European Journal of Plant Pathology **106:** 745–752, 2000.

Survey of fungicide sensitivity in *Colletotrichum gloeosporioides* from different avocado and mango production areas in South Africa

Gina M. Sanders, L. Korsten and F.C. Wehner
Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa (Fax: +27124204588; E-mail: Gina.Swart@fabi.up.ac.za)

Accepted 26 June 2000

Key words: benomyl, post-harvest decay, prochloraz, thiabendazole

Abstract

Pre-harvest fungicidal treatments aimed at reducing inoculum levels in the field include copper oxychloride and benomyl. Pre-harvest applications of benomyl are currently restricted if used on fruit destined for certain export markets. Isolates of *Colletotrichum gloeosporioides* collected during a three-year market survey were used to determine the incidence of resistance to benomyl, thiabendazole and prochloraz using an *in vitro* assay. A total of 17.7% of all isolates tested were resistant to benomyl, of which 8.5% were highly and 9.2% moderately resistant. Isolates from certain production areas were less sensitive to benomyl and thiabendazole, and mango isolates were generally more sensitive than avocado isolates. No isolates were resistant to thiabendazole or prochloraz.

Introduction

In South Africa, avocados and mangos comprise a large portion of the subtropical fruit industry, with total production of the two crops for the 1996–1997 season being 55 782 and 24 584 tonnes respectively (Abstract of Agricultural Statistics, 1998). Seventy per cent of the avocados produced in South Africa is exported, 95% of it to Europe (South African Avocado Growers' Association). Mango production is directed at the local market and only about 30% of the crop is exported (Donkin and Oosthuyse, 1996). Because the South African subtropical fruit industries are highly export-orientated, a high premium is placed on ensuring fruit quality. One of the most serious threats to the maintenance of these standards are post-harvest diseases such as anthracnose on avocados and mangos caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (Jeffries et al., 1990). This pathogen was consistently isolated from stem-end rot lesions on avocados and mangos as well as from soft brown rot on mangos (Swart, 1999). Control of *C. gloeosporioides* and other post-harvest avocado and mango pathogens focuses mainly on inoculum reduction and prevention of latent infections. It is therefore crucial to have an adequate pre-harvest spray programme in place for this purpose (Darvas et al., 1987). In South Africa, previously registered fungicide programmes included monthly pre-harvest applications of benomyl, followed by cupric hydroxide or copper oxychloride for avocados, and copper oxychloride alone or with alternate mancozeb or benomyl sprays for mangos (Vermeulen et al., 1992). With the exception of benomyl, the above compounds are all contact fungicides and timing of application should therefore coincide with periods of high rainfall when inoculum is dispersed (Lonsdale, 1992a).

The introduction of benzimidazole fungicides such as benomyl, carbendazim and thiophanates in the early 1960s revolutionised disease control (Russell, 1995). However, extended use of these compounds resulted in selection for resistant pathogen genotypes, which remained predominant for several years after discontinued use (Moorman and Lease, 1992; Ziogas and Girgis, 1993). The first cases of resistance were reported in fungi with short life cycles such as *Botrytis cinerea* in vineyards (Leroux and Clerjeau,

1985). Resistance has subsequently been reported for fungi isolated from a variety of sources, e.g. *Venturia inaequalis* from apple (Sholberg et al., 1989), *Penicillium digitatum* and *P. italicum* from citrus (Bus et al., 1991) and *C. gloeosporioides* from rambutan and mango (Farungsang and Farungsang, 1992).

Currently, only prochloraz and thiabendazole are registered for post-harvest treatment of avocados, the former also for post-harvest use on mangos (Nel et al., 1999). In areas where cercospora spot is a serious problem, avocado growers sometimes include an early-season benomyl treatment (Korsten, pers. comm.). However, it has been recommended that the use of benomyl as a pre-harvest treatment should be limited to no more than one spray per season (Lonsdale and Kotzé, 1989) to prevent build-up of resistance to the compound (Darvas et al., 1987). Despite this, reduced control of stem end rot on avocado (Lonsdale and Kotzé, 1989) and anthracnose on mango (Saaiman, 1995) with benomyl has been reported. In the 1990 season, the use of benomyl for post-harvest treatment of mangos was abandoned (Lonsdale, 1992b). At present, there are no registered pre-harvest chemical treatments for anthracnose on avocados, although benomyl remains registered for pre-harvest cercospora spot control (Nel et al., 1999).

Since prochloraz and thiabendazole are still registered for use and benomyl is still used to a limited extent, the purpose of this study was to determine the incidence and distribution of fungicide resistance in *C. gloeosporioides* isolates from avocado and mango production areas in South Africa.

Materials and methods

Collection of isolates

Fuerte avocado fruit were collected from the Pretoria National Fresh Produce Market in 1994, 1995 and 1996 from Louis Trichardt (Northern Province), Tzaneen, Levubu, Nelspruit (Mpumalanga) and KwaZulu–Natal midlands (KwaZulu–Natal) and Sensation mango fruit from Letsitele, Tzaneen, Levubu, Hazyview (Mpumalanga), Kaapmuiden, Hoedspruit and Malelane (Northern Province) in 1995 and 1996. Fruit were left to ripen at ambient temperature and isolations made at three stages of ripeness, viz. eating-ripe, slightly overripe and overripe. At each stage, fruit were removed and surface-disinfected for 30 s with 1% sodium hypochlorite. Isolations were made from the periphery of anthracnose, stem end rot and stem end rot lesions on oatmeal agar (20 g oatmeal, 20 g agar (Biolab), 1 l distilled water) (OA). Plates were incubated at ambient temperature under constant mixed irradiation from near-ultraviolet and daylight type fluorescent tubes (Phillips TL 40W/08RS, F40 B43 and TL 40W/33RS respectively) until sporulation occurred. *C. gloeosporioides* isolates were preliminarily identified from all fungi obtained and a subsample from these were used for further study. Monoconidial cultures of *C. gloeosporioides* isolates were prepared from this subsample and preserved for further use by freezing in 50% glycerol at $-78\,^{\circ}$C, as well as on potato-dextrose agar (PDA) (Biolab) slants and under sterile water. Identity of isolates from this subsample were confirmed using inoculation studies, morphology and molecular analysis (Swart, 1999). One-hundred and fifty-eight monoconidial *C. gloeosporioides* isolates were used in this study, of which 126 were from avocado and 25 from mango. Six papaya isolates and a spinach isolate were also included for comparative purposes.

Fungicide resistance studies

Starter cultures were prepared by incubating each *C. gloeosporioides* isolate on OA for five days. Resistance studies were carried out according to Bernstein et al. (1995) and De Lapeyre de Bellaire and Dubois (1997). Briefly, PDA (Biolab) was amended with either 1, 2, 3, 4 and 5 $\mu g\,ml^{-1}$ benomyl (Benlate, Du Pont), 0.5, 1, 1.5, 2 and 2.5 $\mu g\,ml^{-1}$ thiabendazole (Tecto, Logos Agvet SA) and 10, 50, 110, 150 and 200 ppm prochloraz (Omega, AgrEvo). Unamended PDA served as control. Plugs (4 mm diameter) were cut from the edge of starter cultures and plated onto three replicate plates per fungicide concentration. Plates were incubated at $25\,^{\circ}$C in the dark and radial growth on amended and unamended PDA was recorded daily for seven days. The percentage growth of each isolate on amended relative to unamended medium was calculated. To compare isolates with regard to all fungicides tested, only the recommended concentration was considered, i.e. 2 $\mu g\,ml^{-1}$ benomyl, 1.5 $\mu g\,ml^{-1}$ thiabendazole and 110 ppm prochloraz. Values obtained were categorised as follows: category 1: 0–5%, 2: 6–10%, 3: 11–15%, 4: 16–20%, 5: 21–25%, 6: 26–30%, 7: 31–35%, 8: 36–40%, 9: 41–45%, 10: 46–50%, 11: 51–55%, 12: 56–60%, 13: 61–65%, 14: 66–70% and 15: more than 71% growth. Resistance was ascertained according to Farungsang and

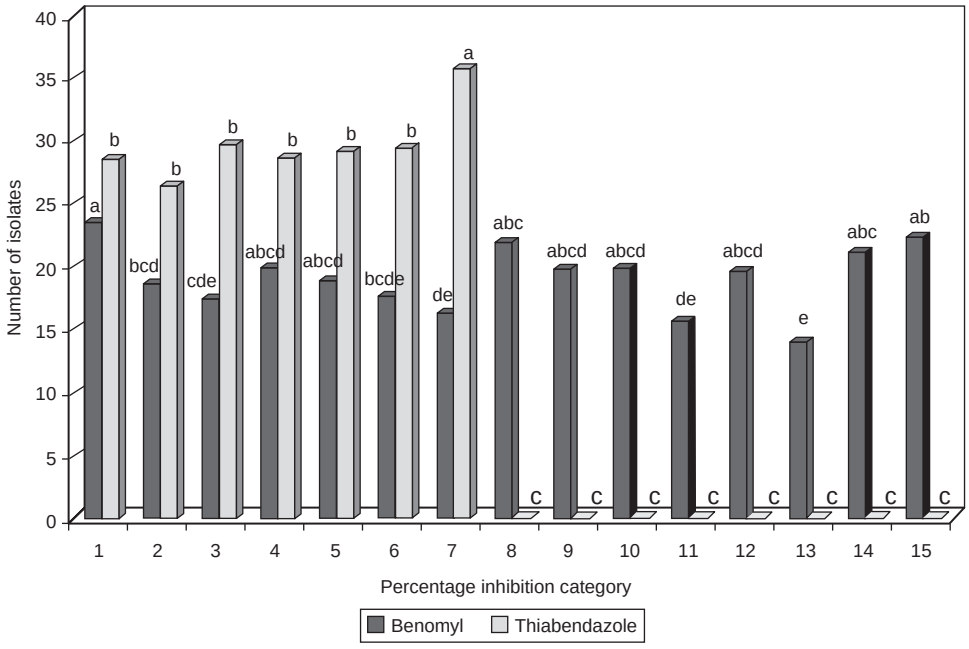
Farungsang (1992). Isolates were termed highly resistant if growth on amended PDA was 66% or greater that on unamended PDA, i.e. from category 14, moderately resistant if growth was 36% or greater (from category 8) and sensitive if growth was less than 35% (category 7 and lower). Data were analysed statistically by Duncan's multiple range test and Student's *t*-test.

Results

From the total of 158 isolates representative of the various areas and hosts, viz. avocado, mango, papaya and spinach, 17,7% were resistant to benomyl, 8.5% highly and 9.2% moderately so. No isolates showed resistance to thiabendazole or prochloraz. All isolates tested were highly sensitive to prochloraz, with no growth being observed at any concentration tested, except for two isolates. The first, a papaya isolate from Tzaneen had a percentage growth of 13% of that of the control at the lowest concentration. The second, a mango anthracnose isolate from Letsitele, showed 72.0%, 40.8%, 19.7%, 19.5% and 18.2% growth at 10, 50, 110, 150, and 200 ppm respectively.

When considering all isolates, irrespective of host or locality, numbers of isolates with different reactions to the recommended rate of benomyl were distributed throughout all the categories, with the most isolates in category 1 and the least in category 13 (Figure 1). Since no isolate was resistant to thiabendazole, distribution of isolates was limited to categories 1–7. Significantly more isolates were grouped into the last category than in any other category ($P = 0.0001$).

No isolate from any specific lesion type fell into a category regarded as resistant, although isolates from anthracnose and stem end rot lesions from both avocados and mangos were significantly less affected by benomyl and thiabendazole than isolates from soft brown rot on mango ($P = 0.0001$). Mango isolates were significantly more sensitive to benomyl ($P = 0.0001$) and thiabendazole ($P = 0.0001$) than avocado isolates. Fruit ripeness when isolations were

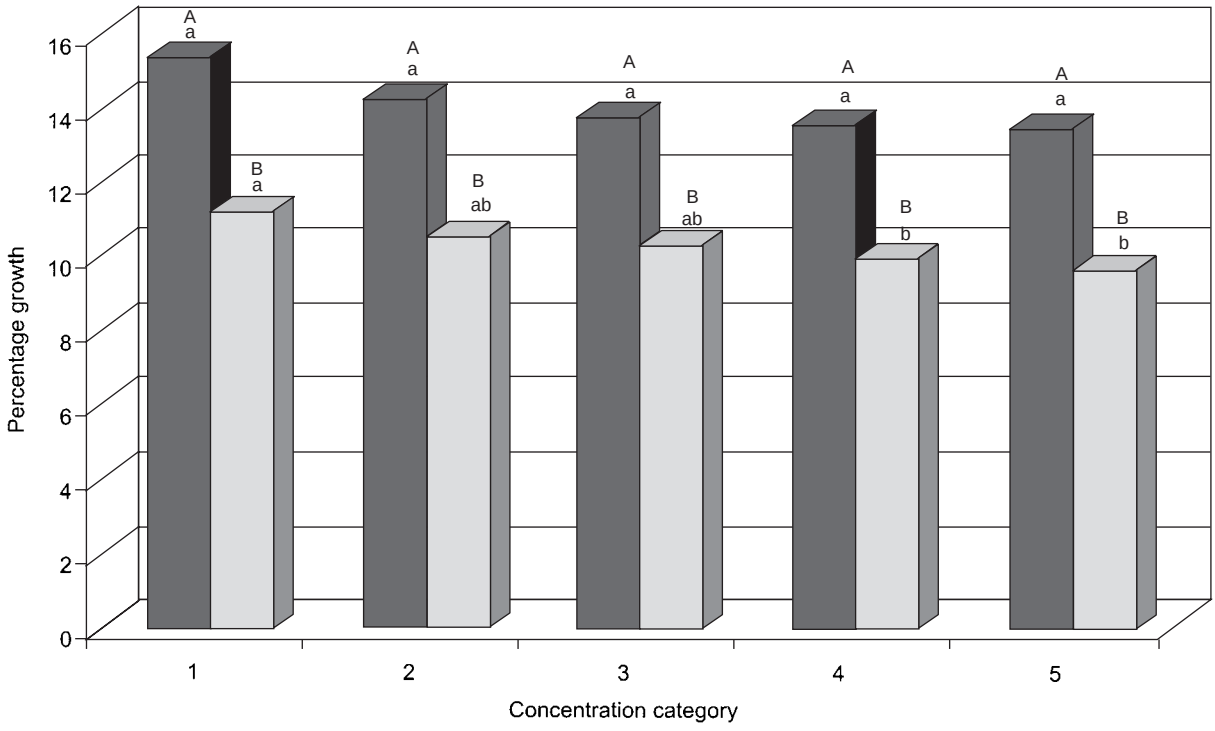


Only registered rates were considered, i.e. 2 μg ml^{-1} benomyl, 1.5 μg ml^{-1} thiabendazole. Category 1: 0–5%, 2: 6–10%, 3: 11-15%, 4: 16-20 %, 5: 21-25 %, 6: 26-30 %, 7: 31-35 %, 8: 36-40 %, 9: 41-45 %, 10: 46-50 %, 11: 51-55 %, 12: 56-60 %, 13: 61-65 %, 14: 66-70 % and 15: greater than 71 %. Bars with the same pattern and letter do not differ significantly according to Duncan's multiple range test (Benomyl: P=0.0001; Thiabendazole: P=0.0001).

Figure 1. Distribution of *C. gloeosporioides* isolates according to categories of benomyl and thiabendazole sensitivity.

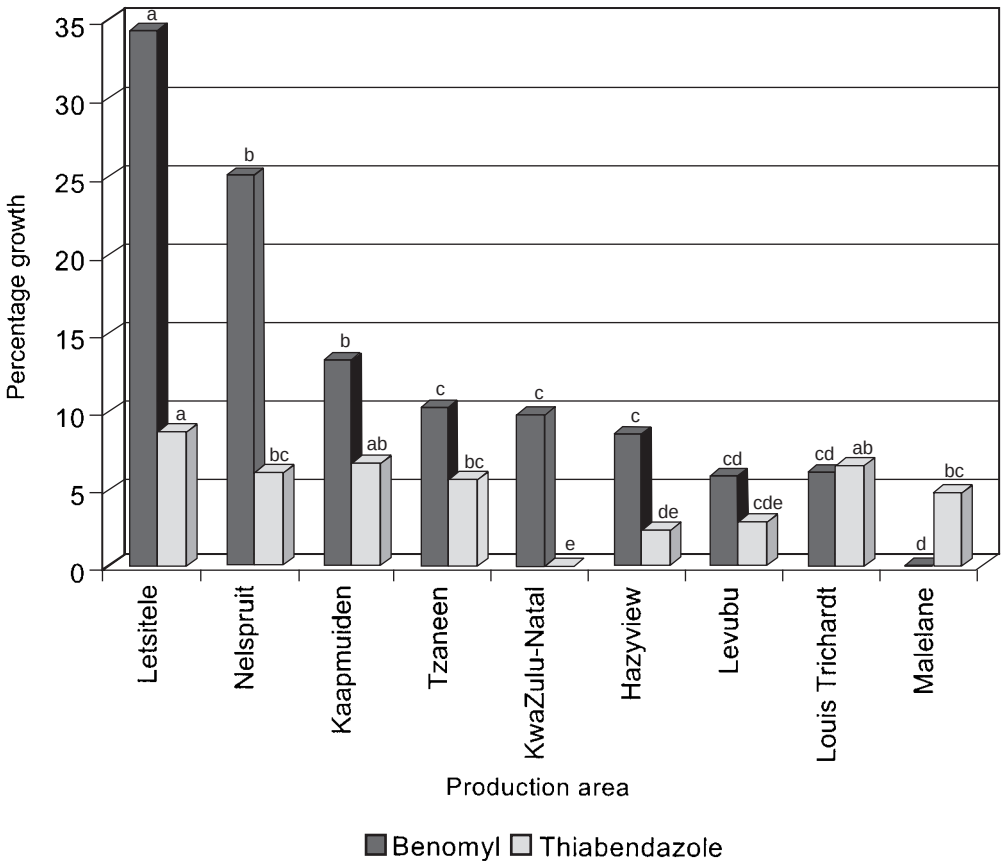
made had a significant effect on benomyl sensitivity and isolates from overripe fruit, followed by slightly overripe and eating-ripe fruit, were more sensitive to benomyl ($P = 0.0001$). Stage of ripeness from which isolates were obtained had no effect on thiabendazole sensitivity ($P = 0.0001$).

Percentage growth of all isolates tested did not differ significantly at the different concentrations of benomyl (Figure 2). Percentage growth on 0.5 μg ml^{-1} thiabendazole was significantly higher than on 2 and 2.5 μg ml^{-1} thiabendazole. Isolates were consistently more sensitive to thiabendazole than benomyl, although differences were only significant at the two highest concentrations (concentration categories 4 and 5) (Figure 2). Due to the lack of a gradient response to concentrations of particularly benomyl, only data from the registered fungicide rates were considered for obtaining a picture of the geographic distribution of isolate sensitivity. Data from avocado and mango isolates were pooled for this purpose. Isolates from Letsitele, followed by those from Nelspruit and Kaapmuiden, were the least sensitive to benomyl (Figure 3). However, only the isolates from Letsitele were considered resistant to benomyl according criteria defined by Farungsang and Farungsang (1992). Isolates from Malelane were the most sensitive to benomyl



Concentration categories are defined as potato-dextrose agar amended with benomyl 1: 1 μg ml^{-1}, 2: 2 μg ml^{-1}, 3: 3 μg ml^{-1}, 4: 4 μg ml^{-1}, and 5: 5 μg ml^{-1} or thiabendazole: 1: 0.5 μg ml^{-1}, 2: 1 μg ml^{-1}, 3: 1.5 μg ml^{-1}, 4: 2 μg ml^{-1} and 5: 2.5 μg ml^{-1}. Bars within the same category with the same upper case letter do not differ significantly according to Student's t-test (P=0.0001 for all categories). Bars with the same pattern and lower case letter do not differ significantly according to Duncan's multiple range test (Benomyl: P=0.0001; Thiabendazole: P=0.053).

Figure 2. Comparison of fungicide sensitivity of avocado and mango isolates of *C. gloeosporioides* to different concentrations of benomyl and thiabendazole amended PDA.



Bars with the same pattern and letter do not differ significantly according to Duncan's multiple range test (Benomyl: P=0.0001; Thiabendazole: P=0.0001). Only registered rates of the fungicides were considered, i.e. 2 μg ml^{-1} benomyl, 1.5 μg ml^{-1} thiabendazole.

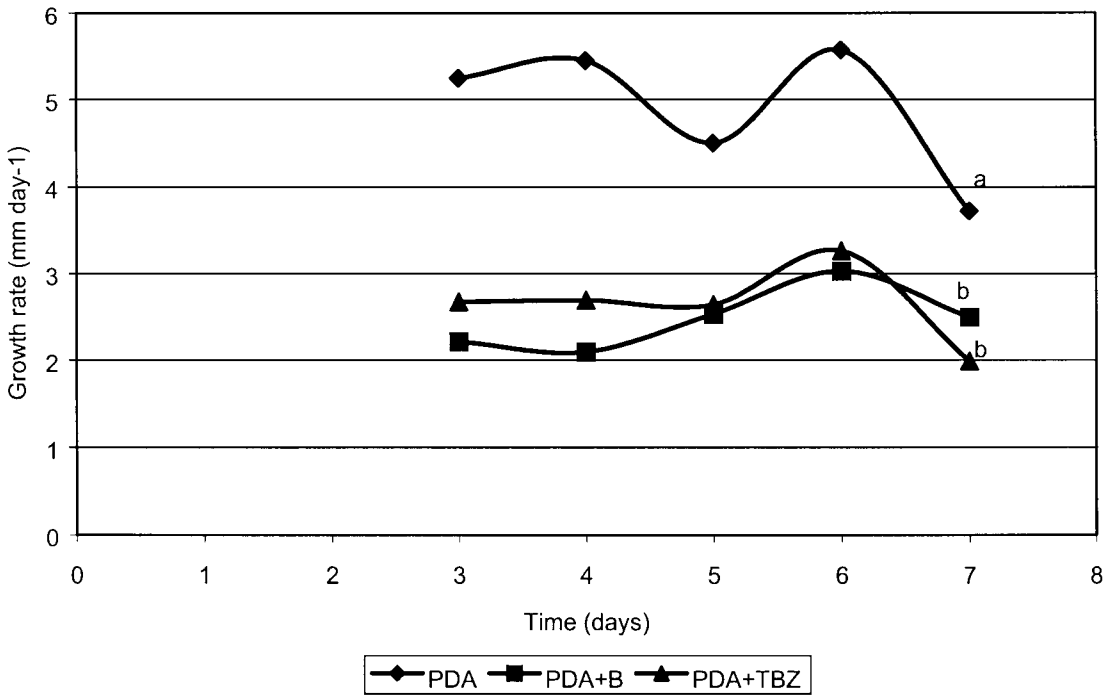
Figure 3. Distribution of *C. gloeosporioides* isolates from different production areasbased on benomyl and thiabendazole sensitivity.

although not more so than those from Levubu and Louis Trichardt. Isolates from Letsitele, Kaapmuiden and Louis Trichardt were the least sensitive, albeit not resistant, to thiabendazole, whereas isolates from KwaZulu–Natal were particularly sensitive.

Mean daily growth rate on unamended medium was 4.9 mm day^{-1} (Figure 4) and growth rates of isolates from the areas evaluated did not differ significantly, except for isolates from Hazyview, which grew significantly slower than isolates from all other production areas ($P = 0.0001$). This, however, appeared to have no effect on fungicide resistance. Mean growth rates of isolates on both fungicide-amended media were similar, viz. 2.7 and 2.5 mm day^{-1} on thiabendazole and benomyl amended media respectively. Mean daily growth rate on unamended PDA was 4.9 mm day^{-1}and was significantly higher than amended media ($P = 0.0001$) (Figure 4). Percentage growth of isolates on thiabendazole amended PDA was significantly lower than that of benomyl, i.e. isolates were much more sensitive to thiabendazole than benomyl ($P = 0.0001$).

Discussion

Circumstantial evidence has for a long time suggested the existence of resistance to benzimidazole fungicides in *C. gloeosporioides* associated with post-harvest



PDA: Unamended potato-dextrose agar control

PDA+B: Potato-dextrose agar amended with 2 μg ml^{-1} benomyl

PDA+TBZ: Potato-dextrose agar amended with 1.5 μg ml^{-1} thiabendazole

Points at day seven with the same letter do not differ significantly according to Duncan's multiple range test (P=0.0001).

Figure 4. Comparison of daily growth rates of *C. gloeosporioides* isolates on benomyl and thiabendazole amended PDA.

decay of avocado and mango. Using confirmed *C. gloeosporioides* isolates, this study quantified and further corroborated this evidence.

Benzimidazole fungicides act by inhibition of tubulin biosynthesis (Davidse, 1973). This is due to mutations in the β-tubulin gene and has been related to specific amino acid substitutions at several distinct regions within the β-tubulin molecule (Fujimura et al., 1992). Prochloraz is part of a group of fungicides which act by inhibiting sterol biosynthesis (Hassal, 1982) by C14-demethylation of lanosterol (Bus et al., 1991). According to Russell (1995), benzimidazole resistance is an established fact, and extensive monitoring is no longer carried out. However, no reports of prochloraz resistance could be traced.

The number of benomyl resistant isolates from avocado and mango were determined with almost similar numbers being highly (15) and moderately (13) resistant. Resistance of 12.9% was recorded for *Colletotrichum* spp. isolated from mangos in Thailand (Farungsang and Farungsang, 1992), 19% for *Pseudocercosporella herpotrichoides* isolated from cereals (Murray, 1996) and 27% for *B. cinerea* isolated from wild blackberry (Johnson et al., 1994). The incidence of 17.7% resistance in the subtropical fruit industry indicates similar levels of resistance to those of *C. gloeosporioides* from Thailand (Farungsang and Farungsang, 1992) and *P. herpotrichoides* (Murray, 1996). It has been reported world-wide that there is an increase in the number of isolates of *C. gloeosporioides* that are resistant to benomyl. It has been speculated that the teleomorph stage, *Glomerella cingulata* (Stoneman) Spaulding & v. Schrenk may play a role at a genetic level in how the pathogen adapts to its host and environment (Dodd et al., 1992). Interestingly, no isolates were resistant to thiabendazole, although

most isolates grouped in the category just below the cut-off point for resistance. This could be in part due to previous exposure to benomyl which also increased chances of resistance to thiabendazole, since the two fungicides are closely related (Delp, 1980). This lack of resistance may possibly be ascribed to the fact that, although thiabendazole is registered, it is applied only post-harvest, and is not widely used.

Resistance levels of isolates to benomyl found in this study were approximately evenly distributed. However, two groups of resistance as defined by Farungsang and Farungsang (1992) could be determined from the total population tested, viz. highly resistant (8.5% of the population tested) and moderately resistant (9.2% of the population tested). Different classes of resistance of *V. inaequalis* to benomyl have been described based on growth responses on amended media (Katan et al., 1983). A study at the molecular level revealed that codons for lysine and alanine at position 198 were always associated with very highly resistant and highly resistant phenotypes of *V. inaequalis*. Base substitutions resulting in a tyrosine codon at position 200 were always associated with the moderately resistant phenotype (Koenraadt and Jones, 1992). However, such analyses were beyond the scope of this study and this could not be confirmed.

Isolates from Letsitele were less sensitive to both benomyl and thiabendazole. Because of the decreased sensitivity to benomyl and thiabendazole, it is important to note that the only prochloraz-resistant isolate was from a mango produced in this area. Isolates from KwaZulu–Natal and Malelane were relatively sensitive to thiabendazole and benomyl respectively and also moderately cross resistant. There appeared to be no connection between increased fungicide sensitivity and disease incidence. On the contrary, fruit from KwaZulu–Natal in during the 1996 survey had the highest incidence of anthracnose of all areas evaluated. It should therefore be kept in mind that fungicide efficacy is not the only factor determining disease incidence. Factors such as inoculum load and prevailing weather conditions play an important role in symptom expression. Keinath and Zitter (1998) found that areas with a higher rainfall had a higher incidence of benomyl resistance in *Didymella bryoniae* a pathogen of curcubits, and was possibly due to more favourable conditions for rapid reproduction of the pathogen.

Differences in virulence, morphology, physiology and genotype between isolates of *C. gloeosporioides* obtained from various hosts have been widely reported (Agostini et al., 1992; Davis et al., 1992; Sreenivasaprasad et al., 1993; Freeman et al., 1998). It is therefore not surprising that differences in fungicide sensitivity were found in isolates from avocados and mangos. This differential reaction of *C. gloeosporioides* to benomyl is consistent and has been used from comparison of *C. gloeosporioides* isolates from different hosts (Liyanage et al., 1992; Brown et al., 1996; Freeman et al., 1998).

Although prochloraz provides excellent control of post-harvest diseases, it is not cleared for use on many overseas markets (Anderson, 1986) and is therefore not widely used. It is therefore not surprising that all isolates tested were highly sensitive. Since a single highly resistant isolate was detected, it might be prudent to monitor subtropical fruit populations of *C. gloeosporioides* if use of this this fungicide increases. However, potential for resistance development is small since it is applied post-harvest.

At this stage, the use of benomyl in South Africa is limited and is no longer used on mangos (Lonsdale, 1992b). Avocado *C. gloeosporioides* populations on the other hand, have been exposed to regular benomyl sprays in the past and reduced control was noted by Darvas et al., (1987), who also proposed a build-up of resistance to benomyl. Several alternatives to benomyl for pre-harvest use on avocado (Lonsdale, 1992a) and mango (Saaiman, 1995) are being investigated. However, successful disease control could still be obtained with judicious use of benomyl in combination with another low-risk fungicide (Russell, 1995). Further post-harvest control measures such as detergent sanitisers (Boshoff et al., 1995) and biocontrol agents (Korsten et al., 1998) are being investigated and show great promise for control of anthracnose and stem end rot on avocados and mangos.

References

Agostini JP, Timmer LW and Mitchell DJ (1992) Morphological and pathological characteristics of strains of *C. gloeosporioides* from citrus. Phytopathology 82: 1377–1382

Anderson MD (1986) Omega (prochloraz), a fungicide for post-harvest control of anthracnose, the *Dothiorella*/*Colletotrichum* complex and stem-end rot in avocados. South African Avocado Growers' Association Yearbook 9: 27–30

Bernstein B, Zehr E, Dean RA and Shabi, E (1995) Characteristics of *Colletotrichum* from peach, apple, pecan and other hosts. Plant Disease 79: 478–482

Boshoff M, Slabbert MJ and Korsten L (1995) Effect of detergent sanitisers on post-harvest diseases of avocado. South African Avocado Growers' Association Yearbook 18: 96–98

Brown AE, Sreenivasaprasad S and Timmer LW (1996) Molecular characterisation of slow- growing orange and key lime anthracnose strains of *Colletotrichum* from citrus as *C. acutatum*. Phytopathology 86: 523–527

Bus VG, Bongers AJ and Risse LA (1991) Occurrence of *Penicillium digitatum* and *P. italicum* resistant to benomyl, thiabendazole, and imazalil of citrus fruit from different geographic origins. Plant Disease 75: 1098–1100

Darvas JM, Kotzé JM and Wehner FC (1987) Effect of treatment after picking on the incidence of post-harvest fruit diseases of avocado. Phytophylactica 22: 93–96

Davis RD, Boland RM and Howitt CJ (1992) Colony descriptions, conidium morphology, and the effect of temperature on colony growth of *Colletotrichum gloeosporioides* isolated from *Stylosanthes* spp. growing in several countries. Mycological Research 96: 128–134

Davidse LC (1973) Antimitotic activity of methyl benzimidazole-2-yl carbamate (MBC) in *Aspergillus nidulans*. Pesticide Biochemistry and Physiology 3: 317–325

De Lapeyre de Bellaire L and Dubois C (1997) Distribution of thiabendazole resistant *Colletotrichum musae* isolates from Guadeloupe banana plantations. Plant Disease 81: 1378–1383

Delp CJ (1980) Coping with resistance to disease control agents. Plant Disease 64: 654–657

Dodd JC, Estrada A and Jeger MJ (1992) Epidemiology of *Colletotrichum gloeosporioides* in the tropics. In: Bailey, J.A. and Jeger, M.J. (eds.) *Colletotrichum*: biology, pathology and control. (pp 308–323) CAB International, Wallingford

Donkin DJ and Oosthuyse SA (1996) Quality evaluations of sea-exported South African mangos in Europe during the 1995/96 season. South African Mango Growers' Association Yearbook 16, 1–5

Farungsang U and Farungsang N (1992) Benomyl resistance of *Colletotrichum* spp. associated with rambutan and mango fruit rot in Thailand. Acta Horticulturae 321: 891–897

Freeman S, Katan T and Shabi E (1998) Characterisation of *Colletotrichum* species responsible for anthracnose diseases of various fruits. Plant Disease 82: 596–605

Fujimura M, Oeda K, Inoue H and Kato T (1992) A single amino acid substitution of the beta-tubulin gene of *Neurospora* confers both carbendazim resistance and diethofencarb sensitivity. Current Genetics 21: 399–404

Hassal A (1982) The Chemistry of Pesticides. MacMillan Press, New York

Jeffries P, Dodd JC, Jeger MJ and Plumbley RA (1990) The biology and control of *Colletotrichum* species on tropical fruit crops. Plant Pathology 39: 343–366

Johnson KB, Sawyer TL and Powelson ML (1994) Frequency of benzimidazole- and dicarboximide-resistant strains of *Botrytis cinerea* in western Oregon small fruit and snap bean plantings. Plant Disease 78: 572–577

Katan T, Shabi E and Gilpatrick JD (1983) Genetics of resistance to benomyl in *Venturia inaequalis* isolates from Israel and New York. Phytopathology 73: 600–603

Keinath AP and Zitter TA (1998) Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. Plant Disease 82: 479–484

Koenraadt H and Jones AL (1992) The use of allele-specific oligonucleotide probes to characterise resistance to benomyl in field strains of *Venturia inaequalis*. Phytopathology 82: 1354–1358

Korsten L, Towsen E and Claasens V (1998) Evaluation of avogreen as post-harvest treatment for controlling anthracnose and SE on avocado fruit. South African Avocado Growers' Association Yearbook 21: 83–87

Leroux P and Clerjeau M (1985) Resistance of *Botrytis cinerea* Pers. and *Plasmopara viticola* (Berk. & Curt.) Berl. and de Toni to fungicides in French vineyards. Crop Protection 4: 137–160

Liyanage HD, McMillan RT and Kistler HC (1992) Two genetically distinct populations of *Colletotrichum gloeosporioides* from citrus. Phytopathology 82: 1371–1376

Lonsdale JH (1992a) Evaluation of systemic fungicides as pre-harvest treatments of avocados. South African Avocado Growers' Association Yearbook 15: 35–38

Lonsdale JH (1992b) In search of an effective postharvest treatment for the control of postharvest diseases of mangos. South African Mango Growers' Association Yearbook 12: 32–36

Lonsdale JH and Kotzé JM (1989) Chemical control of pre- and post-harvest diseases of avocados. South African Avocado Growers' Association Yearbook 12: 68–70

Moorman GW and Lease RJ (1992) Benzimidazole- and dicarboximide-resistant *Botrytis cinerea* from Pennsylvania greenhouses. Plant Disease 76: 477–480

Murray TD (1996) Resistance to benzimidazole fungicides in the cereal eyespot pathogen, *Pseudocercosporella herpotrichoides*, in the pacific Northwest 1984-1990. Plant Disease 80: 19–23

Nel A, Krause M, Ramautar N and Van Zyl K (1999) A guide for the control of plant diseases. Directorate: Agricultural Production Outputs, National Department of Agriculture, Pretoria, Republic of South Africa

Russell PE (1995) Fungicide resistance: occurrence and management. Journal of Agricultural Sciences 124: 317–323

Saaiman WC (1995) Evaluation of fungicides applied pre-harvest on the incidence of post-harvest diseases. South African Mango Growers' Association Yearbook 15: 80–83

Sholberg PL, Yorston JM and Warnock D (1989) Resistance of *Venturia inaequalis* to benomyl and dodine in British Columbia, Canada. Plant Disease 73: 667–669

Sreenivasaprasad S, Brown AE and Mills PR (1993) Coffee berry disease pathogen in Africa: genetic structure and relationship to the group species *Colletotrichum gloeosporioides*. Mycological Research 97: 995–1000

Swart GM (1999) Comparative study of *Colletotrichum gloeosporioides* from avocado and mango. PhD Thesis, University of Pretoria

Vermeulen JB, Krause M, Nel A, Hollings N and Greyling J (1992) A guide to the use of pesticides and fungicides in the Republic of South Africa. Directorate of Livestock Improvement and Agricultural Resources, Pretoria

Ziogas BN and Girgis SB (1993) Cross-resistance relationships between benzimidazole fungicides and diethofencarb in *Botrytis cinerea* and their genetical basis in *Ustilago maydis*. Pesticide Science 39: 199–205