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CONTROL OF BENZIMIDAZOLE-TOLERANT *PENICILLIUM EXPANSUM* IN POME FRUIT

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ABSTRACT

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Benzimidazole-tolerant *Penicillium expansum* has been detected in pear fruit in New South Wales. Cross tolerance to benomyl, carbendazim, thiophanate methyl and thiabendazole was demonstrated in vitro. The tolerant strain was highly pathogenic to pear and apple fruit and resembled sensitive strains in appearance in fruit and in culture.

The tolerant strain was controlled in apple fruit in short term storage at room temperature by imazalil, iprodione and 2-aminobutane. For long term storage at low temperature, imazalil and iprodione were adequate, but 2-aminobutane was ineffective. Benomyl provided the best control of the sensitive strain.

Mixtures of imazalil or iprodione plus benomyl are suggested as a suitable treatment for fruit where tolerance has not been detected, because of their efficacy against the sensitive strain and because a degree of control could be provided should benzimidazole tolerance arise.

INTRODUCTION

Blue mould (*Penicillium expansum* Link ex. S.F. Gray) is the major storage disease of apples and pears in New South Wales. To control this disease, growers dip fruit routinely in either benomyl, thiabendazole or thiophanate methyl fungicides prior to storage.

In September 1976 specimens were received from an orchard where blue mould had caused considerable losses of stored pears, in spite of pre-storage dipping in benomyl. Ogawa et al. (1975) had found benzimidazole tolerant *P. expansum* causing post harvest rot of cherries following application of benomyl and thiophanate methyl in overhead irrigation systems. Since our work commenced, Wicks (1977) has reported finding a benzimidazole-tolerant strain of *P. expansum* in pears in South Australia.

Our investigations were carried out to determine the sensitivity to benzimidazole fungicides in vitro and in vivo of isolates of *P. expansum*, to examine their pathogenicity, and to find alternative fungicides for control of blue mould in stored apples and pears.

MATERIALS AND METHODS

Sensitivity of P. expansum to fungicides in vitro

The fungi were cultured from rotted pear fruit on potato dextrose agar (PDA) using standard techniques. The isolates examined were:

B226 from pear (cultivar 'Beurré Bosc'), Orange, N.S.W.
B229 from pear (cultivar 'Packham's Triumph'), Orange, N.S.W.
B235 from pear (cultivar 'Winter Cole'), Orange, N.S.W.
B231 from culture collection (DAR27044) ex apple (cultivar 'Granny Smith'), Bathurst, N.S.W.

The fungicides tested were: benomyl, methyl 1-(butylcarbamoyl) benzimidazole-2-ylcarbamate, Benlate (R = registered trade name); carbendazim, (methyl benzimidazole-2-ylcarbamate) (MBC), Bavistin (R); thiophanate methyl, 1,2-di-(3-methoxycarbonyl-2-thioureido) benzene, Topsin M (R); thiabendazole, 2-(4-thiazolyl)benzimidazole, Tecto 40 Flowable (R); 2 amino butane, Decottane (R).

The effects of the fungicides on the germination and growth in vitro and pathogenicity to fruit, were studied largely following methods used previously with *Sclerotinia fructicola* (Penrose and Koffmann, 1977). Spore suspensions from cultures of *P. expansum* were prepared in sterile water, placed on fungicide amended PDA and observations of germination made after 1 day. For growth studies, PDA plates prepared as above, were inoculated with 5 mm discs of potato dextrose agar (PDA) in which *P. expansum* spores had been incorporated whilst the agar was still molten (Muirhead, 1974). The diameters of resulting colonies were measured along 2 previously marked axes, after 12 days incubation at 25°C.

Pathogenicity to pear fruit and effect of fungicides

Groups of 20 pear fruit (cultivar 'Packham's Triumph') per treatment were wounded 3 times at the calyx end with a blunt nail to a depth of 2 mm, dipped for 30 s in a suspension of spores (isolate B226) suspected of being tolerant to the benzimidazoles and allowed to dry. After dipping for 30 s in a fungicide suspension, the fruit were incubated at 21 ± 1°C for 10 days, then the number of wounds which developed rot was counted.

Post-harvest mould control in artificially inoculated apples

Short term storage at room temperature. — Sound, ripe 'Granny Smith'

apples were artificially inoculated with spores of 2 *P. expansum* isolates (B226, tolerant and B231, sensitive to benzimidazole fungicides). The fruit was wounded 3 times at the calyx end with a blunt nail to a depth of 2 mm. After wounding, the fruit was dipped for 30 s in either of the spore suspensions, and allowed to dry. The fruit was then dipped in the fungicide suspensions and incubated for 8 days at 21 ± 1°C.

The fungicide suspensions were prepared in 10 l of water, to which diphenylamine (DPA), a scald inhibitor (4 cc l^{-1}), and Tween 20, a non-ionic wetting agent (0.12 cc l^{-1}) were added. The suspension was then divided into two 5 l aliquots. The experiment was designed as a split plot, with fungicides as the main plot treatments and *P. expansum* isolates as the split plot treatments. Three replicates were used with 25 fruit in each split plot.

Counts of the number of wounds which developed rot were made after 8 days of incubation at 21 ± 1°C.

The fungicides used were, benomyl; captan, N-trichloromethyl thio-4-cyclohexene-1,2 dicarboximide, Captan (R); iprodione, 1-isopropyl carbamoyl-3-(3,5-dichlorophenyl) hydantoin, Rovral (R); imazalil, 1-(β-allyloxy)-2,4-dichlorophenethyl imidazole, experimental fungicide, Janssen Pharmaceutica; 2 aminobutane; mancozeb, zinc ion and manganese ethylenebisdithiocarbamate, Dithane M45 (R); chlorthalonil, 2,3,5,6-tetrachloroisophthalonitrile, Bravo Flowable (R); DPX 164, experimental fungicide, Du Pont Australia Ltd; RH3928 mixture B, furophanate plus mancozeb, experimental fungicide, Rohm and Haas, Australia Pty.Ltd.; and procymidox, Sumisclex (R), I.C.I. Australia Ltd.

Long-term cold storage. — Healthy ripe Granny Smith apples were inoculated and treated with fungicides as in the previous experiment. Fruit was stored at 0 ± 2°C and examined after 14 weeks. Counts were made of the number of wounds which developed blue mould.

RESULTS

Sensitivity to fungicides in vitro

Germination. — Benomyl, carbendazim and thiabendazole at a concentration of 1 μg/ml caused a reduction in spore germination, and a distortion of germ tubes of the sensitive isolates B229 and B231. Affected germ tubes were short, twisted and thickened, and these reactions became progressively more severe as the concentration of fungicide increased. At a concentration of 500 μg/ml spore germination was reduced to about half that of the controls. Thiophanate methyl at a concentration of 1 μg/ml had no effect on the germ tubes. At 10 μg/ml and above, this fungicide caused effects similar to those caused by benomyl, carbendazim and thiabendazole, although at a concentration of 1000 μg/ml germination was reduced to only 85% of the controls.

The germ tubes of tolerant isolates B226 and B235 were shortened at a

concentration of 1 μg/ml benomyl or carbendazim, although severe shortening and twisting was only noted at 100 μg/ml and above. Percentage germination of spores was also reduced at this concentration. Thiophanate methyl and thiabendazole had no effect on percentage germination at any concentration to 1000 μg/ml, and germ tubes were normal on 10 μg/ml thiophanate methyl and below. Germ tubes were slightly shortened at a concentration of 10 μg/ml thiabendazole, and at 100 μg/ml thiophanate methyl and thiabendazole germ tubes were severely shortened and twisted.

Germination was normal with all isolates on 10 μg/ml 2-aminobutane. Germ tubes were shortened somewhat, but the germination percentage was unaffected at a concentration of 100 μg/ml. At 500 μg/ml and 1000 μg/ml 2-aminobutane, germination was reduced from 95% to less than 50%. With 2000 μg/ml germination was less than 1% and at 3000 μg/ml and above, germination was completely prevented.

Growth of Colonies. — The effect of the fungicides on the radial growth of the colonies is recorded in Table 1. Sensitive isolates failed to grow at a concentration of 1 μg/ml benomyl or carbendazim, and of 10 μg/ml thiophanate methyl and thiabendazole.

TABLE 1

Effect of fungicides incorporated in PDA on the radial growth (mm) of *P. expansum* after 12 days incubation at 25°C. Each observation is the mean of 5 replicates

Fungicide	Isolate	Concentration (μg/ml)					
		0	1	10	100	500	1000
Benomyl	B231	64	0	0	0	0	0
	B229	56	0	0	0	0	0
	B226	46	47	33	16	0	0
	B235	67	65	53	19	0	0
Carbendazim	B231	64	0	0	0	0	0
	B229	56	0	0	0	0	0
	B226	46	42	34	24	20	20
	B235	67	63	54	38	32	28
Thiophanate methyl	B231	64	48	0	0	0	0
	B229	56	45	0	0	0	0
	B226	46	48	48	45	36	30
	B235	67	67	67	61	48	46
Thiabendazole	B231	59	17	0	0	0	0
	B229	58	15	0	0	0	0
	B226	50	45	43	37	19	16
	B235	68	63	44	19	0	0
2-aminobutane	B231	59	65	56	34	24	27
	B229	58	61	65	36	27	32
	B226	50	50	57	53	37	29
	B235	68	63	71	44	33	26

Of the benzimidazoles, benomyl had the greatest effect on the growth of tolerant colonies, which on low concentrations of benomyl and carbendazim were green in colour, and which became progressively more brown—mauve from 10 μg/ml as fungicide concentration increased. Sporulation was inhibited above 10 μg/ml of either fungicide. Thiophanate methyl and thiabendazole at 100 μg/ml caused colonies to tend to brown—mauve, but sporulation was not greatly affected even at 1000 μg/ml thiophanate methyl. Sporulation of tolerant colonies was inhibited at 100 μg/ml thiabendazole.

Growth of all cultures was similar on 2 aminobutane, and colony diameter was not greatly reduced. At a concentration of 10 μg/ml, colonies were light brown in colour and produced few spores. At higher concentrations colonies were brown to white in colour, less dense and closely appressed to the surface of the agar.

Pathogenicity to pear fruit and effect of fungicides

The suspected tolerant isolate (B226) was highly pathogenic to pear fruit (Table 2). None of the benzimidazole group of fungicides provided adequate control of blue mould. 2-aminobutane reduced rot considerably at 5000 μg/ml and at 15 000 μg/ml.

Post-harvest control of mould in artificially inoculated apples

Short-term storage at room temperature. — The results of the post-harvest dip treatments on blue mould are presented in Table 3. Control of the benzimidazole sensitive strain was good with benomyl, and the non-benzimidazole fungicides iprodione, imazalil, and 2-aminobutane. Mixtures of a non-benzimidazole with benomyl generally gave a higher degree of control

TABLE 2

Percentage of wounds in pear fruit cultivar 'Packham's Triumph' developing rot after inoculation with isolate B226 of *P. expansum*. Twenty fruit per treatment, 3 inoculation sites per fruit

Fungicide	Concentration (μg/ml)	Percentage rot
Control		92
Benomyl	250	87
Benomyl	750	87
Thiabendazole	1000	95
Thiabendazole	3000	90
Thiophanate methyl	700	80
Thiophanate methyl	2100	73
2-aminobutane	5000	22
2-aminobutane	15000	8

TABLE 3

Short-term room temperature storage trial. Percentage of lesions in apples cultivar 'Granny Smith' which developed blue mould infections after inoculation and incubation at 21 ±1°C for 8 days. Each observation is the mean of 75 fruit divided into 3 replicates

Fungicide	Concentration (µg/ml)	Sensitive isolate B 231		Tolerant isolate B 226	
		Angular transformation	Retransformed percentage	Angular transformation	Retransformed percentage
Iprodione	250	40.0	41.3	40.8	42.7
Iprodione plus benomyl	250 250	24.9	17.8	40.2	41.7
Imazalil	400	42.1	45.1	47.0	53.3
Imazalil plus benomyl	400 250	39.7	40.8	42.5	45.7
2-aminobutane	5000	44.6	49.4	38.3	38.4
2-aminobutane plus benomyl	5000 250	26.3	19.6	35.7	34.1
Captan	1100	61.6	83.6	61.0	76.5
Captan plus benomyl	1100 250	44.6	49.4	64.2	81.1
Chlorthalonil	1500	70.6	89.0	72.4	90.9
Chlorthalonil plus benomyl	1500 250	47.4	54.2	69.5	87.8
Mancozeb	1200	72.5	91.0	66.9	84.6
Mancozeb plus benomyl	1200 250	38.4	38.6	67.8	85.7
DPX 164	1500	32.8	29.4	65.2	82.4
RH 3928	1500	62.4	78.6	67.9	85.9
Benomyl	250	42.6	45.8	72.1	90.6
Control		77.3	95.2	74.7	93.2
L.S.D. 5% between strains for same chemical		7.5			
L.S.D. 5% between chemicals for same or different strains		6.9			

than the non-benzimidazole alone, which appeared to be due to the benomyl present. The experimental fungicide DPX 164, a mixture of mancozeb and carbendazim, gave control similar to that found with mancozeb plus benomyl. The other products tested show no promise for blue mould control in pome fruit.

As expected, benomyl failed to control the tolerant strain. Control with mixtures of a non-benzimidazole plus benomyl indicated that control was due only to the non-benzimidazole fungicide.

Iprodione, imazalil, and 2-aminobutane controlled both tolerant and sensitive strains of blue mould with equal efficiency.

Long-term cold storage. — The results obtained in this trial are presented in Table 4. Benomyl failed to control the tolerant strain during long-term cold storage, but was outstanding in its effectiveness against the sensitive

TABLE 4

Long term cold storage trial. Percentage of lesions in apples cultivar 'Granny Smith' which developed blue mould infections after inoculation and storage at 0 ± 2°C for 14 weeks. Each observation is the mean of 75 fruit divided into 3 replicates

Fungicide	Concentration (μg/ml)	Sensitive isolate B 231		Tolerant isolate B 226	
		Angular transformation	Retransformed percentage	Angular transformation	Retransformed percentage
Iprodione	250	50.1	58.9	61.0	76.5
Iprodione plus benomyl	250 250	15.8	7.4	54.6	66.5
Iprodione	500	38.6	38.9	44.4	49.0
Iprodione plus benomyl	500 250	12.0	4.3	42.8	46.2
Imazalil	400	34.6	32.3	39.2	40.0
Imazalil plus benomyl	400 250	13.3	5.3	41.2	43.4
Imazalil	800	36.6	35.6	31.1	26.7
Imazalil plus benomyl	800 250	7.6	7.8	32.0	28.1
2 aminobutane	2500	62.2	78.3	68.9	87.1
2 aminobutane plus benomyl	2500 250	17.9	9.5	59.6	74.4
2 aminobutane	5000	67.3	85.1	72.5	91.0
2 aminobutane plus benomyl	5000 250	14.1	5.9	64.3	81.2
Procymidox	250	54.2	65.8	67.7	85.6
Procymidox plus benomyl	250 250	8.3	2.1	58.0	73.2
Procymidox	500	58.2	72.3	53.4	64.5
Procymidox plus benomyl	500 250	20.8	12.6	54.9	66.9
Benomyl	250	16.5	8.1	60.3	75.5
Control		60.8	76.2	62.2	78.3
L.S.D. 5% between strains for same chemical		14.8			
L.S.D. 5% between chemicals for same or different strains		18.8			

strain. No cross tolerance occurred to the other chemicals tested. Mixtures of benomyl with other fungicides provided excellent control of the benomyl-sensitive strain, but gave no improvement in control of the tolerant strain.

Benomyl-tolerant strains were best controlled with imazalil at 400 and 800 μg/ml and iprodione at 500 μg/ml. Control was poor with 2-aminobutane.

DISCUSSION

The effect of benomyl on the germination and growth of the sensitive isolates was similar to those reported with other fungi (Richmond and Pring, 1971; Penrose and Koffmann, 1977).

Our work has confirmed the presence of benzimidazole tolerant *P. expansum* in pear fruit in New South Wales. The number of orchards affected has not yet been determined. Cross tolerance of this strain to benomyl, carbendazim, thiophanate methyl and thiabendazole has been confirmed, as has been found with other benzimidazole tolerant fungi (Muirhead, 1974; Wicks, 1977). The tolerant strain was found to be highly pathogenic to fruit and in culture is indistinguishable from the sensitive strain in colony shape, colour and sporulation.

The occurrence of tolerance to benzimidazole fungicides in *P. Expansum* is of major concern because of the lack of registered alternative fungicides for pome fruit blue mould control.

The short term storage trial at room temperature showed the effectiveness of imazalil, iprodione and 2-aminobutane in prolonging shelf life of fruit infected with the benzimidazole-tolerant strain. The level of control was similar to that provided against the benzimidazole-sensitive strain by benomyl in this test.

Mixtures of iprodione or 2-aminobutane plus benomyl provided superior control to either chemical alone when used against the benzimidazole-sensitive strain, but the addition of benomyl was of no advantage against the tolerant strain.

Under long-term cold storage conditions benomyl was by far the best material against the sensitive strain. Mixing benomyl with iprodione, imazalil and 2-aminobutane improved their control of the sensitive isolate considerably.

Imazalil performed well in both trials, but 2-aminobutane and iprodione were less effective in the long term trial, which suggests a lack of persistence when compared with the results of the short-term trial.

The use of mixtures of imazalil or iprodione with benomyl would seem to be the best treatment in a non-tolerant situation. Such mixtures, as well as providing excellent control of benzimidazole sensitive strains, may also delay the occurrence of tolerance, since the lack of cross resistance between the components suggests that their mode of action is different. Further, if tolerance to benzimidazole does arise, a measure of control can be expected from the other fungicide present. The possibility of tolerance arising to other new, non-benzimidazole fungicides cannot be ignored; Harding (1976) has detected tolerance in *P. digitatum* and *P. italicum* to 2-aminobutane.

The high degree of fruit infection recorded with all chemicals in our trials is related to the severity of the test method. The introduction of spores into a large wound places a harsh test on the effectiveness of a chemical. Nevertheless, results of such tests indicate which material will perform best under conditions where fruit is damaged and spore numbers high, and these chemicals can be expected to provide an even higher level of control in uninjured fruit.

Cultures used in this study, together, in some cases, with the specimens from which they were obtained, are lodged in the Department of Agriculture

Herbarium, Biological and Chemical Research Institute, Rydalmere (DAR), under the accession numbers DAR 27963, DAR 27044, DAR 27965.

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