Development of New Fungicides for Management of Anthracnose and other Diseases of Strawberries, Year 3

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Summary

Previously we provided experimental results to develop and register the biofungicide Zivion™-S 10SC (active ingredient natamycin) for use as a pre-plant treatment for managing strawberry anthracnose caused by *Colletotrichum acutatum*. Consequently, this product is currently labeled and available for use on strawberry. The focus of this report is the control of Botrytis gray mold on transplants during long-term cold storage. For purposes of this report, we are calling the disease "box rot." Zivion was ineffective against box rot and had phytotoxic effects as evidenced by blackening of plant tissues and increased plant mortality. However, several other treatments were effective. Effective treatments were Meteor™ (iprodione), Ph-D (polyoxin-D), Switch (cyprodinil + fludioxonil) and Scholar® (fludioxonil). These treatments were highly effective against Botrytis box rot and caused little to no phytotoxicity for up to four months of cold storage. Increased plant mortality occurred with Ph-D at six months of cold storage. Meteor is already registered for use against box rot, while Switch and Scholar master labels would allow this use pattern and could be added to the labels through a FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) section 24(c) (special local needs).

Introduction

The California strawberry nursery (plant production) industry is concentrated at low elevation locations (Manteca and Turlock area) while high elevation nurseries are centered around Macdoel in the Butte Valley at 4,000 ft elevation. In general, the initial increase of plants takes place at the low elevation nurseries, while the final increase and supply most of the plants for fall planting along the Central Coast happens at the high elevation nurseries. The September low temperatures at high elevation provide adequate chill hours (between 28 and 45°F) for improved plant vigor in time for planting in fruit production fields from Oxnard to Watsonville.

Plants are harvested at high elevation nurseries from early September to early November. Most of the plants are destined for Fall planting, first in Oxnard (late September to late October), then in Santa Maria (early October to late November) and finally in Watsonville-Salinas (mid-October to mid-November). In contrast, low elevation nurseries harvest plants from late December to late January. Most of the plants harvested at low elevation nurseries are destined for increase at high elevation nurseries. Because planting at high elevation doesn't begin until the third week of March, a six-week gap between harvest and planting exists. During this period, plants are stored frozen at 28°F. Transplants stored this way are called "frigo" plants.

Plants destined for summer planting in Oxnard (late June to mid-July) or Santa Maria (late May to early July) are typically produced at the low elevation nurseries because of the shorter storage period required compared to high elevation nurseries. Summer plantings use plants that have been stored at 28°F for four to seven months. During this long-term cold storage, molds (e.g., Botrytis gray mold) may develop on boxed transplants (box rot) leading to reduced quality and death of transplants. A fungicide treatment prior to long-term cold storage would be useful to protect plants from decay during storage.

The goal of this study was to evaluate the effectiveness of several treatments for the control of box rot during long-term cold storage.

OBJECTIVES

- Determine the efficacy of existing and new fungicide treatments against box rot during long-term cold storage and document any phytotoxic effects.
- Identify the causal organisms involved in box rot of strawberry.

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Materials and Methods

Fungicide efficacy against box rot

Plants of cultivar 'PE-7.2059' (Plant Sciences, Inc.) were harvested at a low-elevation nursery on January 21, 2020, trimmed and placed into three industry standard storage boxes (1,000 plants per box). Plants were stored at 34°F (1.1°C) until treated with fungicide on January 29. To mimic wounding that occurs during plant mowing prior to digging, 0.5 cm of petiole tissue was removed using hand pruning shears one day prior to fungicide application. Transplants were washed to remove soil, except for the non-washed control, and drip dried for 5 min. Washed transplants were submerged and agitated in fungicide suspensions for 5 min and drip dried for 5 min. Table 1 lists the fungicide treatments and rates. Treatments were replicated four times and transplants were placed inside 1-gal plastic bags (20 transplants/bag), except for the four-month storage time which included three extra plants (i.e., 23 plants) for an eight-week grow-out to observe any phytotoxic treatment effects.

Transplants were inoculated by misting with a Botrytis cinerea spore suspension (1×10^5 spores/ml). Inoculum was applied using spray bottles to mist one side of 20 transplants using 3 ml, flipping the transplants over to spray the other side with an additional 3 ml of spore suspension. The two lower corners of the plastic Ziplock bag were cut off to allow any excess moisture to drain and allow some ventilation during storage. Plants were placed into cardboard boxes lined with plastic bags. Treatment placement was randomized within the box and boxes were stored in a chest freezer (Excellence, Tampa, FL) at 28°F (-2.2°C) for one, two, four, and six months.

At the end of each storage duration, transplants were removed from the freezer and stored at 45°F (7.2°C) for 12 days and evaluated for incidence and severity of Botrytis gray mold on plant surfaces (box rot). Box rot severity was recorded on a scale of 0 to 100 (0=no mold; 100=all plant surfaces moldy).

Table 1. Fungicide treatment list..

Product (rate)	Active ingredient
Non-washed, non-inoculated	---
Zivion (250 ppm)	Natamycin
Switch (8 oz/100 gal)	cyprodinil + fludioxonil
Meteor (2 pt/100 gal)	Iprodione
Scholar (16 fl oz/100 gal)	Fludioxonil
Scholar (10 fl oz/100 gal) + Zivion (250 ppm)	fludioxonil + natamycin
Ph-D (6.2 oz/100 gal)	polyoxin-D
Ph-D (6.2 oz/100 gal) + Zivion (250 ppm)	polyoxin-D

Phytotoxicity evaluations

After transplants were evaluated for disease incidence and severity, they were planted in 23 in. × 16.25 in. × 6 in. plastic bins with CB 1294 potting mix (Sungro, Santa Maria, CA) and grown for 14 days in a high plastic tunnel and assessed for plant mortality and any obvious signs of phytotoxicity (stunting, yellowing, etc.). For the four-month evaluation, three of the healthiest plants from each bag were removed and planted in 6 in. pots with CB 1294 potting mix immediately following removal from cold storage. These plants were observed for eight weeks and evaluated for stunting and phytotoxicity. Data was subjected to analysis of variance and Fisher's LSD mean separation test.

Fungal identification

Fungal isolations were made by cutting 2 cm long root pieces from plant roots with visible mycelium. Samples were surfaced sterilized with 1,000 ppm sodium hypochlorite for 3 min and plated onto potato dextrose agar amended with ampicillin (130 mg/L) and rifampicin (20 mg/L). Identifications were made by visual observations of growth habit, color, and micromorphology. A few of the most prevalent fungi were submitted for DNA sequencing for identification to species. DNA was extracted using Qiagen Microbial DNA extraction kit (Hilden, Germany), PCR products were cleaned using ExoSapIT (Applied Biosystems, Waltham, MA) and sent to McLab, Molecular Cloning Laboratories (San Fransisco, CA) for sequencing using ITS1F/ ITS4 primers. Forward and reverse DNA sequences were analyzed for quality parameters using FinchTV 1.4.0 software and generated consensus sequences. Contiguous sequences with ≥ 40 quality scores were selected. These sequences were compared against NCBI DNA library of known fungal DNA sequences using Nucleotide BLAST to obtain matches with 100% identity.

Results and Discussion

Efficacy. Transplants inoculated with *B. cinerea* developed signs of box rot as visible fungal mycelium during and after low-temperature storage (Figure 1). Disease severity increased with increasing storage time for inoculated, water-washed plants and was 1.8%, 53.8%, 76.6% and 100% for storage durations of one, two, four, and six months, respectively. Switch, Scholar, Ph-D, and Meteor were highly effective at controlling box rot with complete inhibition of Botrytis gray mold for Switch, Scholar and Switch + Zivion. Zivion alone was ineffective and when mixed with Ph-D, was less effective than Ph-D alone (Figure 2).

Plant mortality. Plant mortality was near zero until plants were stored for four months. At four and six months of cold storage, significant differences among treatments were detected with treatments containing Zivion having the highest plant mortality. In general, treatments with lower disease incidence resulted in less plant mortality (Figure 3).

Phytotoxicity. Plants in the Zivion treatment turned black and increased the rate of decay. Severity of the phytotoxicity symptoms increased as storage time increased and led to higher plant mortality.

Causal organisms. Fungal isolations from roots with fungal signs at each sampling time yielded mostly *B. cinerea*. In addition, *Trichoderma harzianum*, five species of Penicillium, and four species of Fusarium were isolated with the greatest frequency. Other species identified were *Cladosporium cladosporioides*, *Alternaria alternata*, *Clonostachys rosea*, *Plectosphaerella cucumerina* and *Pseudogymnoascus* sp. (Table 2). These fungi are common soil inhabitants involved in the breakdown of organic matter. *Botrytis cinerea* often produced copious amounts of sclerotia during cold storage, likely as a response to low temperature. These sclerotia serve as an excellent survival mechanism for *Botrytis* and are seen occasionally on transplant material. These results are added to those published by Anderson (1980), Lockhart (1965) and Lockhart and MacNab (1966).

Figure 1. Photographs of each treatment on June 1, 2020, showing the level of disease present after four months of storage at 28°F.

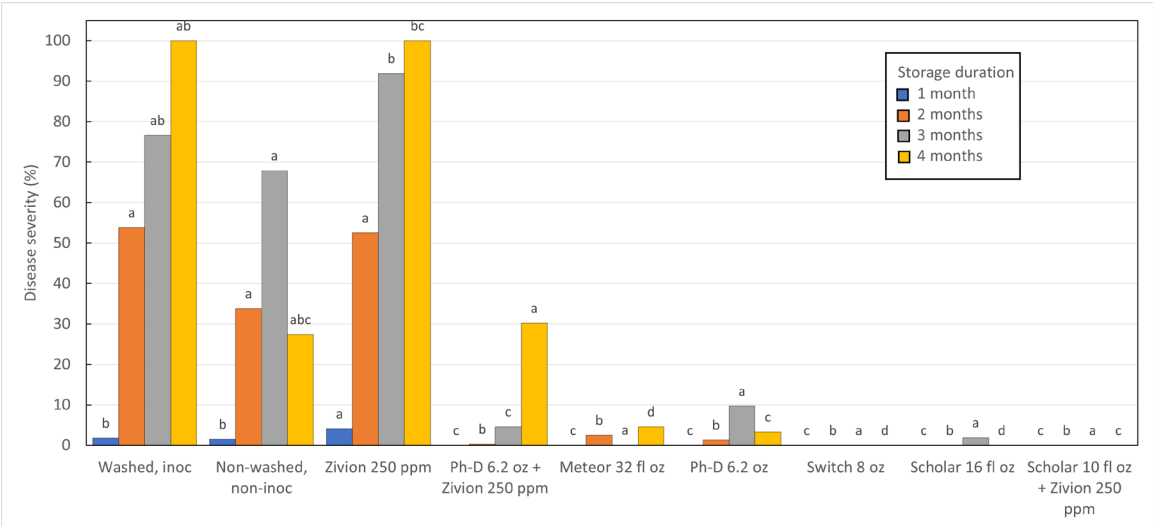

Figure 2. Efficacy of fungicides against Botrytis box rot incidence after one, two, four and six months of storage at 28°F. Bars with the same letters within a storage duration are not significantly different by Fisher's Protected LSD (P = 0.05).

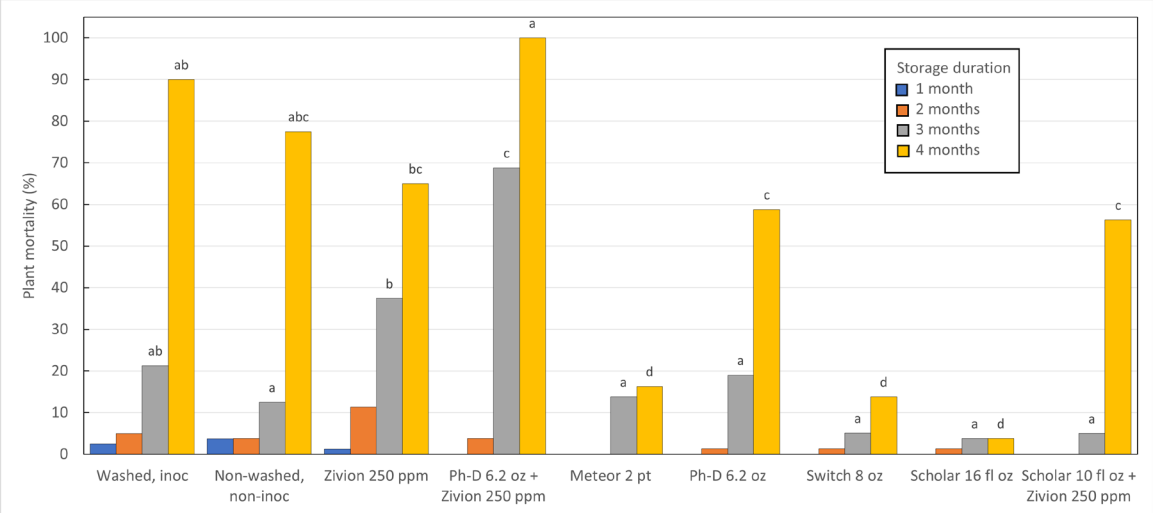

Figure 3. Plant mortality two weeks after transplanting for one, two, four and six months of storage at 28°F. Bars with the same letter within a storage period are not significantly different by Fisher's Protected LSD (P = 0.05).

Table 2. Fungal species other than Botrytis cinerea isolated from strawberry transplants after one to six months of storage at 28F.

Fungal species	Frequency of isolation
Trichoderma harzianum	7
Penicillium crustosum	4
Penicillium expansum	4
Clonostachys rosea	3
Fusarium acuminatum	3
Fusarium proliferatum	3
Cladosporium sp.	2
Fusarium fujikuroi	2
Penicillium brevicompactum	2
Alternaria alternata	1
Cladosporium cladosporioides	1
Fusarium circinatum	1
Penicillium chrysogenum	1
Penicillium ochrochloron	1
Plectosphaerella cucumerina	1
Pseudogymnoascus sp.	1

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