Varnish Spot, Destructive Disease of Lettuce in California Caused by Pseudomonas cichorii

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ABSTRACT

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A destructive disease caused by *Pseudomonas cichorii* on mature head lettuce has occurred sporadically during the past 4 yr in the Salinas Valley of California. The disease, which is referred to as "varnish spot," is characterized by dark-brown, shiny, firm, necrotic spots a few millimeters in diameter, that occur on the blades and petioles of leaves underneath the two or three outermost leaves of the head. Isolations from the lesions consistently yielded fluorescent pseudomonads. The disease was reproduced consistently within 24 to 36 hr at 23 C in mature Calmar lettuce plants that were inoculated by spraying with bacterial suspensions (10^5 cells/ml). Significantly ($P = 0.01$) more infection occurred in the field on Calmar lettuce plants that were inoculated 3 wk before harvest than on those inoculated 1, 2, 4, or 5 wk before harvest. The pathogen was identified as *P. cichorii* by comparison of some of its biochemical and physiological characteristics with *P. cichorii* and other nomenspecies, and by the similar pathogenicity of the varnish spot pathogen and *P. cichorii* strains on lettuce and several other hosts. The pathogen was isolated from soil and root samples from fields in the Salinas Valley with a history of the disease.

Additional key words: *Lactuca sativa* L.

A destructive bacterial disease of mature head lettuce has occurred sporadically during the past 4 yr in several fields in the Salinas Valley of California. During the 1974 and 1975 seasons, the disease resulted in partial or total loss of crops in at least 10 fields (a total of approximately 80 hectares). The disease, referred to by growers as "varnish spot," is characterized by shiny, dark-brown, firm, necrotic spots a few mm in diameter, that occur on the blades and petioles of leaves underneath the second or third outermost head leaves (Fig. 1); the outermost leaves of the head usually were not affected. Thus, symptoms were evident only after the heads were cut open. This hidden symptomatology prevented selective harvest of noninfected heads; thus, affected fields usually were abandoned. The disease consistently has been associated with nearly mature plants and has occurred only in sprinkler-irrigated fields in the Salinas Valley. This study was conducted to determine the cause of the disease and some of the factors affecting disease development and severity.

MATERIALS AND METHODS

Isolation of causal organism and disease production.—Microscopic examination of discolored tissue from diseased samples received initially in our laboratory revealed the presence of abundant rod-shaped motile bacteria. Initially we inoculated some detached leaves of head lettuce (cultivar Calmar) by sprinkling with a bacterial suspension obtained by allowing bacteria from a few lesions to ooze out into several ml of water. The inoculation was done at about 1700 hours and the leaves were incubated overnight at about 21 C in a closed glass dish lined with wet paper towels. Upon examination at about 0830 hours the following morning, dark-brown lesions were evident on inoculated leaves, but control leaves sprinkled with sterile water were symptomless. Microscopic examination of bits of tissue from the brown lesions on the inoculated leaves revealed the presence of numerous rod-shaped bacteria similar to those that had been observed in the original samples.

Ten fluorescent strains derived from single colonies obtained by plating wash water from diseased lettuce tissue on King's Medium B (MB) agar plates (4) were tested for pathogenicity. Mature heads of lettuce (cultivar Calmar) were cut into quarters and placed in a covered plastic container lined with wet paper towels. These sections were inoculated by drenching with 5 ml of bacterial suspensions containing about 10^7, 10^6, 10^5, and 10^4 live cells/ml from each of the strains, as determined by combination of nephelometry and colony counts on MB plates. Control sections were treated similarly with 5 ml of sterile water. Inoculated and control sections were rated

for disease severity after 3 days at 24 C.

In three fields in the Salinas Valley, head lettuce plants (cultivars Calmar and White Boston) were inoculated about 1 wk before harvest. About 5 ml of bacterial suspension (approximately 10^8 cells/ml) were injected by means of hypodermic syringes at the depth of about 5 cm into 10 heads of each cultivar; noninoculated control plants were injected with sterile water. The plants were rated for disease severity about 1 wk after inoculation.

Identification of the causal bacterium.—To identify the causal bacterium, 10 pathogenic strains from diseased lettuce samples from the Salinas Valley were compared in a number of biochemical and pathological tests with strains of the following *Pseudomonas* nomenspecies: four strains of *P. cichorii*, NCPPB (National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Harpenden, Herts., England) 90652, 90651, 1039, and 1512; two strains of *P. viridiflava*, NCPPB 1248 and 1810; ten strains of *P. marginalis*, NCPPB 667 and 1689, and eight un-numbered strains isolated by us from soil samples collected in the Salinas Valley, California; two strains of *P. marginata* [*P. gladioli* (3)], NCPPB 1891, and 316; eight strains of *P. fluorescens* ATCC (American Type Culture Collection, Rockville, MD 20852) 17498, 17387, and 17825, and five un-numbered strains isolated by us from soil samples from the Salinas Valley, California; and 10 strains of *P. syringae*, 5D4105, 5D428, 5D429, 5D4136, 5D425, 5D443, 5D4113, 5D421, 5D413, and 5D46, from the culture collection of J. E. DeVay, University of California, Davis. Included in these tests were cytochrome oxidase and arginine dihydrolase, production of 2-ketogluconate, production of levan, soft-rotting of potato, growth at 36 C, and hypersensitivity on tobacco (5). Tests were conducted as described previously (8).

Fig. 1. Symptoms of the varnish spot disease caused by *Pseudomonas cichorii* on a naturally infected head of lettuce (cultivar Calmar). The three outermost leaves were removed to reveal the dark-brown lesions on the interior leaves of the head.

Host range.—Three-wk-old seedlings of greenhouse-grown tomato (*Lycopersicon esculentum* Mill.), cabbage (*Brassica oleracea* var. *capitata* L.), Pak-choi (*B. chinensis* L.), celery (*Apium graveolens* var. *dulce* DC), soybean [*Glycine max* (L.) Merr.], dark-eye sunflower (*Helianthus atrorubens* L.), safflower (*Carthamus tinctorius* L.), sowthistle (*Sonchus oleraceus* L., jimson-weed (*Datura stramonium* L.), groundsel (*Senecio vulgaris* L.), head lettuce (*Lactuca sativa* var. *capitata* L.), and endive (*Cichorium endiva* L.) were inoculated by gently spraying (hand-held atomizer) with bacterial suspensions containing about 10^8 cells/ml of the varnish spot bacterium. Noninoculated control plants were sprayed with sterile water. Inoculated plants were placed in a mist chamber for 36 hr after inoculation, and then were kept on an open greenhouse bench for 6 days prior to symptom evaluation. In other tests, detached leaves of mature cabbage, endive, chrysanthemum, and lettuce, and leaves and stems of celery were spray-inoculated with bacterial suspensions containing about 10^6 cells/ml of the varnish spot bacterium. Inoculated tissues were kept at 24 C in covered plastic boxes lined with wet paper towels for 2 days prior to recording results. The pathogenicity tests were repeated twice.

Sources of inoculum.—Isolations were made from soil and plant samples collected from four fields where the disease had occurred previously, and from two fields with no history of the disease (all fields had produced at least one crop of lettuce each year for the past 20 or more years). Sampling was done by collecting approximately 10-g samples of soil from the top 2 cm from 10 different locations in each field. The samples from each field were combined, mixed thoroughly, and stored at 5 C for 4-8 days prior to isolation. To isolate the bacterium, 0.1 g of each soil sample was added to 0.5 ml of sterile water in a test tube at 22-23 C, and mixed thoroughly by placing the test tubes on a rotary shaker for 30 min. Tenfold dilutions were made from the soil suspensions and 0.1 ml of each dilution was spread evenly on the surface of MB agar plates. Within 24 to 48 hr, discrete fluorescent colonies selected at random from the culture plates, were transferred to MB culture slants and tested for pathogenicity after 24 to 48 hr of incubation at room temperature (22 C).

Samples of head lettuce, bean, and several kinds of weeds also were collected from the above fields. Roots from these plants were cut into 5-cm sections, washed under tap water to remove soil, and rinsed several times with sterile water. Approximately 50 g of root sections were added to 100 ml of sterile water in 250-ml flasks and were shaken on a rotary shaker for 2 hr at 22 C. Dilutions from the root washings were used to inoculate MB plates as described above. Screening for pathogenicity of about

277 fluorescent strains recovered from soil and plant sources was accomplished by inoculating small portions of lettuce leaves taken aseptically from the center of mature heads. These leaf sections were placed in plastic petri dishes containing 2 ml of sterile water and were inoculated by mixing one loopful of bacterial suspension into the water surrounding the leaf sections.

Pathogenic strains produced typical brown varnish spot lesions within 24 to 48 hr at 24 C; the tissue was firm and not macerated. Thus, the varnish spot bacteria were readily distinguishable from strains of soft-rotting *Pseudomonas* sp. which comprised about 4% of the total number of strains tested.

Effect of timing of field inoculation on disease development.—Each of 60 plants in four 15-plant replicates in a commercial field in the Salinas Valley was sprayed with about 1.5 ml of a suspension of the varnish spot bacterium containing 10^5 cells/ml, at 1, 2, 3, 4, or 5 wk before harvest. Sixty plants in four 15-plant replicates in the same plot also were inoculated by injecting 1 ml of the same bacterial suspension inside each head at a depth of about 5 cm, 1 wk before harvest. Noninoculated control plants were sprayed or injected with equivalent amounts of sterile water. At harvest time, inoculated and noninoculated heads were cut into quarters and rated for disease severity on a scale of 1 to 4 (very mild to severe). Results were subjected to Duncan's multiple range test.

Effect of age of plants on susceptibility.—To determine the effect of plant age on susceptibility, 2-, 4-, 6-, 9-, 12-, 14- and 16-wk-old, greenhouse-grown Calmar lettuce plants were spray-inoculated with bacterial suspensions containing about 10^6 cells/ml of the varnish spot bacterium. Inoculated plants were covered with a plastic bag for 24 hr, and then were left on a greenhouse bench and observed periodically for disease development during 7 days.

RESULTS

Isolation of the pathogen and production of the disease.—The disease was reproduced consistently within 24 to 36 hr at 23 C in Calmar lettuce plants drenched with washings from leaves with varnish spot symptoms or by inoculation with suspensions (10^5 cells/ml) of various strains isolated from diseased lettuce. Some strains, however, induced disease symptoms only when inoculum levels of 10^7 cells/ml were used. Thus, the virulence of the pathogenic population in the field apparently was variable. Pathogenic strains were recovered consistently from the inoculated tissues in which symptoms developed. Injection of inoculum beneath the outermost leaves of Calmar and White Boston heads in three different fields resulted in disease development in > 90% of the inoculated plants and the symptoms were typical of naturally occurring varnish spot.

Identification of causal bacterium.—The reactions of all strains of the varnish spot bacterium in the several tests were identical with those of the *P. cichorii* strains and differed from the other nomenspecies (Table 1). Moreover, identical disease symptoms were produced on detached leaves of mature head lettuce, endive, cabbage, celery, and chrysanthemum that were spray-inoculated with suspensions (10^6 cell/ml) of the 10 varnish spot strains and the four strains of *P. cichorii*. Similar inoculations with *P. viridiflava*, *P. marginalis*, *P. marginata* [*P. gladioli* (3)], *P. fluorescens*, and *P. syringae* did not produce varnish spot symptoms on head lettuce. Thus, the causal bacterium was identified as *P. cichorii*.

Host range.—Inoculation of 3-wk-old, greenhouse-grown plants of tomato, cabbage, chinese cabbage, celery, soybean, sunflower, safflower, *Sonchus* sp., *Datura* sp., *Senecio* sp., head lettuce, and endive with a suspension of the varnish spot bacterium (10^8 cells/ml) caused no symptoms. However, detached leaves of mature cabbage, endive, chrysanthemum, lettuce and leaves and stems of mature celery developed symptoms similar to those of varnish spot on lettuce after inoculation with a strain of the varnish spot bacterium (10^6 cells/ml).

Sources of inoculum.—Among 196 bacterial strains obtained from soil samples from four fields with a history of disease and from two fields with no previous history of the disease, there were four oxidase-positive fluorescent

TABLE 1. Biochemical and physiological tests of the lettuce varnish spot pathogen, *Pseudomonas cichorii*, and five other *Pseudomonas* nomenspecies

			Comparative tests[a]							
Nomenspecies	Number of strains	Oxidase	Tobacco hypersensitivity	Potato soft rot	Arginine dihydrolase	2-keto-gluconate	Pectate	Growth at 36 C	Levan	Varnish spot on lettuce
Varnish spot[b]	10	10	10	0	0	0	0	0	0	10
P. cichorii	4	4	4	0	0	0	0	0	0	4
P. viridiflava	2	0	2	2	0	0	2	0	1	0
P. marginalis	10	10	0	10	10	10	10	10	7	0
P. gladioli[c]	2	0	0	0	0	0	0	2	0	
P. fluorescens	8	8	0	1	8	8	1	8	7	0
P. syringae	10	0	10	0	0	0	0	0	2	0

[a]Figures indicate number of strains of each nomenspecies that reacted positively in the respective tests.

[b]Varnish spot strains were obtained from varnish spot-diseased lettuce from the Salinas Valley, California; other nomenspecies were received from J. E. DeVay, C. I. Kado, R. Y. Stanier, or M. N. Schroth. Except for pathogenicity, all tests were conducted as described earlier (8). Pathogenicity tests were done by drenching quarter-sections of head lettuce (cultivar Calmar) with 5 ml of bacterial suspensions containing about 10^5 cells/ml. Inoculated sections were placed in a covered plastic container lined with wet paper towels and rated for disease severity after 3 days at 24 C.

[c]Cultures were labeled *P. marginata* when received, but are listed here under *P. gladioli* as recommended by Hildebrand et al. (3).

strains capable of causing soft-rotting of lettuce and potato slices, as did *P. marginalis*, and two oxidase-positive, nonsoft-rotting fluorescent strains capable of producing varnish spot symptoms on head lettuce. The latter two strains were recovered from soil in two of the four fields with a history of disease. The other 190 strains were not pathogenic on lettuce. Among 44 fluorescent strains isolated from roots of several plants, including head lettuce from the above fields, 37 were not pathogenic on lettuce; six oxidase-positive strains caused soft rotting of head lettuce and potato slices, and one oxidase-positive nonsoft-rotting strain similar to *P. cichorii* produced varnish spot symptoms on inoculated head lettuce.

Effect of timing of field inoculation on disease severity.—Timing of inoculation influenced the incidence of the disease. There was a higher percentage of infection ($P = 0.01$) on lettuce plants that were inoculated by spraying 3 wk before harvest (35%) than from inoculations that were done 1, 2, 4, or 5 wk before harvest (1, 15, 13, and 10%, respectively). However, between 94 to 100% infection developed in similar-aged plants inoculated by injection of a bacterial suspension into the heads. Thus, the lower incidence of disease in spray-inoculated plants may be the result of the lesser accessibility of internal tissues to the inoculum.

Effect of plant maturity on susceptibility to the disease.—Of greenhouse-grown lettuce plants inoculated at different stages of development, only 12-, 14-, and 16-wk-old plants (nearly mature) developed mild-to-severe varnish spot symptoms on the middle leaves. Plants inoculated at 2, 4, 6, and 9 wk of age developed no symptoms. These results are consistent with field observations that the disease has occurred only on nearly mature plants.

DISCUSSION

Pseudomonas cichorii has been reported to cause diseases on celery (12, 13), cabbage (9, 11, 13), tomato (14), chrysanthemum (6), and Gerbera (7). However, we know of no report of *P. cichorii* causing a disease of lettuce similar to varnish spot. Burkholder (1) reported that *P. cichorii*, *P. marginalis*, and *Xanthomonas vitians* caused rots of head lettuce in the field in New York. However, he reported that the rots caused by *P. cichorri* and *P. marginalis* were so similar in appearance that it was difficult to distinguish one from the other. Thus, because the symptoms of lettuce varnish spot are distinctly different from the soft rotting symptoms caused by *P. marginalis*, it seems unlikely that the lettuce rot observed in New York State by Burkholder (1) is the same as varnish spot in California. *Pseudomonas cichorii* has been reported to cause a disease on lettuce in Germany (10) and in Brazil (2), but the symptoms that were described differ from the symptoms of varnish spot. Especially different is their description of external symptoms on old leaves because the symptoms of varnish spot always were inside the head.

Celery and cabbage apparently are susceptible to the varnish spot pathogen, but we have never observed brown spot symptoms on these crops in the Salinas Valley where both crops are grown extensively.

The increased incidence and severity of disease in plants inoculated with hypodermic needles versus spray-inoculated plants probably were not due to increased infection as a result of injury, because *P. cichorii* readily infected unwounded lettuce tissues.

The bacterium is soil-borne and apparently is spread by splashing water from sprinkler irrigation. Although we have not tested this means of spread experimentally, the occurrence of the disease only in sprinkler-irrigated fields provides indirect evidence. Thus, in fields where the disease has occurred previously, furrow irrigation, especially during the latter part of the growing season, is suggested as a possible control procedure.

At present, occurrence of the varnish spot disease is sporadic and has occurred mainly in the northeastern portion of the Salinas Valley. The reason for this restricted distribution is not known. Apparently, the disease does not always occur even though inoculum is present, as indicated by our isolation of the pathogen from roots of lettuce in a field with no evidence of or history of the disease.

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