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Arabidopsis thaliana as a Host for *Xanthomonas campestris* pv. *campestris*

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One of the most challenging areas in plant pathology is the isolation of genes that control a plant's interaction with pathogens. The difficulty in isolating these genes stems from the complex nature of most higher plants. We suggest that the cruciferous weed *Arabidopsis thaliana* may serve as a model organism in plant pathology since its biological and genetic properties and genomic structure are particularly amenable to the isolation of plant genes. We report here that *Arabidopsis* was a host for the phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris*, the causal agent of black rot in cruciferous crops. Furthermore, two ecotypes of *Arabidopsis* showed differential responses to *X. c.* pv. *campestris*, suggesting that disease resistance genes are present in the *Arabidopsis* gene pool and may be isolated in a straightforward manner.

Additional keywords: plant-pathogen interaction.

Little is known about the molecular basis of bacterial interactions with plants. There has been rapid progress in isolating bacterial genes important in these interactions (Daniels *et al.* 1988). In contrast, there has been little progress in isolating the corresponding plant genes. Synthesis of a number of enzymes and other molecules accompanies the infection of plants by pathogens. The plant genes mediating these responses are thought to be part of a general defense reaction, but definitive proof is lacking (Collinge and Slusarenko 1987). A major obstacle to obtaining definitive proof has been the difficulty in studying the molecular genetics of plants. A solution is the use of a model such as *Arabidopsis thaliana* (L.) Heynh. where molecular genetic research is relatively easy.

A. thaliana is a weed from the mustard family that has been proposed as a model organism for studies in plant molecular genetics, development, physiology, and biochemistry. Compared with other higher plants, its small size allows the screening of hundreds of thousands of plants in a limited area. A short generation time, self-fertility, and fecundity facilitate classical genetic research. Extensive genetic studies have resulted in a detailed genetic map. The small and simple genome is well-suited for the molecular cloning of genes for which only a variant phenotype and a genetic map position are known (Meyerowitz 1987). As a result of these unique characteristics, this plant appears to be well-suited for pathological studies, yet very little is known about the interaction of *Arabidopsis* with plant pathogens (Susnova and Polak 1975; Aerts *et al.* 1979).

Xanthomonas campestris pv. *campestris* (Pammel) Dowson is the cause of black rot, the most important disease of crucifers worldwide (Williams 1980; Naegely 1988). Hosts include radishes and cultivated brassicas such as cabbage, cauliflower, and oil-seed rape. The bacteria enter the plant through water pores at the leaf margins or through wounds. The symptoms include V-shaped chlorotic lesions originating from the point of bacterial entry, darkened veins resulting from the movement of the bacteria through the plant's vascular tissues, and eventual yellowing and drying of the tissues that were affected.

Since *Arabidopsis* is in the same family as the hosts for *X. c.* pv. *campestris*, we investigated the interaction between *Arabidopsis* and this bacterium. We found that *Arabidopsis* was a host for *X. c.* pv. *campestris*.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The R14-A strain of *X. c.* pv. *campestris* and the Rcar-B strain of *X. c.* pv. *carotae* (Kendrick) Dye are spontaneous rifampicin-resistant mutants of strains described by Cook and Robeson (1986). The KXCC and XCCS strains of *X. c.* pv. *campestris* were gifts from Jan Leach and Brian Staskawicz, respectively. A culture grown overnight in nutrient broth at 30° C from a single colony inoculum was pelleted and then washed and resuspended in 10 mM $MgSO_4$ at approximately 10^9 colony forming units per milliliter.

***Arabidopsis* ecotypes and cultivation.** *A. thaliana* ecotypes Ob-1 (Oberursel, Federal Republic of Germany), No-O (Nossen, East German Democratic Republic), Pn-O (Pontivy, France), Uk-2 (Umkirchen, Federal Republic of Germany), Bu-20 (Burghaun, Federal Republic of Germany), and Edi-0 (Edinburgh, United Kingdom) were obtained from the *Arabidopsis* Information Service seed bank (Kranz and Kirchheim 1987). To increase genetic uniformity within each ecotype, we used seed from at least two cycles of single-seed descent and performed experiments with seeds from one individual. Plants were grown in a 1:1:1 mixture of perlite:vermiculite:standard potting mix. Plants were maintained in growth chambers

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at 25° C and about 75% humidity on a 6-hr day (125–200 $\mu E \cdot m^{-2} \cdot sec^{-1}$), 18-hr night cycle. The short-day conditions delayed flowering and senescence. The plants were inoculated 4 to 6 wk after sowing.

Spray inoculation protocol. After 4 hr in a dew chamber, guttating plants were moved to an enclosed inoculating area, sprayed with the bacterial suspension described above, and covered with clear plastic domes to maintain high humidity. Plants were then returned to the growth chamber. Domes were removed after 2 days.

Wound inoculation protocol. Inoculum and plants were prepared as described above. A leaf was inoculated such that a narrow razor cut a slit through the midvein, 1 cm from the tip, and into bacteria-soaked cotton. After inoculation, the plants were covered with a dome for 2 days and treated as described above.

Infiltration inoculation protocol. Inoculum and plants were prepared as described above. Using a 1-ml plastic syringe without a needle, a single site on each leaf was inoculated as previously described (Collinge *et al.* 1987; Tsuji and Somerville 1988).

Enumeration of bacteria to test Koch's postulates. Leaves were harvested, surface-sterilized by submersion in 70% ethanol for 40 sec, rinsed in water, and blotted dry. A disk with a diameter of 4.8 mm was isolated from each leaf using a No. 1 cork borer and macerated in 0.4 ml of 10 mM $MgSO_4$. Colony forming units were determined by plating the serially diluted suspension on Bacto nutrient agar (Difco Laboratories, Detroit, MI) plates.

Bacterial growth curves. At various times after wound inoculation, one leaf from each of 10 plants was harvested, surface-sterilized by submersion in 70% ethanol for 40 sec, rinsed in water, and blotted dry. A single disk with a diameter of 4.8 mm was isolated from each leaf using a No. 1 cork borer placed immediately distal to the wound. A sample consisted of 10 disks macerated in 0.3 ml of 10 mM $MgSO_4$. Colony forming units were determined by plating the serially diluted suspension on Bacto nutrient

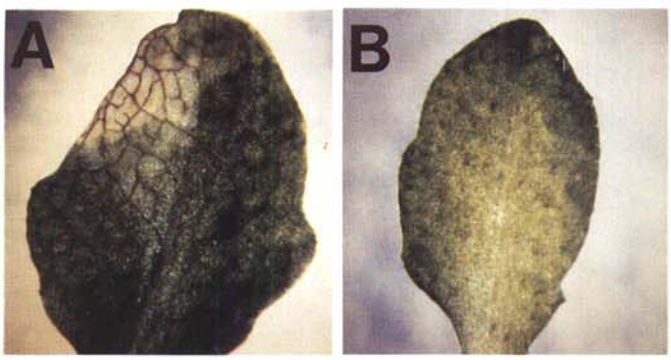

Fig. 1. Symptoms of the Ob-1 ecotype of *Arabidopsis thaliana* 12 days after inoculation by spraying with the R14-A strain of *Xanthomonas campestris* pv. *campestris* (**A**) or with *X. c.* pv. *carotae* (**B**).

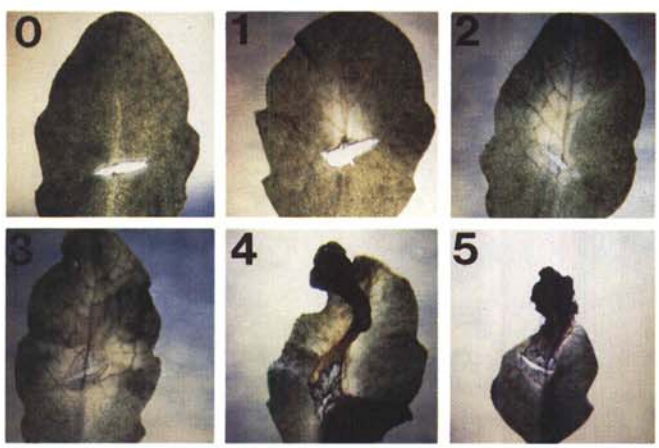

Fig. 2. The disease rating system. The Ob-1 ecotype of *Arabidopsis* was inoculated with the R14-A strain of *Xanthomonas campestris* pv. *campestris* by wounding. The slit is visible in each leaf. Five days later leaves were examined macroscopically for symptoms of black rot (chlorotic regions with blackened veins). Scores of zero to five were assigned based on the area of symptomatic tissue distal to the slit as illustrated in the panels. The average score for each ecotype was based on the observation of at least 90 leaves.

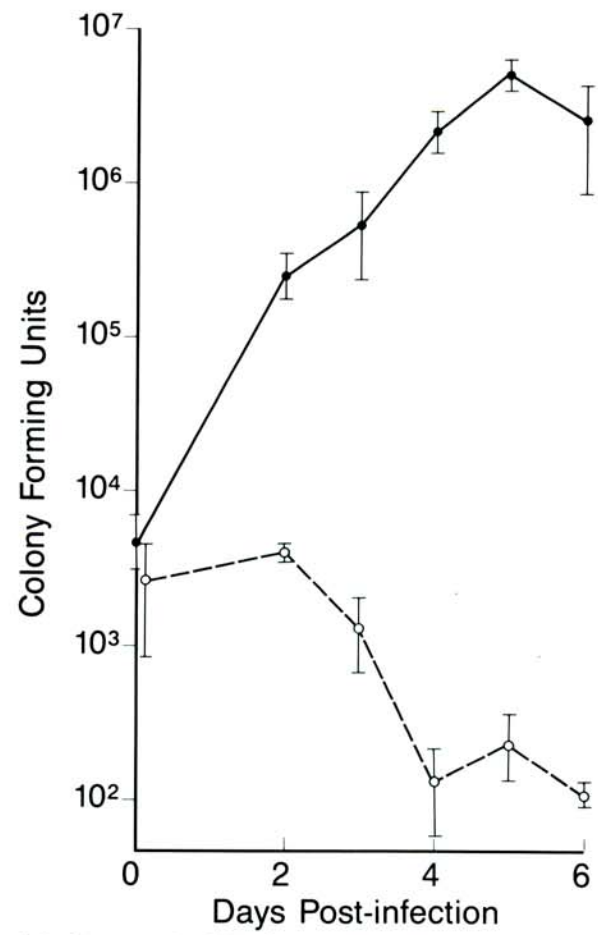

Fig. 3. *In planta* growth of *Xanthomonas campestris* pathovars. The Ob-1 ecotype of *Arabidopsis* was inoculated by wounding with the R14-A strain of *X. c.* pv. *campestris* (solid line) or *X. c.* pv. *carotae* (broken line). At various times, disks from surface-sterilized leaves were macerated, and the average number of colony forming units per disk was determined by serial dilution and plating. The average of three samples is plotted, and the bars indicate the standard deviation of the mean. Similar results were obtained in three independent experiments.

agar plates containing 50 μg/ml of rifampicin. This procedure varies slightly from that described above for the test of Koch's postulates.

RESULTS

Symptom expression. When we spray-inoculated a suspension of the R14-A strain of *X. c.* pv. *campestris* onto *A. thaliana* plants, symptoms typical of black rot were produced (Fig. 1A); the V-shaped pattern suggested that the water pores were the site of entry. Symptoms were first observed 7 days after inoculation. Typical black rot symptoms were also produced (Fig. 2, panels 1–5) when we introduced the bacteria by wounding. In this case the symptoms were first observed 3 days after inoculation. By 6 days after inoculation, symptoms were observed on about 40% of the inoculated leaves. With both the wound inoculation and spray inoculation procedures, the number of leaves showing symptoms of black rot increased as the bacterial concentration in the inoculum increased.

Koch's postulates. Koch's postulates, the conditions that must be met before an organism can be confirmed as the cause of a disease, have been fulfilled in the case of the *X. c.* pv. *campestris-Arabidopsis* interaction. A suspension of bacteria derived from a pure culture of *X. c.* pv. *campestris* was sprayed onto *Arabidopsis* plants. Two weeks after inoculation, disks were isolated from the symptomatic leaves of five plants. In each case, about 1,000,000 bacteria were detected in the disks isolated from leaf lesions. No bacteria were detected in disks isolated from asymptomatic leaves of the same plant. These bacteria had the same characteristics as those derived from the original culture of *X. c.* pv. *campestris*; the bacteria formed yellow,

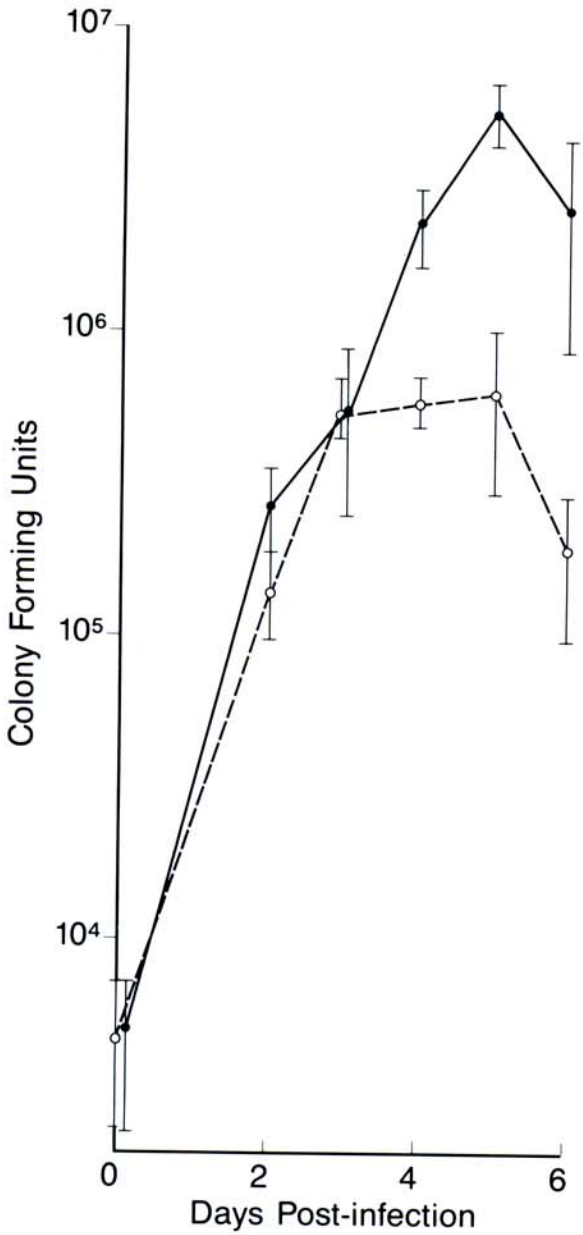

Fig. 4. Growth of *Xanthomonas campestris* pv. *campestris* in two ecotypes of *Arabidopsis*. The Ob-1 (solid line) and Pn-O (broken line) ecotypes of *Arabidopsis* were inoculated by wounding with the R14-A strain of *X. c.* pv. *campestris*. At various times, disks from surface-sterilized leaves were macerated, and the average number of colony forming units per disk was determined by serial dilution and plating. The average of three samples is plotted, and the bars indicate the standard deviation of the mean. Similar results were obtained in three independent experiments.

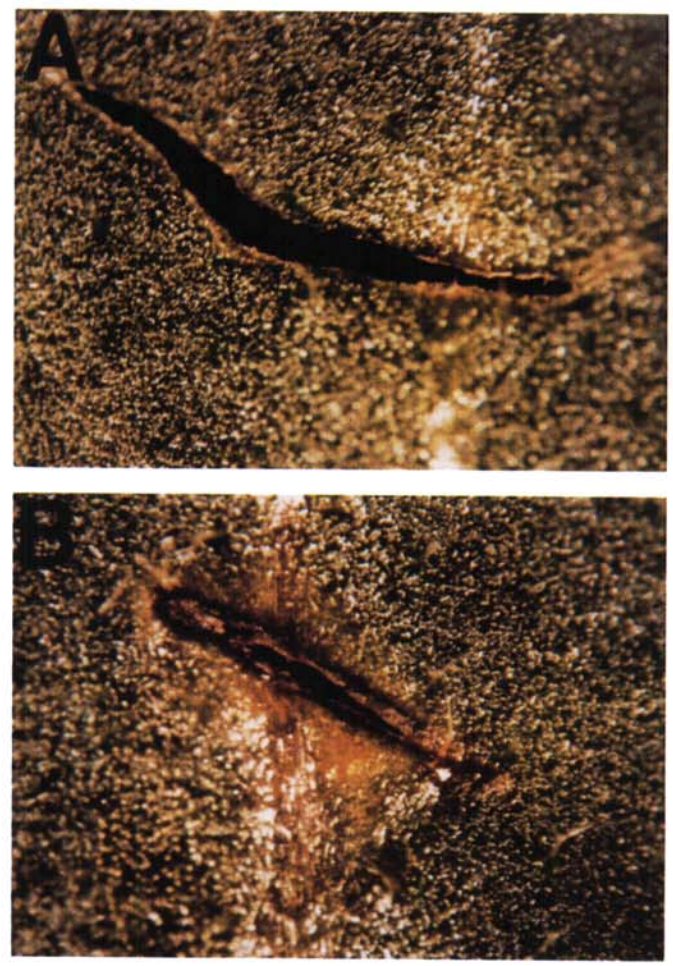

Fig. 5. Microscopic examination (×25) of leaves from the Edi-0 ecotype of *Arabidopsis* three days after inoculation with 10 mM $MgSO_4$ (**A**) or the KXCC strain of *Xanthomonas campestris* pv. *campestris* (**B**).

mucoid colonies on nutrient agar plates and showed resistance to rifampicin. Finally, pure cultures of the bacteria obtained from *Arabidopsis* were able to produce black rot on both *Arabidopsis* and cabbage (*Brassica oleracea* L.). Thus, we concluded that *X. c.* pv. *campestris* was pathogenic to *Arabidopsis*. The symptoms induced were typical of those of black rot caused by the bacterium on other cruciferous hosts.

Pathovar specificity. To test the specificity of the interaction with respect to the pathogen, we inoculated six *Arabidopsis* ecotypes (Ob-1, No-O, Pn-O, Uk-2, Bu-20, and Edi-0) with a different pathovar of *X. campestris* (Pammel) Dowson. Pathovars of *X. campestris* are distinguished by their ability to cause disease on different host species (Bradbury 1984). For example, *X. c.* pv. *carotae*, which causes bacterial blight of carrot, is known to be nonpathogenic to cabbage, inducing a limited necrosis rather than black rot (Cook and Robeson 1986). As expected, when we inoculated *Arabidopsis* with *X. c.* pv. *carotae*, black rot was not visible even 4 wk after inoculation. The edges of the wounds turned brown with no further spread of necrosis while leaves inoculated by spraying appeared unchanged (Fig. 1B). To further characterize the specificity of the interaction, we followed the growth *in planta* of *X. c.* pv. *campestris* and *X. c.* pv. *carotae*. For this work, we chose to introduce the bacteria by wounding. The progress of the disease was more rapid using the wound inoculation rather than the spray inoculation procedure, and the wounding procedure allowed us to follow each inoculated leaf. After inoculation by wounding, *X. c.* pv. *campestris* showed marked growth *in planta* while the viable count of *X. c.* pv. *carotae* rapidly decreased (Fig. 3). Thus, the responses of *Arabidopsis* to infection by *X. c.* pv. *campestris* and *X. c.* pv. *carotae* were similar to the respective responses of cabbage (Cook and Robeson 1986). We concluded that *Arabidopsis* was a host for *X. c.* pv. *campestris*.

Ecotype specificity. We tested three of the geographical races (ecotypes) of *Arabidopsis* and showed that there were differences in their responses to inoculation with *X. c.* pv. *campestris*. We used a disease rating system that is based on the area per leaf expressing black rot symptoms (Fig. 2) and in which scores ranged from zero to five. Inoculations with a control, *X. c.* pv. *carotae*, resulted in scores of zero. The Ob-1 ecotype of *Arabidopsis* with an average score of 1.6 was more susceptible to *X. c.* pv. *campestris* than was the Pn-O ecotype with an average score of 0.18. This differential response with respect to symptom expression was positively correlated with bacterial growth *in planta*. The maximum pathogen population was significantly greater in the infected leaves of the Ob-1 ecotype than the Pn-O ecotype (Fig. 4). The third ecotype tested, No-O, showed responses similar to those of the Pn-O ecotype.

Encouraged by these results, we observed the interaction of two different strains of *X. c.* pv. *campestris* with three different ecotypes of *Arabidopsis* (Table 1). The interaction of the Uk-2 ecotype with either bacterial strain resulted in an average score of greater than 2.0 while the interaction of the Bu-20 ecotype with either strain resulted in an average score of less than 0.10, further illustrating ecotypic differences in symptom expression. In contrast, the Edi-0 ecotype showed differential responses with two bacterial strains. The inoculation of the Edi-0 ecotype with the KXCC strain resulted in an average score of 0.03 while inoculation with the XCCS strain resulted in an average score of 2.1.

To see if a low score was accompanied by a hypersensitive response, we observed with the aid of a microscope the leaves of the Edi-0 ecotype inoculated with the KXCC strain. The resulting localized necrosis (Fig. 5B) was distinct from the control wound response (Fig. 5A). The localized necrosis suggested that a hypersensitive response may be involved, but proof requires further characterization.

Table 1. Disease ratings for three ecotypes of *Arabidopsis* inoculated with two strains of *Xanthomonas campestris* pv. *campestris*[a]

	KXCC			XCCS		
Ecotype	**Expt. 1**	**Expt. 2**	**Average**	**Expt. 1**	**Expt. 2**	**Average**
Uk-2	1.9	2.4	2.2	2.5	1.7	2.1
Bu-20	0.06	0.06	0.06	0.00	0.14	0.07
Edi-0	0.04	0.02	0.03	1.9	2.2	2.1

[a] Each ecotype was inoculated with either the KXCC strain or the XCCS strain of *X. c.* pv. *campestris*. Responses were scored 6 days after inoculation using the disease rating system illustrated in Figure 2. The score for each experiment was based on at least 45 leaves. The results of two independent experiments and the average scores are reported.

DISCUSSION

The symptoms produced by *X. c.* pv. *campestris* on *A. thaliana* using three different inoculation procedures were similar to the symptoms produced by this pathogen on *Brassica*. Koch's postulates were fulfilled indicating that this bacterium was the cause of the symptoms. The first two inoculation procedures, spraying and wounding, produced symptoms characterized by V-shaped chlorotic lesions extending out from the site of inoculation and many blackened veins within the chlorotic region. When *Arabidopsis* was inoculated via spraying or wounding with a control pathovar, *X. c.* pv. *carotae*, the symptoms produced were similar to the incompatible reaction of cabbage to this pathovar (Cook and Robeson 1986). The third inoculation procedure, stomatal infiltration, is not considered to be a natural route of infection for *X. c.* pv. *campestris* (Williams 1980), is not suitable for black rot studies (Shaw and Kado 1988), and fails to elicit typical black rot symptoms on *Brassica* or *Arabidopsis* (Collinge *et al.* 1987; Tsuji and Somerville 1988; our data, not shown). When we inoculated the Ob-1 ecotype of *Arabidopsis* with the R14-A strain of *X. c.* pv. *campestris* via the stomatal infiltration procedure, we also failed to observe typical black rot symptoms. The similar responses of *Arabidopsis* and *Brassica* to *X. campestris* using the three inoculation procedures suggest that *Arabidopsis* may be a good model for the study of black rot of *Brassica*.

The use of *Arabidopsis* as a model is further supported by the similarities of *in planta* bacterial growth between *Arabidopsis* and *Brassica*. For both, there is marked growth of *X. c.* pv. *campestris* (Fig. 3, this work; Cook and Robeson 1986). In addition, *X. c.* pv. *carotae* has a significantly lower maximum population than does *X. c.* pv. *campestris*

in both *Arabidopsis* and cabbage (Fig. 3, this work; Cook and Robeson 1986). There are significant differences in the growth of *X. c.* pv. *campestris* between cabbage cultivars (Staub and Williams 1972). Likewise, we have shown differences in the growth of *X. c.* pv. *campestris* between ecotypes of *Arabidopsis* (Fig. 4). These similarities confirm that *Arabidopsis* is a good model for black rot investigations.

The first step in these investigations is to characterize the differential responses of *Arabidopsis* ecotypes to *X. c.* pv. *campestris*. We observed that the interaction of one ecotype with either of two bacterial strains resulted in low scores indicating a low level of symptom expression. In contrast, the interaction of another ecotype with the same two bacterial strains resulted in high scores. In addition, we observed an ecotype that interacted with one strain resulting in a high score yet interacted with the other strain resulting in a low score. This differential response showed that differences in susceptibility need not be due to differences in leaf morphology.

Ecotypic differences such as these form the basis for further black rot investigations. A major goal would be to determine the molecular mechanisms of resistance to *X. c.* pv. *campestris*. The differential response of *Arabidopsis* ecotypes to *X. c.* pv. *campestris* suggests that this determination may be feasible. Several hundred ecotypes of *Arabidopsis* have been collected from Europe, Asia, Africa, and North America (Kranz and Kirchheim 1987). Currently we are screening a large number of these ecotypes for their response to *X. c.* pv. *campestris* to locate an ecotype expressing a high level of resistance to black rot. We will then determine the genetic basis of this resistance and isolate the genes. In addition to its usefulness in black rot investigations, we suggest that *Arabidopsis* may be a good model for the study of plant interactions with other pathogens.

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