African Journal of Biotechnology Vol. 6 (11), pp. 1289-1292, 4 June 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Effect of benzimidazole fungicides and calcium chloride on *Alternaria alternata* and *Penicillium expansum* rot during storage of pears

Abdelfettah Maouni[1], Ahmed Lamarti[2], Anouar Aidoun[1], Mustapha Khaddor[1] and Alain Badoc[3]

[1]Département de Sciences Naturelles, École Normale Supérieure, BP 202, 93150 Martil, Morocco.
[2]Département de Biologie, Unité de Biotechnologie et d'Amélioration des plantes, Faculté des Sciences M'hannech II, BP 212, 93002 Tétouan, Morocco.
[3]Laboratoire de Mycologie et Biotechnologie végétale, Faculté des Sciences Pharmaceutiques, Université Victor Segalen Bordeaux 2, 146, rue Léo Saignat, 33076 Bordeaux Cedex, France.

Accepted 25 January, 2007

The present study assayed the effect of the fungicides benomyl, methyl-thiophanate, thiabendazole and calcium chloride on fungal decay of pears caused by *Alternaria alternata* and *Penicillium expansum*. Both *in vitro* and *in vivo*, the efficacy of fungicides alone against the two fungi was shown to be weak and without any practical interest. *In vitro*, $CaCl_2$ alone was tolerated by both species at more than 4%. *In vivo* and at low temperature, $CaCl_2$ significantly reduced fungal decay when used at 4 and 6%. The association fungicides - $CaCl_2$ (4%) allowed a better control of *A. alternata*.

Key words: Pears, conservation, fungi, calcium chloride, fungicides.

INTRODUCTION

During storage of pears, certain fungi lead to problems with serious economical consequences (Palazón et al., 1984). Blue mold rot caused by *Penicillium expansum* Link and black spot caused by *Alternaria alternata* (Fr.) Keissler are among the main diseases found on pears and apples in storage (Amrani and Najim, 1985; Bondoux, 1969, 1992; El Hassani, 1991; Maouni, 1980; Ramdani, 1989; Selmaoui and Douira, 1999).

When they were first introduced, chemical treatments by fungicides benzimidazoles considerably reduced losses caused by these fungi. Nevertheless, the latter again began to appear during storage even after intense chemical protection and resistance phenomena developed (Bondoux, 1992; Mc Phee, 1980; Ramdani, 1989). To solve this problem of resistance and to fight against certain physiological disorders of apples and pears linked to calcium deficiency such as bitter pit, calcium chloride was used (Mason and Welsh, 1970; Perring, 1986; Raese and Stahly, 1988) and the association fungicide-$CaCl_2$ was proposed to control *Alternaria* sp. (Biggs et al., 1993).

The aim of this work was to test *in vitro* and *in vivo* the association of three benzimidazole fungicides against two of the main fungal pathogens.

MATERIALS AND METHODS

Pathogens

Isolates of A. alternata and P. expansum were selected for by their aggressiveness among several isolates found in different pears cultivars: these isolates developed well on Malt-Extract-Agar (MEA, Biolife Italiana s.r.l.) and Potato-Dextrose-Agar (PDA, Biolife Italiana s.r.l.) media and produced the largest lesions on inoculated pears. The fungi were maintained on PDA, with periodic transfers through pears.

Fungicides and calcium chloride tested

The fungicides tested in this work are largely marketed in Morocco and were used at authorized doses (AD): benomyl (B) 50% (benlite, 60 g/hl), methyl-thiophanate (MT) 70% (Pelt 44, 350 g/hl), and thia-

*Corresponding author. E-mail: lamarti.ahmed@menara.ma. Phone: (212) 39960569. Fax: (212) 39994500.

bendazole (TB) (Decco 20S, 150 g/hl), alone or with $CaCl_2$ 96% purity at 4% (w:v). The calcium chloride was tested alone at 2, 4 and 6%.

In vitro tests

To study conidial germination, a spore suspension (10^5 spores/ ml) of *A. alternata* and *P. expansum* was spread everyone out over the surface of three petri plates containing an agar – fungicide mixture and/or $CaCl_2$. Germinated spores were counted on 200 spores after 24 h incubation at 25°C in darkness.

To evaluate the mycelial growth, a solution of each fungicide and/or $CaCl_2$ was incorporated in the MEA medium still in fusion and the mixture was placed in petri dishes. In each petri dish three inoculations points were done with mycelial discs issued from culture of 10 to 15 days. Mycelial growth was evaluated after seven and ten days of incubation at 25°C by measuring the diameter respectively of *P. expansum* and *A. alternata* colonies.

As for the sporulation, four of 5 mm discs diameter, taken at the edge of each of the 27 cultures colonies used to study mycelial growth was placed in a tube containing 1 ml of distilled water. After 30 s shaking with a vortex, the spores were counted by a Malassez cell at a rate of three counting per suspension.

The efficacy of each treatment was given according to the following formula:

$$E(\%) = \frac{X - Xi}{X} \times 100$$

X: Estimation of germination, growth or sporulation in a medium without fungicide or $CaCl_2$ (Control). Xi: Estimation of germination, growth or sporulation in a medium with fungicide and/or $CaCl_2$.

In vivo tests

'Williams' pears were surface sterilised by dipping them into 1% (w:v) sodium hypochlorite for 1 min, rinsed 3 times with sterile distilled water and blotted dry on sterile filter paper. The fruits were wounded by a 1 mm diameter needle three points on their equatorial area and dipped into the solution of fungicide and/or $CaCl_2$. After 24 h, they were inoculated by 1 mm diameter mycelium fragments. Thereafter, the fruits were distributed separately under black plastic sheeting to maintain a relative humidity at 100% (Maouni et al., 2001) and were preserved for two months at 4°C or 10 days at 25°C.

The efficacy (inhibition percentage) of each treatment is given according to the following formula:

$$E(\%) = \frac{D - Di}{D} \times 100$$

D: Diameter of lesion without treatment (control). Di: Diameter of lesion after treatment by fungicide and/or $CaCl_2$.

Statistical analysis

Each treatment was replicate three times. All data were submitted to analysis of variance and the means were separated with Duncan's multiple range test (P = 0.05, n = 27). Before the analysis, the percentage was converted into arc sin angles to produce approximately constant variance.

RESULTS

Efficacy of $CaCl_2$ *in vitro* and *in vivo*

Up to 6% (Table 1), $CaCl_2$ had little efficacy on the three developmental stages of the two species *in vitro*. In most cases, the inhibition percentage did not exceed 50%. The inhibition seemed greater for sporulation and increases with $CaCl_2$ concentration. *In vivo* (Table 2), efficacy against the two species increased with the $CaCl_2$ concentration and was clearly greater at 4°C than 25°C. From 4% concentration, $CaCl_2$ was efficient for conservation at 4°C.

Efficacy of fungicides and $CaCl_2$ *in vitro*

The efficacy of the fungicides tested alone is weak and without any practical interest on the conidial germination of the two species (Table 3). In the presence of $CaCl_2$, the efficacy of B and MT increases remarkably for both species, whereas those of TB remain unchanged. The three tested fungicides (Table 3) had little action on the mycelial growth of *A. alternata*. On the contrary, the efficacy of TB on *P. expansum* was quit high (about 70%). In the presence of $CaCl_2$, the efficacy of B and MT significantly increases, contrary to that of TB. These associations were without practical interest against *A. alternata*. All the fungicides tested had little effect on the sporulation of the two studied species (Table 3) and were less active than $CaCl_2$. The association of B and TB with calcium chloride gave better results, especially on *P. expansum*. All in all, the efficacy of the fungicides alone was relatively low on the three developmental stages of the two species studied and tended to increase in the presence of calcium chloride.

Efficacy of fungicides and $CaCl_2$ *in vivo*

In vivo (Table 4), the fungicides had little effect against the two species whatever the conservation temperature and had less effect than $CaCl_2$ at 4°C. The association fungicides - $CaCl_2$ was significantly positive against *A. alternata*, whatever the conservation temperature, and gave satisfactory result even at 4°C. With regard to *P. expansum*, the association appeared effective only for the B on fruits stored at 25°C.

DISCUSSION

The results obtained *in vitro* and *in vivo* show that *A. alternata* and *P. expansum* tolerate benzimidazole fungicides at the authorised doses. Similar results were obtained with *P. expansum* on apples and pears (Maouni et al., 2002; Ramdani, 1989; Wicks, 1977).

Table 1. Efficacy (%) of calcium chloride on the development of *Alternaria alternata* and *Penicillium expansum in vitro.*

$CaCl_2$ (%)	*Alternaria alternata*			*Penicillium expansum*		
	Germination	Growth	Sporulation	Germination	Growth	Sporulation
2	8.2c	5.2c	17.6b	2.6c	7.3c	25.0b
4	12.4b	8.1b	25.4a	15.0b	19.4b	25.8b
6	24.8a	22.1a	26.4a	21.8a	25.0a	45.9a

Means within a column, followed by the same letter, are not significantly different (P= 0.05) according to Duncan's multiple range test.

Table 2. Efficacy (%) of calcium chloride on the development of *Alternaria alternata* and *Penicillium expansum* on 'Williams' pears.

$CaCl_2$ (%)	*Alternaria alternata*		*Penicillium expansum*	
	4°C	25°C	4°C	25°C
2	57.7a	27.5a	8.2a	4.7a
4	71.5b	35.2b	68.0b	22.5b
6	92.5c	59.0c	72.5b	40.0c

Means within a column, followed by the same letter, are not significantly different (P= 0.05) according to Duncan's multiple range test.

Table 3. *In vivo* efficacy (%) of fungicides (A.D) associated or not with calcium chloride (4%) on the development of *Alternaria alternata* and *Penicillium expansum*.

Conditions	*Alternaria alternata*			*Penicillium expansum*		
	Germination	Growth	Sporulation	Germination	Growth	Sporulation
$CaCl_2$	20.5c	10.6a	43.0c	22.6a	34.0b	53.6cd
B	29.0d	6.6a	10.0ab	31.6b	31.0b	38.0b
B + $CaCl_2$	71.6e	20.0b	50.6d	91.6c	55.6d	61.0d
MT	15.7bc	13.0ab	7.0ab	35.0b	10.9a	35.6b
MT + $CaCl_2$	95.0f	35.6c	14.7b	97.0c	42.6c	44.6bc
TB	3.9a	30.4c	4.9a	33.5b	69.6e	10.6a
TB + $CaCl_2$	7.6ab	35.6c	44.6cd	37.8b	75.0e	83.0e

Means within a column, followed by the same letter, are not significantly different (P= 0.05) according to Duncan's multiple range test.

Table 4. Efficacy (%) of fungicides (A.D) associated or not with calcium chloride (4%) on the development of *Alternaria alternata* and *Penicillium expansum* on 'Williams' pears.

Conditions	*Alternaria alternata*		*Penicillium expansum*	
	4°C	25°C	4°C	25°C
$CaCl_2$	69.0d	34.6b	61.0c	21.6bc
B	15.6a	10.6a	5.9a	3.6a
B + $CaCl_2$	84.0e	74.0d	65.0c	60.0d
MT	51.6c	30.0b	27.0b	10.0ab
MT + $CaCl_2$	88.6e	59.6c	55.6c	32.6c
TB	40.0b	29.7b	14.0a	7.0a
TB + $CaCl_2$	84.0e	58.0c	64.0c	20.7b

Means within a column, followed by the same letter, are not significantly different (P= 0.05) according to Duncan's multiple range test.

Resistance to benzimidazoles has also been reported in *Botrytis cinerea* (Besri and Diatta, 1985; Hmouni et al., 1996). It is very likely that the repetitive uses of these systemic fungicides and their persistence during long periods of conservation (Ben Arie, 1975; Prusky, 1985) have led to considerable selective pressure on both species. Moreover, the single site action of these fungicides could have facilitated the appearance of resistant strains (Lepoivre, 1989).

In vitro, $CaCl_2$ had little efficacy against the two fungi, even at 6% while *in vivo* and at low temperature, it was effective at 4%. Similar results were obtained with *P. expansum* and *Alternaria* sp. on apples (Biggs et al., 1993; Conway, 1982) and pears (Maouni et al., 2001), and with *Leucostoma persoonii* on peaches (Biggs et al., 1994). The Ca^{++} ions might bind with intercellular pectic acids and constitute pectate chloride; which is resistant to the fungal pectolytic enzyme polygalactouronase (Bateman, 1964; Conway and Sams, 1984). At 25°C, the calcium would be insufficient to prevent this enzymatic activity.

The benzimidazoles - $CaCl_2$ association appears profitable *in vitro*, and against *A. alternata in vivo*. Satisfactory results were observed with other fungicides associated with $CaCl_2$ (Biggs et al., 1993). The increase efficacy of benzimidazole fungicides in the presence of $CaCl_2$ can be explained by the fact that $CaCl_2$ is an acid salt. Moreover, they might act by linking with a microtubule protein, thereby preventing cellular division (Davidse and Flach, 1978). It seems that calcium salts would reinforce the bonds of these fungicides with this protein.

In conclusion, the strategy of associating benzimidazoles with $CaCl_2$ could be used to control certain physiological and parasitic diseases in pears. The present findings show that it gives satisfactory results against *A. alternata*.

REFERENCES

Amrani N, Najim L (1985). Contribution to study of microscopical fungal of Morocco. 2. *Alternaria alternata*, microsclerotia and chlamydospores. Cryptogam., Mycol. 6: 265-270.

Bateman DF (1964). An induced mechanism of tissue resistance to polygalactouronase in *Rhizoctonia* infected hypocotyls of beans. Phytopathology. 54: 438-445.

Ben Arie R (1975). Benzimidazole penetration, distribution and persistence in post harvest pears. Phytopathology. 65: 1187-1189.

Besri M, Diatta F (1985). Résistance de *Botrytis cinerea*, agent de la pourriture grise de la tomate aux benzimidazoles, dicarboximides et sulfamides. Bull. OEPP. 15: 379-386.

Biggs AR, El-Kholi MM, El-Neshawy SM (1994). Effect of calcium salts on growth, pectic enzyme activity, and colonization of Peach twigs by *Leucostoma persoonii*. Plant Dis. 78: 886-896.

Biggs AR, Ingle M, Solihati WD (1993). Control of *Alternaria* infection of fruit of apple cultivar Nittany with calcium chloride and fungicides. Plant Dis. 77: 976-980.

Bondoux P (1969). Les maladies cryptogamiques des poires et des pommes au cours de l'entreposage. II. Identification et description. Ann. Phytopathol. 1: 327-352.

Bondoux P (1992). Les maladies de conservation des fruits à pépins : pommes et poires. PHM Revue Horticole. pp. 173.

Conway WS (1982). Effect of post-harvest calcium treatment on decay of Golden Delicious apples. Plant Dis. 66:402-403.

Conway WS, Sams CE (1984). Possible mechanisms by which post-harvest calcium treatment reduces decay in apples. Phytopathology. 74: 208-210.

Davidse LC, Flach W (1978). Interaction of thiabendazole with fungal tubulin. Biochim. Biophys. Acta. 543: 82-90.

El Hassani N (1991). Influence des traitements chimiques au Drencher sur l'incidence des pourritures à *Penicillium expansum* sur poires en conservation. Mémoire de 3e cycle, IAV Hassan II of Rabat, Morocco.

Hmouni A, Hajlaoui MR, Mlaîki A (1996). Résistance de *Botrytis cinerea* aux benzimidazoles et aux dicarboximides dans les cultures abritées de tomates en Tunisie. Bull. OEPP. 26: 697-705.

Lepoivre P (1989). Traité de pathologie végétale. Aspects phytopathologiques de la chimiothérapie. Les presses agronomiques de Gembloux. pp. 492.

Maouni A, Lamarti A, Douira A, Badoc A (2001). Effets de dérivés calciques sur le développement de moisissures lors de la conservation des poires. Bull. Soc. Pharm. Bord. 140: 79-88.

Maouni A, Lamarti A, Douira A, Badoc A (2002). Étude de la résistance d'*Alternaria alternata* et *Penicillium expansum* aux fongicides lors de la conservation des poires. Bull. Soc. Pharm. Bord. 141: 61-72.

Mason JL, Welsh MF (1970). Cork spot (pit) of "Anjou" pear related to calcium concentration in fruit. HortScience. 5: 447-452.

Mc Phee WJ (1980). Some characteristics of *Alternaria alternata* strains resistant to iprodione. Plant Dis. 64: 847-849.

Palazón I, Palazón C, Robert P, Escudero I, Muñoz M, Palazón M (1984). Estudio de los problemas de la conservación de peras y manzanas en la provincia de Zaragoza. Publicación no. 990, Institución Fernando El Católico of Zaragoza, Spain, pp. 68.

Perring MA (1986). Incidence of Bitter pit in relation to the calcium content of apples: problems and paradoxes, a review. J. Sci. Food Agric. 37: 591-606.

Prusky D (1985). Development, persistence, survival and strategy for control of thiabendazole resistant strains of *Penicillium expansum* on pome fruit. Phytopathology. 75: 877-882.

Raese JT, Stahly EA (1988). Timing of calcium sprays to increase fruit calcium, improve fruit quality and control disorders of "Anjou" pears. HortScience. 23: 836 (Abstract).

Ramdani A (1989). Les pourritures à *Penicillium expansum* Link ex. Thom. Des pommes et des poires dans une station frigorifique de la région de Meknès: Problèmes et remèdes. Mémoire de 3e cycle, IAV Hassan II of Rabat, Morocco.

Selmaoui K, Douira A (1999). Microscelerotia in *Alternaria alternate*. Phytopathol. Mediterr. 38: 43-46.

Wicks T (1977). Tolerance to benzimidazole fungicides in blue mold (*Penicillium expansum)* on pears. Plant Dis. Rep. 61: 447-449.