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TITLE Postharvest treatments for decay control

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JUSTIFICATION

Disease control with fungicides is also an important facet of postharvest decay management. Fungicides applied to fruit surfaces prevent the growth and development of pathogenic fungi in the fruit. Currently, thiabendazole (TBZ) and captan are the only registered fungicides for postharvest use on pome fruit. Over 30% of isolates of *Penicillium expansum* and (causal agent of blue mold) recovered from postharvest drenches are insensitive to TBZ. Captan use is limited due to import restrictions in several key export markets, especially Taiwan. New fungicides that show promise for control of postharvest disease must be identified, tested and their development encouraged by the registrants.

OBJECTIVES:

- Assess efficacy of new chemical and biological fungicides for control of postharvest decay in pome fruit

PROGRESS:

Procedures

Chemical fungicides. *Scholar* (fludioxonil), *Stretch, Abound* (azoxystrobin), *ZA-1963, TM-41501, and TN41702.*

In vitro assays. Initial in vitro assays were done to assess the fungistatic activity of the test materials. Different concentrations of fungicide were added to ½ strength PDA (Difco) after autoclaving. Aqueous suspensions made from 1 wk old colonies of *Penicillium expansum, Botrytis cinerea*, or *Mucor piriformis* were spread onto the surface of either three or four replicate agar plates. Plates seeded with *P. expansum* and *B. cinerea* were incubated at room temperature for 48-72 hr, at which time numbers of colonies growing on the plates were counted. Those seeded with *M. piriformis* were incubated at 16 C for 48 hr.

Fruit assays. Fruit tests were done using either Red Delicious, or Golden Delicious apples, or Anjou pears. Fruit were surface disinfested in 150 ppm chlorine for 5 min followed by a fresh water rinse. Two punctures were made in each of 30 fruit for each treatment with a sterile blunt nail. Fruit were dipped in aqueous suspensions of different concentrations of fungicide and conidia of the respective pathogens (2000 conidia/ml). Single isolates of *B. cinerea* and *M. piriformis*, and two isolates of *P. expansum* were used, one sensitive and one insensitive to TBZ at 250ppm, in a 3:1 ratio. Inocula were added to the fungicides and the suspensions were mixed thoroughly. Within 2 min after mixing, fruit were dipped in the inoculum-fungicide suspensions

for about 30 seconds. Fruit then were placed on new pulp trays and incubated at room temperature for 7-10 days at which time the number of wounds with lesions were assessed.

Biological fungicides. Experiments were begun to test the efficacy of EXC9001, a strain of *Cryptococcus infermo-miniatus,* for control of blue mold and Mucor rot apple and pear fruit.

Drench trials. Four replicate bins of fruit each were drenched at receipt at the warehouse with either Water, TBZ (100 ppm), TBZ (528 ppm), EXC 9001 (10^7 cfu/ml) + TBZ (100 ppm), or EXC 9001 (10^8 cfu/ml) + TBZ (100 ppm). Drench mixtures were seeded with 3000 cfu/ml of *P. expansum* in a 1:3 ratio of TBZ resistant and sensitive isolates.

Bins of fruit will be placed in standard CA storage (Pears: 30 F, 1.5% O_2 and <1.0% CO_2, apples: 34 F, 1.0% O_2 and 2.0% CO_2) for about 4 months at which time it will be assessed for gray mold and other decay incidence.

Dip treatments. Surface disinfested fruit were wounded and dipped into aqueous suspensions of either EXC 9001 (10^8 cfu/ml), EXC 9001 (10^8 cfu/ml) + TBZ (528 ppm), EXC 9001 (10^8 cfu/ml) + TBZ (100 ppm), EXC 9001 (10^7 cfu/ml), EXC 9001 (10^7 cfu/ml) + TBZ (528 ppm), EXC 9001 (10^7 cfu/ml) + TBZ (100 ppm), EXC 9001 (10^6 cfu/ml), EXC 9001 (10^6 cfu/ml), EXC 9001 (10^6 cfu/ml) + TBZ (528 ppm), TBZ (528 ppm), TBZ (100 ppm), or water each amended with 3000 conidia/ml of either *P. expansum* or *M. piriformis*. Four 25-fruit replicates will be used for each treatment.

Fruit were disinfested by soaking in 100 ppm free chlorine for 5 min after which it will be rinsed in fresh tap water. Fruit will then be placed on new pulp trays and wounded with a metal punch to simulate stem punctures (5 mm diam. x 3 mm deep). Replicate fruit will then be placed in sterile plastic mesh onion bags and dipped into plastic 5 gal pails containing the suspensions that will be periodically agitated by stirring. After 1 min fruit will be removed from the suspensions, drained and replaced on the pulp trays. Trayed fruit will then be placed in cardboard fruit boxes with poly-liners and stored at 30 F and 34 F for pears and apples, respectively.

The proportion of wounds with lesions in each replicate will be assessed after about 4 months of storage. Comparisons will be made among dip treatments for each pathogen separately. All CIM treated fruit will be destroyed at the conclusion of the experiment.

Line spray treatments. Treatments will be applied to surface disinfested, wounded, fruit that will have been inoculated with either *P. expansum* or *M. piriformis.* Inoculated fruit will then be treated in a line spray either with EXC 9001 ($2x10^8$ cfu/ml), EXC 9001 (10^8 cfu/ml) + TBZ (264 ppm), TBZ (528 ppm), or fresh water. Treatments will be applied to each of three 100-fruit replicates of both Anjou pears and Red Delicious apples.

Fruit used in the experiment will be disinfested and wounded as described above. Fruit will be inoculated by dipping into a suspension of conidia of either *P. expansum* or *M. piriformis* (3000 conidia/ml). Each replicate group of fruit will then be treated with one of the fungicides or water. Treated fruit then will be placed in cardboard fruit boxes with poly-liners and stored at 30 F and 34 F for pears and apples, respectively.

The proportion of wounds with lesions in each replicate will be assessed after about 4 months of storage. Comparisons will be made among line spray treatments for each pathogen separately.

Results and Discussion

Chemical fungicides.

Scholar (fludioxonil). Fludioxonil completely inhibited the growth of all fungi tested in vitro, including *M. piriformis*, at 150 ppm (Table 1). In the first test on fruit, control of decay caused by both species of *Penicillium* and *B. cinerea* was excellent at all concentrations (Table 2). Decay incidence in fruit inoculated with *M. piriformis* also appeared to be somewhat reduced, especially at 150 and 300 ppm. However, in the second test on fruit somewhat less control of decay caused by *P. expansum* was observed than in the first (Table 3). This may be due to the condition of the fruit, however, as it was less firm than those used in the first test. Control of decay caused by *P. solitum* and *B. cinerea* was similar to that observed in the first test. Similarly, some reduction in the incidence of decay caused by *M. pirifomis* was observed at 300 ppm, but it does not appear that fludioxonil can be relied on to reduce losses from Mucor rot.

Stretch. Colonies of all fungi were greatly restricted in plate tests with 0.1% (v:v) Stretch added (Table 4). There was no evidence, however, that spore germination was reduced. No growth was observed at concentrations above 0.5% Stretch. No fruit decay control was observed in Stretch treatments at any concentration (Table 5).

Abound (azoxystrobin). Although Abound showed some effect on germination of spores of *P. expansum*, no effect was observed spores of either *B. cinerea* or *M. piriformis* (Table 6). No further tests were done with this fungicide.

ZA 1963. This fungicide showed little effect on any of the major postharvest decay causing fungi (Table 7). No further tests were done with this fungicide.

TM-41501. In an initial test, TM41501 completely inhibited germination of spores of *P. expansum* and *B. cinerea*, and had an effect on spores of *M. piriformis* at 100 ppm a.i. (Table 8). In a second test, that effect was observed on spores of *P. expansum* and *B. cinerea* at 25 ppm a.i. A large reduction in *M. piriformis* spore germination occurred at 400 ppm a.i.

Tests with this fungicide on fruit have just been done using 0, 250, 500, and 1000 ppm a.i. Results have not yet been fully evaluated.

TM-41702. This fungicide completely prevented germination of spores of *B. cinerea* at 0.1 ppm, but was ineffective against either *P. expansum* or *M. piriformis* at any concentration tested (Table 9). No further tests were done with this fungicide.

Biological fungicides.

EXC 9001. Tests with EXC 9001 have not been evaluated at this time.

Conclusions

- Control of decay caused by both species of *Penicillium* and *B. cinerea* was excellent at all concentrations of fludioxonil tested.
- It does not appear that fludioxonil can be relied on to reduce losses from Mucor rot.
- No fruit decay control was observed in Stretch treatments at any concentration.
- Neither Abound nor ZA1963 appear to effect germination of spores of important postharvest decay causing pathogens of pome fruit.
- TM-41501 shows promise for control of decay causing fungi.

- TM-41702 was very effective against B. cinerea, but not the other major postharvest pathogens.

PROPOSED RESEARCH

Test new chemical and biological fungicides and application technologies as they arise

Table 1. Effect of fludioxonil on germination of conidia of *Penicillium expansum, Penicillium solitum, Botrytis cinerea* and *Mucor piriformis* in vitro

	Colonies per plate			
Fludioxonil (ppm)	*P. expansum*	*P. solitum*	*M. piriformis*	*B. cinerea*
Test 1				
0	13.2	4.4	17.2	--
150	0	0	0	--
300	0	0	0	--
600	0	0	0	--
1200	0	0	0	--
Test 2				
0	13.2	17.0	17.7	54.2
150	0	0	0	0
300	0	0	0	0
600	0	0	0	0
1200	0	0	0	0

Table 2. Effect of fludioxonil and TBZ on decay caused by *Penicillium expansum*, *P. solitum*, *Mucor piriformis*, and *Botrytis cinerea* in wounded Red Delicious apples, trial 1

Fludioxonil concentration (ppm)	Wounds with symptoms (%)[w]			
	P. expansum[x]	*P. solitum*	*M. piriformis*	*B. cinerea*
0	100.0 c[y]	45.0 b	35.0 b	75.0 b
150	1.7 a	0.0 a	11.7 a	5.0 a
300	0.0 a	0.0 a	8.3 a	0.0 a
600	1.7 a	1.7 a	15.0 ab	1.7 a
TBZ	30.0 b	28.3 b	56.7 c	5.0 a
	$P < 0.001$[z]	$P < 0.001$	$P < 0.001$	$P < 0.001$

[w] Apples were wounded (30 replicate fruit, 2 wounds/fruit) and dipped into a suspension containing both fungicide and 2 H 10^3 conidia/ml of each fungus.

[x] 1.5×10^3 conidia/ml of both a thiabendazole sensitive and an insensitive isolate of *P. expansum* were mixed.

[y] Means followed by a common letter are not significantly different according to Tukey's test at $P = 0.05$.

[z] Significance of *F* statistic. Data were transformed to arcsin$\sqrt{}$ values before analysis by oneway ANOVA

Table 3. Effect of fludioxonil and TBZ on decay caused by *Penicillium expansum*, *P. solitum*, *Mucor piriformis*, and *Botrytis cinerea* in wounded Red Delicious apples[x], trial 2

Fludioxonil concentration (ppm)	Wounds with symptoms (%)			
	P. expansum	*P. solitum*	*M. piriformis*	*B. cinerea*
0	100.0 c[y]	68.3 b	89.2 b	90.8 b
150	8.3 a	0.8 a	77.5 b	1.7 a
300	5.0 a	0.0 a	40.0 a	0.8 a
600	11.7 a	0.0 a	44.2 ab	0.8 a
TBZ	11.7 a	54.2 b	66.7 ab	0.8 a
	$P < 0.001$[z]	$P < 0.001$	$P = 0.005$	$P < 0.001$

[x] Apples were wounded (3 replicates of 20 fruit each, 2 wounds/fruit) and dipped into a suspension containing both fungicide and 2 H 10^3 conidia/ml of each fungus.

[y] Means followed by a common letter are not significantly different according to Tukey's test at $P = 0.05$.

[z] Significance of *F* statistic. Data were transformed to arcsin$\sqrt{}$ values before analysis by oneway ANOVA

Table 4. Effect of Stretch on germination of conidia of *Penicillium expansum*, *Botrytis cinerea*, and *Mucor piriformis* in vitro

Stretch concentration (% v:v)	Colonies per plate		
	Penicillium expansum	*Mucor piriformis*	*Botrytis cinerea*
Test 1			
0	75	81	22
0.1*	73	70	20
1.0	0	0	0
Test 2			
0	30	12	--
0.1*	41	12	--
0.5	0	0	--
1.0	0	0	--

* All colonies grown on PDA amended with 0.1% Stretch were greatly restricted in size.

Table 5. Effect of Stretch and TBZ (Mertect 340F) on decay in wounded Red Delicious fruit*

Stretch concentration (% v:v)	Wounds with symptoms (%)	
	*Penicillium expansum***	*Mucor piriformis*
0	100	65
0.1	100	63
0.5	100	53
1.0	100	55
TBZ (528 ppm)	95	77
0.1 + TBZ (528 ppm)	98	62
0.5 + TBZ (528 ppm)	95	82

* Apples were wounded (30 replicate fruit, 2 wounds/fruit) and dipped into a suspension containing both fungicide and 2 x 10^3 conidia/ml of each fungus.

** 1.5 x 10^3 conidia/ml of both a thiabendazole sensitive and an insensitive isolate of *P. expansum* were mixed.

Table 6. Effect of Abound on germination of conidia of *Penicillium expansum, Botrytis cinerea* and *Mucor piriformis in vitro.*

Concentration (ppm)	Colonies per plate		
	Penicillium expansum	*Mucor Piriformis*	*Botrytis cinerea*
0	18.8	4.0	82.7
0.01	6.8	6.5	82.7
0.1	2.5	7.5	76.7
1.0	3.0	5.5	83.5
10	1.5	5.5	60.7
100	1.8	7.8	75.0
1000	3.0	uncountable	uncountable

Table 7. Effect of ZA 1963 on germination of conidia of *Penicillium expansum, Botrytis cinerea and Mucor piriformis in vitro.*

Concentration (ppm)	Colonies per plate		
	Penicillium expansum	*Mucor Piriformis*	*Botrytis cinerea*
0	222.0	0.8	20.5
0.01	156.0	0.0	23.5
0.1	145.0	0.0	18.3
1.0	108.0	0.8	17.0
10	99.0	0.0	21.3
100	103.0	0.8	22.0
1000	uncountable	0.0	20.8

Table 8. Effect of TM-41501on germination of conidia of *Penicillium expansum, Botrytis cinerea* and *Mucor piriformis in vitro.*

	Colonies per plate		
Concentration (ppm)	*Penicillium expansum*	*Mucor Piriformis*	*Botrytis cinerea*
Test 1			
0.00	37.0	11.3	65.0
0.01	45.8	12.3	47.5
0.10	41.3	14.0	63.8
1.00	36.5	15.3	63.0
10.00	12.3	17.3	3.0
100.00	0.0	3.5	0.0
1000.00	0.0	0.0	0.0
Test 2			
0.0	>200.0	8.0	20.3
25.0	0.0	5.3	0.0
50.0	0.0	4.5	0.0
100.0	0.0	6.5	0.0
200.0	0.0	6.3	0.0
400.0	0.0	0.5	0.0
600.0	0.0	0.0	0.0
800.0	0.0	0.8	0.0

Table 9. Effect of TM-41702 on germination of conidia of *Penicillium expansum, Botrytis cinerea* and *Mucor piriformis in vitro.*

	Colonies per plate		
Concentration (ppm)	*Penicillium expansum*	*Mucor Piriformis*	*Botrytis cinerea*
0	182.8	19.0	49.0
0.01	237.0	21.0	21.0
0.1	243.3	13.5	0.0
1.0	158.5	7.0	0.0
10	120.3	17.5	0.0
100	54.3	10.5	0.0
1000	108.0	23.5	0.0