A Red Spot Fruit Blemish in Apricots

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ABSTRACT

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A red spot fruit blemish of apricots, particularly the Moorpark cultivar, is reported and described. It is differentiated from other blemishes, including those caused by Coryneum blight and San Jose scale, by a smooth surface texture and bright red halo. The causal agent was shown to be *Alternaria alternata*, and a hypersensitive host response is suggested.

A previously undescribed apricot fruit blemish has been observed for several years in central Washington. The superficial red spots often are given the common name "apricot freckles" by local fieldmen and growers, a name also used occasionally in this paper. The blemishes most frequently are observed on fruit of cultivar Moorpark (Wenatchee Moorpark) but also have been seen occasionally on fruit of cultivars Perfection and Riland. The symptom is similar to that of blemishes caused by San Jose scale (*Aspidiotus perniciosus* Comst.) and "shothole" or Coryneum blight (caused by *Stigmina carpophila* (Lev.) M. B. Ellis; syn. *Coryneum beijerinckii* Oud.) and infected fruit has been mistakenly culled for these other disorders. This misinterpretation by graders confuses the orchardist who has applied what should be adequate pest control measures for the latter two problems. This paper provides a description of the blemish and a summary of the results of our studies of the problem during several growing seasons.

MATERIALS AND METHODS

General and histological observations. Affected fruit collected by us and by local fieldmen were examined with a dissecting microscope, and thin vertical and tangential sections of affected fruit epidermis were made freehand with a razor blade. These sections were mounted in 0.05% trypan blue in lactophenol before they were examined with a compound microscope.

Isolation and culture procedures. In 1977 attempts were made to isolate the causal organism from 25 thin epidermal and subepidermal tangential sections of the spots. Approximately half of these were surface-sterilized for 10 sec in 0.675% sodium hypochlorite solution, rinsed for 10–20 sec in sterile distilled water and placed on potato-dextrose agar (PDA) plates as described by Lacy and Bridgmon (5), but modified by adjusting the concentrations of dextrose to 15 g/L and the agar to 16.25 g/L. The remaining sections were rinsed only in sterile distilled water prior to placement in the PDA plates. All plates were incubated at 20 C and examined for fungal growth beginning 3 days after inoculation. The more frequently observed organisms were transferred to PDA slants for identification and short-term storage. Sporulating material of each fungal isolate was mounted in 0.05% trypan blue in lactophenol by using the rapid slide mount technique (6). The resulting slides were used for microscopic examination and documentation.

Field plot studies. Field plot studies of cultivar Moorpark apricot fruit were made in 1978. Spore inoculum from a spot-associated isolate of *Alternaria alternata* (Fr.) Keissler was obtained by washing the spores from PDA-grown cultures with sterile distilled water containing one drop of Tween-20 wetting agent per 100 ml of spore suspension. This inoculum suspension was applied via atomizer to immature symptomless fruit to the point of surface beading. All fruit was harvested 11 July, bulked according to treatment, and evaluated for occurrence and frequency of "apricot freckles."

At shuckfall (24 April 1978) 150 symptomless fruits on six trees were individually covered with 11 × 14-cm drawstring-mouth bags of featherweight Pellon interlining material (Pellon Corp., 119 W. 40th St., New York, NY 10018). This was done to reduce the exposure of the fruit to the naturally occurring inoculum. The 120 bags still containing viable symptomless fruit on 2 June (39th day) were arbitrarily divided into three groups of 40 fruits each. Fruits in these groups were treated either with a concentrated spore suspension (2×10^5 spores per milliliter), a dilute spore suspension (2×10^4 spores per milliliter), or a sterile distilled water and Tween-20 blank. The inoculation treatments were repeated 8 June (45th day) following several days of abnormally high temperatures which were assumed to have reduced the inoculum potential of the initial inoculation. The bags were removed from the individually bagged fruit 6 July to allow 5 days of natural light exposure for normal fruit coloration and were harvested 11 July (78th day). An additional 30 nonbagged untreated individual fruit (five per tree)

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were randomly selected, harvested, and bulked to serve as field checks.

Small fruit-bearing terminals left attached to nine trees also were arbitrarily selected and bagged in 20 × 30 cm rice-paper bags at three times during fruit development to determine the timing of natural inoculation. Ten terminals were bagged at shuckfall 24 April (0 day), an additional 15 terminals were bagged 12 May (18th day), and a final three terminals were bagged 8 June (45th day). All paper bags were removed 6 July to allow 5 days of exposure to natural light for normal fruit coloration, and the fruit was harvested 11 July (78th day).

Two groups of 12 fruit-bearing terminals on a total of six trees were arbitrarily selected on 11 May 1978, following the completion of laboratory fungicide screening tests. One group was left untreated, and the second group of terminals was sprayed to runoff with Manzate 200 (Zn + Mn ethylenebisdithiocarbamate 80 WP; E. I. duPont de Nemours & Co., 1007 Market Street, Wilmington, DE 19898) at the rate of 2.4 g of formulated fungicide per liter of water with a small hand-operated sprayer. The treatment was reapplied 8 June (45th day) prior to harvest on 11 July (78th day).

The previously described histological methods were used to examine spots from four individual nonbagged uninoculated fruit and from five individually bagged fruit inoculated with 2×10^5 spores per milliliter. The procedures used in the initial isolation also were used in attempts to reisolate *A. alternata* from 11 spots on these inoculated fruit.

RESULTS

Macroscopic and histological observations. The red spot fruit blemish initially was observed 18 days after shuckfall on naturally infected nonbagged fruit in the field plot; it appeared as very small, solitary-to-numerous scattered red dots, usually located on the upper fruit surface. It finally consisted of small (1–3 mm in diameter), smooth-textured, superficial nonelevated light tan to brownish spots with necrotic centers and red halos (Fig. 1 and 2). Only the fruit epidermis and the immediately underlying cells were affected. Fruit rot was only rarely associated with the spots during post-harvest refrigerated storage of the field-plot fruit; the one instance of fruit-rot caused by *A. alternata* was observed on a single nonbagged, naturally inoculated fruit.

Histological examination of 20 spots on fruit submitted by local fieldmen showed the spots consistently had a stomate at the center regardless of their size or degree of necrosis. Fifteen spots also had a germinated *A. alternata* spore in the immediate vicinity of the central stomate, and the germ tube from these germinating spores typically had grown toward or into the orifice of this central stomate; however, further hyphal growth within the fruit tissues was not detected. The germination process was most clearly visible in the early stages of spot development due to the accumulation of brownish deposits within cells of the central zone as the spots enlarged and aged. In several mounts, the germ tube produced an appressoriumlike structure within the stomatal orifice.

Cultural studies. *A. alternata* was the only organism consistently associated with the 25 tissue sections used in initial isolation attempts. Nonsurface-sterilized sections of epidermal tissues yielded seven colonies of *A. alternata* surface and one colony of *Epicoccum purpurescens* Ehrenb. ex. Schlect. while similar surface-sterilized sections produced two colonies of *A. alternata*; no colonies were found on sections of subepidermal tissues regardless of sterilization treatment. Colonies of *A. alternata* were observed on eight of 14 epidermal sections of small, young, red spots. Eleven epidermal sections of larger, more developed red spots produced only a single colony of *A. alternata*. Identifications of the isolates, including the three selected for subsequent experimental use, were based upon descriptions in Ellis (2) and Neergard (10), and J. Ogawa (*personal communication*) subsequently confirmed our identification of *A. alternata*.

Field studies. Enclosure of individual young fruit in "Pellon" bags greatly reduced the incidence of both "apricot freckles" and Coryneum blight fruit lesions; inoculation of similarly bagged fruit with *A. alternata* spores increased the incidence of "apricot freckles." Specifically, uninoculated individually bagged control fruit averaged 1.3 spots per fruit ($\sigma = 1.9$) in contrast to an average of 20.9 spots per fruit ($\sigma = 14.9$) for naturally inoculated nonbagged fruit. Individually bagged fruit inoculated with the dilute spore suspension averaged 4.2 spots per fruit ($\sigma = 6.5$), and similar fruit inoculated with the concentrated spore suspension averaged 10.0 spots per fruit ($\sigma = 7.3$). The incidence of Coryneum blight lesions on individually bagged fruit was estimated to be 50–70% of that on nonbagged fruit. No evidence of San Jose scale infestation was observed in the plot.

Fruit from bagged terminals with various lengths of exposure to the naturally occurring inoculum exhibited a relationship between

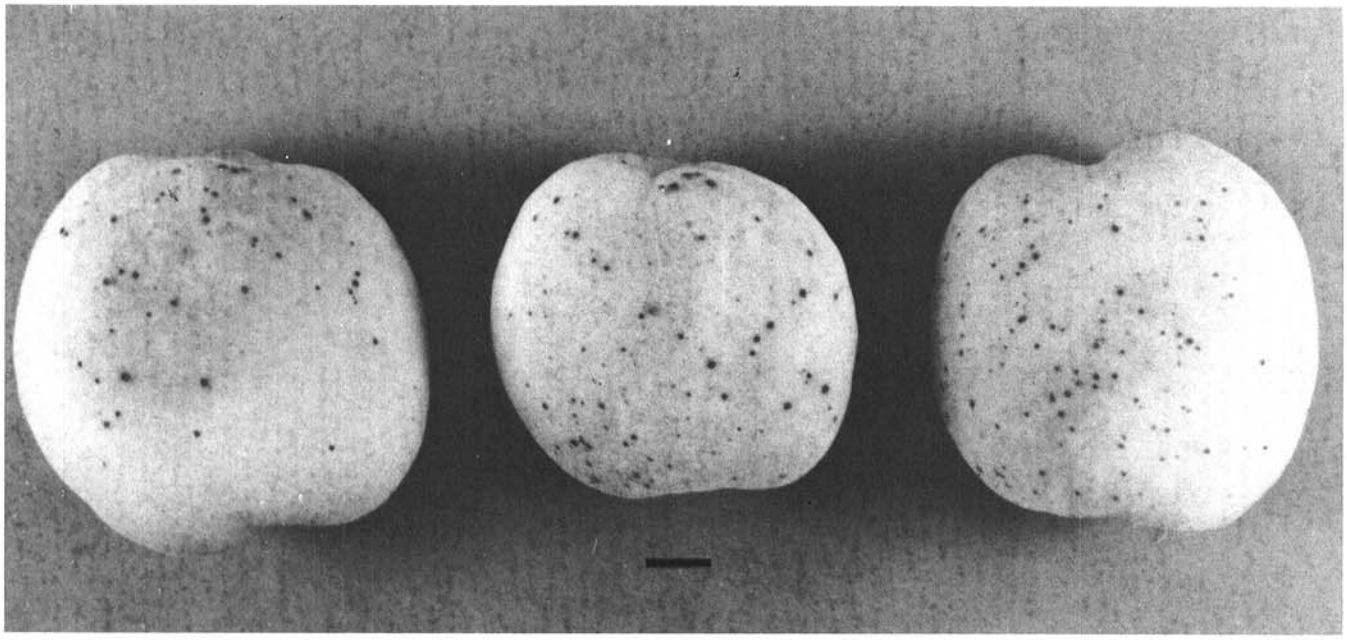

Fig. 1. Apricot fruit (cultivar Moorpark) showing typical "apricot freckles" resulting from severe natural infection by *Alternaria alternata* during the 1976 growing season. Bar = 1 cm.

the length of exposure and red spot incidence at harvest. As shown in Fig. 3, red spot incidence on fruit bagged at shuckfall 24 April (5 days total exposure) was only 19% of that for the nonbagged check fruit (78 days total exposure). Spot incidence on fruit bagged 12 May (23 days total exposure) and 8 June (50 days total exposure) was 46 and 74%, respectively, of that for the check fruit.

Treatment of young fruit at shuckfall and at 45 days with Manzate 200 also substantially reduced the incidence of the blemish on field-grown fruit. Treated fruit averaged only 2.5 spots per fruit ($\sigma = 1.9$), while the untreated nonbagged fruit on the same trees average 20.9 spots per fruit ($\sigma = 14.9$).

Histological examination of affected tissue of fruit from the field plots verified that germinated *A. alternata* spores are consistently associated with the central stomates of the blemish. This was observed for 7 of 10 spots examined from five uninoculated nonbagged fruit and for 6 of 10 spots examined from four individually bagged fruit inoculated with the concentrated (2×10^5 spores per milliliter) spore suspension. The germination tubes from these spores almost invariably grew toward or into the central stomate of the spots, and intrastomatal appressoria were observed in three of the spots examined from uninoculated nonbagged fruit. No intrastomatal appressoria could be found, however, in the 10 spots examined from inoculated individually bagged fruit.

Attempts to culture the pathogen from tissue of 11 spots from individually bagged fruit inoculated with the concentrated spore suspension were partially successful. *A. alternata* was reisolated from four (36%) of the blemishes used in these culture attempts, approximately the same as for naturally inoculated fruit blemishes used initially. The remaining seven produced no bacterial or fungal colonies.

DISCUSSION

Apricot fruit blemishes described previously include peach scab, bacterial spot, Coryneum blight, and San Jose scale damage (1,3,7,8). None of these, however, exhibit the characteristic superficial spots with smooth to only slightly sunken light tan to brown centers and bright red halos associated with the "apricot freckle" blemish. Peach scab, occasionally called "freckles" and caused by the fungus *Cladosporium carpophilum* Thum., differs in that the superficial lesions begin as small greenish spots and become olivaceous-brown in color and velvety in texture as they age and produce conidiospores (1,3). Bacterial spot, which is caused by *Xanthomonas pruni* (E. F. Smith) Dowson, differs in that the dark brown lesions either are accompanied by "cracking" and/or "pitting" of the fruit or are surrounded by a distinctive light-green halo when they are of the "pin-point" type (1,3). Coryneum blight lesions have a darker red to purple-red halo around light-colored, elevated, scablike centers (1,3,7). San Jose scale apricot fruit damage lesions also have a bright reddish halo around a light-colored center which has a scablike texture when the scale insect is present and has a smooth texture when the insect has been removed (8). At least some of the lesions, however, should have insects still attached when examined. Scale damage on apricot fruit is relatively rare in Washington state and is typically associated with an extensive scale infestation (S. C. Hoyt, *personal communication*).

The causal agent of the "apricot freckle" blemish is shown by our data to be *A. alternata*. Initial evidence of this relationship is provided by histological observations of a germinated *A. alternata* spore frequently located near the central stomate of each blemish with the germination tube leading into the stomatal orifice and in several instances producing an appressoriumlike structure within the stomate. Additional supportive evidence is the consistent isolation of *A. alternata* from the young spots and the lack of any other fungi associated with them. Further, the reduction of the blemish incidence by spray application of Manzate 200 implies a biological causal agent, and the rate of Manzate 200 used in the field study has been found to reduce the spore germination rate for *A. alternata* to 2–3% of that for untreated spores in our laboratory studies (authors, *unpublished*). The induction of "apricot freckles" by inoculating young fruit with *A. alternata* spores and the reisolation of *A. alternata* from the resulting spots provides the final conclusive evidence of causality via completion of the conditions stated in Koch's postulates.

The data from the experiment on timing of natural inoculation suggest that most of the effective inoculation occurs early in the period (Fig. 3). Fruit with 23 days of exposure had 46% as many spots as the nonbagged check fruit. Exposure during the final 5 days of maturation may account for aproximately 5–6% of the spots since the rate of spot increase between 23 and 78 days exposure approximates 1% per day (Fig. 3). Additionally, the individually bagged fruit from the other experiment also had 5 days of final exposure, but only 6% as many spots as the nonbagged check fruit. The 13% difference in spot incidence between fruit with the same

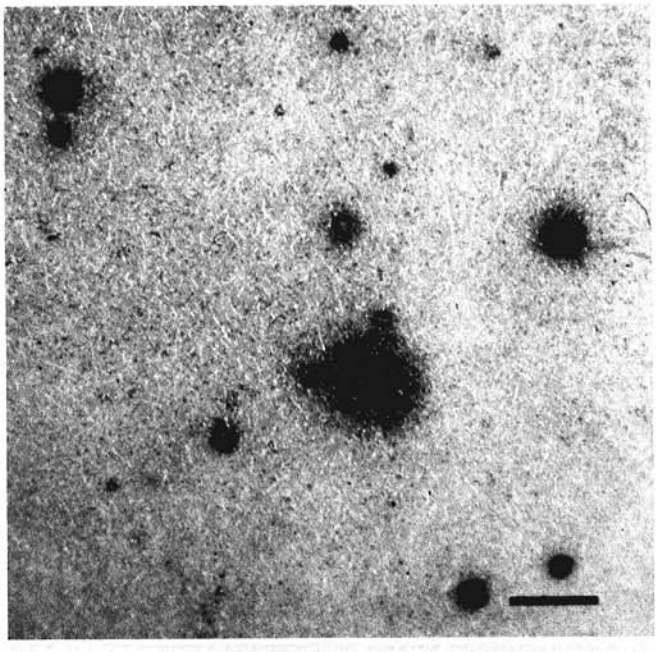

Fig. 2. Close-up of apricot fruit skin surface showing "apricot freckles" of various sizes and ages. Bar = 1 mm.

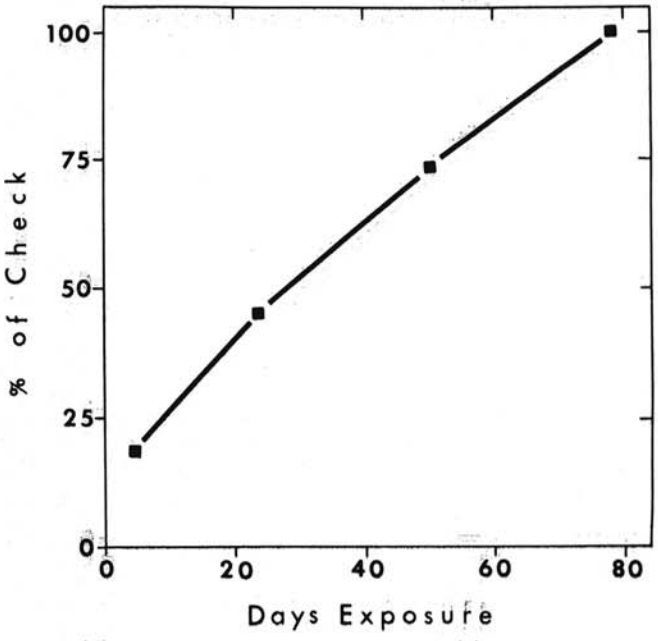

Fig. 3. Relationship of exposure of apricot fruit to natural *Alternaria alternata* inoculum and spot incidence at harvest expressed as percent of spots on nonbagged check fruit.

exposure (5 days) but different bagging procedures (e g, individual fruit vs entire terminals) is probably due either to incomplete protection afforded by the paper bags or to a limited production of natural inoculum on the bagged branches and twigs. The difference in rate of spot increase before and after 23 days of exposure may be due either to a higher level of inoculum or to a greater fruit susceptibility during early stages of development. We have found *A. alternata* to be a relatively common fungus in orchards of central Washington (authors, *unpublished*) and suspect that the level of naturally occurring inoculum may remain approximately constant throughout the period of apricot fruit development. It is not possible, however, on the basis of the present data, to ascertain which of these two alternatives is responsible for the higher rate of spot increase prior to 23 days of total exposure.

Our cytological observations and isolation data are consistent with those that would be expected in a host-pathogen system with a hypersensitivity resistance mechanism (4,9,11). These include: the observed lack of hyphal growth beyond the appressorium, the greater success in isolating *A. alternata* from epidermal sections bearing smaller (younger) lesions with only reddish cell coloration than from those bearing larger (older) lesions with brownish necrotic centers, and the inability to isolate the fungus from subepidermal sections or from most surface-sterilized epidermal sections. The reddish epidermal cell coloration is apparently an early state of host response to the germinating spore prior to the penetration of the stomate and the formation of the appressorium, after which the invading fungus is killed.

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