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Penetration of surface-applied ^{45}Ca into apple fruit†

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SUMMARY

The uptake of ^{45}Ca applied to the skin of apple fruit was examined in the laboratory with regard to the practice of spraying with calcium solutions to prevent bitter pit.

The speed of penetration of ^{45}Ca from a drop on the fruit surface was found to be much higher under conditions of 50–80% relative humidity of the air than at saturation. Evaporation of the drop apparently strongly influenced the absorption of Ca into the apple. Penetration was faster in cv. Cox's Orange Pippin than in James Grieve. The Ca which entered the fruit remained either in the skin or immediately beneath it.

When ^{45}Ca was applied more than once, the subsequent treatments, especially those applied at high relative humidity, were less effective. It is concluded that for practical purposes it would be advisable to spray repeatedly when the relative humidity is low.

To reduce the incidence of the physiological disease bitter pit in different varieties of apple, it has for some time been the practice to spray the trees with solutions of Ca salts in water (Baxter, 1960; Martin *et al.*, 1965; van der Boon *et al.*, 1968; and many other authors). A reduction in bitter pit has also been obtained by dipping apples in Ca solutions after picking (Bangerth, 1970). As the results of spray treatments in orchards have been inconclusive, various laboratories conducted research on the uptake of Ca by the fruit after spraying. In these experiments the radioisotope ^{45}Ca has often been used to obtain data concerning the effect of point of application on the penetration of calcium, on the way in which it is transported in the fruit, and on the speed of absorption.

Schumacher *et al.* (1966) and van der Boon and Das (1969) showed that very little Ca is transported from leaf to fruit. A basipetal movement of Ca from one leaf to another could only be induced by treating the first leaf with chelating agents or acids (Millikan and Hanger, 1965*a*). This means that Ca deposited on the fruits by the spray treatments is particularly important.

Most of the data on penetration into the fruit are qualitative and difficult to compare as they were obtained under different experimental conditions. It may be concluded from various publications that, in general, Ca penetration into young apples extends to about 1 cm, and into older apples on the tree to not more than a few mm under the skin. This is also true for picked apples after a penetration time of a few months. Penetration into the fruit through the calyx and the side of the peduncle is somewhat more rapid (Millikan and Hanger, 1965*b*; Martin and Lewis, 1967; Wieneke, 1967, 1968).

† Dedicated to Prof. Dr H. Veldstra on the occasion of his retirement as Professor of Biochemistry at the University of Leyden on 29th September, 1972.

Little quantitative research has been done on the amount of Ca absorbed by the apple in relation to the time it takes to penetrate. Wieneke (1968) experimented with Cox's Orange Pippin and found that after 10 days 7–20% of the Ca applied to the skin of nearly ripe apples had penetrated to the underlying tissues. From the results of Bangerth (1970), who treated Jonathan apples after harvest, it may be calculated that after 12 days about 10% of the applied Ca had penetrated into a layer 1 cm thick under the skin.

The effect of atmospheric humidity on penetration was investigated by Bangerth (1970) and by Martin and Lewis (1967). They found a higher rate of penetration into the tissue under the skin at a relative humidity of 85–90% than at lower humidities.

The use of higher Ca concentrations or repetition of the spray treatments has been shown further to decrease the incidence of bitter pit (Schumacher and Fankhauser, 1966; Bangerth, 1970). Millikan and Hanger (1965*b*) also found that penetration of Ca increased with the concentration of the solutions applied.

Theoretically the behaviour of Ca penetrating an apple is difficult to explain. The Ca will largely have to pass through an intact skin. This skin contains, among other things, wax, in which strongly lipophylic compounds such as paraffins and esters are found (Martin and Juniper, 1970). Ions such as Ca^{2+}, which are strongly hydrophylic, will pass through with difficulty. The solubility in lipophylic substances is a determining factor in penetration (Darlington and Cirulis, 1963). The cuticle may also be viewed in part as an ion-exchanger. In the tomato cuticle, for instance, fixation of Ca was found to occur, especially on the inner side (Yamada *et al.*, 1965). The process of penetration through the skin is probably a combination of diffusion, dissolution in the organic layer, exchange phenomena with the negatively charged groups in the cuticle as counter-ions and penetration through lenticels and small cracks. In view of these mechanisms it is reasonable to assume that there will be a positive relationship between penetration of Ca and its concentration on the skin surface.

We conducted experiments to investigate: (i) the speed of penetration of Ca into the skin and deeper layers; (ii) the influence of the relative humidity of the air on this rate; and (iii) the influence on penetration of repeated application of Ca.

MATERIALS AND METHODS

Plant material

The experiments were made with the apple cultivars Cox's Orange Pippin, James Grieve, Laxton's Superb and Granny Smith (*Malus silvestris* Mill.). For the study we used (a) detached apples in desiccators, (b) apples on branches in flasks with a nutrient solution and (c) apples on trees growing in soil in 25-litre pots. The Cox, Laxton and Granny Smith apples generally had a diameter of 65–75 mm. The Cox apples on branches in flasks measured 55–75 mm, and the James Grieve apples in the desiccators 70–80 mm and in pots 60–80 mm.

Atmospheric conditions

For the study on detached apples (a) the desiccators were filled with water and anhydrous $CaCl_2$ respectively; the temperature was 20–30 °C, and the relative humidity was 100% under the wet conditions and 50–80% under the dry. The branch experiments (b) were performed in the radiochemical laboratory at a temperature of 20–30 °C and a relative

humidity of 35–70%. The experiment with fruit on the tree (c) was made early in June in a glasshouse at a temperature of 20–30 °C and a relative humidity of 40–50%.

Penetration experiments

A 10 μl drop of solution containing 1% $Ca(NO_3)_2$ in water and 0.05% of a surfactant (an alkylphenolpolyglycolether) was applied with a micropipet to one side of the apple. The drop contained 0.1 μCi ^{45}Ca and had a pH of about 5. In other experiments with Cox's Orange Pippin the application of Ca was repeated respectively on day 1, days 1 and 2, and days 1, 2, 3 and 4 after the first application on day zero. These experiments then lasted 2, 3 and 7 days, repectively. In another experiment a piece of skin with a diameter of 5 mm was removed with a cylindrical borer before applying the Ca solution.

At the end of each experiment the remainder of the applied drop was wiped away with filter paper. A cylinder 18 mm in diameter was then cut out with a corkborer around the place of application down to the core of the apple. For a period of penetration of 5 or more days the diameter of the cylinders was 25 mm.

For determining the total quantity which had penetrated, the layers 0–5 and 5–10 mm of the apple cylinder (T 1 and T 2) were used. For estimating the penetration into the tissue under the skin of the apple the skin was cut away and then two layers of 0–5 and 5–10 mm (t1 and t2) were cut from the cylinder.

The pieces of apple tissue were washed twice for one minute in a 1% solution of $Ca(NO_3)_2$ (50 ml per four samples) and once for one minute in water; this treatment removed the remaining unadsorbed Ca and some exchangeable Ca. Finally the tissue was dried on filter paper.

Ashing and the determination of ^{45}Ca

The pieces of apple tissue were ashed in glass vials for 12 hours at 500 °C. The ash was taken up in 3 ml of N-HCl, after which 8 ml of a scintillation mixture were added. The scintillation mixture of Bruno and Christian (1961), modified according to Ringoet and De Zeeuw (1968), consisted of a mixture of dioxane and 2-methoxyethanol 5:1 to which 5 g diphenyloxazole and 50 g naphthalene were added per litre.

A Philips liquid scintillation counter PW 4510 was used, a correction for quenching being applied by counting in different channels. Penetration was calculated as a percentage of the total quantity applied to the apple. No radioactivity was found outside the pieces of tissue used for the determinations. The determinations were generally repeated eight times, and the standard deviation was calculated.

Autoradiography

For these experiments, cylinders were cut from the apple to a depth of 1 cm and washed as already described. From the central part of the cylinders, slices about 2 mm thick were cut at right angles to the surface of the apple skin. These were dried first in petri-dishes at 105 °C and then between filter paper at the same temperature. Finally the slices were fixed on paper, covered with a thin plastic sheet and laid upon Kodak X-ray film NS-2T for 2–20 days; the film was then developed.

RESULTS

The total penetration of ^{45}Ca, the penetration into the layers under the skin, the total penetration after removing parts of the skin, and the penetration following repeated application of ^{45}Ca are described below.

Total penetration of Ca in the apple

In Figure 1 are shown the percentages of Ca adsorbed by the apples after different periods of time. It is apparent from Figure 1a and 1b that penetration was much more rapid under dry than under humid conditions. At low relative humidity the drops dried within 1–2 hours, whereas at high humidity they generally remained until the end of the experiments. The difference in adsorption rate between the two treatments occurred mainly during the early stages, when the drops above the $CaCl_2$ evaporated, and shortly after that period.

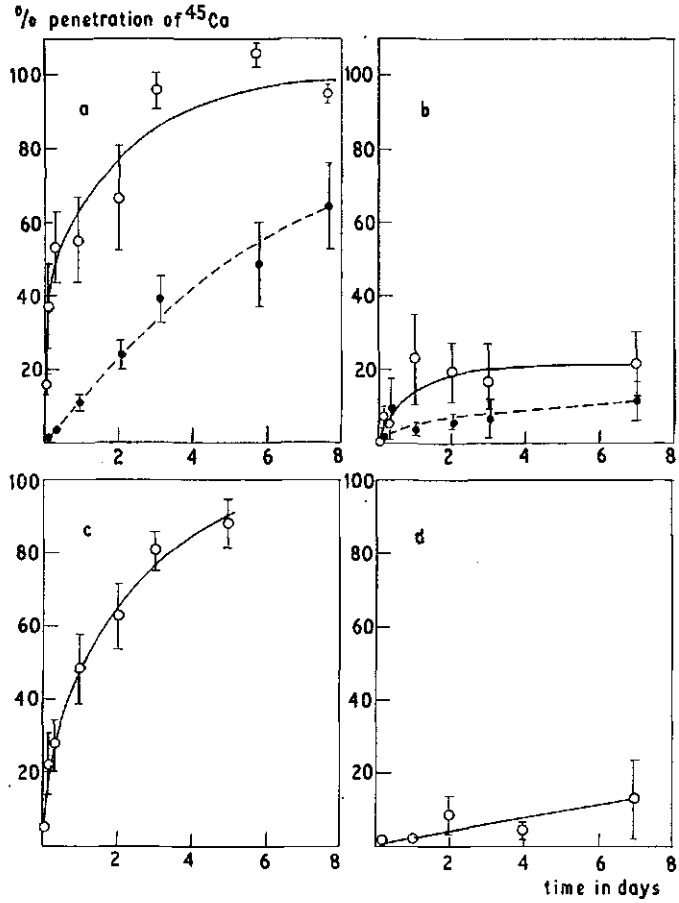

FIG. 1

Total absorption of ^{45}Ca by fruits of (a) Cox in desiccators, (b) James Grieve in desiccators, both under dry (○) and humid (●) conditions, (c) Cox on branches, and (d) James Grieve on trees in pots.

Figures 1a and 1b and Table I show that, in these trials, the penetration of Ca into the apple proceeded much more rapidly in Cox than in James Grieve during the first few hours after application.

TABLE I

Total uptake of ^{45}Ca in % after 12 hours

		Detached apples	Apples on branches on tree
Cox	dry	54	35
	wet	5	—
James Grieve	dry	9	2
	wet	3	—

The behaviour of Cox apples on branches and of apples in desiccators under dry conditions was generally similar (Fig. 1c). For James Grieve adsorption was somewhat lower in fruit on the tree than in fruit in a desiccator at low humidity (Fig. 1d).

Penetration into the tissue under the skin

From Figure 2, in which the curves are almost linear, it is apparent that relatively little Ca penetrated into the tissue under the skin. In Cox, only 1% of the applied radioactivity reached this tissue in 12 hours under dry conditions. Under humid conditions penetration through the skin was even slower, no ^{45}Ca was found under the skin until 2 days after application. In the deeper layers t2 and T2 activity never exceeded 1% of the amount applied.

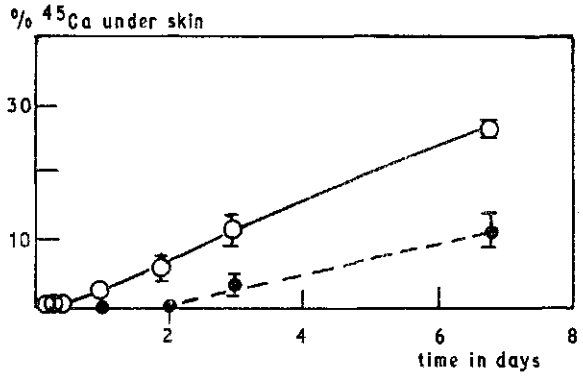

FIG. 2

Penetration of applied ^{45}Ca into the tissue under the skin.
Experiment with Cox in desiccators under humid (●) and dry (○) conditions.

The autoradiographs also demonstrate that penetration was very slow and, at most, about 1 mm in depth (Fig. 3). The same picture was obtained when more non-radioactive Ca was added and observations were made during a period up to 4 weeks. The autoradiographs under humid conditions are less deeply black. The lateral movement of Ca through the skin was only slight.

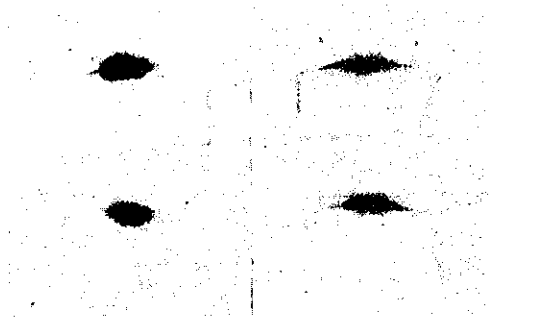

FIG. 3
Autoradiograph of cylinder of Granny Smith apple in desiccators.
Left: dry conditions; right: humid conditions. Penetration time 9 days.
The contours of the tissue pieces have been drawn in the figure.

Absorption after removing part of the skin

In Figure 4 the penetration is shown following removal of part of the skin before the application of Ca. As the experiments were made with different apple varieties the results cannot be compared directly with those shown in Figure 1. Penetration into the surface layer was very rapid, but hardly any Ca was found in the layer 5–10 mm deep.

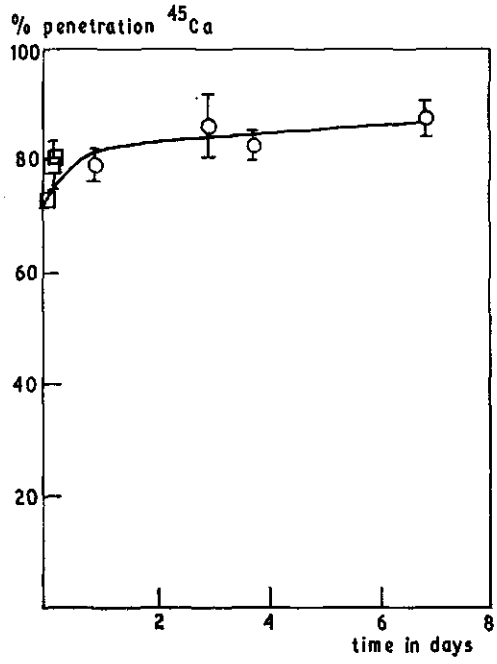

FIG. 4
Total absorption of ^{45}Ca after removing part of the skin of Laxton (○) and Granny Smith (□).

Repeated application of the radioactive solution

From Figure 5 it is apparent that the efficiency of subsequent applications in increasing the absorption of ^{45}Ca by the apple was lower than that of the first application. The difference was particularly large when the humidity of the air was high. In general, however, the total absorption of Ca increased with increasing number of applications. The changes in penetration into the deeper layers were similar.

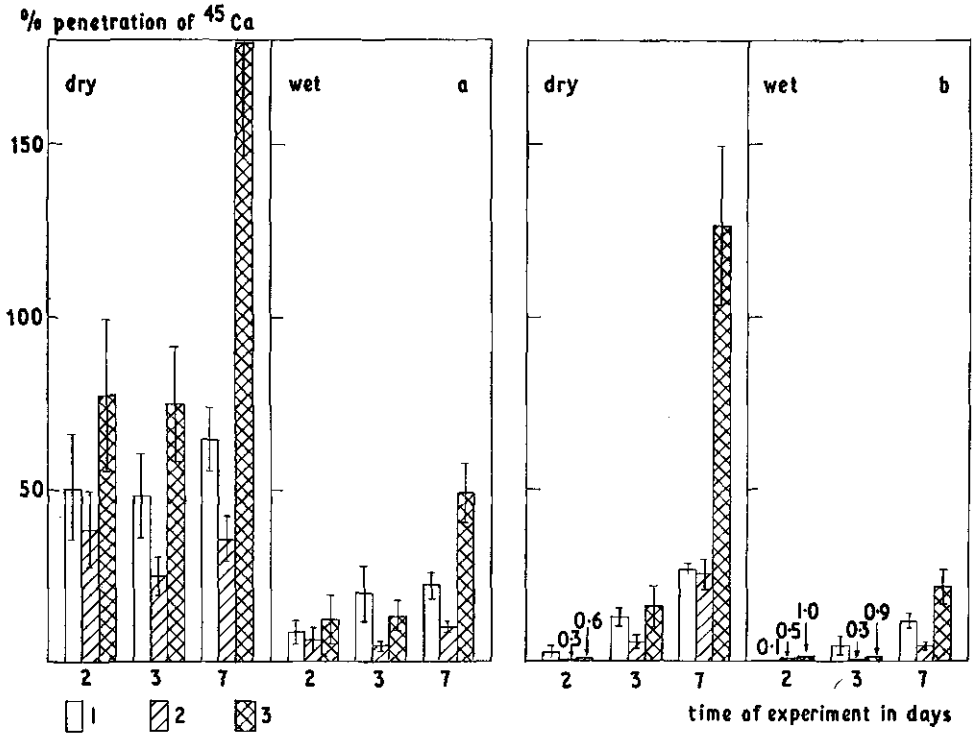

FIG. 5

Uptake of ^{45}Ca with and without repeated application of the radioactive Ca solution to Cox's Orange Pippin. Experiment in desiccators. (a) Total absorption, (b) penetration into the tissue under the skin. (1) Without repeated application; (2) with repeated application, 1, 2 and 4 times for experiments lasting 2, 3 and 7 days, respectively, given as percentage of the total applied quantity; (3) with repeated application calculated as a percentage of the quantity given in a single application.

DISCUSSION

It is apparent from the results with apples in desiccators that under humid air conditions, when the drops on the apple surface do not evaporate, penetration of ^{45}Ca into the fruit of Cox's Orange Pippin and James Grieve proceeds almost linearly with time during the first week after application. Evaporation of the drop under dry conditions results in higher initial rates of penetration. A possible explanation is that, with the higher concentration on the skin surface, there is an increase in diffusion through the skin (Jacobs, 1967). Later the rate of penetration under dry conditions became about equal to that under humid conditions.

Bangerth (1970) and Martin and Lewis (1967), who carried out experiments over a range of humidities lower than we used, found an increased uptake at their highest moisture levels. This could indicate that, with increasing relative humidity up to 90%, ^{45}Ca uptake increases, but that under saturated conditions, when little or no drying of the deposits occurs, uptake may be considerably slower.

Penetration was more rapid in Cox than in James Grieve, possibly because of the more waxy skin of the latter. The results do not agree with those of earlier research, in which no difference was found between the uptake of Ca in Cox and James Grieve after a large number of spray treatments in an orchard experiment (van Goor and van Lune, 1971). However, it is possible that in that experiment the more rapid increase in the surface area of the James Grieve counteracted slower absorption per unit of surface area. There is, however, better agreement with the work of van der Boon (personal communication), who found that it is often difficult to reduce bitter pit in James Grieve by spraying.

The rather large standard deviations in the various experiments can possibly be explained by the presence of small cracks and lenticels. Even under humid conditions the drops eventually disappeared from some of the apples.

Apples on branches and on the tree gave a picture similar to that of detached apples in desiccators.

Penetration into the tissue under the skin in Cox was slow and proceeded in a linear fashion during the first week. After 5 days in dry air, 20% of the ^{45}Ca was present here as compared with 8% in humid air. These results are within the same range as those found by Wieneke (1968) and Bangerth (1970). From the autoradiographs it could be concluded that the applied Ca remained in or directly under the skin during the first four weeks after application. It is possible that the Ca is bound to negative groups in the skin, as described by Yamada *et al.* (1965), or more especially to the cell walls of the exterior cell layers (Rathore *et al.*, 1972). In the parenchyma tissue of the fruit Ca is apparently rather immobile.

Repeated application of ^{45}Ca on successive days gave a larger total absorption of Ca, but the efficiency of the second and of each subsequent application decreased, especially under humid conditions. This can be explained by the reduction in time span between the subequent applications and moment of sampling, especially when under conditions of low evaporation there was no increase in the Ca concentration of the droplet.

For the horticultural practice of spraying trees with Ca solutions to control bitter pit, the conclusion can be drawn that loss of sprayed Ca due to rain is an important factor to consider, especially if it occurs within 24 hours after treatment. Hence frequent spraying under dry conditions can be recommended.

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