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EFFECT OF SORBIC ACID AND POTASSIUM SORBATE ON THE CONTROL OF SOUR ROT OF CITRUS FRUITS

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Abstract. **In agar culture tests, when pH of the media was not adjusted, sorbic acid (SA) was nearly as effective as sodium** o**-phenylphenate (SOPP) to control sour rot** (Geotrichum candidum **Lk. ex Pers.), but potassium sorbate (SK) was less effective. When pH of the media was adjusted, the effectiveness of SA and SK was the same and both were more effective than SOPP at lower pH than 5. Two % SK was effective to control sour rot inoculated artificially to lemons. When it was applied as a 50°C water solution by dipping lemons for 2 min, the effectiveness was the same or more than SOPP.**

Sour rot caused by the fungus *Geotrichum candidum* is one of the most troublesome diseases after harvest. The disease has been found in many imported citrus fruits in Japan, but is especially important to California and Arizona lemons which have been imported from 100,000 to 120,000 metric tons annually. According to our observation it has become a serious disease to Florida grapefruit recently (8).

G. candidum is resistant to biphenyl and thiabendazole. *O*-phenylphenol/sodium *o*-phenylphenate (OPP/SOPP) is the only fungicide to control sour rot to some extent among the fungicides permitted for citrus fruits by the Japanese Government.

While postharvest fungicides are agricultural chemicals in the U. S. and other countries, they are considered as food additives in Japan and are regulated by the food sanitation laws (6). Recently, SOPP was found to be carcinogenic by Hiraga and Fujii (5). Dietary administration of this compound to rats resulted in bladder carcinoma. Fukushima et al. (4) also reported that SOPP has promoting activity towards the urinary bladder. When the Ministry of Health and Welfare confirms the carcinogenesis, OPP/SOPP must be banned.

As OPP/SOPP is the only effective fungicide to control sour rot, decay of imported citrus fruits would be greatly increased if OPP/SOPP is banned. With the loss of OPP/SOPP very likely, we started to evaluate the effectiveness of sorbic acid (SA) and potassium sorbate (SK) on sour rot in agar and inoculation tests.

Materials and Methods

G. candidum was isolated from rotted fruit imported from the U. S. SA, SK and equal molecular mixture of SA and SK and SOPP were uniformly dispersed in potato dextrose agar (PDA) and dispensed in 9 cm diameter petri dishes. The mycelium from the edge of a growing colony was placed on the center of the dish. After 4 days of incubation at 25°C in the dark the colony diameter was measured. The degree of inhibition was calculated using the means of 5 dishes per treatment.

Imported California lemons were used for inoculation tests. Each fruit was injured with 14 separate punctures of 3 mm deep with a nail (1.5 mm diameter). The fruit was dipped in the water suspension of spores of *G. candidum* (4-5 x 10^5/ml) for 10 min. Thiabendazole (TBZ) (3,000 ppm) was added to the inoculum to prevent natural green mold infections. Tween-20 was added to the inoculum. Eight hr at room temperature after inoculation, treatments were conducted by dipping the fruit in test solutions or water (control). The dipping time was 1 min unless noted. At every test, the standard application of SOPP, dipping the fruit in 2% SOPP for 2 min followed by rinsing with fresh water (10), was conducted. The treated fruit were stored at 25°C and high humidity (over 95% RH) for 5 days and the number of infected punctures by sour rot was counted. Thirty fruit were used per treatment.

NaOH and HCl were used to adjust pH of culture media and fungicide solutions. Media pH values were adjusted before autoclaving, but were measured again after autoclaving to check the exact pH.

Results

Agar culture tests. SOPP was the most effective in controlling the growth of *G. candidum*. Complete inhibition was obtained at 300 ppm. SA was next and it needed 350 ppm to achieve complete inhibition. SA + SK and SK were less effective (Fig. 1).

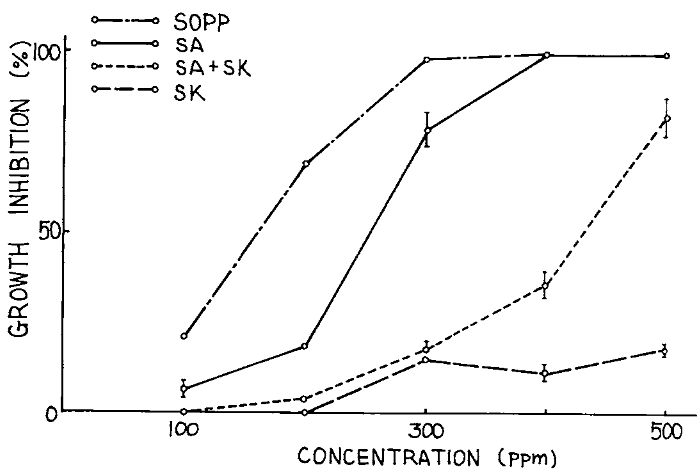

Fig. 1. Effect of sorbic acid (SA), potassium sorbate (SK), an SA+SK mixture, and sodium orthophenyl phenate (SOPP) on the growth of *Geotrichum candidum*.

Growth of *G. candidum* was greatly affected by the pH of the media with the maximum growth at about pH 6 (Fig. 2). The effect of chemicals were also affected by the pH of the media. The effectiveness of SA and SK were much less at pH higher than 7. It was noticed that the effect of 200 ppm SA and 270 ppm SK were nearly same at the same pH because SK at 270 ppm is 200 ppm of SA.

It was also noted that SA and SK were more effective than SOPP in controlling *G. candidum* at pHs lower than 5. The effectiveness of SK at lower pHs was confirmed by the test shown in Fig. 3; i.e. 270 ppm SK completely inhibited at pHs lower than 4.7. These tests were repeated with 3 isolates of *G. candidum* from different citrus fruits and similar results were obtained.

Inoculation tests. In the initial test, the effect of 2% SK at pH 7.9 (unadjusted) and 6.4 (adjusted) on the decay control of lemons inoculated with *G. candidum* were compared (Table 1). pH 6.4 is the lowest pH to dissolve 2% SK in water. Two % SK was effective in controlling sour rot. At pH 7.9, 2% SK was even more effective than 2% SOPP in this test.

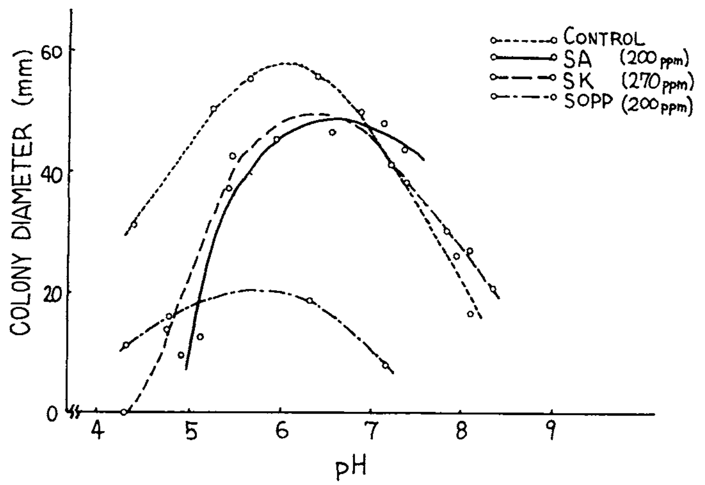

Fig. 2. Effect of sorbic acid (SA), potassium sorbate (SK) and sodium orthophenyl phenate (SOPP) on the growth of *Geotrichum candidum* on the pH-adjusted PDA.

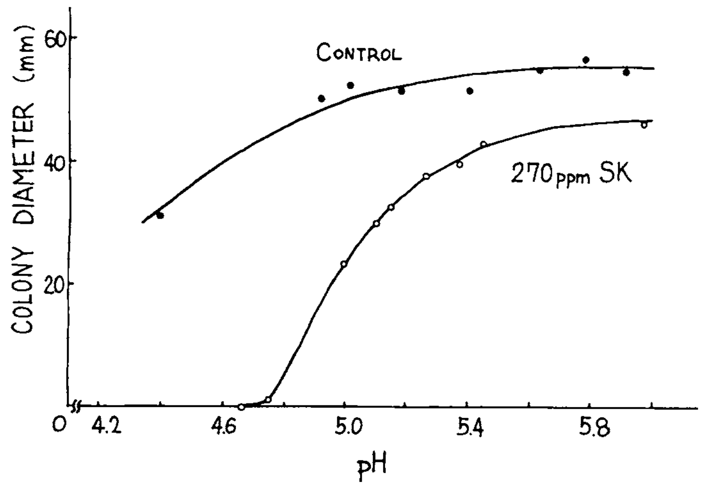

Fig. 3. Effect of potassium sorbate (SK) on the growth of *Geotrichum candidum* on the pH-adjusted PDA.

Table 1. Effect of potassium sorbate (SK) and sodium orthophenyl phenate (SOPP) on the control of sour rot (Inoculation test).

Treatment	Treatment solution (pH)	Decay (%)
Water (control)	5.8	26.4
2% SK	7.9	4.5 **z
Water	6.3 (Adjusted)	26.4
2% SK	6.4 (Adjusted)	6.9 *
2% SOPP	12.5	6.7 *

zSignificantly different from the control at 1% (**) or 5% (*) level.

In the next test, 2% SK and 0.16% SA were compared (Table 2). This is the highest concentration of SA in water at room temperature. It was found that 0.16% SA was not significantly effective in controlling sour rot. In this test, 2% SK was less effective than 2% SOPP.

SA was dissolved in 40% ethyl alcohol, rape oil, and water all at 50°C at 2.0, 0.7 and 0.5%, respectively and their effectiveness was compared with 2% SK at 50°C and water at room temperature (Table 3). Among them, 2% SK in 50°C water was the most effective and was comparable to 2% SOPP.

In the test of Table 4, 2% SK in 50°C water of pH 7.8 (unadjusted) and 5.8 (adjusted) were compared by dipping for 5 min. The control treatment, 50°C water for 5 min, itself was effective to control sour rot, but 2% SK in 50°C water of both pH were equally more effective than both the control and the 2% SOPP.

Five and 2 min treatment of 2% SK in 50°C water were compared in the test of Table 5. Two-min treatment was as effective as 5 min and was comparable to 2% SOPP.

Table 2. Effect of sorbic acid (SA), potassium sorbate (SK) and sodium ortho phenylphenate (SOPP) on the control of sour rot (Inoculation test).

Treatment	Treatment solution (pH)	Decay (%)
Water (control)	8.0 (Adjusted)	46.7
2% SK	8.0	17.0 *z
Water	3.0 (Adjusted)	28.8
0.16% SA	2.7	23.3
Water	12.3 (Adjusted)	47.9
2% SK	12.3 (Adjusted)	16.2 *
2% SOPP	12.4	7.1 **

zSignificantly different from the control at 5% (*) or 1% (**) level.

Table 3. Effect of sorbic acid (SA) potassium sorbate (SK) and sodium orthophenyl phenate (SOPP) in various solutions on the control of sour rot (Inoculation test).

Treatments	Treatment solution (pH)	Decay (%)
Water (control)	5.6	14.5
50°C water	5.7	12.9
2% SA / 50°C 40% ethyl alcohol	3.3	14.0
0.7% SA / 50°C rape oil	—	39.8
0.5% SA / 50°C water	3.1	11.2
2% SK / 50°C water	8.0	4.7 *z
2% SK	8.1	7.4
2% SOPP	12.5	4.5 *

zSignificantly different from the control at 5% level (*).

Table 4. Effect of potassium sorbate (SK) and sodium orthophenyl phenate (SOPP) on the control of sour rot (Inoculation test).

Treatments	Treatment solution (pH)	Treatment time (min)	Decay (%)
Water (control)	5.6	1	16.2
Water / 50°C	5.7	5	5.0 *z
2% SK / 50°C	7.8	5	1.9 **
2% SK / 50°C	5.8 (Adjusted)	5	2.1 **
2% SK	8.1	5	6.4 *
2% SOPP	12.4	2	4.0 *

zSignificantly different from the controls at 5% (*) or 1% (**) level.

Discussion

In agar tests, SA was nearly effective as SOPP in

Table 5. Effect of potassium sorbate (SK) and sodium orthophenyl phenate (SOPP) as related to treatment temperature and time on the control of sour rot (Inoculation test).

Treatments	Treatment time (min)	Decay (%)
Water (control)	1	17.0
2% SK	2	7.8 *z
2% SK	5	9.6 *
2% SK / 50°C	2	2.9 **
2% SK / 50°C	5	5.2 **
2% SOPP	2	3.9 **

zSignificantly different from the control at 5% (*) or 1% (**) level.

controlling sour rot, but SK was less effective (Fig. 1). When pH of the media and concentration as SA was adjusted, however, the effectiveness of SA and SK were the same (Fig. 2). Therefore, the difference in effectiveness of SA and SK in Fig. 1 would result from the difference of pH in water solution and concentration as SA. SA and SK were less effective than SOPP to control sour rot at high pH, but both were more effective at lower pH than 5. Complete inhibition occurred at 200 ppm as SA at pH 4.5-4.7 (Fig. 3), whereas SOPP needed 300 ppm (Fig. 1). Smoot and McCornack (15) tested the effect of SK on sour rot in agar culture of pH 4.5 and found 52% inhibition at 100 ppm. Brown (1) reported that 5,500 ppm was the lowest SK concentration which prevented growth of *G. candidum,* but the pH of the medium was not cited.

It is well known that the fungistatic effects of SA are greater at lower pH, for the undissociated SA is the active mode of SA (12). Therefore, these chemicals are generally used in acid foods as preservatives. As citrus fruits are acid, the pH of peel juice is 5.00 to 5.65 (13), we might expect SA and SK to be effective fungicides on citrus fruits.

In inoculation tests, it was found that SK was effective in controlling sour rot, but the effectiveness varied, i.e. 2% SK was more effective than 2% SOPP in Table 1 and it was not as effective as SOPP in Table 2. The effect of lowering the pH of the dipping solution was not clear. Two % SK of pH 7.9 (unadjusted) was even more effective than 2% SK of pH 6.4 (Table 1). pH of 2% SK water solution is 7.9-8.0 and the solubility decreases at lower pH. pH 6.4 is the lowest pH of 2% SK solution. pH of SA water solution is lower than that of SK, but SA is difficult-to dissolve in water. The highest concentration of SA at room temperature is 0.16%. This was less effective than 2% SK (Table 2). Solubility of SA increases with temperature and by organic solvents. The effectiveness of SA in 40% ethyl alcohol (2%), rape oil (0.7%) and water (0.5%) all at 50°C, however, were less than that of 2% SK in 50°C water (Table 3).

In California, hot water treatment, dipping the fruit for 2 to 4 min at 46 to 48°C, was a standard packinghouse treatment to control citrus brown rot (2). In Florida, hot water treatment, 52.8°C for 5 min, applied just before packing, reduced stem-end rot (14). When the inoculated fruits were dipped in 50°C water with or without 2% SK for 5 min, the hot water alone was effective in controlling sour rot, but at 50°C, 2% SK at pH 7.8 and 5.8 was more effective (Table 4). According to the test in Table 5, a 2-min dip was enough for the treatment of 50°C 2% SK. It was reported that fungicidal effects of SA and SK become greater when they are applied with some heat (9).

As solubility in water of SA is limited, SK seems better to use as a fungicide at packinghouses. All results obtained in this investigation agree that SK is effective to control sour rot; however, its effectiveness was not as consistent as SOPP. SK in hot water increased the effectiveness, but it may physiologically weaken the fruit. More investigation is required to find the method to increase the effectiveness of SK.

In this investigation, the effectiveness of SK was always compared with the dipping treatment in 2% SOPP for 2 min. Recently, however, foam washers have been popular means of treating citrus fruits with SOPP. The foam treatment is known to be less effective in decay control than the bath treatment (3). Therefore, if SK is evaluated with the foam treatment, the effectiveness of SK might be comparable.

It is well known that SOPP often causes severe scalding on the fruit, but SK never caused any injury. This is one of the merits of using SK. Smoot and McCornack (15) showed 2% SK applied to oranges and grapefruit gave as good or better decay control than SOPP. Nelson et al. (11) reported SK applied in combination with benzimidazole reduced benzimidazole resistant green mold. Kitagawa and Tani (7) also reported synergistic effects of TBZ and SK to control green and blue molds in agar culture and inoculation tests.

SK is permitted in many processed foods in Japan, but not on citrus fruits, yet. If SK is permitted on citrus fruits it would be useful in reducing decay of imported citrus fruits.

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