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CHEMICAL INVESTIGATIONS ON BITTER PIT OF GRANNY SMITH APPLES

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SUMMARY

Spraying Granny Smith apple trees on 12 occasions after flowering with solutions of calcium chloride and calcium nitrate resulted in a marked reduction in the incidence of bitter pit. Calcium concentration in the fruit fell continuously throughout the growing season but there was a progressive increase in total calcium per fruit. Spraying had no effect on the calcium levels in fruit or leaves or on fruit weight, fruit diameter or firmness. Calcium nitrate sprays did not affect the nitrogen level of the fruit.

The results indicate that control of bitter pit is not effected by correction of a simple calcium deficiency. Pit reduction due to treatment was associated with higher fruit weight loss during cool storage, suggesting that bitter pit control may result from a physical effect of the sprays.

I. INTRODUCTION

Since Garman and Mathis (1956) reported that calcium was a critical element associated with bitter pit incidence in apples, investigators throughout the world have studied the effect of orchard sprays containing calcium salts in controlling the incidence of the disorder. The results of this research have recently been summarized by the Agricultural Research Service of the United States Department of Agriculture (Anon. 1965). Despite considerable variation in calcium salts used, their concentration, time of application and number of sprayings, complete eradication of bitter pit has not been reported by any investigator. In addition, the role of these sprays in reducing bitter pit incidence is not fully understood.

The experiments reported here were conducted to obtain information on the chemical and physical effects of orchard sprays of calcium chloride and calcium nitrate on Queensland-grown Granny Smith apples in an endeavour to elucidate how control of bitter pit is achieved and the cause of variability in this control.

II. METHODS AND MATERIALS

A block of 16 Granny Smith apple trees on the Granite Belt Horticultural Research Station was selected in 1964 for the purpose of these experiments. These trees were planted in 1951 with a 20 ft x 20 ft spacing. Of the 16 trees selected, 12 had a history of high bitter pit incidence and the remaining four a history of low bitter pit incidence. Date of full blossom of the trees was October 7, 1964, The experimental block was divided into four sections, each section consisting of three trees with a known high bitter pit history and one tree with a known low bitter pit history.

Treatments were applied as follows:—

(a) Untreated control, consisting of one tree in each of the four sections with a known high bitter pit incidence history.

(b) Untreated control, consisting of one tree in each of the four sections with a known low bitter pit incidence history.

(c) Calcium chloride sprays containing 5 lb/100 gal at a rate of 200 gal/ac applied to one tree in each of the four sections.

(d) Calcium nitrate sprays containing 6 lb/100 gal at a rate of 200 gal/ac applied to one tree in each of the four sections.

To aid wetting and to ensure adequate spray coverage, X77 detergent at the rate of 1½ ml/gal was added to each spray.

Spraying was carried out for the first time on October 28, 1964, and was repeated at fortnightly intervals until March 30, 1965. A total of 12 spray applications was made to the experimental trees throughout the season.

In studying the effect of calcium sprays on fruit size, fruit weight, leaf calcium, fruit calcium and skin calcium during the season, the following procedures were adopted.

Samples of five fruit were taken at random from each tree in each treatment. The first picking was made on October 28, 1964, the first occasion on which the trees were sprayed but prior to actual spraying. A further set of samples were picked on November 4, 1964, seven days later and thereafter at 14-day intervals, with the last samples being picked on April 22, 1965. A total of 14 sets of samples was harvested from the experimental trees. After picking, each fruit was weighed and the mean equatorial diameter measured. A transverse equatorial disc, approximately 15-20 mm thick, was cut from each fruit and a number of cylinders of tissue removed with an 8-mm cork borer from the cortex for calcium determinations. The weight of these tissue samples was 4-6 g. This procedure was followed with all pickings except the first and second, where, because of the smallness of the fruit, the samples for analyses were obtained by peeling and removing seeds, with the remaining tissue being used for calcium determination. Calcium was determined in these tissue samples by the method of Williams and Twine (1960), as slightly modified by R. C. Menary (unpublished data), using an Eel flame photometer. Since Martin, Lewis, and Cerny (1960) found in a study of the relationship between calcium and bitter pit in the Cleopatra variety that conversion from dried weight to fresh weight did not affect comparisons of calcium between treatments, fresh tissue weight was used for all determinations and calculations in these investigations. The calcium status of the fruit from each tree was deduced by taking the mean of the five estimations and that of each treatment from the mean of the four trees in each treatment.

For the determination of skin calcium, the sample for analysis was obtained by peeling the equatorial disc used for tissue sampling with a potato-peeler. Skin calcium was estimated in five pickings of fruits harvested at fortnightly intervals from February 25, 1965, onwards. The method of analysis was the same as above. Leaf samples for calcium determinations were taken from each tree on February 25, 1965. Each sample consisted of the sixth and seventh leaf from the apex of a leader from each of five different leaders selected at random on each tree, a total of 10 leaves from each tree. Each sample of leaves was separated at random into two batches each of five leaves and calcium was estimated separately on each of the two batches. The mean of the two determinations was then taken.

The following procedures were used in studying the effect of calcium sprays on bitter pit incidence, fruit firmness, loss in weight during storage, fruit nitrogen content and the calcium content of core tissue, wax free skin and tissue adjacent to skin, of fruit picked at a recommended picking date for overseas export of this variety.

On March 2, 1965, the day the tenth spray application was due, 50 fruit were harvested from each tree of each treatment prior to spraying. This picking date was selected as it is in a period during which this variety is particularly susceptible to bitter bit, as shown by Stevenson (1962) and Stevenson and Carroll (1963). After picking, each batch of fruit was weighed, stored at 32°F for 8 weeks, removed to 70°F for 7 days, and finally weighed and inspected for bitter pit incidence. Firmness was measured on 10 fruit from each tree in each treatment with a Magness penetrometer, using the 7/16 in. plunger. Two readings were made on opposite sides of each fruit and the mean of the 20 readings recorded. Total nitrogen of the cortical tissue was estimated on tissue samples, obtained in the same way as that used for calcium estimations, by the standard Kjeldahl method on five fruit selected at random from each batch of fruit remaining after the fruit affected by bitter pit had been removed. In addition, cores were removed from these fruit with a 25-mm cork-borer, the seeds were removed and calcium was estimated as outlined above. Calcium was also estimated on a slice of tissue taken from the equatorial disc from the above fruit after the skin had been removed. This slice of tissue was 1 mm thick and was about 1 mm inside the fruit from the junction of the skin and flesh.

A further five fruit were selected at random from each batch of fruit, and after weighing were dipped in hexane for 3 min. The fruit was then washed with hexane, dried and the amount of wax removed calculated. A sample of skin was removed from the equator of each fruit and the calcium content determined.

III. RESULTS

The results are summarized in Tables 1 and 2. Table 1 shows that a number of changes occurred in the fruit with increase in time after fruit set, with these changes generally occurring independently of treatment. Fruit weight increased in all treatments, following a similar pattern, and was still increasing when the final picking was made on April 22, 1965. Calcium sprays appeared to have no effect on fruit weight at any stage during the growing season or on the final weight attained by the fruit. A similar effect was encountered with fruit diameter. The calcium content of the tissue, expressed in parts per million, decreased as the season progressed, but when these results were converted arithmetically to milligrams/fruit there was a seasonal increase in the amount of calcium present in the fruit. Skin calcium dropped slowly during the period studied and the results suggest that the calcium content of the skins of the sprayed fruit was slightly higher than that from the unsprayed controls.

The results in Table 2 show a marked decrease in bitter pit in fruit from the sprayed trees. This decrease was highly significant. However, the amount of bitter pit present in fruit from the unsprayed trees with a low bitter pit history was also significantly lower than in the control trees, and there were no significant differences in bitter pit incidence between fruit from the sprayed trees and fruit from those trees with a low bitter pit history. No significant differences in calcium content were found between treatments in either leaves or tissue adjacent to the skin of the fruit. Core calcium in control fruit from trees with a known history of low bitter pit incidence was significantly higher than in fruit from either the trees sprayed with calcium nitrate or the untreated controls with a known history of high bitter pit incidence, but was not significantly higher than in fruit from the calcium chloride treated trees, although the difference in this case was almost significant at the 5% level. When fruit from the experimental trees was dipped in hexane for 3 min to remove cuticle wax, there was significantly more calcium present in the skins of fruit from trees sprayed with calcium chloride than in fruit from the untreated control trees with a known high bitter pit incidence. There were no significant differences in weight loss between treatments after dipping in hexane, or in the nitrogen content of the cortical tissue of the fruit. Fruit from all treatments was firmer than that from the controls with a known high bitter pit incidence, but the differences were only significant with respect to fruit from the calcium chloride sprayed trees and that from trees with a known low bitter pit history. After storage for 8 weeks at 32°F, followed by 7 days at 70°F, weight loss in fruit from the sprayed trees was higher than that in fruit from the controls, but the difference was significant only with respect to fruit from trees sprayed with calcium chloride.

TABLE 1

CHANGES IN MEAN FRUIT WEIGHT, MEAN FRUIT DIAMETER AND CALCIUM CONTENT OF FRUIT AND SKINS BETWEEN TREATMENTS AND HARVESTING DATES

Date of Harvest	Days from Full Blossom	Treatment a					Treatment b				
		Mean Weight (g)	Mean Diameter (mm)	p.p.m. Ca in Tissue	mg Ca per Fruit	p.p.m. Ca in Skins	Mean Weight (g)	Mean Diameter (mm)	p.p.m. Ca in Tissue	mg Ca per Fruit	p.p.m. Ca in Skins
28.x.64	21	2·2	15·7	336	0·6	. .	2·4	16·4	285	0·7	. .
4.xi.64	28	3·2	18·1	280	1·0	. .	2·9	17·1	321	0·9	. .
18.xi.64	42	9·5	24·3	139	1·2	. .	9·4	25·8	126	1·1	. .
2.xii.64	56	26·8	38·1	72	1·9	. .	24·9	37·3	74	1·8	. .
16.xii.64	70	43·6	45·3	62	2·7	. .	38·4	43·5	71	2·7	. .
30.xii.64	84	55·1	49·2	53	2·9	. .	52·9	48·8	60	2·9	. .
13.i.65	98	67·7	53·0	59	3·9	. .	64·1	52·6	60	3·8	. .
27.i.65	112	79·5	56·0	49	3·8	. .	69·7	54·0	55	3·8	. .
10.ii.65	126	97·7	59·7	44	4·1	. .	88·2	58·5	48	4·2	. .
25.ii.65	141	101·0	60·3	41	4·1	98	97·6	59·5	45	4·4	108
10.iii.65	154	116·5	61·8	36	4·2	94	106·6	60·9	37	3·9	109
25.iii.65	169	128·4	65·5	37	4·7	100	111·3	62·6	34	3·8	93
8.iv.65	183	131·6	65·1	29	3·8	76	128·6	66·0	32	4·1	83
22.iv.65	197	155·0	69·6	34	5·3	75	127·1	66·7	35	4·7	82

TABLE 1—continued

CHANGES IN MEAN FRUIT WEIGHT, MEAN FRUIT DIAMETER AND CALCIUM CONTENT OF FRUIT AND SKINS BETWEEN TREATMENTS AND HARVESTING DATES—*continued*

Date of Harvest	Days from Full Blossom	Treatment c					Treatment d				
		Mean Weight (g)	Mean Diameter (mm)	p.p.m. Ca in Tissue	mg Ca per Fruit	p.p.m. Ca in Skins	Mean Weight (g)	Mean Diameter (mm)	p.p.m. Ca in Tissue	mg Ca per Fruit	p.p.m. Ca in Skins
28.x.64	21	2·4	16·3	316	0·7	..	2·2	15·6	322	0·7	..
4.xi.64	28	3·3	17·6	329	1·1	..	3·6	18·1	271	1·0	..
18.xi.64	42	10·1	26·2	163	1·4	..	9·4	25·9	138	1·2	..
2.xii.64	56	26·4	38·2	73	2·0	..	27·4	38·7	72	2·0	..
16.xii.64	70	40·9	44·5	71	2·9	..	39·9	44·4	64	2·6	..
30.xii.64	84	51·9	48·0	62	3·2	..	52·6	48·4	56	2·9	..
13.i.65	98	65·0	52·1	66	4·0	..	68·0	53·0	59	4·0	..
27.i.65	112	70·9	54·0	59	4·1	..	81·2	56·8	50	4·0	..
10.ii.65	126	83·0	56·8	46	4·0	..	95·1	58·5	43	3·9	..
25.ii.65	141	93·2	58·2	50	4·5	126	102·0	60·9	40	4·1	109
10.iii.65	154	103·9	60·8	43	4·3	122	121·1	63·4	37	4·5	101
25.iii.65	169	111·5	60·9	44	4·7	137	119·5	62·6	36	4·3	126
8.iv.65	183	114·9	61·9	37	4·1	109	135·8	66·0	33	4·4	99
22.iv.65	197	129·1	65·0	40	5·0	105	142·2	67·3	37	5·2	99

TABLE 2

MEAN RESULTS OF INSPECTION AND DETERMINATIONS ON FRUIT AFTER 8 WEEKS' STORAGE AT 32°F

Item	Treatment a	Treatment b	Treatment c	Treatment d
Bitter pit (%)	7·5	1·5	0·5	0·5
Ca in core (p.p.m.)	90	109	97	90
Ca in hexane-dipped skins (p.p.m.)	108	125	142	119
Ca in tissue below skin (p.p.m.)	42	47	48	46
Percentage wax removed by hexane dip	0·077	0·064	0·075	0·079
Percentage weight loss after cool storage	1·46	1·47	1·77	1·64
N2 in cortical tissue (mg/100g)	43	40	47	48
Fruit firmness (lb)	15·86	17·11	17·51	16·82
Ca in leaves (p.p.m.)	3,991	4,498	4,085	4,269

Date of harvest Mar. 2, 1965 (148 days from full bloom) for all determinations except Ca in leaves (date of harvest Feb. 2, 1965, 142 days from full bloom).

Percentage bitter pit.—Treatment a significantly greater than treatments b, c, d (1% level).

Ca in core (p.p.m.).—Treatment b significantly greater than treatments a and d (1% level).

Ca in hexane-dipped skins (p.p.m.).—Treatment c significantly greater than treatment a (1% level).

Ca in tissue below skin (p.p.m.).—No significant differences.

Percentage wax removed by hexane dip.—No significant differences.

Percentage weight loss after cool storage.—Treatment c significantly greater than treatments a and b.

N2 in cortical tissue (mg/100g).—No significant differences.

Fruit firmness (lb).—Treatment c significantly firmer than treatment a (1% level). Treatment b significantly firmer than treatment a (5% level).

Ca in leaves (p.p.m.).—No significant differences.

IV. DISCUSSION

Martin, Lewis, and Cerny (1965), in a study of the effect of spray treatments of calcium, barium and strontium nitrates on the Cleopatra, Cox's Orange Pippin, Sturmer and Democrat apple varieties, showed that these salts had no effect on fruit weight. The Queensland investigations gave a similar result with respect to fruit weight and fruit size of Granny Smith apples sprayed with either calcium chloride or calcium nitrate. Falls in the calcium content of cortical tissue with increase in fruit maturity similar to those found in these investigations have been reported in the Cox's Orange Pippin variety by Askew *et al.* (1959) in New Zealand and Wilkinson and Perrin (1964) in Great Britain. In addition, these investigations and those of Wilkinson and Perrin (1964) show that the decrease in calcium is due partly to a dilution effect from growth of the fruit, but this is partly offset by gain in total calcium per fruit, since when calcium content is converted arithmetically to milligrams/fruit there is an increase in calcium per fruit throughout the season.

Repeated application of sprays containing calcium did not give detectable differences in cortical tissue calcium between treated and untreated trees, nor were there any detectable differences in fruit from trees with high or low bitter pit incidence histories. Askew *et al.* (1959) also found no consistent differences between the calcium content of fruit from sprayed and unsprayed trees. However, Martin (1965) reported increased calcium in the cortex of treated fruit. It has been clearly shown by Martin (1961) and Millikan and Hanger (1965) that calcium salts applied to fruit enter the tissue. The former author reported that when $Ca45Cl_2$ was painted on the fruit, Ca45 spread through the flesh by vascular bundles, was passed by the pedicel of the fruit into the spurs and spur leaves and even moved into the leaves of the branch. This movement of calcium from the fruit to the tree could therefore account for the failure to detect increased calcium in the fruit from trees sprayed with solutions containing calcium salts. Nevertheless, in this as in other reported investigations, the use of sprays containing calcium salts significantly reduced the incidence of bitter pit in fruit from the treated trees.

It is not possible from the data obtained in this investigation to consider the control of bitter pit by using calcium sprays to be the result of the correction of a simple calcium deficiency in the fruit. This does not preclude the possibility that the mode of control is a chemical one, but difficulties with tissue sampling and existing analytical techniques make a study of the presence and changes in micro-amounts of calcium in fruit virtually impossible.

D. Martin (private communication 1965), in a discussion on the mode of action of calcium sprays, has suggested that their role may be to control bitter pit susceptibility by purely physical means such as affecting sites and rates of water loss. In these investigations, calcium sprays resulted in greater weight loss by fruit during subsequent cool storage (significant at the 5% level for calcium chloride sprays only) and this was associated with a significant reduction in the incidence of the disorder. However, unpublished work by the author with untreated fruit showed a marked increase in bitter pit with increase in fruit weight losses resulting from storage under a range of relative humidities. The relationship between bitter pit incidence and weight loss requires further study with particular reference to the effect of calcium sprays, since it must be regarded as extremely interesting that the two most effective calcium salts in reducing the incidence of the disorder are the chloride and the nitrate, both of which are highly hygroscopic. The significance of hygroscopicity and its effect on fruit weight loss and bitter pit incidence warrants further study.

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