Xanthomonas Leaf Spot of Catnip: A New Disease Caused by a Pathovar of *Xanthomonas campestris*

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ABSTRACT

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Xanthomonas leaf spot is a new disease that has occurred on catnip (*Nepeta cataria*). Catnip is grown commercially in California for use as herbs, seasonings, and tea. This disease has developed recently on catnip transplants that are produced in enclosed greenhouses. Symptoms consist of small brown flecks that are visible from both sides of a leaf. The flecks later develop into larger, dark brown, angular leaf spots. Severe infection reduced the quality and marketability of the transplants. *Xanthomonas campestris*, as identified by biochemical, physiological, and molecular tests, was consistently isolated from symptomatic plants, and selected strains caused similar symptoms when inoculated onto catnip test plants. However, catnip strains failed to cause any symptoms when inoculated onto nine other plants in the Lamiaceae family and five other hosts of known *X. campestris* pathovars. Catnip plants showed no symptoms when inoculated with *X. campestris* pvs. *campestris*, *carotae*, and *vesicatoria*. Catnip also was not susceptible to the *X. campestris* pathogen isolated from lavender. This is the first report of a bacterial disease of catnip caused by a *Xanthomonas* pathogen, and the catnip strains may be a new and distinct pathovar of *X. campestris*.

Additional keywords: hrp primers

Catnip (*Nepeta cataria*) is a perennial shrub in the Lamiaceae family and is probably best known as a plant that is attractive to cats. In California, the plant is grown and used for herbs, seasonings, and tea. Beginning in 1998, catnip grown commercially as transplants in California greenhouses exhibited symptoms of a previously undescribed foliar disease. Initial symptoms consisted of small brown flecks (1 to 2 mm diameter) on foliage that were visible from both adaxial and abaxial sides of the leaves. These flecks expanded into larger leaf spots (2 to 8 mm diameter) that were dark brown to black and angular in shape. When sections of leaf lesions were cut, mounted in water, and examined with phase-contrast microscopy, bacterial streaming was observed consistently. When grown in greenhouse conditions that included overhead sprinkler irrigation, transplants developed extensive leaf infections and had reduced plant growth and reduced quality and marketability. The purpose of this study was to determine and characterize the causal agent of this disease.

MATERIALS AND METHODS

Isolation of the causal agent. Symptomatic leaves were surface-sterilized (0.525% sodium hypochlorite; 1 min), and small (3 × 3 mm) sections of tissue were excised aseptically from leaf spot margins and macerated individually in 40 µl of sterile distilled water. The resulting suspensions were streaked onto sucrose peptone agar (SPA) (3) plates and incubated at 24°C. After 3 to 5 days, representative bacterial strains were purified by restreaking single colonies onto SPA, then kept in 40% glycerol at -70°C for routine use or lyophilized for long-term storage. To test for possible fungal pathogens, small, surface-sterilized leaf sections were removed aseptically and placed onto acidified potato dextrose agar (2 ml of lactic acid per liter). Plates were incubated under lights at 24°C and examined after 3 to 10 days for fungal growth.

Identification of the bacteria. Isolated strains of recovered bacteria were tested for morphological, biochemical, and physiological characteristics. Gram and flagellar stains were performed as described by Schaad et al. (9). Standard tests necessary for identification of xanthomonads (catalase and oxidase reactions, arginine dihydrolase, ability to induce pitting on polypectate gel, and hypersensitive reaction on tobacco [*Nicotiana tabacum* cv. Turk] leaves) were conducted on the strains (9).

Bacteria also were characterized by determining their carbon source utilization profiles on Biolog GN microplates

(Biolog, Inc., Hayward, CA) and by fatty acid analysis. Bacteria were cultured on trypticase soy broth agar at 28°C for 24 h, then extracted for fatty acid methyl esters using a standard method (10). Fatty acids were analyzed with the Sherlock Microbial Identification System Version 2.11 (MIDI Inc., Newark, DE) that used an automated GC 6890 Hewlett-Packard gas chromatograph fitted with a 25 × 0.2 mm phenyl methyl silicone-fused silica capillary column, an HP 7673 automatic sampler, and HP Chem Station Software (10). *Xanthomonas campestris* pv. *campestris* (strain 0186-1) from cauliflower (*Brassica oleracea* subsp. *botrytis*) and *X. campestris* pv. *vesicatoria* (strain 0788-2) from tomato (*Lycopersicon esculentum*) were used as controls. For this study, we chose to follow the more traditional classification of xanthomonads as restated in Schaad et al. (9) rather than following the revised classification scheme of Vauterin et al. (11).

DNA amplification with hrp-related primers. Total bacterial genomic DNA was isolated by the cetyltrimethylammonium bromide (CTAB) purification method essentially as described by Ausubel et al. (1). The primers (RST21 [5'GCACGCTCCAGATCAGCATCGAGG3'] and RST22 [5'GGCATCTGCATGCGTGCTCTCCGA3']) were designed originally by Leite et al. (8) to delineate a 1,075-bp fragment of the hypersensitivity reaction and pathogenicity (hrp) gene region of *X. campestris* pv. *vesicatoria*. DNA was amplified in a total volume of 100 µl of reaction mixture, which contained 10 µl of Vent DNA polymerase 10× buffer (NE BioLabs, Inc., Beverly, MA), 200 µM each deoxynucleoside triphosphate (NE BioLabs), 0.1 µM of BSA, 1 mM MgSO₄, 5% DMSO, 1 µM of each primer, and 1 µl of Vent polymerase (4). The amount of template DNA added was 25 ng of total bacterial DNA. PCR amplification was performed in an automated thermocycler (EasyCycler TM Series, Ericomp, Inc., San Diego, CA) using the following cycles: an initial denaturation of DNA template at 95°C for 5 min; 24 cycles of denaturation at 95°C for 1 min each; annealing at 57°C for 30 s; extension at 72°C for 1 min; and a final extension at 72°C for 5 min. Detection of amplified DNA was performed according to previously described procedures (1,8).

Pathogenicity to catnip. Pathogenicity to catnip was demonstrated by growing inocula of nine individual strains in nutri-

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ent broth (Difco Laboratories, Detroit, MI; 8 g/liter) shake cultures for 48 h. The resulting bacterial suspensions (10^6 CFU/ml as determined with dilution plating) received either Tween 20 (0.05%) and were sprayed onto potted catnip plants using a hand-held mister, or Carborundum and were inoculated by rubbing onto catnip leaves. Control plants were sprayed or rubbed with sterile nutrient broth plus Tween 20 or Carborundum, respectively. Twelve plants, each 2 months old, were used for each inoculation. After inoculation, plants were incubated in a dew chamber maintained at 18°C for 48 h, then kept in a greenhouse at 22 to 24°C. Plants were examined daily for symptoms. The experiment was done twice.

Pathogenicity to other plants in Lamiaceae. To test the ability of the catnip strains to infect other plants in the Lamiaceae family, inocula of five strains were produced as described previously. Each broth culture was inoculated by spraying or rubbing onto separate sets of 12 plants of each of the following: carpet bugle (*Ajuga reptans* cv. Mahogany), basil (*Ocimum basilicum*), coleus (*Coleus blumei* cv. Rainbow Mixed), English lavender (*Lavandula angustifolia*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), salvia (*Salvia farinacea* cv. Blue Bedder), thyme (*Thymus vulgaris*), and spearmint (*Mentha spicata*). Catnip plants also were inoculated with the strains, and control plants of all species were spray- or rub-inoculated with sterile nutrient broth. After inoculation, plants were incubated in a dew chamber maintained at 18°C for 48 h, then kept in a greenhouse at 22 to 24°C. Plants were examined daily for symptoms. The experiment was done twice.

Host range comparison of *X. campestris* strains from catnip and other *X. campestris* hosts. To compare host ranges of *X. campestris* pathogens from catnip and other plants, bacterial colonies of four catnip strains (O-1, O-12, R-1, R-12), one *X. campestris* pv. *campestris* strain (0186-1) from cauliflower, one *X. campestris* pv. *carotae* strain (1199-54) from carrot (*Daucus carota* subsp. *sativus*), and one *X. campestris* pv. *vesicatoria* strain (0788-2) from tomato were grown on YDC agar for

48 h at 28°C and then suspended in sterile distilled water at a concentration of 10^6 CFU/ml, based on optical density readings with a Klett-Summerson colorimeter. The bacterial suspensions either were infiltrated into leaves using syringes or sprayed onto plants until runoff with an aerosol-propelled spray unit. Three plants each of the following were inoculated by each method: catnip, cauliflower, pepper (*Cap-sicum annuum*), tomato, and stock (*Mat-thiola incana*). Because of its very small leaves, carrot was inoculated only by spraying. These plant species were chosen because catnip transplants were grown in greenhouses that also contained transplants of several of these species (cauliflower, pepper, tomato) or because these species also are hosts of an *X. campestris* pathovar.

In another experiment, inocula were prepared of three strains (0894-4, 1294-6, 1294-9) of an *X. campestris* that was shown previously to be pathogenic on lavender (6). Inocula were prepared as described previously and were infiltrated or sprayed onto different sets of catnip and lavender plants.

All inoculated plants were misted intermittently for 24 h with incubation temperatures at 23 to 26°C. Plants later were transferred to a greenhouse having ambient temperature at 17 to 22°C. The plant reactions to inoculations were recorded every day for 30 consecutive days. All of these host range comparison inoculations were done twice.

RESULTS

Isolation and identification of the causal agent. Fungi were not observed on nor recovered from the leaf sections placed onto acidified potato dextrose agar plates. However, yellow, mucoid bacterial colonies were obtained consistently from symptomatic catnip leaves plated onto SPA. No other bacteria were isolated. Tests indicated that the recovered bacteria were Gram negative, catalase positive, oxidase negative, with single polar flagella. Strains were obligately aerobic and urease, arginine dihydrolase, and nitrate negative. Strains hydrolyzed starch, liquefied gelatin, were unable to accumulate PHB granules, and did not grow on a defined minimal medium lacking growth factors.

Infiltration of tobacco leaves with cell suspensions of the catnip strains all resulted in typical hypersensitivity reactions within 24 h. Based on these characteristics, the bacterium was identified as *Xanthomonas campestris* (7,9).

Biolog was useful for rapid identification of this bacterium. The Biolog data base identified all isolated strains as *X. campestris*, with similarity readings ranging from 57 to 79%. The closest similarity was with *X. campestris* cv. *juglandis*. In cellular fatty acid analyses, the catnip strains were closely related to *X. campestris*, with similarity percentages ranging from 59 to 69%. The closest similarity again was to *X. campestris* cv. *juglandis*.

DNA amplification with hrp-related primers. Using the oligonucleotide primers for the *X. campestris* pv. *vesicatoria* hrp gene cluster, fragments of identical sizes (1 kb) were amplified from all *X. campestris* strains isolated from catnip as well as from *X. campestris* pvs. *campestris* and *vesicatoria* control strains.

Pathogenicity to catnip. When representative catnip strains were inoculated by either method onto catnip, small brown flecks developed on leaves after 4 to 5 weeks. One to 2 weeks later, these flecks developed into larger angular, brown spots. Both symptoms were similar to those observed on diseased transplants in commercial greenhouses. Bacteria morphologically similar to the original strains were isolated consistently from test plants and after characterization were confirmed to be identical to the original strains. Catnip inoculated with sterile broth did not develop any symptoms. Results from the two experiments were the same.

Pathogenicity to other Lamiaceae plants. After 4 to 5 weeks, inoculated catnip developed leaf spot symptoms characteristic of the disease and *X. campestris* was reisolated from leaves. However, none of the other Lamiaceae plants developed any symptoms, regardless of the inoculation method. After 8 weeks, the experiment was terminated for lack of symptoms. Results from the two experiments were the same.

Host range comparison of *X. campestris* strains from catnip and other *X. campestris* hosts. When four *X. campestris*

Table 1. Reaction of various hosts of *Xanthomonas campestris* when inoculated with known *X. campestris* strains or those isolated from catnip²

Plant	Infiltrated strain				Sprayed strain			
	4 from catnip ³	pv. <i>campestris</i>	pv. <i>carotae</i>	pv. <i>vesicatoria</i>	4 from catnip ³	pv. <i>campestris</i>	pv. <i>carotae</i>	pv. <i>vesicatoria</i>
Catnip	+++	+	+	—	+++	—	—	—
Carrot	NT	NT	NT	NT	—	—	+++	—
Cauliflower	+	+++	+	+	—	+++	—	—
Pepper	+	+	+	+++	—	—	—	+++
Stock	+	+	+	+	—	—	—	—
Tomato	+	+	+	+++	—	—	—	+++

² Reactions were evaluated as follows: — = no reaction; + = limited water-soaking, necrosis; ++ = water-soaking, necrosis, leaf spot development; +++ = extensive leaf spot and disease development; NT = not tested. Results from the two experiments were the same and are combined in this table.

³ All four catnip strains responded similarly.

strains from catnip were infiltrated into leaves of catnip, cauliflower, pepper, stock, and tomato, water-soaking occurred on all plants within 24 h. Water-soaked areas on all plant species turned into brown-black necrotic lesions after 48 h. However, only catnip leaf lesions expanded beyond the initial infiltrated area during the test period (Table 1). Because the other plants did not develop any disease symptoms beyond the inoculated area, isolations were not done. However, bacteria morphologically similar to the original strains were isolated consistently from the catnip leaf lesions that formed beyond inoculation areas, and after characterization were confirmed to be identical to the original strains.

Catnip leaves infiltrated with *X. campestris* pvs. *campestris* and *carotae* also developed water-soaked, brown-black necrotic lesions after 48 h; these areas likewise did not expand beyond the initial infiltrated area. Catnip leaves infiltrated with *X. campestris* pv. *vesicatoria* did not develop water-soaking or necrosis. In contrast, cauliflower leaves infiltrated with *X. campestris* pv. *campestris*, and pepper and tomato leaves infiltrated with *X. campestris* pv. *vesicatoria* all developed water-soaked lesions that became brown and necrotic. These lesions later expanded and developed into typical black rot and bacterial spot symptoms, respectively (Table 1). Bacteria morphologically similar to the original cauliflower and tomato strains were isolated consistently from symptomatic cauliflower, pepper, and tomato leaves, and after characterization were confirmed to be identical to the original strains. Results from the two infiltration inoculation experiments were the same.

When plants were inoculated by spraying, the four *X. campestris* strains from catnip caused typical leaf spot symptoms on catnip plants but failed to cause any symptoms on the other plants (Table 1). Catnip plants did not show any symptoms when inoculated by spraying with the *X. campestris* pvs. *campestris*, *carotae*, and *vesicatoria*. Carrot, cauliflower, pepper, and tomato developed leaf spots only in response to their respective pathogens. As with the infiltration experiments, each *Xanthomonas* pathovar was reisolated only from symptomatic leaves of its respective

host. Results from the two spray inoculation experiments were the same.

In the second experiment, the three *X. campestris* strains from lavender caused limited water-soaking when infiltrated into catnip leaves but no symptoms when inoculated by spraying. However, these strains caused brown lesions when infiltrated or sprayed onto lavender plants, and *X. campestris* was reisolated from these lesions. Results from the two infiltration and two spray inoculation experiments were the same.

DISCUSSION

This appears to be the first report of a foliar bacterial disease of catnip caused by *X. campestris*. Named here as *Xanthomonas* leaf spot of catnip, the disease resulted in extensive leaf spotting and loss of quality of catnip transplants. In previous reports, catnip has been reported to be a natural host of *Pseudomonas syringae* pv. *tabaci* (2) and several fungal pathogens, namely *Ascochyta leonuri*, *Botrytis cinerea*, *Cercospora nepetae*, *Didymella catariae*, *Phoma exigua*, and *Septoria nepetae* (5).

Our inoculation experiments demonstrated that the *X. campestris* strains from catnip did not infect several other Lamiales plants or five other hosts of *X. campestris* pathovars, and that catnip is not susceptible to three known *X. campestris* pathovars or to the *X. campestris* pathogen of lavender. These data suggest that the catnip pathogen might be host specific to this plant and hence constitute a new pathovar. Additional studies would be needed to confirm this. It was notable that under our experimental conditions the catnip test plants took an extended time, i.e., 4 to 5 weeks, before showing the onset of symptoms. This delay in symptom expression was consistent throughout our studies.

Commercially produced catnip transplants were grown in enclosed greenhouses that also contained transplants of cauliflower, pepper, and tomato. Based on our inoculation studies, it appears that *X. campestris* pathogens from these other crops are not the cause of the bacterial disease of catnip, and the catnip transplants are not an inoculum source for *Xanthomo-*

nas diseases of cauliflower, pepper, or tomato. These findings have important epidemiological implications, as greenhouse transplant managers will not need to be concerned about disease spreading from catnip onto the sizable cauliflower, pepper, or tomato transplant crops. The occurrence of this disease on catnip seedlings grown in enclosed greenhouses suggests that initial inoculum could possibly be seedborne.

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