, the evidence for the transient nature of increased suckering and burrknotting with micropropagation supports the view that an effective method for exploiting micropropagation with rootstocks such as M.9 would be through the provision of field-grown stock plants for improved conventional propagation (Jones, 1993).

However, the requirement remains for longer term evaluation of rootstocks from improved conventional propagation following micropropagation. To this end, the trees used in the present study have been removed from the nursery to an orchard site where their performance will be monitored for at least five years.

We thank Mr Martin Ridout for statistical analyses and Mr W. C. C. Hadlow for assistance with collecting field data. This work was funded by the AFRC and The Agricultural Genetics Company.

REFERENCES

JONES, O. P. (1993). Propagation of apple *in vitro*. In: *Micropropagation of woody plants*. (Ahuja, R., Ed.), Kluwer Academic Publishers. Dordrecht, The Netherlands, 169–86.

WEBSTER, C. A. and JONES, O. P. (1989). Micropropagation of the apple rootstock M.9: effect of sustained sub-culture on apparent rejuvenation *in vitro*. *Journal of Horticultural Science*, **64,** 421–8.

WEBSTER, C. A. and JONES, O. P. (1992). Performance of field hedge and stoolbed plants of micropropagated dwarfing apple rootstock clones with different degrees of apparent rejuvenation. *Journal of Horticultural Science*, **67,** 521–8.

(Accepted 21 February 1993)

Journal of Horticultural Science (1993) **68** (5) 767–774

Effects of humidity and Ca level on dry-matter and Ca accumulation by leaves of cucumber (*Cucumis sativus* L.)

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SUMMARY

Two cucumber cultivars (Corona and Rebella) were grown under a range of day and night humidity combinations and at two levels of Ca (180 and 270 mg l^{-1}) in the nutrient solution applied to the rockwool substrate. High humidity increased the incidence of Ca deficiency symptoms and decreased the dry weight of the leaves. The concentration of Ca (%) in the leaf dry matter was reduced by high humidity at night in Experiment A and equally by high humidity during the day or night in Experiment B. The response of the leaf tips (% Ca in the terminal 7 cm) was greater than that of the complete laminae, and similar to that of the total amount of Ca (mg) accumulated per complete lamina. Increasing the level of applied Ca increased the concentration of Ca (%) in the complete laminae and leaf tips, but the increase in total Ca per lamina (mg) was more marked. 'Corona' had heavier leaves than 'Rebella' and a higher concentration of Ca (%), resulting in a greater accumulation of Ca (mg) per leaf lamina.

THE occurrence of higher humidities as a consequence of energy saving practices has resulted in the more frequent incidence of symptoms attributed to Ca deficiency on the leaves of protected crops. High humidity reduces Ca movement since the rate of translocation of Ca is largely determined by the rate of transpiration (Mengel and Kirkby, 1987). The affected leaf tissue becomes yellow and later brown, thus reducing the photosynthetic potential of the crop. Invasion of the dead tissue by fungal diseases may cause a more serious reduction in crop potential.

Until the recent interest in humidity, little work was done on this aspect of the environment, and crop responses were uncertain. Armstrong and Kirkby (1979) found that high humidity promoted the growth rate of young tomato plants. They suggested that this was due to the more open stomata, which allowed better assimilation of CO_2 and resulted in faster leaf expansion. This initial stimulation in growth agreed with the findings of Acock *et al.* (1976). Hoffman (1979) observed a detrimental effect of high humidity on the growth of 11 out of 16 crops tested at vapour pressure deficits (vpd) less than 0.3 kPa. More recently, Burrage (1988), Holder and Cockshull (1990), and Bakker (1990) found that the leaf area of tomato decreased at high humidity. The average area per leaf was decreased by high humidity in sweet pepper but the number of leaves increased so that total leaf area was hardly affected (Bakker, 1989). Unfortunately, he did not report the effect of humidity on the dry weight of the leaves, nor did Bakker and Sonneveld (1988) for cucumber. The dry weight of tomato plants in growth chambers increased at high humidity (95–100% r.h.; Swalls and O'Leary 1975) but decreased under similar conditions in the greenhouse. A decrease in dry weight at high humidity was also found by Burrage (1988) and by Adams and Holder (1992).

The Ca content of cucumber leaves decreased with increasing humidity, the margin being more seriously affected than the central part of the lamina (Bakker and Sonneveld, 1988). When they increased the proportion of cations present as Ca, the response to high humidity was the same but the percentage of Ca

in the leaves was considerably greater. High humidity also reduced the Ca content of tomato leaves, the terminal leaflet being affected more than the remainder of the leaf (Adams, 1991). Calculation of the total amount of Ca (mg) accumulated per leaflet showed an even more marked response to high humidity, as the dry weight was also reduced. The magnitude of the response increased with the period of exposure to high humidity (Adams and Holder, 1992).

The present experiments were designed to study the effects of a wide range of day and night humidities on the accumulation of Ca and dry matter by cucumber leaves. Two levels of applied Ca were included so that any interaction between humidity and Ca supply could be evaluated.

MATERIALS AND METHODS

Plots and treatments

The 16 glasshouse compartments (plots) were arranged in four rows of four.

Experiment A: The experimental design had three strata; compartments formed the main plots for the humidity treatments, double rows of plants formed the split-plots for two levels of applied Ca (180 and 270 mg l^{-1}), and the double rows were divided in half as split-split-plots for the two cultivars Corona and Rebella. A fogging system was used to raise the humidity and venting to reduce it; identical air temperatures were maintained at all humidities. Humidities are expressed throughout as vpd (kPa). Three replicates of a 2 × 2 design of day (D) and night (N) humidities (0.1, 0.3 kPa) were provided and two replicates each of two low humidity combinations (0.3/0.7 and 0.7/0.3 kPa, D and N respectively). Thus, six out of the nine possible combinations of the three day and three night humidity treatments were tested (Figure 1a). Day was defined as the 12 h period between 0600 and 1800 hours. After the treatments were applied in early February, the mean vapour deficits achieved up to the date of the last leaf sample (i.e. over 32 d of treatment) are shown in Table Ia.

Experiment B: A new experimental design was used in which there were two strata: compartments formed the main plots for the humidity treatments as before, and double rows of plants formed the split-plots for the two levels of applied Ca (180 and 270 mg l^{-1}). Only one cultivar, Corona, was grown. In the fractional factorial design, described in detail by Edmondson (1991), eight of the 16 possible treatment combinations were tested (Figure 1b). Two replicates of each treatment were provided. The humidity levels were chosen so that the logs of the vapour pressure deficits gave equally spaced treatments. The nominal humidity treatments and the mean vapour deficits achieved up to the date of the last leaf sample (i.e. over 34 d of treatment) are shown in Table Ib.

Glasshouse procedure

Seeds were sown mid-December and mid-November for Experiments A and B respectively, propagated in rockwool cubes, and transferred to their corresponding final positions on

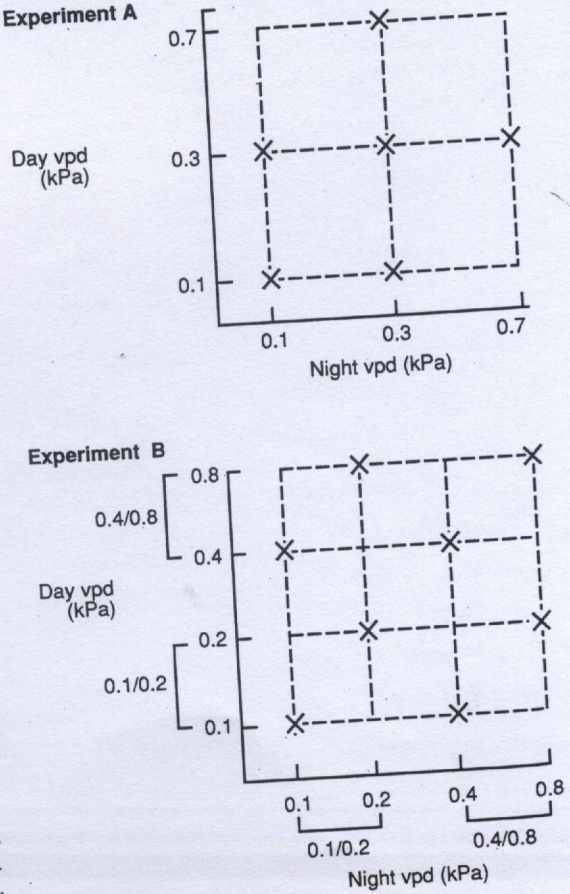

FIG. 1
Treatment matrices for the two experiments showing the humidity combinations tested.

TABLE I

The humidity treatments applied in the two experiments and the actual mean vapour pressure deficits achieved

a) Experiment A

Actual vpd (kPa)	Nominal day/night vpd (kPa) 0.1/0.1	0.1/0.3	0.3/0.1	0.3/0.3	0.3/0.7	0.7/0.3
Day	0.23	0.29	0.34	0.36	0.41	0.55
Night	0.21	0.36	0.24	0.36	0.53	0.39

b) Experiment B

Actual vpd (kPa)	Nominal day/night vpd (kPa) 0.1/0.1	0.1/0.4	0.2/0.2	0.2/0.8	0.4/0.1	0.4/0.4	0.8/0.2	0.8/0.8
Day	0.12	0.14	0.20	0.19	0.26	0.27	0.39	0.40
Night	0.10	0.24	0.18	0.25	0.11	0.25	0.15	0.39

the rockwool slabs in mid-January and early December respectively. Irrigation was applied in relation to solar radiation integrals plus an excess of 50%, which was allowed to run to waste. Equal volumes of nutrient solution were applied to all treatments by trickle irrigation. The glasshouse atmosphere was enriched with carbon dioxide during daylight hours to 1000 vpm.

Sampling and analysis

In both experiments, eight representative leaves (fourth below the head) were collected during the fifth week after the treatments were applied. At the same time the terminal 7 cm was cut from leaves in the same position in Experiment A, and from younger leaves (first below the head) in Experiment B, to represent the tissue furthest from the main stem. In addition, tips were sampled from the fourth leaf below the head 7 d before, and 4 and 14 d after, the treatments were commenced in Experiment A. The tissue was dried at 80°C and then ground to pass a 1 mm sieve. The Ca content of the ashed (560°C) tissue was estimated by atomic absorption spectrophotometry.

RESULTS

Symptoms of Ca deficiency

Yellowing around the leaf margins developed on plants grown at high humidity. When the margins became brown, further expansion of the laminae resulted in cupping of the leaves (Winsor and Adams, 1987). The severity of these symptoms, assessed visually 61 d after planting in Experiment A, increased with the humidity during the day ($P = 0.015$). The same trend (not significant) was found with humidity at night (Table II). The corresponding score for the low humidity levels (0.40) was significantly lower than that of the other treatments ($P = 0.02$). Increasing the concentration of Ca in the nutrient solution from 180 to 270 mg l^{-1} decreased the mean score for all other treatments from 1.00 to 0.84 ($P = 0.05$); this response, though smaller than that to night humidity, was significant as it was based on 32 split-plots. There was no difference between the cultivars in susceptibility to development of the deficiency symptoms.

Ca and dry matter accumulation by the leaves

Experiment A: High humidity decreased the concentration of Ca content in the leaf tips after only four days ($P<0.001$). Continuously high humidity (0.1 kPa vpd) caused the greatest reduction in percentage Ca; high humidity during the night only also depressed Ca content ($P<0.01$), but the response to humidity during the day was not significant (Figure 2). The reason for the sharp decrease in Ca at all humidities in early February was not estab-

TABLE II

Effect of humidity on the incidence and severity of Ca deficiency symptoms, assessed visually after 61 days of treatment; the score increased with the severity and frequency of the symptoms (Experiment A; mean of both cultivars and Ca levels)

Day humidity (vpd, kPa)	Night humidity (vpd, kPa) 0.1	0.3	Mean
0.1	1.67	1.36	1.50*
0.3	0.77	0.59	0.68
Mean	1.21a	0.98a	

SED: *Day humidity, 0.192 (d.f. = 4).

In each table, means of rows or columns that are followed by the same letter do not differ significantly ($P = 0.05$).

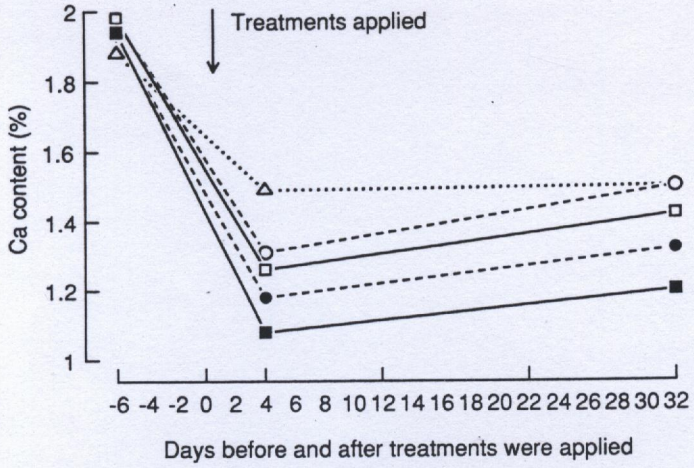

FIG. 2
The concentration of Ca (% of dry matter) in the leaf tips (fourth below the head on each occasion) assessed 6 d before, and 4 and 32 d after the humidity treatments were applied (Experiment A; cv. Corona at 180 mg l^{-1} Ca only). Day/night humidity treatments (vpd, kPa): triangle, mean of 0.7D/0.3N and 0.3D/0.7N; open circles, 0.3D/0.3N; open squares, 0.1D/0.3N; filled circles, 0.3D/0.1N; filled squares, 0.1D/0.1N.

lished. After 32 d of treatment, the responses to humidity were similar to those found after only 4 d, but the difference between the low (0.7/0.3 and 0.3/0.7 kPa) and intermediate (0.3/0.3 kPa) humidity treatments had disappeared. The data presented are for 'Corona' only ('Rebella' had lower Ca contents), and for plants grown at 180 mg l^{-1} only, to show the maximum response to humidity.

Analysis of the complete laminae of leaves sampled after two weeks of treatment confirmed a significant response to high humidity at night ($P<0.01$; Table III). There was no response to the level of humidity during the day. The concentration of Ca in the leaves also increased with the Ca level in the nutrient solution ($P<0.05$), but this response was mainly at 0.3/0.3 kPa. The mean value for the low humidity treatments (0.3/0.7 and 0.7/0.3 kPa), which did not respond to increased Ca in solution, was 1.67% Ca. As the dry weight of the leaves were not recorded, the total Ca content (mg) per leaf could not be calculated.

After 32 d of treatment, the high humidity treatment combinations (0.1 × 0.3 kPa) reduced the dry weight per leaf lamina by 11% compared with the low humidity combinations (0.3 × 0.7 kPa; $P = 0.01$). Increasing the humidity at night decreased the dry weight (Table IV); compared with the low humidity treatments (0.476 g), the reductions were 7 and 15% at 0.3 and 0.1 kPa respectively. Although humidity had no effect on the concentrations of Ca (%) in the laminae, the high humidity treatments (0.1 × 0.3 kPa) reduced the total Ca content (mg) per lamina by 11% ($P<0.05$) when compared with the low humidity treatments (0.3 × 0.7 kPa). Furthermore, the higher (0.1 kPa) and intermediate (0.3 kPa) humidities at night tended to reduce the total Ca content (mg per leaf) by 17 and 6% respectively compared to the low humidity treatments (9.55 mg; $P<0.07$). The Ca concentration (%) in the corresponding leaf tips was decreased similarly (Table IV; 1.58% Ca at low humidity).

TABLE III
Effects of humidity and applied Ca level on the concentration of Ca (% of dry matter) in the laminae of the fourth leaf below the head after 14 days of treatment (Experiment A; cv. Corona only)

		Night humidity, vpd (kPa)		
		0.1	0.3	Mean
Day humidity, vpd (kPa)	0.1	1.23	1.57	1.40a
	0.3	1.38	1.55	1.47a
Applied Ca (mg l^{-1})	180	1.28	1.46	1.37
	270	1.34	1.66	1.50
Mean		1.31	1.56	

SEDs: Day or night humidity, 0.062 (d.f. = 4); Ca level, 0.047 (d.f. = 10).

TABLE IV

Effects of night humidity on the dry weight, Ca content (% of dry matter), and weight of Ca (mg) per leaf in the laminae, and on the Ca content (%) of the leaf tips (fourth leaf on the laterals, counted from the main stem) after 32 days of treatment (Experiment A; means of both cultivars and Ca levels)

	Night humidity (vpd, kPa)		Significance
	0.1	0.3	*P*
Dry weight per leaf (g)*	0.407	0.442	0.05
% Ca*	1.93	1.96	n.s.
Total Ca (mg)*	7.95	8.98	0.07
% Ca in leaf tips	1.33	1.48	0.05

*Laminae only.

The response of the leaf tips to high humidity during the day was not quite significant (1.47 and 1.34% Ca at 0.3 and 0.1 kPa respectively; $P = 0.06$). The dry weights of the tips were not recorded and so the total Ca contents (mg) could not be calculated. However, as the response for percentage Ca in the leaf tips was as large as that for mg Ca per leaf, the response in terms of mg Ca per leaf tip would have been even greater than in the complete laminae, assuming a similar dry matter response.

Considerable differences were found between cultivars. The average dry weight of the leaf laminae from 'Corona' was greater than those from 'Rebella' (21%), as was the concentration of Ca (%) in both the complete leaves and the leaf tips. The leaves of 'Corona' contained 44% more total Ca (mg) than those of 'Rebella' (Table V).

The higher level of Ca in the nutrient solution had no effect on the dry weight of the leaves, but increased the concentration of Ca (%) in the laminae and leaf tips (Table V); the total Ca content per leaf was increased by 17%.

Experiment B: After five weeks of treatment, the concentration of Ca (%) in the complete leaf laminae was decreased equally by high humidity during either day ($P = 0.03$) or night ($P = 0.02$; day x night interaction, $P<0.05$; Table VI). The decrease in dry weight per leaf lamina (5%) due to high humidity during the day was not significant, nor was the corresponding reduction (13%) in the total Ca content (mg) per lamina. The concentration Ca (%) in the leaf tips was reduced by continuously high humidity ($P<0.001$), but the decrease due to high humidity during the day or night only ($P<0.001$ in both cases) was less. In this instance, the interaction between day and night humidity was also significant ($P = 0.02$). The range of values was also greater than those for the complete leaves.

The higher concentration of Ca in solution did not affect the dry weight of the leaves, but increased the concentration of Ca (%) in the complete laminae and leaf tips (Table VII); the total Ca content (mg) of the laminae was increased by 22%.

DISCUSSION

Symptoms of Ca deficiency occurred at high humidity (vpd, 0.21–0.55 kPa; Experiment A), and were most severe where a high humidity (0.21–0.23 kPa) was maintained continuously. The data in Table II show a marked contrast between low humidity during the day with high humidity at night (score, 0.77) and high humidity during the day and low humidity at night

TABLE V

Effects of cultivar and applied Ca concentration on the dry weight, Ca content (% of dry matter) and weight of Ca (mg) per leaf in the laminae and on the Ca content (%) of the leaf tips (fourth leaf on the laterals, counted from the main stem) after 32 days of treatment (Experiment A; means of all humidity treatments)

	Cultivar*		Significance	Applied Ca (mg l^{-1})†		Significance
	Corona	Rebella	*P*	180	270	*P*
Dry weight (g)‡	0.489	0.386	0.001	0.439	0.436	n.s.
% Ca‡	2.28	1.60	0.001	1.79	2.11	0.001
Total Ca (mg)‡	11.23	6.25	0.001	8.04	9.44	0.001
% Ca in leaf tips	1.50	1.41	0.01	1.36	1.54	0.001

*Means of two Ca levels; †Means of two cultivars; ‡ Laminae only.

TABLE VI

Effects of day and night humidity on the concentration of Ca (% of dry matter) in the leaf laminae (fourth below the head) and leaf tips (from first leaf below the head) after 34 days of treatment (Experiment B; cv. Corona only)

Day humidity (vpd, kPa)	Night humidity (vpd, kPa) 0.1/0.2	0.4/0.8	Mean	SED for day or night means
Leaf laminae				
0.1/0.2	1.05	1.08	1.07	0.0937
0.4/0.8	1.06	1.37	1.22	
Mean	1.06	1.23		
Leaf tips				
0.1/0.2	1.01	1.25	1.13	0.0232
0.4/0.8	1.20	1.60	1.40	
Mean	1.11	1.43		

SED for the body of the table: complete leaf, 0.0655; leaf tips, 0.0328.

(score, 1.36), although the 24 h average humidities were virtually identical. These results contrast with those of Bakker and Sonneveld (1988), who found no difference in response to high humidity during the day or night, but suggested that the response depended on the 24 h average. However, their treatments (Experiment I) were in the range 0.37–0.86 kPa , and only the high humidity at night (0.37–0.38 kPa) was within the range of treatments tested here. It is apparent from the data that they were unable to maintain a high humidity during the day (0.54 kPa compared with 0.37 kPa at night under continuously high humidity, and 0.69 kPa in the day for high humidity compared with 0.76 kPa at night for low humidity). Thus, the lower range of humidities used by Bakker and Sonneveld may explain much of the difference in our results.

Previous reports of the incidence of Ca deficiency symptoms on cucumbers grown at high humidities (Bakker *et al.*, 1987; Bakker and Sonneveld, 1988) related the severity of symptoms to the vapour deficit but not to the Ca analyses of the leaves. Unfortunately, in the data reported here, the foliar symptoms were recorded some time after the leaves were sampled. Hence, the symptoms and analyses could not be directly related. However, Bakker and Sonneveld (1988) concluded that Ca deficiency symptoms occur when the Ca content of the leaf margins declined below 2% Ca in the dry matter.

The effect of humidity on the concentration of Ca (%) in complete leaf laminae was generally small. Since humidity may affect dry-matter accumulation, the dry weight of the leaf sampled should be recorded, so that it can be allowed for in the results. For example, no apparent difference in Ca content (%) was found between leaves grown at continuously low or high humidity, but high humidity decreased the *total* amount of Ca (mg) per leaf lamina by 17% (Table IV). Coincidentally, the depression in the Ca content (%) of the leaf tips was of the same order (16%). This suggests that the leaf tips could be useful for rapid assessments of the Ca status of the leaves in relation to humidity. For research purposes, however, particularly when considering the physiological consequences of changes in factors affecting growth (e.g. light, humidity, salinity), the total

TABLE VII

Effect of the concentration of Ca in the nutrient solution on the dry weight, Ca content (% of dry matter) and weight of Ca (mg) per leaf in the laminae (fourth below the head), and on the Ca content (%) of the leaf tips (from first leaf below the head) after 34 days of treatment (Experiment B; cv. Corona only)

	Applied Ca (mg l^{-1}) 180	270	Significance *P*
Dry weight per leaf (g)*	0.329	0.337	n.s.
% Ca*	1.06	1.21	0.01
Total Ca (mg)*	3.37	4.11	0.05
% Ca in leaf tips	1.22	1.31	0.01

*Laminae only.

amount of Ca accumulated is generally more meaningful than the concentration of Ca (%) in the dry matter.

The difference in response between the complete laminae and the leaf tips is also important in assessing the effect of humidity. This difference arises because transport of Ca to the part of the leaf furthest from the main stem is inhibited by high humidity. The uneven distribution of Ca in leaves grown at high humidity was such that the terminal leaflet of tomato always contained a lower concentration and total amount of Ca than the other leaflets (Adams, 1991). As the terminal leaflet contained less than 10% of the total Ca (mg) in the leaf, this response was greatly diluted when all leaflets were combined. The same principle applies to cucumber leaves.

There was a marked difference in the percentage Ca and the total Ca content (mg) of leaves from the two cultivars (Table V); this was greater than that due to any of the treatments. The difference may be associated with leaf size, and should be taken into account when interpreting the data from different cultivars.

The effects of high humidity at night were particularly marked in Experiment A. A similar response was found with tomato (Ho, 1989; Adams and Holder, 1992). Since the cucumber leaves sampled were not fully expanded, cuticular transpiration at night may still have been an important factor in promoting Ca transport into the leaves (Ho, 1989). For example, in controlled environment cabinets, transpiration from young tomato plants during the dark period (12 h) was reduced by 44% at high humidity (0.1 kPa vpd) compared with those at low humidity (0.8 kPa); the corresponding reduction during the light period was 47%. High humidity at night would therefore have been particularly effective in reducing Ca accumulation, as it inhibits both stomatal and cuticular transpiration.

In the data reported here, the effect of high humidity during the day on the Ca content (%) of the leaves was small and not significant in Experiment A, but was similar to that of high humidity at night in Experiment B. This result seems anomalous since the low vapour deficits achieved were similar in both Experiments. However, the total irradiance received during the periods reported on differed markedly; 186 MJ m^{-2} in 32 d in Experiment A compared with 64 MJ m^{-2} in 34 d in Experiment B. Previous work at high humidity by Grange and Loach (1983) showed that transpiration depends mainly on irradiance rather than vapour pressure deficit. This is what would be expected in a glasshouse system where evapotranspiration tends to an 'equilibrium' rate dependent on energy input (Jarvis and McNaughton, 1986). Thus, the plants in Experiment A would have transpired at a higher average rate during the day than those in Experiment B. Since Ca movement into the leaves is closely related to the rate of transpiration, the difference in irradiance between the Experiments may explain the smaller response to high humidity in Experiment A.

The authors wish to thank Mr R. Edmondson for advising on the statistical design of the experiments and for undertaking the analysis of the data. Support for this work by MAFF and DES is gratefully acknowledged.

REFERENCES

ACOCK, B., CHARLES-EDWARDS, D. A. and HAND, D. W. (1976). An analysis of some effects of humidity on photosynthesis by a tomato canopy under winter light conditions and a range of carbon dioxide concentrations. *Journal of Experimental Botany*, **27,** 933–41.

ADAMS, P. (1991). Effect of diurnal fluctuations in humidity on the accumulation of nutrients in the leaves of tomato (*Lycopersicon esculentum*). *Journal of Horticultural Science*, **66,** 545–50.

ADAMS, P. and HOLDER, R. (1992). Effects of humidity, Ca and salinity on the accumulation of dry matter and Ca by the leaves and fruit of tomato (*Lycopersicon esculentum*). *Journal of Horticultural Science*, **67,** 137–42.

ARMSTRONG, M. J. and KIRKBY, E. A. (1979). The influence of humidity on the mineral composition of tomato plants with special reference to calcium distribution. *Plant and Soil*, **52,** 427–35.

BAKKER, J. C. (1989). The effects of air humidity on growth and fruit production of sweet pepper (*Capsicum annuum* L.). *Journal of Horticultural Science*, **64,** 41–6.

BAKKER, J. C. (1990). Effects of day and night humidity on yield and fruit quality of glasshouse tomatoes (*Lycopersicon esculentum*). *Journal of Horticultural Science*, **65,** 323–31.

BAKKER, J. C. and SONNEVELD, C. (1988). Calcium deficiency of glasshouse cucumber as affected by environmental humidity and mineral nutrition. *Journal of Horticultural Science*, **63,** 241–6.

BAKKER, J. C., WELLES, G. W. H. and VAN UFFELEN, J. A. M. (1987). The effects of day and night humidity on yield and quality of glasshouse cucumbers. *Journal of Horticultural Science*, **62,** 363–70.

BURRAGE, S. W. (1988). Growth and ion uptake in tomatoes grown in high and low humidities. In: *The effects of high humidity on plant growth in energy saving greenhouses* (Cockshull, K. E., Ed.). Report EUR 11261, Office for Official Publications of the European Communities, Luxembourg, 9–18.

COCKSHULL, K. E., GRAVES, C. J., HAND, D. W., ADAMS, P., GRANGE, R. I. and HO, L. C. (1988). The effects of humidity on growth and nutrient composition of tomatoes. *Report of the Glasshouse Crops Research Institute for 1986–87*, 76–7.

EDMONDSON, R. N. (1991). Agricultural response surface experiments based on four-level factorial designs. *Biometrics*, **47,** 1435–48.

GRANGE, R. I. and LOACH, K. (1983). Environmental factors affecting water loss from leafy cuttings in different propagation systems. *Journal of Horticultural Science*, **58,** 1–7.

HO, L. C. (1989). Environmental effects on the diurnal accumulation of ^{45}Ca by young fruit and leaves of tomato plants. *Annals of Botany*, **63,** 281–8.

HOFFMAN, G. J. (1979). Humidity. In: *Controlled environment guidelines for plant research* (Tibbits, T. W. and Kozlowski, T. T., Eds). Academic Press, London. 146–55.

HOLDER, R. and COCKSHULL, K. E. (1990). Effects of humidity on the growth and yield of glasshouse tomatoes. *Journal of Horticultural Science*, **65,** 31–9.

JARVIS, P. G. and MCNAUGHTON, K. G. (1986). Stomatal control of transpiration: scaling up from leaf to region. *Advances in Ecological Research*, **15,** 1–49.

MENGEL, K. and KIRKBY, E. A. (1987). Chapter 11, Calcium. In: *Principles of plant nutrition*. International Potash Institute, Bern, 461–6.

SWALLS, A. A. and O'LEARY, J. W. (1975). The effect of relative humidity on growth, water consumption, and calcium uptake in tomato plants. *Journal of the Arizona Academy of Science*, **10,** 87–9.

(Accepted 4 February 1993)

Journal of Horticultural Science (1993) **68** (5) 775–781

Peroxidase isoenzyme genes in the identification of apple cultivars and *Malus* species

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SUMMARY

The peroxidase genotypes of 188 apple cultivars, rootstocks and *Malus* species were recorded. Four loci, **PRX-2**, **PRX-3**, **PRX-4** and **PRX-7** were involved including 15 alleles. Using this information a clear grouping of 83 cultivars, 46 rootstocks and 59 species was achieved through knowledge of the genetic basic of banding patterns, which consequently enabled secondary and inconsistent bands to be recognised and disregarded as relevant factors in identification. The possible selective advantage of the **PRX-2e** allele recognised in descendants of 'Jonathan' (**PRX-2ce**) is discussed. Triploid cultivars showed three different **PRX-2** alleles including 'Ashmead's Kernel' (**PRX-2bce**), providing further evidence of the triploid nature of this cultivar.

PEROXIDASES (PRX; E.C.1.11.1.7) are one of the most widely investigated enzyme systems, being highly variable within higher plants. Peroxidases are highly polymorphic in many genera. Electrophoretic analysis is used to produce zymograms which show discrete bands representing isoenzymes, the products of distinct alleles. Peroxidases are usually characterized as monomeric enzymes and by the occurrence of null alleles. Often the electrophoretic pattern is complicated, this complexity being accentuated by wide differences that can occur between handling patterns in different tissues and at different stages of development.

In spite of these problems phenotypic comparisons of banding patterns observed in zymograms of peroxidase have been used for the identification of apple cultivars and rootstocks (Misic *et al.*, 1980a; Mendendez *et al.*, 1986a and b; Vinterhalter and James, 1983, 1986; Quarta and Arone, 1987). Misic *et al.* (1980b), Chevreau and Laurens (1987) and Bournival and Korban (1987) proposed genetic models determining peroxidase isozymes in apple and recent work (Manganaris and Alston, 1992) has further clarified the genetics of PRX in *Malus*. Six anodal and two cathodal zones of PRX activity are controlled by at least eight genes (**PRX-1–8**); analysis of four (**PRX-2**, **PRX-3**, **PRX-4** and **PRX-7**) revealed 15 alleles including three null alleles. In this paper a reliable approach to the use of peroxidase zymograms in the identification of apple cultivars and *Malus* species is described which is based solely on these genes.

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MATERIALS AND METHODS

Various tissues were used in the preparation of samples including leaves, bark, flower buds and roots. Samples were collected from plants of different ages throughout the year from the gene banks of Horticulture Research International at East Malling and the National Apple Collection at Faversham, UK. The techniques of sample preparation and electrophoresis employed were those for peroxidases described by Manganaris and Alston (1992).

RESULTS

Genetically identical plants yielded the same peroxidase patterns for the key bands in both young and old plants; these bands were not tissue specific.

The genotypes for **PRX-2**, **PRX-3** and **PRX-7** for 83 cultivars are presented in Table I, for **PRX-2** and **PRX-3** in rootstocks in Table II, and for **PRX-2**, **PRX-3**, **PRX-4**, and **PRX-7** for *Malus* species in Table III. Genotypes for