found may, in some cases, favor the production of STB which has not been demonstrated to have any fungicidal activity. This kind of application may even result in strains of mold that are stimulated by benzimidazole fungicides (24). Once resistant molds are established in the packinghouse, they are very difficult to control (13, 15, 23, 25, 29).

Since benomyl is a systemic fungicide (8, 9, 10, 16) whereas TBZ (16) and MBC (8, 9, 10) are not it is important to get the maximum amount of undegraded fungicide onto the fruit. This may be accomplished by applying benomyl in a vehicle that is no more alkaline than pH 8.5 and keeping the amount prepared for application to no more than the amount that may be used up in one day. Since the half life of benomyl, at use strength, is 7 hrs (7), an alternate would be to prepare a strong (3%) suspension that could be then metered into a small reservoir for dilution to use strength. Table 2 indicates that using this up rapidly would give more benomyl at the fruit. Part of the reason for the slower degradation of benomyl in concentrates is probably the build up of BIC concentration which slows down the degradation of benomyl (7). By making up no more than a weeks supply of concentrate at one time, the amount of benomyl available for decay control will be greatly increased.

Benomyl is an excellent fungicide, if used properly, but the trend in the Florida citrus industry is to abuse it in application and handling. If this fungicide is to be handled in a marginal manner it may be wise to increase the amount used in order to maintain good decay control.

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THE ISOLATION OF *PENICILLIUM DIGITATUM* SACC. STRAINS TOLERANT TO 2-AB, SOPP, TBZ AND BENOMYL

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Additional index words. disease resistance, exploratory fungicides.

Abstract. **Sporeload exposure plates and isolates from decayed fruits from citrus packinghouses are routinely used in our laboratory to isolate cultures of green molds and to determine their sensitivity to commercial fungicides. A large body of data has been accumulated that demonstrates the widespread occurrence of** *Penicillium* **strains that are individually tolerant to either 2-AB (2-amino butane), SOPP (sodium orthophenylphenate) or the benzimidazoles (thiabendazole and benomyl). Doubly resistant strains have also been reported by several investigators. However, we have, for the first time, isolated strains of** *Penicillium digitatum* **with tolerance to all three groups of fungicides. These multiple tolerant strains have been found to be sensitive to**

imazalil and CGA-64251. In our *in vivo* tests imazalil gave excellent sporulation control of tolerant strains. The discovery of this multiple tolerance underlines the need to add new fungicides to the approved listing of chemicals available to packinghouses to combat decay.

Various chemicals are available for control of plant pathogens. One of the common problems in the chemical control of plant pathogens is the ability of these pathogens to develop resistance (5, 6, and 21). In December 1979, a joint U.S. and Japanese meeting was held at Palm Springs, California titled, *Pest resistance to pesticides: challenges and prospects,* where development, genetics, mechanism and management of resistance to pesticides were discussed. The summaries of papers were circulated widely among the members of National Agricultural Chemicals Association in May 1980 (7).

A similar week-long post graduate course titled, *Fungicide resistance in crop protection* was held in August 1980 at the Agricultural University of Wageningen and co-sponsored by International Society of Plant Pathology and FAO. The purpose of this course given by international experts was to provide the practicing plant pathologists, information on the biochemical, genetic and epidemiological aspects of fungicide-resistance, to discuss methods to detect and measure fungicide resistance at an early stage, and to study the principles upon which counter measures would be based (1). Ogawa, et al. (22) has thoroughly reviewed methods for detecting and monitoring the resistance of plant pathogens to chemicals. In this report the authors have listed some one hundred chemicals to which no resistance has been detected in plant pathogens (from 1800-1977). Twenty-five chemicals are listed for which resistance in plant pathogens has been detected in laboratory tests but not in the field; some thirty-five chemicals are listed for which resistance to various plant pathogens have been detected under commercial usage or field conditions.

Then, we can depart from the foregoing record of worldwide concern for the increasing incidence of pathogens resistance to the focus of this paper: the resistance of *Penicillium* molds that are pathogenic to citrus. Table 1 presents history of fungicides to protect citrus fruits and the initial reports of resistance to *Penicillium* molds. Subsequent reports of the worldwide occurrence of *Penicillium* resistance problems in citrus have been documented by various researchers (4, 8, 9, 10, 15, 16, 17, 18, 19, 27 and 28). In the past 25 years Decco Research Laboratory has been involved in developing and commercializing several formulated products containing fungicides listed in the table. During this period we have developed several *in vitro* and *in vivo* methods to screen chemicals for their effectiveness against plant pathogens causing decay losses in fresh fruits and vegetables after harvest. In our search for new chemicals as potential fungicides, we typically test antifungal properties of chemicals at 1, 10 and 100 μg/ml levels in amended Potato Dextrose Agar using selected plant pathogens. If a given chemical shows antifungal properties at low levels it is classified as a potential candidate for controlling the sensitive test organism in the appropriate host crop. If the effective level is 100 ppm on another test organism the chemical is considered to be ineffective; such low residue levels of fungicides of 1 to 10 ppm also point to the economics of establishing national and international tolerance levels on crops considering safety, toxicity and other data.

The *in vitro* tests are usually repeated with several intermediate low levels of the chemical to determine minimum inhibitory concentrations (MIC) for the sensitive test organisms. We have arbitrarily selected levels of a fungicide to determine tolerant strains higher than MIC levels. We use such high level fungicide-amended media to constantly monitor the magnitude of the microbial load in packinghouses as part of our integrated pest management program. The present report deals with isolation of strains of *Penicillium digitatum* Sacc. tolerant to 2-AB, SOPP, TBZ and Benomyl (multiple resistance) and presents results of tests that show that there are fungicidal chemicals that could be used to control such tolerant *Penicillium* mold strains.

Table 1. History of fungicides to protect citrus fruits.[z]

Chemical common name	Year introduced	Resistance to *Penicillium* reported by author, year (ref.)
Biphenyl	1944	Harding, 1959 (11)
SOPP/OPP	1936	Harding, 1962 (12)
2-AB	1962	Harding, 1976 (14)
Benomyl	1967	Muirhead, 1974 (20)
		Smoot and Brown, 1974 (24)
		Wild, 1974 (26)
TBZ	1962	Davé/Petrie, 1970 (3)
		Harding, 1972 (13)

[z]Adapted from Ogawa, et al. 1980 (22).

Material and Methods

The *in vitro* procedures for screening chemicals using selected plant pathogens and the MIC methods were described in our earlier report (16). The *in vivo* tests to determine the decay control and sporulation control potential of fungicides were also reported in the same paper. The media used in the monitoring of packinghouses for *Penicillium* molds and for determining the *in vitro* antifungal sensitivity or tolerance to fungicides are reported by J. F. Petrie in Ogawa, et al. (22).

Results

Fungicide-amended plates (a check plate, plus 4 fungicidal plates) were exposed for 15-60 seconds in citrus packinghouses. The number of colonies in each fungicidal plate were recorded as a percent of total colonies observed in the check plates. If a given fungicidal plate contained no colonies, that plate exposure was recorded to have zero resistance or the presence of an entirely sensitive population; if the fungicidal plate showed <20% colonies of those found on the check plate, it was recorded as possessing slight resistance, 20-50% was recorded as moderate resistance, and if the colony count was more than 50% of the check plate tally, the exposure was recorded as being a case of severe resistance. More than 1,200 such plate exposures were made during 33 months covering almost three citrus seasons (1978-80) in packinghouses in California and Arizona. The results presented in Fig. 1 show the extent of the resistance problem in these states. Florida is not immune to this resistance problem. Smoot and Brown (24) reported benzimidazole resistance in 1974. During a 1979 experiment, G. E. Brown again reported isolates of green mold recovered from 31 decayed fruits that were resistant to TBZ and Benomyl in Florida (2).

In our tests isolated suspected resistant colonies or spores from decayed fruits were cross streaked on fungicide amended media and observed for growth or inhibition. Results presented in Table 2 show sensitivity of *Penicillium* molds to imazalil but tolerance to other fungicides. Table 3 gives *in vitro* screening of CGA-64251 against several plant

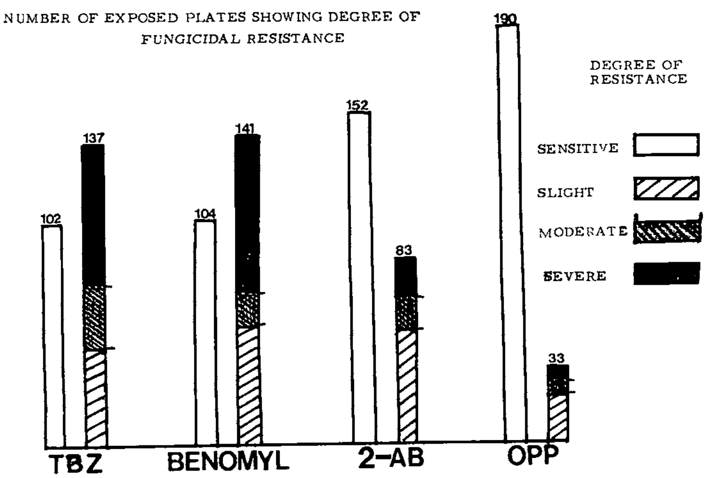

Fig. 1. Monitoring of packinghouses for Penicillium molds.

pathogens of post-harvest significance. Similar data on imazalil was reported earlier by Kaplan and Davé (16). Tables 4, 5 and 6 describes *in vitro* and *in vivo* testing of a selected isolate of *Penicillium digitatum* that was resistant to TBZ, benomyl, 2-AB and OPP but was sensitive to imazalil and CGA-64251.

In the past decade, a period in which not one new chemical defense was introduced into the citrus industry to combat post-harvest pathogens, resistance to all registered fungicides has become widespread. This is now presenting the citrus packer with substantial packout losses due to decay. New chemical tools are here but await the cumbersome registration process to run its course before they can enter commerce and alleviate the state of emergency which

Table 3. *In vitro* screening of CGA-64251.

Test organisms	Growth (—) or inhibition (4+) of organisms in presence of CGA-64251[z] (13.5% EC) µg/ml of CGA-64251 in PDA 0	1	10	100
Alternaria species (kiwi)	—	3+	4+	4+
Aspergillus species (peanuts)	—	—	4+	4+
Aspergillus niger Tiegh	—	—	4+	4+
Botrytis cinerea Pers.	—	—	3+	4+
Cladosporium species	—	4+	4+	4+
Fusarium species (potato)	—	3+	4+	4+
Geotrichum candidum Lk. ex Pers.	—	3+	4+	4+
Monilinia fructicola Honey	—	4+	4+	4+
Penicillium digitatum Sacc.	—	4+	4+	4+
Penicillium expansum Lk. ex Thom	—	2+	4+	4+
Penicillium italicum Wehmer	—	4+	4+	4+

Note: No effect on species of *Rhizopus, Mucor* and *Bacillus* at 10 µg/ml levels.

[z]CGA-64251 is a Ciba-Geigy experimental fungicide.

Table 2. *In vitro* sensitivity of blue/green molds to various fungicides.

Culture source plate/fruit	Growth[z] or inhibition on amended media: Check no fungicide	TBZ[y] 20[y]	Benomyl 10	2-AB 500	SOPP 20	Imazalil[x] 1.0
Packinghouse A (Plate)	—	—	—	—	NT	4+
Packinghouse B (Plate)	—	—	—	—	NT	4+
Packinghouse C (Plate)	—	—	—	—	—	4+
Packinghouse D (Plate)	—	—	—	—	—	4+
Packinghouse B (Orange)	—	—	—	—	4+	4+
Packinghouse E (Lemon)	—	—	—	4+	—	4+
Packinghouse D (Plate)	—	—	—	—	—	4+
Packinghouse D (Plate)	—	—	—	—	—	4+

[z]— = good growth; 4+ = no growth; NT = not tested.
[y]µg/ml of fungicide in the PDA medium.
[x]Imazalil is fungicide manufactured by Janssen Pharmaceutica, Beerse, Belgium.

Table 4. *In vitro* sensitivity of strains of *Penicillium digitatum* to fungicides.[z]

Strain code	Growth (—) or inhibition (4+) on PDA of µg/ml of fungicides: Check	TBZ 20	Benlate 10	2-AB 500	SOPP 20	Imazalil 0.065	0.125	.25	.5	1.0	Strain type
A	—	—	—	1+ BS	—	2+	4+	4+	4+	4+	Triple-R
B	—	—	—	—	4+	2+	4+	4+	4+	4+	Double-R SOPP-S
C	—	—	—	4+	—	2+	3+	4+	4+	4+	Double-R 2-AB-S
D	—	4+	4+	4+	3+	2+	4+	4+	4+	4+	Triple-S
E (LG)	—	—	—	1+	—	2+	4+	4+	4+	4+	Triple-R
F	—	—	—	±	—	2+	4+	4+	4+	4+	Triple-R
G	—	—	—	4+	4+ NS	2+	4+	4+	4+	4+	Single-R
H	—	4+	4+	— LS	—NS	—	—	NT	NT	— NS	Double-R Ben-S TBZ-S

[z]— = growth; 4+ = inhibition; BS = brown spores; LG = light green; NS = no spores; LS = low spores; NT = not tested; R = resistant; S = sensitive.

Table 5. *In vivo* testing of fungicides.

Test no.	Fungicide	Dip concentration PPM	No. decays (total 200 sites) Lemons[z]	Oranges	% Decay	% Decay control
			Decay control of *Penicillium* molds on citrus			
1	None	0	94	73	83.5	—
2	SOPP	3,500	15	6	10.5	87.4
3	TBZ	1,000	84	44	64.0	23.4
4	2-AB	10,000	24	2	13.0	84.4
5	Benomyl	800	63	21	42.0	49.7
6	Imazalil	500	2	4	3.0[y]	96.4
7	CGA-64251	500	1	3	2.0[y]	97.6

[z]Combination results from two replicates.
[y]No spores, good sporulation control.

Table 6. *In vivo* testing of fungicides: sporulation control of *Penicillium* molds on citrus.

Test no.	Fungicide	Dip concentration PPM	Possible sporulation score	Actual score	% Sporulation control
1	None (water)	0	90	87	4
2	SOPP	3,500	90	76	15
3	TBZ	1,000	90	85	6
4	2-AB	10,000	93	87	6
5	Benomyl	800	90	87	3
6	Imazalil	500	93	0	100[z]
7	CGA-64251	500	90	1	99[y]

[z]18 fruits covered with white mycelium only.
[y]2 fruits covered with white mycelium only.

was declared in late 1979 by the states of Arizona and California. This situation is not restricted to the citrus industry. Pome and stone fruit packers are also now observing the onset of resistant strains of plant pathogens (21, 23 and 25).

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