A Leaf Disk Assay for Detecting Resistance to Early Blight Caused by *Alternaria solani* in Juvenile Potato Plants

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ABSTRACT

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Resistance to early blight, caused by *Alternaria solani*, was examined using leaf disks from plants of 10 cultivars of potato (*Solanum tuberosum*) grown in a growth chamber or greenhouse. Disks (14 mm diameter) were cut from five leaves of equivalent maturity per cultivar, 21–80 days postemergence. The leaf disks were spray-inoculated with a conidial suspension of *A. solani*, floated on solutions containing either 1-naphthaleneacetic acid (NAA), indoleacetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP), abscisic acid (ABA), or H_2O, and incubated at 22 C in the dark for 3–5 days. Estimates of susceptibility, which correlated highly with observations of early blight in Wisconsin field trials, were obtained when leaf disks were floated on 10.7 μM NAA ($r = 0.86$) or 4.0 μM 2,4-D ($r = 0.71$).

Early blight of potato (*Solanum tuberosum* L.), incited by *Alternaria solani* Sorauer, occurs wherever potatoes are grown. It is a disease of economic importance in areas of North America where potatoes are grown under sprinkler irrigation, and it causes significant losses in areas such as Africa, Asia, Australia, Central and South America, Europe, India, and Israel (13). Early blight is currently controlled by the application of fungicides, a practice that is not economically feasible in all areas of the world. One means of reducing the need for fungicide treatment is to develop cultivars resistant or highly tolerant to early blight.

Developing potato cultivars with early blight resistance is hindered by the lack of an accurate and efficient method for detecting resistance in juvenile tissue, since most young potato plants appear resistant to early blight. Symptoms typically do not appear until individual leaves begin to reach maturity (14,15,18). In field trials, late-maturing cultivars do not develop extensive symptoms as early in the season as early-maturing cultivars (3,20), requiring an entire growing season for selection of desirable lines. When field trials are used to screen for resistance, the undesirable trait of late maturity may be mistaken for early blight resistance. Environmental conditions that cannot be controlled in the field, such as temperature, humidity, and leaf wetness, play an important role in early blight development (7). In addition, plants being evaluated for early blight resistance in the field may be affected by other plant pests, including insects, mites, and plant pathogens, that may either increase or decrease (2) susceptibility to early blight.

Detached leaf assays have been developed for detecting early blight resistance, many of which have the same disadvantage as field trials in that plants must reach maturity for use in the assay. Even when detached, leaves taken from juvenile plants show few symptoms when inoculated with *A. solani* (11,12), pre-

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cluding accurate evaluation of genetic resistance.

Seedling screens have been used for evaluating early blight resistance in juvenile potato plants (10), but results do not correlate highly with field trials. Poor correlations may be due to temporary resistance to attack by *A. solani* inherent in unmodified juvenile tissue.

Because of these problems, research was conducted to develop a simple, rapid, and reliable assay to quantify resistance to early blight in juvenile plants of *Solanum* species. In the assay described herein, phytohormones are used to simulate senescence as it occurs naturally in the field. Phytohormones were chosen because of their variable effect on senescence. Whereas cytokinins such as BAP retard senescence, abscisic acid accelerates the process (19). The response of inoculated disks to incubation on water is variable, possibly because of differences in phytohormone concentrations within individual leaves. Auxins perform a regulatory function, minimizing the differences between individual leaves within a cultivar and maximizing differences between cultivars.

MATERIALS AND METHODS

Plant growth. Ten potato cultivars (Table 1), representing a range of susceptibility to early blight and time of maturation in Wisconsin field trials (W. R. Stevenson and J. W. Pscheidt, *unpublished*), were tested. Tubers were obtained either from the Hancock Research Station, Hancock, Wisconsin, or from the Lelah Starks Elite Foundation Seed Potato Farm, Rhinelander, Wisconsin. A "melon baller" was used to remove seed pieces (2.5 cm diameter), each containing a single eye or sprout. Seed pieces were incubated overnight at 22 C in the dark, then planted in 10 × 35 × 55 cm flats containing pasteurized Plainfield loamy sand or a 50:50 mixture of Houghton muck and sand. Plants were grown either in a growth chamber at 20 C with a 12-hr photoperiod (350 $\mu E \cdot m^{-2} \cdot s^{-1}$) or in a greenhouse at 20 ± 3 C, under supplemental fluorescent lights with a 14-hr photoperiod. Plants were watered as needed and fertilized with a nutrient solution (6) every third watering.

The same cultivars were grown in tissue culture in 25 × 150 mm test tubes containing 12 ml of Prop medium (5) at 22 C in growth rooms with a 14-hr photoperiod. Rooted nodal cuttings, taken from tissue culture plants, were transplanted into U.C. mix (1) and grown at 20 C for 4 wk under high-intensity lights (430–730 $\mu E \cdot m^{-2} \cdot s^{-1}$) with a 14-hr photoperiod. Transplants were watered as necessary and fertilized once a week with nutrient solution (6).

Twenty plant introduction (PI) accessions were obtained from the IR-1 Potato Introduction Station, Sturgeon Bay, Wisconsin. Twenty-five to 50 true seeds from each accession were grown in Jiffy Mix (Jiffy Products of America, Inc.) in a greenhouse under high-intensity lights with a 14-hr photoperiod for 4 wk, watered as needed, and fertilized with nutrient solution (6) once a week.

***A. solani* isolates.** *A. solani* was isolated from angular necrotic lesions characteristic of early blight (8) on potato leaves grown at Hancock, Wisconsin. The two isolates used in subsequent experiments were selected on the basis of rapid growth on potato-dextrose agar (PDA) and high sporulation capacity. Both isolates induced symptoms characteristic of early blight on susceptible *Solanum* cultivars and were reisolated from inoculated plants showing characteristic early blight lesions.

Inoculum preparation. Inoculum was prepared by culturing single-spore isolates of *A. solani* on PDA in the dark. After cultures were grown to confluence, surface mycelium was removed by scraping with a sterile scalpel. The remaining culture and agar were cut into 1-cm^2 squares and transferred to $CaCO_3$ medium (16). Sporulation was induced on $CaCO_3$ medium according to the method of Shahin and Shepard (16) modified by the addition of a small reservoir containing a pellet of KOH with three drops of water, placed in the center of the $CaCO_3$ plate. After 18–24 hr at 20 C, conidia were harvested by placing the agar squares with sporulation in a 120-ml bottle containing 50 ml of sterile deionized water with 0.05% Tween 20, shaken vigorously for 50 sec, then filtered through a double layer of cheesecloth into 50-ml conical centrifuge tubes. The conidial suspension was concentrated approximately 10-fold by centrifuging at 1,000 rpm for 10 min and removing excess supernatant. Conidial suspensions were counted on a hemacytometer and diluted to a concentration of 2,000 or 3,000 conidia per milliliter using sterile deionized water. An inoculum concentration of 2,000 conidia per milliliter was used for leaf disks taken from plants derived from tissue culture and plants grown from true seed, and an inoculum of 3,000 conidia per milliliter was used for leaf disks taken from plants grown from tuber seed pieces.

Tissue preparation. Leaf tissue was collected from plants at 3 wk to 2 mo postemergence and tested for reaction to *A. solani*. The first fully expanded leaf from the apex was excised from five plants of each cultivar grown from tuber seed pieces or true seed. A 14-mm-diameter cork borer was used to cut leaf disks from each leaflet, avoiding major veins when possible. Leaf disks were floated on 11 ml of one of three phytohormone solutions—10.7 μM 1-naphthaleneacetic acid (NAA), 3.5 μM benzylaminopurine (BAP), and 0.04 μM abscisic acid (ABA)—or H_2O in a 10-cm glass petri dish immediately after excision. Successively higher concentrations of NAA, BAP, and ABA were evaluated until concentrations were found that optimized results. Disks were excised from the third or fourth leaf from the apex of transplants derived from tissue culture and floated on either 10.7 μM NAA, 4.0 μM 2,4-dichlorophenoxyacetic acid (2,4-D), or 25 μM indoleacetic acid (IAA).

Inoculation procedure. Leaf disks were placed on a waterproof template with 12-mm holes centered over the 14-mm disks. The template served to immobilize the leaf disks during the inoculation procedure and to prevent cut edges of disks from becoming directly inoculated. Leaf disks were then spray-inoculated with a conidial suspension of

Table 1. Susceptibility to *Alternaria solani* of potato leaf disks from tuber-derived plants incubated on 10.7 μM naphthaleneacetic acid (NAA), 3.5 μM 6-benzylaminopurine (BAP), 0.4 μM abscisic acid (ABA), or H_2O

		Mean early blight rating of leaf disks (SE)[x]			
Cultivar[v]	AUDPC[w]	NAA	BAP	ABA	H_2O
Ontario (VL)	0.47 a	2.4(1.2) ab	3.3(1.5) b	1.2(0.0) a	1.8(1.0) a
Nooksack (VL)	0.52 b	2.0(0.8) a	···	···	···
Langlade (L)	0.54 bc	4.2(0.8) c	2.6(0.4) a	···	3.5(0.3) c
Kennebec (ML)	0.54 bc	2.6(1.7) b	3.5(1.8) b	1.8(0.6) b	3.5(0.3) c
Russet Burbank (L)	0.59 cd	6.0(1.2) e	···	···	···
Atlantic (M)	0.60 d	5.1(0.5) d	···	···	···
Monona (M)	0.62 c	5.8(0.6) e	3.5(1.4) b	4.1(0.3) d	5.2(0.2) d
Superior (E)	0.68 e	5.0(1.8) d	5.3(1.9) d	1.0(0.8) a	2.8(1.0) b
Early Gem (E)	0.70 d	5.2(0.8) d	3.9(2.0) c	2.4(0.4) c	5.9(0.7) e
Norchip (E)	0.74[y]	4.4(1.6) c	3.5(2.2) b	1.9(0.3) b	3.1(0.9) bc
r[z]		0.62(0.13)	0.10(0.37)	0.19(0.29)	0.27(0.01)

[v] Letters in parentheses represent maturity, with VL = very late, L = late, ML = medium late, M = medium, and E = early.

[w] Values from 1985 field trials.

[x] Data represent six experiments with NAA, five with BAP, and two each with ABA and H_2O. Rating is on a scale of 0–9, with 0 = no symptoms and 9 = surface covered by lesions. Means in a column followed by a common letter do not differ significantly ($P = 0.05$) according to Fisher's least significant difference.

[y] Norchip was not grown in 1985, so value was estimated from 1983 and 1984 values.

[z] Correlation of mean leaf disk rating with AUDPC obtained from field trials at Hancock, Wisconsin, in 1985.

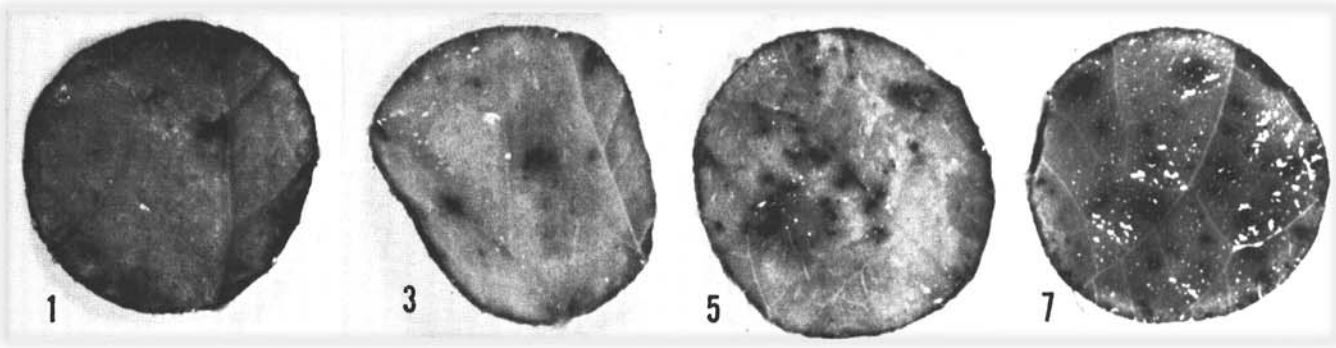

Fig. 1. Examples of ratings 1, 3, 5, and 7 of early blight on potato (*Solanum tuberosum*) leaf disks infected by *Alternaria solani*, where 0 = no symptoms, 1 = 1–3% of leaf area affected (necrotic specks), 2 = 4–6% (larger lesions with some chlorosis), 3 = 7–10%, 4 = 11–15%, 5 = 16–25%, 6 = 26–40%, 7 = 41–50%, 8 = 51–55%, and 9 = 56–100% of leaf affected with lesions.

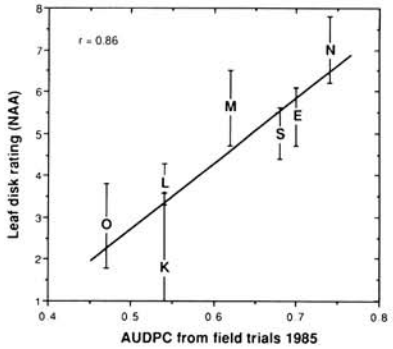

Fig. 2. Correlation of mean early blight ratings obtained from potato leaf disks floated on 10.7 μM 1-naphthaleneacetic acid (NAA), with area under disease progress curves (AUDPCs) from 1985 field trials ($r = 0.86$). Cultivars: O = Ontario, L = Langlade, K = Kennebec, M = Monona, S = Superior, E = Early Gem, and N = Norchip.

A.solani (3,000/ml) using a squeeze-bulb atomizer held approximately 4 mm from the leaf surface. Inoculated disks were returned, by use of a forceps, to the petri plates containing a phytohormone solution or water. Uninoculated leaf disks of each cultivar, marked with water-insoluble ink for identification as negative controls, were floated on the incubation solution along with inoculated disks.

Symptom evaluation. Lesion development was evaluated after 3 days (5 days for BAP). Inoculated leaf disks were rated visually on the basis of area covered by lesions, darkness of lesions, and presence of chlorosis, relative to negative controls. Each leaf disk was rated on a scale from 0 to 9, with 0 indicating no symptoms and 9 indicating complete coverage with lesions (Fig. 1). The area covered by one large lesion was given the same rating as an equivalent amount of area covered by several small lesions (12). The 10 ratings can be assigned to four categories of susceptibility, where 0 = immune (−); 1–3 = moderately resistant, with a few small lesions (+); 4–6 = moderately susceptible, with approximately one-third of the leaf disk covered by lesions (++); and 7–9 = highly susceptible, with over one-half the leaf disk covered with lesions (+++).

Cultivars having the lowest early blight susceptibility in Wisconsin field trials were used as resistant standards. Ratings given to the resistant standards in the leaf disk assay were arbitrarily used to establish the "resistance threshold" against which test material was compared. The resistance threshold is the highest early blight rating given to plants considered to have useful early blight resistance.

Accuracy of the leaf disk assay was further evaluated by comparison of leaf disk ratings with disease progress on each cultivar from Wisconsin field trials during 1985. Disease severity was evaluated weekly in field trials from early July to vine kill for each cultivar (J. W. Pscheidt and W. R. Stevenson, *unpublished*), using the 0–11 Horsfall-Barratt rating system (9). The area under the disease progress curve (AUDPC) (17), a numerical value representing cumulative loss of foliage to early blight over the season, was calculated for each cultivar.

RESULTS

Effect of water. Allowing senescence to occur naturally in excised tissue by floating inoculated leaf disks on deionized water in the dark for 3 days did not result in accurate assessment of early blight susceptibility as it appears in the field. Susceptible cultivars such as Superior and Norchip with high AUDPCs from field trials received low leaf disk ratings (Table 1). Cultivar reaction was highly variable among experiments, and correlations with 1985 field results were low ($r = 0.26$ and 0.28).

Effect of cytokinin. Amending deionized water with phytohormones resulted in a range of reactions. Incubation on the cytokinin BAP delayed symptom expression and decreased lesion development. Prolonged incubation periods (5 days) on BAP were required for lesion development. Susceptible cultivars did not consistently receive higher leaf disk ratings than resistant cultivars (Table 1). For example, mean ratings for the susceptible cultivar Norchip and the more resistant cultivar Kennebec, respectively, in five separate experiments were 1.2 and 1.4, 4.8 and 4.4, 7.0 and 1.4, 1.4 and 5.2, and 3.0 and 5.2. Susceptibility of the leaf disks on BAP sometimes correlated well with AUDPCs from 1985 but was not reproducible among the five experiments ($r = -0.46$, 0.43, 0.54, 0.15, and −0.18). A correlation as high as 0.54 was obtained only when leaf disks were derived from cultivars that were beginning to senesce at 11 wk postemergence.

Effect of abscisic acid. Incubation of leaf disks on ABA caused a rapid breakdown of tissue, interfering with normal lesion development. Lesions developed slowly on both resistant and susceptible cultivars before tissue necrosis. Correlations between leaf disk assays and field evaluations were variable ($r = -0.10$ and 0.48) (Table 1).

Effect of auxins. Disease expression was most similar to that observed in the field when leaf disks were incubated on the auxin NAA. Data were reproducible (Table 1) and highly correlated with field AUDPCs (Fig. 2). Correlation of mean leaf disk ratings with AUDPCs ranged from 0.45 to 0.86 in six separate experiments, with an average of 0.62 when the age of plants being assayed was 4–11 wk postemergence. In six experiments at 4 wk or more after emergence, leaf disks from the cultivars Russet Burbank, Atlantic, Monona, Superior, Early Gem, and Norchip, which are moderately to highly susceptible in the field, had higher early blight ratings than those from the cultivars Ontario, Nooksack, Langlade, and Kennebec, which are more resistant in the field (Table 1). All susceptible cultivars had mean early blight ratings significantly higher than the resistance threshold ($P = 0.05$), as determined by the resistant standards, when leaf disks were floated on NAA (Fig. 3). Resistance could not be distinguished from susceptibility using the resistance threshold when leaf disks were incubated on either H_2O, BAP, or ABA but was easily distinguished when leaf disks were incubated on NAA.

Reaction of tissue culture plants. Leaf disks assayed from plants taken directly

from tissue culture were severely attacked by *A. solani*, giving the appearance of high susceptibility (*personal observation*). Leaf disk ratings similar to field results were obtained by growing tissue culture transplants for 4 wk in a greenhouse and lowering the inoculum concentration to 2,000 conidia per milliliter.

In addition to NAA, successively higher concentrations of the auxins 2,4-D and IAA were evaluated until the assay was optimized. Accurate and reproducible estimates of early blight susceptibility were obtained when leaf disks were incubated on 10.7 μM NAA or 4.0 μM 2,4-D but not on 25 μM IAA. Highly susceptible cultivars could not be distinguished from the resistant standards when leaf disks from tissue culture plants were incubated directly on the auxin IAA. Susceptible cultivars such as Atlantic and Early Gem had lower leaf disk ratings than the resistant cultivars Ontario and Kennebec (Table 2). Mean leaf disk ratings on IAA correlated with AUDPCs ($r = 0.41$) from 1985.

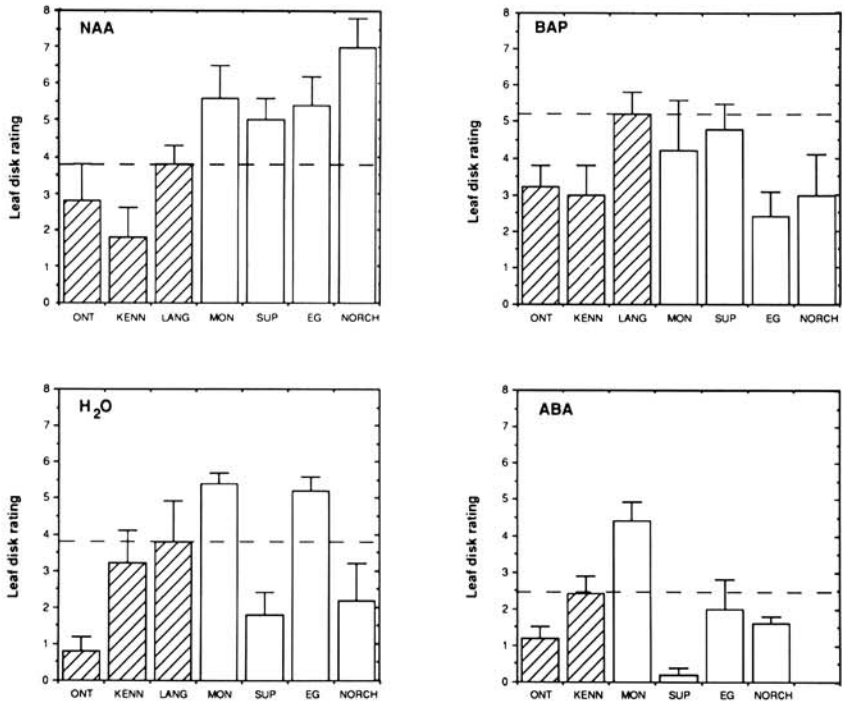

Fig. 3. Comparison of early blight ratings obtained by incubating potato leaf disks on 10.7 μM 1-naphthaleneacetic acid (NAA), 3.5 μM 6-benzylaminopurine (BAP), H_2O, or 0.04 μM abscisic acid (ABA). The dashed line represents the resistance threshold. Bars with diagonal lines represent resistant cultivars and open bars represent susceptible cultivars as determined by Wisconsin field trials. Cultivars: ONT = Ontario, KENN = Kennebec, LANG = Langlade (not tested with ABA), MON = Monona, SUP = Superior, EG = Early Gem, and NORCH = Norchip.

Table 2. Susceptibility to *Alternaria solani* of potato leaf disks 3.5 wk (2,4-D and IAA) or 5 wk (NAA) after transplanting from tissue culture to the greenhouse, when incubated on different auxin solutions

	Mean early blight rating of leaf disks (SE)[x]			
Cultivar	**2,4-D**	**IAA**	**NAA**	**AUDPC[y]**
Ontario	1.4(0.2) ab	1.4(0.5) bc	2.5(0.5) b	0.47 a
Kennebec	1.0(0.3) a	0.7(0.3) ab	0.5(0.3) a	0.54 bc
Langlade	1.4(0.2) ab	0.0(0.0) a	···	0.54 bc
Russet Burbank	2.6(0.4) bc	0.9(0.2) ab	4.8(0.7) c	0.59 cd
Atlantic	3.0(0.6) c	0.8(0.3) ab	3.8(0.5) c	0.60 d
Monona	4.7(0.4) d	2.7(0.8) c	4.0(0.4) c	0.62 d
Early Gem	2.6(0.6) bc	0.7(0.2) ab	5.0(0.6) c	0.70 ef
Norgold Russet	4.1(0.7) cd	1.7(0.5) bc	6.3(0.4) d	0.72 f
Norland	3.9(0.6) cd	2.1(0.6) c	3.5(0.2) bc	0.78 g
r[z]	0.71	0.41	0.57	

[x]Rating ($n = 7$) is on a scale of 0–9, with 0 = no symptoms and 9 = surface covered by lesions. 2,4-D = 2,4-Dichlorophenoxyacetic acid (4 μM), IAA = indoleacetic acid (25 μM), NAA = 1-naphthaleneacetic acid (10.7 μM). Means in a column followed by a common letter do not differ significantly ($P = 0.05$) according to Fisher's least significant difference.

[y]Values from 1985 field trials.

[z]Correlation of in vitro early blight ratings with 1985 AUDPCs obtained from field trials at Hancock, Wisconsin.

Early blight susceptibility was accurately determined when either 2,4-D or NAA was used as an incubation medium. Although cultivars susceptible to early blight in the field had higher leaf disk ratings than the resistant standards on both NAA and 2,4-D, greater distinction was obtained using 2,4-D (Table 2). Mean leaf disk ratings for cultivars that originated in tissue culture correlated highly with AUDPCs when incubated on 2,4-D ($r = 0.71$) and NAA ($r = 0.57$).

Reaction of plants grown from true seed. The assay was also applied to *Solanum* species grown from true seed. Inoculated leaf disks taken from true seed-derived plants varied from highly resistant to highly susceptible, compared with standards derived from tissue culture, when incubated on NAA (Table 3).

Soil effect. Growth of cultivars in two different soil types had no observable effect on evaluation of leaf disks for early blight susceptibility. Results were not significantly different in five of seven experiments when material tested was grown in a greenhouse vs. a growth chamber.

Plant age effect. Plant age did not have a significant effect on evaluation of early blight resistance when plants were older than 3 wk postemergence. Susceptibility could not be detected in the in vitro assay when leaf disks were taken from plants 3 wk postemergence.

DISCUSSION

Modification of juvenile leaf tissue by incubating inoculated, excised disks on NAA or 2,4-D resulted in accurate, reproducible quantification of early blight resistance. The method requires substantially less time for implementation than field trials, is less subject to concomitant infections under controlled conditions, may be more independent of cultivar maturity than field trials, and is low in cost. The leaf disk assay effectively reproduced the resistance reaction of cultivars ranging from early to very late maturity with varying levels of susceptibility to early blight in the field. The cultivars Ontario, Kennebec, Langlade, and Nooksack are least susceptible in Wisconsin field trials and were used as resistant standards to establish an arbitrary resistance threshold to which other lines could be compared. Evaluation of susceptibility using this assay is accurate when all susceptible cultivars have an average leaf disk rating higher than the resistance threshold.

The resistance threshold can be used to determine the sensitivity, specificity, and predictive value (4) of the leaf disk assay. Sensitivity is defined here as the percentage of cultivars with significant early blight resistance in the field that are evaluated as resistant in the leaf disk assay, or true positives. Specificity is the percentage of cultivars appearing relatively susceptible in the field that are

Table 3. Susceptibility to *Alternaria solani* of leaf disks from *Solanum* species derived from true seed, when incubated on 1-naphthaleneacetic acid (10.7 μM)

Solanum species	PI accession or cultivar	No. of plants evaluated	Early blight rating (SE)[y]
S. tarijense	473228	7	2.6(0.3) bcde
	473220	8	1.9(0.5) bc
	473222	15	0.0(0.0) a
	414148	8	3.0(0.6) cdef
	414149	6	3.5(1.0) defg
	414150	8	2.2(0.7) bcd
	414152	7	2.6(0.6) bcde
	458364	4	2.2(1.0) bcd
	217457	5	2.2(0.6) bcd
	217458	8	1.4(0.3) b
	265577	8	1.8(0.2) bc
S. chacoense	WRF320	14	3.6(0.3) efg
	472826	11	3.0(0.5) cdef
	472824	15	2.9(0.4) cde
	472825	11	4.2(0.5) fg
	WRF324	11	2.5(0.4) bcd
	WRF363	7	5.6(0.6) h
	WRF369	15	2.7(0.3) cde
	WRF378	7	2.6(0.5) bcde
	WRF888	8	3.5(0.6) defg
S. tuberosum[z]	Kennebec (resistant)	10	3.0(0.3) cde
	Norgold Russet (susceptible)	7	4.6(0.4) gh
	Norland (susceptible)	8	4.5(0.5) gh

[y] Rating is on a scale of 0–9, with 0 = no symptoms and 9 = surface covered by lesions. Means followed by different letters are significantly different ($P = 0.05$) according to Fisher's least significant difference.

[z] Plants were derived from tissue culture.

evaluated as susceptible in the assay, or true negatives. The predictive value, or accuracy of the assay, is the percentage of correct evaluations out of the total number of determinations.

Plants originating from tuber seed pieces should be grown in either a growth chamber with a 12-hr photoperiod (350 $\mu E \cdot m^{-2} \cdot s^{-1}$) or in a greenhouse under supplemental fluorescent lights with a 14-hr photoperiod. Plants originating from tissue culture or true seed should be grown at least 4 wk under high-intensity lights (430–730 $\mu E \cdot m^{-2} \cdot s^{-1}$). All plants should be grown at 20 ± 3 C, watered as needed, and fertilized with a nutrient solution (6) every third watering. Evaluation should be made on the third or fourth leaf from the apex by excising a 14-mm leaf disk, inoculating it with *A. solani* (3,000 conidia per milliliter for tuber-grown plants and 2,000 conidia per milliliter for plants grown from tissue culture or true seed), and floating it on a solution of 10.7 μM NAA (or 4 μM 2,4-D for true seed and tissue culture plants). Leaf disks can be rated for lesion development after incubation at 22 C in the dark for 3 days. The mean leaf disk rating for each accession should be compared to ratings given to resistant and susceptible standards.

Incubation of leaf disks on water caused a variable response possibly because of differences in phytohormone concentrations within individual leaves. The auxins NAA and 2,4-D acted similarly in their ability to regulate senescence. The leaf disk assay using NAA or 2,4-D distinguished resistance from late maturity as well as or better than field trials. True resistance (resistance detected in assay on cultivars that are resistant in field trials) was detected with a sensitivity of 82%. True susceptibility (susceptibility detected in assay on cultivars that are susceptible in field trials) was detected with a specificity of 95%. The overall predictive value of the leaf disk assay using NAA as an incubation medium is 90%, as determined on nine different cultivars in six replicate experiments. The very late-maturing cultivar Ontario, which is highly resistant in the field, was consistently rated slightly more susceptible in the leaf disk assay when incubated on NAA than cultivars with higher susceptibility in the field, suggesting that the in vitro assay may be less influenced by cultivar maturity than field trials.

A high AUDPC value is indicative of high susceptibility to early blight. For example, the susceptible cultivar Norchip had an AUDPC of 0.74, whereas the cultivar Ontario, which is highly resistant to early blight in the field, had an AUDPC of 0.47. AUDPCs vary slightly from year to year because of variations in environmental conditions affecting disease development.

This assay is more accurate than previously described detached leaf and seedling screens. The results of this study indicate, however, that certain precautions are necessary. Standards of known high and low susceptibility should be run simultaneously with test material in each assay. Standards should consist of cultivars commonly grown in the area in which the new breeding material will be employed. The cultivar chosen as the "resistant" standard should show low levels of susceptibility in areas where the potatoes will be grown. Plant material used for standards should be the same age as test material. The uninoculated "negative" control monitors the effects of incubation conditions on each cultivar. It is important that test material be free from injury, since lesions caused by insects or physical or chemical damage are easily confused with necrotic early blight lesions on leaf disks.

Preliminary studies indicate that resistance to early blight can be evaluated in *Solanum* species grown from true seed. The delicate physical nature of plants grown from true seed is similar to that of plants transplanted from tissue culture, so tissue culture transplants were used as resistant and susceptible standards for comparison. The assay permits preliminary selection of desirable plants from large quantities of germ plasm grown from tissue culture, true seed, or tuber seed pieces in the greenhouse. Subsequently, selected lines receiving ratings lower than the resistant standard can be evaluated more extensively in field trials.

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