

## FUNGITOXIC CHEMICAL SUBSTANCES IN AVOCADOS

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### **OPSOMMING**

*Ondersoek is ingestel na die voorkoms van antifungiese verbindings in enkele avokado kultivars. Etanolekstrakte van al die kultivars wat getoets is het oor antifungiese eienskappe beskik. Die geaardheid en antifungiese spektrum van die ekstrakte verskil van kultivar tot kultivar.*

### **SUMMARY**

*The occurrence of antifungal substances in various avocado cultivars was investigated. Ethanol extracts of all the cultivars assayed exhibited antifungal properties. The nature and antifungal spectrum of the extracts varied between cultivars.*

### **INTRODUCTION**

Considerable levels of resistance to root rot caused by *Phytophthora cinnamomi* Rands occur in several species of the genus *Persea* (Zentmyer, 1961). Zaki *et al.* (1973) reported the presence of a potent preformed antifungal agent in tissues of resistant species but not in certain susceptible species. The chemical structure of this substance has recently been reported by (Zaki *et al.*, 1980), who gave it the trivial name borbonol. *In vitro*, borbonol significantly inhibits vegetative growth of *P. cinnamomi* at a minimum concentration of 1  $\mu\text{gml}^{-1}$  and inhibits spore production at concentrations of 5—10  $\mu\text{gml}^{-1}$ . The agent was found to occur in relatively high concentrations in most avocado cultivars which are relatively resistant to *P. cinnamomi* (Zaki *et al.* 1980). Since the occurrence of preformed antibiotic compounds is believed to constitute a disease defence mechanism in some plants (Schonbeck and Schlosser, 1976), it was hypothesized by Zaki *et al.* (1980) that the presence of borbonol in the roots of resistant *Persea* spp. might prevent their infection by *P. cinnamomi* and that species with low concentrations or no borbonol in their roots would be susceptible.

This paper reports on a preliminary investigation of various avocado cultivars grown in South Africa for the presence of borbonol and other fungitoxic substances.

## MATERIALS AND METHODS

### Borbonol, plant material and extraction procedures

A sample of crude borbonol was kindly supplied by Prof GA Zentmyer, University of California. Leaves and/or branches from avocado cultivars Edranol, Duke 6, Duke 7 and an "escape tree" (Fuerte scion) were obtained from Westfalia Estates, Duiwelskloof. Fresh leaves or pulverized air dried branches were homogenized in 96% ethanol with an ATO-Mix blender and the filtered extracts were concentrated *in vacuo*. The concentrates were partitioned with ethyl acetate, the organic layers dried with anhydrous MgSO<sub>4</sub>, concentrated *in vacuo* and redissolved in methanol.

### Thin layer chromatography bioassay

Plant extracts were spotted on thin layer chromatography (t.l.c.) plates and developed in chloroform/methanol (97/3). The presence of a 254 nm ultraviolet absorbing spot at R<sub>F</sub> 0,55 was taken as presumptive evidence for the presence of borbonol (Zaki et al. 1980). Air dried plates were sprayed with a spore suspension of *Cladosporium herborum* in molten potato dextrose agar (PDA) at 45°C and incubated in a moist chamber for 48 h at 25°C as described by Keen et al. (1971). Antifungal substances appeared as light spots on a dark background of fungal growth.

### Disc bioassay

Filter paper-discs (10 mm diameter) were impregnated with the various extracts, as well as with crude borbonol. After evaporation of the solvent, the discs were placed on PDA or nutrient agar in Petri dishes in which propagules suspensions of the following organisms were incorporated. *Root pathogens: Fusarium oxysporum, Macorhominia phaseolina, Phytophthora cinnamomi, Pythium ultimum, Rhizoctonia solani; Post-harvest pathogens: Botryodiplodia theobromae, Fusarium sambucinum, Pestalotiopsis versicolor; Bacteria: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus.* The plates were incubated at 25°C (37°C in the case of the bacteria) and were examined regularly for zones of inhibition surrounding the filter paper discs.

## RESULTS

The t.l.c. bioassay of crude borbonol revealed zones of fungus inhibition of various R<sub>F</sub> values with chloroform/methanol (97/3) as the developing solvent. A zone of inhibition was present at R<sub>F</sub> ca 0,55, but the fungitoxicity at this ultraviolet light absorbing spot was weak compared to that of other zones. A pronounced streak of inhibition was especially evident at the base (Fig. 1). Weak fungus toxicity occurred on the t.l.c. plates of Duke 6 and Duke 7 extracts. However, in the case of Edranol, a pronounced zone of inhibition was present at R<sub>F</sub> 0,3 (Fig. 2). Not one of these fungus-inhibiting spots absorbed 254 nm ultraviolet light. In the case of the "escape tree", an ultraviolet absorbing spot was evident at R<sub>F</sub> 5,5 on the t.l.c. plate (Fig. 3). Although fungitoxicity did occur at this spot in the bioassay, inhibition also occurred at other R<sub>F</sub> values, especially at R<sub>F</sub> 7,2 (Fig. 4).

All the extracts tested, including crude borbonol, had a slight inhibitory effect on *P. cinnamomi*, but varied in toxicity towards the other fungi incorporated in the disc assay (Table 1). The Edranol extract inhibited growth of the widest range of fungi. Crude borbonol inhibited growth of *Staphylococcus aureus* only. No other bactericidal effect was evident.

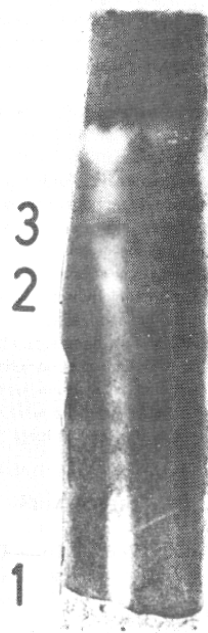


FIG. 1: T.l.c. bioassay of crude borbonol

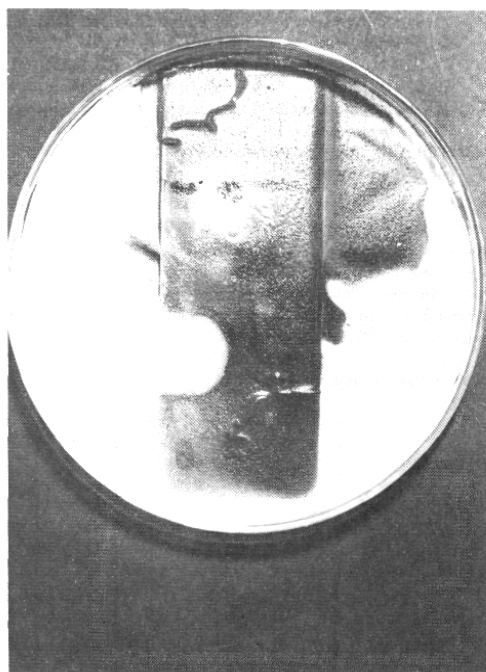


FIG. 2: T.l.c. bioassay of ethanol extracts of Edranol, Duke 6 and Duke 7

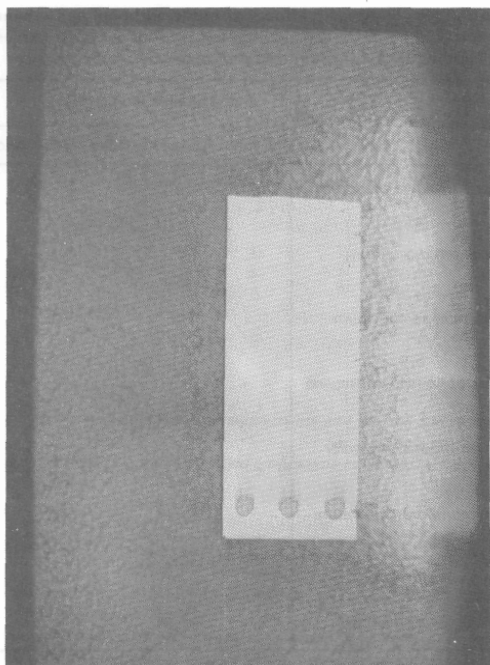


FIG. 3: T.l.c. of ethanol extract of "escape tree" showing ultraviolet absorption at  $R_f$  0,55

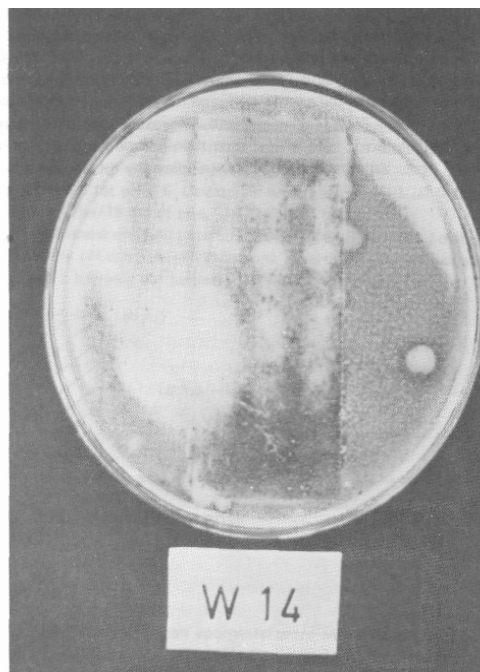


FIG. 4: T.l.c. bioassay of ethanol extract of "escape tree"

## DISCUSSION

The evidence presented by Zaki *et al.* (1980) does not entirely support the hypothesis that the presence of borbonol in the roots of *Persea* plants confers resistance to *P. cinnamomi* and that its absence leads to susceptibility since the agent was found to occur in the susceptible avocado cv. Topatopa (Zaki *et al.* 1980). The results reported here indicate that fungitoxic substances other than borbonol are present in avocado trees. Whether these substances play any role in resistance to *P. cinnamomi* is not clear at this stage. Nevertheless, the agent responsible for the pronounced fungitoxic effect at  $R_f$  P,3 on the Edranol t.l.c. plate might be of significance since it is known that Edranol is comparatively resistant to root rot. However, till a more comprehensive survey has been undertaken, such conclusions can only be presumed.

The differences in the antifungal spectra of the various extracts indicate the presence of fungitoxic substances of different structure and activity in the avocado cultivars and thus confirms the results obtained with the t.l.c. bioassays. Further research is being done to assay all available avocado pathogens for sensitivity to extracts from various avocado cultivars and to determine whether the substances responsible for the fungitoxicity have any effects *in vivo*.

The bactericidal effect of borbonol on *S. aureus* is of no significance as far as plant pathology is concerned. However, it is of interest to note that the Indians use avocado seed for dysentery and diarrhoea (Uphof, 1968). The therapeutic effects of this remedy may partly be ascribed to the presence of bactericidal agents like borbonol in the avocados. Borbonol has the same lactone structure basically as i.e. patulin, which is active against a wide spectrum of bacteria, including *S. aureus*, as well as certain fungi (Ciegler *et al.* 1971).

**TABLE 1: Fungitoxic effects of crude borbonol and ethanol extracts of various avocado cultivars on root and post-harvest pathogens of avocado as determined by the disc assay**

Pathogen	Inhibition of fungal growth			
	Edranol	Duke 6	Duke 7	Borbonol
<i>Botryodiplodia theobromae</i> (P)	+	-	-	-
<i>Fusarium oxysporum</i> (R)	-	-	-	-
<i>Fusarium sambucinum</i> (P)	-	-	-	-
<i>Macrophomina phaseolina</i> (R)	+	-	-	-
<i>Pestalotiopsis versicolor</i> (P)	+	-	-	±
<i>Phytophthora cinnamomi</i> (R)	±	±	±	±
<i>Pythium ultimum</i> (R)	+	-	-	±
<i>Rhizoctonia solani</i> (R)	±	-	-	7/9

P: Post-harvest pathogen  
R: Root pathogen  
+: Pronounced inhibition  
±: Slight inhibition  
-: No inhibition

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