Chemicals Plus Heat as Seed Treatments for Control of Angular Leaf Spot of Cucumber Seedlings

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ABSTRACT

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Control of disease in seedlings was obtained by treating cucumber seeds for 20 min in aqueous preparations (50 C) of calcium propionate or tartaric acid, each at 33 g/L, or with acidic cupric acetate at 50 g/L. Seeds had been vacuum-inoculated with *Pseudomonas syringae* pv. *lachrymans*. Seed germination was not affected by chemical-heat treatments. Tests were conducted in the growth room and a field trial. Disease control also was obtained in growth room tests when inoculated seeds were treated 20 min with cupric acetate in 95% ethanol at 50 C.

Treatment of seeds for eradication of plant-pathogenic bacteria usually is not completely reliable. Common treatments, such as with chlorine salts, antibiotics, or hot water, appear to eliminate bacteria on the seed surface but not all bacteria within the seed. These escapees, even though they may be few, can initiate seedling disease if conditions are favorable. Inoculum from a few diseased seedlings may then spread throughout the field to cause serious loss.

The pathogen cells that survive within the seed are not only in protected positions that are hard to reach by usual treatment procedures, but they also are in a hypobiotic condition—cells are in a state of reduced metabolism (3). Hypobiotic bacteria are particularly resistant to inhibitory agents. Thus, the effective seed treatment must kill the interior hypobiotic pathogen cells.

Our original work, a short account of which has been published (4), was aimed at finding gaseous chemicals that would kill hypobiotic bacteria and would not harm soybean (*Glycine max* L.) seeds. Propionic acid appeared promising. Bacterial blight of soybean (*Pseudomonas syringae* pv. *glycinea*) that originated from naturally infested seeds was partly reduced in a laboratory test (12) by treating seeds with propionic acid. Disease control was greater when a small amount of water was added, but the addition usually lowered seed germination unacceptably. These results prompted work with salts of propionic acid. Additionally, other chemicals that had shown some promise during the course of the work were reinvestigated. Part of the results are reported in this paper. The aim was to devise effective procedures that could be applied practically, at least on a small scale.

This paper describes studies with three chemicals applied with heat to eradicate the angular leaf spot pathogen, *P. syringae* pv. *lachrymans* (*Psl*) from cucumber (*Cucumis sativum* L.) seeds. A short account of the work has been published (6).

MATERIALS AND METHODS

Seed inoculation. Early work was done with soybean seeds naturally infested with *P. syringae* pv. *glycinea*, but because bean seeds are usually harmed by aqueous preparations (7,10), the cucumber-angular leaf spot combination was used. Wild-type *Psl* cells taken from cultures 1–2 wk old, an age favoring survival (16), were vacuum-infiltrated (7) into 15-g lots of cucumber seeds.

Because no naturally infested seeds were available for comparison with inoculated seeds, it was not possible to be certain that results with naturally infested seeds would be the same. The inoculation method, however, is a severe one that places pathogen cells within as well as on seeds (7). Usually, 60–80% of the resulting seedlings were diseased.

Disease assessment. The method for assaying seeds in the growth room has been described (7). Briefly, a 15-g lot of seeds (500–600, depending on the cultivar) was planted in moist vermiculite. After 7–10 days at 24 C, cotyledons of each seedling were inspected for characteristic lesions incited by *Psl*. A seedling was considered diseased if there was one lesion (inoculated controls often bore 10–15 lesions). When a treatment produced a high level of disease control (>99%), isolations from suspect lesions were made on medium M72 (*Psl* colonies are distinctive on this medium—see color photograph [5]). Medium M72 is the same as M71 (2), except boric acid is used at 2 g/L. For verification, cells from representative presumed colonies of *Psl* were used in a pathogenicity test. Colonies were taken up in a wet cotton swab and rubbed on leaves of cucumber seedlings previously dusted with Carborundum; in 5–8 days, typical angular leaf spot lesions were produced.

A field test was made at Wooster, OH. Rows (replicates) were 15 m long and 1 m apart in randomized blocks. Test rows were separated by uninoculated buffer rows. To lessen early spread of the

Table 1. Effects of treating cucumber seeds with chemicals in water at 50 C for 20 min on seed germination and angular leaf spot control in growth room tests

Chemical	Seeds inoculated	Seedlings germinated[a,b] (no.)	Seedlings diseased[a] (%)
None	No	516	0
None	Yes	453	63
Calcium propionate	Yes	457	0.2[c]
None	No	505	0
None	Yes	473	78
Tartaric acid	Yes	472	0.3[c]
None	No	497	0
None	Yes	496	81
Cupric acetate	Yes	501	<0.1[c]

[a] Means of three different experiments. About 570 seeds of cultivar National Pickling (lot 1) planted per experiment.

[b] There were no significant differences ($P = 0.05$) according to Duncan's new multiple range test among treatments in tests with a given chemical.

[c] Pathogen isolated in culture and pathogenicity proved.

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Table 2. Effects of treating three cultivars of cucumber seeds with chemicals in water at 50 C for 20 min on seed germination and angular leaf spot control in growth room tests

Chemical	Seeds inoculated	Cultivar 1[a] Seedlings germinated[b] (no.)	Cultivar 1[a] Seedlings diseased (%)	Cultivar 2[a] Seedlings germinated[b] (no.)	Cultivar 2[a] Seedlings diseased (%)	Cultivar 3[a] Seedlings germinated[b] (no.)	Cultivar 3[a] Seedlings diseased (%)
None	No	448	0	478	0	536	0
None	Yes	410	74	428	72	536	59
Calcium propionate	Yes	446	0	394	0	526	0.4[c]
Tartaric acid	Yes	378	0	329	0.2[c]	548	0.3[c]
Cupric acetate	Yes	408	0	404	0.1[c]	524	0

[a] Means of two experiments. Approximate number of seeds planted in each experiment: cultivar 1 (SMR 58) 525, cultivar 2 (Imperial Long Green) 534, and cultivar 3 (Producer) 597.
[b] Numbers of seedlings germinated not significantly different ($P = 0.05$) according to Duncan's new multiple range test.
[c] Pathogen isolated in culture and pathogenicity proved.

Table 3. Effects of treating cucumber seeds with chemicals in water at 50 C for 20 min on seed germination and angular leaf spot control in a field test

Chemical	Seeds inoculated	Cultivar National Pickling[x] Seedlings germinated[y] (no.)	Cultivar National Pickling[x] Seedlings diseased (%)	Cultivar Marketer[x] Seedlings germinated[y] (no.)	Cultivar Marketer[x] Seedlings diseased (%)
None	No	204 a	0	173 b	0.2[z]
None	Yes	196 a	15	154 c	33
Calcium propionate	Yes	196 a	0	180 b	0.2[z]
Tartaric acid	Yes	211 a	0.2[z]	168 b	0
Cupric acetate	Yes	191 a	0.2[z]	172 b	0.2[z]

[x] Means of three replicates. About 250 seeds of National Pickling (lot 2) and 268 seeds of Marketer planted per replicate.
[y] Column means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.
[z] Pathogen isolated in culture and pathogenicity proved.

Table 4. Effects of treating cucumber seeds with chemicals in ethanol at 50 C for 20 min on seed germination and angular leaf spot control in growth room tests

Chemical	Seeds inoculated	Cultivar National Pickling[w] Seedlings germinated[y] (no.)	Cultivar National Pickling[w] Seedlings diseased (%)	Cultivar Marketer[x] Seedlings germinated (no.)	Cultivar Marketer[x] Seedlings diseased (%)
None	No	465 a	0	500	0
None	Yes	443 a	71	532	97
Ethanol alone	Yes	444 a	6	532	48
Calcium propionate	Yes	464 a	0.3[z]	512	6
Tartaric acid	Yes	268 b	21	...	...
Cupric acetate	Yes	437 a	0	544	0

[w] Means of three experiments. About 570 seeds (lot 2) planted per treatment per experiment.
[x] One experiment. About 536 seeds planted per treatment.
[y] The same letters following the means are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.
[z] Pathogen isolated in culture and pathogenicity proved.

pathogen by insects, which usually takes place in this region, inoculated untreated seeds were planted in one plot and uninoculated untreated seeds and inoculated treated seeds were planted in another plot separated by 300 m. To minimize pathogen spread by insects within plots, seedlings with lesions were removed when detected and no data were taken after the second seedling leaf was wider than 5 cm. As in growth room tests, isolation and pathogenicity tests were made from presumed *Psl* lesions.

Seed treatments. Fifteen-gram samples of seeds were immersed in the treatment preparation for 20 min at 50 C. To ensure uniformity, treatment preparations, vessels, and seeds were preheated to 50 C before seeds were added to the preparation. Seeds were dried quickly (1) by passing a gently flowing stream of air at 43 C through them for 1 hr. Commercial cucumber seeds are often dried this way after the fermentation step used to free seed from pulp.

RESULTS

Calcium propionate was the most frequently studied chemical in early tests. For example, when seeds were soaked for 1 hr at 24 C in 16 g of calcium propionate per liter of water, disease was usually 5–10% compared with 60% in the control. Similar results were obtained with D-tartaric acid. Although these materials showed promise, disease control was not sufficient.

Temperatures were elevated and treatment times shortened in attempts to improve control. Studies on survival of bacteria at 75% RH (7) and work with chemicals (9,15) indicated that this would be worthwhile.

With calcium propionate at 16 g/L of water and a treatment time of 20 min, the temperature resulting in the best disease control and seed germination was 50 C. This temperature was adopted as standard. The best calcium propionate level, 33 g/L, was selected as a result of another test; this amount was selected arbitrarily for tartaric acid tests. Acidic cupric acetate, 50 g/L, made as described by Schaad et al (15), was the third treatment used for detailed study.

All three treatments gave excellent disease control in growth room tests with four cultivars (Tables 1 and 2) and in a field test with two cultivars (Table 3). The inoculation method was severe, as shown by the incidence of disease in inoculated control plants. Seed germination was not reduced by the chemical treatments. With the cultivar Marketer in the field test, inoculation reduced germination, but this was not observed with the cultivar National Pickling. Because a low level of disease was found in uninoculated control Marketer plants in the field test (Table 3) but not in growth room tests (Table 4), some pathogen spread by insects may have taken place in the field despite precautions.

Hot water alone was not used in the tests described because in two early growth room experiments, it did not result in acceptable disease control. When inoculated seeds were treated for 20 min in water at 50 C, an average of 51% diseased seedlings were produced compared with 61% from inoculated untreated seeds. In other tests with vacuum-inoculated seeds and somewhat different conditions, the figures were 29 and 44%, respectively (7). Therefore, the combination of chemicals and heat seems essential.

Because organic solvents may improve performance of chemicals for treating

seeds (8), growth room tests were made with the chemicals applied in ethanol (95% ethanol, 5% water) instead of water alone. Seeds were treated for 20 min at 50 C and dried as described. With National Pickling, calcium propionate and cupric acetate gave good disease control of *Psl* in three tests, but with tartaric acid, germination was reduced and there was poor control (Table 4). Calcium propionate did not control disease well in the test with Marketer. It is noteworthy that hot ethanol alone gave substantial control.

DISCUSSION

In these studies, it was necessary to apply heat to calcium propionate in water to achieve a high level of disease reduction in seedlings from *Psl*-infected seeds. There were several reports early in this century describing addition of chemicals in hot water treatment of seeds for control of internal pathogens. The additions apparently had little advantage over hot water alone and were not used widely. Other methods of control were also used for seeds carrying internal fungi, but hot water continued to be recommended for reducing internal pathogenic bacteria. Recently, however, treatment of *Brassica* sp. seeds carrying the black rot bacterium was improved by adding an antibiotic (9) or acidified cupric acetate (15) to hot water. The usefulness of the cupric acetate treatment was verified with the *Psl*-cucumber combination in these studies. Hot organic solvents as carriers of chemicals merit more study, but results with ethanol in these tests indicate that there may be more limitations than with aqueous systems. Heat is probably essential because it increases solvent uptake (11).

It would be helpful to examine other chemicals plus heat for eradication of bacterial pathogens from seeds and other propagative parts, particularly to replace materials toxic to humans. Calcium propionate, for example, is used widely as a human food preservative in the United States. Tartaric acid is found in some foods, so it may also fall into the same category.

More studies are needed to determine if chemicals will lessen the detrimental effects often associated with seed thermotherapy (1). These depend on such factors as plant species and cultivar and size, age, vigor, and moisture content of seeds. Where heat treatment times are short, as in this work, careful scale-up studies must follow small-scale experiments to be sure that large amounts of seed are treated accurately and uniformly; there may well be a narrow temperature range between death of the pathogen and life of the seed (1). Nevertheless, the realization that heat and chemicals can produce a high level of disease control opens the way for development of more reliable seed treatments that can play an important part in the overall control of bacterial diseases (13). Because pathogen-contaminated seeds are hard to detect (14), even a method that could not be used economically with large volumes of seeds would be useful for routinely treating small lots for special purposes.

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LITERATURE CITED

1. Baker, K. F. 1962. Thermotherapy of planting material. Phytopathology 52:1244-1255.
2. Leben, C. 1972. The development of a selective medium for *Pseudomonas glycinea*. Phytopathology 62:674-676.
3. Leben, C. 1974. Survival of plant pathogenic bacteria. Ohio Agric. Res. Dev. Cent. Spec. Circ. 100. 21 pp.
4. Leben, C. 1978. A method for testing volatile toxicants against plant pathogenic bacteria. (Abstr.) Phytopathol. News 12:188-189.
5. Leben, C. 1981. How plant-pathogenic bacteria survive. Plant Dis. 65:633-637.
6. Leben, C. 1982. Calcium propionate and tartaric acid at 50 C for the control of *Pseudomonas syringae* pv. *lachrymans* in cucumber seed. (Abstr.) Phytopathology 72:991.
7. Leben, C., and Sleesman, J. P. 1981. Bacterial pathogens: Reducing seed and in vitro survival by physical treatments. Plant Disease 65:876-878.
8. Lewis, J. A., Papavizas, G. C., and O'Neill, N. R. 1979. Effect of seed immersion in organic solvents on germinability. J. Agric. Sci. Camb. 52:563-570.
9. Lockhart, C. L., Gourley, C. O., and Chipman, E. W. 1976. Control of *Xanthamonas campestris* in brussel sprouts with hot water and aureomycin seed treatment. Can. Plant Dis. Surv. 56:63-66.
10. Moore, R. P. 1972. Effects of mechanical injuries on viability. Pages 94-113 in: Viability of Seeds. E. H. Roberts, ed. Syracuse University Press, Syracuse, NY.
11. Murphy, J. B., and Noland, T. L. 1981. Temperature effects on seed imbibition and leakage mediated by viscosity and membranes. Plant Physiol. 69:428-431.
12. Parashar, R. D., and Leben, C. 1972. Detection of *Pseudomonas glycinea* in soybean seed lots. Phytopathology 62:1075-1077.
13. Ralph, W. 1977. Problems in testing and control of seed-borne bacterial pathogens: A critical evaluation. Seed Sci. Technol. 5:735-752.
14. Schaad, N. W. 1982. Detection of seedborne bacterial plant pathogens. Plant Dis. 66:885-890.
15. Schaad, N. W., Gabrielson, R. L., and Mulanax, M. W. 1980. Hot acidified cupric acetate soaks for eradication of *Xanthomonas campestris* from crucifer seeds. Appl. Environ. Microbiol. 39:803-807.
16. Sleesman, J. P., and Leben, C. 1976. Bacterial desiccation: Effects of temperature, relative humidity, and culture age on survival. Phytopathology 66:1334-1338.