Assessment of Greenhouse and Laboratory Screening Methods for Evaluating Potato Foliage for Resistance to Late Blight

Anne E. Dorrance and Debra Ann Inglis, Washington State University, Mount Vernon-Research and Extension Unit, 1468 Memorial Highway, Mount Vernon 98273

ABSTRACT

Dorrance, A. E., and Inglis, D. A. 1997. Assessment of greenhouse and laboratory screening methods for evaluating potato foliage for resistance to late blight. *Plant Dis.* 81:1206-1213.

Greenhouse and laboratory screening methods for assessing potato foliage for resistance to late blight were compared using 15 cultivars and advanced breeding selections with known field response to late blight. Screening methods included greenhouse inoculation of plants in several age classes, and laboratory assays of detached leaflets, leaf disks, and stem cuttings. Greenhouse inoculation of plants 7 to 11 weeks after planting, near the time of flowering, corresponded best to results obtained in field evaluations, but there were significant differences in disease severity between separate greenhouse tests. This is consistent with variation in late blight severity on a year-to-year basis when cultivars are compared in the field. The greenhouse inoculation method allowed for testing of several components of partial resistance, such as infection efficiency and lesion growth rate, which may exist for each cultivar. Laboratory assays proved less reliable than greenhouse assays for overall ratings of partial resistance, but could be useful for measuring specific components of resistance. Screening evaluations for late blight resistance should include standard cultivars with known reaction to *Phytophthora infestans* to reference the disease potential within the screening evaluation.

Additional keywords: partial resistance, *Solanum*

Recent potato (*Solanum tuberosum* L.) breeding efforts in the United States have emphasized resistance to *Streptomyces scabies* (Thaxt.) Waksman & Henrici, *Alternaria solani* (Sorauer), and *Verticillium dahliae* Kleb. in addition to desirable yield potential and tuber qualities for processing (36). In contrast, screening for resistance to late blight caused by the fungus *Phytophthora infestans* (Mont.) de Bary has been an essential part of potato cultivar development in many other areas of the world. As a result, numerous screening techniques have been developed for the assessment of resistance to late blight in *Solanum* germ plasm, and the study of parasitic fitness of *P. infestans* populations. The arrival of aggressive strains of *P. infestans*, including the A2 mating type

(18,19,33), and the simultaneous loss in effectiveness of a major fungicide (metaxyl) used in the management of this disease (9,16) have placed a new demand on United States potato breeding programs to develop cultivars with long-lasting, or durable, resistance to late blight. It would be advantageous to utilize previously developed screening techniques, particularly in early stages of selection when a large number of clones need evaluation, and to employ techniques appropriate for selection of partial resistance. Partial resistance or rate-reducing resistance in potatoes is thought to be durable (5,25,35,52). The purpose of this investigation was twofold: (i) to review the methodology currently reported for screening germ plasm for late blight resistance, and (ii) to employ some of the methods on a selected set of cultivars to determine whether known field reactions are validated. Results of these studies are being used to develop rapid greenhouse and laboratory screening techniques to enhance the selection of late blight resistance in germ plasm and future potato cultivars.

LITERATURE REVIEW

From 1920 to 1950, single genes or R-genes for late blight resistance were transferred from *Solanum demissum* into potato cultivars (39,50,53). This type of resistance

was quickly overcome when resistance in the host selected for new virulence loci in the fungus (reviewed by Toxopeus [50],53). This dynamic is typical of plant pathosystems that operate in a gene-for-gene system as first described by Flor (11). Hence, potato breeding efforts that incorporate partial or rate-reducing resistance into new commercial cultivars are now widely preferred. While new selections may not be immune, where there is no interaction between the pathogen and the host to any specific race of *P. infestans*, they do limit disease development through a variety of mechanisms. Characters that have been associated with partial resistance include reduced infection efficiency (limitation to infection and penetration), reduced lesion size, and reduced sporulation capacity (6,27,46,52). There are also many sources of partial resistance components to late blight in wild *Solanum* spp., and several evaluation techniques have been used to identify them (6,22,37,48). In addition, phenotypes similar to partial resistance have been identified in other host systems (55) and reviewed by Parlevliet (35).

The most effective and reliable methods for screening germ plasm are generally accepted to be natural infections or inoculated test plots under field conditions (22). However, this requires a site where late blight epidemics occur reliably year after year, and a *P. infestans* population that is complex not only in regard to virulence loci (*sensu* Flor; 12), but representative of fungal populations where the potato cultivars will eventually be produced. Such requirements pose a challenge for plant pathologists and breeders. Laboratory or greenhouse screening techniques that rapidly and accurately assess resistance, particularly the effects of partial resistance components on large numbers of seedlings early in the selection process, could be useful and time-saving if they accurately predict future reactions under field epiphytotics.

There are other challenges to consider in the development of germ plasm screening assays for late blight resistance. Tuber infection is a second type of crop loss occurring at the end of a field epidemic. Several studies have reported that tuber and foliage susceptibilities to *P. infestans* are

Corresponding author: D. A. Inglis
E-mail: dainglis@coopext.cahe.wsu.edu

This work was supported, in part, by the National Potato Council. Plant Pathology New Series no. 0253, project 11D 3461 5123, College of Agriculture and Home Economics Research Center, Washington State University, Pullman.

Accepted for publication 23 July 1997.

Publication no. D-1997-0820-03S
© 1997 The American Phytopathological Society

not well correlated (7,14,17,24,26,47). Therefore, it is necessary to utilize screening assays which will assess resistance in tubers and foliage separately.

The resistance response may be influenced by many variables. Stewart (40) reported that age of the plant, spore concentration, and method of inoculation can all influence the resistance response in foliage. She indicated that plants in bud or early stages of flowering expressed resistance consistently between tests. Fry and Apple (13) found the rate of the epidemic was influenced by the age of the plants, and disease progression did not occur as fast in intermediate-aged plants when compared to older plants. Reactions were also influenced by maturity characteristics of the cultivars. Cultivars with longer vegetative (late-maturing) periods are generally more resistant to late blight, presumably because the foliage is physiologically younger than shorter season (early-maturing) cultivars. Carnegie and Colhoun (4) reported that changes in disease reaction were dependent on plant age and leaf position. Resistant cultivars were susceptible as young plants, but susceptibility decreased prior to flowering and increased during senescence. Other factors that have been reported to affect the expression of partial resistance include day length, plant nutrition (46), and inoculum densities (28).

Tables 1 and 2 outline some of the seedling, detached leaflet, detached leaf, and

leaf disk methods that have been utilized to identify susceptible and resistant germ plasm in early screening efforts for late blight resistance. The seedling assay has been utilized to assess resistance or susceptibility to late blight (31,51), parasitic fitness of *P. infestans* isolates (26), genetic components that comprise resistance, and to identify the gene action in potential parents (1,32,44). Umaerus (51) measured infection efficiency by limiting the incubation period following seedling inoculations, and reported that late blight outbreaks are delayed under field conditions in potato cultivars having low infection efficiency. This resistance component is, however, not closely associated with late maturity. Caligari et al. (2) reported seedling blight screens were useful in eliminating susceptible material from a population and found greenhouse assays correlated well with field tests. Seedlings scored for leaf lesion size, leaf lesion frequency, petiole lesion size, petiole lesion frequency, stem lesion size, and stem lesion frequency plus an overall rating were used to identify potential parental material with general combining ability (GCA). Parents with the highest GCA often expressed high levels of partial resistance. Bradshaw et al. (1) used the seedling assay to measure GCA and specific combining ability (SCA) effects in progenies from a diallel cross. They reported that R-gene resistance could be separated from partial

resistance because the GCA would increase for a partially resistant parent in all progenies tested. R-genes tend to increase SCA, while partial resistance contributes to the magnitude of GCA when compatible virulence genes are present in the fungal population.

Hodgson (23) was the first to report a screening assay using leaf disks. The advantage to this technique is that plants can be used in more than one assay and challenged with more than one isolate. Leonard-Schippers et al. (30) were able to map quantitative trait loci for late blight resistance using this technique. Detached leaflet assays are routinely used to identify virulence loci in *P. infestans* populations (9,37,49). Tooley (48) demonstrated variation in the resistance response among a collection of *Solanum verrucosum* PI's using the detached leaflet method. However, Hodgson (23) cautioned that plants and leaves to be used in screening evaluations must be the same age, and tests must be made when the plants are at the same stage of growth and when they are normally attacked in the field. Stewart (40) identified two types of responses in potato differentials when inoculated with *P. infestans* in order to identify virulence genotypes: lesions produced at all plant ages and spore concentrations, and a variable response resulting from plant age, spore concentration, and inoculation method ranging from immunity to spreading sporulating lesions.

Table 1. Summary of some greenhouse (GH) methods used in screening seedlings of *Solanum* germ plasm and potato cultivars for foliar late blight resistance

Plant material; age; size	Inoculum	Environment ^a	Rating	Citation
2 plants/clone; prior to flowering; 12 cm pots	Sprayed sporangia/zoospore suspension	7 d at 15-18°C; high humidity; shaded GH	1-9 scale; 1 = susceptible, 9 = resistant	31
True seed; 6-8 weeks after sowing	5 × 10 ⁴ sporangia/ml; complex race 1,2,3,4,5,7,8,9,10,11	24 h at 15-18°C; high humidity; followed by 6 d at 15°C	1-4 scale based on percent leaf area with blight; 1 = > 50%, 4 = little to no blight	3, 41
True seed; 15-20 cm height	Sprayed with 4,000 sporangia/ml	16-18 h at high humidity; rated after 7 d	Susceptible or resistant; no. of lesions/rate of spread	44
Fitness test for isolates on cv. Norchip; 4-8 weeks old	50 µl of 25,000 sporangia/ml on adaxial surface of 4 subterminal leaflets	24 h dark; 100% RH; lesions allowed to expand for 48 h; followed by 100% RH to induce sporulation		49
True seed; 20 cm height; 8 weeks old	Sprayed with an aerosol delivery, sporangia and zoospores	Automatic mist; shaded GH; 7 d	Scored for size and frequency of lesions on leaves and petioles; overall score 1-9	32
12-15 in tall; 6-10 true leaves	Atomized 3,300, 10,000, or 30,000 sporangia/ml; 10 ml per plant	12 h at 21°C; high humidity	No. of lesions per leaf	27
True seed; 4-6 weeks old; 4-5 true leaves	Race 1,2,3,4; 150 × 10 ⁴ zoospores/mm ³ ; 30 ml/1000 plants	15 h at 15-17°C; 100% RH; rated after 4-5 d	Presence/absence of lesions	51
Minitubers; 45 cm tall	Complex race, zoospores	100% RH	% disease compared to controls; scale 1-9	29
True seed; 5-6 weeks old	5 × 10 ⁴ zoospores/ml; 2 isolates race 1,4,10,11 & race 1,2,3,4,7	7 d	1-4 scale; 1 = > 50% disease, 4 = < 10% disease	1

^a d = day; RH = relative humidity.

The existence of new immigrant strains of *P. infestans*, and metalaxyl-resistant *P. infestans* populations throughout the United States (9,18,19), have placed a renewed emphasis on defining durable resistance to late blight for future cultivars. There is a continuing need for greenhouse and laboratory tests that could expedite cultivar and germ plasm evaluation for late blight resistance particularly if partial resistance components could be measured.

MATERIALS AND METHODS

Field evaluations. The western Washington *P. infestans* population has been reported to consist of complex races (8,9,20). Virulence factors 9 and 11 have not been identified to date and, in 1995, virulence factor 10 was very rare (A. E. Dorrance and D. A. Inglis, *unpublished data*). Only two genotypes of *P. infestans*, US-6 and US-11, have been detected, 1990 to 1996 (18,24). Both are metalaxyl-insensitive and A1 mating type.

The field design used in this study was reported earlier (24). Cultivars were planted in a randomized complete block design, with three replications each consisting of 10 plants per plot. Plots consisted of four record rows bordered on the outside by potato cultivar White Rose, which is intermediate in resistance to late blight (Table 3). In 1996, cultivars Norchip and Alpha were planted in a neighboring trial in four plant rows. Once the onset of disease occurred, naturally infested leaves from an adjoining field trial were moved along the border rows to ensure uniform

inoculum dosage. Late blight was recorded weekly (percent blighted foliage per plot) throughout the 1995 and 1996 growing seasons. Percentages were used to calculate area under the disease progress curve (AUDPC). The AUDPC values were analyzed using the PROC RANK procedure in SAS (SAS Institute, Cary, NC). Correlation coefficients were calculated using the PROC CORR procedure from the ranked mean response from each greenhouse and laboratory assay and the ranked AUDPC means for each cultivar.

Inoculum production. One isolate, *P. infestans* 95WWA (US-11, sensu Goodwin and Fry; 24) with virulence genes 1,2,4,5,7 (Dorrance and Inglis, *unpublished data*) was used in all experiments. Of the *P. infestans* isolates we have identified from western Washington to date, 95WWA is one of the most complex race types. Inoculum was produced on potato leaflets, tuber slices, or rye agar. A 40- μ l-drop of a sporangial suspension (1×10^4) was placed on potato leaflets in moist chambers and incubated at 20°C under 12 h light (Philips Fluorescent TL70 or Philips 60W Fluorescent F48T12 and Philips Tungsten 60W) for 7 days. A 5-mm colonized plug was placed on the center of a potato tuber slice or rye agar plate and incubated at 20°C in the dark for 5 to 7 days. Potato leaflets, tuber slices, and rye agar plates were washed with distilled water, sporangia counted with a hemacytometer, and final concentrations were adjusted according to the assay protocol described below.

Greenhouse cultivation. Potato cultivars and advanced breeding selections used in this study are shown in Table 3. Tubers of potato cultivars were received from the USDA-ARS, University of Idaho Aberdeen Research and Extension Center. Approximately 500 ml of Sunshine mix (SunGro Horticulture, Bellevue, WA) was placed in 16-cm-diameter pots, one eye was cut approximately 10 to 15 cm in diameter from a tuber, the seedpiece was covered with mix, and more mix was added as the sprouts developed. Plants were fertilized when watered with 100 ppm N-P-K and 2.5 ppm Peters soluble trace element mix (Scotts Sierra Horticultural Products Co., Marysville, OH). Supplemental fertilizer (23N-19P-17K) with RAPID GRO (San Ramon, CA) was applied as needed. Supplemental lighting from high intensity lights (Sylvania 1000, Danvers, MA) provided a 12-h light:dark cycle.

Greenhouse seedling/whole plant assay. Eleven cultivars were planted on 14 April and 15 May, 14 cultivars on 31 May, and 16 cultivars on 14 June and 1 July. Three plants from each cultivar and planting date were inoculated with a *P. infestans* sporangia suspension (8×10^3 /ml) with an atomizer on 6 June, 6 July, 1 August, and 1 September. Immediately after inoculation, plants were transferred to a mist chamber where they were kept under 100% relative humidity for 2 days at temperatures ranging from 10 to 22°C. Ratings of infection were begun 4 or 5 days later. To induce sporulation, plants were placed in the mist

Table 2. Summary of laboratory methods utilizing leaf disks (LD), detached leaflets (LL), or detached leaves (LV) in screening *Solanum* germ plasm and potato cultivars for foliar resistance to late blight

Plant material; age	Inoculum	Environment ^y	Rating	Citation
LL; estimated resistance to invasion; flowering stage	Standardized zoospore suspension; 60-200 per drop	5 d at 15°C; 95-100% humidity; continuous light	Measured diameter of lesions	34
LL; flowering stage	4 isolates; 1 drop of 5,000-10,000 zoospores	3 d	Hypersensitive response; sporulation	37
LL; youngest fully expanded pair of leaflets, abaxial surface	Zoospores, spray and drop; after 24 h disks inverted	15°C; high humidity; 16 h light	1-3: 1 = no symptoms, 2 = spreading but no sporulation, 3 = spreading sporulating lesion	40
LL; 4 weeks after transplanting; 3 leaflets/plant; abaxial surface	40 μ l of 50,000 sporangia/ml	Plastic boxes; 7 d at 18°C	Sporulation rated 0-3 scale; % area colonized	48
LL; lateral leaflets of the first terminal pair	2,500 sporangia/50 mm ³ one drop/leaflet near midrib	Plastic trays; 6 d at 16°C; high humidity; 16 h light	3 categories based on lesion development	43
LL; 4 weeks; R- gene differential genotypes	50 μ l of 40,000 sporangia/ml or a 4 mm mycelial plug	5 d at 18-20°C	Susceptible if sporulating	9
LL ^z	10,000 sporangia/ml, atomized onto surface	5 d at 18°C; high humidity	Presence of lesion and sporulation	15
LD, 15 mm	50-100 zoospores/45 μ l	7 d at 20°C; high humidity	Presence/absence sporulation	23
LD; 8 mm in diameter; adaxial surface; 8 LD per genotype; collected from same or directly adjacent leaf; third internode from top; same plant used for all experiments	3-10 μ l of 30,000 sporangia/ml and 40,000 zoosporangia/ml	3-4 d at 17°C; 14 h light		30
LD; 100 disks	Zoospores, sporangia	7d	Mycelial growth and sporulation	54
LV		7 d at 18°C; 100% RH		21

^y d = day; RH = relative humidity.

^z Assay used to detect redistribution of air-applied fungicides.

chambers for 24 h, 6 to 8 days after inoculation. Ratings included: (i) overall rating of 1 to 9, where 9 = resistant and 1 = susceptible (based on percent foliage with lesions, detailed description in Malcolmsen (31), (ii) number of lesions per plant, and (iii) percent plant diseased. Ratings were taken periodically over a period of 1 to 2 weeks or until the most susceptible plant died. Ratings from each experiment were pooled and analyzed for differences between experiments, among cultivars and plant ages, and interactions between cultivars and plant age, cultivars and experiments, cultivars and plant age, and experiments using SAS. Data were sorted by test, cultivar, and plant age. Cultivar mean late blight ratings were analyzed using Fisher's least-significant-difference test (LSD) for each experiment and plant age.

Laboratory detached leaflet and leaf disk assays. The two top fully expanded leaves from 3 to 4 plants were collected from 6- to 8-week-old plants. Side leaflets were detached with scissors and used for detached leaflet assay. Leaf disks were cut with a cork borer (15-mm) from the primary leaflet on both sides of the vein. Four leaflets and 3 to 4 disks per cultivar were placed in the lids of inverted, large (15-cm) petri dishes containing 25 ml (1.5%) water agar (49). Three plates per cultivar were kept at 20°C with 12 h light in a growth chamber (Philips 60W Fluorescent F48T12 and Philips 60W incandescent) or in an incubator, also at 20°C and 12 h light (Philips Fluorescent TL70), for 7 days. Percent colonization by the fungus was determined at 5, 6, and 7 days after inoculation with a 40- μ l droplet of 4,000 sporangia per ml. Statistical analysis was done on the final reading of percent colonization and the number of leaflets and leaf disks that developed blight lesions. This assay was repeated three times. The data from

the three experiments for both detached leaflets and leaf disks were pooled and analyzed for differences between experiments, detached leaflets, and leaf disks for the cultivars. The mean percent late blight of the detached leaflets and leaf disks was then analyzed separately for each experiment using LSD.

Laboratory stem assay. Three above-ground stems from each of 14 cultivars were cut to 100 to 140 mm in length from 4- to 6-week-old potato plants 6 July and 16 July, and immediately placed in distilled water and designated Test 1 and Test 2. The test was repeated with 16 cultivars on 16 August and designated Test 3. Each stem was transferred to a 50-ml tube containing a nutrient solution of 660 ml distilled water, 4.5 g sucrose, and 0.3 g Peters Fertilizer 20-20-20, (Scotts Sierra Horticultural Products Co., Marysville OH; 15). Filter paper squares (5 mm²) were soaked in a sporangial suspension of 1.4 to 1.5 $\times$ 10⁴ sporangia/ml for 30 min. One filter paper square was placed on each stem approximately 30 to 50 mm from the top of the cutting with forceps. Tubes with stem cuttings were placed in a randomized block design in a growth chamber at 20°C with 12 h light (Philips 60W Fluorescent F48T12 and Philips 60W incandescent) for 7 days. Nutrient solution was added to the tubes as needed to prevent desiccation of stems. Presence of stem blight was noted and lesion length was measured. Lesion length data were pooled from the three experiments and analyzed by the general linear model procedure (GLM) of SAS for experiments and cultivars. Cultivar mean separation using LSD was completed on the experiments separately.

RESULTS

Field evaluations. Potato cultivars and advanced selections used for this study and

their field reactions to late blight are shown in Table 3. Members of this group have had a similar reaction to late blight when ranked against each other at Mount Vernon and in other locations in the US (24,42,45,47,53). Field analysis near Mount Vernon, Washington in 1993 to 1996 indicated that Elba, COO83008-1, and White Rose were less susceptible to late blight than Russet Burbank; Kennebec and Ranger Russet were similar in susceptibility; and Shepody and Russet Norkotah were more susceptible than Russet Burbank. Bzura, Brador, and Brodick were the most resistant of those tested.

Greenhouse seedling/whole plant assay. The data for each greenhouse inoculation were pooled and analyzed. There were highly significant differences for cultivars ($P = 0.0001$), ages ($P = 0.0001$), experiments ($P = 0.0001$), cultivar by age ($P = 0.0001$), and cultivar by test interaction ($P = 0.0001$). Hence, each test and cultivar age experiment was analyzed separately (Table 4).

Differences in greenhouse experiments were attributed mainly to variation in overall disease severity due to different environmental conditions in June, July, and August. For example, mean disease rating of 4- to 6-, 7- to 8-, and 9- to 11-week-old plants in three tests were 7.3, 8.2, and 4.5; 6.6, 7.9, and 4.1; and, 7.7, 4.4, and 4.7, respectively. Temperatures in the mist chambers were higher (13 to 17°C) during the August test compared to the other three inoculations, where temperatures sometimes fell below 10°C following inoculation.

Plants of Alpha, Russet Burbank, Elba, and Kennebec had more foliage blight following inoculation on 4- to 6-week-old than on 7- to 8-week-old plants in the August test. Russet Norkotah, Norchip, Ranger Russet, Shepody, A84118-3, and

Table 3. Area under the disease progress curve (AUDPC) values for 13 potato cultivars and 2 advanced selections obtained from field studies near Mount Vernon, Washington, and used to assess reliability of greenhouse and laboratory screening methods for late blight resistance

Potato cultivar and advanced selections	Reported resistance ^z	AUDPC ^y			
		1993	1994	1995	1996
Bzura	R genes and FR	...	...	304 a	225 a
Brador	R genes and FR	...	...	1,050 bc	465 b
Brodick	General FR	...	...	...	606 b
A84118-3	unknown	...	...	890 b	1,202 c
Alpha	FR	...	...	...	1,285 c
Elba	FR	527 a	135 a	880 b	1,498 d
COO83008-1	unknown	...	362 a	915 b	1,600 d
White Rose	IS	1,060 b	834 bc	...	1,539 d
Kennebec	MR	...	247 a	1,000 b	1,674 de
Russet Burbank	IS	1,035 b	650 b	1,243 cde	1,776 ef
Ranger Russet	S	1,101 bc	389 a	1,108 bcd	1,890 f
Shepody	S	1,169 bc	807 bc	1,303 de	1,868 f
Red LaSoda	S	...	1,030 c	...	...
Norchip	HS	...	...	...	2,189 b
Russet Norkotah	S	1,214 c	1,423 d	1,377 e	2,479 a

^y AUDPC calculated from % blighted foliage; ranks determined according to PROC RANK procedure in SAS (SAS Institute, Cary, NC); means followed by the same letter in a column do not differ significantly at $P \leq 0.05$ according to Fisher's least significant difference test; ... designates that there is no data for that cultivar in that year.

^z Ratings for resistance: FR = field resistant, MR = moderately resistant, IS = intermediate susceptible, S = susceptible, and HS = highly susceptible (22,24,42,45,47,53).

COO83008-1 had more foliage blight 7 to 8 weeks than 4 to 6 weeks after planting in the August test. Disease reactions were similar, though, for most cultivars inoculated when either 7 to 8 or 9 to 11 weeks old. Plants that were 15 weeks old or older senesced in the mist benches and the data was considered unreliable for inclusion in the final analysis.

Plants inoculated at 7 to 8 weeks and 10 to 12 weeks in age in the greenhouse corresponded most closely in resistance ratings to the field rankings for both percent foliage blight (data not shown) and Malcolmson's (31) late blight rating scale. The 7- to 8-week-old plants in our greenhouse study were flowering or had just completed the flowering stage. The number of lesions that developed on the cultivars following inoculations was not related to field AUDPC (data not shown). In general, resistant cultivars developed fewer lesions than intermediate and susceptible cultivars which varied in number of lesions.

Laboratory detached leaflet and leaf disk assays. Data from two of the experiments were pooled and analyzed for differences in percent leaf colonized among experiments, cultivars, detached leaflets, and leaf disks. There were significant differences among the experiments ($P = 0.0001$), cultivars ($P = 0.0001$), and detached leaflets versus leaf disks ($P = 0.0004$). For each test, leaflets and leaf disks were then analyzed separately (Table 5). Again, different amounts of disease developed between the experiments; the mean percent disease was 45.9, 30.6, and 12.7 for detached leaflets and 28.6, 73.5, and 19.9 for the leaf disks in tests 1, 2, and 3, respectively. The third test (data not

shown) followed an insecticide treatment of resmethrin which is reported to inhibit foliar infection (38).

The cultivars did not respond the same way within each experiment or between experiments in regard to the amount of blight on the detached leaflets compared to the leaf disks. For example Russet Burbank had 82.5 and 27.6% colonization in the detached leaflet and 8.3 and 96.9% in leaf disk assays (Table 5). However, Bzura, Brador, and Brodick means ranged from 0.0 to 10.3% colonization, indicating high levels of resistance. The rest of the cultivars were all colonized by *P. infestans* to some extent, indicating susceptibility. Due to high variability between experiments, detached leaflet and leaf disk assays when read 7 days following inoculation could only reliably separate cultivars into two groupings: resistant or susceptible.

Laboratory stem assay. The data from all three tests were pooled and analyzed. Test 1 and 2 were not significantly different from one another ($P = 0.96$). Test 3 differed from the first two based mainly on the change in reaction of cultivar Elba and longer lesion length for all cultivars. In the first two assays, Elba failed to develop stem lesions, but in the last assay stem lesions developed. The results from each experiment are presented in Table 6. These results are similar to the foliage response in that the cultivars fall into similar groupings of resistant and susceptible, but there is considerable variability of the individual rank for each cultivar. For example, Kennebec had small mean lesion length of 8.0 mm in test 2, indicating resistance, but in test 3 was susceptible, with lesion length of 46.3 mm.

Correlation of assays. Correlation coefficients for comparisons with the ranked means of the field 1996 AUDPC (the most complete set of cultivars and advanced selections) and with the ranked means from each greenhouse and laboratory assay are presented in Table 7. In most cases, the greenhouse inoculation results for plants inoculated 7 to 11 weeks after planting were more highly correlated ($r \geq -0.84$) with the field AUDPC values from 1996 than were results for plants inoculated 4 to 6 weeks after planting (r values ranged from -0.57 to -0.82) and all of the laboratory assays (r values ranged from 0.44 to 0.77). The one exception was in the July greenhouse inoculation of plants 7 to 8 weeks old, in which the correlation coefficient was the lowest ($r = -0.28$). In this trial, one plant of the cultivar Shepody may have escaped disease during the inoculation process, and Elba had a lower disease rating than expected. The final mean rating (Malcolmson's scale, 31) for this assay was also higher than for the other inoculations. This illustrates that greenhouse assays must reach a sufficiently high disease severity level to provide a reliable indication of field response. The laboratory assays for the detached leaflet, leaf disk, and stem assays were variable in their correlation with the field response (Table 7).

DISCUSSION

The above assays demonstrate possible ways to assess potato foliage and stems for resistance to *P. infestans*, and are validated by established field responses of the cultivars tested. Each laboratory and greenhouse assay separated cultivars into resistant and susceptible groups, although

Table 4. Greenhouse ratings^x of late blight foliage resistance for 13 potato cultivars and 2 advanced selections in three age classes from separate inoculation experiments

Cultivar	4- to 6-week-old plants ^y			7- to 8-week-old plants			9- to 11-week-old plants			
	June 16 days ^z	July 8 days	August 7 days	June 16 days	July 8 days	August 7 days	July 8 days	August (9 week) 7 days	August (11 week) 7 days	September 15 days
Bzura	...	9.0 a	9.0 a	...	...	8.7 a	...	8.3 a	...	9.0 a
Brador	9.0 a	9.0 a	9.0 a	9.0 a	9.0 a	8.7 a	9.0 a	8.7 a	9.0 a	9.0 a
Brodick	...	9.0 a	9.0 a	...	...	9.0 a	...	9.0 a	...	9.0 a
A84118-3	7.5 a	8.3 abc	4.7 bcd	7.7 ab	8.3 abc	3.0 cde	8.3 ab	6.0 b	7.0 ab	8.0 ab
Alpha	...	...	3.0 cdef	...	...	3.7 bcd	...	--	...	6.5 bc
Elba	...	8.3 abc	2.0 ef	...	7.0 e	5.3 b	7.7 bc	4.7 bc	4.7 bcd	4.2 de
COO83008-1	7.7 a	7.0 d	6.0 b	7.5 ab	8.7 ab	4.0 bc	8.3 ab	3.7 cd	7.0 ab	5.7 cde
White Rose	8.0 a	8.0 bc	5.3 bc	6.7 bc	7.5 de	4.0 bc	7.7 bc	5.0 bc	5.3 bc	5.7 cde
Kennebec	...	8.7 ab	2.0 ef	...	7.0 c	4.0 bc	7.0 cd	3.3 cde	5.0 bcd	5.5 cde
Russet Burbank	7.3 a	...	1.3 f	6.7 bc	7.3 de	2.3 cde	7.3 cd	2.3 def	3.3 cde	5.3 cde
Ranger Russet	5.7 a	7.7 cd	4.7 bcd	5.0 c	7.7 cde	3.0 cde	7.3 cd	1.3 f	3.5 cde	5.2 cde
Shepody	6.3 a	7.7 cd	4.0 bcde	5.0 c	8.3 abc	2.0 cde	7.0 cd	2.0 def	1.0 e	4.7 cde
Red LaSoda	7.3 a	8.0 bc	4.3 bcde	6.3 c	7.7 cde	1.3 e	6.5 d	3.5 cd	1.3 e	3.7 e
Norchip	...	...	2.7 def	...	...	1.7 de	...	--	...	1.5 f
Russet Norkotah	7.0 a	8.0 bc	4.3 bcde	5.3 c	8.0 bcd	1.3 e	7.0 cd	1.5 ef	2.0 de	6.2 bcd
Means	7.3	8.2	4.5	6.6	7.9	4.1	7.7	4.4	4.7	6.1

^x Ratings are on a scale from 1 to 9, where 9 = no blight, 1 = 100% blight as described by Malcolmson (31); mean ratings are separated by Fisher's least significant difference test; ratings within columns followed by the same letter are not significantly different at $P \leq 0.05$.

^y Greenhouse inoculations; plants were atomized with a suspension of 8,000 sporangia/ml and placed in a mist chamber for approximately 40 h; ratings were taken periodically for 1 to 2 weeks.

^z Days following inoculation when the rating was taken.

assays done at different times did not always have the same disease pressure. Typically, field results also vary from year to year because of the different severity of late blight epidemics. The importance of routinely using a set of known cultivars as standards in all experiments as a measure of the late blight epidemic potential, in order to impartially screen and compare germ plasm or fungicides, has previously been emphasized (10). Differences in disease severity among the plant age classes for specific cultivars also confirms previous reports of differences in susceptibility of potato cultivars with respect to age (13,23,40,41).

Assessment of cultivars for late blight resistance in the whole plant greenhouse assays included a disease severity rating which was an overall plant assessment and an estimate of the percentage of the plant blighted, where 9 = no blight and 1 = 100% blighted. These ratings are similar to AUDPC values from field studies, in that they summarize resistance, the sum total of specific partial resistance components. For example, the number of lesions that develop, lesion growth rate, and latent period (time required from infection to sporulation) all contribute to the AUDPC and the parameters measured in the greenhouse ratings. The whole plant greenhouse assay evaluates infection efficiency for specific inoculum dosages, rate of lesion development, and growth. To a limited extent, sporulation capacity can be measured by manipulating the humidity levels.

The detached leaflet and leaf disk assays may not be useful for overall measurements of partial resistance. Colon et al. (6) reported that measuring individual components was very time consuming, and each component was influenced by the test conditions. Our results reflect a considerable change in reaction of some cultivars between experiments and variation in disease severity within experiments. These types of assays would require numerous repeated experiments and a large number of replications to verify results, both of which limit the usefulness in large segregating populations. Wenzel and Foroughi-Wehr (54) arrived at a similar conclusion when they reported that accurate measures of resistance to *Phytophthora* required 100 leaves, plant material as uniform as possible, and a large number of replicates.

One of the inherent risks in any screening evaluation is the presence of escapes or false designation of resistance when, in fact, there is inoculum failure. In the case of a detached leaf or disk in a plate, losing the inoculum is a particular danger. However, if disease escapes are a problem we would see that the most susceptible lines would sometimes have no blight. This occurred only to a limited extent in our study. The variation we recorded in these experiments likely resulted from the percentage of blight on each leaflet or leaf

disk. The detached leaflet assay has proven effective for identifying virulence loci in specific *P. infestans* isolates, and separating germ plasm into distinct resistant or susceptible categories (Table 2). The detached leaflet or leaf disk assay may also be useful in examining infection efficiency under controlled environmental conditions or sporulation capacity on a known leaf area. Even though these laboratory assays may not be useful for overall disease evaluation, they would be helpful for as-

sessing individual components of partial resistance.

Occurrence of stem lesions is sometimes attributed to the new immigrant and metalaxyl-resistant *P. infestans* genotypes. Although potato plants may differ in stem resistance to late blight (17), not all researchers are in agreement (J. S. Niederhauser, *personal communication*). The stem assay described here does illustrate differences in resistance among the cultivars tested to the development of stem

Table 5. Comparison of detached leaflet and leaf disk laboratory assays for assessing potato foliage for resistance to late blight in twelve cultivars and two advanced potato selections

Cultivar and advanced potato selections	Percent colonization ^x			
	Detached leaflet ^y		Leaf disk ^z	
	Test 1	Test 2	Test 1	Test 2
Bzura	...	2.1 ab	...	10.3 a
Brador	0.0 a	0.0 a	0.0 a	0.0 a
Brodick	...	0.0 a	...	0.0 a
A84118-3	17.9 ab	59.1 e	...	91.3 bcde
Alpha	...	25.7 cd	...	90.0 bcde
Elba	15.0 ab	60.0 e	75.0 d	98.8 de
COO83008-1	31.9 bc	32.3 d	33.3 abc	82.2 bcde
White Rose	56.7 cde	12.3 abc	8.3 ab	75.0 b
Kennebec	88.8 f	19.4 bcd	47.5 bcd	91.7 bcde
Russet Burbank	82.5 ef	27.6 cd	8.3 ab	96.9 cde
Ranger Russet	33.8 bc	63.1 e	...	100.0 e
Shepody	51.8 cd	56.7 e	25.0 abc	92.5 bcde
Norchip	...	35.0 d	...	93.8 cde
Russet Norkotah	64.2 def	23.5 cd	50.7 d	98.5 de
Means	45.9	30.6	28.6	73.5

^x Percent colonization is based on the average of the visual estimate of 4 detached leaflets or 4 leaf disks (15-mm-diameter) per plate. Means within columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's least significant difference test.

^y Detached leaflet assay = side lateral leaflets removed from greenhouse-grown plants (approximately 6 to 7 weeks old, just prior to flowering) and placed abaxial surface up in moist chambers, inoculated with 40 μ l of 4,000 sporangia/ml and incubated for 7 days at 20°C under 12 h light:dark.

^z Leaf disk assay = leaf disks (15-mm-diameter) cut from primary leaflet of four separate leaves and placed adaxial surface up in moist chambers, inoculated with 40 μ l of 4,000 sporangia/ml, and incubated for 7 days at 20°C under 12 h light:dark.

Table 6. Differences in stem lesion length in 13 potato cultivars and 2 advanced selections challenged with a sporangial suspension of *Phytophthora infestans*^y

Cultivar	Stem blight lesion (mm) ^z		
	Test 1	Test 2	Test 3
Bzura	0.0 a	2.4 a	0.0 a
Brador	0.0 a	0.0 a	0.0 a
Brodick	0.0 a	0.0 a	0.0 a
A84118-3	28.5 cd	44.4 bc	14.7 ab
Alpha	...	...	50.0 cde
Elba	0.0 a	0.0 a	58.7 de
COO83008-1	2.3 ab	4.2 a	27.3 bc
White Rose	43.6 de	2.1 a	0.0 a
Kennebec	26.8 cd	8.0 a	46.3 bc
Russet Burbank	17.6 abc	34.7 bc	33.0 bc
Ranger Russet	55.1 a	38.8 bc	57.7 de
Shepody	35.3 cde	39.3 bc	21.7 ab
Red LaSoda	31.3 cde	52.5 c	70.0 e
Norchip	...	...	35.7 bcd
Russet Norkotah	19.0 abc	22.0 bc	29.7 bc
Means	20.1	20.5	29.7

^y Stem cuttings 100-140 cm in length were taken from greenhouse-grown plants (6-8 weeks old) and placed in nutrient solution (14). Filter paper squares with sporangia suspension (16-18,000 sporangia/ml) were placed on the stem. Cuttings were incubated for 7 days at 20°C with 12 h light:dark cycle.

^z Means of three cuttings were separated using Fisher's least significant difference test, means followed by the same letter in the same column are not significantly different at $P \leq 0.05$.

Table 7. Correlation coefficients for the relationships between area under the disease progress curve (AUDPC) measured in the field in 1996, and disease ratings in greenhouse and laboratory assays using 13 potato cultivars and 2 advanced selections for foliage resistance to *Phytophthora infestans*

Plant age classes	AUDPC ^x and greenhouse assays ^y				AUDPC and laboratory assays ^z			
	June	July	August	September	Assay	Test 1	Test 2	Test 3
4 to 6 weeks	-0.82*	-0.75*	-0.57*	...	Detached leaflet	0.68*	0.49	...
7 to 8 weeks	-0.88*	-0.28	-0.84*	...	Leaf disk	0.44	0.77*	...
9 weeks	...	-0.85*	-0.95*	-0.74*	Stem	0.62*	0.56	0.52*
11 weeks	...	...	-0.84*	...				

^x Correlation coefficients are from comparisons with the ranked means of the field AUDPC (see Table 3). Numbers followed by an asterisk are significant at $P \leq 0.05$.

^y Greenhouse assays utilized a disease rating scale of 1 = 100% disease and 9 = no disease, described by Malcolmson (31) for different age classes (weeks after planting) of potatoes (see Table 4).

^z Laboratory assays measured percent colonization of detached leaflets, leaf disks, and stem lesion length (see Tables 5 and 6); numbers followed by an asterisk were significant at $P \leq 0.05$.

blight. A majority of cultivars developed stem lesions to our 95WWA *P. infestans* isolate. Several replications of this assay may be necessary to accurately assess resistance of potato stems.

We found significant differences between tests within each assay, not necessarily in the final resistance ranking of the cultivars tested but in how quickly the disease progressed and the final disease severity. The way in which temperature influences the rate of epidemic development and changes the resistance response needs to be further defined for greenhouse and laboratory assays. Knowledge in these areas will improve environmental controls for assay conditions, thus improving the quality of the assay. Cultivars Elba and Ranger Russet might be good candidates for studies that explore environmental effects on the expression of late blight resistance, because their disease rankings tend to fluctuate in different field and laboratory assays.

The greenhouse whole plant inoculation is useful for studies of inheritance of resistance, identification of resistance components and for choosing potential germ plasm to be used as parents (1,2,3,51). Greenhouse-grown *Solanum* progeny could be selected from *P. infestans*-challenged plants based on percent blight or a rating scale, thus eliminating the need for large-scale field tests. Challenges with complex *P. infestans* genotypes or isolates known to colonize a wide range of potato genotypes in these laboratory assays would help avoid selection of single R-gene resistance.

This study confirms previous reports that greenhouse studies on plants inoculated 7 to 11 weeks after planting can reliably aid screening efforts to assess germ plasm for resistance to late blight. Inoculations at or near flowering corresponded best with overall 1996 field AUDPC ratings when there was a high amount of disease in the test. The greenhouse inoculations 7 to 11 weeks after planting also provided a wide range of disease responses for separation into distinct resistant classes: highly susceptible, moderately susceptible, moderately resistant, and resistant. Greenhouse and laboratory assays cannot replace the value of evaluating

germ plasm for foliar resistance to late blight under field conditions. If repeated under controlled environmental conditions, they can help expedite the selection process and allow for evaluation of the partial resistance components needed in potato cultivars at this time.

ACKNOWLEDGMENTS

We thank Charles Brown, Dennis Corsini, Dennis Johnson, and Roland Line for providing comments and suggestions regarding this manuscript, and Marc Evans, Larry Madden, Nancy Callahan, and Babette Gundersen for statistical and technical support.

LITERATURE CITED

- Bradshaw, J. E., Stewart, H. E., Wastie, R. L., Dale, M. F. B., and Phillips, M. S. 1995. Use of seedling progeny tests for genetical studies as part of a potato (*Solanum tuberosum* subsp. *tuberosum*) breeding programme. *Theor. Appl. Genet.* 90:899-905.
- Caligari, P. D. S., Mackay, G. R., Stewart, H. E., and Wastie, R. L. 1984. A seedling progeny test for resistance to potato foliage blight (*Phytophthora infestans* (Mont.) de Bary). *Potato Res.* 27:43-50.
- Caligari, P. D. S., Mackay, G. R., Stewart, H. E., and Wastie, R. L. 1985. Confirmatory evidence for the efficacy of a seedling progeny test for resistance to potato foliage blight (*Phytophthora infestans* (Mont.) de Bary). *Potato Res.* 28:439-442.
- Carnegie, S. F., and Colhoun, J. 1982. Susceptibility of potato leaves to *Phytophthora infestans* in relation to plant age and leaf position. *Phytopathol. Z.* 104:157-167.
- Colon, L. T., and Budding, D. J. 1988. Resistance to late blight (*Phytophthora infestans*) in ten wild *Solanum* species. *Euphytica* 5:77-86.
- Colon, L. T., Budding, D. J., Keizer, L. C. P., and Pieters, M. M. J. 1995. Components of resistance to late blight (*Phytophthora infestans*) in eight South American *Solanum* species. *Eur. J. of Plant Pathol.* 101:441-456.
- Davila, E. 1964. Late blight infection of potato tubers. *Am. Potato J.* 41:103-112.
- Deahl, K. L., and Inglis, D. A. 1995. Occurrence of metalaxyl-insensitive *Phytophthora infestans* on *Solanum sarachoides* in northwestern Washington. *Plant Dis.* 79:540.
- Deahl, K. L., Inglis, D. A., and DeMuth, S. P. 1993. Testing for resistance to metalaxyl in *Phytophthora infestans* isolates from northwestern Washington. *Am. Potato J.* 70:779-795.
- Dowley, L. J., O'Sullivan, E., and Kehoe, H. W. 1991. Development and evaluation of blight resistant potato cultivars. Pages 373-382 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, S. S. Shaw, and L. R. Cooke, eds.

Cambridge University Press, Cambridge.

- Flor, H. H. 1955. Host parasite interaction in flax rust-its genetics and other implications. *Phytopathology* 45:680-685.
- Flor, H. H. 1971. Current status of the gene-for-gene concept. *Ann. Rev. Phytopathol.* 9:275-296.
- Fry, W. E., and Apple, A. E. 1986. Disease management implications of age-related changes in susceptibility of potato foliage to *Phytophthora infestans*. *Am. Potato J.* 63:47-55.
- Gallegly, M. E. 1968. Genetics of the pathogenicity of *Phytophthora infestans*. *Annu. Rev. Phytopathol.* 6:375-396.
- Geary, B., Johnson, D. A., Cummings, T., and Miller, J. S. 1996. Redistribution of air applied fungicides in potato canopies. (Abstr.) *Am. Potato J.* 73:356.
- Gisi, U., and Cohen, Y. 1996. Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annu. Rev. Phytopathol.* 34:549-572.
- Glendinning, D. R. 1989. Late blight resistance in the British neo-tuberosum potato population. *Potato Res.* 32:321-330.
- Goodwin, S. B., Sujkowski, L. S., Dyer, A. T., Fry, B. A., and Fry, W. E. 1995. Direct detection of gene flow and probable sexual reproduction of *Phytophthora infestans* in northern North America. *Phytopathology* 85:473-479.
- Goodwin, S. B., Sujkowski, L. S., and Fry, W. E. 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. *Phytopathology* 86:793-800.
- Goodwin, S. B., Sujkowski, L. S., and Fry, W. E. 1995. Rapid evolution within clonal lineages of the potato late blight disease fungus. *Phytopathology* 85:669-676.
- Goth, R., and Keane, J. 1996. Use of detached leaf assay for evaluating late blight reactions of potato and tomato. (Abstr.) *Am. Potato J.* 73:357.
- Guzmán, N. J. 1964. Nature of partial resistance of certain clones of three *Solanum* species to *Phytophthora infestans*. *Phytopathology* 54:1398-1404.
- Hodgson, W. A. 1961. Laboratory testing of the potato for partial resistance to *Phytophthora infestans*. *Am. Potato J.* 38:259-264.
- Inglis, D. A., Johnson, D. A., Legard, D. E., Fry, W. E., and Hamm, P. B. 1996. Relative resistances of potato clones in response to new and old populations of *Phytophthora infestans*. *Plant Dis.* 80:575-578.
- James, R. V., and Fry, W. E. 1983. Potential for *Phytophthora infestans* populations to adapt to potato cultivars with rate-reducing resistance. *Phytopathology* 73:984-988.
- Kadish, D., Grinberger, M., and Cohen, Y.

1990. Fitness of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* on susceptible and resistant potato cultivars. *Phytopathology* 80:200-205.
27. Knutson, K. W. 1962. Studies on the nature of field resistance of the potato to late blight. *Am. Potato J.* 39:152-161.
28. Kroll, R. E., and Eide, C. J. 1981. Effect of inoculum concentration of *Phytophthora infestans* on potato late blight. *Am. Potato J.* 58:153-161.
29. Lacey, C. N. D., Jellis, G. J., Starling, N. C., and Currell, S. B. 1987. A joint cyst nematode/late blight test for early generation screening of potato clones. Pages 81-83 in: *The Production of New Potato Varieties: Technological Advances*. Cambridge University Press, Cambridge.
30. Leonards-Schippers, C., Gieffers, W., Schafer-Pregl, R., Ritter, E., Knapp, S. J., Salamini, F., and Gebhardt, C. 1994. Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. *Genetics* 137:67-77.
31. Malcolmson, J. F. 1976. Assessment of field resistance to blight (*Phytophthora infestans*) in potatoes. *Trans. Br. Mycol. Soc.* 67:321-360.
32. Malcolmson, J. F., and Killick, R. J. 1980. Breeding values of potato parents for field resistance to late blight measured by whole seedlings. *Euphytica* 29:489-495.
33. Miller, J. S., Johnson, D. A., and Hamm, P. B. 1996. Aggressiveness of *Phytophthora infestans* isolates in the Pacific Northwest. (Abstr.) *Phytopathology* 85:1127.
34. Nilsson, B. A. 1981. Component analysis of general resistance to *Phytophthora infestans* in clones from the Colombian potato collection. *Potato Res.* 24: 239-244.
35. Parlevliet, J. E. 1979. Components of resistance that reduce the rate of epidemic development. *Annu. Rev. Phytopathol.* 17:203-222.
36. Pavek, J. J. 1987. Some interesting aspects of recent and expected developments in potato breeding in North America (Canada and the United States of America). *Acta Hortic.* 213:61-65.
37. Rivera-Peña, A. 1990. Wild tuber-bearing species of *Solanum* and incidence of *Phytophthora infestans* (Mont.) de Bary on the western slopes of the volcano Nevado de Toluca. 5. Type of resistance to *P. infestans*. *Potato Res.* 33:479-486.
38. Schumann, G., and Thurston, H. D. 1976. Foliar infection of potato by *Phytophthora infestans* inhibited by two insecticides. *Plant Dis. Rep.* 60:734-735.
39. Simmonds, N. W., and Wastie, R. L. 1987. Assessment of horizontal resistance to late blight of potatoes. *Ann. Appl. Biol.* 111:213-221.
40. Stewart, H. E. 1990. Effect of plant age and inoculum concentration on expression of major gene resistance to *Phytophthora infestans* in detached potato leaflets. *Mycol. Res.* 94:823-826.
41. Stewart, H. E.; Taylor, K., and Wastie, R. L. 1983. Resistance to late blight in foliage (*Phytophthora infestans*) of potatoes assessed as true seedlings and as adult plants in the glasshouse. *Potato Res.* 26:363-366.
42. Swiezynski, K. M., Domanski, L., Sieczka, M. T., and Zarzycka, H. 1993. Specific resistance to *Phytophthora infestans* in the potato. *Genet. Pol.* 34:327-336.
43. Swiezynski, K. M., Sieczka, M. T., Sujkowski, L. S., Zarzycka, H., and Zimnoch-Guzowska, E. 1991. Resistance to *Phytophthora infestans* in potato genotypes originating from wild species. *Plant Breeding* 107:28-38.
44. Tai, G. C. C., and Hodgson, W. A. 1975. Estimating general combining ability of potato parents for field resistance to late blight. *Euphytica* 24:285-289.
45. Tarn, T. R., Tai, G. C. C., Murphy, A. M., DeJong, H., Platt, H. W., Bagnall, R. H., Arsenault, W. J., Thorpe, J. H. E., Young, D. A., and Davies, H. T. 1995. AC Brador: A new late-maturing cultivar with a high degree of field resistance to late blight. *Am. Potato J.* 72:401-408.
46. Thurston, H. D. 1971. Relationship of general resistance: Late blight of potato. *Phytopathology* 61:620-626.
47. Thurston, H. D., Plaisted, R. L., Brodie, B. B., Jones, E. D., Loria, R., Halseth, D., and Sieczka, J. G. 1985. Elba: A late maturing, blight resistant potato variety. *Am. Potato J.* 62:653-656.
48. Tooley, P. W. 1990. Variation in resistance to *Phytophthora infestans* among 21 *Solanum verrucosum* plant introductions. *Am. Potato J.* 67:491-498.
49. Tooley, P. W.; Sweigard, J. A., and Fry, W. E. 1986. Fitness and virulence of *Phytophthora infestans* isolates from sexual and asexual populations. *Phytopathology* 76:1209-1212.
50. Toxopeus, H. J. 1956. Reflections on the origin of new physiologic races in *Phytophthora infestans* and the breeding for resistance in potatoes. *Euphytica* 5: 221-237.
51. Umaerus, V. 1969. Studies on field resistance to *Phytophthora infestans* 2. A method of screening young potato seedlings for field resistance to *P. infestans*. *Z. Pflanzenzüchtung* 61:167-194.
52. Umaerus, V., Umaerus, M., Erjefält, L., and Nilsson, B. A. 1983. Control of *Phytophthora* by host resistance: problems and progress. Pages 315-326 in: *Phytophthora Its Biology, Taxonomy, Ecology, and Pathology*. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. American Phytopathological Society, St. Paul, MN.
53. Wastie, R. L. 1991. Breeding for resistance. Pages 193-223 in: *Advances in Plant Pathology Phytophthora infestans, the Cause of Late Blight of Potato V7*. D. S. Ingram and P. H. Williams, eds. Academic Press, New York.
54. Wenzel, G., and Foroughi-Wehr, B. 1990. Progeny tests of barley, wheat, and potato regenerated from cell cultures after in vitro selection for disease resistance. *Theor. Appl. Genet.* 80:359-365.
55. Wilcoxson, R. D. 1981. Genetics of slow rusting in cereals. *Phytopathology* 71:989-993.