Source of Resistance to Black Rot of Cabbage Expressed in Seedlings and Adult Plants

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ABSTRACT

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About 350 accessions of cabbage (*Brassica oleracea* var. *capitata*) in the U.S. Plant Introduction Station collection were screened as seedlings for resistance to black rot by spraying a suspension of bacterial cells of *Xanthomonas campestris* pv. *campestris* onto leaves with guttation droplets in a greenhouse mist chamber. The bacterial cells were drawn into the leaves via the hydathodes when drying conditions were provided. Seedlings were inoculated 2.5 wk after sowing when the first true leaf was about 2.5 cm long. Several lines with excellent resistance as adult plants were highly susceptible under these conditions. However, PI 436606, an introduction from China, was resistant at this early stage as well as at later stages of maturity. The resistant introduction was challenged by 16 strains of *X. c.* pv. *campestris* that induced typical black rot symptoms, leaf blight, or symptoms intermediate between those of black rot and leaf blight on susceptible plants and was found effective against all strains. PI 436606 also was resistant against a closely related pathogen, *X. c.* pv. *armoraciae*, that induces leaf blight and hydathode necrosis symptoms on susceptible plants.

Black rot is considered the most important disease of crucifers, occurring worldwide on all cultivated brassicas and radishes and on numerous cruciferous weeds (14). The pathogen, *Xanthomonas campestris* pv. *campestris* (*X. c.* pv. *campestris*), was first discovered on cabbage (*Brassica oleracea* var. *capitata*) in the United States in 1898 (7). The bacterium is seedborne and moves systemically in the plant after germination (12,14). The pathogen can spread to adjacent plants by windblown rain, mechanical means, and possibly by insects (5,9,14). Invasion can occur through wounds made by insects, hail, or equipment (14,15), but hydathodes at the margins of the leaves are the primary site of entry (4). The bacterium colonizes the vascular system. This restricts water flow and typically leads to the formation of V-shaped chlorotic-necrotic lesions on the margins of the leaves and blackened veins (11,14). In addition, strains have been identified by Yuen and Alvarez (16) that induce a leaf blight symptom characterized by rapid necrosis of tissues with no vein blackening and others that induce a reaction intermediate between those of typical black rot and leaf blight. Stomatal infection can be induced in cabbage leaves that have been artificially water-congested, but this seldom occurs in nature (4).

Another bacterium closely related to *X. c.* pv. *campestris* but which invades stomata and causes both leaf spot and hydathode necrosis on cabbage was identified by Black and Machmud (3) as *X. c.* pv. *armoraciae*. They considered *X. c.* pv. *armoraciae* to be identical to and to have priority over *X. c.* pv. *raphini* (3,6,13).

In 1952, Bain (1) found resistance to black rot in the Japanese cultivar of cabbage Early Fuji, but the importance of plant age on the expression of resistance was not reported. Studies conducted later with this resistance also did not determine the importance of plant age because these studies involved older plants (2,10,15). Recently, however, H. Humaydan (*personal communication*) indicated that this resistance is not expressed in young seedlings. In this regard, Sharma et al (8) indicated a need to develop screening techniques for inoculating young seedlings. Straub and Williams (10) had reported successfully screening 8-wk-old plants by placing them in a dew chamber to induce formation of guttation droplets, spraying the plants with a bacterial suspension after formation of droplets, and then allowing the plants to dry slowly. This enabled the bacterial cells that had landed in the guttation droplets to enter the hydathodes by capillary action (4). They also inoculated veins directly by injuring them, and in this way, they were able to conclude that hydathodes are the site where resistance normally operates.

The purpose of our study was to develop a procedure that does not require expensive dew chambers to inoculate large numbers of young cabbage seedlings through the hydathodes and to identify a source of resistance to *X. c.* pv. *campestris* and *X. c.* pv. *armoraciae* that is expressed in both seedlings and adult plants.

MATERIALS AND METHODS

About 350 accessions of the cabbage germ plasm collection of the U.S. Plant Introduction Station were evaluated for resistance to *X. c.* pv. *campestris*. Twelve

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seeds of each accession were planted in Cornell mix in Speedling trays (34 × 67 cm) divided into 72 cells. These were kept for about 11 days in a greenhouse with fluorescent lights, then held for an additional 7 days at 24 C under metal halide lights. Maximum energy during midday was 300 μE m^{-2} s^{-1} in the winter when these studies were conducted. About 18 days after sowing, most seedlings had one leaf 2.5 cm long.

A totally enclosed greenhouse mist chamber was used to induce formation of guttation droplets. The chamber (1.3 m high) was built with opaque fiberglass sheets, except for the front, which consisted of a plastic curtain that could be opened for easy access and could be sealed tightly to hold moisture. Mist was provided by three nozzles (air atomizer nozzles 1/4 J with spray setup no. 13, Spraying Systems Company, Wheaton, IL) near the top of the chamber directed at a splash pan at the rear. The amount of air entering one side was adjusted with mechanical valves to produce a foglike mist. Solenoid valves for the air and water were connected to a timing device that could be set to operate the mist system intermittently for any length of time. The frequency and length of the mist period required to obtain maximum formation of guttation droplets without excess water was determined by trial and error.

Accessions from the Plant Introduction collection were evaluated by inoculating plants with a strain of *X. c.* pv. *campestris* obtained from the Wisconsin collection (PHW-117, originally isolated from cabbage in Louisiana). Cultures were grown for 48 hr at 25 C on YDCP medium (9), and the cells were then suspended in sterile distilled water. The cell suspension was adjusted to 80% transmittance at 600 nm on a Bausch & Lomb model 20 spectrophotometer, which resulted in a concentration of about 1.25×10^8 colony-forming units per milliliter. The suspension was sprayed onto plants with a DeVilbiss 152 atomizer (DeVilbiss Company, Somerset, PA) attached to an air-pressure line in the greenhouse and adjusted to 103 kPa to obtain small droplets. Later a Preval sprayer (Precision Valve Corporation, Yonkers, NY) was used. This does not need a central source of pressurized air, and it provides a fine aerosol.

Seedlings to be inoculated in the routine screening phase of the study were placed in the chamber at 5:00 P.M. and watered thoroughly to saturate the soil. The curtain was closed, and the mist system operated intermittently as required for good droplet formation on the leaf edges. The curtain was opened at 9:00 A.M. and the inoculum sprayed from a height of about 1 m above the plants. This permitted the atomized spray droplets to fall gently onto the plants without dislodging the guttation droplets. After inoculation, the chamber was left open until 5:00 P.M. to ensure that all droplets had been drawn into the leaves through the hydathodes. The seedlings were then watered and the trays rotated, the chamber closed, and the mist system reactivated. The inoculation procedure was repeated for three consecutive days, then the plants were returned to a greenhouse bench under metal halide lights at about 24 C.

The disease reaction was recorded 7–10 days after the seedlings were removed from the mist chamber and before it became difficult to distinguish senescent from diseased leaves. A rating scale of 0–4 was used (0 = no symptoms, 1 = trace amount, and 2 = 1.0, 3 = 1.5, and 4 = ≥2.0 cm^2 of diseased tissue).

After PI 436606 was identified as resistant, a replicated trial (five replicates of six plants per line) was conducted with 2.5- and 5-wk-old seedlings (weeks after sowing) of this introduction, four lines resistant as adult plants (BI-20, LAWI-3, Hancock, and NY-1414), and a fully susceptible cultivar (Round-up). Seedlings of both ages were grown for 1 wk under the metal halide lights before being inoculated for three consecutive days with the PHW-117 strain of *X. c.* pv. *campestris*.

To ensure that PI 436606 was resistant to a wide range of strains, 2.5-wk-old seedlings were inoculated with 15 other strains of U.S. origin: seven *X. c.* pv. *campestris* strains that induce typical black rot symptoms, two *X. c.* pv. *campestris* strains that induce leaf blight symptoms, two *X. c.* pv. *campestris* strains that induce symptoms intermediate between those of typical black rot and leaf blight, and four *X. c.* pv. *armoraciae* or *X. c.* pv. *armoraciae*-like strains that induced leaf spot and hydathode necrosis symptoms. Seeds for this test were planted in Cornell mix in plastic trays cut into cells for 12 seedlings, which were grown as described previously. At the time of inoculation, each tray was removed from the mist chamber and inoculated with one strain to avoid contamination with other strains. After a single inoculation, they were held on greenhouse benches (inside open plastic bags supported on four sides with stakes to avoid splashing inoculum from one tray to another when watering) until disease reactions were scored. The cabbage cultivar Round-up was used as a susceptible check. In a subsequent test, the first strain (XLS-2) of *X. c.* pv. *armoraciae* reported to cause disease (leaf spot and hydathode necrosis) on cabbage was used to challenge PI 436606. However, in an effort to obtain penetration and infection via the stomata, inoculum was first sprayed at 70 kPa from a distance of about 20 cm from the leaves with a DeVilbiss 152 atomizer after the plants were held overnight in the greenhouse mist chamber. The next day, the same seedlings were inoculated again by spraying a bacterial suspension over leaves with guttation droplets.

In 1984, a field test was conducted to determine if PI 436606 is also resistant when inoculated as adult plants. Seedlings were first inoculated in the greenhouse, and those that gave a zero disease reaction were transplanted into a field where a black rot trial was being conducted by a commercial seed company. In August, when the plants were 9 wk old, they were inoculated several times by spraying them with a bacterial suspension early in the morning, when the plants were covered with dew and guttation droplets. They were observed for disease symptoms late in September.

Table 1. Reactions of cabbage seedlings when inoculated with *Xanthomonas campestris* pv. *campestris* in greenhouse mist chamber tests[a]

Line or cultivar	Age (wk)[b] 2.5	5.0
PI 436606	1.58[c]	1.66
LAWI-3	3.46	2.96
BI-20	3.92	3.56
NY-1414	3.82	3.12
Hancock	3.70	2.92
Round-up	3.96	4.00
LSD ($P = 0.05$)	0.347	0.473
LSD ($P = 0.01$)	0.474	0.645

[a] A 10^8 cfu/ml suspension of *X.c.* pv. *campestris* strain PHW-117 was sprayed over plants with guttation droplets. The plants were subsequently allowed to dry in order to draw the bacterial cells into the hydathodes. This inoculation procedure was repeated for three consecutive days.

[b] Age refers to weeks after sowing.

[c] Disease reaction was scored 8 days after seedlings were removed from the mist chamber (0 = no symptoms, 1 = trace amount, 2 = 1.0, 3 = 1.5, and 4 = ≥2.0 cm^2 of diseased leaf tissue); ratings are average of five replicates, six plants per replicate.

RESULTS

The greenhouse mist chamber proved to be an excellent means of inducing the formation of guttation droplets. They could be induced in as little as 3 hr, but generally the seedlings were left in the chamber overnight with the timer programmed to increase the frequency of misting for 3–4 hr before inoculation in the morning. Within a few hours after the mist chamber was shut off and the front curtain of the chamber opened, the guttation droplets were all drawn into the leaves through the hydathodes.

Young seedlings of PI 436606 always showed resistance, but the line was not immune because some seedlings usually showed symptoms. However, these were minimal compared with other lines (Table 1). Also, the symptoms were restricted to the inoculated leaf or leaves, and after senescence of these leaves, the plants remained healthy when held in the greenhouse for 2 mo. Among the four

lines with adult plant resistance, LAWI-3 appeared to be the most resistant when inoculated at the seedling stage based on the lack of subsequent development of symptoms in uninoculated leaves. Most plants of the fully susceptible cultivar Round-up were killed rapidly.

PI 436606 showed resistance against a range of strains of *X. c.* pv. *campestris*, including strains causing symptoms of black rot, leaf blight, or symptoms intermediate between those of black rot and leaf blight, and against *X. c.* pv. *armoraciae* and *X. c.* pv. *armoraciae*-like organisms (Table 2) causing leaf spot and hydathode necrosis.

In a test with the strain of *X. c.* pv. *armoraciae* (XLS-2) isolated from cabbage in Louisiana, PI 436606 was found to have excellent resistance. Fourteen days after inoculation, 10 of 11 Round-up seedlings showed extensive symptoms, whereas eight of 12 PI 436606 seedlings were infected but lesions were restricted. When the disease reaction was scored again 15 days later, the bacterium had spread systemically and caused severe disease in eight of 11 Round-up seedlings. In contrast, all infected PI 436606 leaves that resulted from direct inoculation had fallen off and there was no evidence of systemic spread when the PI 436606 plants were dissected to look for blackening of the veins and when isolations were made on YDCP medium. In contrast, XLS-2 was present in stem tissue in Round-up check plants. Similarly, with PHW-117, which was used as a control to inoculate PI 436606 and Round-up, there was no visual evidence of its systemic movement and the bacterium could not be isolated from stem tissue of PI 436606, but it had spread systemically in Round-up.

All plants that had survived a seedling test with complete absence of symptoms before transplanting in the field, and which were inoculated three times in the field as adult plants, remained free of disease. In contrast, many plants of other lines being tested by a seed company in the same plot showed typical black rot symptoms.

Table 2. Reactions of 2.5-wk-old seedlings of PI 436606 and Round-up cabbage when inoculated with strains of *Xanthomonas campestris* pv. *campestris* (Xcc) and *Xanthomonas campestris* pv. *armoraciae* (Xca)[a]

Pathovar	Strain	Symptom type	No. plants infected/ no. plants inoculated		Av. no. leaves/plant with >1 cm^2 leaf area diseased	
			PI	Round-up	PI	Round-up
Xcc	PHW-117	Typical	1/12	9/10	0.2	2.1
	H-M		2/12	12/12	0.4	2.2
	Cabaret		5/11	7/7	0.7	1.9
	GAC-111		0/9	10/11	0.0	1.6
	GAC-137		3/12	9/10	0.3	1.8
	EEXC-114		0/12	11/12	0.0	1.3
	A-249		1/9	6/9	0.1	1.0
	G2-12	Blight	0/11	9/9	0.0	0.8
	G2-17		0/12	11/12	0.0	1.2
	GAC-17	Intermediate	0/12	10/10	0.0	2.1
	GAC-20		2/11	11/11	0.2	1.7
	G3-38A		0/12	8/10	0.0	1.2
					Av. no. lesions/plant	
Xca	756	Leaf spot, hydathode	3/11	9/9	1.5	17.1
	417	necrosis	0/11	5/7	0.0	3.7
Xca-like	A-342	Leaf spot, hydathode necrosis	3/9	10/10	0.9	24.0
	G3-27		2/11	12/12	0.2	3.4

[a] A 10^8 cfu/ml suspension of cells of each strain was sprayed over plants with guttation droplets. The plants were subsequently allowed to dry in order to draw the bacterial cells into the hydathodes.

DISCUSSION

PI 436606 is considered an excellent source of resistance to black rot at all stages of plant maturity. Although a few seedlings became infected, this was not unexpected considering the severity of the inoculation procedure and the fact that these were unselected seed sources that could have been segregating for resistance. PI 436606 was obtained from the People's Republic of China. Finding the resistance in an introduction from China in this instance and from Japan in the case of Early Fuji is not surprising because the greatest diversity of crucifer germ plasm is found in these countries, and *X. c.* pv. *campestris* is endemic in this region (14). Fortunately, the resistance in PI 436606 is expressed as early as when only one true leaf is present. Thus, this source of resistance could be a valuable addition to the excellent adult plant resistance that has been incorporated into advanced breeding lines and cultivars (14,15) from Early Fuji. PI 436606 is horticulturally similar to Early Fuji in that it also has a flat, loose head.

A major concern with using only adult plant resistance is that young seedlings grown in the southern region of the United States, where *X. c.* pv. *campestris* is endemic, may become infected before they are shipped as transplants. Thus, infected seedlings with adult plant resistance might serve as a source of inoculum for susceptible cultivars being grown in areas where *X. c.* pv. *campestris* is not endemic. Visual inspection to detect infected seedlings in the seedbed is inadequate to prevent this problem (14). Therefore, expression of resistance in the seedling stage is very important.

The greenhouse method of evaluating seedlings for resistance to black rot is easy to do and results in very reproducible results. However, care must be taken to maintain plants in a physiological state favoring the expression of resistance (10). Conversion of a greenhouse bench 5–6 m long to a mist chamber would permit screening 1,000–1,500 seedlings twice per week; therefore, the limiting factor is regular greenhouse space with adequate lighting and temperature to grow seedlings and hold them for 10–14 days after inoculation. Susceptible genotypes could be eliminated by this process, and only those seedlings expressing resistance would have to be transplanted to the field for later evaluation for adult plant resistance. The current procedure used by most plant pathologists and breeders consists primarily of screening plants only as adults. Without using a seedling-screening procedure, it appears that plants may be selected that only have adult plant resistance. Possibly, this has already occurred with breeding programs using Early Fuji as a source of resistance, because P. Williams (*personal communication*) has indicated that the original selection in his black rot breeding program had resistance at the seedling stage.

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