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IMAZALIL — A NEW SYSTEMIC FUNGICIDE FOR CITRUS POST HARVEST DISEASE CONTROL

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Summary

Imazalil, a new systemic fungicide chemically unrelated to the benzimidazoles and possessing differences in biological activity, is a member of a class of substituted imidazoles including miconazole and clotrimazole used in medicine. It is active against several ascomycete fungi such as *Erysiphe*, *Podosphaera*, *Sclerotinia* and *Venturia*, as well as a number of fungi imperfecti including *Alternaria*, *Cercospora*, *Fusarium*, *Helminthosporium*, *Mycosphaerella* and *Penicillium*. Imazalil is also effective against fungal strains which have developed resistance to benzimidazole fungicides. Imazalil has shown high activity towards blue (*Penicillium italicum*) and green (*P. digitatum*) moulds as well as *Geotrichum* sour rot under New Zealand conditions.

INTRODUCTION

Imazalil was discovered in 1969 by Janssen Pharmaceutica, Belgium. It has been tested in all of the citrus growing areas of the world including Australia, France, Israel, Japan, Spain and U.S.A. Post-harvest decay control in citrus has, over recent years in New Zealand, been exclusively with benzimidazole fungicides, primarily benomyl. The systemic action of these materials not only provided improved citrus mould control but had the advantage of remaining active against infections occurring after application. However, as has been the case with other diseases in other crops, exclusive use of these fungicides has led to the development of resistant strains. An investigation (Hartill pers comm) in 1978 from the citrus growing areas in New Zealand, has shown benomyl resistant strains to be predominant in all the main growing areas including areas where few citrus are grown, such as Nelson.

Imazalil at rates of 250-1,000 ppm as a post-harvest dip or 2,000 ppm in a wax emulsion, has given excellent control of *Penicillium* moulds overseas and in New Zealand (Hartill *et al* 1977) and other citrus diseases such as *Alternaria citri*, *Diplodia natalensis*, *Geotrichum candidum* and *Phomopsis citri* (Laville *et al* 1977), some of which occur in New Zealand.

Chemical and physical properties:

Chemical structure:

Empirical formula: C₁₁H₁₀Cl₂N₂O
Molecular weight: 297.18

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Types and Formulations:

Imazalil exists in three types:

As a free base (R23979) as a nitrate salt (R18531) and as a sulphate salt (R27180) and previously as a hydrosulfate XE 326S, now withdrawn, belongs to a chemically related group. Imazalil is formulated as an 80% and 20% emulsifiable concentrate (free base) and a 50% soluble powder (sulphate salt).

Biological properties:

Toxicology:

Animal studies have shown imazalil to be moderately toxic with an acute oral LD₅₀ for male rats of 320 mg/kg and 640 mg/kg for beagle dogs; the acute dermal LD₅₀ for male rats of 4,200 mg/kg. Imazalil is not toxic to wildlife; Japanese quail acute oral LD₅₀ is 510 mg/kg; Harlequin fish 2.6 mg/kg. Imazalil is non toxic to bees. Long term (two year) feeding studies showed no adverse effects on rats when fed orally, at daily levels of 80 mg/kg. In beagle dogs the level causing no toxicological effect is considered to be 5 mg/kg/day. Imazalil presents little acute inhalation hazard and at a dilution of 1000 ppm did not produce any eye irritation.

Resistance:

The possibility of resistant strains occurring has been considered from the very beginning. Several attempts to obtain *P. digitatum* strains resistant in vitro to imazalil were unsuccessful even though the same method frequently produced benomyl resistant strains. With *Aspergillus nidulans* it is possible to obtain, in vitro, resistant strains to imazalil, this resistance being bound to a polygenic system, including 21 genes on 8 loci and linked with six groups (Van Tuyl 1976). Thus in all probability, the occurrence of *Penicillium* strains resistant to imazalil is an extremely rare phenomenon and if this event did occur it must involve many genes without certainty that the new mutant strain will be pathogenic to citrus fruit.

Residues:

Imazalil is very active at low rates, particularly when incorporated into a wax. New Zealand lemon residue results have shown:

Rate mg/litre	Method of application	Time lapse (days)	Residues mg/kg	
			Skin	Whole fruit
480	Spray in wax	43	0.61	0.51
960	"	43	0.96	0.72
1440	"	43	1.05	0.91
1920	"	1	23.6	10.4
1920	"	7	6.4	2.6
1920	"	16	2.8	1.8
1920	"	28	0.97	0.56
1920	"	43	1.03	0.71
500	Dip in water	1	14.3	4.3

Kodex international has set a tolerance of 5 ppm for citrus (whole fruit) with an ADI of 0.01 ppm (temporary). A residue tolerance of 5 ppm has now been established in New Zealand, 2 ppm in Spain and 10 ppm in USA.

METHOD

Trials with imazalil on citrus have been conducted in New Zealand since 1977.

Trial 1 1977:

Freshly picked lemons (cv Meyer) from an orchard in Welcome Bay, Tauranga, were first dipped in an inoculating bath of mould infected lemons from the same property. After these had been allowed to dry they were dipped in the various fungicide

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treatments and divided so that each treatment had four replicates of approximately 200 fruit each. The fruit was transhipped to Nelson where regular disease assessments were carried out (Table 1). Diseased fruit were removed at each inspection.

Trial 2 1979:

An assortment of growers lines of lemons (cv Lisbon) were dipped in the standard calcium hypochlorite bath after arrival at the Citrus Marketing Authority Depot, Tauranga. Two hundred fruit were selected at random for each treatment, of which one hundred were scratched on some part with a hand-held, coarse metal scraper and then inoculated on that area with a highly concentrated inoculum obtained from diseased fruit at the depot. The two hundred fruit, either "clean" or inoculated, were put through the waxer separately, then through the drier and finally placed into separate boxes each holding 25 fruit.

All imazalil treatments were carried out using an 80% EC formulation of the free base (R23979) incorporated in with the wax (pre-diluted at 400 ml wax : 1600 ml water) with the foam waxing unit set at 2 litres wax/945 kg of fruit. Benomyl, being immiscible with the wax, was applied as a separate water bath treatment prior to waxing. Fruit was stored at ambient temperatures (10 - 15 °C) for the 6 weeks trial duration. Regular disease evaluations were carried out (Table 2 and 3).

RESULTS AND DISCUSSION

TABLE 1: Control of mould and *Phytophthora* brown rot in lemons (cv Meyer)

Treatment	Rate g/100 litres	% Fruit infected with mould				% Brown rot fruit	
		19 days	40 days	90 days	120 days	19 days	120 days
XE326S	25	0.8c	1.8c	2.7c	3.8d	1.6a	5.8ab
guazatine & imazalil	50.1 4.2	1.7bc	2.7c	3.8c	6.7c	0.3b	1.8d
guazatine	57.1	1.6bc	4.3bc	5.2c	5.8c	2.1a	5.3abc
triforine	60	4.6ab	7.5b	10.3b	12.1b	1.2ab	3.7abcd
benomyl	25	1.8bc	2.9c	3.2c	3.2d	1.3ab	6.1a
inoculated	—	8.4a	15.1a	18.1a	22.7a	1.9a	3.2cd
CV %		30.4	27.1	22.3	17.3	47.5	25.7

This initial screening trial showed that even though all treatments gave significantly better mould control than untreated, only XE326S was as good as benomyl after 120 days. The addition of imazalil (free-base) to guazatine gave no improved mould control over guazatine alone. Although guazatine/imazalil obviously reduced the number of fruit with brown rot, this result would appear to be non-conclusive as neither guazatine alone nor XE326S gave control.

As expected, scratched inoculated fruit resulted in over 50% disease loss in 2 weeks. Benomyl only slightly reduced this level while imazalil gave excellent control at all rates tested. Where healthy fruit was used, the level of disease remained very low in all treatments with very few differences being present.

Levels of fruit infected with sour rot were considerably higher where the fruit was scratched and inoculated. Only imazalil at the two highest rates considerably reduced the levels of infection but little infection continued after 15 days. Uninoculated fruit showed few differences due to the low level of infection. Neither imazalil nor benomyl would be expected to control brown rot of citrus and the results confirm this.

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TABLE 2: Control of mould in lemons (cv Lisbon)

Treatment	Rate		% Fruit infected		
	mg/kg fruit	mg/litre wax	15 days	28 days	42 days
imazalil	1.0	480	2.0c* (0.3a)**	2.0c (0.3ab)	3.1c (0.3bc)
imazalil	2.1	960	0.0c (0.0a)	0.0c (0.0b)	0.0c (0.0c)
imazalil	3.1	1440	1.0c (0.0a)	1.0c (0.0b)	2.0c (0.0c)
imazalil	4.2	1920	0.0c (0.0a)	0.0c (0.0b)	3.1c (0.0c)
imazalil	6.2	2880	0.0c (0.0a)	0.0c (0.0b)	1.2c (0.0c)
benomyl	0.25 g/litre as dip		37.0b (0.3a)	38.0b (0.8a)	40.0c (0.8ab)
wax only	0.4	—	46.5a (0.0a)	47.4a (0.0b)	50.5ab (0.3bc)
untreated	—	—	52.4a (0.3a)	53.4a (0.5a)	58.4a (1.3a)
CV %			17.5 (32.2)	18.5 (25.4)	26.7 (27.1)

* Unbracketed figures give results of lemons scratched and inoculated prior to treatment.

** Bracketed figures give results of standard healthy lemons.

TABLE 3: Control of sour rot and brown rot in lemons (cv Lisbon)

Treatment	Rate mg/kg fruit	% Sour rot infection		% Brown rot	
		15 days	42 days	After 42 days	
imazalil	1.0	*15.6abc (0.5a)**	17.6ab (0.5ab)	3.3a (0.0b)	(0.0b)
imazalil	2.1	11.5bcd (0.0b)	13.5abcd (0.8a)	2.0a (0.0b)	(0.0b)
imazalil	3.1	19.4ab (0.0b)	20.4ab (0.3ab)	1.4a (0.0b)	(1.0a)
imazalil	4.2	5.2d (0.0b)	5.2d (0.0b)	1.1a (0.0b)	(0.5ab)
imazalil	6.2	6.2cd (0.0b)	6.2cd (0.8a)	5.0a (0.5ab)	(0.3ab)
benomyl	0.25g/litre	20.0a (0.0b)	20.0a (0.5ab)	2.0a (0.0b)	(0.0b)
wax only	0.4ml	13.3abc (0.0b)	13.3abc (0.0b)	5.2a (0.0b)	(1.0a)
untreated	—	9.8bcd (0.0b)	9.8bcd (0.3ab)	5.0a (0.3ab)	(0.3ab)
CV		31.8 (22.8)	33.1 (29.4)	76.3 (34.0)	

*, ** As per Table 2.

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