Effect of Imazalil on Pathogenicity of *Penicillium* spp. Causing Storage Rots of Pome Fruits

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ABSTRACT

Prusky, D., and Ben-Arie, R. 1985. Effect of imazalil on pathogenicity of *Penicillium* spp. causing storage rot of pome fruits. Plant Disease 69:416-418.

Imazalil failed to control storage rot of pome fruits caused by *Penicillium* spp. when the compound was first introduced into commercial use. Isolates of *Penicillium* obtained from decayed, treated fruit were pathogenic and almost four times less sensitive to imazalil than was the common storage rot fungus, *Penicillium expansum*. These isolates, identified as *P. crustosum*, could not compete with *P. expansum* unless imazalil was present but caused fruit decay when imazalil at 500 μg a.i./ml of water treatment or water plus diphenylamine was applied to harvested fruit.

Additional key words: biological imbalance

The development of fungal resistance to postharvest treatments with benzimidazole fungicides in citrus (3) and pome fruits (6) has stimulated the search for new fungicides effective in postharvest decay control. One of these fungicides, imazalil, efficiently controls *Penicillium digitatum* L. and *P. italicum* L. in citrus (3) and *P. expansum* L. in pome fruits (2). After the development of resistance to benzimidazole fungicides in Israel, imazalil was tested for control of *P. expansum* (4); however, when imazalil (Fungaflor 20EC) was tested for the first time under commercial conditions, it failed to control decays caused by *P. crustosum*, a new pathogen in pome fruits. This work describes a set of conditions that allowed the inherent pathogenicity of *P. crustosum* to become manifest in stored pome fruits treated with imazalil.

Contribution from the ARO, No. 938-E, 1983 series.

Accepted for publication 8 October 1984.

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MATERIALS AND METHODS

Commercial trials were conducted with naturally infected apples (cultivar Calville de San Sauveur). The experiment consisted of 10 bulk bins, each containing about 280 kg of fruit per treatment. Within 2 hr of harvest, fruit was immersed for 20 sec in a fungicide solution containing 2,000 μg/ml of diphenylamine (DPA) to prevent apple scald. The fungicides tested were imazalil at 800 μg a.i./ml and thiabendazole at 600 μg a.i./ml (Tecto 60WP). Untreated control fruits were dipped only in DPA. After treatment, the fruit was stored in a controlled atmosphere for 5 mo. After removal from storage, the fruit from each treatment was sorted on the packing line and all decayed fruit per treatment was weighed. One hundred rotten fruits from each treatment were tested for presence of *Penicillium* spp. resistant to thiabendazole by determining the growth of the fungus isolated from the tissue on potato-dextrose agar (PDA) amended with thiabendazole at 40 μg a.i./ml of medium (D. Prusky, M. Bazak, and R. Ben-Arie, *unpublished*). Isolates of *Penicillium* spp. less susceptible to imazalil than the common storage rot, *P. expansum*, were obtained from fruit that decayed during 5 mo of storage after commercial imazalil treatment. Decayed tissue was placed on PDA amended with imazalil at 20 μg a.i./ml (Fungazil 75WP). This formulation was used in all the following laboratory experiments. *Penicillium* spores developing on this medium were tested for pathogenicity on apples (*Malus communis* DC. 'Calville de San Sauveur' or 'Delicious') and pears (*Pyrus communis* L. 'Spadona') that had been surface-disinfected with ethanol (90%). Inoculation was done by placing a square block of imazalil-amended PDA with the unidentified *Penicillium* spores in a window-shaped wound followed by incubation at 25 C and 98% RH. Abundant sporulation was observed after 1 wk. Newly produced spores from infected fruits, reisolated on PDA media amended with imazalil at 20 μg a.i/ml, were used for preparing single-spore cultures. Cultures were maintained continuously on imazalil-amended media. A number of the isolates showing relative resistance to imazalil were sent to the Commonwealth Mycological Institute (CMI) for identification (CMI Report

No. 4684/83/417) by A. H. S. Onions. All experiments were performed with single-spore cultures. In competition experiments, a quantitative inoculation was performed by injecting 15 μl of a suspension containing a 1:1 mixture of *P. expansum* and *P. crustosum* at a concentration of 10^6 spores per milliliter in a window-shaped wound (5×5 mm) and incubating as described before. Spore dilutions were prepared and spread on PDA containing various concentrations of imazalil. The number of colonies developing on amended PDA was compared with that counted on the fungicide-free PDA.

RESULTS AND DISCUSSION

The commercial trial in which apples were immersed in an 800-μg a.i./ml solution of imazalil for control of thiabendazole-resistant isolates of *P. expansum* had three times the incidence of fruit decay found in the untreated control fruits (Table 1). In contrast to a previous report (2), imazalil treatment did not cause visible phytotoxic effects that might have enhanced decay. However, it is possible that microscopic phytotoxicity affected fungal development. The thiabendazole treatment was also ineffective in controlling decay. *P. crustosum* Thom. isolated from rotted imazalil-treated fruit in commercial storage was able to grow on PDA media amended with imazalil at 20 μg a.i./ml. Wound inoculation of apple fruits with the newly produced conidia collected from these cultures showed that the *P. crustosum* isolates were pathogenic and induced disease symptoms similar to those of the commonly observed rot fungus (wild type), *P. expansum* (Fig. 1). Single-spore cultures of three pathogenic isolates of *P. crustosum* showed ED_{50} values for response to imazalil ranging from 4×10^{-3} to 10^{-2} μg a.i./ml of medium, whereas the ED_{50} of *P. expansum* was 2.5×10^{-4} μg a.i./ml of medium (Fig. 2). Isolates of *P. crustosum*, numbered 1, 2, and 2-3, were still able to infect pears and sporulated copiously (Fig. 3). The development of *P. expansum* on inoculated pears was almost completely prevented by dipping in imazalil at 500 μg a.i./ml 24 hr after inoculation. However, the same treatment only partially inhibited the development of the *P. crustosum* isolates in inoculated pears (Fig. 3).

When competition between the isolates of *P. crustosum* and the imazalil-susceptible strain of *P. expansum* was tested in a 1:1 mixture, 99% of the new population of spores produced on apples 10 days after inoculation was sensitive to imazalil at 0.1 μg a.i./ml of PDA and was identified as *P. expansum*. However, when the inoculated fruit was treated with imazalil at 500 μg a.i./ml, 1 day after inoculation with the mixed spores, only 2.7% of the new population of spores was sensitive to imazalil and was identified as *P. expansum*. *P. crustosum* was also identifiable on unamended PDA by the brown-yellow color that developed, 4–5 days after seeding, on the bottom of the plate compared with the light brown color occurring beneath *P. expansum* cultures.

The isolates of *P. expansum* and *P. crustosum* identified by A. H. S. Onions looked similar when grown on malt agar. However, *P. crustosum* was crusting and produced rough conidiophores and less elongated heads than *P. expansum*, which did not crust and produced smooth conidiophores and more elongated heads. Also, the color of the tissue decayed by *P. crustosum* was usually darker than the decay caused by *P. expansum*. No further differences were observed in disease symptoms.

P. crustosum has been previously isolated from decaying apples in Israel (1) and would be expected to produce a limited rot of apples (1,5). In our experiments, however, the species was decidedly pathogenic, although decay development and sporulation were slightly delayed compared with that caused by *P. expansum*. The reduced sensitivity of *P. crustosum* to imazalil treatment has created a new situation where a usually weakly pathogenic (1), poorly competitive species of *Penicillium* may become a serious pathogen as a result of biological imbalance after

Table 1. Effect of imazalil on control of a thiabendazole-resistant population of *Penicillium expansum* on stored apples (cultivar Calville de San Sauveur) after 5 mo of storage at 0 C in a commercial trial

Treatment	Rate (μg a.i./ml)	Quantity of treated fruit (kg)	Decay (%)
Control	0	2,823	10.2
Thiabendazole	600	2,780	14.9
Imazalil	800	2,731	31.6

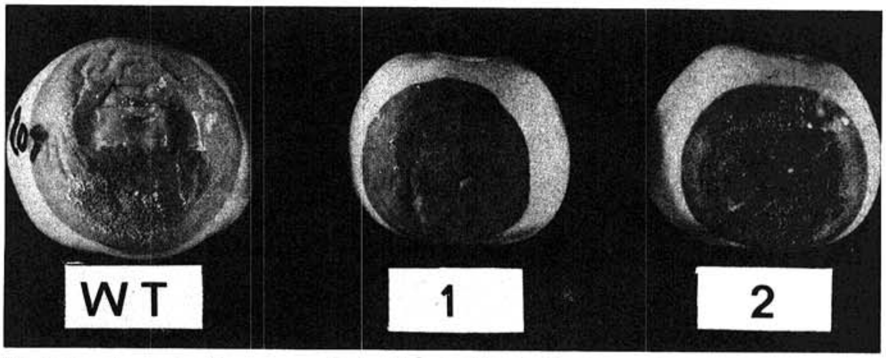

Fig. 1. Development of decay on apples (cultivar Calville de San Sauveur) caused by the common storage rot fungus *Penicillium expansum* (WT) and isolates 1 and 2 of *P. crustosum* obtained from imazalil-treated, decayed fruits.

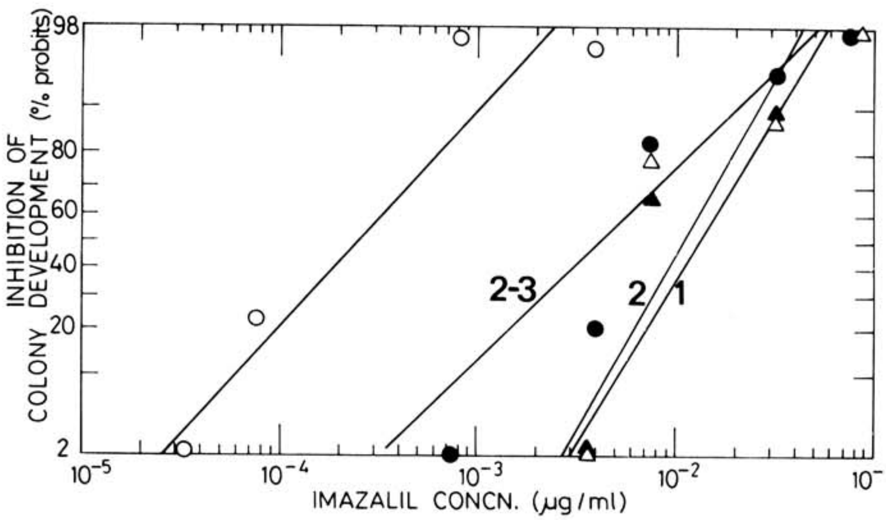

Fig. 2. Fungicide dosage response curves of *Penicillium expansum* (o) and three single-spore isolates of *P. crustosum* obtained from imazalil-treated fruits: ● = isolate 2-3, Δ = isolate 1, and ▲ = isolate 2.

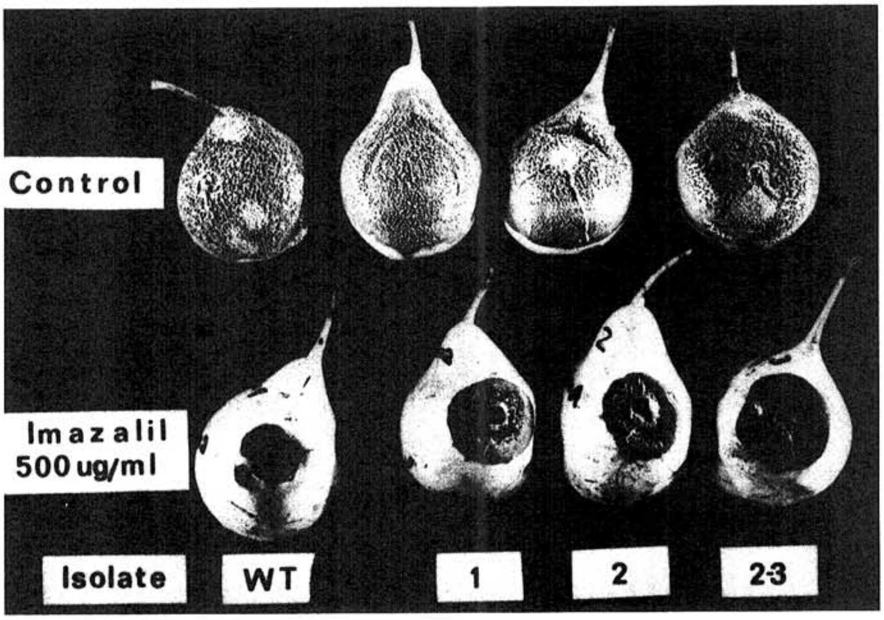

Fig. 3. Effect of imazalil on development of *Penicillium* decay on pears (cultivar Spadona). Dip treatment was applied 24 hr after inoculation and fruit was stored at 25 C. WT is the common storage rot fungus, *Penicillium expansum* (wild type); 1, 2 and 2-3 are single-spore culture strains of *P. crustosum* obtained from imazalil-treated fruits.

imazalil treatment. Under natural conditions without any selection pressure, *P. crustosum* cannot compete with the wild type of *P. expansum*. However, since *P. expansum* is effectively controlled by imazalil and pome fruits are stored for long periods of time with a possible decrease in fungicide concentration, *P. crustosum* might easily develop on wounded imazalil-treated fruits and become a serious pathogen.

The high incidence of rot in imazalil-treated fruit in the commercial experiment might indicate the prevalence of *P. crustosum* spores in the atmosphere of that location that under normal conditions are held under control either by competition with the wild type of *P. expansum* or by the commonly used thiabendazole treatment.

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