Internal Mold Caused in Sweet Pepper by *Alternaria alternata*: Fungal Ingress

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ABSTRACT

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The ingress of the fungus *Alternaria alternata*, which causes internal mold of ripe red pepper fruit without external signs of infection, was investigated. Information obtained in field surveys and inoculation experiments suggested that the fungus entered the developing fruit at the flowering stage via the stigma and the style. The fungus developed compact masses of mycelia and spores on seeds when the fruit was biologically mature. Infection by the parasite, damaged flower and young fruit tissues causing necrosis on the blossom end, on the seed, and placenta tissue. Hyperplasia of seed and placenta tissues was also observed. Fungal infection induced flower drop in greenhouse experiments. Disease incidence was affected by the age of the flower at the time of infection. The mode of ingress of *A. alternata* and the type of damage it causes to pepper fruit tissue under local conditions in Israel are unlike those reported by investigators in other countries.

Additional key words: *Capsicum annuum* 'Yellow Y,' 'Gambo,' and 'California Wonder.'

Damage of pepper fruit by the fungus *Alternaria alternata* (Fr.) Keissler has been reported from most countries where this crop is grown (1,4–6,8,9). The damage was described by researchers as black spots on the fruit surface, fruit rot, internal mold of fruits, and blossom end rot. Most of the above authors characterize the fungus as a weak pathogen and report that infection was not established unless the fruits were mechanically injured.

In Israel, the pathogen infects fruits of various cultivars of sweet pepper (*Capsicum annuum* L.) (10). The damage caused is called "internal mold of fruit" by the local growers. Compact masses of mycelium and spores are formed on the seeds of the ripe red fruit (Fig. 1). Contrary to reports from abroad, in Israel no symptoms or signs were found on the surface of infected fruit and these were detected only by cutting the fruit.

The objective of the present study was to determine and to elucidate how and when *A. alternata* invades and develops inside the ripe fruit without causing external symptoms or signs of infection. A preliminary report on the subject was published in 1973 (3).

MATERIALS AND METHODS

Examination and inoculation procedures. Fruits of pepper cultivars Yellow Y (YY), California Wonder (CW), and Gambo were sampled in commercial fields where internal mold had been detected. Each sample comprised 100–180 fruits of various ages ranging from fruit setting to the red-ripe stage. Each fruit was cross sectioned and observed for damage to the pericarp, seeds, and placental tissue. The damage was described and the tissue from each sample of cultivar CW were sown on potato-dextrose agar (PDA) for fungal isolation. The fruits of each sample were divided into six groups (30 fruits in each group), according to weight, from 5 to 80 g per fruit. The following techniques were applied for isolation of the fungus: the fruits were soaked for 3 min in 0.5% $HgCl_2$ solution, rinsed in distilled water, and dried. Sections, measuring 4–6 mm in diameter, were cut from the pericarp, just below the style, and from the middle of the placenta. The pericarp just below the style henceforth will be identified as the "blossom end." The surface-sterilized tissue pieces, sorted into fungus-damaged and undamaged, were sown on PDA and incubated for 6 days at 20 C, with white fluorescent lamp illumination (12,500 lux) for 8 hr per day.

Inoculation studies were carried out on potted plants in the greenhouse. At anthesis, the flowers were tagged so that their age could be determined when inoculated. In all inoculation trials an isolate, designated 2A, of *A. alternata* was used. It was isolated from a naturally infected fruit and cultured from a single spore. The fungus was grown for 8–12 days on PDA at 20 C with 8 hr of fluorescent illumination per day. The concentration of fungal suspension for inoculation was 2×10^5 spores per milliliter of water.

Inoculation was performed by smearing the suspension of spores on the flower parts with a brush; sterile water was applied similarly to control plants. Following inoculation the plants were covered for 48 hr with polyethylene bags. The temperature in the greenhouse at the time of inoculation and during growth was 15–18 C during the night and 29–31 C during the day.

In the various inoculation trials ovaries of dropped flowers, marketable green and red fruits were cut for observation of disease incidence on the blossom end, placenta, and/or seed. The technique described above was applied in isolating the fungus from the tissue.

Site and mode of fungal ingress. Various parts of the flowers were inoculated 1 day after anthesis. To prevent inoculation of other flower parts, the uninoculated parts of the flowers were covered with plastic capsules. Treatments, each applied to 40 flowers, included: inoculation of the stamens and the base of the style, both followed by covering of the style, and the inoculation of the stigma, without any covering.

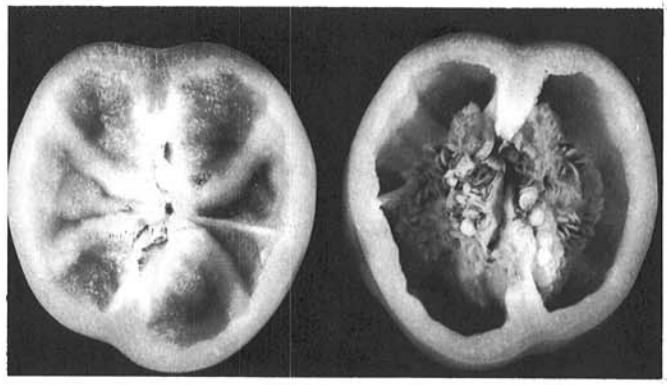

Fig. 1. Cross-sectioned red pepper fruit showing internal mold caused by *Alternaria alternata* on the right half bearing seeds.

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Effect of inoculation at various stages of ovarial development on disease incidence. Two experiments were carried out on different cultivars. Flowers of CW were inoculated on the stigma at various stages of ovary development. One day before anthesis, the flower petals were opened and the fungal suspension was placed on the stigma. Additional inoculations were done on the day of anthesis, 5–6 days after anthesis, and 20 days after anthesis. Uninoculated flowers were used as controls. Sixty to 70 flowers on a total of five plants were inoculated in the first three treatments and the control and 20 young fruits in the fourth one. Marketable green fruits and ovaries of dropped flowers were examined for infection. In the second experiment, the stigma of cultivar Yellow Y was inoculated 2 and 7 days after anthesis. For each treatment and for the uninoculated control 160–200 flowers of 11 plants were used. However, due to flower drop, only 50–90 marketable green and red ripe fruits were examined in the various treatments.

Effect of pollination and fertilization on ingress of the fungus. At the budding stage (1 day before anthesis) stamens were removed from flowers or left intact. In each case 70–80 flowers were inoculated on the stigma. All flowers were covered with paper bags until harvest time. Disease incidence was rated by observing the ovaries of dropped flowers and cross-sectioned marketable green fruits.

RESULTS AND DISCUSSION

Examination of naturally infected fruits. Necrosis of fruit tissue (pericarp, placenta, and seeds) was detected in the young fruits weighing only a few grams and in green and red fruits of cultivars YY, CW, and Gambo that were ready for harvest. The necrosis of the pericarp was restricted to the inner pericarp below the style; the damage was manifested as a brown lesion of indefinite form in the blossom end tissue (Fig. 2). Fungal hyphae could sometimes be detected by microscopic examination of the lesion.

The necrosis in seeds appeared as brown spots of variable size, which frequently spread over the seed surface. The affected seed were generally located in the center of the placenta (Fig. 2). Sometimes, hyphae were seen ramifying among the seeds. The necrosis in seeds was generally accompanied by necrosis of the blossom end, but the latter was not always accompanied by seed necrosis.

Necrosis in the placenta was conspicuous especially in parthenocarpically produced fruits. *A. alternata* was isolated in 80–90% of the cases from the necrotic tissue of the blossom end, seeds and placenta in fruits of all ages. The fungus also was isolated from 8–20% of the fruits without necrosis.

Hyperplasia of the tissue of seeds and sometimes in the placenta was also observed in fruits infected with *A. alternata*. This damage was characterized by increased and irregular growth of the endosperm tissue, which emerged through the seed coat, giving an irregular shape to the damaged seed (Fig. 3). In the anatomical section of the injured seed of Fig. 3, the cells of the damaged part differed conspicuously from those of its undamaged part (Fig. 4). The phenomenon of hyperplasia in affected fruits was common in a commercial field; hyperplasia was detected in 900 of the 1,800 fruits affected by the fungus.

Fungal colonization of the style. Spores of *A. alternata* were detected in dense masses on the styles of fruits of all ages, although the styles of pepper fruits remain attached to the fruit even after harvest. In checking the styles of a sample of red fruits with 20–40% of internal mold, 90% of the styles were covered with mycelium and spores of *A. alternata* and 5% of the latter also were covered with spores of *Cladosporium* spp. Spores of *Alternaria solani*, *Fusarium* spp., and *Stemphylium* spp. were observed in the styles of a few fruits.

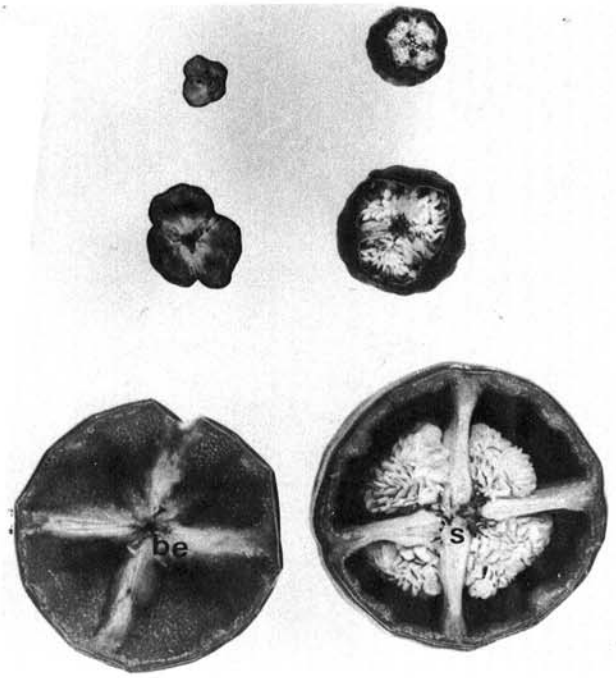
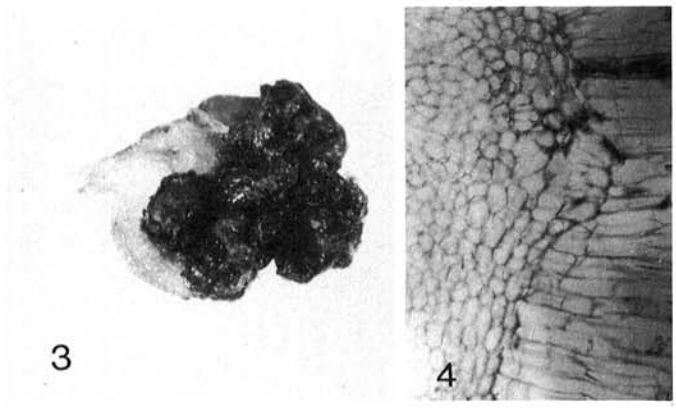


Fig. 2. Necrosis appearing at center of halved fruit of various ages. Larger size indicates greater fruit age. Left—necrosis on blossom end (be). Right—necrosis on seeds (s).



Figs. 3 and **4.** Hyperplasia of pepper seed caused by *Alternaria alternata*. **3,** Macroscopic aspect showing hyperplasia in the right part of the seed. **4,** Microscopic aspect at the juncture of normal (left) vs abnormal (right) tissue.

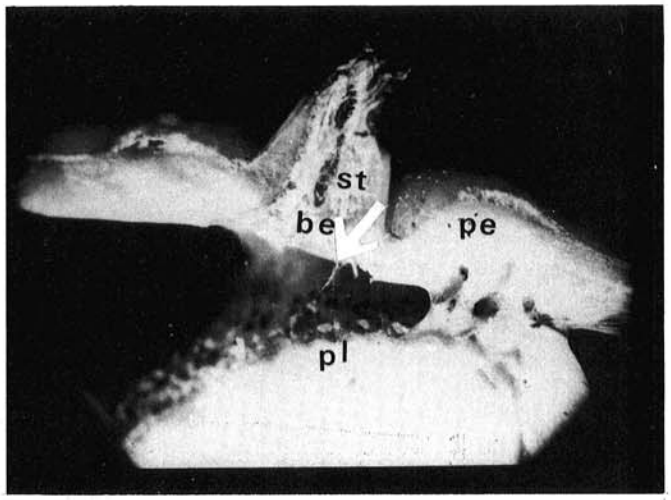


Fig. 5. Vertical section of young fruit, inoculated 1 day after anthesis: style (st); blossom end (be); pericarp (pe); and placenta (pl). Note the *Alternaria alternata* hyphae (arrow) reaching the placenta.

TABLE 1. Effect of inoculation with *Alternaria alternata* in various stages of ovary development on flower drop and disease incidence in ovaries and fruits of pepper cultivar California Wonder

	Percentage of the total number tested				
		Necrosis in ovaries of dropped flowers		Necrosis in fruits	
Time of inoculation	Flower drop	Blossom end	Blossom end and placenta	Blossom end	Blossom end and placenta
Uninoculated control	57.3	0	1.4	0	0
1 day before anthesis	87.3	27.0	46.0	37.5	62.5
Day of anthesis	88.2	20.4	42.6	25.0	50.0
5–6 days after anthesis	77.2	4.0	34.0	13.3	26.7
20 days after anthesis		0	0	0	0

Site and mode of fungal ingress. Necrotic lesions on the blossom end and seeds of fruit were observed in 50% of the fruits developing from flowers previously inoculated on the stigma. The fungus was not detected in any of the fruits developing from flowers inoculated on their stamens or style base. The damage detected in the affected fruits in this experiment was similar to that observed in naturally infected fruits and only *A. alternata* was isolated from the necrotic tissues. The first symptom in the inoculated flowers appeared on the 1 or 2 days after inoculation as a browning or blackening of the stigma tissue associated occasionally with fungal hyphae on the apex of the stigma. Browning appeared later in the upper part of the style and sometimes extended over the style of the inoculated flowers. Fungal hyphae invading from the style into the ovary were conspicuous in longitudinal sections of young fruit (Fig. 5). Ingress of fungi via the stigma was reported also by Strobel and Hawitt (11) in grape and Werner (12) in tomato.

Effect of inoculation at various stages of ovary development, on symptoms development. Greatest disease incidence both in the ovaries of the dropped flowers and the picked fruits of cultivar California Wonder was obtained by inoculating the stigma 1 day before anthesis or at day of anthesis (Table 1). Disease incidence decreased when flowers were inoculated 5–6 days after anthesis and no necrosis was observed when fruits were inoculated 20 days after anthesis. In some ovaries and fruits necrosis was detected at the blossom end only; in others, it also was found in placenta and seed. In several ovaries and fruits, hyphae of *A. alternata* were observed on the necrotic tissue. Frequency of flower drop was increased in comparison with controls when flowers were inoculated 1–6 days after anthesis. An average of 7.2 marketable green fruit per plant was picked in the control, as compared with 3.0 and 1.6 fruits developed from flowers inoculated 5–6 days after anthesis and on the day after anthesis, respectively.

Disease incidence in seed of both green and red fruits of cultivar Yellow Y was higher in fruits developing from flowers inoculated 2 days after anthesis compared with flowers inoculated after 7 days (Table 2). In more than half of the affected red fruits of this trial, compact masses of mycelium were detected on the seeds in addition to necrosis in the seed tissue. In the green fruits, necrosis only was observed. In the visual examination, disease incidence in red fruits was higher than that of fruit picked still green. When sections of the central part of the placenta including the seeds of all green fruits (damaged and undamaged) were sown on PDA, the fungus was isolated as frequently as from red fruits (Table 2). The pathogen was isolated from fruits with symptoms and also from some fruits in which no necrosis was detected by visual examination.

It seems that in some cases the mycelia entered into the ovary and reached the placenta without causing damage. Later, at the stage of fruit ripening, it renewed its growth as mold on the seed.

In cultivar Yellow Y, similarly to cultivar CW, more flower drop occurred in inoculated flowers than in the control. The highest amount of flower drop was observed in flowers inoculated 2 days after anthesis; it reached 52% compared with 16% in the control. Hyperplasia in seed of green fruits was 84 and 74% in fruit inoculated at early and late stages, respectively. The hyperplasia observed in the injured tissues was presumably caused by fungal secretion substrata. By injection of the seed surface of young fruit with a fungal suspension or a sterilized fungal extract, we induced

TABLE 2. Disease incidence in pepper cultivar Yellow Y after inoculating flowers at various stages of ovary and fruit development with *Alternaria alternata*

	Disease incidence (%)		
	Visual examination		Fungal isolation from green fruits
Time of inoculation	Green fruits[a]	Red fruits[b]	
Control fruits			
2 days after anthesis	0	2	0
7 days after anthesis	0	2	0
Inoculated fruits			
2 days after anthesis	36.6	52.6	58.3
7 days after anthesis	9.4	39.0	34.7

[a] Necrosis on seed.
[b] Necrosis and mycelia on seed.

TABLE 3. Disease incidence in pepper ovaries and fruits inoculated with *Alternaria alternata* when flowers were or were not emasculated

	Necrosis in placenta and/or seeds (%)			
	Ovaries of dropped flowers		Fruits	
Treatment	Emasculated flowers	Non-emasculated flowers	Emasculated flowers	Non-emasculated flowers
Control	0	2	0	0
Inoculated	64	44	68	40

hyperplasia in the seed's tissue (A. Meiri, *unpublished*). The fungus invaded the fruit via the style when pollination did or did not occur (Table 3); however, the disease incidence was higher in the absence of pollination than when pollination occurred. It seems that the pollen did not facilitate the *A. alternata* ingress as otherwise reported for *B. cinerea* in strawberry (2).

Our findings suggest that the mode of ingress of *A. alternata* and the damage induced in tissues as a result of the mode of entrance, differ from those described by researchers in other countries. According to Mathur and Agnihotri (5) and Quebral (9), the fungus is a weak parasite and invades the fruit via the injured pericarp only. In our experiments inoculations on the stigma induced subsequent necrosis of live tissue. To the best of our knowledge, such damage caused by *A. alternata* on sweet pepper fruits has not been reported previously. The different behavior of the fungus under Israeli conditions may be a result of either the different ecological conditions or the activity of different fungal strains, or both.

LITERATURE CITED

1. Bremer, H. 1945. On pod spots in peppers. Phytopathology 35:283-287.
2. Chu Chou, M., and Preece, T. 1968. The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. Ann. Appl. Biol. 62:11-22.
3. Halfon-Meiri, A., and Rylski, I. 1973. Penetration of *Alternaria tenuis* into pepper fruit. (Abstr.) Phytoparasitica 1:57.
4. Leyendecker, P., Jr. 1950. Blossom end rot of pepper (*Capsicum annuum* L.) in New Mexico. Phytopathology 40:746-748.

5. Mathur, K., and Agnihotri, J. P. 1961. Internal mold of chillies caused by *Alternaria tenuis* Auct. Indian Phytopathol. 14:104-105.
6. Melikova, S. 1960. The susceptibility of pepper varieties to spot diseases. Rest. Zashch. Moskova 5:57 (From Rev. Appl. Mycol. 40:444 [1961].
7. Powelson, R. 1960. Initiation of strawberry fruit rot caused by *Botrytis cinerea*. Phytopathology 50:441-444.
8. Pucci, A. 1947. Una grave alternariosis sobre pimento y Berenjena en la Republica Argentina. Publ. Tec. Irec. Agropec. B. Aires 4:7. (From Rev. Appl. Mycol. 27:458. [1947]).
9. Quebral, F. 1966. A study of Alternaria rot of pepper in Illinois caused by *Alternaria tenuis* Auct. Ph.D. thesis, Univ. of Illinois, Urbana-Champaign.
10. Rylski, I., Halfon-Meiri, A., and Kempler, H. 1975. (The susceptibility to internal mold of fruit). Hassadeh 55:1630-1631 (in Hebrew).
11. Strobel, G., and Hewitt, W. 1964. Time of infection and latency of *Diplodia viticola* in *Vitis vinifera* var Thompson Seedless. Phytopathology 54:636-639.
12. Werner, E. 1936. Black rot of tomato, *Lycopersicon esculentum*, caused by *Alternaria* sp. Phytopathology 26:530.