Comparison of thiabendazole formulations and application methods for control of green mold of oranges.

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COMPARAISON DE QUELQUES FORMULATIONS DE THIABENDAZOLE ET DE MODES D'APPLICATION POUR LA LUTTE CONTRE LA MOISISSURE VERTE DES ORANGES

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Fruits, Jan. 1982, vol. 37, nº 1, p. 27-31.

RESUME - Différentes formulations du thiabendazole et des modes d'application après récolte ont été évalués pour la lutte contre la moisissure verte des oranges navels pendant la saison 1979-1980.
La lutte contre cette altération avec du thiabendazole dans la cire n'a pas été aussi bonne que son application dans l'eau.
La solution de ce fongicide dans le solvant + cire n'a pas été aussi efficace que sa suspension dans une émulsion eau + cire. Ceci est dû à la couverture médiocre résultant de l'application en brouillard du solvant + cire.
Un précédent travail effectué par différents chercheurs, démontrait une plus forte efficacité du thiabendazole dissous dans le solvant + cire; ceci peut être attribué au fait que le solvant + cire était appliqué par trempage et non par l'habituelle pulvérisation.
La sensibilité des oranges à la moisissure verte augmente lorsque le fruit devient mature, particulièrement en fin de saison.

In Egypt, as in other citrus producing countries, fruit is subject to various postharvest decays. These can originate from latent infections that occur in the grove or from infections occuring after harvest, through injuries formed during handling. Treating fruit with an approved fungicide is compulsory in Egypt and some other countries for fruit destined for export.

Different methods of applying fungicides to fruit can be used depending on the properties of the fungicide concerned, the type of formulation and on practical considerations. Water-soluble fungicides e.g., sodium o-phenylphenate tetrahydrate (SOPP), are commonly used as washing solution additives. In most processes it must be rinsed off the fruit to avoid chemical injury to the rind. Orthophenylphenate may also be incorporated in both shipping and storage wax emulsions. On the other hand, solvent wax formulation may contain 1 % o-phenylphenol, providing an alternate means of applying this fungicide to citrus fruit. In Egyptian packinghouse operations, the water-insoluble fungicide thiabendazole (TBZ) is applied as either (i) a water suspension after washing but before waxing, (ii) incorporated as a suspension (emulsion) in waterbase wax, (iii) dissolved in solvent wax, or (iv) after washing but before waxing as an aqueous solution containing lactic acid.

In Egypt the navel orange is the leading early variety. The season starts in late November and the peak of harvest occurs during early January, and the crop lasts for only a

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few months. However, growers usually store the crop on the trees for periods of 3-4 months, untill the latter part of April, with 10 % of the fruit remaining marketable. In this connection, HARDING *et al.*, (1940) stated that ; «Oranges left on the tree remain in good eating condition for a protracted period, however, if left too long granulation or drying out of the flesh, and an aged flavor, indicating senescence, eventually develop. «The tests reported here were conducted during the 1979-1980 season, late February to early March, to determine the effectiveness of various methods of applying thiabendazole for control of green mold caused by *Penicillium digitatum* SACC., and to compare the susceptibility of Egyptian navel oranges to this type of decay. Also to determine the value of chemical treatment for the control of decay at different periods of growing season.

MATERIALS AND METHODS

These trials were conducted at El - Wadi Research Labo ratories, and at Behna and Quesna citrus packing houses in Egypt. The fruit used were navel oranges harvested from commercial groves. All experiments were done using the same lot of fruit at the same time. In addition to natural infection all fruits were artificially inoculated with spores of *P. digitatum* 24 hr before treatment. Inoculation was performed by making a scratch 1.0 cm long and 1.0 mm. deep in the rind on both sides of each fruit and then applying dry spore dust to the scratches with small brush.

The tests using water emulsion wax were carried at Benha packing house using an applicator (Brogdex Co., Pomona, California) in which the oranges are conveyed on 10 soft roller brushes under two No.3.5 Monarch spray nozzles on oscillating «wig-wag» booms. Each nozzle makes 52-62 oscillations per minute, passing over each fruit at least three times. The wax emulsion is deposited on the fruit as a fine spray while the rotating brushes spread the emulsion evenly over the surface and rub it into the inoculation injuries.

Citrashine® water emulsion wax (Decco Co., Catania, Italy) was used as the carrier for the SOPP or TBZ. The stock emulsion was diluted with water to give a final concentration of 16 % wax solids. The correct amount of SOPP (wt/vol) was added to the diluted emulsion. SOPP is completely soluble in aqueous wax emulsion but TBZ wettable powder forms a non-colloidal suspensions and all experimental formulations of TBZ were agitated during use to keep the TBZ in suspension. In the treatment combining TBZ the required amount of TBZ 98 % wettable powder was mixed to a smooth consistency with a small amount of diluted water wax emulsion,which was progressively diluted, with constant mixing, untill the correct dilution was achieved.

For all treatment of the water wax emulsion trial the fruit were thoroughly washed by soaking for three minutes in a bath containing 0.5 % SOPP tetrahydrate at pH 11.8-12.0, heated to 38°c. They were then rinsed and dried before treatment. The treatments prepared in 4 gallon aliquots, were as follows (1) Control, prewashed in 0.5 % SOPP and dried (2) waxed with water emulsion wax (w.w) only. (3) Waxed with water emulsion wax incorporating 1.5 % SOPP. (4) Water emulsion wax incorporating 1500 μg/ml TBZ. (5) Spray of water suspension, 1500 μg/ml TBZ dried and followed by water emulsion wax. (6) Water emulsion wax incorporating 2500 μg/ml. (7) Spray of water suspension 2500 μg/ml TBZ dried and followed by water emulsion wax.

The trials using solvent wax formulations were carried out at Quesna packing house. Prior to wax fungicide treatments all experimental fruit were cleaned with a foam containing 2.0 % SOPP with 0.2 % foam detergent at pH 11.8. The foam-covered fruit were passed over revolving brushes for about 15 seconds before drying. The wax formulation used in this trial was Flavorseal® (F.M. Corporation, San José, Calif) containing waxy materials dissolved in a volatile solvent, and was applied with an F.M.C. spray applicator. The treatments in this trial were : (1) Control foam and SOPP wash and dry. (2) Solvent wax spray only. (3) Solvent wax spray incorporating 1500 μg/ml. (4) Solvent wax spray incorporating 2500 μg/ml TBZ. (5) Spray of 1500 μg/ml aqueous soluble TBZ dried and followed by solvent wax spray. (6) As in (5) but using spray of 2500 μg/ml aqueous soluble TBZ. (7) Dip of 1500 μg/ml TBZ incorporated in solvent wax. (8) As (7) using 2500 μg/ml TBZ. The soluble TBZ used in treatment (5) and (6) was a lactic acid formulation containing 25 % active ingredient.

In the third trial the solvent wax treatments (4) and (6) above were tested at monthly intervals from January to March, 1980 to compare their effect on the control of green mold, which increases in intensity as the citrus season advances in Egypt.

In all experiments each treatment was tested in three replicates of three cartons, each containing 72 oranges. All fruit were wrapped in plain tissue paper wraps, packed in small ventilated cartons and stored at 17°-18°C. They were assessed for decay after two weeks storage. Data were statistically analysed using arcsin transformation of percentage of decay.

RESULTS

The results of the trial using solvent wax (table 1) showed that al TBZ treatments reduced decay as compared with fruit not treated with TBZ, or fruit treated with solvent wax alone. The lower concentration (1500 μg/ml), however, when used in a combined application with wax was not significantly better than the solvent wax applied on its own. At both 1500 μg/ml and 2500 μg/ml, TBZ applied as a combination spray in solvent wax was significantly less effective than equivalent concentrations of an aqueous solution of the fungicide sprayed onto the orange and dried before spray application of the wax. When applied as a dip,

TABLE 1 - Decay control of Navel oranges comparison of TBZ formulations and methods of application in combination with solvent wax.

Treatment (i)

FUNGICIDE	CONC. µg/ml	SOLVENT WAX (ii)	APPLICATION METHOD	MEAN NO. DECAYED FRUIT	DECAY %
1. SOPP Wash Only	-	none	-	43.33	60.18 a
2. SOPP Wash Only	-	wax only	spray	7.33	10.52 b
3. TBZ (iii)	1500	comb.	spray	3.67	5.10 c
4. TBZ	2500	comb.	spray	2.67	3.71 d
5. TBZ Sol. Aq. (iiii)	1500	sep.	spray	0.67	1.39 e
6. TBZ Sol. Aq.	2500	sep.	spray	0.00	0.00 f
7. TBZ	1500	comb.	dip	0.00	0.00 g
8. TBZ	2500	comb.	dip	0.00	0.00 h

(i) All experimental fruit pre-cleaned in 2 % SOPP foam, rinsed and dried before treatment.
(ii) Solvent wax : Sep = applied separately, after fungicide spray - Comb. = combined with fungicide formulation.
(iii) Wax soluble.
(iiii) TBZ Sol. Aq. : water soluble thiabendazole in lactic acid formulation, 25 % active material sprayed in aqueous solution

Statistical analysis (Arcsin transformation) shows :
a. significantly different from all treatments (P : 0.01)
b. not significantly different from C (P : 0.05) but significantly different from all fungicide treatments.
c. and d. significantly less effective than all other fungicide treatments (P : 0.01)
e.f.g.h. not significantly different from each other but significantly better than all other treatments (P : 0.05).

however, the combined TBZ/solvent wax formulation was significantly more effective than when applied as a spray. The tests using water emulsion wax showed that all fungicide treatments, both TBZ, whether combined with or separately from the wax at both concentrations, and SOPP in combination with the water emulsion wax, gave equally good control of decay.

In the trial to compare the effectiveness of different formulations and methods of application of TBZ at 2500 µg/ml in controlling seasonal variation in decay showed that the separate spray application of the fungicide before waxing was more effective than a combined fungicide wax spray. With both types of application, however, the effectiveness of the treatment was less as the season progressed.

DISCUSSION

The trial using solvent wax treatments demonstrated that at concentrations of TBZ used the separate application of the fungicide prior to waxing gave better control of green mold than when it was applied in a mixture with the wax. This correlates well with work done in south Africa by PELSER (1972, 1973) who found that incorporation of TBZ into solvent wax gave poorer control of *Diplodia* stem-end rot than the application of a water suspension applied before waxing, presumably because the solvent evaporated immediately on contact with the fruit before the fungicides could move under the stem button where the latent infection is established. Generally, decay control with benzimidazole fungicides combined with wax has not been as good as when they are applied in water before waxing (GUTTER *et al*, McCORNACK and BROWN, 1967 ; PELSER, 1972, 1973 ; RAYLANDS and STAUNARD, 1975 ; RIPPON and WILD, 1973 ; SMOOT and MELVIN, 1970 ; TUGWELL, 1973). Non-availability of fungicide at the infection site because of incorporation with wax (TUGWELL, 1973) and uneven coverage with the wax applicator have been suggested as the reason for reduced decay control (NORMAN *et al.*, 1972 ; TUGWELL, 1973). Although the combination of TBZ with solvent wax gave good control of green mold when used as a dip (table 1) this method is not practical for commercial use, where spray application is used. Further it was observed that with the dip application the toluene/ethanol solvent caused burning of the orange rind around scratches, thus inhibiting mycelial growth. Also, the effectiveness of the dip treatment may be due to an increase in surface residue and perhaps uptake of the fungicide.

Although the solvent wax and water emulsion wax experiments cannot be directly considered together there are indications from the results (table 1 and 2) that when TBZ is applied as a combined spray with water wax it controls post-harvest decay more effectively than when combined with solvent wax. It has been shown by many workers that solutions of benzimidazole fungicides in solvent wax are not as effective as suspensions in emulsion waxes (PELSER, 1972, 1973 and SMOOT and MELVIN, 1974). This may be due to poorer coverage (PELSER,

TABLE 2 - Decay control of Navel oranges. Comparison of fungicide formulations in combination with water emulsion wax (all applications by sprays).

Treatment (i)

FUNGICIDE	CONC. g/ml	WATER EM. WAX SPRAY	MEAN NO. DECAYED FRUIT	DECAY %
1. SOPP wash only	-	-	40.33	56.03 a
2. SOPP wash only	-	wax only	26.00	36.11 b
3. SOPP in wax	15.000	comb.	1.00	1.40 c
4. TBZ w.p.	1.500	comb.	2.00	2.80 d
5. TBZ w.p.	1.500	sep.	0.00	0.00 e
6. TBZ w.p.	2.500	comb.	0.00	0.00 f
7. TBZ w.p.	2.500	sep.	0.00	0.00 g

(1) All experimental oranges were pre-washed in soak tank containing 0.5 % SOPP and dried before treatment.
(ii) Water wax emulsion spray : sep : wax emulsion spray applied separately after fungicide spray
comb. : fungicide combined with water wax in a single spray.

Statistical analysis (Arcsin transformation) indicates :
a. Significantly different from all other treatments
b. Significantly better than a. but less effective than all other treatments.
c. to g. All fungicide treatments are significantly better than a. or b. but do not differ significantly from each other (P : 0.5).

TABLEAU 3 - Effectiveness of thiabendazole in controlling green mold of Navel harvested at different periods of Navel season.

Months	2500 µg TBZ/ml			
	In HOH spray, dried + solvent wax (F) spray		Dissolved in (F) spray	
	Mean No. of decayed fruit	% Decay	Mean No. of decayed fruit	% Decay
January	1.33	1.85	2.67	3.71
February	1.67	2.32	6.33	8.79
March	3.00	4.17	10.33	14.35

Statistical analysis indicated :
The months differed significantly at the 0.05 level of significance. January was better than the other two months and February was better than March in reducing the percentage decay.

TBZ sprayed in HOH was more effective than when dissolved in the wax (F) at the 0.01 level of probability.

1972), particularly under the calyx, for stem-end rot control. Work by RISK *et al.*, (1974) and HARDING and SCHADE (1967) reports that thiabendazole in solvent wax was more effective than when incorporated in water wax. This difference however, may possibly be attributed to the fact that the solvent wax formulation was applied as a dip rather than by the usual commercial spray application.

In the trial using water emulsion wax (table 2) the results demonstrate that a spray of TBZ in water is a better treatment than TBZ applied in water emulsion wax. In Israel suspensions of the thiabendazole in water have provided better control of Penicillium decay than suspensions in wax emulsions (GUTTER *et al.*, 1974). This may be explained by the fact that water suspensions of thiabendazole may wet the surface of the fruit and superficial injuries more efficiently than the wax treatment. ECKERT and KOLBEZEN (1977) have concluded that the ingredients of the wax formulation appear to reduce the efficiency of the thiabendazole deposit perhaps by coating the fungicide particles and thereby reducing their availability to fungal mycelium.

The results of the trial reported in (table 3) indicate that susceptibility of oranges to decay increased as the fruit became more mature. SMOOT and MELVIN (1974) found

that incidence of decay increased as the fruit became riper. The results obtained, however, make the effect of ripeness quite evident.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the valuable assistance provided by El-Wadi Co., Egypt, Merck & Co: Inc., Rahway, N.J., U.S.A., FMC Corporation and Decco Company of Italy.

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