Host Range of Strains of *Pseudomonas syringae* pv. *tagetis*

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ABSTRACT

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Using different inoculation techniques, the host range was determined for *Pseudomonas syringae* pv. *tagetis* strains isolated from marigold, sunflower, common ragweed, Jerusalem artichoke, and a presumptive strain from dandelion. Wound inoculation induced apical chlorosis in all hosts except dandelion, which exhibited no symptoms. With spray inoculation, ragweed became infected but only with strains isolated from this host; other hosts, except dandelion, exhibited leaf spots and apical chlorosis with all strains. Dandelion became temporarily chlorotic only when the inoculum was infiltrated into the leaf with a hypodermic syringe. Despite its apparent inability to infect dandelion, this strain appears to be pv. *tagetis* based on the distinctive symptoms it induces on marigold, its microbiological characteristics, and its fatty acid ester profile.

Five species of Compositae have been reported to be naturally infected by *Pseudomonas syringae* pv. *tagetis* (Hellmers) Young, Dye, & Wilkie: African marigold (*Tagetes erecta* L.) (2), dwarf margiold (*T. patula* L.) (10), sunflower (*Helianthus annuus* L.) (1), common ragweed (*Ambrosia artemisiifolia* L.) (8), and Jerusalem artichoke (*H. tuberosus* L.) (6).

We have recently isolated a bacterium from dandelion (*Taraxacum officinale* Weber) that exhibited apical chlorosis and necrotic leaf spots characteristic of the disease. Furthermore, the symptoms on marigold caused by this bacterium were identical to those induced by marigold strains of pv. *tagetis*. On this basis, we felt that the bacterium from dandelion might be another strain of pv. *tagetis*.

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The strains from the five hosts are indistinguishable on the basis of conventional biochemical tests, and they produce in their respective hosts the apical chlorosis typical of the disease (6, 9). On marigold, however, leaf lesions are substantially larger than they are on the other species. A preliminary comparison of five marigold strains failed to resolve the question of whether this is attributable to the strain or host cultivar, but it demonstrated that the strains varied in virulence.

The objectives of this study were to examine the response of each known host to all the strains, including the strain from dandelion, and to complete the microbiological tests necessary to characterize the strain from dandelion.

MATERIALS AND METHODS

Marigold, sunflower (cultivar Dahlgren 716), and Jerusalem artichokes were grown in the greenhouse during the summer. Ragweed and dandelion seed and Jerusalem artichoke tubers were collected locally. Ragweed plants were grown during the winter in a greenhouse (28 C and 18 hr of light); dandelions were grown in a growth chamber (28 C and 12 hr of light).

Marigold cultivars were used that varied in disease resistance to pv. *tagetis*. As previously determined by Styer and Durbin (7), *T. erecta* 'Moonshot,' 'Crackerjack,' and 'Diamond Jubilee' are susceptible and exhibit severe chlorosis and many leaf spots, Orange Jubilee and *T. patula* 'Queen Sophia' are intermediate in reaction, and Viking is resistant, exhibiting mild chlorosis and few leaf spots.

Five marigold strains of the bacterium were isolated from plants at St. Paul, MN; two Jerusalem artichoke strains were provided by W. W. Shane and J. S. Baumer (Department of Plant Pathology, University of Minnesota), and two were isolated from diseased Jerusalem artichoke collected at Madison and Verona, WI. The putative dandelion strain was isolated at Madison. One sunflower strain came from Arlington, WI, and one was provided by T. J. Gulya (Department of Plant Pathology, North Dakota State University). Three ragweed strains came from plants collected at Madison, Hancock, and Arlington, WI.

In all experiments, except the preliminary comparison of marigold strains, five to seven plants of each host were inoculated with representative strains of the pathogen from each host. In the comparison, three marigold plants (cultivar Moonshot) were sprayed with each of five marigold strains. For ragweed susceptibility experiments and assessments of symptoms induced by ragweed strains on marigold (Moonshot),

all three ragweed strains were used. When infection did not result from inoculation, for example in ragweed susceptibility experiments, additional strains were employed.

Ragweed and Jerusalem artichoke plants were inoculated 3 wk after germination, sunflower and dandelion at 4 wk, and marigolds at 4–5 wk. Plants were scored for symptoms at the time of maximal expression—usually 7–10 days after inoculation; however, observations of dandelions were made up to 3 wk after inoculation.

Two inoculation procedures were used; in each, the bacteria were washed and diluted with 0.85% saline to 1×10^9 cfu/ml. The first technique involved spraying plants to runoff with the bacterial suspension. Inoculated plants were then incubated overnight in a mist chamber at 24 C. For the second procedure, a 20-μl droplet of the bacterial suspension was placed in the leaf or cotyledonary axils. The stem beneath each droplet was then punctured with a sterile dissecting needle and the droplet was passively drawn into the plant.

Two additional methods of inoculating dandelion were tried. In one, leaves of 5-wk-old dandelion plants were infiltrated with the bacterial suspension by means of a hypodermic syringe and a 27-gauge needle inserted along the midrib. Routinely, less than 0.1 ml per leaf was infiltrated. In the second method, 3-wk-old dandelion seedlings were gently uprooted, transplanted, and well watered. Fifteen milliliters of the bacterial suspension was then poured onto the plant and allowed to soak into the soil.

Procedures used to characterize the bacterium from dandelion were: Gram strain, tobacco hypersensitivity induction (HR), and tests for arginine dihydrolase, oxidase, catalase, and fluorescine production. The ability to utilize *m*-inositol, sorbitol, succinate, mannose, xylose, fructose, glucose, sucrose, fucose, maltose, or ethanol as a sole carbon source was also tested (5). A marigold strain from our collection of pv. *tagetis* was used for comparison. In addition, fatty acid profiles of all strains were determined by M. Sasser according to the methods described by Sasser and Miller (4).

RESULTS

After wound inoculation, all strains infected each of the hosts except dandelion. Infected plants exhibited apical chlorosis, but no leaf spots ever developed. Chlorosis was severe in all cases; however, the intensity varied slightly with the strain. When inoculated by the spray technique, ragweed developed symptoms only when inoculated with strains from ragweed. Dandelion was not infected, but the other hosts, as with wound inoculation, were infected by all strains of the bacterium. In addition to apical chlorosis, infected plants exhibited necrotic leaf spots, sometimes accompanied by chlorotic halos. The intensity of chlorosis varied among strains, ranging from very mild to severe. There was no correlation between strain virulence and the hosts from which they were originally isolated. By the infiltration method, dandelion was infected by all strains, but the plants began to recover within a week. Dandelion was not infected when inoculated by the soil drench treatment.

The leaf lesions induced on marigolds by two of the three ragweed strains were characteristically different from those produced by the other strains. They were few in number and about 0.5 mm in diameter. In some plants, leaf spots were entirely lacking. The other ragweed strain, isolated at Madison, and those from the other hosts included leaf spots on marigold that were more numerous and typically 2–5 mm in diameter.

Marigold cultivar reaction, in terms of severity of symptoms, was a function of strain virulence. When five marigold strains were compared, symptoms varied from moderate to severe both in the intensity of the chlorotic reaction and the number of leaf spots, but symptom expression within treatments was consistent. After spray inoculation, the same relative differences in strain virulence was obtained as with wound-inoculated plants, but the range of symptom expression was much broader. The reaction of the marigold cultivars after spray inoculation with all strains was in accordance with that previously reported for a single pv. *tagetis* strain from marigold (7).

In the microbiological tests, the bacterium isolated from the dandelion behaved identically to pv. *tagetis*: it was gram-negative, arginine dihydrolase-negative, oxidase-negative, and catalase-positive. It caused HR in tobacco, produced fluorescine, and had a pattern of carbon source utilization identical to that previously determined for pv. *tagetis* (9).

Fatty acid profiles reveal three rough groupings of strains not associated with other pathovars. The strains from marigold, ragweed, and dandelion are indistinguishable, whereas the sunflower and Jerusalem artichoke strains constitute two other closely related but separate groups (M. Sasser, *personal communication*).

DISCUSSION

The results clearly separate the ragweed strains of pv. *tagetis* from the others. Only these can infect ragweed by spray inoculation. Parenthetically, if the original strain of pv. *tagetis* had been isolated from ragweed rather than marigold, it is unlikely that strains subsequently isolated from other hosts, which are unable to infect ragweed, would bear the same pathovar designation.

The differences in host susceptibility and symptom expression based on the spray and wound inoculations illustrate the importance of using an appropriate inoculation technique for assessing a strain's natural host range. By means of wound inoculation techniques, other workers showed that pv. *tagetis* is capable of inducing chlorosis in a relatively broad range of plant species not limited to the Compositae (1,10). When host range is determined by the spray technique, however, differences in host susceptibility are evident. Additionally, the leaf spots obtained are useful in assessing both strain virulence and cultivar susceptibility.

Dandelion was infected by bacterial infiltration but not by any of the other inoculation technique we tried, even when the bacterium originally isolated from dandelion was used. Natural infection of the other hosts is relatively common, but we have found only one instance of natural infection of dandelion. Nevertheless, we believe that the bacterium isolated from dandelion is another pv. *tagetis* strain. Symptoms on the dandelion infected with this bacterium were characteristic of the symptoms incited on other hosts, and the bacterium induces symptoms in marigold that are indistinguishable from those caused by marigold strains of pv. *tagetis*. The results of microbiological procedures were identical to those obtained with the control pv. *tagetis* strain. Furthermore, the fatty acid profiles group the bacterium from dandelion with marigold and ragweed strains of pv. *tagetis*. Perhaps the bacterium infects dandelion only uncommonly; if so, this would expand the known host range of pv. *tagetis* to a third tribe, Lactuceae, in which dandelion is placed (3). Previously, it had been reported to infect hosts within two other Compositae tribes (Tageteae and Heliantheae) (8). This further supports the concept that pv. *tagetis* is restricted to host in the Compositae and suggests that additional economically important Compositae species may be affected. In earlier wound inoculated studies, however, species such as lettuce and chicory showed only slight chlorosis (1).

Shane and Baumer (6) have suggested that infected ragweed may serve as a source of inoculum for infection of Jerusalem artichoke in the field. If this were the case, strains isolated from naturally infected Jerusalem artichokes should infect ragweed. However, of the 11 strains we have tested, including four from Jerusalem artichoke, none will infect ragweed by spray inoculation, except those originally isolated from ragweed. In addition, we have reisolated and reinoculated a ragweed strain through four generations of marigold (Moonshot) to determine if it would lose pathogenicity on ragweed. It did not.

From these results, we conclude that ragweed probably does not serve as an inoculum source for Jerusalem artichokes or the other hosts.

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