European Journal of Plant Pathology **108:** 373–378, 2002.

Short communication

A glasshouse cropping method for screening lettuce lines for resistance to *Sclerotinia sclerotiorum*

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Accepted 29 January 2002

Key words: breeding, disease, lettuce drop, susceptibility

Abstract

A soil-based glasshouse crop procedure was developed to screen lettuce lines for resistance to *Sclerotinia sclerotiorum*. Six sequential crops of 19 different lettuce lines with a range of cultural morphologies, reported previously to exhibit some form of resistance to *S. sclerotiorum*, were planted in a glasshouse infested with *S. sclerotiorum* and natural disease development compared with a standard susceptible commercial butterhead cultivar, Rachel. Concomitantly, the same lettuce lines were planted in pots in a nearby glasshouse, were artificially inoculated with ascospores of *S. sclerotiorum*, assessed for infection and scored for disease severity. Most of lines exhibited resistance in at least one of the crop or direct inoculation assessments with wild form, PI 251246, and stem lettuce, Taiwan, exhibiting resistance in three of the assessments and wild form, PI 271938 (*Lactuca serriola*), and Iceberg (crisp) line, 74-1076, exhibiting resistance in all four assessments. Cos line, PI 250427, was less resistant than the standard control in all assessments. The crop based screen with predictable, natural disease development was the most discriminating overall assessment and enabled growth habit to be taken into account during the screening process which was not possible through the direct inoculation procedures. Nevertheless, the novel ascospore inoculation screening process provided information on the type of resistance expressed that could not be identified from the cropping procedure.

Sclerotinia sclerotiorum is a widespread pathogen infecting over 400 species of plants including many important crop species (Boland and Hall, 1994). It is the major cause of sclerotinia disease in lettuce (*Lactuca sativa*) in the UK, although *Sclerotinia minor* can be the major causal agent elsewhere (Subbarao, 1998; Melzer et al., 1997). All commercial cultivars of lettuce are susceptible to both pathogens. However, there are reports that some accessions or breeding lines of *L. sativa*, and the closely related *Lactuca serriola,* exhibit some resistance or tolerance to *S. sclerotiorum* or *S. minor* (Newton and Sequeira, 1972; Hawthorne, 1974; Abawi et al., 1980; Madjid et al., 1983; Subbarao, 1998).

Some workers have used field trials to assess lettuce germplasm for resistance to *Sclerotinia* species (Newton and Sequeira, 1972; Hawthorne, 1974) which allows plant habit and disease escape to be taken into account in the screening process. However, in the field, because of variation in environmental conditions throughout the year, predictable and reproducible levels of disease needed in screening tests are not always achieved. Consequently, most screening tests of lettuce lines for resistance to *Sclerotinia* species have been done in the glasshouse and involved direct inoculation with the pathogen in the form of mycelial macerates, mycelium contained in agar blocks, infested cereal grains or infected plant tissue, or sclerotia (Newton and Sequeira, 1972; Abawi et al., 1980; Madjid et al., 1983). These tests are relatively quick and easy to do but are limited in that they do not take account of growth habit, which is an important field resistance

factor, and also fail to recognise that most infections by *S. sclerotiorum* under European conditions occur through airborne ascospores (Ben-Yephet et al., 1993; Twengström et al., 1998).

This paper reports a novel and more realistic procedure to screen lettuce germplasm for resistance to *S. sclerotiorum* in the glasshouse. A crop based system exposing the test plants to natural, reproducible and increasing levels of disease, enables the influence of growth habit on disease to be assessed, and a pot-based infection system using ascospore suspensions allows tissue based resistance to be determined.

Small seed samples of 16 *L. sativa* or *L. serriola* genotypes and three breeding lines of *L. sativa* reportedly exhibiting resistance to *S. sclerotiorum* were obtained from Dr. E.J. Ryder, USDA-Agricultural Research Service, 1636 East Alisal Street, Salinas, CA 93905, USA (Table 1). The accessions were multiplied by seeding six plants per accession in the glasshouse of summer 1996. Seed from all six plants was bulked to provide a seed stock of each genotype for resistance testing. Observations were made of the growth habit of each genotype (Table 1). Seeds of cv. Rachel, a butterhead growth type used as a *S. sclerotiorum*

Table 1. Accession numbers and description of reported Sclerotinia resistant lettuce genotypes used in this study

Morphological type and description	Accession number or name	Reference
Wild form		
Oilseed type, rapid bolting, long narrow dark green leaves with smooth margins, red stems	PI 251246	E.J. Ryder, pers. comm., Subbarao (1998)
L. serriola, fairly rapid bolting, long narrow mid green leaves with serrated margins	PI 271938	Abawi et al. 1980
Stem lettuce		
Non hearting, very vigorous, large smooth mid green leaves	Taiwan	Madjid et al. 1983
Iceberg (crisp)		
Great Lakes type, dark green with wavy leaf margin	Great Lakes 54	E.J. Ryder, pers. comm.
Breeding line, mid green, smooth leaf	74-501-1	E.J. Ryder, pers. comm.
Breeding line, dark green, wavy leaf margin	74-1076	E.J. Ryder, pers. comm.
Breeding line, dark green, small frame	74-1077	E.J. Ryder, pers. comm.
Batavian (crisp)		
Pale green, blistered leaf	Batavia blonde de Paris	Chupp and Sherf (1961)
Pale green, blistered leaf	Holborn Standard	E.J. Ryder, pers. comm.
Butterhead		
Mid green, large frame	PI 184787	Newton and Sequeira (1972), Abawi et al. (1980), Madjid et al. (1983)
Mid green, large frame	PI 187239	Newton and Sequeira (1972), Abawi et al. (1980), Madjid et al. (1983)
Small frame, pale green	PI 250429	Newton and Sequeira (1972), Madjid et al. (1983)
Open butterhead/leaf type, segregating for large mid-green and small dark green plants	PI 255568	Newton and Sequeira (1972), Abawi et al. (1980), Madjid et al. (1983)
Open butterhead/leaf type, segregating for large mid-green and small dark green plants	Bibb	Madjid et al. (1983)
Cos		
Erect, mid green, smooth leaf	PI 165063	Newton and Sequeira 1972; Abawi et al. (1980), Madjid et al. (1983)
Prostrate, smooth leaf, segregating for anthocyanin	PI 206965	Newton and Sequeira (1972), Madjid et al. (1983)
Erect open, mid green, smooth leaf	PI 229761	Newton and Sequeira (1972)
Erect open, mid green, smooth leaf	PI 250427	Newton and Sequeira (1972), Abawi et al. (1980), Madjid et al. (1983)
Erect, light green	PI 251790	Newton and Sequeira (1972), Madjid et al. (1983)
Standard		
Butterhead, Sclerotinia susceptible	Rachel	JM Whipps, EE Jones and SP Budge (HRI)

susceptible standard, were obtained from commercial suppliers.

The soil-based glasshouse trial consisted of six sequential crops of lettuce grown in soil infested with *S. sclerotiorum*. Each experimental crop (run) consisted of 24 plots, arranged in three rows of eight and oriented in an East–West direction. Each plot contained 60 plants. The trial was designed using two three-by-three semi-Latin squares, one for the first three crops, the other for the last three. Each block of eight plots contained the standard Rachel either once or twice, with each of the 19 test lines occurring once in each run and twice in each row position (once in the first three crops and once in the last three). Disease was established initially by artificially inoculating a crop of lettuce (cv. Rachel) that was planted throughout the chamber. After harvest of the healthy plants, the remaining infected material was spread evenly across the soil surface, to provide uniform distribution of sclerotia. The first experimental crop was planted in early July 1997 and the final experimental crop harvested in October 1998. Diseased plant debris was left on the soil surface between crops and evenly distributed throughout the chamber before preparation of the soil for the next experimental crop. At harvest of each crop, the number of infected plants was noted and percentage of diseased plants calculated.

Pot-based direct inoculation tests were carried out in a nearby chamber when disease began to appear in each of the glasshouse trials. Each test was arranged as a randomised block design with four replicate blocks, each block containing 23 plots, one plot of each of the 19 test lines and four plots of the standard Rachel. Leaves of the five plants in each plot, which had been planted individually in pots at the same time as the glasshouse trials, were pierced with a needle in a localised area five times. A drop (100 μl) of ascospore suspension in water containing 10^6 conidia per ml was applied to the damaged area and the whole plant enclosed in a polyethylene bag for 48 h to ensure high humidity, necessary to allow initiation of infection. Ascospores were collected dry from apothecia and stored in a desiccator at 4 °C prior to use following the procedures of Hunter et al. (1982). The presence of disease was then recorded when the bags were removed and disease severity was subsequently assessed after 12 days. Severity was scored using the following scale: 0, no infection; 1, initiation of infection, showing water-soaked brown lesion; 2, initial lesion spreading along leaf petiole; 3, inoculated leaf collapsed, infection spreading onto stem; 4, stem infected, other leaves beginning to collapse; 5, total plant collapse. Disease assessments were summarised in three ways – the percentage of infected plants in each plot, the mean disease severity score per plot for infected plants, and the mean disease severity score per plot across all plants.

To cope with the unbalanced nature of the experimental design, the glasshouse crop results were analyzed using the residual maximum likelihood (REML) approach after angular transformation of the percentage data. This approach allows a proper allowance to be made for the differences between runs and rows, whilst still allowing estimation of differences between treatments. A combined analysis of the six pot-based direct inoculation experiments was achieved using analysis of variance (ANOVA), again after angular transformation of the percentage infection data. Significant differences between treatments were assessed using least significant difference (LSD) ($P < 0.05$). For both experiments, additional analyses were performed to assess for differences between the different morphological groups (Table 1). For the REML analysis of morphological group differences, the residual variance includes both variation between replicate plots of each line and variation between lines within morphological groups.

In the glasshouse cropping procedure, six lines (wild forms, PI 251246 and PI 271938 (*L. serriola*); stem lettuce, Taiwan; Iceberg (crisp), 74-501-01 and 74-1076; and Butterhead, Bibb) had mean percentage disease values (11–19%) significantly lower than the standard control (34%) and two lines (Cos PI 165063 and PI 250427) had significantly greater disease levels (54–63%) (Table 2). When the lines were considered as morphologically similar groups (Table 1), the wild forms, the stem lettuce, and Iceberg (crisp) had significantly smaller levels of disease (14–19%) than the standard control (Table 2).

Following artificial inoculation, nine lines (wild forms, PI 251246 and PI 271938 (*L. serriola*); Iceberg (crisp), Great Lakes 54 and 74-1076; Batavian (crisp), Batavia blonde de Paris; Butterhead, PI 184787 and PI 255568; and Cos, PI 165063 and PI 229761) had mean percentage infection values (30–89%) significantly less than standard butterhead control (97%) and two lines (Butterhead, Bibb; and Cos PI 250427) had mean percentage infection values (100%) significantly greater than the control (Table 3). When the lines were considered as morphologically similar groups, only the wild forms and Iceberg (crisp) groups gave significantly smaller levels of infection (45% and 91%, respectively) than the standard control.

Table 2. Mean percentage Sclerotinia disease in lettuce lines from six consecutive crops in a glasshouse trial

Morphological group	Accession number	% Disease[1]		% Disease (groups)[2]		% Disease (groups)[2]	
Wild form	PI 251246	11.4	(19.7)	13.5	(21.6)	13.5	(21.6)
	PI 271938	14.2	(22.1)				
Stem lettuce	Taiwan	16.4	(23.9)	15.6	(23.3)	15.6	(23.3)
Iceberg (crisp)	Great Lakes 54	21.7	(27.7)	19.0	(25.9)	21.7	(27.4)
	74-501-1	13.6	(21.7)				
	74-1076	17.7	(24.9)				
	74-1077	26.3	(30.8)				
Batavian (crisp)	Batavia blonde de Paris	23.9	(29.3)	25.3	(30.3)		
	Holborn Standard	26.3	(31.4)				
Butterhead	PI 184787	48.3	(44.0)	28.6	(32.3)	28.6	(32.3)
	PI 187239	27.9	(31.9)				
	PI 250429	30.1	(33.3)				
	PI 255568	20.3	(26.8)				
	Bibb	18.8	(25.7)				
Cos	PI 165063	62.6	(52.3)	40.4	(39.4)	40.4	(39.4)
	PI 206965	31.0	(33.9)				
	PI 229761	29.2	(32.7)				
	PI 250427	54.0	(47.3)				
	PI 251790	27.0	(31.3)				
Standard control	Rachel	33.9	(35.6)	34.2	(35.8)	34.2	(35.8)
LSD[3]			(8.84)		(9.58)		(9.70)
LSD[4]			(11.42)				

[1]Values are back transformed means from angular transformed data in parentheses.
[2]Individual morphological types considered as groups with Iceberg and Batavian types considered as a single crisp group in the final column.
[3]Significant difference between means calculated from LSD, where LSD $= t \times$ SED from ANOVA and $t =$ critical value ($P = 0.05$) of Student's t distribution for appropriate degrees of freedom (107 between treatments and 120 between groups); between the standard and any treatment and is the maximum value for comparison between the standard and any group.
[4]Between any treatment.

When disease score was examined with all plants included in the analysis, 14 lines gave disease scores (0.98–3.7) significantly smaller than the standard control (4.09) and three lines (Iceberg (crisp), PI 74-1077; Butterhead, Bibb; and Cos, PI 250247) gave disease scores (4.48–4.71) greater than the standard control. When the lines were considered as morphologically similar groups, all groups exhibited a significantly lower disease score than the standard control (4.09). When the disease score was examined with the uninfected plants excluded from the analysis, the results were largely similar to those with the infected plants included except that the disease score for wild type, PI 251246 (4.39) was no longer significantly dfferent to the standard control and the disease score for Butterhead, PI 187239 (4.74) became significantly greater than the control. In addition, the Butterhead group disease score (3.77) became significantly smaller than the standard control.

The cropping procedure and direct inoculation tests provided complementary information on the resistance sources in the lettuce lines studied. For example, the Butterhead variety, Bibb, exhibited significant levels of resistance in the cropping trial, but no resistance in any of the direct inoculation assessments, suggesting that either plant morphology or disease escape mechanisms were important in the resistance in this line. In contrast, wild form, PI 271938 (*L. serriola*), and Iceberg (crisp) line, 74-1076, were significantly more resistant than the standard control in all assessments, clearly indicating that some resistance features are consistently exhibited by these lines. However, wild form, PI 251246, showed a very low level of disease in the cropping trial, low percentage infection and disease score, based on total number of plants, but was no different to the standard control when disease score based on infected plant only was considered. Here, resistance acted at the infection stage as once within the plant, the pathogen spread to

Table 3. Percentage Sclerotinia infection and disease score (either including or excluding non-infected plants) following direct inoculation in pot-based tests in the glasshouse. Values are means from six repeat experiments

Morphological group	Accession number	% Infection[1]						Disease score (all plants)			Disease score (infected plants only)		
			Groups[2]		Groups[2]				Groups[2]	Groups[2]		Groups[2]	Groups[2]
Wild form	PI 251246	29.7	(33.0)	44.7	(42.0)	44.7	(42.0)	1.45	1.22	1.22	4.39	3.00	3.00
	PI 271938	60.2	(50.9)					0.98			1.60		
Stem lettuce	Taiwan	94.9	(76.9)	94.9	(76.9)	94.9	(76.9)	3.62	3.62	3.62	4.11	4.11	4.11
Iceberg (crisp)	Great Lakes 54	79.4	(63.0)	90.7	(72.2)	91.4	(72.9)	2.13	3.10	3.14	2.93	3.66	3.69
	74-501-1	94.8	(76.8)					3.70			4.19		
	74-1076	80.8	(64.0)					2.08			2.80		
	74-1077	99.3	(85.0)					4.48			4.68		
Batavian (crisp)	Batavia blonde de Paris	88.6	(70.3)	92.7	(74.4)			2.82	3.22		3.45	3.75	
	Holborn Standard	96.0	(78.4)					3.63			4.06		
Butterhead	PI 184787	89.4	(71.0)	93.4	(75.2)	93.4	(75.2)	3.43	3.74	3.74	4.19	4.3	4.3
	PI 187239	96.3	(78.9)					4.34			4.74		
	PI 250429	94.0	(75.8)					3.44			3.97		
	PI 255568	76.6	(61.1)					2.8			3.84		
	Bibb	99.9	(88.9)					4.71			4.75		
Cos	PI 165063	78.9	(62.7)	92.9	(74.5)	92.9	(74.5)	2.31	3.30	3.30	3.08	3.77	3.77
	PI 206965	98.9	(83.9)					3.33			3.49		
	PI 229761	70.5	(57.1)					1.96			2.98		
	PI 250427	99.6	(86.2)					4.69			4.84		
	PI 251790	98.4	(82.8)					4.24			4.47		
Standard control	Rachel	96.5	(79.2)	96.5	(79.2)	96.5	(79.2)	4.09	4.09	4.09	4.52	4.52	4.52
LSD[3]			(6.59)		(6.60)		(6.60)	0.289	0.289	0.289	0.148	0.148	0.148
LSD[4]			(8.35)					0.366			0.187		

[1]Values are back transformed means from angular transformed data in parentheses.
[2]Individual morphological types considered as groups with Iceberg and Batavian types considered as a single crisp group in the final column.
[3]Significant difference between means calculated from LSD, where LSD= $t \times$SED from ANOVA and t =critical value ($P = 0.05$) of Student's t distribution for appropriate degrees of freedom (414); between the standard and any treatment and is the maximum value for comparison between the standard and any group.
[4]Between any treatment.

give a disease score equivalent to the control. In another case, the Stem lettuce variety, Taiwan, exhibited very low levels of disease in the glasshouse cropping test and significantly lower levels for the disease scores but had a similar value for percentage infection to the standard control. In this line, resistance must act within the tissues.

The standard Butterhead variety, Rachel, was chosen as the control for these assessments as it was a known susceptible variety used routinely in glasshouse-based biocontrol trials for *S. sclerotiorum* at HRI (JM Whipps and EE Jones, unpublished). Indeed, only Cos line, PI 250427 gave consistently greater levels of disease or infection in all assessments. This explains the observation that when the lettuce lines were compared as morphological groups to the standard control, so many gave significantly greater resistance in the assessments, even though different lines within each group may have exhibited significantly greater or less disease resistance than the standard when compared individually.

In conclusion, the glasshouse cropping procedure proved to be the most discriminating test for resistance investigated. It took account of disease escape related to plant morphology, resistance due to the infection process and tissue resistance. Using such continued cropping under glass also enables resistance to assessed more rapidly and reproducibly than trials done in the field which can have environmental limitations. The direct inoculation tests are the first resistance screening tests done on lettuce using ascospores as inocula, which is more realistic than the use of infested grain, agar plugs or sclerotia used previously. These tests also provide information on how the resistance is being expressed that may aid breeders to decide which lines to use for subsequent breeding work.

Acknowledgements

We would like to thank Andrew Mead for the biometrics advice and statistical analyses and acknowledge funding from the Department for Environment, Food and Rural Affairs (DEFRA). We would also like to thank Dr. E.J. Ryder for provision of the seed samples.

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