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Survey of fungicide resistance of *Colletotrichum gloeosporioides* from different avocado production areas

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ABSTRACT

Registered treatments for the control of avocado anthracnose caused by *Colletotrichum goeosporioides* are post-harvest applications of prochloraz and thiabendazole. Pre-harvest treatments to reduce inoculum levels in the field are also an important factor for successful anthracnose disease control. Pre-harvest application of benomyl was previously registered for this purpose, although at this stage it is only registered for *Cercospora* control. However, it is still generally recommended that the copper spray programme be preceded by a benomyl treatment. Isolates of *C. 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 resistant and 9.2% moderately resistant. No isolates were resistant to thiabendazole or prochloraz.

INTRODUCTION

The success of post-harvest control measures for anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. is largely dependent on the reduced incidence of latent infections (Saaiman, 1995). It is therefore crucial to have an adequate pre-harvest spray programme in place to reduce inoculum levels in the field (Darvas & Kotzé, 1987). Previously registered control measures for this disease included monthly pre-harvest applications of benomyl, cupric hydroxide and copper oxychloride (Vermeulen *et al*., 1992). With the exception of benomyl, these pre-harvest treatments are all contact fungicides and timing of application is important and should coincide with periods of high rainfall when inoculum is dispersed (Lonsdale, 1992). The use of benomyl as a pre- and post-harvest treatment was limited to no more than one pre-harvest spray (Lonsdale & Kotzé, 1989) due to reports of a build-up of resistance (Darvas & Kotzé, 1987). Since then, reduced control of stem-end rot on avocados (Lonsdale & Kotzé, 1989) and anthracnose on mango (Saaiman, 1995) has been reported. At present, there are no pre-harvest treatments for anthracnose, although benomyl is still registered as a pre-harvest treatment for *Cercospora* control (Krause *et* *al*., 1996). Only prochloraz and thiabendazole are currently registered as post-harvest treatments for anthracnose (Krause *et al*., 1996).

The introduction of benzimidazole fungicides such as benomyl, carbendazim and thiophanates from 1964 revolutionised disease control (Russell, 1995). Extended use of these fungicides resulted in selection for resistant genotypes, which remained predominant for several years after withdrawal of the fungicide (Ziogas & Girgis, 1993). The first cases of resistance were reported in fungi with short life cycles such as *Botrytis cinerea* in vineyards (Leroux & Clerjeau, 1985). Resistance has subsequently been reported from fungi isolated from a variety of sources, such as *Venturia inaequalis* on apples (Sholberg *et al*., 1989), *Penicillium digitatum* and *P italicum* on citrus (Bus *et al.,* 1991) and *C*. *gloeosporioides* on rambutan and mango (Farungsang & Farungsang, 1992).

The purpose of this study was to determine the incidence and distribution of fungicide resistant *Colletotrichum gloeosporioides* isolates.

MATERIALS AND METHODS

Benomyl, thiabendazole and prochloraz resistance studies using amended PDA were carried out as described by Bernstein *et al.,* (1995) and de Lapeyre de Bellaire & Dubois (1997). Plugs were cut from edges of five-day-old cultures of isolates cultured on oatmeal agar (20g oatmeal; 20g agar; *1ℓ* distilled water) (OA) and incubated at ambient temperature under mixed irradiation from near ultraviolet and daylight type fluorescent tubes (Phillips TL 40W/08RS, F40 B43 and TL 40W/33RS respectively) and plated onto three replicate plates per treatment. PDA amended with 1-5ug/mℓ benomyl (Benlate, Du Pont), PDA amended with 0.5-2.5 μg/mℓ thiabendazole (Tecto, Logos Agvet SA) and PDA amended with 10-200 ppm prochloraz (Omega, AgrEvo) comprised the three treatments with unamended PDA as control. Plates were incubated at 25°C in the dark and radial growth was determined by measuring colony diameters of amended and unamended PDA at 24h intervals for seven days. The percentage growth of each isolate on amended medium relative to unamended medium was calculated as follows: (Amended PDA)/PDA x I00. For comparison of isolates with regard to both fungicides tested, all 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; greater than 71%. Resistance was determined according to Farungsang and Farungsang (1992). Isolates were termed highly resistant if growth on amended PDA was greater than 65% of unamended PDA, i.e. from category 14, moderately resistant if growth was greater than 35% (from category 8) and sensitive if growth was less than 35% of the unamended control. Data was statistically analysed with the SAS system using analysis of variance. All statistical analyses with probability values equal to or less than 0.05 were regarded as an indication of significant differences between variables.

RESULTS

A total of 155 isolates, representative of the various areas and both hosts, viz. avocado and mango were tested. Of these isolates tested, a total of 17.7% were resistant to benomyl, of which 8.5% were highly resistant and 9.2% were moderately resistant. No isolates were resistant to thiabendazole or prochloraz according to described categories.

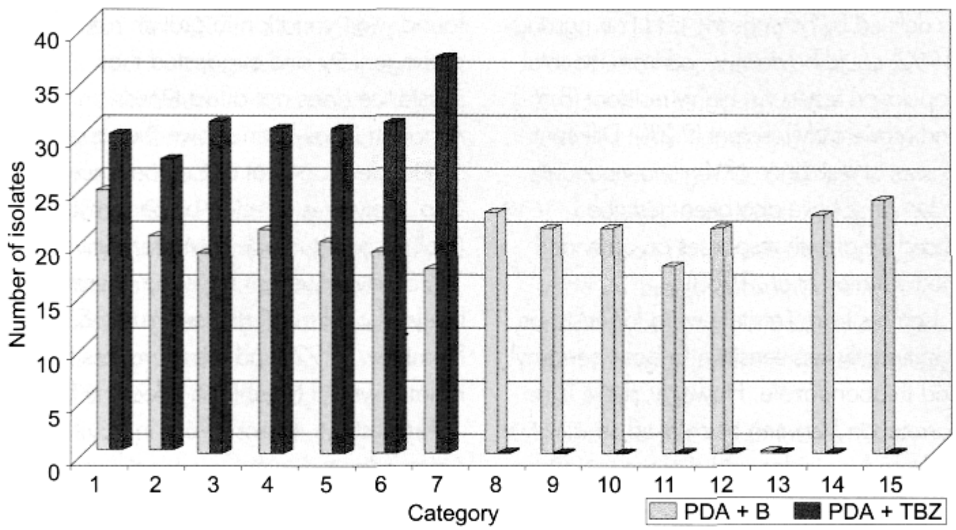

Figure 1: ***Distribution of isolates of* Colletotrichum gloeosporioides *tested for fungicide resistance according to growth on fungicide amended media***

Isolates with different reactions to benomyl were evenly distributed through-out all the categories, with the most isolates being in category 1, and the least in category 7 and 11 (Fig 1). No isolates were grouped in category 13 (Fig 1). There were no significant differences in distribution of isolates between the other categories (P=0.0001) (Fig 1). Because no isolates were resistant to thiabendazole, distribution of isolates for this fungicide were limited to categories one to seven. Significantly more isolates were grouped into category seven than all the other categories (P=0.0001) (Fig 1).

Average growth rates of isolates on both amended media were similar, with growth rates of 2.66 mm/day and 2.54 mm/day on thiabendazole and benomyl amended media respectively. No growth was observed on media amended with prochloraz. Average daily growth rate on unamended PDA was 4.9 mm/day. Daily growth rates on unamended PDA were significantly higher than those on both amended media (P=0.0001). Percentage growth of isolates on thiabendazole amended PDA was significantly lower than that of benomyl, i.e. isolates are much more sensitive to benomyl than thiabendazole (P=0.0001)

Isolates from Letsitele, followed by Nelspruit, Kaapmuiden and Tzaneen were the least sensitive to benomyl, with the highest average percentage growth on benomyl amended PDA (Fig 2). Isolates from Letsitele were considered to be resistant to benomyl according to the categories defined by Farungsang and Farungsang (1992). Isolates from all the other areas were not resistant to benomyl according to these categories (Fig 2). Isolates from Malelane, however, were most sensitive to benomyl, since none of the isolates from this area grew on the benomyl amended medium (Fig 2). Isolates from Letsitele, Kaapmuiden and Louis Trichardt were less sensitive to thiabendazole, but were not resistant (Fig 2). Isolates from Natal were most sensitive to thiabendazole, with no growth on thiabendazole amended medium (Fig 2).

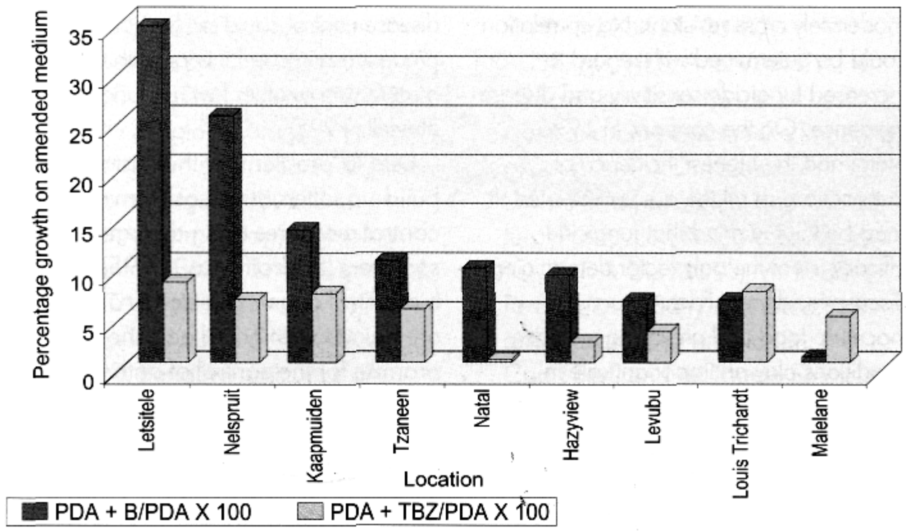

Figure 2: ***Comparison of benonyl and thiabendazole sensitivity of*** **Colletotrichum gloeosporioides** ***isolates according to origin***

Mango isolates were significantly more sensitive to benomyl (P=0.0001) and thiabendazole (P=0.0001) than avocado isolates. Isolates obtained from anthracnose and stem-end rot lesions from avocados were significantly less sensitive to benomyl and thiabendazole than isolates obtained from soft brown rot lesions from mangoes (P=0.0001). Fruit ripeness at which isolations were made had a significant effect on the benomyl sensitivity of the isolates (P=0.0001). Isolates from overripe fruit were significantly more sensitive to benomyl followed by isolates from slightly overripe and eating ripe fruit, although none were resistant according to the resistance categories described. Stage of fruit ripeness from which isolates were obtained had no effect on thiabendazole sensitivity (P=0.0001).

DISCUSSION

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 ß-tubulin molecule (Fujimura *et al.,* 1992). According to Russell (1995), benzimidazole resistance is an established fact, and extensive monitoring is no longer carried out. Since few surveys have recently been carried out to determine the incidence of benzimidazole resistance, it was deemed important to determine the extent of resistance in the subtropical fruit industry in South Africa, since certain compounds are still registered for use.

The amount of benomyl resistant isolates from avocado and mango were determined with similar numbers being highly and moderately resistant. Resistance of 12.9% was recorded for *Colletotrichum* spp. isolated from mangoes in Thailand (Farungsang & Farungsang, 1992), 19% resistance in *Pseudocercosporella herpotrichoides* isolated from cereals (Murray, 1996) and 27% resistance in *B. cinerea* 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 & Farungsang, 1992). It has been reported world-wide that there is an increase in the number of isolates of *C. gloeosporioides* that are resistant to benomyl (Dodd *et al.*, 1992). However, no isolates were resistant to thiabendazole, although the most isolates grouped in the category were just below the cut-off point for resistance. This is cause for great concern in the avocado industry since this indicates that there could be a build up of resistance due to its use as a post-harvest treatment. Furthermore, exposure to benomyl in the past also increases chances of resistance to thiabendazole, since the two fungicides are closely related (Delp, 1980).

Resistance severity of isolates to benomyl found in this study was approximately equally distributed, with the highest distribution being in category 1, (0-5%). Therefore, the largest group was sensitive to benomyl with almost no growth on amended PDA. Two groups of resistance as defined by Farungsang and Farungsang (1992) could be determined from the total population tested viz. highly resistant (8.5%) and moderately resistant (9.2%). Different classes of resistance of *Venturia inaequalis* to benomyl have also been described based on growth responses on amended media (Katan *et al*., 1983).

Isolates from Letsitele were found to be significantly less sensitive to both benomyl and thiabendazole. However, there is no correlation between decreased sensitivity to these fungicides and disease incidence over the three year period evaluated. Isolates from Natal and Malelane are the most sensitive to thiabendazole and benomyl respectively and are also moderately cross resistant. No correlation could be determined with regard to increased fungicide sensitivity and disease incidence. On the contrary, in 1996, Natal had the highest incidence of anthracnose of all the areas evaluated. It must 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. Differences in fungicide sensitivity were observed in the different production areas in South Africa albeit not significantly. This supports findings by Koenraadt & Jones (1992) who provided further evidence that mutations are responsible for resistance by showing independent selection of identical codon conversions in β-tubulin DNA from diverse geographical regions. This has also been found for other pathogens where isolates of *Helminthosporium solani* showed differential sensitivity to thiabendazole and geographic differences regarding sensitivity were observed (Bains *et al*., 1996). This was also found to be the case in Canada where distribution of resistance severity of *V inaequalis* to benomyl differed in different locations (Sholberg *et al*., 1989)

Isolates from overripe fruit were significantly more sensitive to benomyl and grew slower than isolates from eating ripe and slightly overripe fruit. These findings may be ascribed to differences in fitness of the isolates, although Moorman & Lease (1992) found wide variation in growth rates and pathogenicity and suggested that resistance does not affect fitness. Although it has been shown that appressorium development of *C. gloeosporioides* f. sp. malvae is affected by benomyl (Holmstrom-Ruddick & Mortensen, 1995), overripe fruit have the lowest levels of antifungal dienes (Prusky & Plumbley, 1992) and therefore less fit isolates would be able to infect the fruit.

At this stage, benomyl use in South Africa is limited, although it is still used to a limited extent (L. Korsten, pers comm.). Since several alternatives to the pre-harvest use of benomyl are being investigated on avocados (Lonsdale, 1992), successful disease control could still be obtained with prudent use in combination with or as a mixture with another low risk fungicide (Russell, 1995).

Due to problems with resistance build-up, alternative post-harvest control measures using detergent sanitisers (Boshoff *et al*., 1995) and biocontrol agents (Korsten *et al*., 1998) are being investigated and show great promise for the control of anthracnose and stem-end rot.

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