Assessment of Latent Infections as a Basis for Control of Postharvest Disease of Mango

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ABSTRACT

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Alternaria alternata, the causal organism of black spot disease in mango, *Mangifera indica*, penetrates the fruit through lenticels. After infection in the orchard, the hyphae remain latent until postharvest ripening of the fruit, then develop intercellularly. Assessment of the latent infections during fruit growth indicated a continuous increase in infected surface from fruit set until harvest. Protectant fungicide sprays begun after fruit set decreased the latent infected surface by 46%, resulting in a significant reduction of 37% in the incidence of black spot disease during storage. A significant correlation coefficient of 0.92 ($P < 0.05$) was obtained between the latent infected surface of mature mango fruits by *Alternaria* in the field and the incidence of black spot disease in storage. These results indicate the possibility of using preharvest assessment of latent infections as a basis for a control program of postharvest treatments.

Additional key words: iprodione, storage disease

As a result of efforts to prolong storage life to its physiological limits, latent infection of fruits by *Alternaria* sp. has become one of the important factors in decay of stored fruits and vegetables. *Alternaria* decay symptoms have been described for stored oranges and lemons (2), persimmons (11), blueberries (4), apples (3), and tomatoes (1).

Because of the high incidence of black spot decay on stored mango, a project was initiated to identify the fungal agent involved in the decay and to examine possible approaches to control it. Anthracnose, the best known disease of mango, caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. (6), is rarely found in stored mangoes in Israel. This paper describes *Alternaria alternata* (Fr.) Keissler as a causal organism of a black spot disease of mango fruits. A quantitative method for latent infection assessment as a basis for programming the application of suitable treatments to prevent postharvest diseases of mango is also presented.

MATERIALS AND METHODS

Mango fruit (*Mangifera indica* L.), cultivars Haden, Maya, and Nimrod, was used for isolating and inoculating the causal organism of this black spot disease. Fruits were obtained from trees in an orchard near Rehovot (in the coastal plain of Israel). Before isolation, the fruits were surface-disinfested with 90% ethanol. Small slices of peel or large black spots were further surface-disinfested with 1% NaOCl for 2 min and incubated at 25 C on potato-dextrose agar (PDA). Single-spore techniques were used for starting cultures. Cultures were kept on PDA at 25 C. For fruit inoculation, conidia were harvested by adding 1–2 ml of sterile distilled water and gently rubbing the sporulating mycelial mat with a bent glass rod. The suspension of conidia was decanted into sterile distilled water containing 0.1% polyoxyethylene sorbitan monolaurate (Tween 20) as a wetting agent to prevent spore clumping and adjusted to 10^6 conidia per milliliter. Detached, surface-disinfested, unwounded fruits were inoculated by placing a drop of conidial suspension on the surface. After inoculation, the fruit were held at 25 C in polyethylene bags.

Fixation and dehydration of specimens for scanning electron microscopy (SEM) were done in a series of ethanol and acetone solutions. For SEM observations, the dehydrated tissue was handled according to the method of Cohen (5). Samples were observed in a JEOL JSM-35C scanning electron microscope. For light microscope observations, the dehydrated tissue was embedded in Spurr's low-viscosity medium (14). Thin (5-μm) slices were collected in distilled water drops on a glass slide and mounted in immersion oil. Five naturally infected lenticels were observed during the growing season with a phase-contrast microscope.

Assessment of latent infections by *Alternaria* in naturally infected mango fruits from trees in the orchard during the growing season and at harvest was made according to the method described by Prusky et al (12). During the growing season, fruits were harvested for latent infection assessment five times at intervals of 10–20 days beginning 3 wk after fruit set. One fruit was sampled from each tree, each representing a replicate of the experiment. Eight or 10 fruits, varying according to the experiment, were sampled for latent infection assessment. The harvested fruits were washed thoroughly and disks of peel and flesh (5 mm diam. and 4–6 mm thick) were sampled. Disk sampling was done in six circular zones around the fruit at different distances from the stem end. The disks were disinfested as described before. Ten to 13 disks from each zone were placed, peel side down, in a petri dish containing PDA and 20 μg thiabendazole per milliliter as a culture medium and incubated at 25 C for 4 days. Estimation of the latent-infected surface was obtained by the product of the mean infected disks of the fruit and $W^{2/3}$ (the weight of the single fruit, W, transformed into values proportional to its surface area) (12).

Rate of infection during fruit growth was determined quantitatively on untreated and protectant fungicide-treated fruits. For this experiment, 9-yr-old mango trees, cultivar Haden, were chosen in a random block design with eight replicates. The row of trees chosen for one block of the experiment was separated by untreated rows of trees. Within the row (block of experiment), the treated trees were separated by one or two untreated trees. A single tree constituted a replicate. The fungicide maneb was applied as an aqueous spray at a concentration of 0.3% and at a rate of about 8 L per tree, starting 19 days after fruit set; the treatment was repeated every 14 days until harvest. Field experiments were repeated three times, once in each of three consecutive years.

To determine the correlation between latent infections assessed at harvest and incidence of the disease in stored fruits, cultivar Haden fruits were taken from different regions of Israel for the experiment. Different groves were chosen according to previous knowledge of differences in disease incidence in storage in each region. Two or three groves were

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sampled in each region. In each orchard, 10 trees were chosen at random and 20 mature green fruits were harvested from each tree at the end of the growing season. Half of them were treated by immersion in a suspension of the fungicide iprodione at a concentration of 1.5 g/L, and the other half served as controls. These experiments were repeated twice, once in each of two consecutive years.

Harvested fruits were stored for 21 days at 14 C, then transferred to 20 C for five additional days. The percentage of surface covered by black spot was evaluated by comparing the infected area that developed during storage with a prepared scale of black spots covering areas ranging from 0 to 20% of the surface. It was observed that when more than 1% of the surface was randomly covered with black spots, a mango fruit was not marketable.

RESULTS

Causal organism and mode of penetration. The organism isolated from infected mango fruits (Fig. 1) showed oboclavate spores borne in long chains in culture, with three to five cross-septa and within the limits of 20–35 × 8.5–9 μm per spore. This fungus was identified as *A. alternata* (9,13). Fruit inoculation with conidia at 25 C showed symptoms of infection 3–4 days after inoculation. These symptoms consisted of either small black spots 0.5–1.0 mm in diameter with a dark center and diffuse borders or dark lenticels when water drops containing conidia were placed directly on the lenticels. Reisolation from the infected fruits showed *A. alternata* was the causal organism of this black spot disease in mango.

SEM observations of naturally infected fruits showed germinating conidia of *Alternaria* penetrating the lenticels (Fig. 2A). The fungus develops in the lenticels and penetrates intercellularly, resulting in a darkening of intercellular spaces and cell collapse (Fig. 2B). Mycelium of *A. alternata* observed in this state throughout the growing season renewed its development after harvest, resulting in black spot development (Fig. 1).

Sources and time of infection. The possible source of inoculum for fruit infection was identified on mango leaves (Fig. 3). Hundreds of small black limited spots were observed on mango leaves during the year. New leaves were infected in early spring. *Alternaria* isolated from leaves was able to infect fruits, and isolates from rotten fruits were also able to infect detached mango leaves at about 100% RH and 25 C.

Samples of apparently healthy disks taken from fruit peel at very early stages

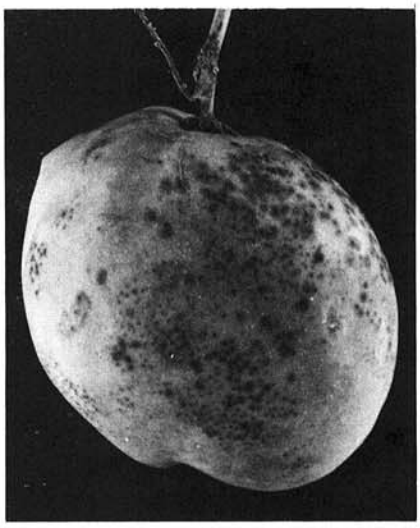

Fig. 1. Symptoms of infection by *Alternaria alternata* on fruit of mango cultivar Nimrod.

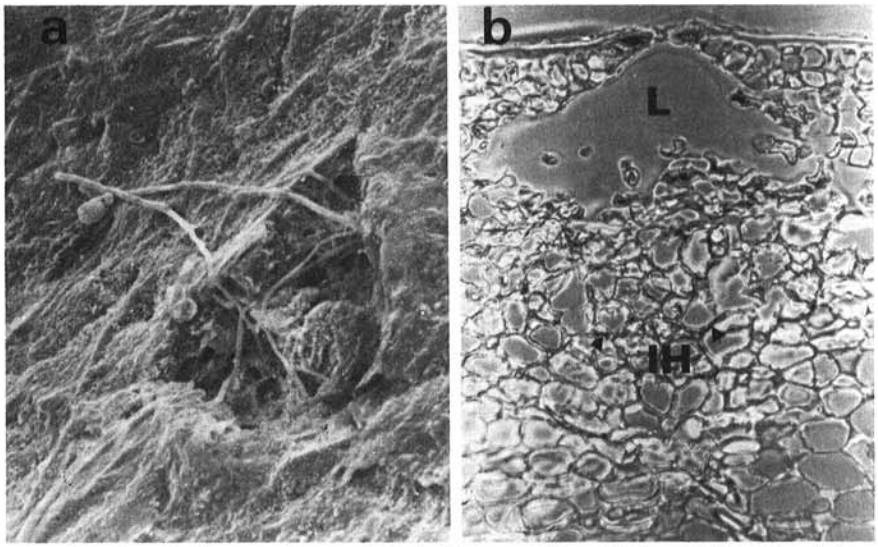

Fig. 2. Infection of *Alternaria alternata* in fruit of mango cultivar Haden. **(A)** Scanning electron micrograph of germinating conidium penetrating a lenticel (×7,500). **(B)** Light micrograph of intercellular hyphae (IH) of the fungus after penetrating lenticel (L) (×700).

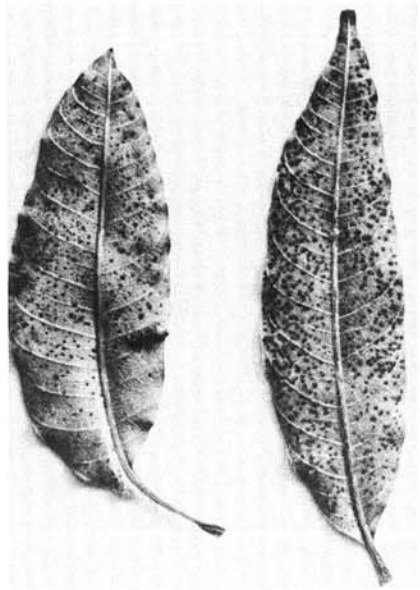

Fig. 3. Symptoms of infection by *Alternaria alternata* on leaves of mango cultivar Haden.

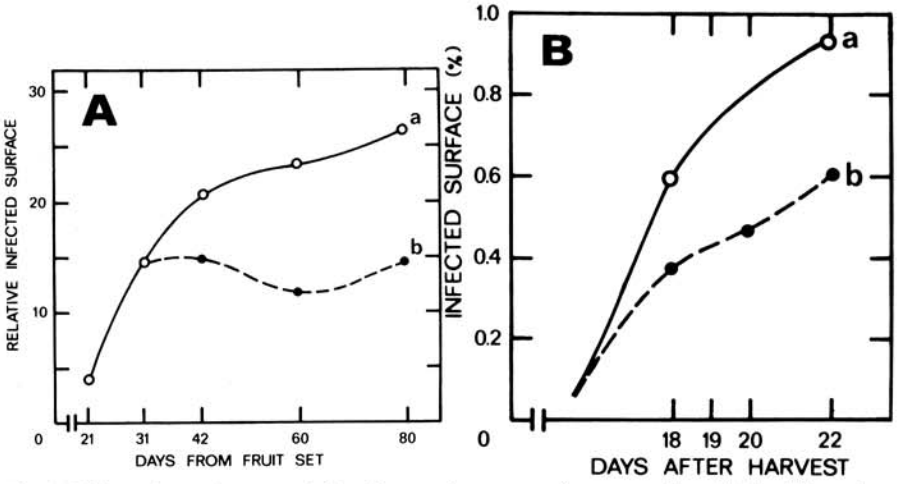

Fig. 4. Effects of maneb sprays during the growing season of mango cultivar Haden **(A)** on the latent infected surface of the fruits and **(B)** on the postharvest percentage of infection (black spot development) in storage for 19 days at 14 C and 3 days at 20 C. o——o = Untreated; •------• = fruits treated with maneb four times during the growing season as described in Materials and Methods.

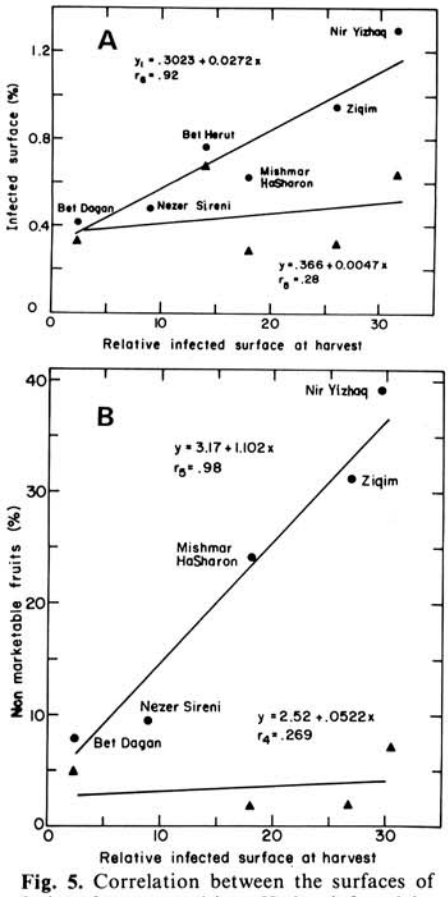

Fig. 5. Correlation between the surfaces of fruits of mango cultivar Haden infected by latent *Alternaria alternata* at harvest and **(A)** visually estimated percentage of infected surface of the fruit and **(B)** percentage of unmarketable fruits after 21 days of storage at 14 C and 5 days at 20 C. Results were obtained from different values of infection from orchards all over Israel during one single year. •——•, Control; △——△, iprodione-treated fruits, 1.5 g/L.

of the growing season, 2 wk after fruit-set, already showed the presence of latent hyphae of *A. alternata* when isolated on PDA. An evaluation of latent infections in mango during the 10-wk growing season indicated a continuous increase in the incidence of *Alternaria* infections on the fruit until harvest (Fig. 4A). Protective treatments during fruit growth with the fungicide maneb resulted in a lower incidence of latent infections (Fig. 4A) and consequently a lower percentage of infected surface after 22 days of storage (Fig. 4B).

Relation between latent infection assessment at harvest and development of Alternaria black spot after harvest. A significant correlation coefficient of 0.92 ($P < 0.05$) (Fig. 5A) was obtained between the relative latent infected surface in different groves and the visually estimated percentage of infected area after storage in the first year of experiments. The regression equation was $y = 0.302 + 0.027x$. A significant correlation, $r = 0.98$ ($P < 0.05$), was also found between the relative latent infection surface and the percentage of unmarketable fruits (Fig. 5B). Similar results were found in the second year of experiments.

The efficacy of postharvest dip treatments with 1.5 g/L iprodione on fruits of each orchard was demonstrated by a decrease in the incidence of Alternaria rot (Fig. 5A) and the percentage of unmarketable fruits after harvest (Fig. 5B) between the regression lines of the control and the iprodione-treated fruits.

DISCUSSION

The fungus isolated from these black spots on stored mangoes was identified as *A. alternata* and not anthracnose, *Colletotrichum gloeosporioides*. *Alternaria* has been increasingly reported as an important pathogen of various stored fruits (8,11,12). This pathogen is related to early infections in the growing season and enters a latent or quiescent state soon after the initial stages of infection are completed (12). After harvest, the fruits gradually lose their resistance as they ripen and the pathogen resumes growth, giving rise to an active decay lesion on the host (7).

Alternaria conidia from mango leaves were able to infect fruit and produce typical disease symptoms in the laboratory. In the field, conidia of *Alternaria* possibly reach the fruit by air or runoff of dew from dried or newly infected leaves (10). Different paths of penetration have been described for *Alternaria*. The pathogen penetrates the stem end and may remain latent in the stylar end of citrus fruits (6). It penetrates stomata in apricots (8); through wounds produced at different periods of fruit life in tomatoes, blueberries, and apples (1,3,4); and directly through the cuticle over the entire surface of persimmon fruits (11). The lenticels of mango fruit seem to be the main penetration path with further intercellular development.

Latent infection assessment by isolation from peel tissue indicated that *Alternaria* infection occurred immediately after fruit set and during the entire period of fruit growth (Fig. 5A). A decrease in the incidence of black spot in storage was achieved by preharvest or postharvest treatments. Preharvest treatments reduced the number of latent infections, whereas postharvest treatments inhibited the development of latent infections already present in the fruit. Latent infection assessment allows determination of the infection period and timing of application of protective fungicides. *Alternaria* infection of mango starts soon after fruit set, and because the fruit growth period is relatively short, continuous protectant treatments are necessary to reduce the latent infected surface significantly. Results with mangoes had indicated previously that delay of protectant treatment after fruit set decreased its effectiveness (12). The significant correlation between the relative infected surface at harvest with the visually estimated percentage of infected surface ($r = 0.92$) (Fig. 5B) and with the percentage of unmarketable fruits ($r = 0.98$) (Fig. 5B) in different groves indicates that quantitative assessment is a reliable way to compare severity of infection, estimate postharvest development of black spot, and program postharvest treatment. Differences in disease incidence of stored mangoes in different regions of Israel are not based on cultural practices, rainfall, or topography. They may be based primarily on the hours of dew in each region (D. Prusky, *unpublished*) and on the density and infestation of the grove.

We have shown the use of the quantitative assessment method together with efficient postharvest treatments like iprodione to be a basis for determining how fruit should be handled. Our data indicate that mango fruit with a latent infected surface with values around 5 should not be treated with iprodione because postharvest treatments will not significantly reduce disease incidence. Higher values of latent infected surface indicate the need for postharvest treatments, and the treatment is expected to be effective.

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