Trichoderma harzianum: A Possible Cause of Apple Decay in Storage

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ABSTRACT

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Fungi recovered from dump-tank water at a large apple storage and packing facility were assayed for their ability to cause decay. *Trichoderma harzianum*, a fungus not usually associated with apple maladies, was found to cause a brown firm rot in apples that were wound-inoculated and kept in storage (20 C) for 1 wk.

Unsanitary conditions in apple storage and packing facilities provide an ideal environment for postharvest pathogen survival. Apple dump-tank water is an especially attractive site for pathogen survival and dissemination onto fruit that pass through. Because apples are often bruised or wounded to varying degrees during packing and handling, many would be especially susceptible to infection and subsequent storage decay as a result of immersion in contaminated water. This additional source of infection could lead to increased postharvest losses because bruises and wounds provide ideal points of entry for pathogens that cannot invade intact fruit (3).

In a study undertaken to determine the presence of possible postharvest pathogens in apple dump-tank water, it was found that *Trichoderma harzianum* Rifai can cause rot in fruit. Rot caused by an unidentified species of *Trichoderma* was reported in Washington in 1944 (1). It was found on Jonathan apples (*Malus sylvestris* Mill.) held in storage for 2.5 mo. The rot in this case had its inception at the calyx or core of the fruit, and in some cases, almost half of the apple was rotted. The pathogenicity of this fungus to Delicious apples was established by artificial inoculation. The symptoms, however, were not fully described. A postharvest decay of citrus fruits caused by *T. lignorum* has also been reported (2). This is the first report, however, that associates *T. harzianum* with fruit decay.

MATERIALS AND METHODS

Water samples were taken from dump tanks in a large storage and packing facility in southern Pennsylvania. These samples were serially diluted, then 1 ml of each dilution was pipetted into sterile plastic petri dishes and molten (46 C) potato-dextrose agar (PDA) was added. The plates were incubated at 20 C and the resulting mixed cultures were subcultured to eventually obtain pure cultures of the fungi present in the apple dump-tank water. The resulting fungi were then identified. Stayman apples (*Malus domestica* Borkh.) were wounded by inserting a transfer needle about 2 mm into the fruit on two sides and placing a small amount of mycelium from one of the recovered fungi into each wound. Each of the recovered fungi was used to inoculate 10 fruit. After 1 wk of storage (20 C), the fruit were removed and examined to determine which of the fungi caused rot. To reisolate the fungi that caused fruit rot, a small amount of tissue at the margin of the rotted area on the fruit was removed aseptically and placed on PDA plates. After 1 wk of incubation (20 C), the recovered fungi were again identified. In order to more fully investigate the rot caused by the *Trichoderma* sp., three sets of 50 Delicious apples that had been in storage (0 C) for about 2.5 mo were inoculated by three different methods. One set of fruit was surface-sterilized, then inoculated by inserting a plug of mycelium 6 mm in diameter and 2 mm deep into wounds of the same size on two sides of the fruit.

Another set of fruit was inoculated by wounding on two sides to a depth of 2 mm by pressing the fruit down on a 2-mm-diameter nailhead. The apples were then immersed for 15 sec in a spore suspension (1×10^6 spores per milliliter) in nutrient broth containing 0.5% Tween 20. The

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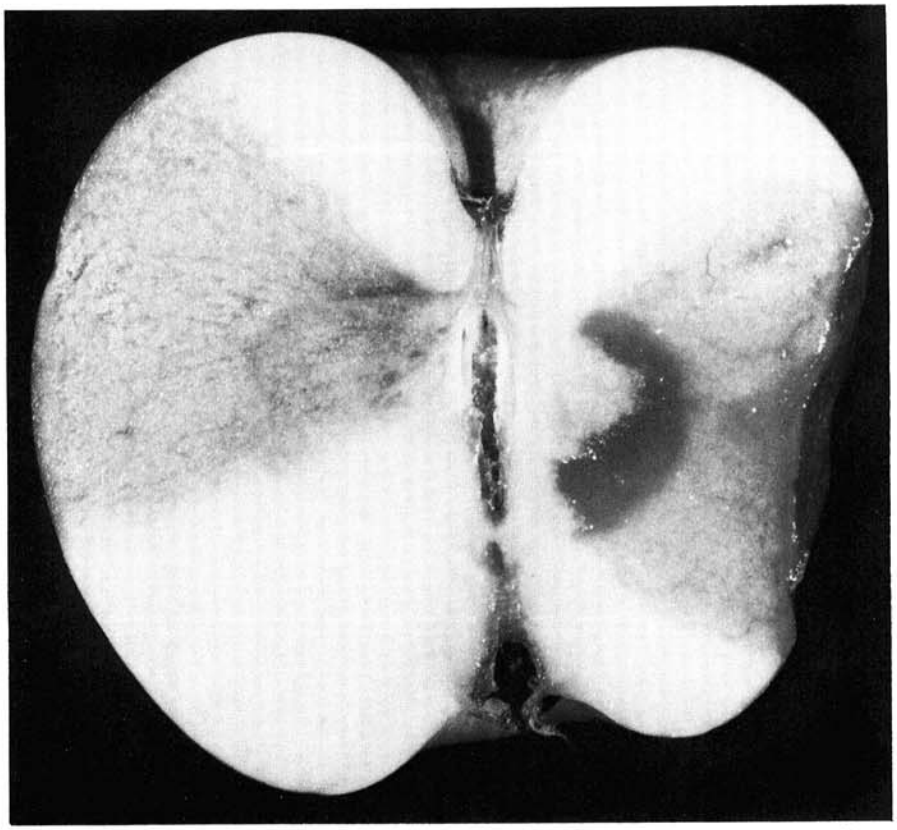

Fig. 1. Decay of Delicious apple caused by (left) *Trichoderma harzianum* and (right) *Penicillium expansum*.

final method involved immersing unwounded and unbruised fruit in a spore suspension similar to that described previously. All inoculum was taken from 2-wk-old cultures. Fruit were placed in tray pack boxes, stored at 20 C, and rated for disease 21 days after inoculation.

RESULTS AND DISCUSSION

Seven genera of fungi were recovered from the apple dump-tank water. They included at least one species each of *Cladosporium, Phoma, Sporathrix, Rhodotorula, Aureobasidium*, and *Trichoderma* and five species of *Penicillium*. Of these fungi, only two, further identified as *Penicillium expansum* Lk. ex Thom and *T. harzianum*, produced symptoms in the fruit. Subsequent reisolation, identification, and inoculation with these two fungi yielded similar results. Because blue mold rot caused by *P. expansum* is the most common and usually the most destructive of all the rots found on apple (3) and because it was found in the same apple dump tank, it is used here as a comparison with the symptoms caused by *T. harzianum*.

Of the three sets of 50 fruit each inoculated by the different methods, those inoculated with the 6-mm plug of mycelium resulted in 97% of the inoculation sites showing symptoms typical of those caused by *T. harzianum*, whereas the other 3% yielded no symptoms. Fruit that were wounded and immersed in the spore suspension resulted in 79% of the inoculation sites yielding typical symptoms, whereas 11% of the sites showed no symptoms and 10% of the sites had symptoms similar to those of *P. expansum*. Subsequent isolation from these areas indicated that *P. expansum*, probably from surface contamination, was indeed responsible for the infection. *P. expansum* may be a more aggressive pathogen than *T. harzianum* because apples were inoculated with a rather heavy spore load of the latter fungus.

In mixed culture, the growth habit of *P. expansum* also resembles that of *T. harzianum* and may be mistaken for it. If indeed the *P. expansum* is more competitive than *T. harzianum*, in the majority of cases, the typical soft-rotting symptoms caused by the blue mold fungus would be seen and those caused by *T. harzianum* would rarely be seen. Finally, fruit inoculated by immersing unwounded apples in the spore suspension had no symptoms, indicating that a wound may be necessary for infection.

The symptoms of both fungi tend to be brownish (Fig. 1). Those caused by *P. expansum* are the typical sunken, soft, water lesions that extend well into the cortex of the fruit. There is a definite border between the healthy and decayed portion of the fruit and the decayed area can be easily scooped out from the healthy tissue. In contrast, the rotted area caused by *T. harzianum* may be moist but remains firm and not sunken and may extend into the cortex. There is not a distinct border between the healthy and rotted tissue and no glassy or watery appearance.

T. harzianum is capable of causing rot of fruit through artificial inoculation and has been found in apple dump-tank water. Any natural infections may be rare or masked by the more virulent and ubiquitous *P. expansum*.

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