Reaction of Lettuce Germ Plasm to Artificial Inoculation with *Sclerotinia minor* Under Greenhouse Conditions

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ABSTRACT

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More than 100 *Lactuca* accessions were evaluated for resistance to *Sclerotinia minor* under greenhouse conditions. Plants 5–6 wk old were inoculated with rye seeds colonized by *S. minor* placed in contact with the taproot 1–2 cm below the soil surface. Surviving plants were reinoculated 3 wk later. All accessions of *L. sativa*, including introductions from 28 countries, were susceptible. However, several selections of wild *Lactuca* spp., including *L. dentata, L. perennis, L. serriola, L. virosa*, and unidentified species, were intermediately to highly resistant to *S. minor*. Some plants that survived the *Sclerotinia* test evidently were not genetically resistant, since their selfed progeny were highly susceptible. Although not all testing has been completed, the selfed or sibbed progenies of selections of *L. dentata, L. serriola*, and an unidentified *Lactuca* sp. have remained highly resistant. No correlation was found between the area of origin or horticultural type and resistance to *S. minor*.

Additional key words: lettuce drop

Severe epidemics of lettuce drop incited by *Sclerotinia* (= *Whetzelinia* Korf & Dumont) *minor* Jagger have occurred repeatedly in recent years in the lettuce-growing areas of central New York. Infection of lettuce (*Lactuca sativa* L.) by the large sclerotial form, *S. sclerotiorum* (Lib.) d. By. [= *W. sclerotiorum* (Lib.) Korf & Dumont], has been insignificant. About 1,100 acres of the muck soil in this area are double-cropped to lettuce annually. The last three seasons were unusually wet, particularly during the second lettuce crop, and about 10% of the planted acreage was affected by drop. Disease incidence varied considerably among fields, ranging from 5 to 50%.

Infection of lettuce with *S. minor* results from the eruptive mycelial germination (2) of sclerotia in the top 10 cm of the soil surface (2,5). Penetration occurs through senescent lower leaves, taproot, and/or secondary fibrous roots. Marcum et al (5) recently demonstrated in California that lettuce drop was controlled with as little as 2.5 kg/ha of 2,6-dichloro-4-nitroaniline (DCNA). At present, fungicide application to control lettuce drop is used only on a limited scale, if at all, and no other effective measures are available for New York growers.

An extensive lettuce-breeding program recently was initiated at Geneva, NY, to develop cultivars that are adapted and resistant to the major lettuce viruses in the area. As losses from lettuce drop increased, resistance to *S. minor* was sought with the aim of incorporating any resistance genes into advanced virus-resistant germ plasm. This paper reports on the evaluation of selected lettuce germ plasm for resistance to *S. minor* through artificial inoculations under greenhouse conditions.

MATERIALS AND METHODS

The isolate SS-57 of *S. minor* obtained from field-infected lettuce from Oswego County, NY, was used throughout the study. The fungus was maintained by periodic transfers on Difco potato-dextrose agar (PDA) at 20–22 C. Infected rye (*Secale cereale* L.) seeds were used as the source of inoculum. About 10 g of rye seeds was placed in 500-ml flasks with 20 ml of distilled water and autoclaved. After cooling, each flask received three disks (6 mm diam) from the margins of advancing fungal colonies on PDA plates. All flasks were incubated for 5–7 days at 25 C before use.

Table 1. Reaction of selected germ plasm of *Lactuca* species to artificial inoculations with *Sclerotinia minor* under greenhouse conditions[a]

Cultivar or PI number	Origin	Species	No. infected/ no. tested	Killed (%)
Ithaca	United States	*L. sativa*	74/77	96
Minetto	United States	*L. sativa*	74/78	95
Vanguard 75	United States	*L. sativa*	22/25	88
Arctic King	United States	*L. sativa*	20/20	100
Big Boston	United States	*L. sativa*	23/23	100
Calmar	United States	*L. sativa*	24/24	100
Celtuce	United States	*L. sativa*	24/24	100
Grand Rapids	United States	*L. sativa*	22/22	100
Great Lakes	United States	*L. sativa*	24/24	100
Oakleaf	United States	*L. sativa*	24/24	100
Paris White Cos	United States	*L. sativa*	23/23	100
Slo Bolt	United States	*L. sativa*	24/24	100
Stokes Evergreen	Canada	*L. sativa*	23/23	100
Valmaine	United States	*L. sativa*	23/23	100
Avon Defiance	England	*L. sativa*	24/24	100
Avoncrisp	England	*L. sativa*	24/24	100
165063	Turkey	*L. sativa*	23/24	96
176579	Turkey	*L. sativa*	13/21	62
184787	Netherlands	*L. sativa*	29/33	88
250427	Czechoslovakia	*L. sativa*	22/24	92
255568	Yugoslavia	*L. sativa*	6/10	60
268405	Afghanistan	*L. sativa*	24/24	100
288244	Egypt	*L. sativa*	15/20	75
120938	Turkey	*L. sativa*	23/24	96
121935	India	*L. sativa*	24/24	100
140392	Iran	*L. sativa*	24/24	100
234624	South Africa	*L. sativa*	24/24	100
183234	Egypt	*L. sativa*	24/24	100
184113	Yugoslavia	*L. sativa*	22/22	100
184786	Netherlands	*L. sativa*	24/24	100
187239	Belgium	*L. sativa*	24/24	100
193489	Ethiopia	*L. sativa*	24/24	100
198733	Israel	*L. sativa*	24/24	100
211600	Afghanistan	*L. sativa*	24/24	100
249536	Spain	*L. sativa*	24/24	100

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Table 1. *(continued from preceding page)*

Cultivar or PI number	Origin	Species	No. infected/ no. tested	Killed (%)
181946	Syria	*L. sativa*	24/24	100
258814	USSR	*L. sativa*	22/22	100
263869	Greece	*L. sativa*	23/23	100
264670	Germany	*L. sativa*	24/24	100
269500	Pakistan	*L. sativa*	22/22	100
273205	England	*L. sativa*	23/23	100
274358	Poland	*L. sativa*	20/20	100
274366	Malta	*L. sativa*	24/24	100
321012	Taiwan	*L. sativa*	22/23	96
289046	Hungary	*L. sativa*	22/23	96
284702	Sweden	*L. sativa*	20/20	100
274416	Rumania	*L. sativa*	24/24	100
274379	France	*L. sativa*	24/24	100
249566	Thailand	*L. sativa*	3/3	100
289015	Hungary	*L. altaica*	8/13	62
190906	Hungary	*L. augustana*	28/28	100
274898	Netherlands	*L. augustana*	9/9	100
234204	Japan	*L. dentata*	3/59	5
273574	Italy	*L. dregeana*	23/25	92
273585	Denmark	*L. livida*	21/28	75
274378	France	*L. perennis*	2/21	10
251798	Italy	*L. saligna*	30/34	88
253229	Turkey	*L. saligna*	23/25	92
258813	USSR	*L. saligna*	22/26	85
261653	Portugal	*L. saligna*	14/18	78
273577	Italy	*L. saligna*	17/18	94
273582	England	*L. saligna*	32/35	91
281876	Iraq	*L. saligna*	26/32	81
281877	Iraq	*L. saligna*	20/31	65
202349	Netherlands	*L. serriola*	21/28	75
204753	Netherlands	*L. serriola*	21/29	72
251247	Egypt	*L. serriola*	23/28	82
253467	Yugoslavia	*L. serriola*	24/24	100
253468	Yugoslavia	*L. serriola*	27/29	93
271938	Belgium	*L. serriola*	0/7	0
273431	France	*L. serriola*	20/26	77
273596	Germany	*L. serriola*	27/29	93
273617	Netherlands	*L. serriola*	12/25	48
274355	Germany	*L. serriola*	23/26	89
274359	Poland	*L. serriola*	21/26	81
274372	USSR	*L. serriola*	24/25	96
274457	Germany	*L. serriola*	26/28	93
274807	India	*L. serriola*	18/21	86
289063	Hungary	*L. serriola*	24/29	83
289064	Hungary	*L. serriola*	24/24	100
289065	Hungary	*L. serriola*	19/23	83
236396	Japan	*L. squarrosa*	24/24	100
261651	Portugal	*L. virosa*	8/10	80
271939	Belgium	*L. virosa*	13/23	57
273579	Italy	*L. virosa*	9/20	45
273597	Germany	*L. virosa*	6/15	40
273607	Germany	*L. virosa*	24/29	83
273614	Belgium	*L. virosa*	3/8	38
274375	Poland	*L. virosa*	23/37	62
274901	Netherlands	*L. virosa*	18/22	82
255665	Afghanistan	*Lactuca* sp.	33/33	100
274376	Poland	*Lactuca* sp.	4/59	7

[a] Plants were grown in steam-treated soil mix at 20–24 C. At 5–6 wk, each plant was inoculated with three *S. minor*-infected rye seeds placed in contact with the stem about 1–2 cm below the soil surface; surviving plants were reinoculated 3 wk later. Check plants receiving three noninfected rye seeds remained healthy.

Lettuce cultivars of different growth habit were screened for resistance to *S. minor*. To broaden the base of the *L. sativa* sampled, introductions from 28 countries were tested. Plant introductions of wild *Lactuca* species also were included. The USDA Regional Plant Introduction Station at Pullman, WA, provided seed and the botanical identity for these introductions (Table 1).

Seeds of the lettuce accessions and related species were planted in steamed coarse sand. Seedlings were transplanted 2 wk later into 10-cm diameter plastic pots (four seedlings per pot) filled with steam-treated soil mix. The plants were grown in a greenhouse at 20–24 C and fertilized weekly with a complete nutrient solution (Start-N-Gro, 16-32-16, Agway Inc., Syracuse, NY 13201). Then, 3–4 wk after transplanting, each seedling was inoculated with three rye seeds colonized by *S. minor*. The inoculum was placed in contact with the taproot about 1–2 cm below the soil surface. Seedlings of each selection that received three autoclaved and noncolonized rye seeds served as check plants. All plants were maintained in the same greenhouse and watered as needed, once or twice daily. The number of infected plants was recorded 7, 14, and 21 days after inoculation.

All surviving plants from each accession were reinoculated 3 wk after the first inoculation. Survivors of the double inoculation were either selfed or sibbed, and their progenies were tested similarly.

RESULTS

Infected plants showed macroscopic symptoms as early as 5 days after inoculation, and typical drop symptoms were usually evident after 10–14 days. First symptoms to appear on plants infected with *S. minor* were water-soaked lesions on the main stem near the point of inoculation. These lesions continued to enlarge and move upward above the soil surface on the stem and lower leaves. On *Lactuca* lines with hard stem tissues, the fungus grew to several centimeters above the soil line. Infected leaf and crown tissues became covered with cottony mycelium of *S. minor*, especially near the soil surface. Later, numerous small sclerotia were produced on the mycelial mat. Infected seedlings often became yellow, wilted, and died within 14 days after inoculation (Fig. 1).

All cultivars and introductions of *L. sativa* evaluated for resistance to *S. minor* were susceptible; very few plants, if any, remained healthy (Table 1). However, one selection each of *L. dentata* (PI 234204), *L. perennis* (PI 274378), *L. serriola* (PI 271938), and *Lactuca* sp. (PI 274376) were highly resistant to *S. minor* (Table 1). Also, several selections of *L. virosa* appeared to be heterogenous populations (intermediately resistant), as suggested by the number of plants surviving two inoculations. All check plants remained healthy.

The reactions of the progenies of selected plants (selfed or sibbed) surviving the initial double inoculation by *S. minor* are summarized in Table 2. Of the progenies tested to date, only those of *L. dentata* (PI 234204), *Lactuca* sp. (PI 274376), and *L. serriola* (PI 271938) have remained resistant.

DISCUSSION

The inoculation method used was easy and fast and gave consistent and reproducible results with lettuce cultivars known to be susceptible to *S. minor*. However, the form of inoculum—a mass of vigorously growing hyphae—and the constantly favorable soil moisture and temperature throughout the test may have reduced the chance for detecting low to medium levels of resistance to *S. minor*.

Adams and Tate (2) showed that preconditioned sclerotia of *S. minor* undergoing eruptive mycelial germination are capable of infecting lettuce directly, with no need for an exogenous energy source. They also reported that the incidence of lettuce drop was closely correlated with the number of germinable sclerotia of *S. minor* in the soil. This suggests that each germinable sclerotium, if near susceptible parts, is capable of infecting lettuce plants. Sclerotia within

Table 2. Reaction of progenies of selfed plants surviving artificial inoculations with *Sclerotinia minor* under greenhouse conditions[a]

Inbred	Parent	*Lactuca* spp.	No. infected/ no. tested	Killed (%)
77-780	Ithaca	*L. sativa*	20/20	100
77-751	PI 289015	*L. altaica*	20/20	100
77-752	PI 289015	*L. altaica*	20/20	100
77-753	PI 190906	*L. augustana*	20/20	100
77-754	PI 190906	*L. augustana*	20/20	100
77-355	PI 234204	*L. dentata*	4/20	20
77-756	PI 273574	*L. dregeana*	19/10	95
77-757	PI 273585	*L. livida*	20/20	100
77-758	PI 273585	*L. livida*	20/20	100
77-759	PI 258813	*L. saligna*	20/20	100
77-760	PI 258813	*L. saligna*	20/20	100
77-761	PI 258813	*L. saligna*	20/20	100
77-762	PI 261653	*L. saligna*	13/20	65
77-763	PI 261653	*L. saligna*	20/20	100
77-764	PI 261653	*L. saligna*	19/20	95
77-779	PI 261653	*L. saligna*	16/20	80
77-765	PI 273582	*L. saligna*	19/20	95
77-766	PI 281876	*L. saligna*	17/20	85
77-767	PI 281876	*L. saligna*	17/20	85
77-768	PI 204753	*L. serriola*	20/20	100
77-769	PI 273431	*L. serriola*	20/20	100
77-770	PI 273617	*L. serriola*	19/20	95
77-771	PI 274359	*L. serriola*	19/22	86
77-584	PI 271938	*L. serriola*	14/30	47
77-776	PI 236396	*L. squarrosa*	19/20	95
77-777	PI 236396	*L. squarrosa*	19/20	95
77-778	PI 273597	*L. virosa*	20/20	100
77-772	PI 274376	*Lactuca* sp.	6/20	30
77-773	PI 274376	*Lactuca* sp.	1/20	5
77-774	PI 274376	*Lactuca* sp.	2/20	10
77-775	PI 274376	*Lactuca* sp.	7/20	35

[a] Plants were grown in steam-treated soil mix at 20–24 C. At 5–6 wk, each plant was inoculated with three *S. minor*-infected rye seeds placed in contact with the stem about 1–2 cm below the soil surface; surviving plants were reinoculated 3 wk later. Check plants receiving three noninfected rye seeds remained healthy.

Fig. 1. Lettuce germ plasm **(A)** not inoculated and **(B)** inoculated with rye seeds colonized by *Sclerotinia minor.* **(Left to right):** *Lactuca sativa* cv. Ithaca, *L. sativa* (PI 176579), *L. virosa* (PI 271939), *L. livida* (PI 273585), and *L. dentata* (PI 234204).

the top 10 cm of soil can infect lettuce (1,5). Thus, preconditioned sclerotia should be used to evaluate selected lettuce germ plasm for resistance to *S. minor*. In addition to using the natural infective propagule, such a method is less severe and may permit identification of germ plasm with different levels of resistance to *S. minor*. We are retesting lettuce germ plasm, especially lines that showed promise, using germinable sclerotia in soil as the infective propagule.

The most resistant species to *S. minor* were *L. dentata, L. perennis, L. serriola,* and *Lactuca* sp., obtained from Japan, France, Belgium, and Poland, respectively. Thus, resistance to *Sclerotinia* was not associated with any geographic area. In addition, no resistance was found in accessions from the Mediterranean basin, believed to be the center of origin for *L. sativa* (7).

The germ plasm we evaluated included all the horticultural types of lettuce known: erect, open rosette, bibb, romaine, stem, butterhead, and crisp-head. Under our greenhouse conditions and using the colonized-seed method of inoculation, no correlation was found between the horticultural type of lettuce and resistance to *S. minor*. Hawthorne (4) and Newton and Sequeira (6) reported that lettuce lines with an upright growth habit were more resistant under field conditions than lines with lower leaves in contact with the soil. However, lines resistant and susceptible in the field were equally susceptible to artificial inoculation in the greenhouse. They concluded that the apparent resistant reaction was due to an escape mechanism through differential wounding from windblown soil particles and modification of the microclimate. Several plant introductions reported by Newton and Sequeira (6) to be resistant to *S. sclerotiorum* under field conditions were susceptible to *S. minor* under our greenhouse test conditions. Furthermore, we have observed that romaine-type cultivars with an upright growth habit, such as Valmaine, do not escape lettuce drop in the field in New York but appear as susceptible as crisp-head cultivars. Consequently, a cultivar with an escape mechanism against *S. minor* through the modification of the microclimate will not be useful under the extreme conditions favoring severe lettuce drop annually in New York. At the same time, the market demands a crisp-head type.

Every accession of *L. sativa* tested had a high incidence of infection with *S. minor*. Several accessions of other *Lactuca* species, however, had lower percentages of infected plants and were considered resistant. Final conclusions as to the relative degree of resistance of these introductions cannot be drawn from the data of Table 1, however, since many of the plant introductions were morphologically heterogenous and may also have been variable for reaction to *S. minor*. The relative degree of resistance needs to be determined by progeny-testing the selections. Of the progeny tests conducted to date, the only selections to breed true for resistance to *S. minor* were from *L. dentata* (PI 234204), *Lactuca* sp. (PI 274376), and *L. serriola* (PI 271938).

Unfortunately, attempts at reciprocal crosses of the highly resistant *L. dentata* (PI 234204) and *Lactuca* sp. (PI 274376) with *L. sativa* lettuce cultivars have thus far failed. Isozyme analyses of both introductions indicate they are closely related to each other but distantly related to *L. sativa* (J. T. Puchalski and R. W. Robinson, *unpublished*). Crute and Davis (3) reported that *L. dentata* is resistant to downy mildew but is not closely related to *L. sativa*. Crosses between *L. sativa* and the *S. minor*-resistant *L. perennis* also were unsuccessful, and these species do not appear to be closely related. Thompson et al (8) also was unable to cross *L. perennis* and *L. sativa*.

Transfer of *S. minor*-resistant factor(s), if found, from *L. serriola* or *L. virosa* to *L. sativa* would be considerably easier, since these species can be crossed with lettuce (7,8). An accession labeled *L. serriola* (PI 271938) appears to be highly resistant to *S. minor*, but the test population was small. This accession differed from all other accessions of *L. serriola* tested, not only in being the only one resistant to *S. minor* but also in morphology and bolting resistance. It resembled the biennial form of *L. virosa*, but its botanical identity needs to be verified. Several other accessions identified as *L. virosa* also had low percentages of plants killed by *S. minor*. Although *L. virosa* appears promising as a source of resistance to lettuce drop, this resistance needs to be verified by progeny tests in the field as well as in the greenhouse.

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