

## Inheritance of Resistance in Lettuce to *Plasmopara lactucae-radicis*

G. J. Vandemark, M. E. Stanghellini, S. L. Rasmussen, and R. W. Michelmore

First, second, and third authors: Department of Plant Pathology, University of Arizona, Tucson 85721; fourth author: Department of Vegetable Crops, University of California, Davis 95616.

We thank S. E. Smith (Department of Plant Science, University of Arizona, Tucson) for his comments on the manuscript. We also thank E. J. Ryder (USDA-ARS, Salinas, CA) for providing seed of *Lactuca sativa* 'Calmar'.

Accepted for publication 5 August 1991.

### ABSTRACT

Vandemark, G. J., Stanghellini, M. E., Rasmussen, S. L., and Michelmore, R. W. 1991. Inheritance of resistance in lettuce to *Plasmopara lactucae-radicis*. *Phytopathology* 82:272-274.

*Plasmopara lactucae-radicis* recently has been described as the causal agent of downy mildew on roots of cultivated lettuce. This fungus is the only known causal agent of downy mildew that is restricted to the roots of its host. Thirty-seven cultivars of *Lactuca sativa* were screened for resistance to *P. lactucae-radicis*. Two-week-old lettuce plants grown under hydroponic conditions were challenged with this fungus and evaluated 2 and 3 wk after inoculation for resistance. Root necrosis and production of sporangia on roots was considered a susceptible reaction.

*Additional keyword:* cultivar screen.

Five cultivars were determined resistant to *P. lactucae-radicis*. Resistant cultivars were colonized by this fungus, but the fungus was unable to sporulate on infected roots. Data from F<sub>2</sub> and F<sub>3</sub> progenies demonstrated that resistance in the cultivar Cobham Green was conferred by a recessive allele at a single locus (*plr*). Fungal infection of susceptible plants by *P. lactucae-radicis* resulted in significant decreases in fresh root and shoot weights and leaf number compared with decreases in these characters associated with infection of resistant plants.

In 1987, a new causal agent of downy mildew, *Plasmopara lactucae-radicis* Stang. & Gilbn., was discovered on lettuce (*Lactuca sativa* L.) cultivars Ostinata and Salina in a commercial hydroponic greenhouse in Rapidan, VA, that employed the nutrient film technique of cultivation (8). This fungus, which systemically colonizes the roots and produces sporangia on indeterminate sporangiophores, is restricted to the root system in which intercellular hyphae and intracellular haustoria are produced.

A preliminary screening of the susceptibility of three commercial lettuce cultivars, Sitonia, Ostinata, and Salina, demonstrated extensive root necrosis and sporangia formation on the roots of Ostinata and Salina but not Sitonia, which was resistant to *P. lactucae-radicis* (7). The objectives of the present study were to screen additional lettuce cultivars for resistance to *P. lactucae-radicis* and to determine the genetic basis for resistance in lettuce to this fungus. A preliminary report has been published (10).

### MATERIALS AND METHODS

**Inoculum.** Cultures of *P. lactucae-radicis*, an obligate parasite, were maintained on lettuce plants (cv. Ostinata) grown under hydroponic conditions in a Phototron growth chamber (Pyraoponic Industries, Inc. II, San Diego, CA). Healthy Ostinata seedlings were rotated into the chamber every 3–4 wk in order to maintain stock cultures of the fungus. Infected plants served as the source of inoculum in all studies as described below.

**Cultivar screen.** The susceptibility of 37 lettuce cultivars (Table 1) to *P. lactucae-radicis* was evaluated in a greenhouse under hydroponic conditions (9). Lettuce seeds were germinated on water agar and transferred to Oasis horticultubes (Smithers Oasis, Kent, OH). Single 1-wk-old seedlings of each cultivar were transplanted into holes cut into a styrofoam flotation board. The board then was placed in a plastic tub containing 13.3 L of a continuously aerated nutrient solution. Tubs were placed in a temperature-controlled box and the nutrient solution temperature was maintained at 26 C (7). One week after transplanting, an Ostinata

plant infected with *P. lactucae-radicis* was placed into each tub to serve as a source of inoculum.

**Evaluation.** The root system of each cultivar was examined microscopically for the presence of sporangia on roots as well as necrosis of the root system 2 and 3 wk after inoculation. Extensive root necrosis and production of sporangia on roots was considered a susceptible reaction, whereas lack of visible root necrosis and no sporulation on roots was considered a resistant reaction. Additionally, a portion of the root system (three or four 1-cm-long root segments) of each cultivar was excised, stained with acid fuchsin, and examined microscopically for the presence of intercellular hyphae and intracellular haustoria as previously described (7). The experiment was replicated three times for each cultivar and was repeated on three separate occasions.

**Genetic studies.** The genetic basis of resistance in lettuce to *P. lactucae-radicis* was evaluated by screening one F<sub>2</sub> family (200 plants) and four F<sub>3</sub> families (104 plants). F<sub>2</sub> progenies resulted from a cross between a resistant cultivar, Cobham Green, and a susceptible cultivar, Calmar.

F<sub>2</sub> seedlings were grown hydroponically and inoculated as described above. The roots of each F<sub>2</sub> plant were examined microscopically for the presence of necrosis and sporangia 2 and 3 wk after inoculation, and the plants were classified as susceptible or resistant. Chi-square analysis of the segregation ratio was performed using 50 plants from each of four separate trials.

Four resistant F<sub>2</sub> plants from the cross Cobham Green × Calmar were transferred to 10-cm-diameter pots containing sterile vermiculite and grown to maturity in a greenhouse. Self-pollination of these plants produced four F<sub>3</sub> families. Two-week-old F<sub>3</sub> siblings from each family were assessed for resistance or susceptibility to *P. lactucae-radicis* as described above. The F<sub>3</sub> analysis was repeated twice for each of the four sibling groups, first with 10 plants per group and then with 16 plants per group. Each hydroponic tub also contained an Ostinata plant that served as a susceptible control plant. Control plants were evaluated microscopically for the presence of sporangia on roots 2 and 3 wk after inoculation to verify that hydroponic conditions were conducive to pathogen spread and sporangia production.

**Effect of root infection on yield.** The effect of *P. lactucae-radicis* infection on yield characteristics was measured in the susceptible cultivar Calmar and in two Cobham Green × Calmar

F<sub>3</sub> sibling groups derived from different resistant F<sub>2</sub> plants. Five 2-wk-old Calmar plants and five 2-wk-old plants of each of two resistant F<sub>3</sub> families were grown separately in tubs along with two 2-wk-old *Ostinata* plants that served as susceptible controls. The tubs were inoculated by transfer of an infected *Ostinata* plant into each tub as described above. Fresh leaf and root weights and number of leaves per plant were measured 30 days after inoculation. Roots were excised from the plant at the base of the stem, blotted dry, and weighed. Five Calmar plants and five plants from each F<sub>3</sub> family, grown in separate uninoculated tubs, served as healthy controls in each replication. Each treatment and comparison between inoculated plants and uninoculated plants was repeated twice.

## RESULTS

**Cultivar screen.** The 37 lettuce cultivars screened for resistance to *P. lactucae-radicis* represented the diversity within the major types of cultivated lettuce (3). Five butterhead cultivars, Cobham Green, Sitonia, Mildura, Bourguignonne Gross Blonde d'Hiver, and May King, were classified as resistant. Thirty-two cultivars of all types were classified as susceptible (Table 1).

Microscopic examination revealed that the root systems of both susceptible and resistant cultivars were colonized by the fungus. However, the extent of root colonization was restricted in resistant cultivars. Numerous intercellular "runner hyphae" (8–20 per root) were observed in susceptible cultivars, whereas only two to four such hyphae were observed in resistant cultivars. Additionally, intracellular haustoria in resistant, but not susceptible, cultivars appeared abnormal. Preliminary studies (10) revealed extensive deposits of callose around haustoria in resistant, but not susceptible, cultivars. The relationship, if any, between callose deposition and resistance will be addressed elsewhere.

**Inheritance of resistance.** An F<sub>2</sub> population from a resistant and a susceptible cultivar identified in the initial screen was screened to determine the genetic basis of resistance in lettuce to *P. lactucae-radicis*. F<sub>2</sub> progeny derived from the cross Cobham Green × Calmar clearly segregated with no significant deviation from the ratio of one resistant to three susceptible plants (46:154, chi-square value 0.43, and 31:119, chi-square value 1.51, at 14 and 21 days, respectively) when assayed after inoculation.

Inheritance of resistance was examined further by analysis of F<sub>3</sub> progenies produced by self-pollination of four resistant F<sub>2</sub> plants derived from the cross Cobham Green × Calmar. All F<sub>3</sub> families produced by resistant F<sub>2</sub> plants were uniformly resistant to *P. lactucae-radicis*. Microscopic examination of roots from one plant from each F<sub>3</sub> family revealed restricted hyphae and callose-encased haustoria in the roots of each plant. These results were similar to those observed in resistant F<sub>2</sub> plants. Each family was grown with a single *Ostinata* plant, which served as a sus-

ceptible control. Microscopic examination revealed that sporangia were produced on the roots of all *Ostinata* plants, indicating that conditions during these tests were conducive to pathogen spread and sporangia formation.

**Effect of root infection on yield.** Results of the effect of *P. lactucae-radicis* infection on fresh shoot and root weights and number of leaves for Calmar and the two Cobham Green × Calmar F<sub>3</sub> families are presented in Figure 1. Infected Calmar plants have over 50% reduction in the number of leaves relative to uninoculated controls, while plants from both F<sub>3</sub> families had less than 10% reduction in leaf number relative to healthy controls. Both F<sub>3</sub> families had significant decreases in root and shoot weights due to infection, but these decreases were significantly smaller than the decreases observed for infected Calmar plants. Roots of infected Calmar plants were brown and necrotic, whereas roots of infected F<sub>3</sub> plants were white and healthy appearing.

## DISCUSSION

The cultivar screen identified five resistant cultivars and 32 cultivars susceptible to *P. lactucae-radicis* based on the presence or absence of root necrosis and sporulation on roots (Table 1). The results of three replicates for each cultivar were consistent,

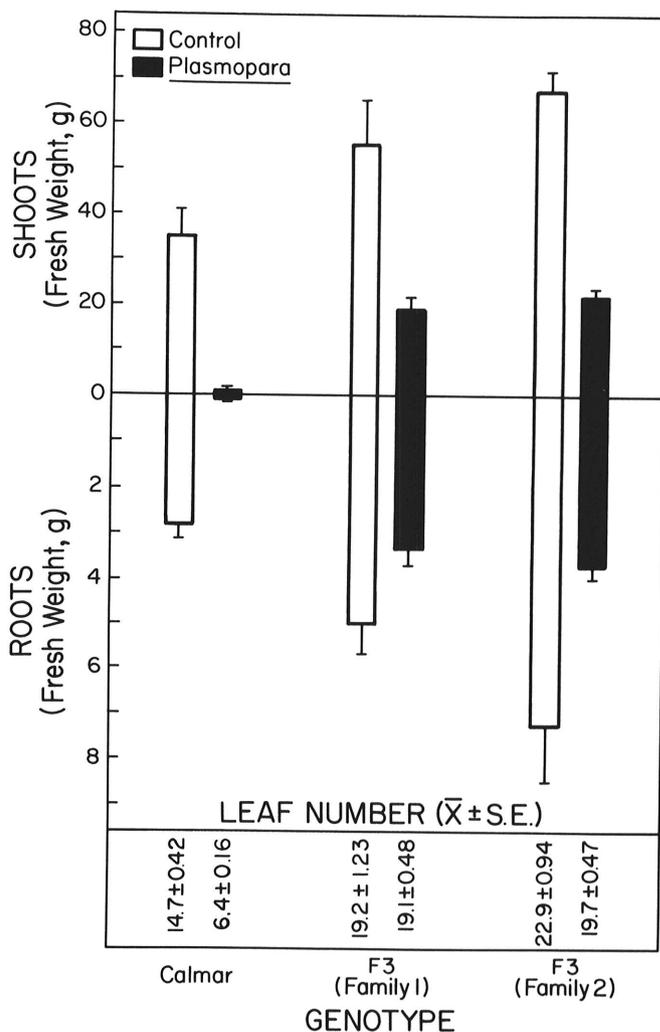


Fig. 1. Effect of *Plasmopara lactucae-radicis* infection on fresh shoot weight, fresh root weight, and leaf number for three genotypes of *Lactuca sativa* L. Two-week-old seedlings of each genotype were inoculated by placing an infected *Ostinata* plant into the hydroponic growth chamber. Plants were assayed 30 days after inoculation. Each mean is the average of five replicates in each of two repetitions;  $n = 10$ . The vertical bar shows the standard error. Calmar = susceptible; F<sub>3</sub> (Cobham Green × Calmar) = resistant.

TABLE 1. Resistance or susceptibility of cultivars of *Lactuca sativa* to *Plasmopara lactucae-radicis*

Susceptible	Type <sup>a</sup>	Susceptible	Type	Resistant	Type
Amplus	B	Lobjoits Cos	S	Bourguignonne GBH	B
Autumn Gold	C	Mariska	B	Cobham Green	B
Avoncrisp	C	Meri	C	Mildura	B
Avondefiance	B	Marquette	C	May King	B
Blondine	B	Mesa 659	C	Sitonia	B
Calmar	C	Montello	C		
Capitan	B	Ostinata	B		
Climax	C	Sabine	B		
Dandie	B	Saffier	B		
Diana	B	Salad Bowl	L		
Empire	C	Salina	B		
Fila	B	Salinas	C		
Grand Rapids	L	Sucrine	B		
Greenlakes	C	Valmaine	S		
Hilde	B	Vanguard 75	C		
Kordaat	B	Winterhaven	C		

<sup>a</sup> B = Butterhead; C = crisphead; L = leaf; S = cos.

with all three replicates producing sporangia on necrotic roots for all susceptible cultivars and all three replicates having no sporangia on asymptomatic roots for all resistant cultivars. These results indicated that there was no variation within a cultivar for resistance, but that there was variation between cultivars for resistance to *P. lactucae-radicis*.

F<sub>2</sub> segregation analysis and F<sub>3</sub> analysis of resistant F<sub>2</sub> families indicated that resistance to *P. lactucae-radicis* was simply inherited and conditioned by a single recessive allele at a single locus in Cobham Green. We designate this gene *plr*.

Our cultivar screen and segregation analysis demonstrated unique features of this host-pathogen interaction. This is the first report of resistance to a fungal root pathogen of lettuce that is determined by a single recessive gene. This differs from resistance in lettuce to leaf downy mildew, *Bremia lactucae* Regel, where resistance is controlled by dominant genes (2). Genetic analysis has identified 13 dominant genes (*Dm*) in lettuce that confer resistance to *B. lactucae* (2). The dominant genes for resistance to *B. lactucae* condition a hypersensitive response. Microscopic observation failed to reveal any evidence for the involvement of hypersensitivity in resistance to *P. lactucae-radicis*, indicating that different mechanisms are present in lettuce for conditioning resistance to these two downy mildews. Although Cobham Green is resistant to *P. lactucae-radicis*, no resistance genes to *B. lactucae* have been identified in this cultivar; therefore, it is used as the universally susceptible cultivar for culturing isolates of *B. lactucae* (2). The determination of resistance to *P. lactucae-radicis* in lettuce by a single recessive gene also is different from resistance to *P. halstedii* (Farl.) Berl. & de Toni (= *P. helianthi* Novotelnova f. *helianthi*) in sunflower (*Helianthus annuus* L.); nine dominant resistance genes have been identified (5). *P. halstedii* is closely related to *P. lactucae-radicis* and is the only other causal agent of downy mildew that produces sporangia on the roots of its host (8).

Microscopic examination of the roots of infected Cobham Green × Calmar F<sub>3</sub> plants demonstrated the absence of sporangia on roots but the presence of hyphae within the roots. This indicated that resistant plants are not immune to infection by *P. lactucae-radicis*, and infection results in reduced shoot and root weights. However, the effects of infection on yield characteristics were measured using an infected *Ostinata* plant as a continual source of inoculum. If F<sub>3</sub> plants had been inoculated and then grown in the absence of an infected *Ostinata* plant, yield reduction would probably have been slight because the fungus cannot sporulate and multiply on resistant plants.

Continuing research includes mapping of the *plr* gene. Linkage to restriction fragment length polymorphism (RFLP) markers is being analyzed in F<sub>2</sub> progeny from Cobham Green × Calmar. This will position *plr* relative to the four clusters of *Dm* genes that condition resistance to *B. lactucae* (1,3,4). We also will investigate the role of callose deposition in resistance to *P. lactucae-radicis*. We previously observed extensive callose deposition around haustoria in resistant plants, but not susceptible plants (10). Callose deposition has been identified as an important component of the resistance to powdery mildew (*Erysiphe graminis* f. sp. *hordei*) conditioned by *ml-o* in barley (*Hordeum vulgare* L.) (6).

#### LITERATURE CITED

1. Farrara, B. F., Ilott, T. W., and Michelmore, R. W. 1987. Genetic analysis of factors for resistance to downy mildew (*Bremia lactucae*) in species of lettuce (*Lactuca sativa* and *L. serriola*). *Plant Pathol.* 36:499-514.
2. Ilott, T. W., Hulbert, S. H., and Michelmore, R. W. 1989. Genetic analysis of the gene-for-gene interaction between lettuce (*Lactuca sativa*) and *Bremia lactucae*. *Phytopathology* 79:888-897.
3. Kesseli, R. V., Paran, I., and Michelmore, R. W. 1989. *Lactuca sativa*. Pages 6.100-6.102 in: Genetic Maps. E. O'Brien, ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
4. Landry, B. S., Kesseli, R. V., Farrara, B., and Michelmore, R. W. 1987. A genetic map of lettuce (*Lactuca sativa*) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. *Genetics* 116:331-337.
5. Sackston, W. E., Gulya, T. J., and Miller, J. F. 1990. A proposed international system for designating races of *Plasmopara halstedii*. *Plant Dis.* 74:721-723.
6. Skou, J. P. 1982. Callose formation responsible for the powdery mildew resistance in barley with genes in the *ml-o* locus. *Phytopathol. Z.* 104:90-95.
7. Stanghellini, M. E., Adaskaveg, J. E., and Rasmussen, S. L. 1990. Pathogenesis of *Plasmopara lactucae-radicis*, a systemic root pathogen of cultivated lettuce. *Plant Dis.* 74:173-178.
8. Stanghellini, M. E., and Gilbertson, R. L. 1988. *Plasmopara lactucae-radicis*, a new species on roots of hydroponically grown lettuce. *Mycotaxon* 31(2):395-400.
9. Stanghellini, M. E., and Kronland, W. C. 1986. Yield loss in hydroponically grown lettuce attributed to subclinical infection of feeder rootlets by *Pythium dissotocum*. *Plant Dis.* 70:1053-1056.
10. Stanghellini, M. E., Michelmore, R. W., Rasmussen, S. L., and Vandemark, G. J. 1990. Inheritance and a possible mechanism of resistance in lettuce to *Plasmopara lactucae-radicis*. (Abstr.) *Phytopathology* 80:966.