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ASSESSMENT OF LATE BLIGHT (*PHYTOPHTHORA INFESTANS*) REACTION AMONG CULTIVARS AND ADVANCED LINES OF POTATO (*SOLANUM TUBEROSUM*)

By

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ABSTRACT

ASSESSMENT OF LATE BLIGHT (*PHYTOPHTHORA INFESTANS*) REACTION AMONG CULTIVARS AND ADVANCED LINES OF POTATO (*SOLANUM TUBEROSUM*)

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The spread of metalaxyl-resistant genotypes of *Phytophthora infestans* from central Mexico in the 1980s and 1990s has led to the re-emergence of late blight as a major threat to potato production worldwide. To assist a breeder in planning crosses to increase resistance in lines for future release as varieties, information on the resistance in available germplasm must be obtained. This study compares data from two years of field evaluation, greenhouse foliage resistance screening, and inoculated tuber reactions among potato lines with various levels of resistance to *P. infestans*. Replicated field trials were grown under irrigation and inoculated with *P. infestans,* then rated throughout the season for defoliation. In the greenhouse, individual plants were grown in pots, moved to an environment chamber for inoculation, then rated for percent defoliation. Tubers were injected with cultured *P. infestans*, incubated, then visually rated for surface degradation and scanned internally for digital analysis. Five lines (AWN86514-2, B0692-4, B0718-3, MSG274-3, and Q237-25) with strong foliar resistance were identified based on field trial results. Greenhouse screens allowed resistant and susceptible lines to be distinguished, but results did not correlate well with field data. Four highly resistant lines (A084275-3, Bzura, MSG007-1, and MSG297-4RD) were found through tuber evaluation, but tuber resistance did not correlate with field foliar resistance. Several breeding strategies and suggestions for future research based on these data are provided.

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GENERAL INTRODUCTION

Origin of the potato

The cultivated potato (*Solanum tuberosum* L.) originated in the Andes mountains of South America (Dean, 1994), where it was domesticated by the natives of Peru and Bolivia as many as 7,000 years ago (Zuckerman, 1998). The potato was valuable as a staple crop in high altitude regions where maize and beans could not be grown, and the hardy plant survived even in thin, low fertility soil under drought conditions (Zuckerman, 1998). Out of over 2,000 *Solanum* species, more than 160 are tuber-bearing wild potatoes, and many can freely interbreed. They can be found in a range of ploidy levels, from the more common diploids (2x = 24), with tetraploid species (including *S. tuberosum*) second most common, to a small percentage of triploids, pentaploids, and hexaploids (Burton, 1989). Potatoes are cross-pollinated by wind or insects and can form fruit containing true seeds; however, when grown as a crop they are mainly sown as small seed tubers or sections of tubers containing one or more eyes.

Spaniards discovered potatoes in the 16th century, most likely around 1537 (Hawkes, 1990), and introduced them to Spain around 1570 (Salaman, 1949). The potato was classified as a member of the Solanaceae in 1596, a family that includes the tomato (*Lycopersicon lycopersicum*), eggplant (*Solanum melongena*), sweet pepper (*Capsicum annuum*), tobacco (*Nicotiana tabacum*), deadly nightshade (*Atropa belladonna*), mandrake (*Mandragorum officinarum*), and henbane (*Hyoscyamus niger*). The association with poisonous (nightshade) and reputedly supernatural (mandrake) plants, combined with its use as a staple by New World slaves, gave the potato an unfavorable

reputation in Europe, and it was not widely grown for two centuries (Zuckerman, 1998). However, the tuber's more valuable qualities eventually became apparent in the poor country of Ireland.

First, the potato is highly nutritious, particularly in terms of usable protein and vitamin C, lacking mainly calcium (Burton, 1989), which could be provided by the addition of milk. Second, it provides more yield per unit area planted than any of the major grain crops, and it can be eaten with a minimum of preparation time and cooking utensils, making it an excellent food source for those with little money or land. The potato thrived under the mild, wet conditions prevailing in Ireland, the short-day adapted South American varieties producing well during the country's long growing season. Finally, unlike other crops with their valuable portion above ground, the tubers were protected from damage by soldiers in the frequent battles of the time (Zuckerman, 1998).

Phytophthora infestans

A new potato disease appeared in Europe and North America in the early 1840s that caused foliage to blacken and tubers to rot. Observers first noted it in the area around New York and Philadelphia in 1843, from which it spread west to the Great Lakes and north into Canada in succeeding years. The disease may have spread from there to Europe in 1844, or both epidemics may have derived from infected tubers imported separately from the same source (Bourke, 1964). In 1845, this blight spread from Belgium westward until it reached Ireland, where it resulted in losses to half the year's crop of potatoes (Robertson, 1991). Another devastating outbreak of late blight in 1846, combined with the societal structure at the time, resulted in a famine in Ireland so severe that one million people died and another 1½ million emigrated (Burton, 1989).

Late blight is considered the most important disease of the potato (Hooker, 1981). *Phytophthora infestans* (Mont.) de Bary, the pathogen that causes potato late blight, is an Oomycete, a member of the order Chromista, and not a true fungus (Fry and Goodwin, 1997). Its asexual cycle begins with lemon-shaped sporangia, which can either infect healthy tissue directly or release zoospores possessing flagella for mobility in water. Once the sporangia or zoospores come into contact with plant tissue, they enter and spread hyphae that can initiate a new round of sporangial production in a matter of a few days (Coffey and Gees, 1991). It is heterothallic, needing two distinct mating types (A1 and A2) in order to undergo sexual reproduction, which results in oospores that can better tolerate winter conditions than the zoospores, in addition to providing a means for genetic recombination (Kirk, 1996).

P. infestans is believed to originate in the central highlands of Mexico. The long-term coexistence of both mating types, the abundance of oospores, and the proliferation of resistant *Solanum* species suggest coevolution with the disease (Bourke, 1964; Goodwin and Drenth, 1997). *P. infestans* prospers in a humid, cool environment, particularly since water helps the spread of its motile zoospores. Primary inoculum is generally from hyphae that overwinter on infected living tissue, such as tubers in storage or cull piles. Tuber lesions are irregular, reddish-brown spots of tissue decay. Infected foliage develops dark, round, water-soaked lesions surrounded by a white fringe of sporangiophores, most easily observed on the lower surface of the leaf (Dean, 1994; Franc, Brown, and Kerr, 1996); stems are also susceptible to infection (Robertson, 1991).

Host-pathogen interaction

Potato species display two kinds of resistance to late blight: vertical or specific resistance and horizontal or general "field" resistance. Lines with vertical resistance, controlled by a few major resistance (R) genes, can show the hypersensitive response, a rapid necrosis of infected and nearby cells. This kind of resistance is highly specific to a virulence gene in the pathogen, conferring strong resistance only to certain pathotypes. Horizontal resistance is regulated by a complex interaction of minor genes and provides partial resistance to all *P. infestans* pathotypes (Ross, 1986). It is more strongly affected than vertical resistance by environmental conditions. Early efforts to breed resistance into cultivated potato from wild relatives focused on the vertical resistance from *Solanum demissum.* However, as backcrossed progeny with specific R genes became exposed to compatible pathotypes, the resistance was quickly overcome (Bradshaw *et al.*, 1995b). Black *et al.* (1953) proposed a system of nomenclature for identifying *P. infestans* pathotypes based on virulence gene composition determined by testing against potato clones with known R genes. A pathotype of *P. infestans* is said to have virulence gene 1 if it can cause infection on a potato clone with gene R1. Eleven R genes have been identified (Malcolmson, 1969). This is different from other systems of R gene interaction, in which pathogens are classified by avirulence genes that provoke a resistance response in a host plant with a corresponding R gene.

Unless both A1 and A2 mating types are present, *P. infestans* undergoes asexual propagation. Lacking sexual recombination, specific strains should form clonal lineages that can be identified with genetic markers. A method has been proposed to identify pathogen genotype using electrophoresis to distinguish two allozyme loci, *Glucose-6-*

phosphate isomerase (*Gpi*) and *Peptidase* (*Pep*) (Goodwin, Schneider, and Fry, 1995). At least 17 of these clonal lineages have been classified using this method in the United States alone. The lineages are named US1, US7, US8, and so on. Detailed characterizations of these lineages to include mating type and resistance to the phenylamide fungicide metalaxyl have been conducted (Fry and Goodwin, 1997).

Prior to the early 1980s, US1, an A1 mating type lineage sensitive to metalaxyl, was the predominant genotype, and so late blight could be controlled with metalaxyl application (Goodwin, Sujkowski, and Fry, 1996). Beginning in 1984, isolates of the A2 mating type resistant to metalaxyl were discovered around the world (Goodwin and Drenth, 1997). The migration of these new genotypes from Mexico posed serious disease management problems (Goodwin *et al.*, 1994) leading to severe economic losses due to late blight epidemics in the United States in the 1990s (Goodwin *et al.*, 1998).

Breeding for late blight resistance

Currently, potato production worldwide is surpassed only by wheat, maize, and rice (Ross, 1986). Despite the crop's importance, there are no acceptable commercial varieties with adequate resistance to late blight (Landeo *et al.*, 1995; Helgeson *et al.*, 1998). Many breeding programs around the world have made the development of resistant cultivars a priority (Bradshaw *et al.*, 1995b; Corsini *et al.*, 1999; Darsow, 1995; Douches *et al.*, 1998b; International Potato Center, 1984; Kankila *et al.*, 1995).

Since varieties of *S. tuberosum* subsp. *tuberosum*, the tetraploid potato most commonly grown in Europe and North America, are susceptible to the disease, breeders must use its cultivated and wild relatives in Mexico and South America for sources of resistance (Bradshaw *et al.*, 1995b; Colon *et al.*, 1995; Darsow, 1995). Introduction of R

genes has largely been abandoned as a means of building resistance in favor of the more complex horizontal resistance because of the ease with which *P. infestans* develops new virulence pathotypes (Black, 1970; Bradshaw *et al.*, 1995a; Colon *et al.*, 1995; Dorrance and Inglis, 1997). One breeding strategy even employs screening and selection to eliminate any R genes present in breeding lines to ensure that all resistance is horizontal (Landeo *et al.*, 1995), although some ineffective R genes may be linked to quantitative horizontal resistance (Ordoñez *et al.*, 1998).

Resistance sources such as *S. tuberosum* subsp. *andigena* (Black, 1970), the cultivated tetraploid from Peru and Bolivia (Burton, 1989), cross readily with commercial varieties through traditional breeding methods. Due to different ploidy levels and endosperm balance number, crosses with other wild species necessitate further measures including embryo rescue, manipulation of parental ploidy level (Bradshaw *et al.*, 1995b), and somatic hybridization (Helgeson *et al.*, 1998). Central to breeding efforts is the ability to screen the available germplasm for resistance so that selection is effective. Many methods are used, including inoculated (Colon *et al.*, 1995a) and naturally infected field trials (Inglis *et al.*, 1996), intensive field trials in Toluca, Mexico (Helgeson *et al.*, 1998), greenhouse seedling tests (Dorrance and Inglis, 1997), detached-leaf evaluation (Goth and Keane, 1997), quantification with transgenic *P. infestans* (Kamoun *et al.*, 1998), and both field and laboratory tuber screens (Dorrance and Inglis, 1998).

In this two-year study, over 200 potato varieties and advanced breeding lines were evaluated under inoculated field conditions. Selected lines were also subjected to greenhouse foliage testing and tuber resistance screens, and the results were compared to the field trial data.

CHAPTER 1: FOLIAGE SCREENING

FOR RESISTANCE TO *PHYTOPHTHORA INFESTANS*

INTRODUCTION

Potato late blight, which caused the Irish potato famine in the 1840s, emerged in the mid-1980s and 1990s as a new threat to global potato (*Solanum tuberosum* L.) production. The pathogen, *Phytophthora infestans* (Mont.) de Bary, had previously been successfully controlled with the systemic fungicide metalaxyl. The migration of metalaxyl-resistant strains of *P. infestans* from central Mexico has caused serious potato production and economic problems worldwide (Fry and Goodwin, 1997).

One of the major goals of the Michigan State University potato breeding program is to introduce new market-quality varieties with greater levels of late blight resistance than are currently available (Douches *et al.*, 1998b). These varieties must also possess agronomic qualities such as high yield, early or moderate maturity, unblemished internal flesh, high specific gravity, and attractive appearance (Dean, 1994). However, sources of strong resistance, especially wild *Solanum* species, do not have commercially desirable attributes, and so many years of backcrossing and selection must be performed prior to release (Bradshaw *et al.*, 1995b).

Since disease response differs due to physiological age, resistance to late blight coincides with extremely late maturity in most sources (Colon et al, 1995; Dorrance and Inglis, 1997; Inglis *et al.*, 1996). When highly resistant varieties are used as parents in a breeding program, they must be crossed to early maturing susceptible lines to generate progeny with acceptable maturity. The result is offspring with a wide range of resistance levels. Varieties with intermediate resistance, partially adapted European lines for example, are less likely to have the agronomic weaknesses of the wild species with the

strongest resistance, and could thus be valuable as parents. Resistance may arise through several different partially effective mechanisms (Black, 1970; Colon *et al.*, 1995b). Progress might be made by intercrossing clones with moderate resistance levels derived from varied sources in an attempt to combine these mechanisms into the same line. Efficient use of this material depends on accurate resistance evaluation.

Although field screening of potato lines is an effective method to evaluate their resistance to late blight, a comparable test would be valuable if it uses less space, takes less time, does not introduce disease inoculum over a large land area, and lacks seasonal restrictions. Greenhouse disease chamber testing can be useful for many reasons. The ability to test plants during the winter (Colon *et al.*, 1995a) in addition to the normal growing season increases the number of lines screened, thereby shortening the selection period. Greenhouse screening allows a breeder to evaluate large numbers of progeny in a short amount of time to determine the general or specific combining ability of the parents (Black, 1970; Bradshaw *et al.*, 1995a) for planning future crosses. Screening progeny of resistance crosses in the greenhouse to predict their performance in the field (Brown *et al.*, 1999) gives the breeder preliminary information to assist in making selection decisions, so that limited field space can be optimized by planting the most promising clones (Bradshaw *et al.*, 1995b). Environmental conditions can be controlled to create uniform infections when performing repeated tests (Helgeson *et al.*, 1998). The controlled conditions reduce the risk of disease spread from an inoculated disease trial (Colon, Budding, and Hoogendoorn, 1995). Finally, the breeder can evaluate varietal resistance to different *P. infestans* genotypes, including both mating types (Inglis *et al.*,

1996), which would introduce the danger of sexual recombination if carried out in the field.

Greenhouse screening is considered an accurate means of predicting a line's field resistance (Dorrance and Inglis, 1997), though differences in lesion growth rate have been reported between field- and greenhouse-grown leaves (Colon *et al.*, 1995b). The environment in an enclosed late blight inoculation chamber, where temperature, humidity, and light can be controlled, is vastly different from that in the field; it is possible that the concentrated conditions of the test might break down the resistance of lines with good field tolerance.

US8 is currently the most prevalent *P. infestans* genotype in the United States (Fry and Goodwin, 1997); however, genotypes such as the original, metalaxyl-sensitive US1 and the metalaxyl-resistant US11 are also found, particularly on the West Coast (Goodwin *et al.*, 1998; Dorrance *et al.*, 1999). A variety with the desired durable resistance should show low infection under all three genotypes of the pathogen.

The primary objective of this study was to conduct a late blight field resistance screen on current MSU potato breeding lines and the germplasm available from other locations for use as parents. These data will provide the basis for decisions about future crosses and for identifying resistant germplasm for commercial release. The second objective was to conduct greenhouse evaluations of select potato lines according to Douches *et al.* (1997) and compare with results obtained in the 1997 and 1998 field trials. The third objective was to gather and compare data on the resistance of eight potato lines when inoculated with US1, US8, or US11 genotypes of *P. infestans*.

MATERIALS AND METHODS

Plant material

About 170 clones were screened in each field trial, although the composition of that total changed from 1997 (Table 1) to 1998 (Table 2) as lines were dropped from or added to the breeding program. The clones fell into the following main categories:

- National Late Blight Trial lines--distributed by Dr. K. G. Haynes (USDA) for the national late blight resistance testing program. These included commercial varieties, European and unadapted material, and lines with known R genes. (Haynes *et al.*, 1998)
- Susceptible controls--commercial varieties that are widely grown in the United States for table or processing markets, including 'Atlantic,' 'Onaway,' 'Russet Burbank,' 'Russet Norkotah,' 'Shepody,' 'Snowden,' 'Superior,' and 'Yukon Gold' (Inglis *et al.*, 1996).
- European varieties--clones imported for evaluation as potential late blight resistance sources or commercial cultivars.
- Breeding lines--clones in the process of agronomic testing and selection from university breeding programs in the North Central region (Douches *et al.*, 1998a).

The US8 greenhouse trials screened a subset of 28 lines from the field trials, including clones with various levels of resistance and susceptible check varieties (Table 3). Eight of those lines (AWN86514-2, Atlantic, B0718-3, Bzura, MSG274-3, Matilda, Snowden, and Zarevo) were further tested against US1 and US11 genotypes.

P. infestans inoculum preparation

Michigan isolates 95-7 (1997) and 97-2 (1998) of the US8 genotype of *P. infestans* were maintained in culture on rye or potato dextrose agar. To prepare an inoculum suspension, each plate of mycelium was flooded with 15-20 ml of distilled water, and the aerial hyphae were peeled from the media with a plastic scraper. The water and hyphae were poured into a beaker, and the plate was rinsed into the same beaker with an additional 10 ml of distilled water to recover as much of the hyphal mass as possible. The hyphae were agitated on a stir plate for 15 min to disperse the sporangia into the water. The water was filtered through four layers of cheesecloth to remove excess hyphae and refrigerated at 4°C for 3 to 4 hr to release the motile zoospores. The concentration of zoospores was estimated with the aid of a hemacytometer and diluted to the desired concentration, 10^3 zoospores/ml. For the field evaluation, the inoculum suspension was administered (100 ml/7.5m row) through the field's irrigation system on July 18 (1997) or July 22 (1998).

Inoculum for the greenhouse screen was prepared from rye cultures as described above, using the following Michigan isolates at 200 ml inoculum per chamber:

- US1: 95-5 and 95-6.
- US8: 94-1, 94-3, 95-7, 97-1, 97-2, and 98-2.
- US11: 96-1.

Field evaluation

Field tests of cultivars and breeding lines were conducted during the 1997 and 1998 growing seasons at the Michigan State University Muck Soils Research Farm, Bath, MI. The trials were planted June 3 (1997) or June 5 (1998) in a randomized complete

block design with three replications. A guard row of susceptible red potatoes bordered each replication to maintain a dense canopy in which the disease could spread. Each 1.5 m plot contained four seed pieces at 30 cm spacing, with eight plots per row. A susceptible red seed piece was planted between plots in 1997; the 1998 trial plots were modified to leave that space bare so the plots could be more easily distinguished. A 1.5 m aisle between every two rows in the 1998 design allowed plot inspection. The field was irrigated frequently with a sprinkler system to promote the humidity favored by the pathogen. No fungicide was applied during the growing season. To ensure that the trial plots would be continuously exposed to the pathogen, 8 kg piles of late blight infected tubers were left in the field's aisles.

Greenhouse evaluation

Greenhouse screening was performed according to the method described by Douches *et al.* (1997). Tubers of the desired lines were hand-harvested from the field trial following vine senescence in late September and stored in paper bags at room temperature (about 20°C) until mid-winter to break dormancy. Seed pieces were planted in 16 cm clay pots in the greenhouse, replicated three times (US8) or ten times (US1/US11) and allowed to grow for approximately six weeks, until just prior to flowering. The late blight disease chamber consisted of a metal bench with a plastic tarp covering it to keep the atmosphere inside the chamber isolated from that of the surrounding greenhouse. The pots were placed inside the chamber on metal trays in a completely randomized design. The foliage was sprayed with the inoculum suspension in the late afternoon or evening, after the tarp cover was closed. A humidifier in the

chamber maintained the high humidity (>90%) favoring disease development. Plants were rated for percent foliar infection after about seven days post inoculation.

Field rating

Beginning on July 30 (1997) or August 6 (1998) and continuing for the next 4 weeks, the percent defoliation of each plot due to the disease was visually estimated every 3 to 7 d. To compare reactions to the disease over time, the Relative Area Under the Disease Progress Curve (RAUDPC) for each line was calculated. This is expressed in terms of the Area Under the Disease Progress Curve (AUDPC, Figure 1), the area under the linear progression of defoliation from inoculation to the end of the evaluation period (Colon *et al.*, 1995a), divided by the maximum AUDPC (100 X the total number of days after inoculation).

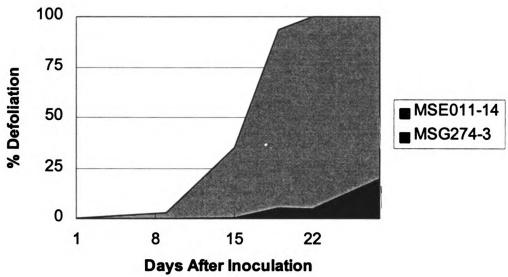


Figure 1. Area Under the Disease Progress Curve after 28 days for the highest (MSE011-14) and lowest (MSG274-3) rated Michigan State University potato breeding lines in the 1998 *Phytophthora infestans* resistance field trial.

Greenhouse rating

Lines were rated about 7 days after inoculation for percent defoliation. Analysis of variance was performed on the results and the least significant differences calculated as above. For the US8 screen, percentages were also converted to a 0-5 scale of increasing severity (Douches *et al.*, 1997) for analysis.

Statistical Methods

Analysis of variance was performed on the RAUDPC values for the field and the percent defoliation values for the greenhouse, and then the least significant differences were calculated using the SAS general linear models procedure (SAS Institute Inc., Cary, NC). Relative rankings of 28 selected lines in 1997 and 1998 were correlated with proc corr in SAS. Correlations of greenhouse and field results were performed as above.

RESULTS

Field results

In 1997 and 1998, *P. infestans* infection spread evenly and rapidly throughout the field, with lesions visible by 9 days after inoculation (DAI). Significant differences were found in 1997 ($p < 0.0001$) and 1998 ($p < 0.0001$). Data from the 76 lines common in both years could not be combined because the variation was significantly greater in 1997 than 1998 ($F_{0MAX} = 4.07$). Among the clones tested, eight showed high levels of resistance in 1997 (Table 1) and eight in 1998 (Table 2). Two of the lines, MSG274-3 and Q237-25, had lesions that were not typical of late blight (small radius, dry appearance, no visible sporulation, leaf wilting and curling) contributing to the total defoliation ratings; an RAUDPC based strictly on late blight defoliation for those two lines would be lower than reported.

The most susceptible line, MSE011-14, reached 100% defoliation by 22 DAI. The commercial varieties used as susceptible controls, such as Atlantic and Russet Burbank, reached 100% defoliation by 28 DAI. Figure 1 illustrates the difference in disease progress between a susceptible (MSE011-14) and a resistant clone (MSG274-3).

Lines with moderate to high levels of resistance or breeding lines with one resistant parent were singled out for repeated testing along with susceptible check varieties (Table 3). Relative field performance remained consistent through two growing seasons (Figure 2).

Table 1. Relative Area Under the Disease Progress Curve (RAUDPC) for the 1997 Field Season

Line	RAUDPC[1]	Line	RAUDPC
B0692-4[2]	**0.4**	B0856-4	45.5
LBRMULTI	**1.1**	ATL	45.5
AWN86514-2	**1.1**	P63-1	45.6
B0767-2	**1.2**	ALPHA	45.7
LBR8	**1.8**	MSG227-2	45.8
B0288-17	**2.5**	MSF068-5	45.9
MSG274-3	**4.1**	MSF019-11	46.1
B0718-3	**5.7**	MSE228-5	46.2
LBR0	13.9	MSG251-10	46.2
BERTITA	15.4	HAMPTON	46.3
DORITA	20.6	MSE220-14	46.4
LBR1R3R4	22.6	ND2676-10	46.5
BZURA	23.4	MSG297-4RD	46.6
C0083008-1	25.4	MSG139-1	46.8
A084275-3	25.6	P32-3	46.9
ROBIJN	25.8	MSG007-1	47.1
LBR3	27.4	MSB057-2	47.2
LBR1R2R4	27.4	MSE226-5Y	47.2
LIBERTAS	28.6	PIKE	47.2
PIMPERNEL	28.6	P84-9-8	47.9
A080432-1	28.8	MSF099-3	48.2
STOBRAWA	29.0	MSE041-1	48.2
B1004-8	29.7	MSD029-3Y	48.4
ZAREVO	29.9	MSG135-12	48.7
KRANTZ	29.9	P84-12-7	49.2
ELBA	30.7	MSB073-2	49.2
B0749-2F	30.9	MSE221-11	49.3
GRETA	31.6	MSG119-1RD	49.3
B0811-13	34.0	MSG010-11	49.3
A84118-3	34.3	MSA110-2	49.3
NORDONNA	34.7	MSF373-A	49.4
OBELIX	35.7	MSA097-1Y	49.4
MSE230-6	35.9	MSG049-4	49.6
MSC120-1Y	36.2	YELLOW FINN	49.7
LILY	36.3	MSE228-9	49.7
MSE018-1	38.1	MSF321-5	49.9
SNOWDEN	38.6	MSE030-4	50.1

Table 1 (cont'd).

Line	RAUDPC[1]
HINDENBURG	39.3
A082611-7	39.3
ONTARIO	39.4
MATILDA	39.5
MSE246-5	39.7
MSG163-1	39.9
MSG083-1RD	40.4
IS. SUNSHINE	40.7
RUSSIAN BLUE	40.8
MSG050-2	40.8
MSB076-2	40.9
LATONA	41.1
MSB040-3	41.1
MSE230-3	41.1
MSB107-1	41.3
MSF019-2	41.3
MSE228-3	41.8
A7961-1	41.9
B0915-3	42.0
MSG170-117	42.1
MSE009-1	42.1
JS111-28	42.1
ALLEGHENY	42.2
DALI	42.2
MSF105-10	42.2
FL1879	42.3
SHEPODY	42.4
MSG135-5	42.8
MSF001-2	42.9
JUL. ROSE	42.9
IS. SUNSET	43.7
MN16489	43.7
MSA091-1	44.4
MSE263-3	44.6
MSE222-5Y	44.9
W1348RUS	44.9
MSE263-10	45.1
MSF165-6RY	45.1

Line	RAUDPC
MSF020-23	50.2
YUKON GOLD	50.6
NY101	50.7
NY103	50.8
MSE215-12	51.0
MSA105-1	51.2
RUS. BURBANK	51.3
P88-13-4	51.4
MSE228-11	51.5
P73-2	51.5
ONAWAY	51.6
MSE221-1	51.7
MSE202-3RUS	51.8
MSG079-2	51.9
MSE230-13	52.0
MSNT-1	52.0
MSE011-10	52.5
W1313	52.5
A8495-1	52.6
CENTURY RUS.	52.9
MSB054-4	53.4
MSC148-A	53.5
MSF014-9	53.8
MSE226-4Y	54.0
MSE247-2	54.4
LONGLADE	54.4
ND860-2	54.9
P88-15-1	55.0
R. NORKOTAH	55.0
MSE149-5Y	55.2
MSE048-1Y	55.6
G8610-2PY	55.6
MSG296-3	55.6
MSB106-7	56.0
MSG049-7	56.1
MSC122-A	56.6
MN16966	57.0
ND2225-1	58.2

Table 1 (cont'd).

Line	RAUDPC[1]	Line	RAUDPC
MSB027-1R	45.2	MSE033-1RD	58.9
MSC103-2	45.2	MSB094-1	59.1
FL1833	45.3	MSF087-03	59.9
MSE213-2	45.4	REBA	61.7
R. NORLAND	45.5	P83-6-18	63.6
DESIREE	45.5	MSE192-8RUS	63.7
MICHIGOLD	45.5	MN16180	66.8

Mean = 42.7

$LSD_{0.05}$ = 10.6

CV% = 33.5

[1]Maximum RAUDPC = 100.

[2]Lines in bold are considered highly resistant (RAUDPC < 10) to *P. infestans* US8 isolate 95-7.

Table 2. Relative Area Under the Disease Progress Curve (RAUDPC) for the 1998 Field Season

Line	RAUDPC[1]	Line	RAUDPC
LBR8[2]	**0.6**	NY112	32.9
LBR9	**1.1**	B0178-34	33.0
MSG274-3	**3.8**	MN17922	33.2
B0692-4	**4.9**	MSF349-1	33.5
Q237-25	**5.1**	MSF060-6	33.7
AWN86514-2	**5.2**	FAMBO	33.8
B0718-3	**8.2**	MSG119-1RD	33.8
LBR0	**8.4**	NY119	34.1
BZURA	10.1	MSF019-11	34.1
ROBIJN	12.1	MSE228-9	34.6
B0288-17	14.1	ATLANTIC	34.6
ZAREVO	16.2	MSG139-1	34.7
ELBA	17.1	MSH381-6Y	34.8
STOBRAWA	17.4	MSF020-23	35.0
LBR5	18.2	MSG007-1	35.0
ND02438-7R	19.1	SNOWDEN	35.0
A084275-3	19.3	MSE274-A	35.1
DORITA	19.4	MSE245-B	35.1
LBR1R2R3R4	19.9	MN16966	35.2
ARS4219-1	20.3	MN16478	35.4
BERTITA	20.5	W1313	35.6
GRETA	20.7	MSE080-4	35.6
A080432-1	21.3	MSE221-1	35.6
A84118-3	21.4	MSNT-2	35.6
LBR7	21.7	YUKON GOLD	35.8
B0811-13	22.2	MSH321-1	35.9
LBR2	24.3	MSH418-1	36.1
A082611-7	24.4	MSG297-4RD	36.4
NORDONNA	25.1	ONAWAY	36.6
B9922-11	25.4	AF1475-20	37.0
PICASSO	25.6	MSH018-4	37.3
P88-5-12	25.8	MSE228-1	37.3
LILY	26.1	MSH106-2	37.4
MSF105-10	26.5	AF1763-2	37.5
MSA091-1	26.6	MSE222-5Y	37.7

Table 2 (cont'd).

Line	RAUDPC[1]	Line	RAUDPC
LBRY	27.0	MSB106-7	38.1
PIKE	27.1	MSE228-11	38.1
B1004-8	27.2	MSNT-1	38.2
MSH120-1	27.2	MS401-1	38.3
LBR3TBR	27.3	R. NORKOTAH	38.4
MSH018-3	27.6	MSB040-3	38.6
MSG124-8P	27.7	NY115	38.9
MSG050-2	28.0	MSE149-5Y	38.9
TURBO	28.5	MSC148-A	39.2
MATILDA	28.7	MSG088-6	39.4
MSG104-6	28.7	SUPERIOR	39.4
MIRAKEL	28.8	SHEPODY	39.5
MSC103-2	29.1	MSE230-6	39.5
W1355-1	29.9	MSE263-10	39.5
ND5084-3R	29.9	A8495-1	40.0
GOLDRUSH	30.0	ACCENT	40.5
AF1753-16	30.3	MSB094-1	41.6
MSF373-8	31.2	MSA097-1Y	41.6
MSF099-3	31.3	ND4093-4RUS	41.7
MSH392-1	31.4	P84-9-8	42.0
A7961-1	31.5	MSE040-6RY	42.1
MSE018-1	31.6	SAGINAW GOLD	42.5
MSH380-3Y	31.7	MSE030-4	42.9
W1151RUS	31.7	MSG141-3	43.0
MSH308-2	31.8	MSE226-4Y	43.4
MSB076-2	32.0	MSE226-5	43.6
DALI	32.2	MSF059-1	43.6
MN17572	32.3	MSH112-6	43.8
ND2470-27	32.3	MSF313-3	44.0
AF1808-18	32.4	MSC086-3	44.0
W1348RUS	32.5	MSC122-1	44.1
ERNTESTOLZ	32.5	MSE192-8RUS	44.2
NORVALLEY	32.5	ARS4152-1	44.5

Table 2 (cont'd).

Line	RAUDPC[1]	Line	RAUDPC
MSC120-1Y	32.7	MSE011-11	44.5
LADY ROSETTA	32.7	MSE033-1RD	45.7
RUS. BURBANK	32.8	MSH351-6	47.8
MSB107-1	32.8	P83-11-5	48.4
MSF420-1	32.8	MSE011-14	50.3

Mean = 31.4
$LSD_{0.05}$ = 6.6

CV% = 33.7

[1]Maximum RAUDPC = 100.

[2]Lines in bold are considered highly resistant (RAUDPC < 10) to *P. infestans* US8 isolate 97-2.

Table 3. Relative Area Under the Disease Progress Curve for Selected Potato Lines

Line	1997 Season[1]	1998 Season
B0692-4[2]	**0.4**	**4.9**
AWN86514-2	**1.1**	**5.2**
B0288-17	**2.6**	**14.1**
MSG274-3	**4.1**	**3.8**
B0718-3	**5.7**	**8.2**
DORITA	20.6	19.4
BZURA	23.4	10.1
A084275-3	25.6	19.3
ROBIJN	25.8	12.1
A080432-1	28.8	21.3
STOBRAWA	29.0	17.4
B1004-8	29.7	27.2
ZAREVO	29.9	16.2
ELBA	30.7	17.1
GRETA	31.6	20.7
B0811-13	34.0	22.2
A84118-3	34.3	21.4
NORDONNA	34.7	25.2
LILY	36.3	26.1
MSE018-1	38.2	31.6
SNOWDEN	38.6	35.0
MATILDA	39.5	28.7
MSG050-2	40.8	28.1
ATLANTIC	45.5	34.7
MSG297-4RD	46.6	36.4
MSG139-1	46.8	34.7
MSG007-1	47.1	35.0
RUS. BURBANK	51.3	32.8
Mean	29.4	21.7
$LSD_{0.05}$	10.1	5.4
CV%	52.7	47.5

[1]Table sorted by 1997 Season. Maximum RAUDPC = 100.

[2]Lines in bold are considered highly resistant (RAUDPC < 10) to *P. infestans* US8 isolate 97-2.

Greenhouse *P. infestans* US8 genotype results

Although the US8 greenhouse inoculation procedure was carried out multiple times, several repetitions of the experiment could not be analyzed due to low levels of infection, abrupt temperature increases that affected plant health, or failure of the humidifier equipment. Results from two separate runs of the experiment, one in 1998 and one in 1999, could be combined ($F_{0MAX} = 2.02$). Significant differences were detected between the most susceptible and most resistant lines (Table 4) both in the individual (1998, $p = 0.0032$; 1999, $p < 0.0001$) and combined ($p = 0.044$) results. No correlation was found between the relative resistance and susceptibility of the lines based on combined greenhouse ratings and field RAUDPC scores (Figure 4), although there was a weak correlation ($r = 0.59$, $p = 0.0009$) between the response of the 1998 field plants and the greenhouse plants grown from their tubers in 1999.

Greenhouse *P. infestans* genotype by potato variety interaction

Eight lines (AWN86514-2, Atlantic, B0718-3, Bzura, MSG274-3, Matilda, Snowden, and Zarevo) were selected from the greenhouse US8 evaluation for inoculation with US1 and US11 (Table 5). Infection with combined Michigan US1 isolates 95-5 and 95-6 was uniformly low across all varieties. Of the eight lines tested, only MSG274-3 developed significantly more than zero infection, with a mean of 5.3% defoliation ($LSD_{0.05} = 1.8$). Inoculation with Michigan US11 isolate 96-1 produced no infection on any variety, even after repeated trials.

Table 4. Greenhouse Defoliation Ratings of 28 Lines Inoculated with *Phytophthora infestans* US8 Genotype

Line	1998 Rating[1]	Scale[2]	1999 Rating	Scale	Combined Rating[3]	Combined Scale
MSG274-3	10.0	1.7	10.7	2.0	10.3	1.8
ZAREVO	1.7	0.3	22.3	2.7	12.0	1.5
MSG297-4RD	38.3	3.7	8.0	1.7	23.2	2.7
B0811-13	33.7	2.7	13.3	2.0	23.5	2.3
B0692-4	48.3	4.0	1.7	0.7	25.0	2.3
B0288-17	46.7	4.0	11.7	2.0	29.2	3.0
AWN86514-2	56.7	4.7	2.0	0.7	29.3	2.7
B1004-8	31.7	3.0	28.3	3.3	30.0	3.2
MSG007-1	31.7	3.0	28.3	3.3	30.0	3.2
ROBIJN	48.3	3.7	11.7	2.0	30.0	3.2
MSG139-1	40.3	3.0	25.0	3.0	32.7	3.0
A84118-3	48.3	4.3	18.3	2.7	33.3	3.5
MATILDA	40.3	3.7	36.7	3.3	38.5	3.5
B0718-3	81.7	5.0	3.3	0.7	42.5	2.8
STOBRAWA	63.3	5.0	22.3	3.0	42.8	4.0
GRETA	90.0	5.0	8.3	1.3	49.2	3.2
ELBA	94.7	5.0	4.3	1.3	49.5	3.2
BZURA	73.3	5.0	30.0	3.0	51.7	4.0
A084275-3	56.7	5.0	50.0	4.7	53.3	4.8
MSG050-2	71.7	5.0	35.0	3.0	53.3	4.0
LILY	78.3	5.0	30.0	3.3	54.2	4.2
RUS. BURBANK	41.7	4.0	68.3	4.7	55.0	4.3

Table 4 (cont'd).

Line	1998 Rating[1]	Scale[2]	1999 Rating	Scale	Combined Rating[3]	Combined Scale
ATLANTIC	34.0	2.3	76.7	5.0	55.3	3.7
MSE018-1	71.7	5.0	40.0	3.3	55.8	4.2
DORITA	86.3	5.0	33.3	3.3	59.8	4.2
NORDONNA	85.0	5.0	35.0	3.7	60.0	4.3
SNOWDEN	65.0	4.0	60.0	4.3	62.5	4.2
A080432-1	85.0	5.0	58.3	4.3	71.7	4.7
Mean	55.5	4.0	27.6	2.8	41.6	3.4
$LSD_{0.05}$	44.5	2.1	31.3	1.8	35.4	1.8
CV%	59.0	39.8	92.8	54.0	78.1	48.8

[1]Rating is a visual estimation of percent defoliation from 0 - 100%.

[2]Percent defoliation converted to a 0 - 5 scale where 0 = no infection, 1 = 1 - 5%, 2 = 6 - 15%, 3 = 16 - 30%, 4 = 31 - 49%, and 5 = 50 - 100%.

[3]List sorted by combined percentage.

Table 5. Greenhouse Defoliation Ratings of 8 Lines Inoculated with *Phytophthora infestans* US1 and US11 Genotypes

Line	US1[1]	US11
ATLANTIC	0.0	0.0
ZAREVO	0.0	0.0
BZURA	0.3	0.0
SNOWDEN	0.4	0.0
B0718-3	0.7	0.0
AWN86514-2	1.1	0.0
MATILDA	1.4	0.0
G274-3[2]	**5.3**	0.0

[1]Rating is a visual estimation of percent defoliation from 0 - 100%.

[2]The value in bold is the only significant rating ($p < 0.05$).

DISCUSSION

Although the inoculum pathotypes of the Michigan isolates have not yet been completely characterized (Niemira, personal communication), the strong resistance exhibited by the USDA late blight differential lines (Black *et al.*, 1953; Malcolmson and Black, 1966) LBR_8 in 1997 (Table 1) and both LBR_8 and LBR_9 in 1998 (Table 2) suggests that the *P. infestans* isolate 95-7 lacks at least virulence gene 8 (LBR_9 was not present in 1997) and isolate 97-2 lacks virulence genes 8 and 9. This is consistent with 1997 findings at five separate late blight field testing locations across the United States (Maine, Minnesota, New York, North Dakota, and Pennsylvania), where LBR_8 was resistant to *P. infestans* US8 genotype inoculation and natural late blight infection (Haynes *et al.*, 1997).

RAUDPC ratings between two field seasons for selected lines of interest are compared in Table 3. There were year-to-year differences in reaction to the disease for each line, but the overall ranking of susceptibility and resistance remained stable (Figure 2). This suggests that field screening can provide an accurate assessment of a clone's level of resistance. When measured against standard varieties, rating should remain consistent regardless of the conditions of the particular growing season (Colon *et al.*, 1995a; Dorrance and Inglis, 1997).

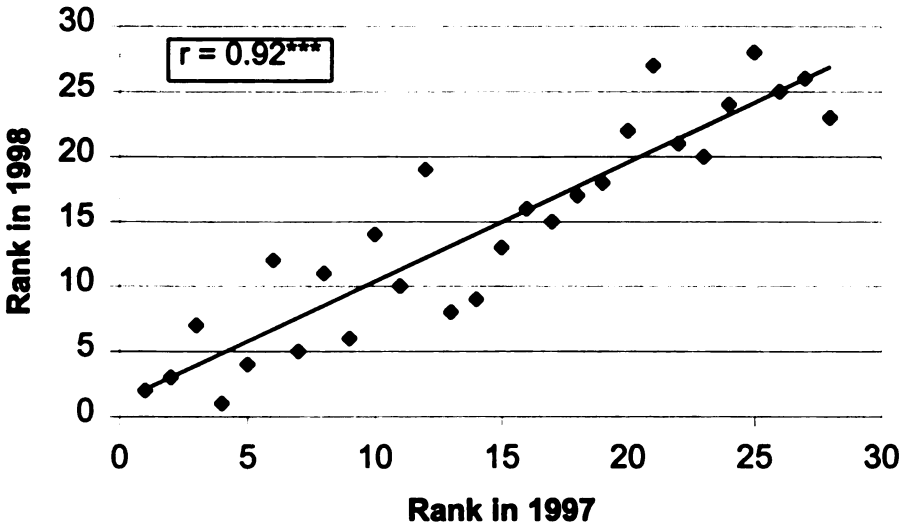


Figure 2. Relative rankings of 28 selected potato lines in 1997 (vertical axis) and 1998 (horizontal axis), where 1 = most resistant to late blight and 28 = most susceptible, based on RAUDPC values.

Lines B0692-4, AWN86514-2 (Corsini *et al.*, 1999), and B0718-3 (Goth and Haynes, 1997) demonstrated strong resistance, but their late maturity and poor agronomic qualities make them unsuitable for commercial use. AWN86514-2 may derive its resistance from the species *S. acaule, S. demissum, S. phureja, S. simiplicifolium, S. stoloniferum, S. stoloniferum*, and *S. tuberosum* subsp. *andigena*, all of which are in the lineage of its female parent, KSA195-96. It is male sterile, but when used as a female can transmit strong resistance to progeny (Corsini *et al.*, 1999). B0718-3 is both male and female fertile and is believed to inherit its resistance from one great-grandparent, PI383470B (Goth and Haynes, 1997). Initial progeny testing indicates that B0718-3 transmits strong resistance to offspring, and that a high proportion of its progeny retain favorable agronomic traits when compared to progeny of other resistance sources

(Bisognin *et al.*, 1998). These clones may prove valuable as parental lines, though the use of B0692-4 and AWN86514-2 is limited by poor fertility.

MSG274-3 and Q237-25 are breeding lines from Michigan State University and Cornell University, respectively. MSG274-3 is the product of a cross between the late-maturing resistant Mexican variety 'Tollocan' and the early maturing susceptible Canadian variety 'Chaleur.' It is unknown whether the resistance transmitted by Tollocan derives from R gene interaction, but current speculation based on greenhouse disease response (below) is that there may be R genes present in its progeny. MSG274-3 is a high yielding advanced selection with good fertility, and it possesses a commercially acceptable intermediate maturity. It produces a visually attractive, bright-skinned, oblong tuber with light yellow flesh and low internal defects. It is an acceptable chipper directly out of the field, but not after storage (Douches *et al.*, 1998a). Q237-25 is derived from a cross with the field resistance source *Solanum tuberosum* subsp. *andigena* (Raman, 1998). Q237-25 has an acceptable appearance, yield, and maturity, and has high levels of resistance to potato cyst nematode, scab, and Potato Virus Y (Raman, 1998). Both are under consideration for direct release as varieties. Because both breeding lines have earlier maturity than most strong resistance sources, their field defoliation scores late in the season reflected natural senescence in addition to disease pressure, while the late-maturing varieties did not suffer from that interaction.

One concern when dealing with strong resistance from a limited number of sources is that the mechanism of resistance in each source, such as B0718-3 and AWN86514-2, may originate from the same genetic locus. It is unknown whether this is the case; speculation is based on observations that the strong resistance in both lines

holds up similarly in regions across the United States (Haynes *et al.*, 1997), and both transmit strong resistance to offspring in a manner suggestive of one or a few major genes (Bisognin *et al.*, 1998). Mapping of resistance genes (Li *et al.*, 1998) and quantitative trait loci that influence resistance (Meyer *et al.*, 1998) is being performed for potato, but until more varieties can be studied, there is a risk that resistance in the above lines may be allelic and crossing them will not lead to further improvement. Varieties that display more moderate levels of resistance, such as 'Bzura,' 'Zarevo,' 'Stobrawa,' 'Bertita,' and 'Greta,' are less likely to derive their resistance from the same genetic loci. They should be crossed with progeny of the strong resistance sources in an effort to combine different resistance genes from a broader background. Further analysis of strong resistance sources such as B0718-3 and G274-3 to determine whether they share markers linked to quantitative resistance (Meyer *et al.*, 1998) will be helpful in devising breeding strategies (see Figure 3 for an example).

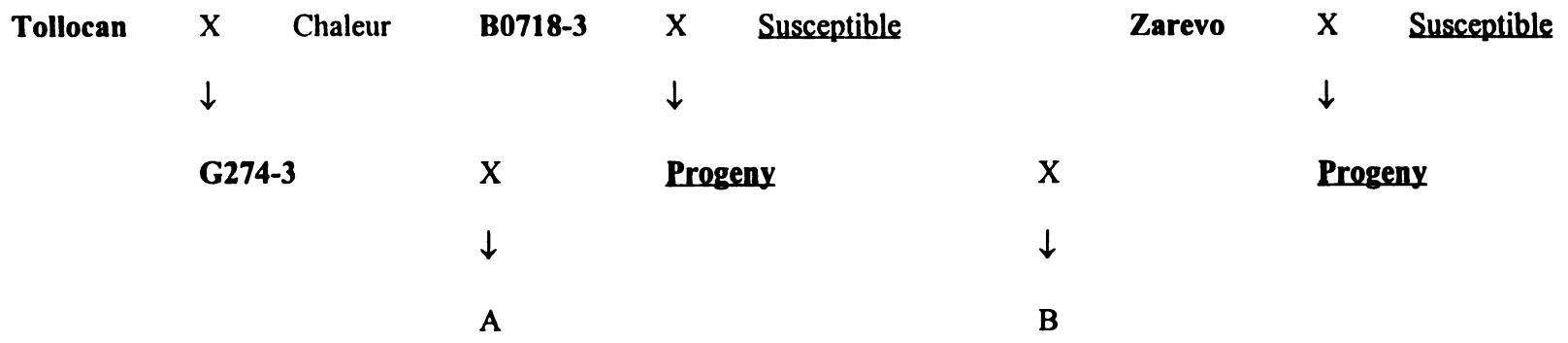


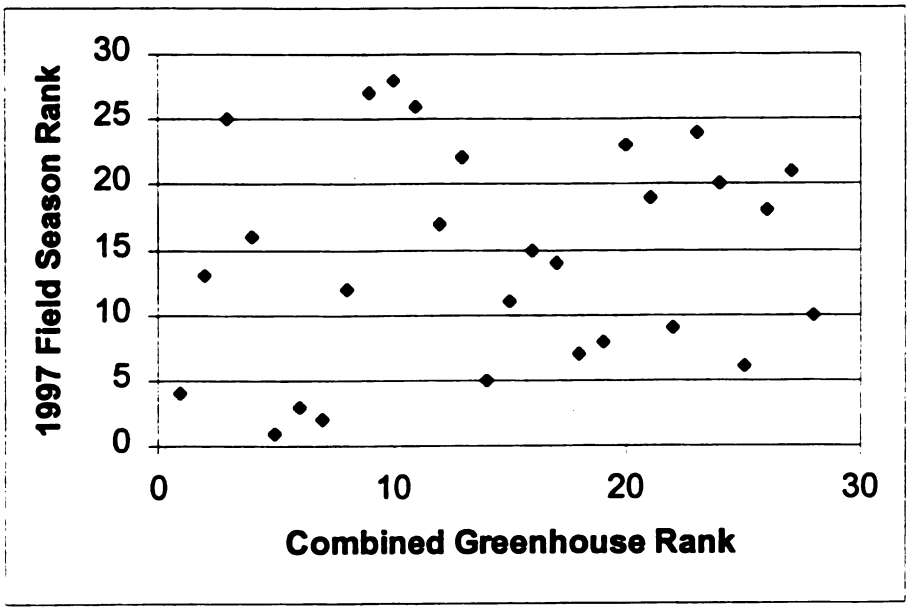
Figure 3. Breeding strategies for introducing durable resistance to *P. infestans* into agronomically acceptable genetic backgrounds. Clones in bold show resistance, and the underlined are unspecified potato lines. A) Cross selected progeny of strong resistance sources with different pedigrees. B) Cross selected progeny of a strong resistance source with that of a moderately resistant source.

The greenhouse US8 late blight resistance screen permitted the separation of resistant and susceptible lines based on percent defoliation (Table 4). Defoliation ratings were also analyzed using a weighted scale developed by Douches *et al.* (1997) in an effort to reduce variation by distinguishing more precisely between lower amounts of defoliation than between higher, undesirable levels; however, the scale did not appreciably improve the separation of means among these data (Table 4).

The results of the greenhouse US8 screen did not correlate well with the field results (Figure 4). One contribution to the scatter of the data points may be the unusual disease severity in the 1998 experiment. When the less severe 1999 experiment alone was compared to the previous season's field ratings, a weak correlation ($r = 0.42$, $p = 0.02$) more consistent with earlier greenhouse data (Douches *et al.*, 1997) was found. There was an interaction between the potato lines tested and the evaluation method. Lines with strong foliar resistance in the field, AWN86514-2 and B0718-3 (Table 3), were intermediate for greenhouse defoliation (Table 4). This difference is probably due to the high disease pressure in the 1998 greenhouse screen, which may have been caused by *P. infestans* cultures with greater virulence than those used in 1999. A second notable variation is between the strong resistance of Zarevo and its progeny MSG297-4RD in the greenhouse compared to intermediate resistance (Zarevo) or susceptibility (MSG297-4RD) under field conditions (Table 6). Two other Zarevo progeny, MSG007-1 and MSG139-1, were intermediate in the greenhouse but highly susceptible in the field (Table 6). The high impact of the environmental conditions on the disease response of Zarevo and its progeny is characteristic of horizontal resistance. Environmental factors in the greenhouse that may influence disease development in these

lines include a lack of direct sunlight that may alter physiological responses, pots that constrain root development, and a more controlled temperature.

Another factor that differed between the field and greenhouse was the number of genotypes of *P. infestans* isolates used during inoculation. For both field experiments, only one isolate was used, while greenhouse inoculation involved a mixture of isolates. The multiple isolate procedure was developed to compensate for lapses in single isolate aggression that resulted in failed runs of the experiment due to poor infection. Some isolates lost virulence over time through standard subculture maintenance, so that even mature, previously virulent cultures did not lead to infection when used as inoculum. Combining multiple isolates increased the chance of sufficient disease development for meaningful evaluation. Another option to maintain isolate virulence would have been to culture the pathogen on living host tissue, but that method required resources unavailable at the time of this study. Since naturally occurring infection by the US8 genotype is not limited to specific isolates, the multiple isolate procedure should in theory produce results that predict field disease response similarly to a single isolate inoculation as long as high enough levels of infection take place to differentiate between resistant and susceptible clones. The usefulness of the US8 classification itself relies upon the genetic identity of the pathogen, so the differences in the US8 isolates used should not have a great effect upon host response.



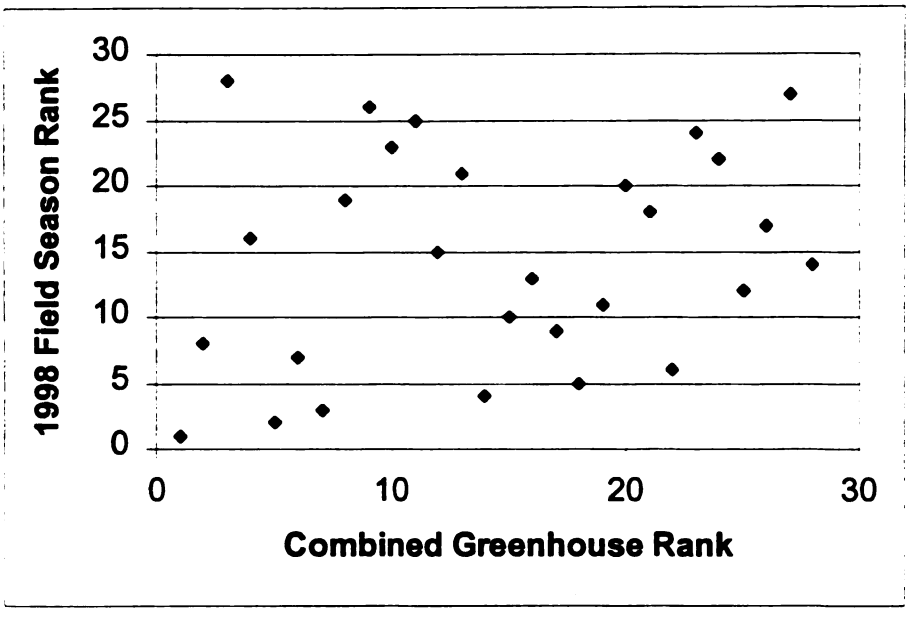


Figure 4. Relative *P. infestans* resistance rankings of 28 selected potato lines in greenhouse tests (horizontal axis) and field trials (vertical axis) where 1 = most resistant to late blight and 28 = most susceptible. Greenhouse rankings are based on percent defoliation and field rankings on the Relative Area Under the Disease Progress Curve for each line. No correlation was found between the greenhouse data and the 1997 field results (top, $p = 0.17$) or the 1998 field results (bottom, $p = 0.08$).

Table 6. Field and Greenhouse Results for 28 Selected Potato Lines

	Field Rating[2]		Greenhouse Rating[3]		
Line[1]	1997	1998	1998	1999	Combined
A080432-1	28.8	21.3	85.0	58.3	71.7
A084275-3	25.6	19.3	56.7	50.0	53.3
A84118-3	34.3	21.4	48.3	18.3	33.3
ATLANTIC	45.5	34.7	34.0	76.7	55.3
AWN86514-2	1.1	5.2	56.7	2.0	29.3
B0288-17	2.6	14.1	46.7	11.7	29.2
B0692-4	0.4	4.9	48.3	1.7	25.0
B0718-3	5.7	8.2	81.7	3.3	42.5
B0811-13	34.0	22.2	33.7	13.3	23.5
B1004-8	29.7	27.2	31.7	28.3	30.0
BZURA	23.4	10.1	73.3	30.0	51.7
DORITA	20.6	19.4	86.3	33.3	59.8
MSE018-1	38.2	31.6	71.7	40.0	55.8
ELBA	30.7	17.1	94.7	4.3	49.5
MSG007-1	47.1	35.0	31.7	28.3	30.0
MSG050-2	40.8	28.1	71.7	35.0	53.3
MSG139-1	46.8	34.7	40.3	25.0	32.7
MSG274-3	4.1	3.8	10.0	10.7	10.3
MSG297-4RD	46.6	36.4	38.3	8.0	23.2
GRETA	31.6	20.7	90.0	8.3	49.2
LILY	36.3	26.1	78.3	30.0	54.2
MATILDA	39.5	28.7	40.3	36.7	38.5
NORDONNA	34.7	25.2	85.0	35.0	60.0

Table 6 (cont'd).

	Field Rating[2]		Greenhouse Rating[3]		
Line[1]	1997	1998	1998	1999	Combined
RUS. BURBANK	51.3	32.8	41.7	68.3	55.0
SNOWDEN	38.6	35.0	65.0	60.0	62.5
STOBRAWA	29.0	17.4	63.3	22.3	42.8
ZAREVO	29.9	16.2	1.7	22.3	12.0
Mean	29.4	21.7	55.5	27.6	41.6
$LSD_{0.05}$	10.1	5.4	44.5	31.3	35.4
CV%	52.7	47.5	59.0	92.8	78.1

[1]List sorted alphanumerically.

[2]Maximum Relative Area Under the Disease Progress Curve = 100.

[3]Rating is a visual estimation of percent defoliation.

In the US1 late blight screen, one clone (MSG274-3) was found with significantly more infection than the other seven tested (AWN86514-2, Atlantic, B0718-3, Bzura, Matilda, Snowden, and Zarevo), although average defoliation of MSG274-3 was only 5.3% (Table 5). Such a low percentage defoliation in itself may not appear to be a matter for concern, but taken in the context of a genotype with low aggression in an experiment with an overall low infection rate, the significant difference across ten replications is worth noting. Since this breeding line generally shows strong resistance to the US8 genotype of *P. infestans* in both the field and greenhouse (Table 6), the higher susceptibility to US1 compared to other lines may be caused by an R gene interaction. The virulence pathotypes of the Michigan isolates used in the US1 inoculation (95-5 and 95-6) are not yet known.

Although US11 is reported to be an aggressive genotype (Miller *et al.*, 1998), Michigan isolate 96-1 caused no visible infection in two separate inoculations. It is possible that this particular isolate has a low level of aggression, or that repeated subculturing since its isolation in 1996 selected for survival on the medium rather than high aggression on the host (Niemira, personal communication). Isolate 96-1 was the only US11 isolate available at the time of this study, so the technique of combining different isolates could not be used. Its virulence had not previously been tested, so the method of increasing virulence by maintenance on a living host plant was not attempted. This method may be useful if further work is anticipated with the 96-1 isolate.

Stewart *et al.* (1983) discuss some of the factors that may cause lower correlation between greenhouse and field tests. Unpredictable disease severity is one of the main

limitations of the greenhouse screening method. Other complications arise from different levels of dormancy among lines, leading to unbalanced replications when some tubers sprout weeks later than others planted on the same day, and the constraints imposed by the limited size of the controlled-environment chamber used for inoculation. Variation is higher in the greenhouse than in the field, so it is preferable to use more replications, yet an experiment conducted with plants crowded in the chamber will have a different canopy density and microclimate than one with fewer replications to be screened, resulting in different rates of disease spread.

A possible strategy to make the most efficient use of the greenhouse screening method is to conduct a preliminary test of a wide range of lines with few replications to determine the most resistant and most susceptible. The second stage would then be to focus on fewer lines based upon those findings and screen them again with more replications for greater precision. Through greenhouse evaluation, the breeder can discard the lines that show the most susceptible response early in the selection process, conserving resources and field space for those that hold the most promise of resistance. The field performance of each line can then be used as the best estimate of *P. infestans* resistance for final selection. This strategy uses the weak correlation of the two separate methods to its best advantage by concentrating on the strong points of each.

CHAPTER 2: TUBER SCREENING

FOR RESISTANCE TO *PHYTOPHTHORA INFESTANS*

INTRODUCTION

Defoliation of a potato field is a readily visible symptom of *Phytophthora infestans* infection. Yield loss due to the death of the plant, however, is not the only damage caused by this pathogen. Spores from the lesions on the foliage can be washed into the soil to infect the commercially valuable tubers (Bradshaw *et al.*, 1995a). Infected tubers pose a serious threat to a potato grower because 1) the diseased tubers become darkened and decayed (Franc *et al.*, 1996), thus decreasing the harvest's marketable value, 2) late blight infection of tubers allows secondary pathogens to invade more effectively, speeding rot and decomposition in storage (Lambert and Currier, 1997), and 3) infected tubers used as seed for the following growing season are a primary source of inoculum, which can spread *P. infestans* to previously uninfected fields, especially when transported over long distances (Fry and Goodwin, 1997).

A cultivar's tuber resistance to late blight is not correlated with its foliar resistance (Black, 1970; Dorrance and Inglis, 1997; Inglis *et al.*, 1996). Therefore, when evaluating a clone's foliar response to late blight, it is essential to test the tubers for resistance as well. The objective of this study was to determine the levels of tuber resistance in selected lines that have exhibited various degrees of foliar resistance and susceptibility in previous field and greenhouse screens (Table 6).

MATERIALS AND METHODS

Plant material

Twenty-six lines were chosen to be included in the tuber resistance screen (Table 6). These lines fall into four general field foliar resistance categories (Table 3):

- Highly resistant: AWN86514-2, B0288-17, B0692-4, B0718-3, and MSG274-3.
- Moderately resistant: A080432-1, A084275-3, Bzura, Dorita, Elba, Greta, Robijn, Stobrawa, and Zarevo.
- Reduced susceptibility: B1004-8, Lily, Matilda, MSG050-2, and Nordonna.
- Susceptible: Atlantic, MSE018-1, MSG007-1, MSG139-1, MSG297-4RD, Russet Burbank, and Snowden.

Tubers were hand-harvested from the 1998 foliage disease trial field plots at the Muck Soils Research Farm (Bath, MI) following vine senescence in late September. Healthy tubers were stored in paper bags at room temperature (about 20° C) until February 1999.

P. infestans inoculum preparation

Tubers were inoculated with a Michigan isolate of the US8 genotype. A mature culture of *P. infestans* grown on rye agar was homogenized by passage through a sterile syringe without a needle. The resulting mycelial homogenate was then re-loaded into the syringe and the needle attached for inoculation.

Tuber inoculation

Tubers were prepared and injected at the apical end with inoculum according to the method of Niemira *et al.* (1999b). Ten replications were inoculated in a completely randomized design. The tubers were then placed in plastic bags and incubated for 420 degree days (14 d at 7° C followed by 33 d at 12° C) in the dark at 95% relative humidity. To provide a basis of reference for healthy tuber flesh, ten tubers of each line were punctured at the apical end with an identical hypodermic needle and incubated under similar conditions for 425 degree days.

Tuber disease rating

Tubers were assessed visually for an estimate of surface degradation on a 1 - 9 scale of increasing disease severity (Table 7). Each tuber was then sliced into sections approximately 2 cm from the apical and terminal ends and through the middle. Sections were immediately placed with the cut surface down on a transparent plate and the plate transferred to a flatbed scanner. Brightness and contrast were manually set at 170 and 190 with the scanner control software (DeskScan II version 2.4; Hewlett Packard Co.) and photograph-quality black and white scans were taken at a resolution of 150 X 150, then saved in tagged image format (.tif). The background of each image was black, with the tuber section images appearing as light reflected from the cut tuber surface. The image files of the sections were analyzed digitally for internal discoloration with image analysis software (SigmaScan version 3.0; Jandel Scientific Software, San Rafael, CA). On the light intensity scale, pure black has an intensity of 0 units and pure white an intensity of 255 units. The area of each tuber section was selected with the "fill" tool with the cut-off threshold set to 10 units so that it would exclude any portion of the image

darker than 10, thereby isolating each section from the black background. The software calculated the light intensity of each pixel in the selected region and returned a value for the average reflective intensity (Niemira *et al.* 1999b). The internal average of each tuber was calculated by summing the apical, middle, and terminal values and dividing by three.

Statistical methods

Analysis of variance was performed on the light intensity values and the least significant differences were calculated using the SAS general linear models procedure (SAS Institute Inc., Cary, NC). Relative rankings of potato lines based on tuber surface rating and internal scan data were correlated with proc corr in SAS.

Table 7. Visual Rating Scale for *Phytophthora infestans* Infection in Potato Tubers[1]

	Visual disease symptoms of whole tubers inoculated with *P. infestans*			
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 diameter)
6	>25	>5	50 - 75	10 - 25 (spots >1cm diameter)
7	>25	>5	>75	25 - 50 (spots >1cm diameter)
8	>25	>5	>75	50 - 75, loss of internal structure
9	>25	>5	>75	75 - 100, complete breakdown

[1]Table from Niemira *et al.*, 1999b.

RESULTS

Surface rating

Individual tubers displayed levels of disease ranging from 1 to 8 on the rating scale used (Table 7), although mean values only varied between 2 and 7 across all lines (Table 8). Visual rating of tuber surface infection severity did allow detection of differences among lines ($p < 0.0001$). There was no correlation between relative tuber surface rating and field foliar late blight resistance ($p = 0.17$). The lines fell into the following surface resistance categories (Niemira *et al.*, 1999a):

- Resistant (< 3): Dorita.
- Moderately resistant (3 - 3.99): B0692-4, Bzura, and A084275-3.
- Moderately susceptible (4 - 4.99): A080432-1, Atlantic, AWN86514-2, Lily, Matilda, MSG274-3, MSG297-4RD, Russet Burbank, and Stobrawa.
- Susceptible (> 5): B0288-17, B0718-3, B1004-8, Elba, Greta, MSE018-1, MSG007-1, MSG050-2, MSG139-1, Nordonna, Robijn, Snowden, and Zarevo.

Table 8. Tuber Late Blight Resistance Ratings for Selected Potato Lines

Line	Surface[1]	Apical[2]	Middle	Terminal	Internal Mean[3]
A084275-3	3.5	175.8	197.1	197.9	190.2
MSG297-4RD	4.5	183.8	193.9	190.5	189.4
MSG007-1	6.1	179.9	190.6	192.8	187.8
BZURA	3.1	170.7	187.9	191.0	183.2
DORITA	2.1	166.9	176.1	174.8	172.6
ZAREVO	5.2	151.7	181.0	181.4	171.3
B0288-17	5.7	153.4	178.8	177.7	170.0
ATLANTIC	4.3	146.2	181.8	181.0	169.6
B1004-8	5.0	163.3	171.9	163.9	166.4
MATILDA	4.6	150.3	170.7	176.9	166.0
LILY	4.6	151.1	170.1	175.8	165.6
B0692-4	3.1	152.8	162.8	159.3	158.3
MSG139-1	6.8	133.1	165.5	164.9	154.5
SNOWDEN	6.0	142.7	154.7	161.6	153.0
AWN86514-2	4.0	137.1	161.5	157.2	151.9
MSG274-3	4.2	136.5	158.8	159.3	151.5
RUS. BURBANK	4.9	146.9	150.1	152.8	149.9
MSE018-1	6.5	131.4	166.7	151.3	149.8
B0718-3	6.4	139.6	151.9	150.7	147.4
A080432-1	4.3	115.2	146.9	156.4	139.5
MSG050-2	6.3	118.5	135.7	134.0	129.4
GRETA	5.8	116.4	133.1	136.3	128.6
STOBRAWA	4.3	117.3	130.4	131.9	126.5
ELBA	5.5	118.8	132.8	123.2	124.9
ROBIJN	7.0	98.6	121.4	117.8	112.6
NORDONNA	5.8	101.6	110.3	122.5	111.5
Mean	5.0	142.3	160.9	160.9	154.7
$LSD_{0.05}$	0.9	20.2	16.8	19.5	16.7
CV%	31.4	22.2	18.1	19.2	18.5

[1]Surface rating is on a 1 - 9 scale of increasing disease severity.

[2]Section rating represents the average light intensity of a scanned image with 0 = black (diseased flesh) and 255 = white (healthy flesh).

[3]Table sorted by internal mean.

Internal section analysis

All potato lines showed signs of infection (Figure 5). Differences were found among lines at the apical, middle, and terminal sections ($p < 0.0001$ for all tests), and the average internal intensity was used for comparisons (Table 8). To compensate for varietal differences in flesh color, average intensities of the diseased tubers were divided by the average intensities of the control tubers (Table 9), which slightly altered the rankings of the lines. A weak correlation (Figure 6) was found between surface ranking and ranking based on mean internal intensity ($r = 0.44$, $p = 0.023$) and between surface ranking and ranking based on percentage of healthy flesh ($r = 0.54$, $p = 0.0041$), consistent with the results obtained by Niemira *et al.* (1999b). There was no correlation between the resistance of the tubers and the resistance of their foliage from the previous field season ($p = 0.9036$). Based on internal mean, the lines can be categorized as follows:

- Resistant (> 180): A084275-3, Bzura, MSG007-1, and MSG297-4RD.
- Moderately Resistant (165 - 179.9): Atlantic, B0288-17, B1004-8, Dorita, Lily, Matilda, and Zarevo.
- Moderately Susceptible (150 - 164.9): AWN86514-2, B0692-4, MSG139-1, MSG274-3, and Snowden.
- Susceptible (< 150): A080432-1, B0718-3, Elba, Greta, MSE018-1, MSG050-2, Nordonna, Robijn, Russet Burbank, and Stobrawa.

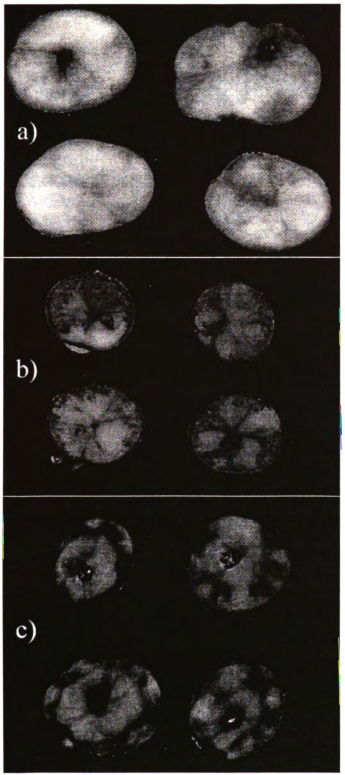


Figure 5. Scanned apical sections of four sample tubers inoculated with *P. infestans* from a) G297-4RD (mean intensity 183.79), b) G274-3 (mean intensity 136.51), and c) Nordonna (mean intensity 101.64).

Table 9. Comparison Between Diseased and Healthy Tuber Flesh Based on Internal Section Mean Light Intensity

Line	Diseased Mean[1]	Healthy Mean	% Healthy[2]
DORITA	172.6	192.7	89.6
MSG297-4RD	189.4	212.1	89.3
A084275-3	190.2	214.6	88.6
BZURA	183.2	210.1	87.2
MSG007-1	187.8	217.7	86.2
ZAREVO	171.3	205.5	83.4
MATILDA	166.0	203.1	81.7
ATLANTIC	169.6	210.0	80.8
LILY	165.6	206.5	80.2
AWN86514-2	151.9	192.0	79.1
B0288-17	170.0	214.9	79.1
B1004-8	166.4	218.9	76.0
MSG274-3	151.5	204.0	74.3
RUS. BURBANK	149.9	202.0	74.2
MSG139-1	154.5	209.7	73.7
B0692-4	158.3	215.8	73.4
SNOWDEN	153.0	213.3	71.7
B0718-3	147.4	206.4	71.4
MSE018-1	149.8	211.4	70.9
GRETA	128.6	193.5	66.5
STOBRAWA	126.5	197.5	64.1
A080432-1	139.5	221.0	63.1
MSG050-2	129.4	213.6	60.6
ELBA	124.9	210.3	59.4
ROBIJN	112.6	193.7	58.1
NORDONNA	111.5	199.5	55.9
Mean	154.7	207.2	
$LSD_{0.05}$	16.7		
CV%	18.5	5.4	

[1]Section rating represents the average light intensity of a scanned image with 0 = black (diseased flesh) and 255 = white (healthy flesh).

[2]List sorted by percent healthy flesh light intensity.

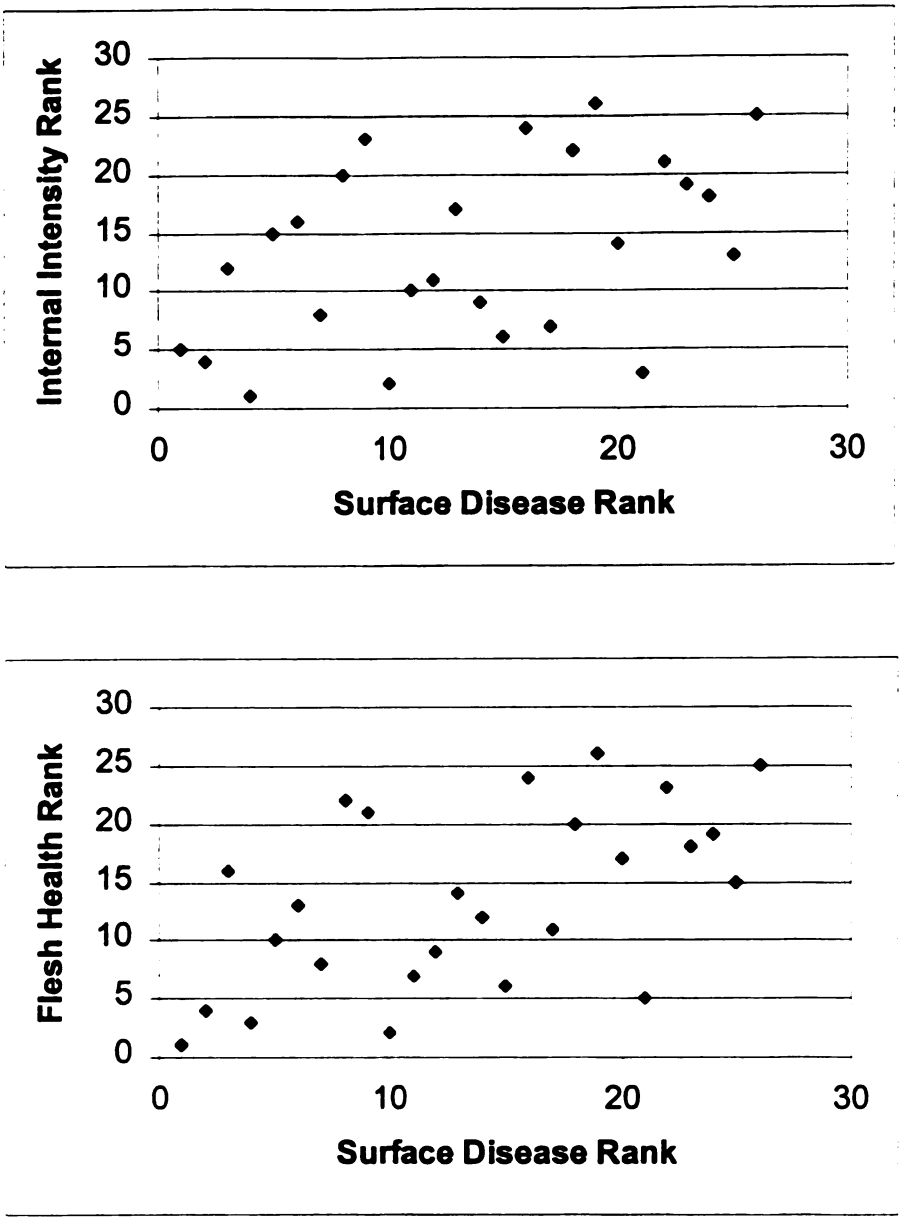


Figure 6. Relative *P. infestans* tuber resistance rankings of 26 selected potato lines according to surface disease (horizontal axis) and internal light intensity (vertical axis, top) or percent intensity of healthy flesh (vertical axis, bottom) where 1 = most resistant to late blight and 26 = most susceptible. Surface rankings are based on a 1 - 9 scale of increasing disease severity and internal rankings on average light intensity where 0 = black and 255 = white. Top, r = 0.44, p = 0.023. Bottom, r = 0.54, p = 0.0041.

DISCUSSION

The difference in internal tuber appearance between a clone highly resistant to *P. infestans* (MSG297-4RD), a moderately susceptible line (MSG274-3), and a highly susceptible line (Nordonna) is illustrated in Figure 5. The resistant MSG297-4RD is an advanced breeding line derived from the foliar resistance source cv. 'Zarevo.' Another highly resistant line, MSG007-1 (Table 8), is an advanced breeding line selected from a cross between Atlantic and Zarevo. Zarevo itself was only moderately resistant in this study. The third progeny line from Zarevo, MSG139-1 (Snowden X Zarevo), was significantly more susceptible than its half-siblings as well as its resistant parent (based on internal mean). A more extensive tuber screen among progeny of Zarevo would be important to determine its value as a source of tuber resistance to *P. infestans*. The Zarevo progeny in this study were lines selected both for tuber appearance and potential foliar resistance, not for tuber resistance, so the segregation of tuber resistance levels should be random among the three.

Different methods have been developed to screen tubers for late blight resistance, including assessment of natural field infection, whole tuber assays, and screens involving tuber pieces or slices (Dorrance and Inglis, 1998). Most common is the whole tuber assay (Stewart *et al.*, 1992; Wastie *et al.*, 1993), in which an undamaged tuber is dipped in or sprayed with inoculum to simulate field conditions, where zoospores are washed from stem and leaf lesions into the soil to infect tubers (Bradshaw *et al.*, 1995a). However, laboratory inoculations can have higher disease pressure than field tuber resistance trials (Platt and Tai, 1998), and other environmental factors like the positions of the tubers under the soil can produce effects that make field resistance vary from

laboratory screenings (Dorrance and Inglis, 1998). The whole tuber assays generally discount any disease due to wounding (Stewart *et al.*, 1994), relying on integrity of the periderm as a component of resistance, a factor that can alter as tubers age in storage (Dorrance and Inglis, 1998). The tuber slice technique, in contrast, does not involve the periderm, but it is also too variable to be a reliable general test (Dorrance and Inglis, 1998).

Another tuber evaluation method involving injection of inoculum at the apical end measures disease spread through the tuber flesh to differentiate between *P. infestans* isolates based on aggressiveness (Lambert and Currier, 1997). In that method, the extent of the damage is quantified by tracing the outline of the rotted portion on filter paper and then weighing the paper to determine the area affected. Although such a method is simpler than digital analysis in that it does not necessitate expensive equipment and software, it also relies more on the observer's subjective judgment when producing the outline and requires more time to perform than the computer's analysis, which can be accomplished quickly for each scanned tuber.

One main weakness of the digital analysis technique is that it is not well suited for evaluating tubers with dark-colored flesh. Niemira *et al.* (1999b) detected no significant differences among varieties for light intensity of healthy flesh, but the three varieties screened were all white-fleshed cultivars. The average light intensity of control tubers for each line tested should be measured to account for natural differences. The surface score does not correlate well with the internal rating, since some lines darken through the center and in others the visible symptoms are confined to the surface, yet each gives

valuable information about varietal response to *P. infestans* infection. The two assessments should be used to complement rather than predict one another.

Further work in this area needs to be conducted on a wider range of varieties and breeding lines, as well as progeny studies with lines that display high levels of resistance. This is especially important for varieties such as Zarevo that also possess moderate or high foliar resistance, as there is some evidence that general combining ability for foliar and tuber resistance may be correlated (Stewart *et al.*, 1994). More extensive screening is crucial to developing commercially acceptable cultivars with adequate resistance to late blight in both the foliage and tubers.

The limiting factor for using tuber screening in the selection process is the availability of enough intact tubers for the desired replications, which can only be attained after several years of tuber increase. The concern when postponing selection for tuber resistance long enough to increase tuber number sufficiently for each clone is that highly tuber resistant lines may be unknowingly discarded in early stages of selection. The example of the Zarevo progeny in this study is positive evidence that selection for unrelated characteristics, such as agronomic quality and foliar late blight resistance, still leaves the breeder with a variety of tuber resistance levels from susceptible to resistant among the remaining breeding lines. Therefore, it seems the most advisable strategy to screen for foliar resistance in the earlier stages of selection, which can be done using a smaller number of tubers, and reserve tuber resistance screening for advanced lines.

SUMMARY

The field trial design was modified between 1997 and 1998 to facilitate the process of rating the infection levels of the plots. The changes included eliminating susceptible guard plants from between adjacent plots, originally intended to separate plots and maintain infection, so that they would not be mistaken for plants in the experimental units and rated. Another difference was the widened path between every two rows in the 1998 trial that allowed researchers to evaluate the plots without damaging the spreading foliage. These changes reflect an attempt to reduce variation in the results due to the rating procedure itself. Further seasons of investigation should reveal whether the measures taken to improve the design are effective.

Field results allowed the identification of highly and moderately foliar resistant lines. By taking advantage of pedigree information and agronomic quality data, a breeder can plan crosses with these lines that will maximize the likelihood of producing commercially acceptable cultivars with strong, durable late blight resistance.

Greenhouse screens were widely variable and provided limited information about the most resistant and susceptible varieties rather than measuring intermediate levels of resistance. Such screens could be preliminary tools to help focus resources on the lines showing the most promise, thereby increasing efficiency in field screening.

The most difficult factor to control in the greenhouse testing procedure was the virulence of the *P. infestans* isolates used to inoculate the plants being screened. Infection was greatest when several different isolates could be combined in the inoculum, as in the experiment with the *P. infestans* US8 genotype, instead of only one (US11) or

two (US1) isolates. Although the US1 and US11 genotype experiments did not produce strong infection, the results obtained point to a possible influence of R genes in the potato breeding material tested that should be investigated further.

Tuber late blight resistance results did not correlate with field foliar resistance data, which reinforces the need for both tests to be performed. It is difficult to conduct a tuber resistance screen during the early stages of selection, when the number of tubers available for each line is limited, so the procedure is best suited to evaluating advanced lines and cultivars. The method of assessing surface and internal disease progress separately provides complementary sets of data about each line's disease response. Digital analysis of flesh discoloration due to late blight infection removes the element of subjectivity from the evaluation.

Two progeny selections of the cultivar 'Zarevo' were among the lines displaying the strongest tuber resistance. A more detailed progeny test for Zarevo as well as a wider screen of available breeding material is needed to determine which lines would be good parents when breeding for improved tuber resistance.

APPENDIX

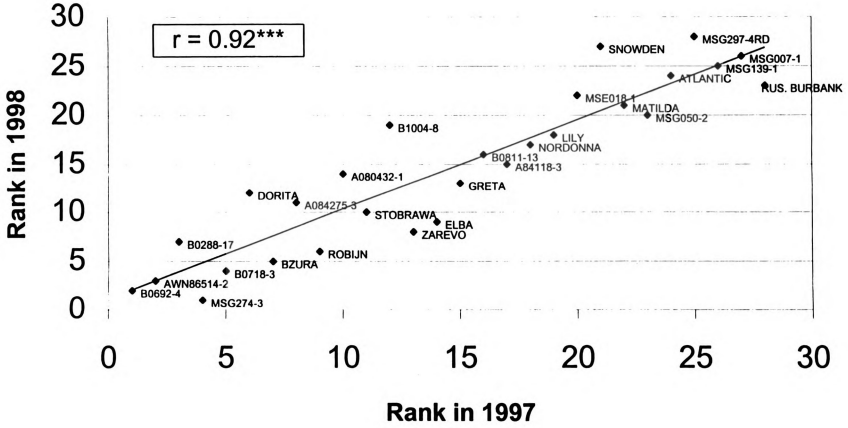


Figure 7. Relative rankings of 28 selected potato lines in 1997 (vertical axis) and 1998 (horizontal axis), where 1 = most resistant to late blight and 28 = most susceptible, based on Relative Area Under the Disease Progress Curve values.

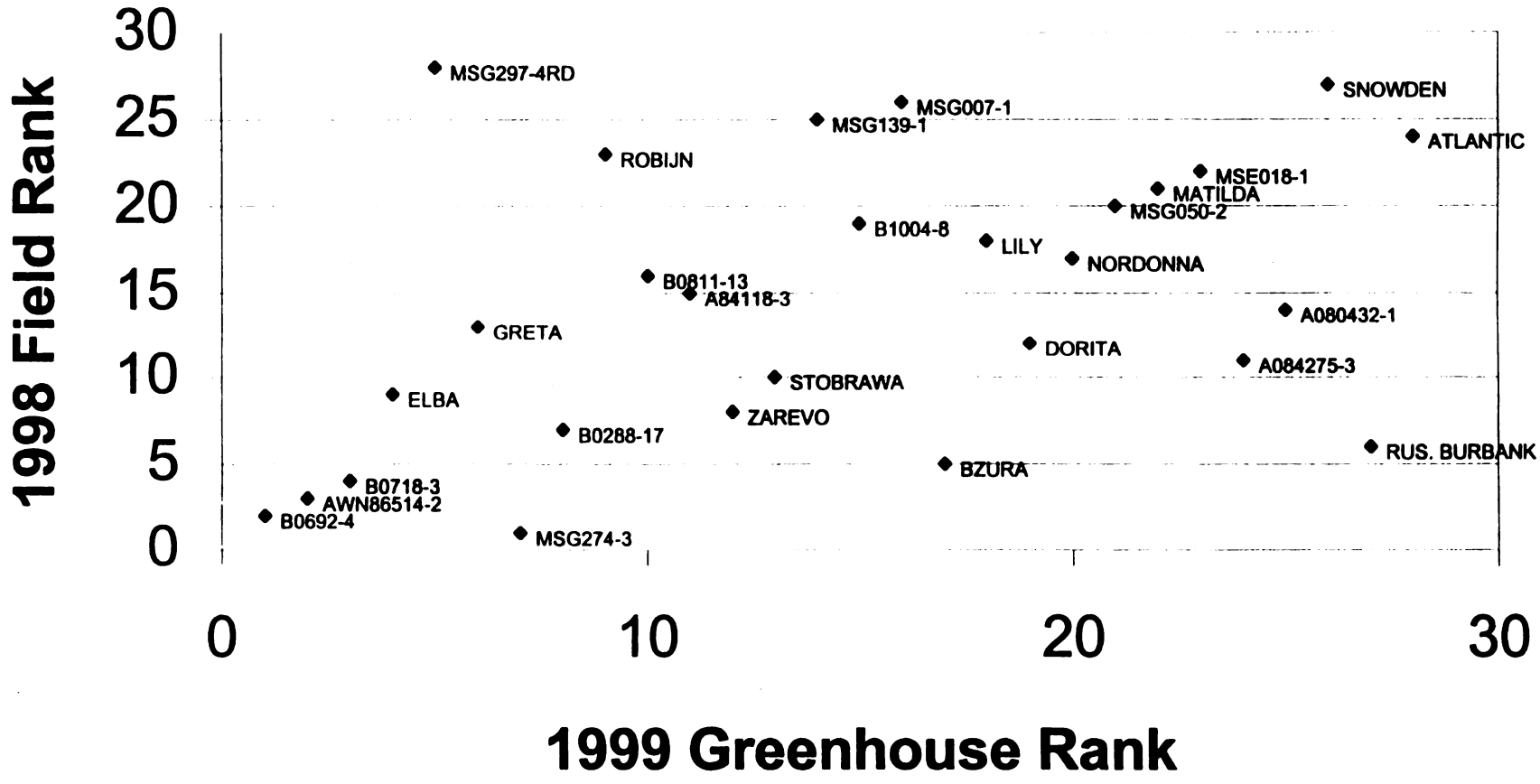


Figure 8. Relative *P. infestans* resistance rankings of 28 selected potato lines in greenhouse tests based on percent defoliation (horizontal axis) and the 1997 field trial based on Relative Area Under the Disease Progress Curve (vertical axis) where 1 = most resistant to late blight and 28 = most susceptible.

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