Symptomless infections in alternaria leaf blight of cotton

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A symptomless phase of Alternaria macrospora leaf blight that occurs in commercial cultivation of cotton between visible disease outbreaks was studied to determine its cause and effect. Most plants between the two outbreaks of leaf blight epidemics in the field were infected but symptomless; yet during the outbreaks the number of infected symptomless plants was minimal. Drought, high temperature, low humidity, and high salinity increased plant resistance to leaf blight by decreasing visible symptoms and causing symptomless infections. Symptomless plants provided inoculum to infect and produce a visible disease in healthy plants when favorable conditions for disease development resumed. Plant age had minimal influence on symptomlessness, and sunlight exposure had none. Defoliation was related to the density of spots on leaves and to the virulence of the strain. Polyphenoloxidase activity and phenol accumulation were similar in both disease phases. Plants having visible symptoms produced more spores, but spores from symptomless plants were detached more easily by air currents. Symptomlessness apparently is a common manifestation of alternaria leaf blight of cotton. It is probably influenced by agro-environmental parameters that induce the two phases of disease expression.

Key words: Alternaria macrospora, cotton leaf blight, spot of cotton, symptomless infections.


Afin d’en déterminer la cause et les effets, l’auteur a étudié une phase sans symptôme de la brûlure des feuilles du coton provoquée par l’Alternaria macrospora, laquelle survient dans les cultures commerciales entre deux épisodes visibles de la maladie. Entre deux épisodes épidémiques de la brûlure foliaire, la plupart des plants sont infectés sans toutefois montrer de symptôme; pendant les éruptions, le nombre de plantes infectées sans symptôme est négligeable. La sécheresse, les hautes températures, l’humidité faible et la forte salinité augmentent la résistance à la brûlure foliaire en diminuant les symptômes et conduisant à des infections sans symptôme. Les plants sans symptôme, fournisrent l’inoculum conduisant à l’infection et produisent une maladie visible chez les plants sains lorsque les conditions favorables au développement de la maladie reviennent. Il y a peu de liens entre l’absence de symptôme et l’âge, et l’exposition au soleil est sans effet. La défoliation est reliée à la densité des taches sur les feuilles et à la virulence de la souche. L’activité des polyphénolases et l’accumulation de phénols sont semblables dans les deux phases de la maladie. Les plantes qui montrent des symptômes produisent plus de spores, mais les spores provenant de plants sans symptôme, se détachent plus facilement sous l’effet du vent. L’absence de symptôme est une manifestation apparente courante de la brûlure foliaire du coton causée par A. macrospora. Ce comportement est probablement influencé par les paramètres agro-environnementaux qui induisent les deux phases de l’expression de la maladie.

Mots clés : Alternaria macrospora, brûlure foliaire du coton, tache foliaire du coton, infections sans symptôme.

[Intaduit par la rédaction]

Introduction

Alternaria leaf blight, caused by Alternaria macrospora, affects almost exclusively Pima cotton plants. Field management of the disease is difficult because registered protectant fungicides are ineffective in controlling epidemics. Yield losses in Israel reached 15% in 1990 (23, 42).

Alternaria macrospora is not considered a major pathogen that jeopardizes cultivation, but it still causes leaf blight in high quality Pima cotton (Gossypium barbadense L.) (2, 7). Most cultivars of the lower quality Acala cotton (Gossypium hirsutum L.) are resistant (7, 17). This pathogen presumably disperses primarily by wind. However, the pathogen is seedborne and can be transferred locally by a variety of biotic vectors and abiotic vehicles (1, 3, 8). The pathogen can modify and increase its aggressiveness over a period of time under monoculture (29). Recently, Alternaria alternata was proposed as an additional pathogenic agent of alternaria leaf blight of cotton (9). It has been further proposed that so-called alternaria leaf blight of cotton is essentially a disease complex caused by two pathogens, A. macrospora and A. alternata (7, 10).

Symptomless disease is common in both crop and wild plants and can be caused by various pathogenic agents: bacteria (5, 6, 13, 15, 21, 25, 27, 38, 46), fungi (12, 14, 19, 20, 24, 26, 30, 32, 33, 34, 36, 44, 45), and viruses (16, 35). Although symptomlessness is known to occur in alternaria blight of cotton (10, 37), factors affecting symptomless infections have not been studied.

The objective of this study was an in-depth evaluation of the symptomless phase of alternaria leaf blight of cotton.

Materials and methods

Organisms

Alternaria macrospora Zim (ATCC 62363) was used in all experiments. The highly virulent strain BS 8-1987 (29) and the weakly virulent strain BS 9-1982 (29) were used in one experiment. Cotton plants (G. barbadense cv. Pima 5-5) were used as host plants.

Procedures

Plant growth conditions in pots in both the greenhouse and the growth chamber, soil growth mixture and fertilization, isolation of the inoculated strains from infected tissue (4, 29), fungal culturing and inoculum production, plant inoculation procedure (4, 11), pathogenicity tests (3, 4), estimation of disease severity (4, 11), polyphenoloxidase and phenol determinations (2), geographic location for field observations (9, site a), experimental design, and statistical analysis (29) were as described elsewhere.

Inoculation levels

Cotton plants having three to five true leaves were inoculated with A. macrospora at a rate of 1.2 × 10^4 spores/mL suspended in deion-
ized water (11). A lower inoculum level of 10^2 spores/mL was used in one experiment. Control plants were treated identically with dead r-irradiated spores (25 kQy) or with sterile water.

Determination of symptomless plants

Symptomless plants were determined by one of the following methods: (i) Cellulose acetate prints of leaf surfaces (22). After the film was removed and transferred to the laboratory, it was analyzed for the presence of germinating spores and hyphae by light microscopy (9). (ii) Leaf prints on fungal growth medium. Intact leaves were printed on fungal growth medium by the method of Sharon et al. (39) using modified Czapek medium (described later). After spore induction (1), the developing colonies were identified according to their spore morphology (31, 43). (iii) Scanning electron microscopy (SEM). Leaf disks (5 mm in diameter) were taken with a cork borer and prepared for SEM as previously described (11). Hyphae, germinating spores, and hyphal penetration via stomata were recorded and compared with samples taken immediately after inoculation, with samples inoculated with dead spores, and with samples treated with water. As the images recorded from symptomless plants were not different from those that were recorded and published previously by us (8, 11), no attempt was made to preserve the images.

Leaves collected at random from 200 field plants that exhibited no visible symptoms (out of a sampling area of 1,500 plants) were analyzed once a month by the cellulose acetate technique. The first sampling was done when the plants had two or three true leaves and the last, 1 week before the commercial harvest. The rest of the plants were monitored for spot formation.

Application of stress

Irrigation stress

The automatic drip irrigation system was programmed to maintain 20–30% water field capacity of the soil mixture. Further fine tuning to induce water stress (expressed as slightly wilted plant appearance) was done daily and manually in late morning. Nonstressed plants were maintained at levels of field capacity and above. On hot days, the automatic irrigation system was shut off, and the plants were continuously irrigated at a flow rate of 4 L/h. Since the soil mixture contained high levels of porous volcanic dust, the pots were never flooded. Excess water leaking from the drainage holes fell through the iron-net tables and collected in containers with a disinfectant.

Temperature and low relative humidity stress

Plants growing at temperatures ranging from 15° (dark) to 32°C (light) were transferred for 6 h into a temperature-controlled growth chamber at 42 ± 3°C. Low relative humidity (≈10%) in the growth chamber was achieved by placing large perforated plastic columns (80 × 20 cm) filled with oven-dried silica gel, closing the external ventilation, and covering the wet surface of the pots with aluminum foil sealed with paraffin.

Brackish water stress

Plants were irrigated with brackish water obtained from the municipal water treatment facility having conductivity of 15 S/cm at 25°C. This salinity diminishes cotton yield by 50%.

Method of field survey

Each field survey included 1,500 plants per sampling (10). A plant was considered visibly diseased if it contained at least two typical spots of alternaria.

Reduced sunlight illumination

The plants were placed outdoors under commercial plastic shades of various translucence that reduced illumination from 1,100 to 150 μmol · m^-2 · s^-1. Other plants were grown in growth chambers under illumination of 100 μmol · m^-2 · s^-1 with a 14-h day and 10-h night cycle.

Detection of airborne spores and artificial wind

In the greenhouse five highly infected cotton plants (before flowering stage, mean disease index of 3.8) and five similar symptomless plants were placed (separately) on a stand 0.5 m above ground level in front of the greenhouse ventilation system that produced a constant wind of 4 ± 1 m/s. Two traps containing five Petri dishes per trap were exposed 4 and 6 m downwind. The medium in the dishes was Czapek medium supplemented with 250 mg/L chloramphenicol and 1 mg/L of the surfactant Triton N-101 (alkylphenylpolyethylenebiglicol) to prevent overgrowth of the colonies on the medium (9). After exposure of the medium, lids were returned, sealed with Parafilm, transferred to the laboratory, incubated at 25 ± 2°C in the dark, and spore formation was induced (1). Three days after sampling each small colony that developed was examined under a stereoscopic microscope. Results are given as the number of spores trapped on 60 cm^2 of medium surface per 2 h.

Results

Survey of symptomless appearance in the field

A field survey carried out in 1987 in Bet Shean valley in Israel over the entire cotton season showed the appearance of symptomless plants to be uneven. The largest number of symptomless plants were recorded in June and July. During the two outbreaks of leaf blight epidemics in April–May and August–September (9), nearly all plants were visibly diseased (Fig. 1). The random sampling of symptomless plants for microscopical analysis proved to be a practical tool, since nearly all plants sampled showed evidence of Alternaria infections, mainly in the form of spore production.

Conditions for inducing symptomless plants

After the initial 24-h humid period required to initiate infection in the plants, stress conditions were applied either by irrigation, high temperature, extremely low relative humidity, or high salinity. These conditions produced fewer symptoms in the infected plants, and a large percentage of the inoculated plants remained symptomless (Table 1). On the other hand, plants growing at optimal water and temperature conditions showed severe symptoms. Low inoculum levels without stress conditions also produced symptomless plants (Table 1).

Defoliation in symptomless plants

Defoliation was directly related to the appearance of spots in inoculated plants. The percentage of defoliation was proportional to the number of spots per plant. Symptomless plants resembled noninoculated ones by expressing negligible defoliation. Only when symptomless plants later developed symptoms did defoliation occur at a level similar to that in plants expressing symptoms (Table 2).
TABLE 1. Plant growth conditions affecting induction of symptomless plants

<table>
<thead>
<tr>
<th>Predispensing factors</th>
<th>Disease index after 8 days (1–5)</th>
<th>Symptomless plants (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum level of 1.2 \times 10^6 spores/mL</td>
<td>2.4b, 46b</td>
<td></td>
</tr>
<tr>
<td>Plants growing under water stress</td>
<td>3.7a, 11a</td>
<td></td>
</tr>
<tr>
<td>Plants growing under continuous irrigation</td>
<td>1.2c, 53b</td>
<td></td>
</tr>
<tr>
<td>Plants growing under temperature stress</td>
<td>2.3a, 62b</td>
<td></td>
</tr>
<tr>
<td>Plants growing under brackish water stress</td>
<td>0.4d, 68b</td>
<td></td>
</tr>
<tr>
<td>Inoculation in humid condition and transfer to complete dryness</td>
<td>0.03d, 93c</td>
<td></td>
</tr>
</tbody>
</table>

Note: Numbers within each column followed by the same letter do not differ significantly at P ≤ 0.05 in ANOVA.

*Data in percentage were arcsine transformed before analysis.

TABLE 2. The effect of symptom expression on the number of defoliated leaves

<table>
<thead>
<tr>
<th>Plant type</th>
<th>No. of defoliated leaves/plant 25 days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninoculated healthy plants</td>
<td>0.1b</td>
</tr>
<tr>
<td>Plants expressing visible symptoms*</td>
<td>15.4a</td>
</tr>
<tr>
<td>Symptomless plants</td>
<td>0.2b</td>
</tr>
<tr>
<td>Symptomless plants that later developed symptoms*</td>
<td>13.7a</td>
</tr>
</tbody>
</table>

Note: Numbers followed by the same letter do not differ significantly at P ≤ 0.05 in ANOVA.
*After irradiation stress.
*24 days after transferring to optimal plant growth conditions.

TABLE 3. Transfer of A. macrospora from symptomless plants to healthy plants in growth chamber

<table>
<thead>
<tr>
<th>Type of inoculation</th>
<th>Disease index (1–5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninoculated plants</td>
<td>0c</td>
</tr>
<tr>
<td>Artificially inoculated plants</td>
<td>3.6a</td>
</tr>
<tr>
<td>Symptomless plants</td>
<td>0c</td>
</tr>
<tr>
<td>Healthy plants in the presence of: symptomless plants*</td>
<td>2.7a</td>
</tr>
<tr>
<td>visibly diseased plants*</td>
<td>3.5a</td>
</tr>
<tr>
<td>symptomless plants under irrigation stress</td>
<td>1.1b</td>
</tr>
</tbody>
</table>

Note: Numbers followed by the same letter do not differ significantly at P ≤ 0.05 in ANOVA.
*After 1 week of incubation together and transfer to conditions conducive to leaf blight.

Infection of healthy plants by symptomless plants

Symptomless plants gave rise to infection of healthy plants in their proximity, as long as plant growth conditions, such as drought, were not restricted to the spread of the disease (Table 3). Disease severity was statistically similar whether plants were infected artificially, from plants with symptoms, or from symptomless plants (Table 3).

Symptom severity in relation to plant age or water stress

Plant age significantly affected disease severity. Very young and mature plants were the most susceptible. The percentage of symptomless plants also corresponded to plant age being highest during the vegetative growth period (Table 4). Water stress decreased disease severity and increased the percentage of symptomless plants regardless of plant age.

TABLE 4. Symptom severity in relation to plant age and to water stress

<table>
<thead>
<tr>
<th>Plant age*</th>
<th>Disease severity (1–5)</th>
<th>Symptomless plants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–2</td>
<td>2.46b, 3.34a</td>
<td>15b, 5c</td>
</tr>
<tr>
<td>5–7</td>
<td>0.72d, 2.21c</td>
<td>45a, 15b</td>
</tr>
<tr>
<td>10–15</td>
<td>1.05d, 1.77c</td>
<td>53a, 20b</td>
</tr>
<tr>
<td>&gt;15</td>
<td>3.1a, 3.9a</td>
<td>1c, 0c</td>
</tr>
</tbody>
</table>

Note: Numbers within each parameter tested (disease severity and symptomless appearance) followed by the same letter do not differ significantly at P ≤ 0.05 in ANOVA.
*Measured by number of true leaves.

Changes in polyphenoloxidase and phenol content in symptom expressing and symptomless plants

No significant differences in the increases of polyphenoloxidase activity and phenol content were observed between symptom-expressing plants and symptomless plants during the presymptomatic period or afterwards (Fig. 2). About 10-fold increases occurred in both enzymatic activity and phenol accumulation during the 8 days following inoculation.

The effect of the pathogenic strain and (or) sunlight on symptom expression

The degree of virulence of the pathogenic strains had a statistically significant effect on symptom appearance (Fig. 3). Exposure of inoculated plants to four declining sunlight illumination regimes or to relatively low artificial light intensity in a growth chamber had no effect on disease severity. No interaction between sunlight and virulence has been revealed by the statistical analysis.

Number of spores produced and released during the symptom and symptomless phases of disease development

Plants exhibiting symptoms produced more viable spores than symptomless plants (Fig. 4a). The amount of spores released from leaves at air currents of 4 ± 1 m s⁻¹ was similar in both symptom-expressing and symptomless plants (Fig. 4b).
Fig. 2. Changes in polyphenoloxidase (A) and phenol content (B) of infected cotton plants. Points representing a specific day followed by the same letter do not differ significantly at $P \leq 0.05$ in ANOVA (A) or Student’s $t$-test (B). ■, symptoms; ☐, symptomless; ◆, healthy.

**Discussion**

Chemical control of alternaria leaf blight of cotton is recommended at the appearance of the first visible symptoms. Timing is a major consideration, weighing the economical cost versus the increased yield obtained by the treatment (41). Although epidemics are not a yearly event, they occur in Israel in two very defined periods of the growing season, at the cotyledon and first-leaf stage in late March to early May, and late in the season before harvest time in August—September (9). In these periods, most leaves are visibly infected and many shred. The reason for low disease occurrence between these periods and only slight disease incidence in certain years has not been explained. Symptomless infection could be an explanation. The disease may always exist in cotton fields but not be expressed with visible symptoms. This study demonstrates the existence of a symptomless phase in alternaria leaf blight and identifies factors that affect it.

To obtain a profitable yield in developed countries, it is necessary to grow a large quantity of the high-quality Pima cotton that is susceptible to leaf blight. Furthermore, it must be cultivated using optimal fertilization, pest control, and irrigation regimes (7). Unfortunately, these agrotechniques also favor disease development expressed by spots and defoliation symptoms. Stressed plants develop fewer visible symptoms, regardless of the stress source. Apparently the stress required to produce symptomlessness is agroenvironmental and not physiological. Although the plants differ in their susceptibility during their life span (2, 40; Table 4), water stress always has an arresting effect on symptom development. Defoliation of blighted cotton plants in Zimbabwe was enhanced by potassium deficiency (28). In Arizona both alternaria leaf blight and defoliation were suppressed by high temperatures (18). Yield stress has the opposite effect; it increased disease severity. The most severe infection of leaf blight in Israel occurred when the plants were heavily fruited (7, 41). The main danger from the symptomless phase is that these plants produce inoculum that infects healthy plants, resulting in visible symptoms in the latter during favorable conditions. Plants exhibiting symptoms can reduce yield (41, 42).

The cause(s) of symptom expression is (are) still unclear. In leaf blight of cotton caused by *A. alternata*, exposure to sunlight was thought to serve as a trigger (37). This was not the case in cotton leaf blight caused by *A. macrospera*. Various sunlight intensities compared with artificial light in a growth chamber had no effect on symptom expression. Furthermore, high light intensity during the cotton season is commonplace. Thus, significant variations in susceptibility cannot be attributed
Fig. 4. Spore production (A) and spore release by artificial wind (B) during symptom and symptomless phases of disease development. Columns with the same letter do not differ significantly at $P \leq 0.05$ in Student’s $t$-test.

to light intensity. The virulence of the pathogenic strain had a significant effect on symptom expression; the more virulent the pathogen, the more severe symptoms it produced. Additionally, *A. alternata* may function as a trigger for symptom expression in leaf blight caused by *A. macrospora* (4).

Visible disease and symptomless disease apparently coexist during the growing season. Symptomless plants can eventually develop visible symptoms. Plant response to infection, as expressed by an increase in polyphenoloxidase activity and phenol accumulation, was similar in both disease phases. Because these markers of cotton plant infection (2) were significantly higher than those found in noninoculated plants, both symptom-expressive and symptomless plants probably are diseased. Plants with the two disease phases differ in the defoliation of infected leaves (7, 42). Symptomless leaves do not shed.

Although visibly diseased plants produce more spores of *A. macrospora*, the spores produced during the symptomless phase are more easily detached by air currents. This allows considerable airborne inoculum from symptomless plants to infect healthy plants. Increased spore release might be a compensation mechanism created by the pathogen for low spore production during this phase.

In conclusion, the symptomless phase of alternaria leaf blight of cotton is a common phenomenon that should be taken into consideration when control strategies are being considered. Symptomless plants may later show symptoms. The symptomless phase is probably controlled by agroenvironmental parameters, not directly related to the age of the plants or to sunlight. These findings, mostly gathered under greenhouse conditions, should be confirmed under field conditions.

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