Alfalfa Sprout Rot Caused by *Erwinia chrysanthemi*

LUELLEN PIERCE, Staff Research Associate, and ARTHUR H. McCAIN, Extension Plant Pathologist, Department of Plant Pathology, University of California, Berkeley 94720

ABSTRACT

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Erwinia chrysanthemi caused a soft rot disease of alfalfa sprouts, a relatively new crop in California. Temperature greatly affected disease severity. Little or no disease occurred at 15 or 18 C, but severe rot occurred at temperatures higher than 28 C. Disease severity was related to inoculum concentration; disease occurred when seeds were soaked in a bacterial suspension of at least 10^2 cfu/ml. Soaking inoculated seeds for 2 hr in 0.5% sodium hypochlorite or calcium hypochlorite controlled the disease. *E. chrysanthemi* did not survive longer than 1–2 wk on inoculated seeds or in infected alfalfa sprouts.

Use of alfalfa sprouts (*Medicago sativa* L.) in salads and sandwiches has become popular in recent years. The culture of alfalfa sprouts is fast becoming an important commercial industry. In 1970, there were only three major growers in California; by 1979, there were 125 growers in California using 65,910 kg of seed per year. The cash value of the alfalfa sprout crop exceeds the value of such crops as parsley, eggplant, and radish (4). Because alfalfa sprouts are a relatively new crop, little information is available on their diseases. In a preliminary report, we described a disease of alfalfa sprouts caused by *Erwinia chrysanthemi* (8). The only report of bacteria on sprouts concerns the presence of human pathogens on healthy sprouts (7). Fecal coliforms including *Klebsiella pneumoniae* are part of the normal flora. Lactobacilli and fecal streptococci counts are low.

Alfalfa sprouts are grown commercially in an indoor environment without added nutrients or soil and often without temperature control. Growers prefer temperatures of about 22–27 C. In one common method, seeds are soaked for 2–4 hr in water, then placed on screened trays under intermittent mist for germination. The soak and mist water is not usually recycled. After 4–5 days, the crop is harvested and placed in plastic bags for sale. Growers frequently experience a soft rot disease of the sprouts in the summer, when maximum temperatures range from 24 to 32 C. The first symptom of the disease is a translucent yellowish coloring of the radicles as they emerge from the seed. Within 24–48 hr, the seeds have stopped growing and have turned into a yellowish, odiferous mass containing numerous bacteria. The disease first occurs in a few trays in one location. Within a few days, it can easily spread through all the trays and destroy the grower's entire production of alfalfa sprouts.

The objective of this study was to identify the cause of the disease and to determine the temperature requirements for disease initiation. Control measures were also investigated.

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MATERIALS AND METHODS

Bacterial isolations and identifications. Rotted alfalfa sprouts obtained from a grower with a severe disease problem were macerated in a mortar and pestle, and loopfuls were streaked onto Miller-Schroth medium (MS) (6). Small (1–2 mm) orange bacterial colonies with a "fried-egg" appearance were selected for investigation because of their *Erwinia*-like appearance. The bacteria were

identified by standard physiological tests (Table 1). These strains were used in the subsequent experiments.

Pathogenicity trials. In the pathogenicity trials, alfalfa (*Medicago sativa* L. 'Moapa') seeds (Northrup King) were soaked in tap water for 2–4 hr. A Whatman No. 4 paper filter (90-mm circle) was placed into the bottom of a plastic petri dish 100 × 15 mm and moistened with 8 ml of sterile water. Inoculum was grown on King's medium B for 24 hr at 28 C. Unscarified seeds were inoculated by soaking for 1 min in a bacterial suspension of 10^8 colony-forming units (cfu) per milliliter. Controls were soaked in water for 1 min. One-fourth of a teaspoon (1.25 cm^3) of hydrated seeds (about 188 seeds) was spread on the moistened filter paper in each petri dish. The petri dishes were placed in incubators at 15, 18, 21, 25, 28, 30, and 34 C. There were two to four petri dishes of inoculated seeds and uninoculated seeds at each temperature, and the entire experiment was repeated three times.

Disease severity was quantified by measuring the lengths of the radicles and the hypocotyls from the bases of the leaves to the tips of the roots after 2 days. The length measurement was obtained by placing the sprouts from each petri dish on a grid where 10 plants were chosen by random numbers for measurement. The 10 lengths were then averaged to give a value for the plate. The same procedures were used in all trials except as noted.

Inoculum concentration. The effect of inoculum concentration on disease was investigated at 28 C. Seeds were soaked for 1 min in concentrations of 10^1–10^7 cfu/ml. Controls were soaked in tap water. There were two to four petri dishes of seeds at each concentration, and the entire experiment was repeated three times.

Disease control. Three surface-sterilizing agents, sodium hypochlorite, calcium hypochlorite, and hydrogen peroxide, were tested as seed treatments. Seeds were inoculated by soaking for 1 min in a bacterial suspension of 10^8–10^9 cfu/ml and immediately treated with an agent. Dry seeds (15 cm^3, about 6,000 seeds) were soaked for 2 hr in 40 ml of a 0.5% solution of the hypochlorite or peroxide; controls were soaked in tap water. Ten sprouts per plate were measured. There were three plates per treatment, and the entire experiment was repeated three times. The seeds were incubated at 28 C for 2 days. The length of the radicle was used as a measure of disease. Uninoculated seeds were soaked in the same surface-sterilizing agents to ascertain the effect in the absence of bacteria.

Pathogen survival. Survival of the pathogen on and in seed was tested by soaking seed in a bacterial suspension of 2×10^8 cfu/ml for 1 min and drying overnight. This experiment was run once. The inoculated seeds were stored at room temperature (20–23 C). The next day and at 1-wk intervals, three 1-g samples (about 440 seeds) were placed in 9 ml of water and soaked for 2 hr, then the supernatant was diluted and plated on MS medium. At each time interval, three petri plates of inoculated and three plates of uninoculated seeds were grown at 28 C to check for disease development.

To study pathogen survival in infected shoots, seeds were inoculated in the usual manner. Rotted sprouting seeds were agitated in sterile water (10 ml), and the wash liquid was diluted and plated at day 2 and at weekly intervals. The experiment was run at 28 C with four replicates.

RESULTS

Bacterial identification. The bacterium causing soft rot of alfalfa sprouts was identified as *E. chrysanthemi* by standard physiological-biochemical tests (Table 1). Colonies on King's medium B (6) were light cream-colored, translucent, round, slightly umbonate, with margins becoming undulate. Colonies, when viewed with transmitted light, appeared somewhat zoned and reached 4–5 mm within 3 days at 28 C. A strong fragrant "banana odor" was evident.

On MS medium, colonies have a fried-egg appearance, becoming orange in the center and lighter toward the margin. Colonies reached 1–2 mm within 3 days. The colonies and surrounding media turned orange within 1–3 days and returned to green after 4 days.

Pathogenicity tests. *E. chrysanthemi* was pathogenic to alfalfa sprouts. Disease severity was correlated with temperature (Fig. 1). Inoculated shoots appeared healthy and had an average radicle length of 9 mm at cooler temperatures (15 C). This is 97% of the control length. At 20 C, the radicles were 10.2 mm long (67% of control length) and appeared yellowish and water-soaked. The rot was severe at 28 C, with the sprouts attaining an average length of only 6 mm (24% of control length). At 34 C, the seed rotted shortly after germination. *E. chrysanthemi* was consistently reisolated from diseased sprouts.

The control sprouts all remained

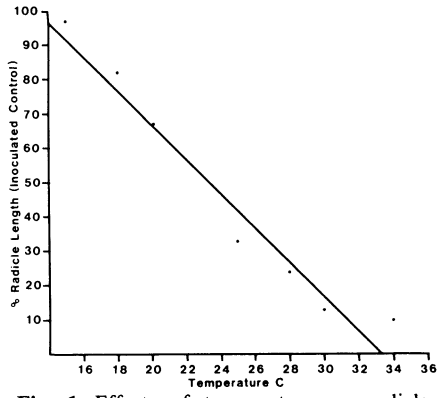

Fig. 1. Effects of temperature on radicle length of inoculated and uninoculated alfalfa sprouts expressed as percent radicle length (uninoculated/control). Each data point represents the mean of three trials ($Y = 1.67 - 0.05x$, $r^2 = 0.954$).

Table 1. Biochemical, physiological, and cultural characteristics of *Erwinia chrysanthemi* from alfalfa sprouts[a]

Characteristic	*E. chrysanthemi* (alfalfa strain)	*E. chrysanthemi*[b]
Cell	Rod	Rod
Gram[c]	–	–
Oxidase[c]	–	–
Hugh-Leifson[c]	+	+
Growth at 37 C[c]	+	+
Sucrose reduction[c]	+	v
Growth in 5% NaCl[d]	Weak +	–
Indole production[d]	+	+
Lecithinase[d]	+	+
Phosphatase[e]	+	+
Erythromycin[c]	Sensitive	Sensitive
Potato soft rot[c]	+	+
Gas from glucose[c]	+	+
Pectate degradation[c]	+	+
Gelatin liquefaction[c]	+	v
Catalase[e]	+	+
Urease[d]	–	–
Acid from (Ayer's method)[d]		
Maltose	–	–
α-Methyl glucoside	–	–
Lactose	–	–
Trehalose	–	–
Dulcitol	–	–
Palatinose	–	–

[a] + = Positive, – = negative, and v = variable.
[b] According to Fahy and Persley (1) and Schaad (10).
[c] By methods in Schaad (10).
[d] By methods in Fahy and Persley (1).
[e] By methods in Gerhardt (2).

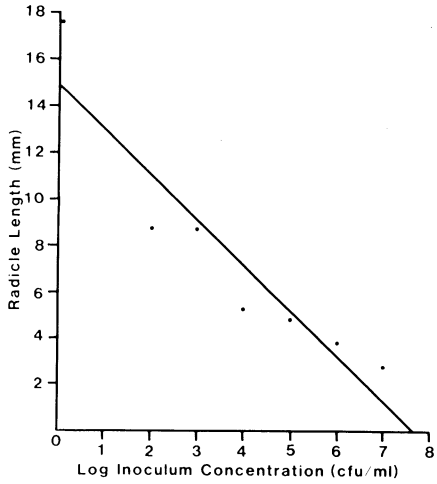

Fig. 2. Effects of inoculum concentrations on radicle length of alfalfa sprouts. Each data point represents the mean of three trials ($Y = 15.0 - 1.97x$, $r^2 = 0.879$).

healthy; sprout lengths of radicles generally increased with temperature, except at 34 C, where there may be heat stress. The average lengths were as follows: 9.1 mm at 15 C, 15.4 mm at 18 C, 15.9 mm at 20 C, 24.2 mm at 25 C, 25.6 mm at 28 C, 26.6 mm at 30 C, and 23.5 mm at 34 C.

Inoculum concentrations. Alfalfa seeds soaked in tap water (28 C) or in a suspension of *E. chrysanthemi* (10 cfu/ml) were healthy and grew to an average length of 17.7 and 18.0 mm, respectively (Fig. 2). Sprouts turned yellowish and translucent and began to rot at an inoculum concentration of 10^2 cfu/ml. The average radicle length was 8.8 mm. The rot became progressively more severe with greater bacterial concentration, until at 10^7 cfu/ml, the sprouts rotted soon after germination and attained an average radicle length of 2.7 mm.

Disease control. Inoculated seeds soaked in water as controls became diseased and grew to an average length of 2.9 mm. Soaking inoculated seeds in calcium hypochlorite prevented the disease, and the average length of the radicles was 29.6 mm. Inoculated seeds soaked in sodium hypochlorite also appeared healthy, and the radicles attained an average length of 23.7 mm. Some disease was apparent in the hydrogen peroxide treatment, and the average radicle length was 13.3 mm. The treatments were all significantly different from each other at $P = 0.05$.

Soaking healthy seeds in the surface-sterilizing agents for 2 hr increased radicle length and did not reduce germination. The average length of radicles in the water control was 24.1 mm, whereas the average length of radicles treated in surface-sterilizing agents ranged from 29.9 to 31.5 mm. The average length of radicles in each of the three treatments was significantly greater than the water control at $P = 0.05$.

Pathogen survival. *E. chrysanthemi* remained viable at a level of 5×10^2 cfu/g of seed and caused disease on inoculated seeds for less than 2 wk at room temperature. Survival was also short on infected shoots, with the population peaking at 2×10^9 cfu/shoot at day 2. The bacterium was not detectable after 1 wk.

DISCUSSION

This is the first report of *E. chrysanthemi* causing a rot of alfalfa sprouts. It is not known to be a disease of mature alfalfa plants. The conditions of high moisture and close contact of seeds during sprout culture present an ideal environment for growth of soft-rotting bacteria. Also, the highly succulent conditions of the sprouts make them very susceptible to bacterial infection.

How the bacterium is introduced into a sprouting house is unknown. *E. chrysanthemi* did not survive on dried seeds for more than 2 wk in our experiments. This contrasts with findings that *E. chrysanthemi* survived on dried *Philodendron selloum* seeds for 13 mo. *E. chrysanthemi* is also known to survive on numerous nonhost plants for 5–6 mo (3). Soft-rotting Erwinias (*E. carotovora* subsp. *carotovora* and subsp. *atroseptica*) have been isolated in water from irrigation ditches, rivers, lakes, snow, and rainwater (5). They have also been detected in open air during rainstorms. There is evidence that airborne spread of viable bacteria could cause major contamination of *Erwinia*-free potato stocks (9). So it is quite possible that sprout houses could become contaminated through the water supply or via the air. Alternatively, greenhouse workers may be a major source of introduction and spread (11). Merely touching an infected sprout tray and then touching a "clean" tray can spread bacteria.

Several control measures are possible once the disease is present in a production area. Temperature-controlled sprouting rooms would reduce disease, because it is not severe below 21 C, although this would slow germination. Sanitation is also an important control measure. Although diseased sprouts may only be infectious for a few days, they could easily infect other sprouts through careless procedures. The bacteria probably survive in water remaining in tanks used for soaking seeds. Because soaking seeds in 0.5% sodium hypochlorite or calcium hypochlorite controls the rot, it would probably be helpful to routinely soak the seeds in a solution of one of these surface-sterilizing agents. Hypochlorite, however, is not currently registered for alfalfa seed treatment in the United States. The reason for the increase in radicle growth in healthy seeds treated with surface-sterilizing agents is not known. It is not related to seed death, because germination was not reduced. Perhaps the surface-sterilizing agents kill detrimental microflora, allowing for increased growth.

Alfalfa sprout rot is a devastating bacterial disease that can best be controlled by a combination of vigorous sanitation and cool growing conditions.

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