Apical Chlorosis of Sunflower Caused by *Pseudomonas syringae* pv. *tagetis*

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ABSTRACT

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Pseudomonas syringae pv. *tagetis* was determined to be the causal agent of an apical chlorosis of sunflower, *Helianthus annuus*, in Minnesota and North Dakota. No other fungal or viral pathogens were implicated. No other field crops commonly grown in the two states were susceptible; the host range of the pathogen appears to be limited to members of the Compositae. The bacterium was seed-transmissible. Resistance was found in several USDA sunflower inbred lines.

A disease of unknown etiology, characterized by extreme apical chlorosis, was observed on oilseed and confection sunflower (*Helianthus annuus* L.) in Minnesota and North Dakota. The symptoms did not resemble those caused by known pathogens of sunflower (1,7,10,16) or those of previously reported chlorophyll deficiencies (3). The symptoms did resemble those of apical chlorosis of marigold (*Tagetis erecta* L.) (5) incited by *Pseudomonas syringae* pv. *tagetis* (*P. s.* pv. *tagetis*; ISPP List, 1980) (2).

The objective of this study was to determine the etiology of this disease, the host range of the causal agent, mode of transmission, and sources of resistance. A preliminary report (4) has been published.

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MATERIALS AND METHODS

Pathogen identification. Chlorotic sunflower leaf and petiole tissues were surface sterilized in 70% ethanol for 10 sec, macerated in sterile distilled water, and streaked onto nutrient agar and King's medium B (8). Resulting bacterial isolates were restreaked to ensure purity and inoculated onto 2-wk-old hybrid 894 sunflower plants by six techniques on different plants: injecting a washed suspension of bacteria into the growing point, stem, or cotyledons; spraying an atomized suspension of bacteria onto the leaves with or without subsequent incubation in a mist chamber for 72 hr; or immersing washed roots in a bacterial suspension for 5 min. Known cultures of *P. s.* pv. *tagetis*, isolated from marigold by R. D. Durbin; *P. s.* pv. *helianthi* (ISPP List, 1980; ATCC 19866); and *P. s.* pv. *syringae* (ISPP List, 1980) (2) isolated from wheat were also inoculated onto sunflower by the same six techniques.

Bacteria reisolated from chlorotic leaf tissue were compared with the original strains from sunflower and with *P. s.* pv. *tagetis* from marigold. Identification was based on the following tests: Gram stain, oxidase reaction, fluorescence on King's medium B, arginine dihydrolase production, levan formation, potato soft rot,

tobacco hypersensitivity, production of hydrogen sulfide and indole, hydrolysis of starch and gelatin, denitrification, growth at 41 C, and the ability to utilize various compounds as sole carbon sources (6,8,11).

Host range. Thirty-two species of crop plants and weeds were tested for susceptibility to both the marigold and sunflower strains of *P. s.* pv. *tagetis*. One to six cultivars represented each crop species. The species tested included the following: adzuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi), barley (*Hordeum vulgare* L.), bean (*Phaseolus vulgaris* L.), broad bean (*Vicia faba* L.), broccoli (*Brassica oleracea* var. *botrytis* L.), buckwheat (*Fagopyrum esculentum* Moench), cardoon (*Cynara cardunculus* L.), chicory (*Cichorium intybus* L.), cocklebur (*Xanthium pensylvanicum* Wallr.), corn (*Zea mays* L.), cucumber (*Cucumis sativus* L.), endive (*Cichorium endivia* L.), field pea (*Pisum sativum* var. *arvense* (L.) Poir.), flax (*Linum usitatissimum* L.), jimson weed (*Datura stramonium* L.), lambsquarters (*Chenopodium album* L.), lettuce (*Lactuca sativa* L.), African marigold (*Tagetes erecta*), French marigold (*T. patula* L.), black mustard (*Brassica nigra* (L.) Koch), white mustard (*B. hirta* Moench), potato (*Solanum tuberosum* L.), rape (*Brassica napus* L.), safflower (*Carthamus tinctorius* L.), salsify (*Tragopogon porrifolius* L.), soybean (*Glycine max* (L.) Merr.), sugar beet (*Beta vulgaris* L.), sunflower, tomato (*Lycopersicon esculentum* Mill.), durum wheat (*Triticum durum* Desf.), winter wheat (*T. aestivum* L.), and zinnia (*Zinnia elegans* Jacq.).

Four pots of each cultivar with three to 10 plants per pot were tested in each of two greenhouse experiments, with one additional pot as a check. Plants were inoculated when 10–14 days old by infiltrating the youngest leaf or cotyledons with a hypodermic syringe. Inoculum consisted of 10^8 colony-forming units (CFU) per milliliter of a washed suspension of *P. s.* pv. *tagetis* from 24-hr-old cultures on nutrient agar with 5% (w/v) dextrose. Control plants were injected with sterile distilled water. Plants were evaluated for apical chlorosis 1 and 2 wk after inoculation.

Seed transmission. The potential of seed transmission of *P. s.* pv. *tagetis* from infected sunflower was tested with seven separate seed lots. One lot consisted of bulked seed collected from infected plants in a commercial breeding nursery. Other lots consisted of seed from individual plants of three different commercial hybrids. Seeds from all lots were surface sterilized in 1% sodium hypochlorite for 5 min. A total of 150 seeds of the bulked sample and 50 seeds from each of six individual heads were planted in steam-sterilized soil in 75-mm pots, one seed per pot. The number of plants showing symptoms of apical chlorosis was recorded weekly for 4 wk.

Evaluation of sunflower germ plasm for resistance. Twenty-four released USDA inbred lines, including six nonoil lines, were evaluated in the greenhouse for resistance to *P. s.* pv. *tagetis*. The experiment was designed as a randomized complete block with three replicates. The experimental unit consisted of five pots of an individual line with two plants per pot for a total of 60 plants per line in both experiments. Plants were inoculated when 10 days old by injecting a washed suspension of bacteria (10^8 CFU/ml) into both cotyledons. Symptoms were evaluated weekly for 5 wk after inoculation.

RESULTS

Symptoms. Symptoms consisting solely of leaf chlorosis without discernible lesions were observed on sunflower in all vegetative growth stages, although they were more frequent and severe on seedlings. Often only a portion of the initially affected leaf was chlorotic, but subsequently formed leaves were uniformly chlorotic, including the veins (Fig. 1). Infected seedlings were frequently stunted and occasionally died; however, infection of older plants rarely resulted in any stunting. With seedling infection, systemic chlorosis lasted up to 8 wk and spanned eight to 10 leaves, but chlorosis on older (prebloom) plants was frequently limited to a few leaves. Symptoms were never observed on subapical, fully expanded leaves of plants past the bud stage, nor were chlorotic leaves ever observed to recover. Chlorotic plants were usually scattered throughout a field, occurring singly or in small groups within a row or occasionally spanning two to three rows. The distribution of diseased plants did not appear to be associated with any variation in topography.

Pathogen. Gram-negative, oxidase-negative, fluorescent bacteria were consistently isolated from leaves and petioles of chlorotic sunflowers, and all such strains produced apical chlorosis 4–5 days after inoculation of healthy sunflowers. All pathogenic sunflower strains and the marigold strain of *P. s.* pv. *tagetis* behaved identically in biochemical tests and conformed to previous descriptions (5,15), with the exception of an inability to utilize ethanol as a carbon source. Strains from either host produced apical chlorosis in sunflower when inoculated by injection or root immersion. Injection into the growing point or cotyledons produced the most pronounced symptoms and was judged the most reliable technique. Neither apical chlorosis nor any leaf lesions were produced when bacterial suspensions were sprayed onto sunflower, with or without subsequent incubation in a mist chamber. In contrast, *P. s.* pv. *helianthi* produced angular lesions on sunflower leaves after spray inoculation but no apical chlorosis with any inoculation method. *P. s.* pv. *syringae* was not pathogenic on sunflower.

Fig. 1. Symptoms of apical chlorosis on a hybrid sunflower plant naturally infected by *Pseudomonas syringae* pv. *tagetis*.

Host range. Sunflower, zinnia, and marigold exhibited apical chlorosis when inoculated with either the sunflower or marigold strains of *P. s.* pv. *tagetis*, as did other species within the Compositae, including chicory, cocklebur, endive, and salsify. Limestone lettuce and Sidwill safflower, also in the Compositae, exhibited only slight chlorosis. None of the other species exhibited chlorosis with either strain, with the exception of tomato, cucumber, and some *Brassica* species (mustard, rape, and broccoli). Chlorosis on *Brassica* species, however, was generally limited to one or two leaves and was very mild and ephemeral on tomato and cucumber.

Seed transmission. Plants from six of seven sunflower seed lots developed symptoms of apical chlorosis. Of 140 plants from the bulk seed sample, 15% were chlorotic 2 wk after planting. From 0 to 63% of the seedlings derived from single heads developed apical chlorosis; overall, 21% of the 330 plants were systemically chlorotic 4 wk after planting.

Evaluation of sunflower resistance. None of the inbred lines tested exhibited immunity to *P. s.* pv. *tagetis*. Significant differences in susceptibility among lines were evident, however, when the percentage of systemically chlorotic plants was compared 5 wk after inoculation (Table 1). Two weeks after inoculation, some of the developing leaves of the more resistant lines were visibly less chlorotic than older leaves on the same plant. By 5 wk, systemic chlorosis was evident in the developing leaves of only 2% of the plants of these lines. Systemic chlorosis persisted through 5 wk on 61% of the plants of the most susceptible lines. Recovery of individual chlorotic leaves was never

Table 1. Disease severity of selected sunflower inbred lines inoculated with *Pseudomonas syringae* pv. *tagetis*

Inbred	Plants systemically chlorotic after 5 wk (%)[a]
HA 234	2 a
HA 124	2 a
RHA 299	4 a
RHA 298	6 ab
HA 99	8 abc
HA 232	9 abc
RHA 282	13 abcd
HA 89	15 abcde
RHA 273	16 abcde
HA 303	19 abcdef
HA 285	30 bcdefg
RHA 278	31 cdefg
RHA 271	31 cdefg
HA 290	35 defg
RHA 297	36 defgh
RHA 296	40 efgh
RHA 293	40 efgh
RHA 274	40 efgh
HA 300	44 fgh
HA 288	44 fgh
HA 292	47 gh
HA 113	61 h
RHA 280	61 h
HA 291	61 h

[a] Average of six replicates (60 plants) in two separate experiments. Means with the same letter are not significantly different at the 5% level, according to Duncan's multiple range test.

observed; a leaf, regardless of the degree of chlorosis, remained chlorotic until senescence. Plants of the more susceptible lines were visibly stunted compared with control plants, and systemic chlorosis persisted until flowering on several plants grown to maturity. Chlorosis was never observed on bracts or ray flowers.

DISCUSSION

This is the first report of *P. s.* pv. *tagetis* on sunflower in the United States, although the bacterium has been found previously on marigold in many countries (5,14,15) and on common ragweed (13). Trimboli et al (15) were able to infect sunflowers in the greenhouse but did not report it occurring naturally on sunflower in Australia. We have observed apical chlorosis on sunflower in Florida, and it has been seen on sunflower in Mexico and South Africa (G. N. Fick, *personal communication*).

None of the other *P. syringae* pathovars produced apical chlorosis in sunflower. In preliminary work (*unpublished*), we were unable to produce apical chlorosis with any fungus isolated from chlorotic sunflower. Electron microscope examination of sunflower leaf-dip preparations or of sections of embedded chlorotic sunflower leaves failed to reveal any viruslike particles or mycoplasmalike organisms. Disease transmission with the green peach aphid (*Myzus persicae* Sulzer) or the aster leafhopper (*Macrosteles fascifrons* Stål) was unsuccessful.

The host range of *P. s.* pv. *tagetis* appears limited to members of the Compositae. The inoculum concentration used in the host range study was higher than normally suggested (12) to avoid possible interference from epiphytic bacteria. Even at 10^8 CFU/ml, none of the tested species exhibited the extreme chlorosis seen on sunflower and marigold. The mild symptoms on the safflower and lettuce cultivars indicated that there may be varietal differences within these species. No other field crops commonly grown in this area were susceptible to the bacterium, although the chlorosis-producing toxin itself is not host specific (9). No differences were noted in the host range between the marigold and sunflower strains of *P. s.* pv. *tagetis*. The sole difference in symptomatology between the two hosts was the absence of necrotic leaf lesions on sunflower. Trimboli et al (15), however, did note sparse leaf lesions following spray inoculations of sunflower, but this never resulted in apical chlorosis.

Transmission of the disease in nature is mainly through seed or possibly via soilborne inoculum (15). Failure to obtain symptoms by spray inoculation and the highly localized occurrence of the disease within fields suggest that rain is not as efficient in disease spread in sunflower as it is in marigold (5,14).

Several USDA sunflower inbred lines were found with high levels of resistance to *P. s.* pv. *tagetis* in greenhouse tests, although none was immune. Attempts at field evaluations of sunflower hybrids and inbreds have been unsuccessful. From our experience, disease development and thus resistance evaluation is best when plants are inoculated in the seedling stage (1–2 wk after emergence) by injection of the cotyledons or growing point.

The incidence of sunflower apical chlorosis has been sporadic in North Dakota and Minnesota, and severity within individual fields generally has not exceeded 1–2%; thus the disease does not appear to pose a major threat to sunflower production in this region. Suggested control measures would include thorough roguing of chlorotic plants in seed production fields, the use of crop rotation to avoid increasing the soilborne population of *P. s.* pv. *tagetis*, and the use of resistant hybrids once they are identified.

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