Wild beets as an important inoculum source of *Alternaria alternata,* a cause of leaf blight of cotton in Israel

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Alternaria alternata, isolated from the leaves of wild beets, infected cotton as well as wild and cultivated beets. Scanning electron microscopy of wild beet and cotton leaves infected by an aggressive isolate of *A. alternata* revealed that conidiophores of the pathogen emerged only from necrotic areas of leaf tissues. Sporulation occurred on leaves only during periods of high relative humidity (>95%) and temperatures ranging from 20 to 28°C. Under low relative humidity (60% at 22-25°C), mycelium penetrated into internal tissues of the leaf or emerged through the stomata. A less virulent isolate did not develop surface mycelium on inoculated leaves, but sporulation was detected on the leaf veins. Plants in several cotton fields adjacent to the diseased wild beet plants were infected by the pathogen early in the growing season. This study proposes that isolates of *A. alternata* that are virulent on cotton may overwinter on wild beet plants, making them an important source of the pathogen inoculum in epidemics of altenaria blight of cotton.

Key words: Alternaria, beet, cotton diseases, fungi overseason transfer, fungi overwintering, survival.

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Les auteurs ont isolé l'*Alternaria alternata* A partir de feuilles de coton infectées ainsi que de bettraves sauvages et cultivées. L'examen en microscopie électronique par balayage des feuilles de betterave sauvage et de coton infectés par un isolat agressif de 1'*A. alternata* montre que les conidiophores du pathogène ne proviennent que des régions nécrotiques du tissus foliaire. La sporulation s'effectue sur les feuilles uniquement pendant les périodes d'humidité relative élevée (> 95°10) et des températures allant de 20 à 28°C. Sous des conditions d'humidité relative faible (60% à 22-25°C), le mycelium pénètre à l'intérieur des tissus foliaires où emerge à travers les stomates. Un isolat moins virulant n'a pas développé de mycélium sur la surface des feuilles inoculées, mais la sporulation a pu être repérée sur les nervures foliaires. Dans plusieurs champs de coton, les plantes qui sont adjacentes aux plants de betteraves sauvages malades deviennent infectées par le pathogène au debut de la saison de croissance. Cette étude propose que les isolats de 'A. *alternata* qui sont virulents Sur le coton pourraient passer l'hiver Sur les plants de betteraves sauvages, en faisant ainsi une importante source d'inoculum du pathogène reliée aux épidémies de la flétrissure altemarienne du coton.

Mots clés : Alternaria, betterave, maladies du coton, survie des champignons à l'hiver.

[Traduit par la redaction]

Introduction

Alternaria blight caused by *Alternaria macrospora* Zimm. is the main foliar disease of high-quality cotton (*Gossypium barbadense* cv. Pima) cultivars in Israel (2, 18, 20). The disease initially appears during the seedling stage and especially on the more susceptible cotyledons (4). Infections are also common late in the crop season (8). Dispersal of *A. macrospora* propagules during the growing season has been attributed to both biotic and abiotic factors (3). Overwintering of the pathogen has been suspected of occurring in seeds and plants left in the field at the end of the crop season (2,6,20). A recent study identified *Alternaria alternata* on the infected cotton leaves of a resistant cotton cultivar (*G. hirsutum* cv. Acala) in the Bet-Shean valley (9). In this region of Israel, cotton is grown as a monoculture (3600 ha of *G. barbadense* cv. Pima and 2100 ha of *G. hirsutum* cv. Acala in 1987).

The fact that *A. macrospora* is seedborne and that diseased postseason cotton plants are left at field borders during the winter season (2, 20) does not adequately explain severe, early, growing season epidemics for the following reasons. Seeds are produced by the seed industry from alternaria-free fields (usually by visual inspection) and are subjected to surface disinfection by sulfuric acid (*G. hirsutum* only). This process destroys most of the seed surface microflora that are the major seed contaminants. All the seeds are further treated by a fungicide (Teracot, Agan Co., Israel). Few internal *Alternaria* propagules, which had previously penetrated the seeds, were detected following these procedures (2, 20). Although cotton plants left over after the growing season may carry pathogens, these plants are very rare (2) and are probably not an important source of inoculum in the epidemics. Recently, it was shown that dispersal of *A. alternata* and *A. macrospora* in field air currents is restricted to short distances (8). Therefore, it is possible that a common weed, grown at cotton field edges, may serve as an overseason carrier for at least one of the pathogens causing alternaria blight of cotton.

The objectives of this study were to (i) identify a wild weed carrier, growing abundantly overseason, which is potentially

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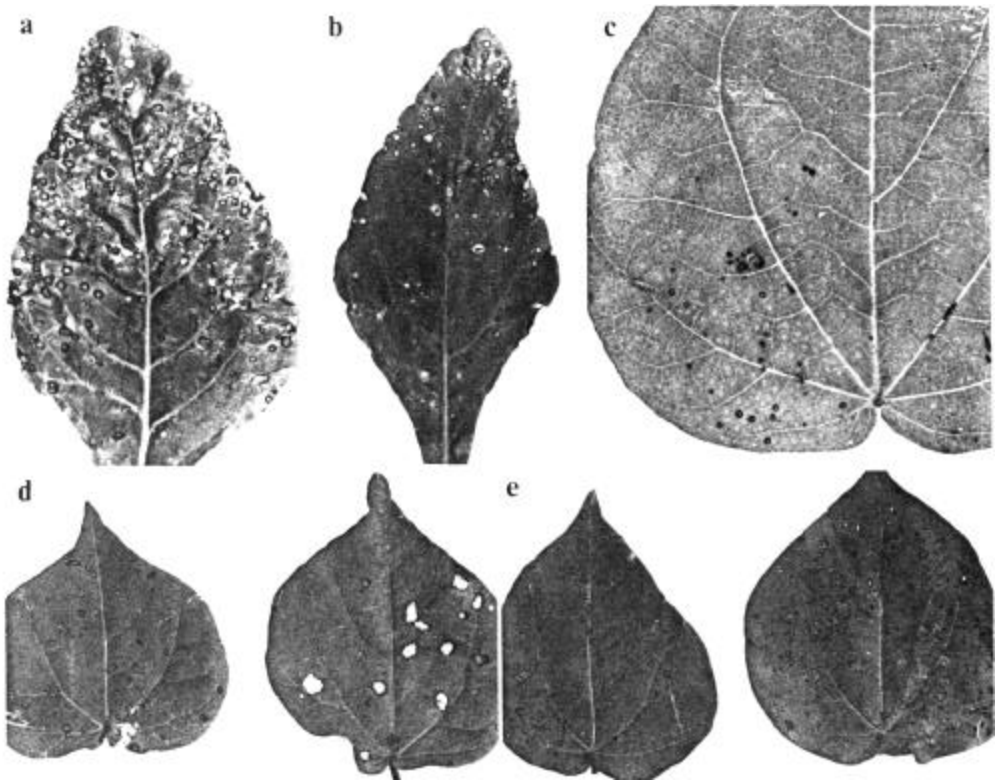

FIG. 1 (*a*) Altemaria blight on a wild beet leaf collected in the field. Leaves of cotton and beet inoculated With different isolates of *A. alternata* isoalted from beets. (*b*) Infection with isolate S-1 on wild beet No. WT-7 (*b*) and on cotton cv. Pima (*d*). Infection with less virulent isolate (S-3) on cotton leaves (c); note the limited symptoms. (*e*) *Alternaria macrospora* infection on cotton leaves cv. Pima. In *d* and *e* , the leaf on the left shows early symptoms. and the one on the right shows mature symptoms. All leaves are 85% of life size.

a major source of *Alternaria* propagules in the absence of cotton plants and (*ii*) follow symptom formation on beet and cotton leaves by *A. alternata* using scanning electron microscopy. A preliminary account has been presented elsewhere (23).

Materials and methods

Organisms

The following fungi were used: *Alternaria macrospora* Zimm. (ATCC 62363) and *A. alternata* (Fr.) Keissler (S-1; S-2; S-3; isolation procedure described later) isolated from wild beets (*Beta vulgaris* L.) in the Bet-Shean valley of Israel. Cotton plants (Gossypium *barbadense* cv. Pima S-5 and *G. hirsutum* cv. Acala SJ-2) and wild and cultivated beet plants (*Beta vulgaris* L.) of the following lines were used as hosts: wild beet Nos. WT-1, WT-2, and WT-7; seeds were collected in the spring of 1987 from noncultivated areas in the fringe of the Bet-Shean valley, and No. 32-360 was obtained from the Plant Introduction Service of Israel, Bet Dagan; forage beet No. 683/1 (ibid) and sugar beet seeds of cv. Kauemegrapoly (KWS, Germany) and ev. Monnac (Florimand Besprez, France) were obtained from commercial sources.

Isolation and inoculation procedure.,

Diseased leaves of wild beets (Fig. la) were collected during January 1987. The site of the first detection was at the borders of a cotton field in Kibbutz Shluchot in which cotton was recently grown. The kibbutz is located on the lower slopes of the Gilboa mountains, 9 km west from the Jordan River in the Bet-Shean valley, in northeastern Israel (Fig. 6, arrow). The collected leaves were young, the 3rd to 10th leaves from the top of the plant, green and without apparent chlorosis and exhibiting small lesions at < 10 lesions/leaf. The leaves were immersed in 1% NaOCl for 5 min, thoroughly rinsed in sterile deionized water, and placed in glass Petri dishes in a laminar flow hood. Each lesion was cut at its center using a sterilized razor blade. Pieces of leaf tissue (approximately 5 x 5 mm) containing a half lesion and the surrounding tissue were rinsed twice in sterilized deionized water, blotted on sterilized Whatmann No. 1 filter paper, and placed (five tissue pieces/dish) on the surface of Czapek's medium (11), supplemented with chloramphenicol (250 mg/L). Dishes were incubated in the dark at 25 ± 1°C for 48 h until the development of fungal colonies on some of the leaf pieces. Spore formation was induced as previously described for *A. macrospora* (2). Two hundred and twenty-five lesions were examined for the presence of the pathogen. Each fungal colony on the medium was tentatively identified by conidial morphology (21, 29. 30). Spores produced by isolates on

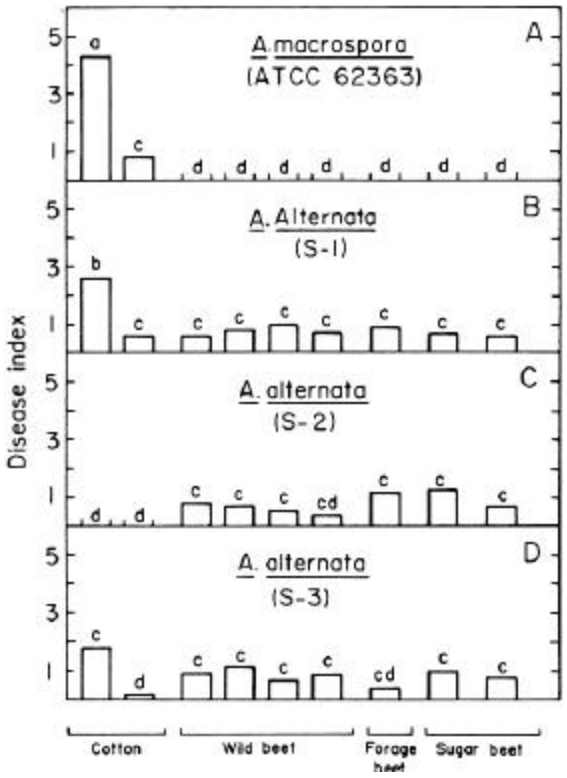

FIG. 2. Disease development in cotton and in wild and cultivated beet plants after inoculation with *A. macrospora* (A) and with three isolates of *A. alternata* (B-D). Disease index scale was as described in Materials and methods. Results are means of triplicates. Columns followed by a different letter differ significantly at $P \leq 0.05$ according to Fisher's least significant difference (LSD) analysis.

TABLE 1. Effect of pretreatments on severity of Alternaria blight symptoms on cotton (cv. Pima) and on wild beet (No. WT-7) leaves inoculated with *A. macrospora* (ATCC 62363) and *A. alternata* (S-1)

	Disease index (0-5)			
	A. macrospora		*A. alternata*	
Pretreatment before inoculation	Cotton	Beet	Cotton	Beet
24 h under mist	1.6*b*	0	0.6*b*	0*c*
42°C for 45 min to 4 h	0.9-1.1*c*	0	0.3-0.4*b*	0*c*
Drying up to wilting point	1.1*c*	0	0.2*c*	0*c*
Carborundum	4.2*a*	0	2.7*a*	0*c*
Carborundum + 4-6°C for 14 h after inoculation	3.8*a*	0	2.8*a*	1.1*a*
4-6°C for 14 h after inoculation	1.2*c*	0	0.5*b*	0.5*b*
Controls				
Mist	0*d*	0	0*c*	0*c*
42°C for 4 h	0*d*	0	0*c*	0*c*
Drying up to wilting point	0*d*	0	0*c*	0*c*
Carborundum	0*d*	0	0*c*	0*c*
Inoculated only	1.4*bc*	0	0.4*b*	0*c*
4-6°C for 14 h	0*d*	0	0*c*	0*c*

NOTE: Values are means of triplicates using the disease index as follows: 0, no symptoms; 1, 1-3 lesions/leaf: 2, 4-10 lesions/leaf; 3, 11-20 lesions/leaf; 4, 21-30 lesions/leaf: and 5, more then 30 lesions/leaf. Numbers within each column followed by a different letter differ significantly at $P \leq 0.05$ in LSD analysis.

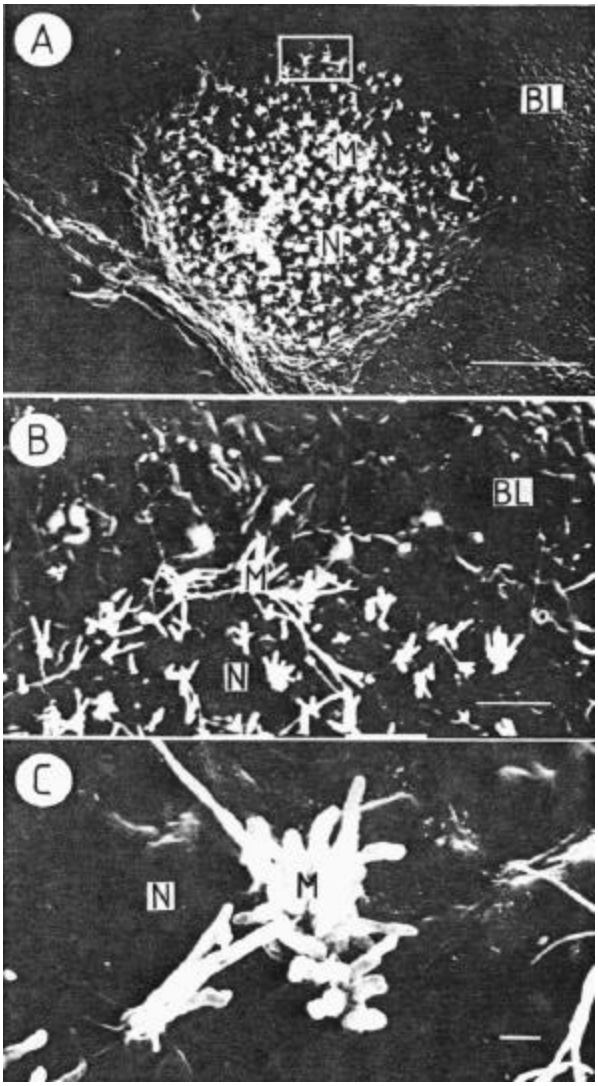

FIG. 3. Scanning electron micrographs (SEMs) of wild beet leaves infected by *A. alternata*. (A) emergence of hyphae from lesion site after 8 h in humid chamber. Bar = 0.5 mm. (B) Enlargement of 3A shows the border between dead and living tissue. Bar = 0.1 mm. (C) Typical emergence of mycelium (possibly conidiophores) from a necrotic lesion. Bar = 10 μm. BL, wild beet leaf surface; M, *A. alternata* mycelium; N, necrotic area.

the Czapek medium were serially diluted in deionized water and used as inoculum at a concentration of 1.2×10^4 spores/mL.

Cotton and beet plants were grown to the three to four true leaf stage in 5-L pots containing autoclaved field soil. The plants were inoculated at night by gentle brushing of the leaves with a soft brush previously dipped in spore suspension containing 0.1 g/L of a very fine carborundum powder (BDH, 300 grit). After inoculation, each pot was covered with a wet, polyethylene bag for 48 h at 25 ± 2°C and 22 ± 3°C (light:dark). The bags were then removed and the plants were kept in a greenhouse at about 60% relative humidity (but without condensed water on leaf surfaces) and under the same temperature regime for an additional 4 d, after which lesion development

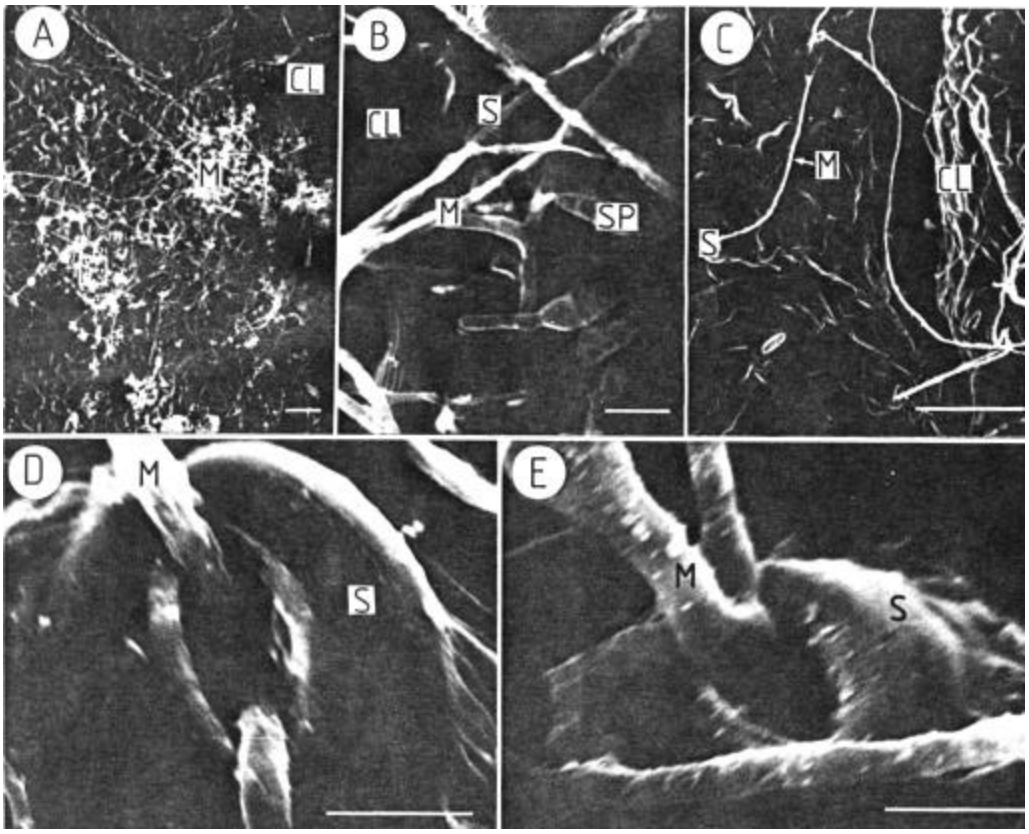

FIG. 4. SEMs of inoculated cotton leaves with aggressive isolate of *A. alternata* (S-1). (A) Mycelium development in restricted areas of the leaves under high relative humidity. Bar = 0.1 mm. (B) Enlargement of 4A showing sporulation on a leaf surface. Bar = 10 μm. (C) Mycelium on leaf surfaces between stomata under low relative humidity. Bar = 0.1 mm. (D) Penetration of open stomata by hyphae. Bar = 10 μm. (E) Hyphae emerging through open stomata and branched. Bar = 10 μm. CL, cotton leaf surface; M, *A. alternata* mycelium, S. stomata; SP, *A. alternata* spore.

was scored. The induction of symptoms in symptomless infected beet plants was performed by exposing the plants to low temperatures (4-6°C) for 14 h in the dark 8 days after inoculation. Similar low temperatures prevail during ma ny nights of the winter season in the BetShean valley (25). Control plants were brushed only with sterile deionized water and carborundum powder, and were incubated identically. All plant species were inoculated with each of the fungal isolates.

Several additional inoculation techniques were used as follows: before inoculation, plants were incubated under mist at 25 ± 3°C for 24 h, or under the normal atmospheric conditions of Bet-Shean valley at 42°C for periods of 45 min to 4 h. Under these conditions, the plants did not wilt. Additional pretreatment included leaving the plants without water until wilting began (7). After the pretreatment, the leaves were inoculated with the pathogens using an atomizer and incubated as described above. After symptom develo pment, the pathogens were reisolated and tentatively identified using a Zeiss light microscope.

Estimation of disease severity

Disease severity was estimated using the following index: 0. no symptoms; 1, 1-3 lesions/leaf; 2, 4-10 lesions/leaf; 3, 11-20 lesions/leaf; 4, 21-30 lesions/leaf; and 5, more than 30 lesions/leaf, indicating heavy infection (2, 4). Lesions were counted on each of the inoculated leaves. The mean number of lesions per leaf represented the disease severity. Following inoculation and incubation, symptomless wild beet leaves were examined at random for spores of *A. alternata* (21, 29, 30) with both light and scanning electron microscopes.

Scanning electron microscopy preparations

Leaf pieces (approximately 0.5 cm^2) were cut and fixed for 2 h in 5% (v/v) glutaraldehyde solution in 0.2 M potassium phosphate buffer supplemented with 0.15 M NaCl (PBS), pH 7.4, under vacuum, and then washed twice in the same buffer. The preparations were dehydrated by passing through an acetone gradient at 4 ± 1°C. The samples were finally dried in a critical point dryer (Tousimis, U.S.A.),

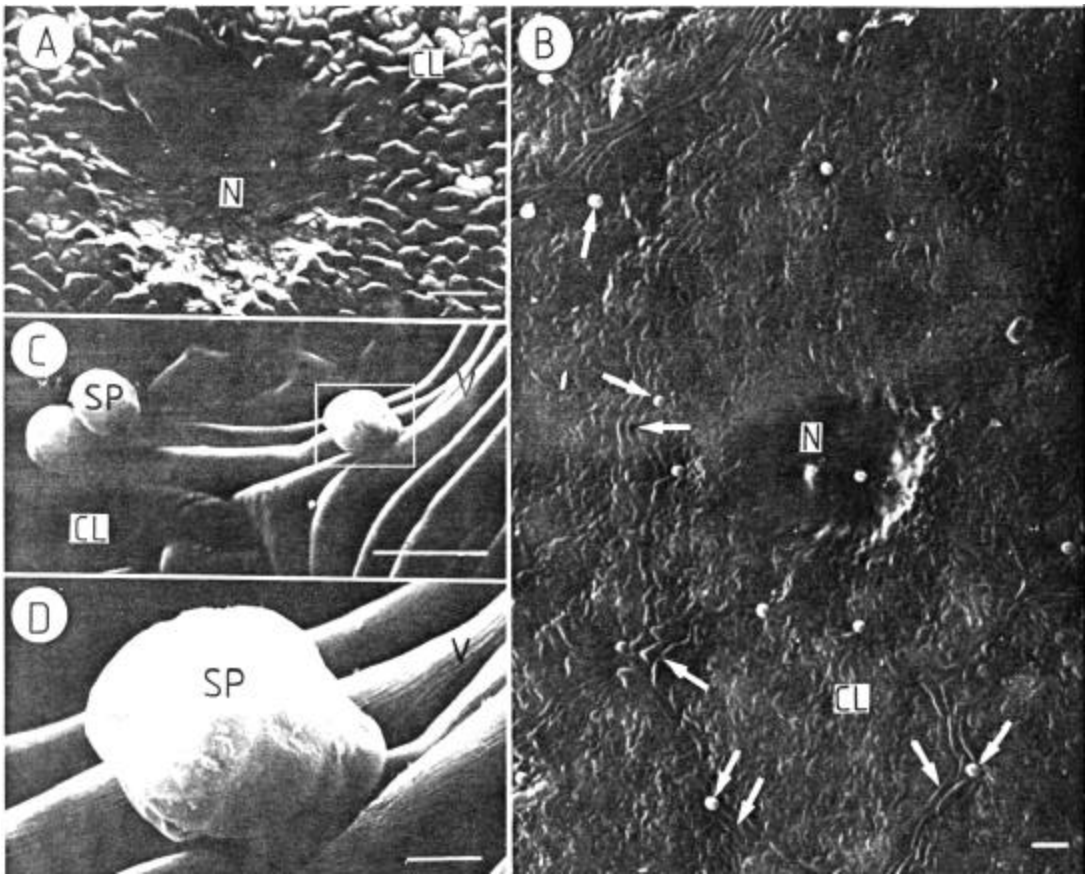

Fig. 5. SEMs of inoculated cotton leaves with a less virulent isolate of *A. alternata* (S-3) from beets. (A) Small lesion on leaf surface. Bar = 0.1 mm. (B) Sporulation on leaf veins; arrows indicate both veins and spores. Bar = 0.1 mm. (C) Spores originating from internal vein mycelium. Bar = 0.1 mm. (D) Enlargement of C showing the multicell spore of *A. alternata.* Bar = 10 μm. CL, cotton leaf surface: N. necrotic area: SP, *A. alternata* spore; V, leaf vein.

first in freon. and then udner CO_2 atmosphere (20 min CO_2 washing). The dry leaves were mounted on stubs using silver paint, coated with gold (100-150 Å (l Å = 0.1 nm), 10 mA. 12 min, using a S-150 spotter coater (Edwards), and examined under a Phillips SEM 505 scanning electron microscope at 25-30 kV (6).

Experimental design and .statistical anal N-sis

All inoculation experiments were performed in a completely randomized design in three replicates using two plants as a replicate. Significance is given by Fisher's least significant difference (LSD) at $P \leq 0.05$.

Results

Isolation of A. alternata *froth wild beet plants*

Three isolates of *A. alternata,* differing slightly in colony morphology but identical in their spore morphology, were isolated from the numerous Alternaria lesions that appeared on the leaves of wild beet plants (Fig. 1*a*). At the time of detection. no planted cotton plantation or postseason cotton was reported in the Bet-Shcan valley (Extension Service personnel responsible for cotton growth in Israel, unpublished data).

Inoculation with A. altemata *from wild beet plants*

Evaluation of several preinoculation treatments to increase the incidence of symptoms on leaves revealed that the most efficient pretreatment was carborundum. It produced minute injuries on cotton plants; however, for the treatment of beets. it also required a period of low temperature after inoculation (Table 1). Response of cotton and beet plants to inoculation with *Alternaria* isolates differed. *Alternaria macrospora* induced a severe disease in susceptible cotton plants (cv. Pima), mild disease in the resistant cultivar (cv. Acala), and no symptoms in any of the beet accessions (Fig. 1*e* and Fig. 2A). One isolate of *A. alternata* (S-1) induced typical alternaria lesions on cotton and beet plants and was considered an aggressive isolate (Figs. 1*b,* 1*d,* and 2B). Another isolate (S-3) was less virulent and resulted in fewer local lesions on cotton leaves (Figs. 1*c* and 2D), whereas the third isolate

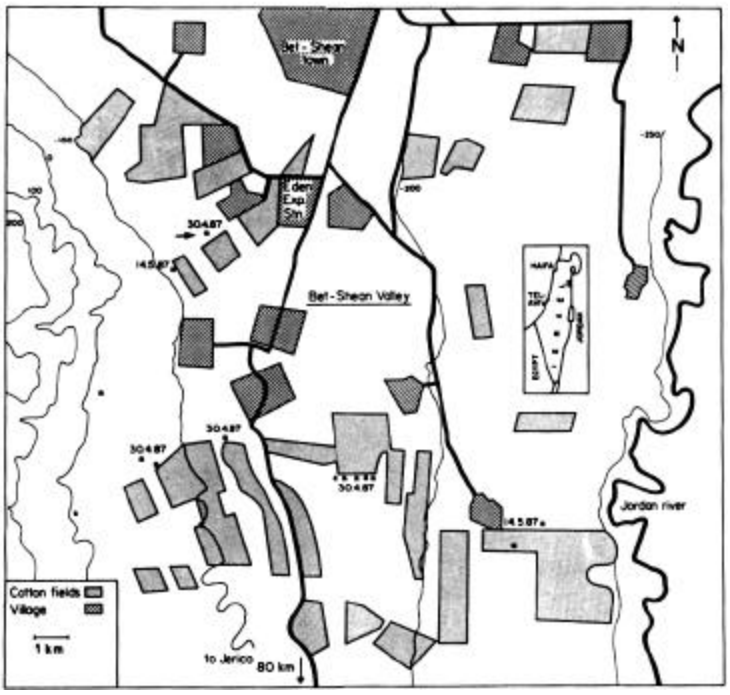

FIG. 6. Location of several wild beet plant populations, heavily infected with *A. alternata*, in the Bet-Shean valley (*) and the nearby infected cotton fields (dates indicate the detection of disease in the cotton fields). Heavy lines, paved roads; light lines, ground altitude above and below sea level (in metres). Insertion shows location of the Bet-Shean valley in Israel and is located in 35°30'E and 32°30'N. Arrow indicates the site of the first detection of infected wild beets.

(S-2) did not induce any visible lesions on cotton leaves and was considered avirulent (Fig. 2C). Inoculation of wild and cultivated beet accessions with *A. alternata* isolates resulted in limited but typical lesions, though only after the plants were exposed to a period of low temperature. Without the low temperature treatment, the plants remained symptomless. Scanning electron microscopy confirmed that the leaves were free from symptoms (data not shown).

Scanning electron microscopy, of infected plants

Numerous hyphae emerged from lesions on beet leaves that were infected naturally by *A. alternata* and kept in high humidity for 8 h (Fig. 3A). Less than 0.5 mm from the lesions, no mycelium on the leaf surface was observed. The hyphae emerged in bundles from specific sites on the lesions (Figs. 31B, 3C), which is probably a weak point of the dead tissue. Typical *A. alternata* spores, at low density ($7 \pm 2/mm^2$), were observed on the leaf surface. When cotton plants were inoculated with an aggressive isolate of *A. alternata* (S-1), two types of leaf colonization occurred, depending on the availability of condensed water on the leaf surface. In the presence of condensed water, massive mycelium was observed on the surface of cotton leaves (Fig. 4A), similar to the spreading of this isolate on wild beet leaves (compare Figs. 3A and 4A). Spores formed on the superficial mycelium (Fig. 4B). In the absence of condensed water on the leaves (RH < 80%), only a small amount of mycelium was observed. This mycelium was associated with the plant stomata (Fig. 4C); superficial hyphae penetrated the stomata (Fig. 4D) and the internal leaf mycelium emerged (Fig. 4E) and divided into two hyphae.

After cotton plants were inoculated with a less virulent isolate of *A. alternata* from beets (S-3), no mycelium was observed on the leaf surface. The lesions were small and usually lacked spores (Fig. 5A). Sporulation was restricted to the leaf veins (Figs. 5B-5D) and was observed even on very small veins. Transfer of these infected plants to high relative humidity, and including condensed water on the leaf surface, did not result in development of hyphae on the leaf surface.

Disease outbreaks near infected wild beet plants

Two field surveys were carried out during February and March of 1987, before the cotton growing season began in the Bet-Shean valley. Areas around cotton fields of *G. hirsutum* were explored for sites with wild beet plants and several were located. Most sites that contained heavily infected beet plants with *A. alternata* lesions in their older leaves were located at the edges of the previous year's cotton fields. Pathogen identity was confirmed by laboratory analysis. Two sites were located on the slopes of Gilboa mountain far from the cultivated cotton area (Fig. 6). Two additional field surveys were

performed later in the crop season after the cotton had begun to grow. This timing corresponded to the first of the two epidemic outbreaks of Alternaria blight in Israel (5, 8).

These surveys revealed that near each of the infected wild beet sites, over 70% of the cotton seedlings were infected with *A. alternata* (Fig. 6).

Discussion

Various strains of *A. alternata* are known pathogens of various plant species (1, 10, 12, 13, 17, 19, 22, 24, 26, 31, 32, 33, 34, 35). These occur as common saprotrophs or are ubiquitous in lesions caused by other pathogens (21). This study, together with the previous one (9), suggests that one of the causal agents of Alternaria blight disease of cotton is a member of the *A. alternata* complex of species (30) and that *A. macrospora* together with *A. alternata* produced a disease composite commonly known as Alternaria blight of cotton (5, 9).

The common development of Alternaria blight of cotton at the cotyledon stage is attributed to *A. macrospora* in *G. barbadense* plants and to *A. alternata* in *G. hirsutum* plants (2, 4, 9, 14), but the source of the pathogen infecting seedlings is unclear. In Israel, there is a law that cotton fields must be plowed deeply at the end of the crop season to control the dormant stages of insects. Survival of the fungus in buried or partially decomposed cotton plant debris was found to be minimal (2). In addition, nondegraded residues of cotton plants are rarely found on the soil surface late in the winter rainy season in Israel (2). Long-term survival of alternaria blight agents in the soil has not yet been demonstrated.

Wild beet is a very common weed widely spread over all regions of Israel near the edges of cultivated crops (15). This weed has been specifically and efficiently controlled in the past since it contaminated sugar beet fields. Since sugar beet cultivation has been abandoned in Israel, this weed is no longer controlled. Thus it has been prolific during periods without cotton cultivation as well as in the beginning of the cotton growth season. However, it was not known whether beet plants, either wild or cultivated, were infected by *A. alternata* (27, 28). The present study demonstrates that wild beet are possible carriers of *A. alternata* pathogens. Therefore, these data may provide a possible explanation for early season, widespread epidemics of *A. alternata* in cotton plants. Furthermore, this study presents field observations that indicate that whenever diseased wild beet plants were present at the edges of cotton fields, many nearby cotton plants were infected at their cotyledon growth stage. However, since the evidence presented in this study is circumstantial or is from the laboratory, this claim will require further field studies.

Chemical control of Alternaria blight of cotton is complicated and often ineffective (Y. Zachs, unpublished data). On the other hand, wild beet plants can be easily controlled by the common herbicides used during cotton cultivation. Thus, this study proposes that an intensive control of wild beet plants in the field should be evalauted as a method of controlling Alternaria blight outbreaks in cotton cotyledons.

In conclusion, wild beet plants may serve as an overseason carrier for *A. alternata* that causes Alternaria blight of cotton. The dissemination of *A. alternata* from the wild beet to the cotton plantation and the exact role of beet plants in maintaining sizable infectious populations for cotton need further study.

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