# Reactions of *Helianthus annuus* and *H. tuberosus* Plant Introductions to *Alternaria helianthi*

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**ABSTRACT**

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Of 497 *Helianthus annuus* plant introduction accessions evaluated during 3 yr of field tests, only eight had equal or significantly ($P = 0.05$) less leaf blight than a commercial *H. annuus* hybrid when inoculated with *Alternaria helianthi*. No differences were detected between the commercial hybrid and the eight *H. annuus* accessions when inoculated in the greenhouse with conidial concentrations of 150, 300, 3,000, or 20,000/ml. Of 13 *H. tuberosus* accessions tested during 2 yr of field tests, all had significantly less leaf blight than the commercial *H. annuus* hybrid. In greenhouse tests, using 20,000 conidia per milliliter, all *H. tuberosus* accessions had significantly lower disease indices (0.8–2.4) than the *H. annuus* hybrid (5.0), which was killed. *H. tuberosus* accessions developed four distinct lesion types dissimilar to those produced on *H. annuus*. Results indicate that *H. tuberosus* may be a good source of resistance to *A. helianthi*.

Alternaria leaf blight and stem spot, incited by *Alternaria helianthi* (Hansf.) Tubaki & Nishihara, is a common disease of sunflower (*Helianthus annuus* L.) in the United States (3,4,6,8) and other regions of the world (1,2). Although little information is available on the economic importance of Alternaria blight, its common occurrence in sunflower-growing areas and yield-loss studies conducted in South Dakota (3) and Australia (2) indicate that it is a potentially serious disease. The most economical means for reducing disease losses would be to develop commercial hybrids with genetic resistance.

Resistance in *H. annuus* to *A. helianthi* has been reported (1,4). Carson (4) indicated, however, that even though significant differences could be detected among sunflower genotypes, the usefulness of the level of resistance in reducing disease loss was uncertain. This conclusion was supported by evidence that an inbred line (HA89), judged resistant to *A. helianthi*, sustained yield losses as great as 60% after field inoculations (3,4). Morris et al (8) reported that three perennial *Helianthus* spp., including *H. tuberosus* L., were moderately resistant to *A. helianthi* in greenhouse tests. They suggested that resistance could be transferable to cultivated sunflower by backcross breeding of inbreds with resistant perennial species.

The purpose of this study was to evaluate the reactions of *H. annuus* and *H. tuberosus* plant introductions (PI) and two local *H. tuberosus* clones to *A. helianthi* under field and greenhouse conditions.

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## MATERIALS AND METHODS

***Helianthus* spp.** Seeds of *H. annuus* and tubers of *H. tuberosus* PI accessions were obtained from the USDA North Central Regional Plant Introduction Station, Iowa State University, Ames. Two additional *H. tuberosus* clones, designated OH red and OH white, were collected from a roadside in Wayne County, Ohio. A commercial *H. annuus* hybrid, Stauffer S3101, was included in all greenhouse and field tests for comparison. This hybrid was susceptible to *A. helianthi* but was no more susceptible than any of the 46 commercial hybrids examined in preliminary tests.

**Inoculum and inoculations.** Two single-conidial isolates of *A. helianthi* were used. One, obtained from overwintered sunflower residue collected from a commercial field in Wayne County in 1980, was used in the initial field screening of *H. annuus* PI accessions in 1981. The second isolate (29-82), obtained from overwintered residues in Preble County, Ohio, in spring of 1982, was used in all other greenhouse and field tests. Isolates were chosen on the basis of their virulence in preliminary tests on hybrid Stauffer S3101.

Inoculum for field tests was produced by growing *A. helianthi* on fresh potato-dextrose agar (FPDA) (5) at 24 ± 2 C under continuous fluorescent light (18 $\mu E/m^2/sec$) in 9-cm-diameter petri plates. Inoculum was prepared by comminuting one 3- to 4-wk-old culture per 100 ml of sterile distilled water (SDW) in a Waring Blendor at low speed. Inoculum was poured into a hand-pump sprayer, and about 4–8 ml of inoculum was sprayed on the top leaves (rosette) of each plant. *H. annuus* accessions were inoculated at growth stage V6–V8 and again at V12–V14 (11) in all tests. *H. tuberosus* accessions were inoculated when the tallest stems had six to 10 leaves and again when the stems had 12–16 leaves.

Inoculum for greenhouse tests consisted of conidial suspensions prepared by adding 10 ml of SDW to each 3- to 4-wk-old culture on V-8 juice agar (7). The agar surface of flooded cultures was scraped, and the resulting suspension was strained through two layers of cheesecloth. Conidial concentrations were determined with a hemacytometer and adjusted by dilution with SDW to 150, 300, 3,000, or 20,000/ml. Plants were inoculated by atomizing (0.7 $kg/cm^2$ pressure) conidial suspensions for about 4 sec onto the top four expanded leaves of each plant. *H. tuberosus* and *H. annuus* accessions were

inoculated when plants had six to 10 and six expanded leaves, respectively.

**Field test site.** Field plots were established at the Ohio Agricultural Research and Development Center, Wooster, on Wooster silt loam soil. Fields were spring plowed, fertilized by broadcasting 112, 84, and 84 kg/ha N, P, and K, respectively, and disked before seedbed preparation. Weeds were controlled by preplant incorporation of trifluralin (1.12 kg a.i./ha). Seeds of *H. annuus* were planted with a cone-type seeder or by hand. Experimental units consisted of single rows 6 m long and 0.75 m apart with 25–30 seeds planted per row. Tubers of *H. tuberosus* accessions were planted by hand, three tubers per 1.5-m row with 0.75 m between rows.

**Field tests.** A preliminary screening trial consisting of 476 *H. annuus* PI accessions was planted on 8 June 1981 in single-row plots (no replicates). Not all *Helianthus* accessions in the USDA collection were tested. The small-seeded, wild *H. annuus* accessions (PIs 413009–413170) and *Helianthus* sp. (PIs 41317–413181) were not tested because of poor emergence in this field trial. In 1982, 45 *H. annuus* PI accessions tested in 1981 plus 21 additional accessions not previously tested were planted on 24 June. The 1983 trial, planted on 17 June, consisted of eight accessions selected from the 1981 and 1982 trials. The 1982 and 1983 trials were planted in a randomized complete block design with four blocks (replicates). *H. tuberosus* accessions were tested in the field for 2 yr. Thirteen accessions were planted 17 June 1983, and 11 of those were planted 12 May 1984 in a randomized complete block design with four blocks. The susceptible *H. annuus* hybrid, Stauffer S3101, was planted in each trial for comparison with the *H. tuberosus* accessions.

Because growth of different *H. annuus* accessions varied, disease severity was assessed when most plants in the experiment were in the R5–R6 growth stages. Disease severity was determined on the basis of a visual rating of all plants in a row. The leaf spot index was calculated as the product of two disease assessment scores. The first score was based on the percentage of leaves on plants affected (0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%). The second score was based on percentage of leaf area affected on inoculated leaves (0 = 0%, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, and 4 = 51–100%). The stem spot rating was determined at the same time as the leaf spot index and was based on the relative size and number of stem lesions (0 = no spots, 1 = few flecks, 2 = many flecks, 3 = few blotches, and 4 = many blotches).

A different disease severity rating system was used on *H. tuberosus* accessions because symptoms were only observed on inoculated leaves. The disease index was based on the percentage of leaf area with lesions (1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%). Disease was assessed on inoculated leaves at the R2–R3 growth stages.

Sporulation on leaves of *H. tuberosus* accessions and the *H. annuus* hybrid S3101 from the 1984 field test was evaluated. Three leaves with lesions were collected from different plants per row the day plants were evaluated for disease severity. Leaves were incubated in a plastic bag with a moistened paper towel at 22–25 C under fluorescent lights (650 $\mu E/m^2/sec$) for 10 hr/day. After 4 days, lesions were examined microscopically (60×) and the relative amount of sporulation assessed as 0 = none, 1 = few, 2 = moderate, or 3 = many conidia per lesion.

**Greenhouse tests.** Greenhouse tests were conducted at different times during 1982 and 1983. All tests were a completely randomized design with five replicate pots per accession, and all tests were repeated twice. Five seeds of *H. annuus* were planted per 15-cm-diameter pot containing a mixture of sand and Marengo silty clay soil (1:1, v/v). Emerging seedlings were thinned to two plants per pot. One tuber of *H. tuberosus* was planted per 15-cm-diameter pot of the same soil mixture. Plants were grown on a greenhouse bench at 23 ± 5 C with

**Table 1.** Reactions of *Helianthus annuus* to *Alternaria helianthi* during 2 yr of field tests at Wooster, OH

| | | 1982 | | 1983 | |
|---|---|---|---|---|---|
| PI no. or line | Origin | Leaf spot index[a] | Stem spot rating[b] | Leaf spot index | Stem spot rating[b] |
| 162675 | Argentina | 7.5 | 2.0 | 5.0 | 2.5 |
| 170390 | Turkey | 7.3 | 1.8 | 6.3 | 1.5 |
| 175731 | Turkey | 9.0 | 2.0 | 6.3 | 2.0 |
| 228345 | Iran | 8.3 | 2.0 | 6.5 | 2.5 |
| 250855 | Iran | 8.3 | 1.8 | 6.0 | 1.5 |
| 250856 | Iran | 8.3 | 1.3 | 6.8 | 1.3 |
| 323279 | Pakistan | 9.0 | 0.3 | 9.0 | 1.5 |
| 377530 | Kenya | 9.0 | 1.5 | 7.8 | 1.3 |
| S3101 | Commercial hybrid[c] | 11.0 | 2.7 | 10.0 | 3.0 |
| LSD ($P = 0.05$) | | 2.0 | 0.6 | 2.6 | 1.2 |
| Test mean | | 10.9 | 2.6 | 7.1 | 1.7 |
| No. of entries | | 67 | | 9 | |

[a] Leaf spot index equals the first score multiplied by the second score, where the first score is based on percentage of leaves affected per plant (1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%) and the second score is based on percentage of leaf area affected on inoculated leaves (1 = 1–10%,, 2 = 11–25%, 3 = 26–50%, and 4 = 51–100%).

[b] Stem spot rating is based on size and number of stem lesions, where 1 = few small flecks, 2 = many small flecks, 3 = few blotches, 4 = many blotches.

[c] *H. annuus* hybrid used for comparison.

**Table 2.** Reactions of *Helianthus tuberosus* to *Alternaria helianthi* during 2 yr of field tests at Wooster, OH

| | | | 1984 | |
|---|---|---|---|---|
| PI no. or line | Origin | 1983 Disease index[a] | Disease index | Lesion sporulation rating[b] |
| 357297 | USSR | 0.0 | 4.0 | 0 |
| 357298 | USSR | 0.0 | 1.3 | 0 |
| 357299 | USSR | 0.0 | | |
| 357300 | USSR | 0.0 | 0.8 | 1 |
| 357301 | USSR | 0.0 | 1.4 | 1 |
| 357302 | USSR | 0.0 | 1.1 | 0 |
| 357303 | USSR | 0.0 | 1.4 | 0 |
| 357304 | USSR | 0.0 | 1.0 | 0 |
| 451980 | North Dakota | 0.0 | | |
| 458544 | Germany | 1.0 | 1.4 | 1 |
| 461518 | USSR | 0.0 | 1.2 | 0 |
| OH white | Ohio | 0.0 | 1.7 | 0 |
| OH red | Ohio | 0.0 | 1.4 | 1 |
| S3101 | Commercial hybrid[c] | 4.0 | 4.2 | 3 |
| LSD ($P = 0.05$) | | ...[d] | 1.5 | ...[d] |

[a] Disease index based on percentage of leaf area with lesions on four inoculated leaves, where 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%.

[b] Lesion sporulation rating determined by placing three leaves with lesions per replicate in a moist chamber for 4 days and visually assessing sporulation within lesions microscopically (60×), where 0 = none, 1 = few, 2 = moderate, and 3 = many conidia per lesion.

[c] *H. annus* hybrid used for comparison.

[d] No statistical analysis performed due to obvious differences in data obtained.

supplemental fluorescent lighting (16 $\mu E/m^2/sec$) for 12 hr/day. Because of space limitations, each test consisted of accessions inoculated with only one conidial concentration (150, 300, 3,000, and 20,000/ml). After inoculation, plants were immediately placed in a mist chamber at 24 C for 48 hr, then returned to the greenhouse, and disease severity was assessed 5–7 days later. Lesions on each of four inoculated leaves per plant were counted in tests using concentrations of 150 and 300 conidia per milliliter. The number of lesions per leaf could not be determined in tests using 3,000 and 20,000 conidia per milliliter because of extensive coalescence of lesions and necrosis. The same disease index (based on percentage leaf area with lesions) for assessing disease severity on *H. tuberosus* in field tests was used. Data from both field and greenhouse tests were analyzed by analysis of variance, and Fisher's LSD ($P<0.05$) was used to compare treatment means, except where noted in the tables.

## RESULTS

**Field tests: *H. annuus*.** Of the 476 *H. annuus* PI accessions tested in 1981, only 45 had less than 10% of the surface of inoculated leaves covered with lesions. Most PI accessions had 25–50% of the leaf area affected, with up to 75% of the leaves per plant bearing lesions. The 45 accessions with less disease were tested in 1982 with 21 additional PI accessions and a susceptible commercial hybrid (S3101). Eight of these accessions had statistically ($P=0.05$) lower leaf spot indices and stem spot ratings than S3101 (Table 1). In 1983, only six of the original eight PI accessions tested had lower ($P=0.05$) leaf spot indices and five had lower stem spot ratings than S3101. Leaf lesions on all *H. annuus* PI accessions had dark brown

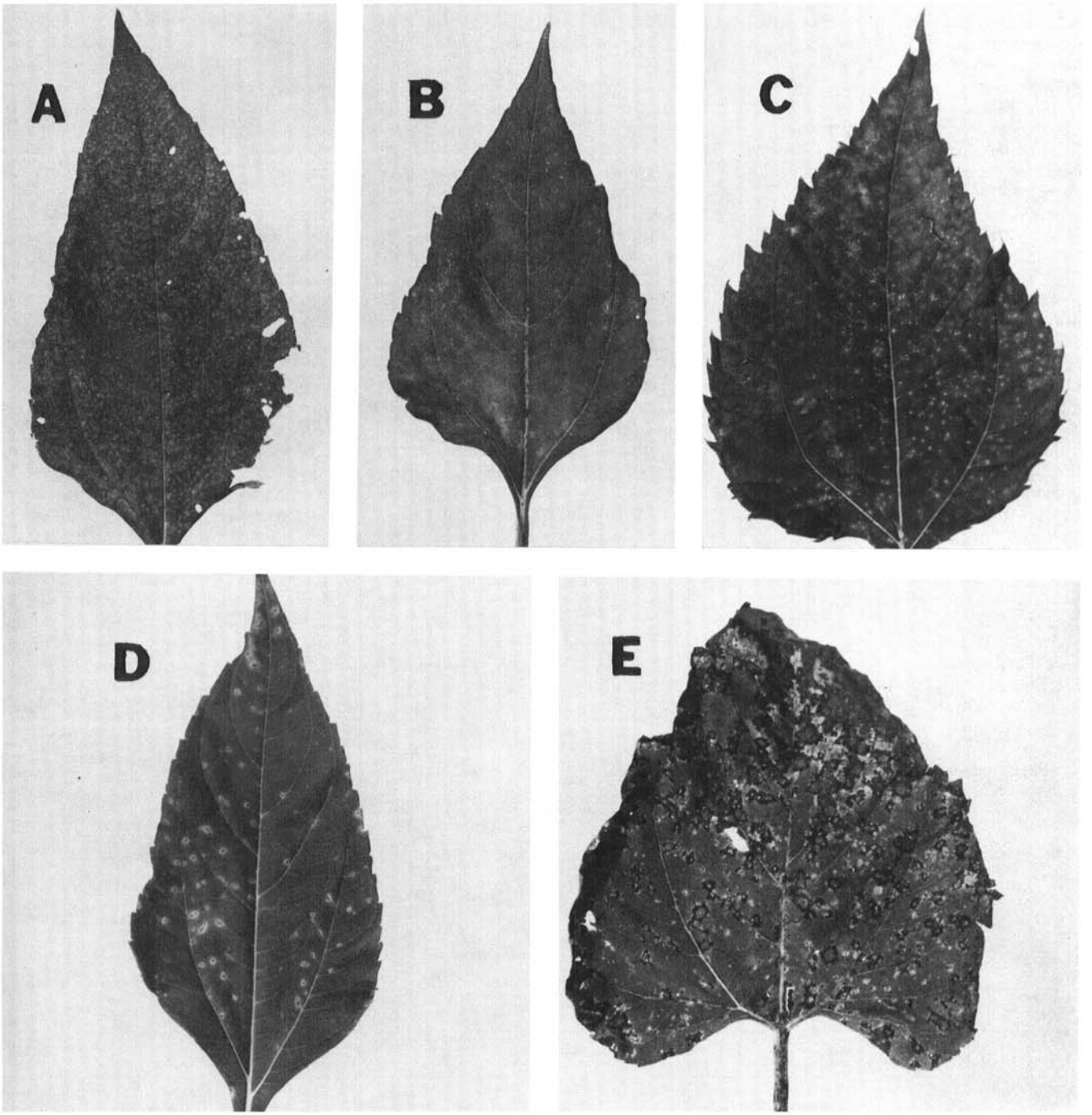


**Fig. 1.** Lesion types observed on *Helianthus tuberosus* and *H. annuus* inoculated with *Alternaria helianthi* in the field. *H. tuberosus* **(A)** PI 357297 with numerous small chlorotic flecks, **(B)** PI 357298 with numerous small necrotic flecks, **(C)** OH white with necrotic flecks and chlorotic halos, and **(D)** OH red with larger necrotic lesions and chlorotic halos and *H. annuus* **(E)** Stauffer S3101 hybrid with large necrotic lesions and brown to gray centers and dark brown borders frequently surrounded by chlorotic halos.

borders with brown to gray centers, frequently surrounded by chlorotic halos when small and enlarging.

**Field tests: *H. tuberosus*.** Only one of the 13 *H. tuberosus* accessions tested in 1983 developed recognizable lesions (Table 2). This clone (PI 458544) developed few small necrotic flecks with chlorotic halos. The *H. annuus* hybrid (S3101) developed characteristic large necrotic lesions on 50–75% of the surface of inoculated leaves. In the 1984 trial, all 11 *H. tuberosus* accessions tested developed lesions. The *H. annuus* hybrid developed extensive leaf lesions on inoculated leaves (disease index 4.2), and one *H. tuberosus* clone, PI 357297, developed numerous flecks on inoculated leaves (disease index 4.0). All other *H. tuberosus* accessions had statistically ($P = 0.05$) lower disease indices than the *H. annuus* hybrid. Although lesions developed on these *H. tuberosus* accessions, four distinct lesion types were detected. One clone (PI 357297) developed numerous small chlorotic flecks (Fig. 1A), six clones (PI 357298, PI 357300, PI 357301, PI 357302, PI 357304, and PI 461518) developed small necrotic flecks (Fig. 1B), three clones (PI 357303, PI 458544, and OH white) developed necrotic flecks with chlorotic halos (Fig. 1C), and one clone (OH red) developed necrotic lesions with chlorotic halos (Fig. 1D). The necrotic lesions on OH red were much smaller than those developing on S3101 (Fig. 1E). No lesions developed on uninoculated *H. tuberosus* leaves throughout the rest of the season, but lesions developed on uninoculated leaves and on the uppermost leaves of the *H. annuus* hybrid by plant maturity. Only a few conidia developed in four of the 11 *H. tuberosus* accessions tested after incubation of leaves under moist conditions (Table 2). Neither conidia nor conidiophores developed on lesions of the other seven *H. tuberosus* accessions, but profuse conidial development occurred on lesions of the *H. annuus* hybrid.

**Greenhouse tests: *H. annuus*.** At the two lower conidial concentrations (150 and 300/ml), the number of lesions per leaf could be easily counted, but at the higher inoculum levels (3,000 and 20,000/ml), lesions coalesced and the number of lesions per leaf could not be determined accurately. A disease index based on percentage leaf area covered by lesions therefore was used to evaluate disease severity at the two higher inoculum levels. No significant ($P = 0.05$) differences were detected among the *H. annuus* accessions tested at any inoculum level (Table 3). Leaves of all plants inoculated at 20,000 conidia per milliliter were flaccid when taken from the mist chambers, and plants died shortly after being placed on the greenhouse bench. Death of plants was due to development of stem lesions that caused necrosis of the growing point and stems. None of the accessions appeared to have less disease than the *H. annuus* hybrid S3101.

**Greenhouse tests: *H. tuberosus*.** Preliminary tests indicated that *H. tuberosus* accessions were much less susceptible to *A. helianthi* than *H. annuus* accessions; therefore, only the highest inoculum level (20,000 conidia per milliliter) was used in tests. All 11 *H. tuberosus* accessions had significantly ($P = 0.05$) lower disease indices than the *H. annuus* hybrid (S3101) used for comparison (Table 4). In both trials, all S3101 plants died after inoculation with 20,000 conidia per milliliter. The percentage of leaf area with lesions on the *H. tuberosus* accessions ranged from 3 to 40 depending on the accession tested. Two clones, OH white and OH red, had the lowest mean disease indices, 0.8 and 1.0, respectively, for the two tests. The accessions produced lesion types similar to those that developed on leaves in the field tests.

## DISCUSSION

Of the 497 *H. annuus* PI accessions tested during 3 yr of field tests, only eight were identified with equal or lower disease severity than the susceptible commercial hybrid chosen for comparison. When PI accessions were tested under greenhouse conditions with four levels of inoculum, no differences in disease severity could be detected among them or the commercial hybrid. This may indicate that the *H. annuus* PI genotypes may have had some degree of field or mature plant resistance compared with the commercial hybrid or that greenhouse test conditions were unsatisfactory to determine the differential response observed in the field. The type of resistance observed in field tests with *H. annuus* was expressed quantitatively as a reduction in percentage of leaf area affected (lesion number), and there was no indication of a qualitative or lesion-type resistance among the accessions tested. Because the level of resistance detected in *H. annuus* was not great, or only slightly improved over the commercial hybrid, the search for better sources of resistance in *H. annuus* should continue before starting a breeding program. The North American wild *H. annuus* accessions should be examined.

*H. tuberosus* appears to be a promising source of resistance to *A. helianthi;* *H. tuberosus* accessions showed a high degree of resistance in both field and greenhouse studies (Tables 2 and 4). They not only had significantly less leaf area affected than the *H. annuus* hybrid but also restricted or prevented sporulation within lesions. The *H. tuberosus* accessions developed four distinct lesion types quite different from those of the *H. annuus* hybrid. More information is needed on the genetics and physiology of resistance in *H. tuberosus* before the significance of these lesion types can be understood. The possibility exists that the resistance in *H. tuberosus* could be transferred to agronomically acceptable

**Table 3.** Reactions of *Helianthus annuus* to *Alternaria helianthi* at four conidial concentrations in the greenhouse

| PI no. or line | No. lesions per leaf[a] | | Disease index[b] | |
|---|---|---|---|---|
| | 150 Conidia/ml | 300 Conidia/ml | 3,000 Conidia/ml | 20,000 Conidia/ml |
| 162675 | 5.8 | 9.6 | 4.3 | 5.0 |
| 170390 | 5.4 | 9.8 | 4.5 | 5.0 |
| 175731 | 4.3 | 8.3 | 3.8 | 5.0 |
| 228345 | 5.1 | 9.1 | 4.0 | 5.0 |
| 250855 | 4.5 | 9.0 | 4.0 | 5.0 |
| 250856 | 4.5 | 9.7 | 4.0 | 5.0 |
| 323279 | 4.2 | 6.8 | 3.6 | 5.0 |
| 377530 | 5.3 | 6.6 | 3.6 | 5.0 |
| S3101 | 5.3 | 11.1 | 3.7 | 5.0 |
| LSD ($P = 0.05$) | NS[c] | NS | NS | NS |

[a] Mean number of lesions per leaf of four inoculated leaves per plant, two plants per pot, five pots per PI accession of two tests per inoculum concentration.
[b] Mean disease index based on percentage of leaf area covered by lesions, where 0 = none, 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%.
[c] NS indicates no statistical difference ($P = 0.05$) among PI accessions according to Fisher's least significant difference test.

**Table 4.** Reactions of *Helianthus tuberosus* to *Alternaria helianthi* (20,000 conidia/ml) in the greenhouse

| PI no. or line | Disease index[a] |
|---|---|
| 357297 | 2.0 |
| 357298 | 2.1 |
| 357299 | 1.8 |
| 357300 | 2.0 |
| 357301 | 1.7 |
| 357302 | 1.9 |
| 357303 | 2.0 |
| 357304 | 2.0 |
| 451980 | 2.4 |
| 458544 | 2.3 |
| 461518 | 2.0 |
| OH white | 0.8 |
| OH red | 1.0 |
| S3101 (*H. annuus*) | 5.0 |
| LSD ($P = 0.05$) | 0.6 |

[a] Mean disease index of two trials based on percentage of leaf area with lesions on four inoculated leaves per plant, two stems from one tuber per pot, five pots per accession, where 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%.

hybrids by interspecific crosses. Interspecific crosses with different *Helianthus* spp. has been accomplished in the Soviet Union (9), and cultivars have been developed with immunity to several diseases through interspecific hybridization with *H. tuberosus* (10). Interspecific hybridization of *H. annuus* and *H. tuberosus* for obtaining high levels of resistance to *A. helianthi* should be explored further.

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