Effects of Leaf Age, Host Growth Stage, Leaf Injury, and Pollen on the Infection of Sunflower by *Alternaria helianthi*

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ABSTRACT

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Laboratory and glasshouse experiments were conducted to investigate the effects of various host factors on the growth and development of *Alternaria helianthi* on sunflower (*Helianthus annuus*). Lesions caused by *A. helianthi* developed most rapidly on leaves of plants that were at the anthesis or seed-filling stages of growth and on the older leaves of plants at the vegetative or budding stages of growth. Chlorotic halos were most prominent surrounding lesions on younger (vegetative or budding stage of growth) sunflower plants and were almost absent from lesions on older plants. The presence of artificially induced injuries of the leaf surface increased the number of lesions per square centimeter caused by *A. helianthi* relative to the number of lesions on uninjured leaves. The germination of conidia and the branching of germ tubes was increased when sunflower pollen was mixed with conidia of *A. helianthi*. The data indicate that the susceptibility of sunflower plants is greatest at the anthesis or seed-filling stages of growth. Consequently, it is suggested that sunflower be planted so that the crop matures when environmental conditions (temperature, leaf wetness, etc) are least suitable for disease development.

Additional key word: environment.

Alternaria blight of sunflowers (*Helianthus annuus* L.), caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara, is regarded as an important disease of sunflower in northeastern Australia (1,14). The disease is characterized by dark, necrotic lesions on the leaves, stem, petioles, and capitulum. Lesions are irregular in outline with a grey center surrounded by a very dark margin. A well developed chlorotic halo frequently surrounds leaf lesions. Initially the lesions are small (1–2 mm across), but during extended periods of wet weather they may enlarge and eventually cause death of leaves. The development of lesions on petioles may also cause death of leaves. Infection of the stem or capitulum results in large black spreading lesions and, under optimal conditions for the

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development of the pathogen, the stem may be completely girdled and the capitulum completely blackened. Alternaria blight of sunflowers is usually most obvious on lower leaves, but under favorable conditions, the disease advances rapidly up the plant and causes death of all plant parts.

Apart from a few brief references to the effects of host growth stage (4,11) and interactions with other pathogens (6,10,11), there has been no previous study of the effects of the biological environment on the development of *A. helianthi* in culture or on sunflower leaves.

Islam et al (11) observed that the development of alternaria blight on sunflower was greatest when plants were weakened and senescing. They found that the disease occurred only sporadically on the older leaves of plants before flowering and that the heaviest infections occurred during seed development. Anilkumar et al (4) noted that Alternaria blight was very severe on plants in the later stages of growth.

During anthesis, large quantities of pollen are produced by sunflowers, and deposits of pollen are common on the leaves. Several authors have demonstrated a correlation between the presence of pollen and increased infection in other diseases. Chou and Preece (8) showed a marked increase in infection of strawberry petals and fruits and broadbean leaves by *Botrytis cinerea* Pers. ex Fr. when pollen was incorporated in infection droplets. Fokkema (9) studied the influence of pollen on the infection of rye leaves by the *Drechslera* state of *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dast and found that the addition of pollen to spore suspensions used to inoculate leaves increased the number of germ tubes per conidium, the length of hyphae per square millimeter of leaf surface, the number of lesions produced per unit area of leaf, and the necrotic area on leaves. He also reported that infection of rye leaves by the *Septoria* state of *Leptosphaeria nodorum* Muller was stimulated by the presence of pollen.

The objectives of the present study were to investigate the effects of leaf age, host growth stage, and leaf injury on growth and development of *A. helianthi* on sunflower. The effect of pollen on germination of conidia of *A. helianthi* was also investigated.

MATERIALS AND METHODS

To determine whether leaf age influenced the amount of disease produced by *A. helianthi*, eight 6-wk-old sunflower plants (cultivar Hysun 30) growing in a soil-sand-peat mixture contained in 15-cm-diameter pots, were uniformly inoculated (10 min at 5 rpm) in a turntable-type inoculation chamber (7) with 20 mg of freshly produced conidia of *A. helianthi* that had been grown on filter paper in the laboratory (3). Four plants were placed in a dew chamber at 22 C (suboptimal for growth of *A. helianthi*) and four were placed in a dew chamber at 25 C (near optimal). After 12 hr of incubation, the plants were placed in a glasshouse at 20–30 C. The numbers of lesions per square centimeter of leaf area, on the second and third leaf pairs (from the base of the plant) were determined 8 days after inoculation. The second and third pairs of leaves were used because of their uniformly horizontal orientation at the time of inoculation.

A second experiment was conducted to investigate the effect of leaf age and host growth stage on lesion size. Glasshouse-grown plants at vegetative, budding, late anthesis, and seed-filling growth stages were inoculated with a conidial suspension (20 mg of conidia per 100 ml in water) using an atomizer. Plants were incubated for 10 days in a glasshouse equipped with a Walton SW5 humidifier that maintained 70–100% RH and 10- to 16-hr dew periods each day. The mean size of 10 lesions on selected leaves on each of four plants (replicates) was determined by measuring the diameter of necrotic lesions and the width of chlorotic halos. The leaves selected were (from the base of the plant) leaves 5 and 9 on plants at the vegetative stage; leaves 9 and 15 on plants at the budding stage; leaves 14 and 19 at the flowering stage; and leaves 19 and 24 at the seed development stage. These leaves were selected to allow a comparison of leaves from plants at different stages of growth.

The effect of leaf injury on infection of sunflower leaves by *A. helianthi* was investigated. One side of leaves 3, 4, 5, and 6 on each of eight sunflower plants was wiped with cotton wool that had been dipped in an aqueous suspension of Celite (diatomaceous earth). The uninjured half of the leaf served as a control. The eight plants were uniformly inoculated with conidia of *A. helianthi* in an inoculation chamber (6) 4 hr after injury and incubated in a dew chamber at 25 C for 12 hr before being returned to the glasshouse. The numbers of lesions per square centimeter on each leaf half (control and injured) was determined 7 days after inoculation.

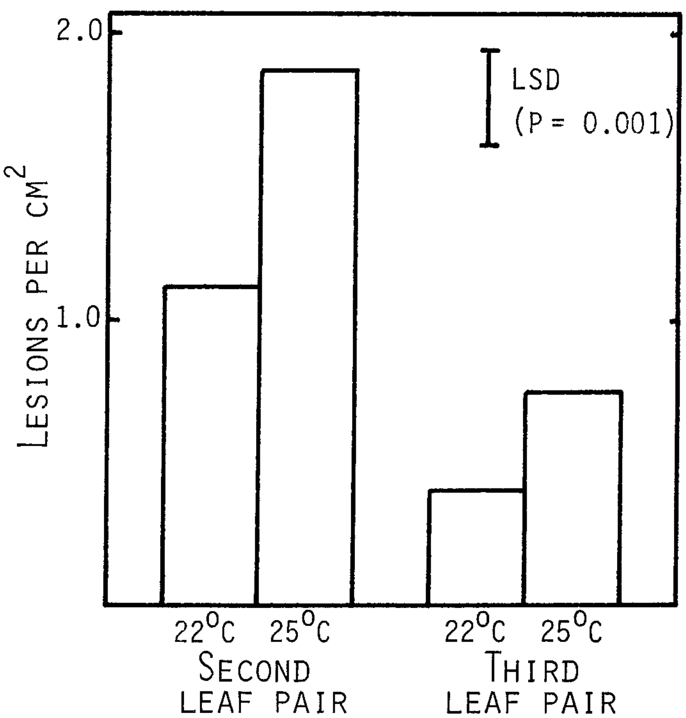

Fig. 1. The effect of leaf age on the infection of sunflower seedlings by *Alternaria helianthi* at 22 C and 25 C. Leaves were numbered from the base of the plant.

TABLE 1. The effect of leaf age and host growth stage on the size of necrotic lesions[a] and width of chlorotic halos produced by *Alternaria helianthi* on sunflower leaves[b]

		Host growth stage			
	Leaf age	Vegetative (leaves 5 and 9)	Budding (leaves 9 and 15)	Late anthesis (leaves 14 and 19)	Seed filling (leaves 19 and 24)
Lesion diameter (mm)	Older	2.22	1.88	2.00	2.05
	Younger	0.84	1.61	1.84	1.99
Halo width (mm)	Older	1.30	1.41	0.15	0.20
	Younger	0.60	1.14	0.30	0.52

[a]For comparing diameter of necrotic lesions in columns, LSD ($P = 0.05$) = 0.23 mm; for comparing width of chlorotic halos in columns, LSD ($P = 0.05$) = 0.17 mm.
[b]Leaves numbered from the base of the plant.

TABLE 2. The effect of sunflower pollen on the germination of conidia of *Alternaria helianthi* in vitro[a]

Treatment	Germination (%)	Branches per 50 germ tubes (no.)
Conidia	77 a	12 a
Conidia and pollen (1 : 1)	81 b	33 b
Conidia and pollen (1 : 2)	83 b	43 c

[a] Values in the same column followed by the same letter are not significantly different ($P = 0.01$).

The effect of sunflower pollen on germination of conidia of *A. helianthi* was investigated. Freshly collected sunflower pollen and conidia of *A. helianthi* were combined on a w:w basis to give the following combinations: conidia alone, conidia and pollen (1:1), and conidia and pollen (1:2). The conidia and conidia-pollen mixtures were brushed onto wet filter paper disks (10-mm diameter) that were placed on wet filter papers in petri dishes. The percentage germination of conidia in each treatment was determined after incubation in darkness at 25 C for 6 hr. One hundred conidia were counted in each of the eight replicates. The mean number of branches per 50 germ tubes was also determined for each treatment.

RESULTS

The results (Fig. 1) show that older sunflower leaves were more susceptible to infection by *A. helianthi* than younger leaves at both optimal and suboptimal temperatures. The number of lesions per square centimeter of leaf that developed on leaves 3 and 4 was approximately double the number that formed on the younger leaves 5 and 6. This finding was supported by the results presented in Table 1, which indicate that the lesions produced on the older leaves of plants that were inoculated at the vegetative or budding stages of growth were significantly larger than the lesions produced on younger leaves of the same plants. In contrast, there was no significant difference between the size of lesions produced on leaves of different ages on plants inoculated at the late anthesis or seed-filling stages of growth. The amount of chlorosis induced by *A. helianthi* was greatest in plants that were inoculated at the vegetative or budding stages.

Wounds or injuries to sunflower leaves resulted in a significant increase (from 2.05 to 2.70) in the number of lesions per square centimeter produced by *A. helianthi*. The germination of conidia of *A. helianthi* and the branching of germ tubes was stimulated by the presence of sunflower pollen (Table 2).

DISCUSSION

The results obtained in these studies support the general observations made by Islam et al (11) and Anilkumar (4) that sunflower plants are most susceptible to infection by *A. helianthi* during the anthesis and seed-filling stages of growth. Chlorotic halos were more prominent around lesions on plants at the vegetative and budding stages of growth. Bhaskaran and Kandaswamy (5) showed that phenolic compounds were concentrated in the halo region of lesions caused by *A. helianthi* on sunflower leaves. The accumulation of phenols is often associated with resistance to disease (13). Therefore, the presence of chlorotic halos may indicate a mechanism of partial resistance to infection by *A. helianthi*, which is present in the leaves of young sunflower plants prior to flowering.

A. helianthi usually infects sunflower leaves by producing an appressorium before directly penetrating the cuticle and epidermis. Penetration via stomates and wounds was only occasionally observed by the authors. However, the presence of numerous wounds or injuries significantly increased the severity of Alternaria blight. It is possible, therefore, that injuries to sunflower leaves on plants in commerical crops may contribute to the rapid development of epidemics. Damage by heavy rain and by abrasion between adjacent leaves in densely planted crops may be particularly significant because epidemics of *A. helianthi* are often associated with cyclonic conditions that often include high winds and heavy rain.

Investigations into the effect of pollen on *A. helianthi* were limited to a study of germination on wet filter paper. The abundance of pollen on leaves subsequent to flowering could stimulate the germination of conidia on the leaf surface and thereby increase the severity of Alternaria blight during this growth stage. This effect could accentuate the effect of the growth stage of the host on disease development.

Sackston (12) compared the reductions in yield components resulting from artificial defoliation at the seedling, flowering, and maturing stages of sunflower plant growth and found that plant height, seed weight, and protein and oil content of the seed were reduced most when plants were defoliated at the flowering stage. This growth stage corresponds to that at which sunflower appears to be most susceptible to Alternaria blight.

The results of these investigations suggest that the control of Alternaria blight of sunflowers is most critical during and after flowering. This can be achieved in northeastern Australia by sowing sunflower in late summer instead of spring (2). Spring-sown sunflower crops mature during the summer months when environmental conditions most favor epidemics of Alternaria blight. Sunflower crops planted in late summer are most susceptible during autumn when the cooler temperatures and dryer weather conditions are not suitable for *A. helianthi* (2).

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