

Susceptibility of Potato (*Solanum tuberosum*) Foliage and Tubers to the US8 Biotype of *Phytophthora infestans*

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ABSTRACT

Commercial varieties and advanced breeding lines of potato (*Solanum tuberosum*) were examined for foliar and tuber susceptibility to the US8 biotype of potato late blight (*Phytophthora infestans*) to determine the extent to which these factors are related. In advanced potato breeding material, foliar and tuber susceptibility varied significantly among 19 breeding lines, but did not differ among 4 commercial varieties. In the potato material examined, foliar and tuber susceptibility to potato late blight were not correlated. Low tuber susceptibility was associated both with extremely low (e.g. G274-3) and high (e.g. E202-3Rus) foliar susceptibility. This implies that the biophysical mechanisms controlling foliar and tuber susceptibility are distinct from each other in the potato material examined. In research programs addressing varietal response to modern biotypes of *P. infestans*, foliar and tuber susceptibility should be regarded as separate agronomic characteristics.

Introduction

Potato late blight (*Phytophthora infestans* (Mont.) de Bary) has re-emerged as a significant threat to potato (*Solanum tuberosum* L.) production worldwide in recent years (Andvorn 1995; Fry and Goodwin 1995). *P. infestans* causes severe defoliation and may also infect potato tubers after sporangia or zoospores move through the soil water and penetrate the tuber periderm (Campbell and Madden 1989; Lacey, 1977). Economic losses due to late blight therefore result from tuber as well as foliar susceptibility. Severe storage losses can occur after tubers infected with *P. infestans* are held for processing at temperatures in excess of 7°C (Kirk et al., 1997). Although the production of late blight resistant varieties is a priority for potato breeding programs, no commercial varieties with good foliar resistance to modern biotypes of *P. infestans* are currently available (Douches et al. 1997). The biophysical mechanisms controlling late blight susceptibility in potato varieties are complex, and can involve differences in leaf and tuber cell defense biochemistry, as well as canopy structure, leaf anatomical variation and vine maturation rates (Douches et al., 1997; Gees and Hohl 1987; Kirk et al., 1997). Studies examining foliar and tuber susceptibility are inconclusive regarding the strength of association between these characteristics (Platt and Tai, 1998; Stewart et al., 1992; Stewart et al., 1994). The metalaxyl-insensitive US8 biotype, A2 mating type, has supplanted the metalaxyl-sensitive US1 biotype, A1 mating type, as the dominant biotype in much of North America (Fry and Goodwin, 1995.). The population structure of *P. infestans* in North America is continuing to change as new biotypes emerge (Fry and Goodwin, 1995.). The objective of this study was to examine tuber and foliar susceptibility of commercial potato varieties and advanced breeding lines to an aggressive isolate of the US8 biotype of *P. infestans* to determine the strength of the association between these agronomic characteristics.

Materials and Methods

Plant material

The commercial varieties included Atlantic, Onaway, Snowden and Yukon Gold and advanced breeding lines obtained from the Michigan State University potato breeding program: A091-1, B040-3, B073-2, B076-2, C103-2, E018-1, E202-3Rus, E221-1, E228-11, E230-6, E246-5, F099-3, F373-8, G007-1, G050-2, G227-2, G274-3, NY101 and NY103. Tuber material was produced in 1997 field plots which were maintained with a chlorothalonil-based fungicide program. Tubers were determined to be disease-free and stored at 8°C, 90% relative humidity for 3 months prior to testing. Ten matured tubers from each variety/breeding line were surface sterilized by soaking in a 10% bleach solution for 30 minutes and rinsed 5x with distilled water.

Tubers were inoculated with *P. infestans* isolate 97-2 (US8, A2 mating type). Axenic cultures of *P. infestans* 97-2 were grown on rye agar plates for 14 days at 18°C in the dark (Dhingra and Sinclair, 1985). A mycelial homogenate was prepared from the mature culture (Schmitthenner and Bhat, 1994). Approximately 0.1ml of the mycelial homogenate was injected into the apical end of each tuber. The homogenate was injected 3-5 mm into the tuber periderm, 1-2cm from the apical meristem. Tubers were transferred to controlled environment chambers (12°C, 95% relative humidity) and stored in the dark for 40 days. Inoculated tubers were removed from storage and surface development of disease was visually rated on a 1-9 scale (Table 1). The tubers were then sectioned transversely, exposing the apical, middle and terminal regions of the internal tuber tissue. The internal surfaces were digitally scanned and computer image analysis was used to quantify internal disease (Niemira et al., 1998). The average reflective intensity (ARI) of the apical, middle and terminal regions yielded the Apical, Middle and Terminal disease metrics, respectively. These metrics were averaged to produce Mean ARI, a metric of overall tuber disease severity. Data were analyzed with ANOVA ($P < 0.05$) to detect differences between varieties. The experiment was performed twice. Data for the first trial is presented in Table 2.

Seed tubers of the described varieties/breeding lines were stored at 10°C, 90% r.h. for at least 8 weeks. Seed tubers were cut and planted in field plots as part of larger variety trials at the MSU Muck Soils Research Station in 1997 and 1998. Plots consisted of five cut seed tubers planted in May of each year. The seed piece size varied among the breeding lines due to tuber size; each seed piece had at least three eyes. Tubers were planted 0.25m apart and at 1m spacing between rows. Within rows, varieties were separated by 0.5m. The varieties were arranged in a complete randomized block design and replicated in three blocks ($n=3$). Plants were hilled immediately after emergence. No foliar fungicides were applied. Weeds were controlled by hilling and with metolachlor at 2.3 l/ha 10 dap (days after planting), bentazon salt at 2.3 l/ha, 20 and 40 dap and sethoxydim at 1.8 l/ha, 58 - 60 dap. Insects were controlled with imidacloprid at 1.4 kg/ha at planting, carbaryl at 1.4 kg/ha, 31 and 55 dap, endosulfan at 2.7 l/ha, 65 and 87 dap and permethrin at 0.56 kg/ha, 48 dap. The dates of application were similar for 1997 and 1998. Immediately prior to row closure, the foliage was sprayed with a suspension of zoospores of the previously described US8 biotype of *P. infestans*. The volume of inoculum applied was calibrated to deliver approximately 1000 zoospores per plant based on the canopy density of a typical variety, e.g. Snowden. In order to promote disease, rainfall was supplemented with mist sprinkler irrigation to maintain high humidity within the canopy. Visual foliar disease ratings were taken every 5 to 7 days beginning immediately following inoculation until approximately 30 days after inoculation. The relative area under the disease progress curve (RAUDPC) was calculated for each plot. Data were

analyzed with ANOVA for differences between varieties. The experiment was performed twice. Data for the first (1997) trial is presented in Table 2.

The field foliar susceptibility trial examined approximately 150 varieties and breeding lines in addition to those presented. To identify possible sampling bias, a chi-square analysis was performed on the quartile rankings of the identified varieties and breeding lines within the 170 varieties in the entire 1997 field trial.

The ratings of tuber and foliar infection were analyzed with Pearson's product moment correlation test to determine the strength of relation between these characteristics. The rank order by tuber susceptibility for each tuber disease metric was compared with the rank order by foliar susceptibility using chi-square analysis. Varieties and breeding lines were described as Susceptible (S), Moderately Susceptible (MS), Moderately Resistant (MR) or Resistant (R) according to defined criteria for each susceptibility metric. The criteria for each category are presented in Table 3.

Results

Tuber susceptibility of the varieties examined was significantly different ($P < 0.05$) only between the most susceptible (e.g. G050-2) and least susceptible (e.g. G274-3) breeding lines (Table 2). The visual rating of tuber surface disease development is significantly ($P < 0.05$) correlated with the digital ratings of internal disease development. The correlation between the surface and internal disease metrics are moderate (Table 2). By the surface disease rating metric, there were significant differences among the breeding lines ($P < 0.05$), but commercial varieties did not differ. The digital measures of internal late blight severity indicated significant ($P < 0.05$) differences among commercial varieties in the Apical and Mean ARI metrics. The Apical, Middle, Terminal and Mean ARI metrics showed significant differences ($P < 0.05$) among the breeding lines. As a result of the apical inoculation site, ARI ratings from apical sections generally tended to be lower (more disease) than ARI from middle or terminal sections. In some lines, this difference was more pronounced (e.g. E221-1, G227-2), while in others this difference was minimal (e.g. A091-1, E228-11). The results from each run of the experiment were similar.

The breeding line G274-3 had a foliar susceptibility (RAUDPC) rating significantly ($P < 0.05$) lower than most other breeding lines (Table 2). All other varieties and breeding lines did not differ from each other. The results from each run of the experiment were similar. The breeding lines and varieties examined for tuber susceptibility were distributed evenly among the quartiles of the foliar susceptibility rankings of the larger foliar susceptibility trial based on chi-square analysis, indicating that there was no selection bias in the varieties chosen for tuber susceptibility studies.

Foliar susceptibility ratings are not correlated with any tuber susceptibility metric (Table 2). Chi-square analysis of the rank orders showed that the ranking by foliar susceptibility did not match the ranking by any tuber susceptibility metric. The classification by susceptibility class is generally consistent between the tuber susceptibility metrics, while the foliar susceptibility metric classifies most varieties as Susceptible, with F373-8 as Moderately Susceptible and G274-3 as Resistant (Table 3).

Discussion

In this study, foliar and tuber susceptibility were not correlated. The rank of a given breeding line or variety with regard to one type of susceptibility allowed no reliable inference to be drawn regarding the other type of susceptibility. The two breeding lines with the lowest tuber susceptibility had the lowest (G274-3) and the highest (E202-3Rus) foliar susceptibility. The breeding lines and varieties examined were classified variously based on the tuber susceptibility metrics, but nearly all were classified as Susceptible based on foliar susceptibility. This also suggests that foliar and tuber susceptibility are governed by different mechanisms. This has implications for the process of breeding a late blight resistant potato variety, in that the two types of susceptibility must be assessed independently. The overwhelming categorization of the 23 breeding lines and varieties examined as Susceptible indicates that more research and breeding is required to produce a commercially acceptable variety with resistance to late blight.

The association between tuber and foliar susceptibility to late blight is a matter of ongoing research. Dorrance and Inglis (1998) concluded that foliar and tuber susceptibility were not correlated, while a separate report indicated that a strong correlation exists (Platt and Tai, 1998). The isolates of *P. infestans* used in these studies included older biotypes such as US1 (Platt and Tai, 1998; Stewart et al., 1992; Stewart et al., 1994) and modern biotypes such as US8 (Dorrance and Inglis, 1998). This also suggests that the pathogen response to resistance mechanisms may be biotype specific. The mechanisms by which late blight development is slowed are different in tubers vs. foliage. These mechanisms may be related to histological and/or cytological variations in the tuber or canopy, biochemical defense responses or a combination of these and other factors (Gees and Hohl, 1987.). The interactions of these underlying mechanisms may change with changes in plant maturity related to canopy development, tuber development and maturation of tubers in storage (Plissey, 1993; Rowe and Secor, 1993).

It is clear that more work is needed to fully elucidate the association between foliar and tuber susceptibility. Potato lines which have been genetically engineered to express anti-fungal agents, such as glucose oxidase (Wu et al., 1995), may have a more tightly coupled response to late blight development if the same anti-fungal agent is expressed both in foliage and tubers. Research is ongoing to determine the extent to which genetically modified potato plants which systemically express glucose oxidase demonstrate changes in foliar and tuber susceptibility to late blight (K. Walters, MSU, personal communication). Foliar and tuber resistance that is native to a breeding line results from different native biophysical mechanisms. Development and implementation of systemically expressed genetic constructs should address the extent to which the introduced resistance mechanism may augment or supercede native resistance mechanisms, both in foliage and tubers. In assigning a variety to a class such as Susceptible or Moderately Resistant, care should be taken, therefore, to specify whether the description is based on foliar or tuber susceptibility.

The North American population of *P. infestans* is evolving, with new biotypes such as US11 increasing in prominence (Fry and Goodwin, 1995.). The role that sexual recombination may play in future population dynamics of *P. infestans* is not fully understood. In light of the emergence of new biotypes and the increasing complexity of the North American *P. infestans* population, susceptibility screening of breeding material and introduced commercial varieties should include tests using modern biotypes of *P. infestans* in order to maximize the utility of the information for breeding and genotype development programs.

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Additional Keywords

Late blight, breeding, variety

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Table 1. Visual rating scale for late blight (*Phytophthora infestans*) in potato tubers (reprinted from Niemira et al., 1998)

Visual disease symptoms of whole tubers inoculated with <i>P. infestans</i>				
Rating	Skin discoloration (% of surface)	Sprout damage (% total sprouts)	Sporulation (% of surface)	Physical degradation (% of surface)
1	0	0	0	0
2	<10	0	0	0
3	>10	0-5	0	0
4	>25	>5	<10	0
5	>25	>5	10-50	<10 (spots <1cm in diameter)
6	>25	>5	50-75	10-25 (spots >1cm in diameter)
7	>25	>5	>75	25-50 (spots >1cm in diameter)
8	>25	>5	>75	50-75, loss of internal structure
9	>25	>5	>75	75-100, complete physical breakdown

Table 2. Susceptibility of potato foliage and tubers of advanced breeding lines and commercial varieties to potato late blight, (*Phytophthora infestans*) US8 biotype.

Variety	Tuber disease					Foliar disease
	Surface Rating ^a	Internal rating ^b			Mean ARI ^c	RAUDPC ^d
		Apical	Middle	Terminal		
Atlantic ^e	4.5 cdef ^f	160.21 abcde	189.69 ab	184.13 abc	178.01 abcd	0.455 a
Onaway	4.8 abcdef	145.65 bcde	153.43 bc	148.82 c	149.30 cd	0.516 a
Snowden ^e	4.3 def	173.34 abcde	185.56 ab	194.01 abc	184.30 abcd	0.386 a
Yukon Gold ^e	4.3 cdef	176.95 ab	202.44 a	210.06 abc	196.48 ab	0.506 a
A091-1	6.0 abcde	142.32 bcde	146.94 bc	146.37 c	145.21 c	0.444 a
B040-3	4.0 ef	163.41 abcde	174.69 abc	162.15 abc	166.75 abcd	0.411 a
B073-2 ^e	5.4 abcdef	154.49 abcde	176.88 abc	174.32 abc	168.56 abcd	0.492 a
B076-2 ^e	6.2 abce	137.27 cde	156.40 bc	150.46 c	148.04 d	0.409 a
C103-2	6.4 abce	151.69 abcde	172.31 abc	185.89 abc	169.30 abcd	0.452 a
E018-1	4.8 bcdef	176.45 abc	190.21 a	191.42 abc	186.03 abc	0.381 a
E202-3 Rus ^e	3.9 f	186.06 a	200.53 a	213.03 a	199.87 a	0.518 a
E221-1	6.0 abcdef	128.00 e	147.67 bc	175.00 abc	150.22 cd	0.517 a
E228-11	4.5 cdef	184.01 a	184.40 abc	186.77 abc	185.06 abc	0.515 a
E230-6	4.4 cdef	164.48 abcde	178.26 abc	178.60 abc	173.78 abcd	0.359 a
E246-5	4.5 cdef	172.79 abcd	178.89 abc	189.78 abc	180.49 abcd	0.397 a
F099-3	4.5 cdef	171.17 abcd	170.37 abc	---	170.77 abcd	0.482 a
F373-8	4.1 cdef	149.33 abcde	170.78 abc	167.80 abc	162.63 bcd	0.272 ab
G007-1 ^e	5.1 abcdef	167.77 abcde	184.03 abc	182.26 abc	178.02 abcd	0.471 a
G050-2 ^e	7.1 ab	160.86 abcde	156.63 bc	162.56 bc	160.02 cd	0.408 a
G227-2	4.6 bcdef	145.95 abcde	155.91 bc	176.37 abc	159.41 cd	0.458 a
G274-3 ^e	3.9 f	156.77 abcde	183.41 abc	187.74 abc	175.97 abcd	0.041 b
NY101 ^g	4.0 f	152.38 abcde	164.30 abc	156.31 c	157.66 cd	0.507 a
NY103 ^g	5.7 abcdef	136.05 de	143.67 c	148.53 c	142.75 d	0.508 a
Correlation coefficient (P<0.05)						
Surface rating		-0.355	-0.404	-0.285	-0.384	NS ^h
Apical			0.736	0.537	0.834	NS
Middle				0.758	0.936	NS
Terminal					0.884	NS
Mean ARI						NS

a Surface ratings are 1 (no disease) to 9 (heavy disease)

b Internal ratings are Average Reflective Intensity (ARI) based on digital scan of internal tuber tissue. Lower value = darker image = more disease

c Mean ARI = (Apical + Middle + Terminal)/3

d RAUDPC = Relative Area Under Disease Progress Curve, based on % diseased foliage (max=1)

e Values for this variety reprinted from Niemira et al., 1998

f Numbers in a given column with different letters are significantly different (P<0.05, Tukey) by ANOVA

g Breeding material produced by Cornell University, obtained through MSU potato breeding program

h NS = no significant correlation (P<0.05, Pearson's product moment)

Table 3. Ranking of varieties and breeding lines according to susceptibility.

Variety	Tuber disease					Foliar disease
	Surface Rating ^a	Internal rating ^b			Mean ARI ^c	RAUDPC ^d
		Apical	Middle	Terminal		
Susceptible (S)	>5	<150	<150	<150	<150	>0.35
Moderately susceptible (MS)	4-4.99	150-164.9	150-164.9	150-164.9	150-164.9	0.20-0.349
Moderately resistant (MR)	3-3.99	165-179.9	165-179.9	165-179.9	165-179.9	0.05-0.199
Resistant (R)	<3	>180	>180	>180	>180	<0.05
Atlantic	MS	MS	R	R	MR	S
Onaway	MS	S	MS	S	S	S
Snowden	MS	MR	R	R	R	S
Yukon gold	MS	MR	R	R	R	S
A091-1	S	S	S	S	S	S
B040-3	MS	MS	MR	MS	MR	S
B073-2	S	MS	MR	MR	MR	S
B076-2	S	S	MS	MS	S	S
C103-2	S	MS	R	R	MR	S
E018-1	MS	MR	R	R	R	S
E202-3 Rus	MR	R	R	R	R	S
E221-1	S	S	S	MR	MS	S
E228-11	MS	R	R	R	R	S
E230-6	MS	MS	MR	MR	MR	S
E246-5	MS	MR	MR	R	R	S
F099-3	MS	MR	MR	NA	MR	S
F373-8	MS	S	MR	MR	MS	MS
G007-1	S	MS	R	R	MR	S
G050-2	S	MS	MS	MS	MS	S
G227-2	MS	S	MS	MR	MS	S
G274-3	MR	MS	R	R	MR	R
NY101	MS	MS	MS	MS	MS	S
NY103	S	S	S	S	S	S

a Surface ratings are 1 (no disease) to 9 (heavy disease)

b Internal ratings are Average Reflective Intensity (ARI) based on digital scan of internal tuber tissue. Lower value = darker image = more disease

c Mean ARI = (Apical + Middle + Terminal)/3

d RAUDPC = Relative Area Under Disease Progress Curve, based on % diseased foliage (max=1)