Role and Survival of Monilinia fructicola in Blighted Peach Branches

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ABSTRACT

A discolored streak in the xylem of peach branches, with attached rotted fruits or blighted blossoms infected with *Monilinia fructicola*, extended from the peduncle for as much as 29 cm toward the tip of the branch and 23 cm toward its base. The streak was shown to be a line of gum-filled lacunae and associated discolored xylem located primarily in the springwood of the current season's growth. Isolations from the discolored tissues in July, 2 to 3 weeks after fruit infection, showed that *M. fructicola* was present in most cases within 3 cm of the peduncle. By the following February, *M. fructicola* was recovered principally from the fruit peduncles on which mummies were attached. The presence of *M. fructicola* in the fruit peduncle in February suggests that the peduncle can serve as an overwintering site. The development of the discolored streak and the subsequent establishment of *M. fructicola* in peach branches appear to be associated with a failure of an abscission layer to form which permits the infected blossom or fruit to remain attached to the branch. A toxic substance may be involved in the death of the cells which form the abscission layer, as well as the xylem cells.

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Brown rot, caused by *Monilinia fructicola* (Wint.) Honey, continues to be one of the most important diseases of the peach, *Prunus persica* (L.) Batsch. Infection of either blossoms or fruits may result in twig blight (4).

Literature prior to 1932 dealing with the twig blight and canker phase of the disease is discussed in detail by Roberts & Dunegan (8). The wood in infected twigs turn brown as they wilt. This discoloration may extend 60 to 90 cm back from the mummy (6). The bark often covers a layer of slimy gum which may extrude through it.

Smith (9) found extensive gum pockets in the region of the cambium and primary phloem of freshly blighted twigs. These gum pockets were full of mycelia of *Monilinia*. The fungus also penetrated the cortical parenchyma and the xylem. According to Roberts & Dunegan (8), the first symptom of canker formation is the presence of a discontinuous narrow brown zone in the region of the cambium. However, no mycelium was found in this zone, and it was not determined whether the discoloration and collapse of cells adjacent to the canker were caused by penetration of the cells by the fungus hyphae or by fungal enzymes.

Jehle (3) reported that *M. fructicola* lives from year to year in cankers, and that these cankers may increase in size. Roberts & Dunegan (8) concluded that the fungus overwintered in a low percentage of the cankers, whereas McCubbin (6) reported that the fungus was rarely present in the twigs.

McCubbin (6) suggested that death of the twigs resulted from something passing into the twigs from rotting fruits. He found that juice from rotting peaches caused twig dieback in the same manner as those with rotting fruits on them. He further noted that acids at the same pH as juice from rotting peaches readily killed the twigs. Hawkins (2) found that the acid content was higher in the rotting portion of the peach, and concluded that either the fungus or peach forms some sort of acid. Mirocha et al. (7) found that fumaric acid, produced in rotted hulls of almond by *Rhizopus*, is translocated into twigs and leaves where it, or some of its metabolic derivatives, are highly toxic. Valenta (10) found that *M. laxa* produced a compound in certain media which caused a rapid wilting of apricot shoots. He determined that the compound was highly acidic, heat stable, and nonvolatile. Wilting was noted after 5 hr, and within 48 hr, all leaves were curved and drying. The youngest leaves first showed the wilting which continued downward to the oldest leaves.

The objectives of this study were to examine the extent of discoloration, the distribution of the fungus, and the histological alterations in branches associated with blighted blossoms or rotted fruits.

MATERIALS AND METHODS.—An isolate (M101) of *M. fructicola*, obtained from a mummified fruit in the spring of 1969, was used throughout this study. In June 1970, 2 to 3 weeks prior to ripening, fruits were injected under the exocarp with ca. 0.1 ml of an aqueous spore suspension of the fungus.

To determine the extent of the discoloration and the location of *M. fructicola* in the discolored tissues, we collected branches with mummified fruit in July 1969, 3 weeks after infection, and in February 1970, toward the end of the overwintering period, from trees that had been inoculated the preceding June. The branches, after surface sterilization for 1 min in 70% ethanol and 5 min in

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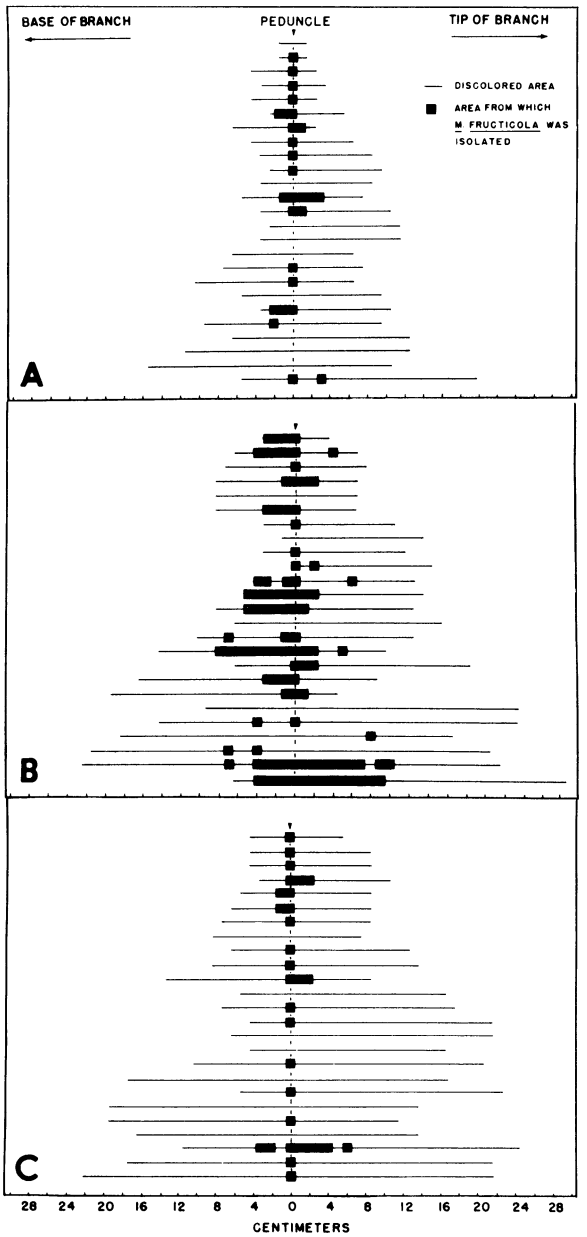

Fig. 1. The extent of discoloration in the xylem of peach branches and the location of *Monilinia fructicola* in the discolored xylem as measured from the peduncle. **A)** Xylem discoloration and the presence of *M. fructicola* in 25 branches with laterally attached blighted blossoms as determined by isolations made in May 1970, 5 weeks after infection. **B)** Xylem discoloration and the presence of *M. fructicola* in 25 branches with laterally attached brown-rotted fruits as determined by isolations made in July 1969, 3 weeks after infection, and in February 1970 (C), 8 months after infection.

0.5% NaOCl, were split and examined for internal discoloration. The length of the discolored streak was measured up and down the branch from the base of the peduncle of the mummified fruit. Portions of the discolored wood, at 1-cm intervals, were removed and plated on potato-dextrose agar. Similarly, measurements and isolations were made in April 1970 from branches with blighted blossoms.

Branches with attached blighted blossoms or mummies that were to be examined histologically were collected in the field, cut into 5-mm sections, and fixed in Formalin-acetic acid-alcohol. The sections were dehydrated in a tertiary butyl alcohol series, infiltrated with paraffin, and embedded in Fisher's Tissuemat. Cross sections, 12 μ thick, were cut using a rotary microtome, fixed to glass slides with Haupt's adhesive, and stained with Johansen's safranin and fast green.

Juice was extracted from naturally infected brown-rotted peaches and also from laboratory inoculated fruits. The flesh from the infected fruits was macerated in a blender. The slurry was filtered through glass wool, 2 thicknesses of Whatman No. 1 filter paper, and a 0.22 mμ Millipore filter into sterile 50 ml Erlenmeyer flasks and stored at 5 C. Juice from healthy fruits was obtained and stored in a similar manner.

Filtrates were also obtained from shake cultures of *M. fructicola*. The fungus was grown on a medium that consisted of 2 g of NH_4NO_3, 1 g KH_2PO_4, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 0.5 g $FeSO_4.7H_2O$, 50 g glucose, and distilled water to make 1 liter (10). Two hundred and fifty ml of the medium were dispensed into 500-ml flasks which were plugged with cotton and autoclaved at 121 C for 15 min. Five flasks of the medium were inoculated with mycelial discs, and were grown at 24 to 26 C for 6 weeks. The contents of the flasks were filtered and stored in the same manner as the peach juice. The hydrogen ion content of the juices was measured with a Beckman pH meter.

A bioassay technique was devised to determine the toxicity of the peach and culture filtrate extracts. Actively growing shoots, ca. 20 cm long, were removed from peach trees and individually placed in sterile 50-ml flasks containing 15 ml of either extracted juice or culture filtrate. Shoots were also placed in sterile medium, juice from healthy fruit, a sodium borate and potassium phosphate buffer at pH 4.0, and distilled water, which was used as a control. A loose cotton plug was used to hold the shoots upright. Flasks were placed in a moist chamber at room temperature and observed daily.

RESULTS AND DISCUSSION.—Discolored streaks often developed within branches with attached blighted blossoms. By late April, or a month after bloom, the discolored streaks reached a length of 23 cm, averaging ca. 13 cm (Fig. 1-A). *M. fructicola* was most often isolated from the flower peduncle and the wood in the branch directly beneath it. The fungus was present in 54% of the peduncles examined.

Similarly, as a peach fruit is decayed by *M.*

fructicola, a brown necrotic streak develops within the branch on which the fruit is attached (Fig. 2-A). By July, the discolored streaks within some peach branches had reached a length of 40 cm with an average of 22.6 cm. By February, discolored streaks averaged 24.4 cm in length, not differing significantly from the July measurements ($P = .05$). However, the location of *M. fructicola* within the streak differed considerably from July to February. In July, *M. fructicola* was consistently isolated from the discolored xylem in a region 3 cm on either side of the peduncle (Fig. 1-B). In one case, it was isolated 9 cm from the peduncle and was often found as far away as 5 or 6 cm. By the following February, *M. fructicola* was most frequently recovered from the fruit peduncle or the tissues just beneath it (Fig. 1-C). Only in one branch was the fungus found as far as 6 cm from the peduncle. In July, just after harvest, *M. fructicola* was isolated from portions of 88% of the branches examined, compared to 72% in the following February.

The greater amount of fungal invasion and internal discoloration in branches associated with rotted fruits, as compared to that from blighted blossoms, might have resulted from increased fungal growth in the fruits or from increased amounts of toxic metabolic products. On the other hand, temperature may play a role in fungal growth in the branch and tissue discoloration. Temperatures during bloom were cooler than during the fruit ripening period. Willison (11) has suggested that the slow growth of *Monilinia* at low temperatures accounts for its failure to become established in branches in the winter and spring.

There is no satisfactory explanation for the decline of *M. fructicola* in the branches from July to

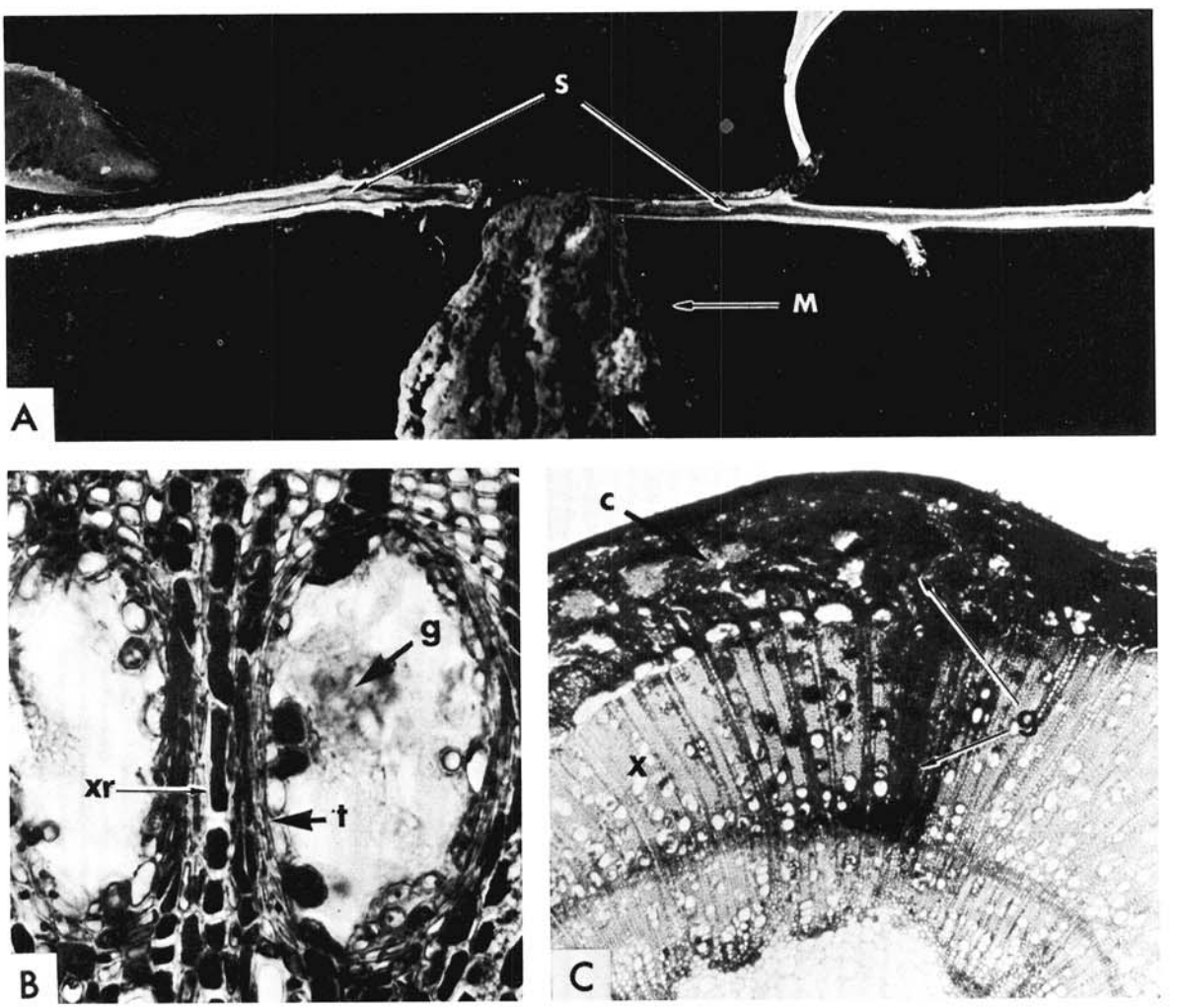

Fig. 2. A) Peach branch with outer tissues removed showing a necrotic streak (s) in xylem in both directions from the attached mummified fruit (m) infected with *Monilinia fructicola*. **B)** Section of lacunae filled with gum (g). Surrounding tracheids (t) have disintegrated or collapsed. Cells of the xylem rays (xr) remain intact. **C)** Cross section of a branch 10 cm from the peduncle of a mummified fruit. Heavy gum deposits (g) are found in the xylem (x), and cortex (c).

February. It is possible that the fungus was killed by desiccation. Willison (11) deduced that *Monilinia* was sensitive to desiccation, and suggested that the dryness of the wound gum region was an important factor in the inability of *Monilinia* to survive in peach branches. In the present study, it was observed that the discolored region in the branch became very dry and hardened.

The presence of the fungus in 72% of the peduncles examined in February proves that *M. fructicola* does overwinter in the peduncle. This supports Kable (5), who found that the fruit peduncle was an important overwintering site of *M. fructicola* in Australia. In the spring, periods of high atmospheric moisture may induce sporulation on peduncles in which the fungus remains alive.

In cross sections of branches with blighted blossoms, the discolored streak was arc-shaped, and was located between the cambium and the summer wood of the preceding year. In branches 1 cm in diam and at 1 cm from the peduncle, the arc often extended one-fourth to one-third of the way around the branch. A series of lacunae, filled with gum, formed the center of the arc (Fig. 2-B). The lacunae usually are located in the region of the large vessels of the current season's springwood, and are often bordered by xylem rays. The cambium in infected branches remains active, and new xylem is laid down next to the row of gum pockets. The new xylem elements have smaller vessels than the corresponding springwood of healthy branches.

Tissue destruction is generally more severe in branches with attached rotted fruits than in branches with blighted blossoms. At 1 cm from the peduncle, and in a branch 1 cm in diam, one-eighth to one-third of the branch may be involved. In cross section, the discolored region often appears wedge-shaped (Fig. 2-C). Not only is the current season's spring wood involved, but in 3-year-old branches, with an infected fruit on a spur, lacunae also form in the springwood of the previous year's growth. The region between the two lines of lacunae stained heavily, and many of the vessels and tracheids were plugged with gum.

Tissue discoloration is inversely related to the distance from the mummified fruit. At 15 cm from the peduncle, the gum pockets involve only several tracheids and vessels. Vessels and tracheids are plugged but not destroyed.

Grosclaude (1) suggests that gummosis in peaches is provoked by toxins produced by microorganisms often far from the affected region. The presence of a necrotic region, apparently in advance of *M. fructicola*, suggests that some toxic substance may be involved in the death of the xylem cells.

Extract from brown rot-infected fruits is very toxic to peach tissues. Shoots placed in the extract (pH 3.9) wilted slightly after 2 hr. After 24 hr, the epidermis of the shoots was shriveled, and a brown discoloration was visible externally 1 to 3 cm up the shoot. Wilting was slightly more pronounced than after 2 hr. After 3 days, the shoots were discolored externally and internally three-fourths of the distance up the stems. Shoots resembled wilted branches on trees in the orchard with attached brown-rotted fruit. As in the orchard, leaves became bleached, necrotic, and remained attached to the shoot. Shoots placed in juice from healthy peaches (pH 4.2) also quickly wilted. The lower 2 to 3 cm of the stems were discolored after 3 days, and gum oozed from the affected area. The epidermis of the shoots shriveled and the shoots began to defoliate. Only terminal leaves remained after 5 days.

The toxic nature of juice from brown-rotted fruits might be due to its low pH, to a toxic substance produced on breakdown of the peach tissue, or to one produced by the fungus.

Juices from infected (pH 3.9), as well as healthy (pH 4.2), fruits are acidic. Shoots placed in a sodium borate and potassium phosphate buffer at pH 4.0 were discolored approximately one half the distance up the stem after 5 days. The stem was shriveled and gum oozed from the affected area. Some wilting and chlorosis of the leaves accompanied the stem discoloration. Only juice from rotted fruits caused a greater amount of discoloration. This substantiates the findings of McCubbin (6) that solutions of low pH cause necrosis of peach tissues. However, the involvement of low pH in the development of a discolored streak appears to be secondary. The formation of an abscission layer across the peduncle of an uninfected ripe peach could restrict the movement of certain materials back into the branch from the ripe fruit. However, if cells involved in the formation of the abscission layer are killed, juice from uninfected or rotted peaches may pass into the branch, and the acidity of the juice could affect the xylem tissues.

Toxic substances may be produced as a result of the breakdown of ripening peach tissues. Because discolored streaks also develop within branches with attached blighted blossoms, although to a lesser extent than in rotted fruits, such a breakdown product is probably not the primary cause of the discoloration. However, it cannot be ruled out as a contributing factor in the development of the streak in branches with attached rotting fruits.

Shoots placed in culture filtrates gave an indication that a toxic substance produced by the fungus may play a significant role in the development of the discolored streak. After 24 hr, some interveinal chlorosis was noticeable in leaves in shoots placed in the filtrate (pH 2.5). This was more pronounced after 36 hr, and most leaves were affected after 5 days. The chlorosis began at the base of the leaves and proceeded toward the tip. Little wilting and defoliation accompanied the chlorosis. After 24 hr, some discoloration had progressed 2 to 3 cm up the stem. The epidermis of the shoot was slightly shriveled. Shoots in the noninoculated culture media (pH 4.8) were unaffected. Much of the discoloration and necrosis is probably due to the low pH of the culture filtrate. However, the patterns of chlorotic development in the leaves more closely resembled its development in shoots in the extract from rotting fruit than in those in the buffer solution at pH 4.0.

Perhaps most significant is the prolonged attachment of many of the leaves on the shoot. This is a distinct characteristic of the disease in the field, and was observed on shoots in the extract from rotting fruits, but not on shoots in the sodium borate and potassium phosphate buffer. This suggests that some toxic substance, produced by the fungus, may be responsible for inhibiting the formation of the abscission layer in the fruit peduncle. As the substance moves into the branch, it could affect abscission layers at the base of the leaves in a similar manner, thus explaining their prolonged attachment. In fact, it was observed that discolored streaks, within branches with attached rotting fruits, often extended into leaf traces. Such a substance may also be responsible for the death of the xylem elements.

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