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Prevention of Abscission of Ponkan, *Citrus reticulata* Blanco, Leaves by Various Calcium Salts

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Introduction

Ponkan, *Citrus reticulata* Blanco, is a specialty fruit grown mainly in Kagoshima prefecture. Fruit are harvested in December and mostly marketed in January and February, although some of them are marketed in December for Christmas and New Year consumption. Maturation of fruit judged by sugar and acid contents often well advanced in December in contrast to immature appearance of rind with not fully orange color. Thus, if degreening and coloration of rind are improved, marketing in December will considerably increase demanding a much higher price.

It is well established that ethephon (2-chloroethylphosphonic acid) enhances degreening and coloration of citrus fruits such as oranges[10], tangerines[10,11], tangelos[11], ponkans and kumquats[2]. The commercial use of ethephon for that purpose, however, is not popular because of possible excessive defoliation[2,8].

Recently, authors found that an addition of calcium acetate to ethephon solution almost completely prevented fruit drop and defoliation with hardly any adverse effect on coloration[3,4]. Martin *et al.* also reported that calcium salts added to ethephon offset the defoliation of pecan trees caused by ethephon. They further compared the relative effectiveness of various calcium salts and studied the effects of pH of the solution on defoliation.

We established a leaf explant system to study abscission of ponkan leaves. Using this system the experiments were initiated possibly to find more effective calcium salts than calcium acetate to prevent the abscission of ponkan leaves.

Materials and Methods

Leaves of spring flush were sampled from 7-year-old ponkan, *Citrus reticulata* Blanco, trees grown in a University orchard. Leaf age ranged 4 months to 1 year in accordance with the season of the experiments, but in this experiment system there were no appreciable differences in the time course of the leaf abscission of control explants in leaf age.

Leaf explants were prepared by cutting the leaf blade at 5 mm from laminar abscission zone. Thus, the leaf explant consisted of petiole, laminar abscission zone and a small portion of leaf blade (Fig. 1).

Various test solutions were filled in a petri dish, 9 cm in diameter, which was covered with a

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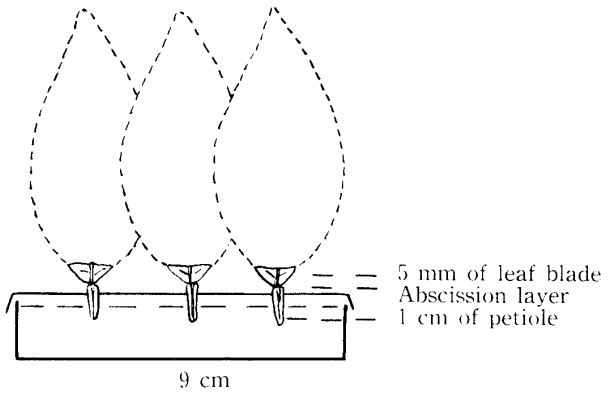

Fig. 1. Schematic presentation of an explant experiment system.

solid plastic sheet with 10 small holes. Leaf explants were placed so that the petiole was passed through the hole and soaked in the solution. Petri dishes with the explants were placed at room temperature for 7 days and every day the explants were examined for abscission with light touching by a forcep.

Each treatment was usually replicated 3 times and mostly the experiments were repeated at least twice. The results were analyzed by the analysis of variance and mean separation by Duncan's multiple range test.

For the measurement of ethylene evolution, the whole system was enclosed in a glass jar, that is, the petri dish filled with the test solution and covered with a plastic sheet, through the hole of which the leaf explants were soaked into the solution. At certain time intervals 0.5 ml air in the jar was sampled through a vaccine cap by a syringe, and analyzed for ethylene by a Shimadzu GC-3BF gas chromatograph with a 1 m by 3 mm stainless column packed with Porapak Q. The carrier gas was nitrogen. Ethylene was detected by dual hydrogen flame detectors. Known concentrations of the reference gas were used for calibration at each time of the measurement.

Results and Discussion

The preventive effects of various calcium salts on the abscission of ponkan leaf explants were investigated using calcium acetate, calcium sulfate, calcium nitrate and calcium chloride, each at the concentration of 0.05 M or 0.01 M in 1000 ppm ethephon (2-chloroethylphosphonic acid) solution (Table 1). Calcium acetate, both at 0.05 M and at 0.01 M, clearly retarded the abscission of leaf explants whereas all the other calcium salts showed only very slight, if any, effects.

In this trial, the pH of 0.05 M and 0.01 M calcium acetate solution with ethephon was 5.72 and 4.86, respectively, while that of ethephon alone and those of other calcium salts with ethephon were around 2. Thus, it was suspected that the difference of pH might be the cause of the different effects shown by the various calcium salts. To explore this possibility, the effects of 0.05 M calcium acetate and calcium chloride, both at pH 3.5 and 6.5, were compared (Table 2). Calcium acetate added to the ethephon solution almost completely prevented the abscission of the leaf explants at both pHs. On the other hand, calcium chloride showed only weak effects on the prevention of the abscission, the effects of the solution of pH 6.5 being slightly superior.

From the above results several other calcium salts were surveyed as for their abscission preventive effects, mainly among the salts of weak acids. Thus, to the ethephon solution were added calcium acetate, calcium lactate, calcium propionate, calcium salicylate, calcium hydroxide and

Table 1. The effect of various calcium salts on abscission of ponkan leaf explant

Treatment	pH	Percentage of abscission	
		3 days	7 days
Control	5.28	85[a]	90[a]
Ethephon 1000 ppm	2.09	30[b]	85[a]
Ethephon 1000 ppm+CaAc 0.05 M	5.72	0[b]	25[b]
Ethephon 1000 ppm+CaAc 0.01 M	4.86	15[b]	35[b]
Ethephon 1000 ppm+$CaSO_4$ 0.05 M	2.26	15[b]	80[a]
Ethephon 1000 ppm+$CaSO_4$ 0.01 M	2.18	5[b]	75[a]
Ethephon 1000 ppm+$Ca(NO_3)_2$ 0.05 M	2.08	25[b]	65[a]
Ethephon 1000 ppm+$Ca(NO_3)_2$ 0.01 M	2.09	20[b]	85[a]
Ethephon 1000 ppm+$CaCl_2$ 0.05 M	2.09	5[b]	65[a]
Ethephon 1000 ppm+$CaCl_2$ 0.01 M	2.08	0[b]	85[a]

Mean separation by Duncan's multiple range test at 5% level.
CaAc: Calcium acetate.

Table 2. The effect of pH of calcium salts on abscission of leaf explant

Treatment	pH	Percentage of abscission	
		3 days	7 days
Control	5.6	47[a]	90[a]
Ethephon 500 ppm	3.5	30[ab]	83[ab]
Ethephon 500 ppm	6.5	20[abc]	80[ab]
Ethephon 500 ppm+$CaCl_2$ 0.05 M	3.5	33[a]	73[bc]
Ethephon 500 ppm+$CaCl_2$ 0.05 M	6.5	23[abc]	63[c]
Ethephon 500 ppm+CaAc 0.05 M	3.5	3[bc]	7[d]
Ethephon 500 ppm+CaAc 0.05 M	6.5	0[c]	0[d]

Mean separation by Duncan's multiple range test at 5% level.
CaAc: Calcium acetate.

calcium chloride, each to make up the concentration of 0.05 M (Fig. 2). Except calcium hydroxide, all the calcium salts tested showed more or less abscission-preventive effects, the order of whose effectiveness was from calcium chloride, through calcium lactate, calcium acetate to calcium propionate and calcium salicylate. The last two ones, that is, the propionate and salicylate, were highly effective and completely suppressed the abscission of the leaf explants even 7 days after the treatment. The leaf explants treated with calcium propionate and calcium salicylate, however, turned brown soon after the initiation of the treatment. Therefore, it was suspected that the failure of the abscission of the leaf explants might be due to the damage caused by the treatment.

To investigate this possibility as well as to find a proper concentration of these calcium salts which prevent the abscission without causing any damage to the leaf explants, lower concentrations of calcium propionate and calcium salicylate were applied. The solutions of calcium propionate at 0.0125 and 0.00625 M, and calcium salicylate at 0.0125, 0.00625 and 0.00312 M in 500 ppm ethephon were prepared by adding the proper amount of each salt to the ethephon solution, and the effects on the abscission were compared with ethephon alone and 0.05 M calcium acetate (Table 3). Judging from the abscission rate at 3 and 7 days after the treatment, 0.0125 M calcium propionate was as effective as 0.05 M calcium acetate in preventing the abscission, while 0.00625 M was

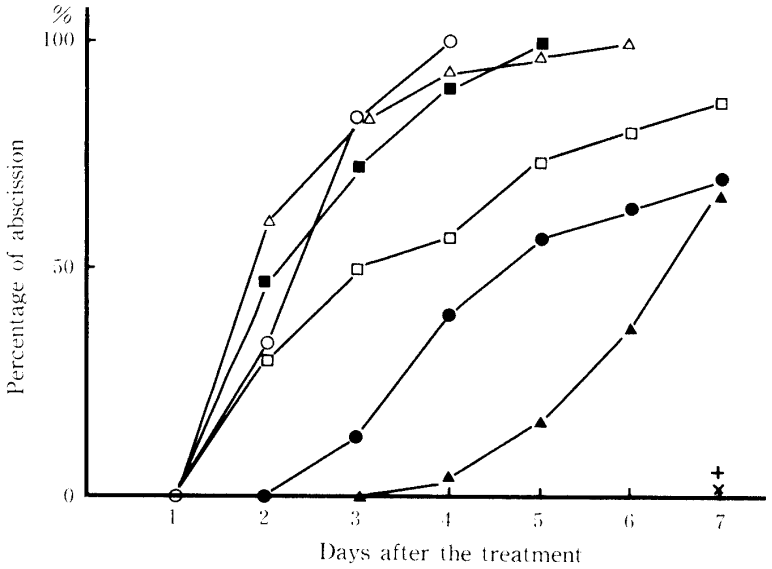

Fig. 2. The effects of various calcium salts on abscission of ponkan leaf explant.
—○— Control
—△— Ethephon 500 ppm
—▲— Ethephon 500 ppm+0.05 M calcium acetate
—●— Ethephon 500 ppm+0.05 M calcium lactate
—+— Ethephon 500 ppm+0.05 M calcium propionate
—×— Ethephon 500 ppm+0.05 M calcium salicylate
—■— Ethephon 500 ppm+0.05 M calcium hydroxide
—□— Ethephon 500 ppm+0.05 M calcium chloride

Table 3. The effect of calcium acetate, calcium propionate and calcium salicylate on abscission of ponkan leaf explant

Treatment	pH	Percentage of abscission	
		3 days	7 days
Control	5.6	90[a]	100[a]
Ethephon 500 ppm	2.9	77[a]	100[a]
Ethephon 500 ppm+CaAc 0.05 M	5.9	8[c]	88[a]
Ethephon 500 ppm+CaProp 0.0125 M	5.4	10[c]	97[a]
Ethephon 500 ppm+CaProp 0.00625 M	5.0	42[b]	98[a]
Ethephon 500 ppm+CaSal 0.0125 M	3.4	2[cd]	3[b]
Ethephon 500 ppm+CaSal 0.00625 M	3.2	3[cd]	10[b]
Ethephon 500 ppm+CaSal 0.00312 M	3.0	0[d]	18[b]

Mean separation by Duncan's multiple range test at 5% level.
CaAc: Calcium acetate, CaProp: Calcium propionate, CaSal: Calcium salicylate.

only slightly effective. On the other hand, calcium salicylate was remarkably effective and even as low as 0.00312 M almost completely prevented the abscission. Calcium propionate at these concentrations did not cause any damage to the leaf explants such as browning. The leaf explants treated with 0.00312 M calcium salicylate showed only a slight browning in tissues 4 days after the initiation of the treatment.

To know the effects of pH, the pH was adjusted at 3.5, 5.0 and 6.5, of the solutions of 0.05 M calcium acetate, 0.0125 M calcium propionate and 0.00312 M calcium salicylate added to 500 ppm

ethephon. The pH of ethephon alone was also prepared in the same order (Table 4). Judging 3 days after the treatment, calcium propionate and calcium salicylate at all pHs prevented the abscission more or less equally, although somewhat weaker than in case of calcium acetate. At 7 days after the treatment calcium salicylate was not effective at all pHs, while calcium acetate and calcium propionate lost their effectiveness at pH 6.5.

Table 4. The effect of pH of calcium salts on abscission of ponkan leaf explant

Treatment	pH	Percentage of abscission	
		3 days	7 days
Control	5.6	90^{ab}	100^{a}
Ethephon 500 ppm	3.5	72^{c}	100^{a}
Ethephon 500 ppm	5.0	82^{bc}	100^{a}
Ethephon 500 ppm	6.5	92^{a}	100^{a}
Ethephon 500 ppm+CaAc 0.05 M	3.5	0^{f}	13^{e}
Ethephon 500 ppm+CaAc 0.05 M	5.0	0^{f}	17^{e}
Ethephon 500 ppm+CaAc 0.05 M	6.5	5^{ef}	82^{c}
Ethephon 500 ppm+CaProp 0.0125 M	3.5	22^{d}	38^{d}
Ehtephon 500 ppm+CaProp 0.0125 M	5.0	10^{de}	28^{de}
Ethephon 500 ppm+CaProp 0.0125 M	6.5	12^{de}	82^{c}
Ethephon 500 ppm+CaSal 0.00312 M	3.5	18^{d}	90^{bc}
Ethephon 500 ppm+CaSal 0.00312 M	5.0	10^{de}	97^{ab}
Ethephon 500 ppm+CaSal 0.00312 M	6.5	7^{ef}	97^{ab}

Mean separation by Duncan's multiple range test at 5% level.
CaAc: Calcium acetate, CaProp: Calcium propionate, CaSal: Calcium salicylate.

Ethylene evolution from the explants treated with 0.05 M calcium acetate without ethephon was extremely low and that from the control explants treated with water was appreciably higher than the former (Fig. 3). The plot with 500 ppm ethephon plus 0.00312 M calcium salicylate (pH 3.26) and that with ethephon alone (pH 2.80) showed rather low ethylene evolution, and some part of it should have come from ethephon itself, although the degradation rate was very low because of lower pH of the solution. The plot with ethephon plus 0.0125 M calcium propionate (pH 5.51) showed a markedly higher ethylene evolution and that with ethephon plus 0.05 M calcium acetate (pH 5.86) showed even more ethylene evolution. Almost all of the ethylene evolved would have come through the degradation of ethephon itself[9].

These results clearly demonstrate that calcium ions were generally highly effective in preventing or retarding the abscission of ponkan leaf explants. The abscission of explants is reported to be similar at least anatomically to the abscission of natural defoliation.[1] Iwahori and Oohata[3,4] also reported that an addition of calcium acetate to the ethephon solution effectively prevented the defoliation and fruit drop of ponkan and kumquat trees. Thus, the explant system used here is useful for screening the effective compounds to prevent the abscission and for studying the mechanisms of abscission processes.

The mechanisms of action of calcium ion to prevent abscission are not fully understood. Poovaiah and Leopold[6] suggested that the effects may be partly due to the cementing effects on the cell walls through the formation of salt bridges between pectic components. They also suggested that besides cementing effects calcium may delay or prevent abscission through a deferral of senes-

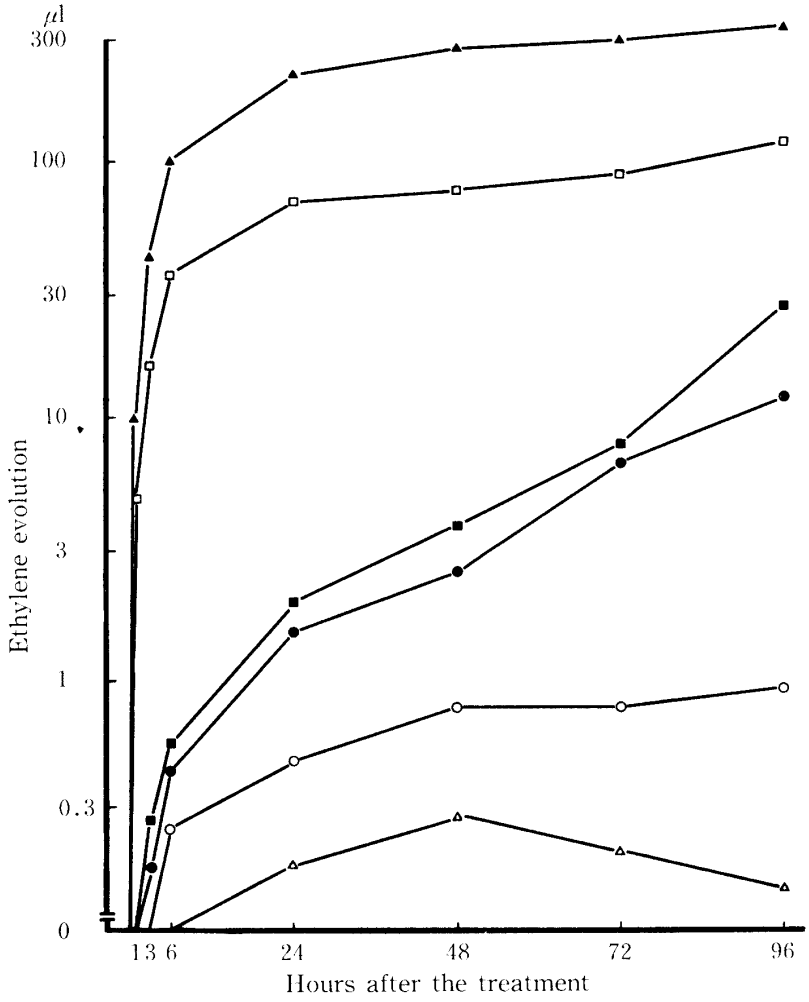

Fig. 3. The effect of calcium acetate alone, ethephon, and various calcium salts added to ethephon on ethylene evolution.

—○— Control
—△— 0.05 M calcium acetate
—●— Ethephon 500 ppm
—▲— Ethephon 500 ppm+0.05 M calcium acetate
—□— Ethephon 500 ppm+0.0125 M calcium propionate
—■— Ethephon 500 ppm+0.00312 M calcium salicylate

cence which is a prerequisite for the onset of ethylene responsiveness. In the present study the explants treated with calcium acetate alone evolved substantially less endogenous ethylene than those treated with water. This may indicate that calcium ions defer the senescence of the tissue of the explants.

The present investigation also indicated that calcium salts of strong inorganic acids were less effective than those of organic acids in preventing the abscission. An addition of calcium salts of inorganic acids to the ethephon solution lowered the pH considerably as well as ethephon solution alone, while that of organic acids raised the pH. Thus, the effects of an addition of calcium salts of organic acids might be regarded as simply raising the pH of the solution, which, in turn, accelerated an ethylene evolution through the degradation of ethephon[9]. However, it does not seem to be the case. Calcium chloride at pH 3.5 and 6.5 was equally less effective than calcium acetate at both pHs (Table 2), although ethephon plus calcium chloride and ethephon plus calcium acetate at a

higher pH evolved a greater amount of ethylene than those at pH 6.5 (Data not shown). Similarly, calcium acetate and calcium propionate added to ethephon solution (at higher pH) caused a markedly higher ethylene evolution than calcium salicylate added to ethephon solution (at lower pH) (Fig. 3), although calcium acetate and calcium propionate prevented the abscission more effectively than calcium salicylate did. Moreover, calcium acetate and calcium propionate at pH 6.5 were less effective than those at pH 3.5 or 5.0 in preventing the abscission (Table 4).

Martin *et al.*[5] also showed that an addition of calcium salts to an ethephon solution effectively offset the defoliation of pecan trees and that calcium acetate was more effective than calcium chloride, calcium sulfate or calcium nitrate.

We suggest that the penetration and the form of calcium in the cells may be different among different calcium salts, and this may be the reason why the effectiveness in preventing abscission is different among the different calcium salts.

Martin *et al.*[5] found that an addition of calcium salts increased the amount of more freely available calcium such as ion form or that adsorbed to protein and pectins. Poovaiah and Rasmussen[7] showed that the abscission zone of nondebladed bean leaves had a higher calcium contents than the petiole and stem tissues. They also showed that when plants were treated with an ethephon solution, calcium content in the abscission zone decreased, with a concomitant accumulation of calcium immediately adjacent to the separation layer on the stem side. Similar and more detailed study is needed on the distribution pattern and the form of calcium in the tissue treated with calcium salts of inorganic and organic acids.

Calcium propionate and especially calcium salicylate were highly effective in preventing the abscission, four times and 16 times more effective than calcium acetate, respectively. Calcium propionate and calcium salicylate at the higher concentration caused damage to the explants such as browning of the tissues, but the lower concentration of calcium propionate did not cause any such damage and calcium salicylate caused only slightest browning, and were still effective in preventing the abscission.

Based on these findings, the field experiments are under progress to see whether an addition of these calcium salts of lower concentration to the ethephon solution is effective in preventing defoliation and fruit drop without sacrificing the effect of ethephon on acceleration of degreening and coloration of ponkan fruit.

Summary

Using leaf explant system, the effects of various calcium salts added to an ethephon solution were investigated, on prevention or retardation of abscission of ponkan leaves. Calcium hydroxide was not effective at all, but all the other calcium salts showed more or less abscission-preventing effects. Among them calcium salts of strong inorganic acids such as calcium chloride, calcium sulfate and calcium nitrate showed rather poor effects, and calcium salts of organic acids such as calcium lactate and calcium acetate were much more effective. Especially, calcium propionate and calcium salicylate were markedly effective, four times and 16 times more effective than calcium acetate, respectively, in the case when 0.05 M calcium acetate was used as a standard. This difference in the effectiveness among the different calcium salts did not seem to be due to the difference in pH, although the solution of ethephon alone, and calcium salts of inorganic acids added to the ethephon solution showed much lower pH.

Calcium chloride added to ethephon at pH 3.5 and 6.5 was equally much less effective, while

calcium acetate at pH 3.5 and 6.5 showed a marked preventive effect, although at the higher pH ethylene evolution from the both solutions was high which was considered to have come mainly from ethephon itself. In another experiment calcium acetate and calcium propionate at pH 6.5 were less effective than those at pH 3.5 and 5.0.

Ethylene evolution from the leaf explants treated with calcium acetate alone was considerably lower than that from control explants treated with water, and it was suggested that calcium ion deferred the senescence of the tissues. Ethylene evolution from the solution containing ethephon was dependent on pH. Thus, solution of ethephon plus calcium acetate (pH 5.86) and ethephon plus calcium propionate (pH 5.51) evolved markedly large amount of ethylene, while ethephon plus calcium salicylate (pH 3.26) and ethephon alone (pH 2.80) evolved only small amount.

The role of calcium ion in the prevention of abscission and the possible practical use of calcium propionate and calcium salicylate for the prevention of defoliation were discussed.

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