Apical Chlorosis and Leaf Spot of Jerusalem Artichoke Incited by *Pseudomonas syringae* pv. *tagetis*

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ABSTRACT

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Pseudomonas syringae pv. *tagetis* causes apical chlorosis, chlorotic and necrotic leaf spots, and stunting of Jerusalem artichoke (JA) plants in Minnesota. More than 80% of the plants in several fields had disease symptoms in 1983. Reduced stands resulted from eventual necrosis and death of many plants that emerged chlorotic. Bacterial strains caused either necrotic spots with slight halos or necrotic spots and chlorosis upon spray inoculation or infiltration of JA. Strains that induced chlorosis were indistinguishable from known strains isolated from sunflower, marigold, or ragweed in pathogenicity on JA, sunflower, marigold, and zinnia, and in biochemical tests. The bacterium is tuberborne.

In 1981, a chlorotic and necrotic disease was observed on Jerusalem artichoke (JA), *Helianthus tuberosus* L., at two University of Minnesota research stations and in several commercial fields in Minnesota. Symptoms similar to those described for *Pseudomonas syringae* pv. *tagetis* Hellmers on marigold (2,13,15,17), sunflower (1), and ragweed (14) included apical chlorosis (Fig. 1); small (1–2 mm diam.), dark, necrotic leaf spots with faint chlorotic halos (Fig. 2); and large chlorotic spots, sometimes with small grey necrotic centers. The objective of this research was to determine the cause of the disease and to ascertain the importance of the disease for the production of JA. A preliminary report has been published (11).

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MATERIALS AND METHODS

Gram-negative, oxidase-negative (5), arginine dihydrolase-negative (16), fluorescin-positive (4) bacteria were consistently isolated in high titer from JA leaf and stem tissue that showed any of the three symptoms mentioned. Comparisons of 15 JA strains from three locations in 1981 were made with known *P. syringae* pv. *tagetis* strains from sunflower, marigold, and ragweed (Table 1). Partial characterizations were made of an additional 25 JA strains collected during 1982 in Minnesota.

The basal medium of Palleroni and Doudoroff (7) was used for carbon compound utilization tests, except ferric chloride (10 mg/L) was substituted for ferric ammonium citrate. Purified agarose (Sigma, St. Louis, MO 63178) (10 g/L) was used to solidify the medium. All carbon sources were adjusted to pH 6.8, filter-sterilized through 0.2-μm filters, and added to the sterile basal medium for

a final concentration of 1 g/L. Test plates were spotted with 10-μl drops of bacterial suspensions, incubated at 27 C, and examined for growth for at least 2 wk. No more than 10 isolates were tested per 9-cm-diameter petri plate. Basal medium plus agar (but no carbon compound) and nutrient agar (NA) (Difco Laboratories, Detroit, MI 48232) plates served as negative and positive controls, respectively. In the case of barely detectable growth, slow-growing isolates were replated on the test media solidified instead with the highly pure Seakem LE agarose (FMC Corporation, Marine Colloids Division, Rockland, ME 04841) at a concentration of 5 g/L.

The plate and tube tests for gelatin liquefaction described by Sands et al (9) were used. Tests for levan production, potato soft-rotting, and nitrate reduction were conducted according to Lelloit et al (6). Additional tests for nitrate reduction were done by the method of Conn (1951) as given by Skerman (12). Ability to degrade pectate was tested according to the method given by Schaad (10). Ability to grow anaerobically was tested by the method of Hugh and Leifson (3) and by the method of deep stabs in Difco phenol red agar plus 10 g/L dextrose (D. F. Ritchie, D. M. Weller, and J. L. White, *unpublished*). Tests for hydrogen sulfide production were conducted by suspending lead acetate paper over bacteria streaked on hydrogen sulfide agar slants. Darkening of the paper within 3 days' incubation was considered positive for hydrogen sulfide production (6). Lead acetate paper was prepared by soaking filter paper strips in a neutral lead acetate suspension (0.1 g/ml) and air-dried. Hydrogen sulfide medium contained 5 g yeast extract, 5 g peptone, 5 g NaCl, 0.1 g cystine, 15 g Difco-Bacto agar, and distilled water to 1 L.

Pathogenicity tests were conducted with bacterial strains grown on NA slants at 27 C for 18–48 hr. Bacterial suspensions were adjusted to 5×10^6 colony-forming units per milliliter based on spectrophotometric readings at 425 nm and a calibration curve determined previously. Suspensions were applied to test plants with a DeVilbiss airbrush so that the foliage was wet but not macroscopically water-soaked. Additional plants were inoculated by infiltration of bacterial suspensions into leaf intercellular spaces using a 10-ml plastic syringe with the needle replaced with a 2-cm-long, 0.5-cm-i.d. rubber tube. A finger wrapped with Parafilm provided support for the plant apex during the infiltration process. Pressure-infiltrated plants were kept in the greenhouse until macroscopic signs of water-soaking dissipated. Inoculated plants were incubated in a chamber (20–24 C) with a 16-hr photoperiod provided by fluorescent lights and intermittently misted (2 min on/3 min off) with deionized water. Plants were removed after 2 days, placed in a greenhouse (22–28 C), and examined for symptoms for 2 wk.

Test plants were the following: JA, *H. tuberosus* 'Columbia,' from Department of Horticulture, University of Minnesota, St. Paul; oilseed sunflower, *H. annus* L. 'DO844' or 'DO704' (Dahlgren & Co., Crookston, MN 56716); African and French marigolds, *T. erecta* L. 'Hawaii' and *T. patula* Cav. 'Janie Gold,' respectively, both from Northrop King, Minneapolis, MN 55413; zinnia, *Zinnia elegans* Jacq., unknown cultivars. All plants were inoculated while still in the vegetative stage because flowering plants fail to show symptoms (13). Plants were sprayed and inoculated by infiltration with each of the 15 JA strains collected in 1981 and seven reference isolates. Pathogenicity of the 25 JA strains collected in 1982 was tested with infiltration inoculations of sunflower seedlings only. In addition, the relative susceptibilities of JA cultivars Sunchoke, obtained from a local grocery store, and Columbia and French White Mammoth, Department of Horticulture, University of Minnesota, St. Paul, were tested with spray and infiltration inoculation with several JA-derived bacterial strains.

JA tubers from a Minnesota commercial field were tested for *P. syringae* pv. *tagetis*. Plants grown from this tuber lot in several commercial fields showed severe chlorosis and necrosis symptoms characteristic of the disease during 1983. Tuber pieces, each with two to three buds, were grown in pasteurized soil under greenhouse conditions (22–26 C) and observed for symptoms. Isolations were made from other tuber sections disinfected for 5 min in 1% NaOCl and rinsed in tap water for 5 min, and from untreated sections. Inner bud tissue was dissected with a sterile scalpel, triturated in sterile distilled water, and 5 min later, streaked on King's medium B. Suspect colonies on the plates were restreaked at least three times for purity. Strains able to cause apical chlorosis in sunflower seedlings, able to fluoresce on King's medium B, and unable to utilize L-lactate and sucrose were considered to be *P. syringae* pv. *tagetis*.

Fig. 1. Apical chlorosis symptoms on Jerusalem artichoke naturally infected by *Pseudomonas syringae* pv. *tagetis*.

Fig. 2. Necrotic spots with slight halos on Jerusalem artichoke naturally infected by *Pseudomonas syringae* pv. *tagetis*.

Table 1. *Pseudomonas syringae* pv. *tagetis* reference isolates

Number of isolates	Original host	Geographic origin	Source
1	Sunflower	Minnesota	R. Urs, Dahlgren & Co.
2	Sunflower	Minnesota	T. Gulya, N.D. State Univ.
1	Ragweed	Wisconsin	R. Durbin, Univ. Wis.
1	Marigold	Rhodesia	R. Durbin, Univ. Wis.
1	Marigold	Minnesota	R. Durbin, Univ. Wis.
1	Marigold	Minnesota	B. Kennedy, Univ. Minn.

RESULTS

Biochemical and physiological tests demonstrated that the bacteria isolated from symptomatic JA tissue were *P. syringae* pv. *tagetis*. The JA strains were levan-negative, aerobic, hydrogen sulfide gas-negative, and potato soft rot-negative and did not pit pectate agar. As with the strains of Trimboli et al (17), all strains from JA utilized mannitol, *m*-inositol, D-sorbitol, quinate, L(+)arabinose, D-xylose, D-gluconate, succinate, and D-glucose and did not utilize L-lactate, sucrose, β-alanine, L-valine, D(+)trehalose dihydrate, or 2-ketogluconate. Unlike the results of Trimboli et al (17), our strains utilized erythritol and some utilized L-tartrate and D-tartrate. In contrast to Trimboli et al (17) and similar to Styer et al (15), none utilized DL-β-hydroxybutyrate or triacetin. In addition, all strains utilized betaine, citrate, and D(+)mannose and did not utilize cellobiose, inulin, L-homoserine, or

fructose. The 25 JA strains collected during 1982 caused apical chlorosis of sunflower seedlings, did not utilize L-lactate or sucrose, and were not characterized further. Colonies of *P. syringae* pv. *tagetis* have slightly irregular margins barely detectable with the unaided eye when grown on NA or King's medium B. Flat colonies with very irregular margins appear with continuous culturing of some strains.

Pathogenicity tests on JA with bacterial strains from this host revealed two strain types: those that induced chlorosis and small necrotic spots (CN strains) and a second class (N strains) that caused only necrosis (Fig. 3). CN and N strains were obtained from all three types of lesions described previously. CN and N strains were indistinguishable in all physiological and biochemical tests. Symptoms induced in JA by spray inoculation with N strains differed somewhat from the original specimens, where varying degrees of chlorosis were always associated with necrotic spots. Six of 40 strains obtained from various JA sources over 2 yr have proven to be N strains. CN- and N-type symptoms have been noticed by other researchers for *P. syringae* pv. *tagetis* on marigold associated with either different strains (17) or in natural versus artificial inoculations (15).

The reference strains from ragweed, marigold, and sunflower were indistinguishable from CN strains from JA in pressure-infiltration inoculations of sunflower, JA, marigold, and zinnia although the chlorosis was more intense with some isolates. Spray inoculations resulted in chlorosis in all combinations including zinnia. Chlorosis in inoculated plants was often associated with slight natural wounds on leaf surfaces that perhaps provided entry points for bacterial invasion. In addition to chlorosis, spray inoculation with CN strains induced dark necrotic spots on JA leaves and to a lesser extent on sunflower leaves. The three JA cultivars tested appeared to be equally susceptible to spray and infiltration inoculations by JA-derived strains.

P. syringae pv. *tagetis* was isolated from buds of both surface-disinfected and untreated tubers. In addition, numerous shoots from similar non-disinfected tuber pieces planted in pasteurized soil in the greenhouse emerged with chlorotic symptoms, as observed for the same tuber lot in several commercial fields.

Fig. 3. Necrotic spots on Jerusalem artichoke spray inoculated with necrotic-type isolate of *Pseudomonas syringae* pv. *tagetis*.

Fig. 4. Jerusalem artichoke sprout emerging from soil completely chlorotic because of natural infection by *Pseudomonas syringae* pv. *tagetis*.

DISCUSSION

JA, like sunflower, is a crop once thought to be relatively free of insects and diseases (8). Occurrence of *P. syringae* pv. *tagetis* on JA is a serious problem primarily because of its effect on stand establishment. Sprouts emerging from the soil nearly or completely chlorotic due to the disease (Fig. 4) often do not survive, based on our observations in commercial fields during 1982 and 1983. The disease caused appreciable losses to at least 15% of the about 25,000 ha of JA planted in Minnesota in 1983. Stand reductions of 50% or more were common; some fields were plowed under. The disease appears to spread readily in the field. This observation is substantiated by the high titers of bacteria in naturally infected tissues and the relative ease of infection of JA in the mist chamber and greenhouse. Infections occurring after the plants are well established in the field, however, generally cause only slight stunting, which is often outgrown as the plants approach flowering.

The pathogenic significance of the N-type strains of *P. syringae* pv. *tagetis* is not certain but appears to be minor because appreciable plant damage has so far been associated with cases where chlorosis was the predominant symptom. Lesions associated with N-type strains in nature were generally no larger than 1–3 mm in diameter.

The finding that the disease is tuberborne is consistent with our observations that certain tuber lots consistently produced plants with high disease incidences and that symptoms are evident on sprouts as they emerge from the soil. Although *P. syringae* pv. *tagetis* could be isolated from surface-disinfected buds, this does not preclude the possibility that the organism may also be present in soil on tuber surfaces.

The disease was common in several Minnesota fields during 1981, somewhat uncommon in 1982, and rampant in 1983 throughout the state and adjoining areas where tubers from Minnesota were sent. Research is needed to elucidate the conditions favoring survival of bacteria on or in tubers and subsequent symptom expression. Practical methods are also needed for treating seed tubers for disease control before planting. At the present time, it seems most advisable to obtain seed stock for future plantings only from fields with no apparent symptoms. Field inspections should be made before flowering, when symptoms may disappear; however, absence of symptoms is no guarantee that the tubers are free of the bacterium.

The bacterium appears to be indigenous to the state on common ragweed. We have isolated *P. syringae* pv. *tagetis* from chlorotic ragweed in rural and metropolitan areas of Minnesota, apparently where no susceptible crops have been grown. Chlorosis was very common on ragweed growing in several severely infected JA fields. The disease also occurs sporadically in commercial sunflower fields (1). Strains from JA, marigold, sunflower, and ragweed were indistinguishable in physiological and carbon compound utilization tests. The usefulness of erythritol utilization as a distinguishing character is uncertain—all strains we tested, regardless of source, utilized this compound in contrast to the findings of Trimboli et al (17). Most *P. syringae* pv. *tagetis* strains could be tentatively identified by their capacities for inducing chlorosis in sunflower, JA, zinnia, and marigold. Identification of non-chlorosis-inducing strains is aided by their somewhat distinctive colony morphology and the apparent inabilities to utilize L-lactate or sucrose. It is interesting to note that *P. syringae* pv. *tagetis* is unable to utilize inulin, a β-(2-1)fructofuranose that is the major storage carbohydrate in JA tubers, and it could not utilize fructose, the monosaccharide of which inulin is composed. No consistent differences could be detected in biochemical or carbon compound utilization tests for distinguishing *P. syringae* pv. *tagetis* strains originating from different plant species. Further work is needed to determine if these strains are identical in their host ranges.

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