Production of Inoculum and Field Assessment of *Alternaria helianthi* on Sunflower

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ABSTRACT

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Alternaria helianthi grew and sporulated better on potato-dextrose agar amended with a sunflower seed extract than on other media tested. Production of inoculum for field and laboratory studies was achieved by culturing the pathogen on filter paper to which a solution containing sucrose and sunflower seed extract had been added. Inoculum produced on the filter paper was stored intact on the mycelium for up to 6 mo with little loss of viability. A pictorial key based on a percentage scale was developed for the assessment of Alternaria blight on sunflower in the field. Regression analysis showed that the percentage of leaf area infected by *A. helianthi* for the whole plant was directly proportional to the level of infection on the lowest unsenesced leaves.

Additional key words: *Helianthus annuus*

Alternaria helianthi (Hansf.) Tubaki & Nishihara has been described as a serious pathogen of sunflower in Yugoslavia (14), India (1,17), and Australia (8). In recent years, the pathogen has been recorded in North America (11,22).

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Early reports on *A. helianthi* isolated from diseased sunflower plants indicated that the pathogen both grew and sporulated well on potato-dextrose agar (PDA) (24). However, subsequent reports referred to very slow growth and abundant sporulation on the same medium (2,13,21). Preliminary tests undertaken by the senior author confirmed these latter reports. Consequently, an attempt was made to find a medium that allowed rapid growth of *A. helianthi* as well as abundant sporulation.

Dry inoculum of *A. helianthi* was required for use in laboratory, glasshouse, and field experiments. However, the collection of dry conidia growing on agar-based media proved to be difficult, and the media tended to dehydrate within a few weeks. It was thus necessary to develop a method for producing and storing conidia of *A. helianthi.*

Many different techniques have been used to assess the severity of Alternaria blight in sunflower, particularly with respect to the evaluation of fungicides for the control of the disease. Gupta et al (9) allocated diseased leaves to one of three groups: leaves with first blight symptoms, leaves at the half-blight stage, and leaves at the full-blight stage. This simple and arbitrary approach has been expanded and improved by many workers. Kolte and Tewari (17), in a study of the effect of planting dates on the occurrence and severity of sunflower diseases, assessed

the mean number of spots per leaf on the tenth leaf (from the top) at the "50% flowering stage of growth." Bhowmik and Singh (7) based their assessment of Alternaria blight severity on James' (15) scale for Stemphylium leaf spot of red clover. These authors recognized the following five blight severity groups: A = healthy; B = light infection (up to an average of 10% of the leaf area blighted per plant); C = moderate infection (25% of leaf area blighted); D = severe infection (50% of leaf area blighted); and E = very severe infection (75% of leaf area blighted).

Sindhamathar et al (23) used a modified Cobb scale to assess the levels of Alternaria blight in sunflower crops. They recognized the following six grades of blight severity: 0 = no spots; 1 = few, small scattered spots on leaf; 2 = 25% of leaf area infected; 3 = 50% infected; 4 = 75% infected; and 5 = 100% infected.

Several authors (3,6,16) have used a grading system for individual plants based on the percentage of the foliage showing disease symptoms and have then applied the McKinney grouping method (19) to calculate the percentage of disease present in the field or plot. Horsfall and Heuberger (12) applied McKinney's technique to the assessment of a defoliation disease of tomato caused by *A. solani* (Ell. & Mart) Jones & Grout. They concluded that the method was quite precise but considered that difficulties in relating results from different workers and different seasons would have been reduced had a standard, pictorial disease assessment key been used.

One of the purposes of the studies reported in this paper was to develop a standard pictorial key for the assessment of Alternaria blight of sunflower in the field and to identify and evaluate methods of using such a key to obtain quick, consistent, and accurate estimates of disease severity under field conditions.

MATERIALS AND METHODS

Artificial medium for growing *A. helianthi*. The growth and sporulation of *A. helianthi* on five different media were compared. These media included PDA prepared from dehydrated powder (Oxoid CM139); Richard's solution (5), modified by the addition of 20 g of agar per liter of medium; PDA plus Richard's solution; PDA plus a sunflower seed extract that was prepared by placing 100 g of sunflower seed (cv. Peredovik) in 1 L of water in a Waring Blendor for 2 min and filtering the resulting product through one layer of cheesecloth; and Richard's solution plus sunflower seed extract. Richard's solution was used because Gupta et al (10) and Lodha and Prasad (18) had successfully used this medium for their studies with *A. brassicae* (Berk.) Sacc. and *A. solani*, respectively. Petri dishes containing the various media were inoculated with *A. helianthi* and incubated at 23 C for 7 days under continuous light (fluorescent tubes, 3,500 lux). The mean colony diameter was determined for each of six replicates, and the abundance of mycelium and amount of sporulation were recorded as either sparse or abundant.

Production of dry inoculum. Preliminary studies showed that sunflower seed extract stimulated sporulation and growth. Consequently, sunflower seed extract was used to produce conidia of *A. helianthi* on filter paper. Three Whatman No. 1 filter papers were placed in a clean petri dish, and 7 ml of sunflower seed extract (prepared as before) amended with 0.2% sucrose were added. After sterilization in an autoclave at 105 kPa for 15 min, the top filter paper was inoculated by wiping an inverted, small square of agar (from a densely sporulating colony of *A. helianthi*) across the surface. Inoculated petri dishes were incubated at 23–25 C with alternating light and dark (12-hr photoperiod). Numerous conidia were produced within 7 days, although the filter papers were usually allowed to dry out (2–3 wk) before harvesting. Conidia were initially harvested with a miniaturized cyclone spore collector (20). This method, however, caused many spores to break. Subsequently, conidia were removed from the filter paper cultures with a dry brush. Conidia were kept intact on the filter papers within the petri dishes for periods of 3, 6, 10, and 12 mo at room temperature. Percentage of germination was determined by brushing dry conidia onto wet filter paper disks that were then incubated in darkness for 8 hr at 26 C. There were six replications of each treatment.

Field assessment of Alternaria blight. One of the major problems in developing a standard pictorial key for disease assessment is to maintain accuracy. James (15) used an IBM drum scanner system that consisted of a scan head containing a photoelectric cell that recorded black areas in units of 0.0001 cm^2.

In the present study, owing to the unavailability of such equipment, standard pictorial keys showing leaves with 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, and 75.0% infection were prepared using squared graph paper and a standard leaf shape equivalent to 2,000 squares. The desired levels of disease were created by filling in the required number of squares (ie, two squares = 0.1%, 500 squares = 25%). These standard diagrams were reduced photographically to fit on one page for convenience and comparison.

To develop a reliable method for assessing the severity of Alternaria blight in the field, we collected data from trials at Hermitage Research Station (near Warwick in Queensland) during the 1977–1978 and 1978–1979 growing seasons (4). These field trials included comparisons between plants in disease-free plots that were sprayed with the fungicide captafol and diseased plots that were artificially inoculated with conidia of *A. helianthi*. Assessments of disease severity included number of senesced leaves per plant and mean percentage of infection (level of disease on each photosynthetically active leaf as determined by using the pictorial key and averaged for the number of leaves assessed per plant).

RESULTS AND DISCUSSION

PDA plus sunflower seed extract gave the best combination of colony growth (mean colony diameter, 19.1 mm after 7 days at 23 C) and abundant sporulation. Richard's solution and Richard's solution plus sunflower seed extract produced large colonies of *A. helianthi* (mean colony diameter, 30.2 and 30.9 mm, respectively); however, sporulation on both media was poor. Colonies grown on PDA alone produced abundant conidia but achieved a mean colony diameter of only 5.7 mm after 7 days at 23 C.

A high proportion of conidia of *A. helianthi* produced on filter paper and stored intact at room temperatures retained their viability for up to 6 mo. The percentage of germination (8 hr at 26 C) of conidia after 6 mo of storage was 68.3%, compared with 87.5% for fresh conidia and 1.7% for conidia that had been stored for 12 mo.

The pictorial assessment key is shown in Figure 1. The data that were obtained (using this key) from the field trials indicated that the highest levels of disease occurred on the lowest leaves (Table 1). Under favorable environmental conditions, the disease appeared to move up the plant, thereby increasing the apparent rate of senescence relative to natural senescence on disease-free plants.

Calculation of the percentage of tissue showing disease symptoms was very time-consuming and neglected the death of lower leaves that resulted from the Alternaria blight epidemic. Evidence of a disease epidemic earlier in the vegetative life of the plant was masked by senescence, and the percentage of infection only gave an accurate measure of the current disease activity.

A comparison between the number of senesced leaves per plant in diseased and disease-free plots enabled us to calculate a correction factor that estimated the number of leaves that had died as a result of the disease epidemic. The correction factor was calculated as follows for each plot in the trial: correction factor = 100 × [mean number of senesced leaves per plant (diseased plants) minus mean number of senesced leaves per plant (disease-free plants)]. This correction factor, when incorporated into the percentage of infection (mean percentage of tissue showing disease symptoms)

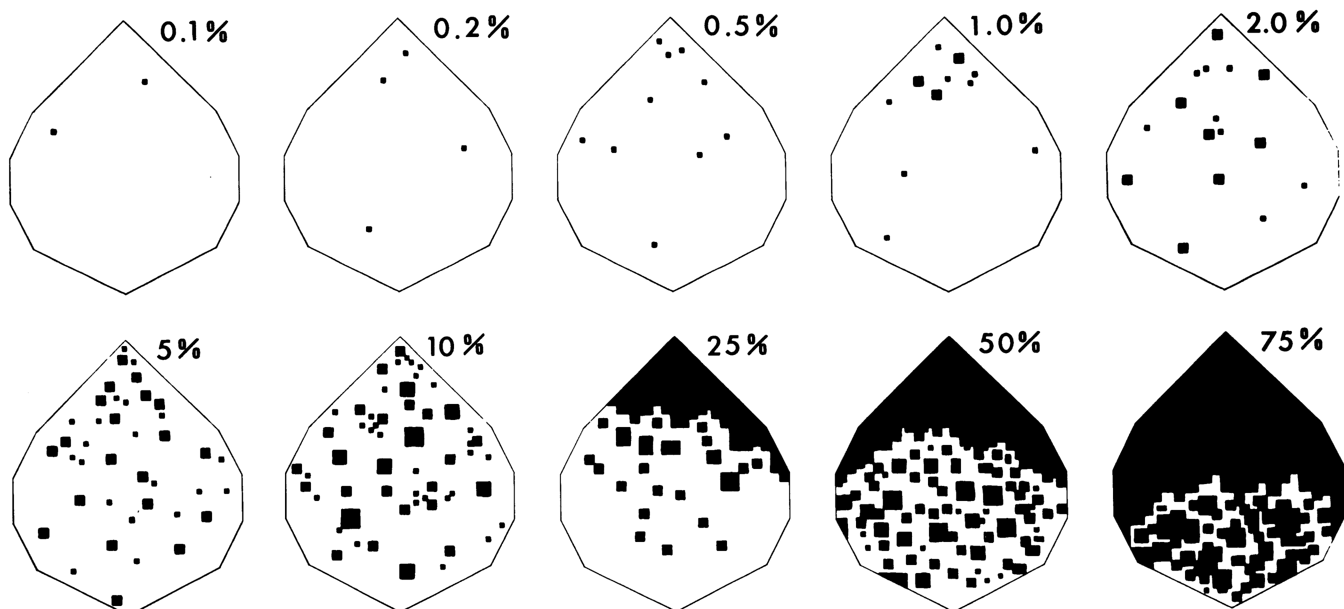

Fig. 1. Pictorial assessment key for Alternaria blight of sunflower.

Table 1. Distribution of leaf blight symptoms[a] caused by *Alternaria helianthi* on sunflower plants at different stages of an epidemic (mean of 50 plants)

Leaf number[b]	Assessment 1[c]	2[d]	3[e]
30	0	0	0
29	0	0	0
28	0	0	0
27	0	0	0
26	0	0	0
25	0	0	0
24	0	0	0
23	0	0	0.1
22	0	0	0.1
21	0	0	0.2
20	0	0.1	0.5
19	0.1	0.2	0.4
18	0.1	0.3	Senesced
17	0.2	0.4	Senesced
16	0.3	1.1	Senesced
15	0.5	1.8	Senesced
14	1.1	2.9	Senesced
13	1.9	5.0	Senesced
12	2.6	9.2	Senesced
11	3.7	16.9	Senesced
10	5.6	22.5	Senesced
9	7.4	27.6	Senesced
8	4.9	44.3	Senesced
7	8.6	62.5	Senesced
6	7.0	Senesced	Senesced
5	9.2	Senesced	Senesced
4	8.7	Senesced	Senesced
3	20.8	Senesced	Senesced
2	32.7	Senesced	Senesced
1	57.7	Senesced	Senesced

[a]Percentage of leaf area infected by *A. helianthi*.
[b]Leaves numbered from the base of the plant.
[c]Late budding stage of growth.
[d]Late anthesis stage of growth.
[e]Seed-filling stage of growth.

data, provided a measure of both past and present disease severity.

When assessing disease in the field, it is usually not possible to measure past disease activity and its effects on the plant because disease-free (fungicide-treated) control plots are not available for comparison. Most field assessments require methods that are accurate and quick and that allow a large number of plants to be assessed in a short time. For these reasons, the use of the mean percentage of diseased tissue per plant and the corrected mean percentage of diseased tissue per plant is not suitable for large-scale disease assessment programs.

The highest levels of infection by *Alternaria* spp. on sunflower occurred on the lowest leaves (Table 1). Therefore, an attempt was made to determine whether the percentage of infection on the lowest leaves was directly proportional to the percentage of infection for the whole plant. Data from each of four assessments made during two growing seasons and at different stages of host growth were subjected to linear regression analysis.

Table 2. Linear regression analyses comparing percentage of infection on the lowest unsenesced leaves (X) with mean percentage of infection for the whole plant (Y)[a]

$Y = 0.432^{***}$	$X_1 + 1.043$	$r^2 = 0.76$
$Y = 0.425^{***}$	$X_2 + 2.099^{*}$	$r^2 = 0.87$
$Y = 0.434^{***}$	$X_3 = 2.857^{*}$	$r^2 = 0.92$

[a]Degrees of freedom = 148. X_1 = percentage of infection of the lowest unsenesced leaf; X_2 = mean percentage of infection of the two lowest unsenesced leaves; and X_3 = mean percentage of infection of the three lowest unsenesced leaves.

The results (Table 2) indicate that there was a good correlation between the percentage of infection on the lowest one, two, or three unsenesced leaves and the percentage of infection for the whole plant.

One would expect the actual relationship ($Y = mX + b$) between the level of disease on the lowest leaves (X) and the percentage of infection for the plant (Y) to vary depending on the duration and intensity of the epidemic and the growth stage of the host. The values (m) and (b) represent indirect measures of such factors as the favorability of the environment and the resistance or susceptibility of the host.

The most accurate measure of Alternaria blight severity is the percentage of infection based on diseased leaf area, especially if this is corrected to include senescence due to disease. Nevertheless, it is very time-consuming and usually impractical to assess independently the level of infection on each leaf of a representative number of plants in a field or trial plot. The rapidity with which an Alternaria blight epidemic can develop precludes the possibility of making one assessment on a particular leaf at a particular growth stage. It would appear that a multiple-point model would be needed to characterize the disease-loss relationship for Alternaria blight on sunflower.

Because the level of Alternaria blight infection on the lowest unsenesced leaves of sunflower plants was directly proportional to the severity of the disease on the whole plant, it is suggested that the assessment of the disease on the lowest leaves should be used to obtain a quick, reliable, and reproducible description of an Alternaria blight epidemic in the field.

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