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BIOLOGICAL CONTROL OF POSTHARVEST DISEASES OF AVOCADO

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ABSTRACT
The unacceptability of chemical residues, environmental pollution by pesticides and eventual pathogen resistance, makes chemical control of postharvest diseases in avocado fruit increasingly undesirable. An alternative control strategy is biological control and in this report, the biological approach is evaluated against standard chemical control measures. Although successful in bioassays against the most important postharvest pathogens, the antagonist, Bacillus lichineformis*, could not compete with copper oxychloride under field conditions. However, effective control was obtained with fruit dips under packhouse conditions.*

OORSIG
Die onaanvaarbaarheid van chemiese residue, omgewingsbesoedeling deur chemiese middels en die opbou van patogeenweerstand, maak chemiese beheer van na-oessiektes van avokadovrugte al hoe meer onaanvaarbaar. Biologiese beheer kan dalk 'n oplossing bied en word in hierdie verslag vergelyk met chemiese beheer. Alhoewel die antagonis, Bacillus llchineformis*, die belangrikste na-oespatogene in vitro inhibeer het, kon dit nie onder veldtoestande opweeg teen koperoksichloried nie. Effektiewe beheer kon egter onder pakhuistoestande verkry word waar 'n vrugdoopmetode gebruik is.*

The avocado *(Persea americana* Mill) is subject to a number of postharvest diseases, the most important being stemend rot, anthracnose and the Dothiorella/Colletotrichum complex fruit rot (Darvas and Kotzé, 1987). Control of these diseases has been achieved by preharvest application of fungicidal sprays (Darvas, 1983). These fungicides, particularly copper oxychloride and captafol, leave visible spray residues on harvested fruit, which are not easily removed by standard packhouse procedures (Denner and Kotzé, 1986). This resulted in rejection of 11,5 19,4 per cent of fruit for export (Pieterse, 1986). Although postharvest fungicidal applications, such as prochloraz, have been successfully tested (Darvas, 1984), product clearance has not been given on the French markets. Public awareness of matters such as environmental pollution by pesticides, and the possible health hazards due to macro and micro residues on consumable items make such fruit unacceptable. The possible build-up of pathogen resistance to chemicals is another important consideration. Delp (1980) reported that chemicals traditionally used for preand postharvest disease control of certain crops, are becoming less effective due to the development of resistance in the pathogens. Disadvantages of the use of chemicals have prompted the investigation of

biological control as an alternative. Although there are numerous reports on the use of antagonists to control plant diseases, most of them deal with the use of such systems in the soil (Corke & Risbeth, 1981). Only a few workers have thus far attempted to manipulate micropopulations on the phylloplane (Knudsen and Spurr, 1987), whereas even fewer have tried biocontrol of postharvest diseases. According to Wilson and Pusey (1985), results obtained with biological control of postharvest diseases have been encouraging, despite the limited number of studies in this field. They list seven examples and describe them as being considerably successful. The present paper reports on the isolation of antagonists from the avocado phylloplane, as well as their evaluation in bioassays and under field and packhouse conditions. The efficiency of the antagonist for the control of postharvest disease in the field was compared to that of existing copper spray programmes used at Westfalia Estate.

MATERIALSAND METHODS

Candidate biological control agents were isolated during March, 1986 from the phylloplane of the Fuerte avocados in an orchard at Westfalia Estate. Trees received preand postharvest sprays of copper oxychloride at concentrations of 0,25 per cent twice a year during November and January. Impressions were made of leaves collected from four sides of ten trees with the aid of a 7 cm diameter weight onto Standard 1 nutrient agar plates (SNI) (Difco). The 250 g weight was applied for 15 seconds. Plates were incubated at 25°C and colonies that developed were picked out and isolated on fresh media. Potential antagonists were first screened for pathogenicity using the tobacco hypersensitive reaction (HR) (Klement, 1963), and possible ice nucleation activity (Süle and Seemüller, 1987). The postharvest decay pathogens *Colletotrichum gloeosporioides* (Penz) Sacc, *Dothiorella aromatica* (Sacc) Petr and Syd, *Fusarium solani* (Mart) Sacc and *Pestalotiopsis versicolor* (Speg) Steyart, were isolated from avocado fruit according to conventional procedures and were used as test organisms. Bacterial isolates were identified using the API 50 CH (PathIdent) system and fungal isolates by the Plant Protection Research Institute, Pretoria.

Bioassays

Pathogenic cultures were inoculated on one side of a PDA plate and incubated at 25°C for three days. The potential antagonists were spot-inoculated at a distance of 2,5 cm from the pathogen. These plates were incubated for a further five days before inhibition zones were measured. Promising antagonists were subsequently evaluated for antagonism against the avocado phylloplane microflora, as described previously. The antagonists were also evaluated for their ability to prevent spore germination, by inoculating a loopful of each antagonist into nutrient broth solutions containing ± 10^4 spores/mℓ of the various pathogens (Baker *et al,* 1983).

To determine the colonisation potential of *Bacillus Hchineformis,* frequently isolated from the avocado phylloplane, ten avocado leaves *ca* 85 95 mm long, were each shaken individually by hand for 30 sec in 100 mℓ sterile distilled Ringers solution (Merck) (sdR), containing three drops of Tween 20. The washing was repeated three

times. After each washing, a dilution series was made according to the method of Leben and Whitmoyer (1979). Subsequently the leaves were dipped for three min in 100 mℓ of a solution containing 1,09 x 10^8 cells/mℓ of *B Hchineformis.* Leaves were then either processed directly, or first air-dried in sterile glass petri-dishes before being processed. The washing and dilution series were repeated as before to determine cell adherence.

Antagonists were also tested for sensitivity to copper oxychloride. Sterile filter paper discs saturated with copper oxychloride solutions of 1, 5, 10 and 20 ppm were prepared. *B Hchineformis* was seeded in STD I plates and the copper oxychloride discs placed on top. Inhibition zones were measured after 48 h incubation at 25°C.

To determine the effect of Tag commercial wax on the antagonist, concentrations of 0, 0,01, 1, 2, 3 and 4 per cent Tag water was inoculated with a loopful of antagonist. Growth was observed after two and four days incubation.

Preparation of field test solution

Starter cultures were prepared by inoculating *B Hchlneformis* into thirty 250 mℓ Erlenmeyer flasks, each containing 200 mℓ STD I broth. After 24 h shakeincubation at 28°C, the starter cultures were added to thirty 2 ℓ Erlenmeyer flasks, each containing 1,8 ℓ STD I broth. After two days incubation, when a density of *ca* 10^8 cells/mℓ had been reached, the antagonist was centrifuged for 15 min at 8 000 rpm in a Sorvall SA 600 rotor. The pellets were then pooled and freeze-dried immediately.

Field tests

Field tests were conducted during the summer of 1986-1987 on a site in block 34 at Westfalia Estate. Thirty Fuerte avocado trees were randomly selected for the preliminary trial. The freeze-dried pellets were dissolved in 600 ℓ water, and Nu Film 16 sticker was added to a concentration of 0,02 per cent before being sprayed onto five trees. A copper oxychloride solution of 2,5 per cent was prepared and supplemented with 0,02 per cent Nu Film 16. Trees were sprayed with high volume ground sprayers, either in mid-November or mid-January or both, with either the antagonist solution or the standard copper oxychloride application. Unsprayed trees were used as controls. Four leaf and fruit samples from each tree were collected one hour after spraying, to determine adherence of the antagonist. Samples were processed according to standard procedures for examination by scanning electron microscope (SEM). All samples were viewed in a Hitachi S-450 SEM at 15 kV. Between 96 and 140 fruit from the various treatments were harvested during May, packed and stored for 28 days at 5°C under commercial packhouse conditions before being transported to the laboratory. After ripening, fruit were analysed for disease incidence and disease severity according to a 1-5 scale (Bezuidenhout and Kuschke, 1982).

Packhouse treatments

B lichineformis was further evaluated under commercial packhouse conditions at Westfalia Estate. Ten boxes containing 14 fruit each were randomly selected for each

treatment. Controls consisted of two treatments, with and without commercial waxes. Two treatments consisted of dipping fruit into 25 ℓ of S *lichineformis* suspension *(ca* 10^{s} cells/mℓ). After drying the fruit in the warm tunnel (part of the commercial packing line) one antagonist and one control treatment were first waxed. Fruit from the various treatments were stored and evaluated as described previously.

RESULTS

Various bacteria, filamentous fungi and yeasts were isolated from the avocado phylloplane. Two bacterial species, identified as *B subtilis* and *B lichineformis,* formed strong inhibition zones against the pathogens (Figure 1) and most of the saprophytic phylloplane colonisers (Figure 2, Table 1). The antagonists gave negative HR and ice nucleation reactions. One strain, designated A6, prevented spore germination of all the pathogens tested. This isolate was selected for further evaluation.

Antagonist A-6 successfully attached to the avocado leaf surfaces (Table 2). Leaves first allowed to air-dry after dipping, yielded higher counts of this organism than leaves processed directly after dipping. The antagonist attached to both fruit and leaf surfaces, but at low numbers, as observed under the SEM (Figure 3).

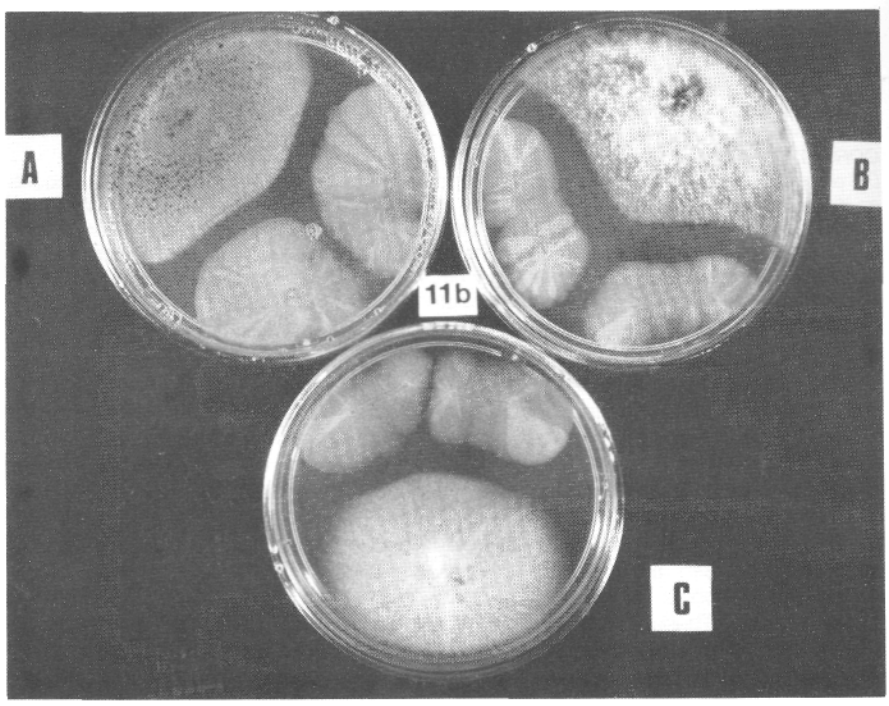


Fig 1 Inhibtion of growth of *Colletotrichum gloeosporioides* (a) *Nigrospora sphaerica* (b) and *Fusarium solani* (c) by *Bacillus lichineformis.*

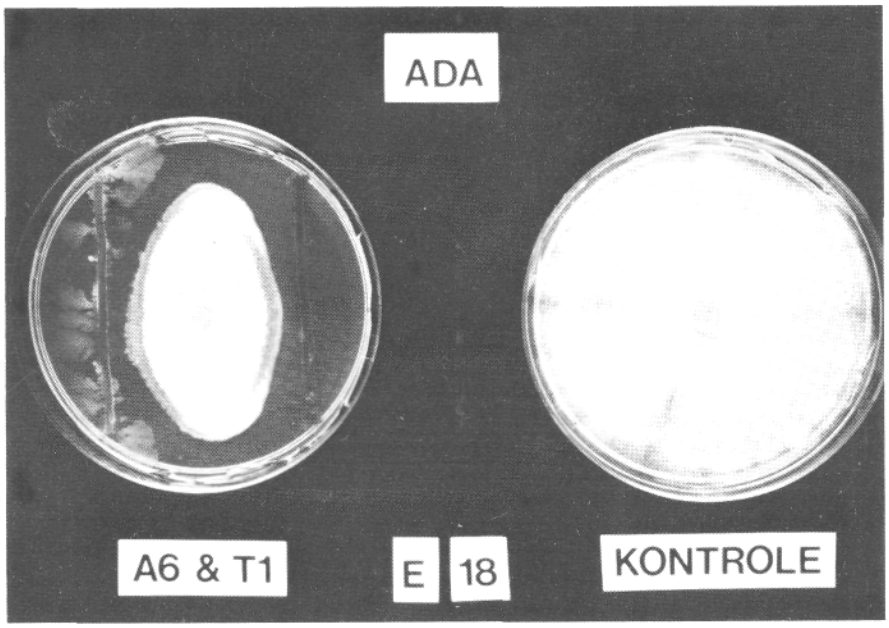


Fig 2 Inhibition of growth of *Penicillium chrysogenum* (E18) by *Bacillus lichineformis* (A6) and *B subtilis* (T1). Control plate on the right.

TABLE 1 *In vitro* inhibition of various avocado fruit pathogens and phylloplane colonisers by two bacterial antagonists

FUNGI	Inhibition zones measured in mm	
	B lichineformis	*B subtilis*
Acrodontium crateriforme	8	15
Alternaria alternata	4	5
Aureobasidium pullulans	15	14
Cladosporium cladosporioides	12	13
Colletotrichum gloeosporioides [a]	4	4
Curvularia senegalensis	3	4
Epicoccum purpurascens	9	14
Fusarium compactum	0	0
Fusarium equiseti	0	0
Fusarium scirpi	0	0
Fusarium semitectum	2	2
Fusarium solani [a]	1	1
Glomerella cingulata	2	2
Nigrospora sphaerica	7	8
Penicillium chrysogenum	5	6
Penicillium janthinellum	9	8
Periconia byssoides	6	11
Pestalotiopsis guepinii	2	2
Pithomyces chartarum	7	7
Sclerostagonospora	11	10

a) Postharvest pathogens

TABLE 2 Successive washing and dilution series of avocado leaves to remove phylloplane micro-organisms followed by dipping 'washed' leaves into suspensions of 1 × 10^8 cells/mℓ *Bacillus lichineformis* to determine the ability of the antagonist to attach to the leaf surface

Wash treatments	Dilution	Numbers of fungi	Numbers of *B lichineformis*
1st wash	10^{-1}	>500	>500[a]
	10^{-2}	>500	>300[a]
	10^{-3}	67	180[a]
2nd wash	10^{-1}	23	58[a]
	10^{-2}	2	20[a]
	10^{-3}	0	130[a]
3rd wash	10^{-1}	0	42[a]
	10^{-2}	0	6[a]
	10^{-3}	0	18[a]
5 × 10^8 cells/mℓ of *B lichineformis* applied to washed leaves	10^{-1}	0	>500
	10^{-2}	0	>500
	10^{-3}	0	>300
(a) Direct wash			
1st wash	10^{-1}	0	>500
	10^{-2}	0	>500
	10^{-3}	0	>300
2nd wash	10^{-1}	0	>500
	10^{-2}	0	>300
	10^{-3}	0	10
3rd wash	10^{-1}	0	>500
	10^{-2}	0	>300
	10^{-3}	0	11
(b) Dry leaf first			
1st wash	10^{-1}	0	>500
	10^{-2}	0	>300
	10^{-3}	0	120
2nd wash	10^{-1}	0	>300
	10^{-2}	0	100
	10^{-3}	0	2
3rd wash	10^{-1}	0	>300
	10^{-2}	0	86
	10^{-3}	0	2

a) Representing various phylloplane bacteria

TABLE 3 Comparison of pre-harvest application of *B lichineformis* with copper oxychloride of physiological and pathological disorders of avocado fruit

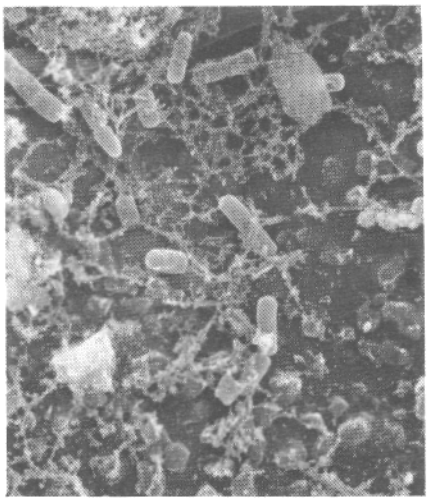
Treatment	No of fruit	Stem-end	Mean disease severity 0-5 scale				
			Anthracnose	C/D complex	Grey pulp	Vascular	Ripening
Nov and Jan : Cu-spray	140	0,057b	0,014a	0,221a	0,157b	0,100b	0,407b
Nov : Cu-spray	126	0,056b	0,071b	0,500b	0,174b	0,111b	0,381b
Jan : Cu-spray	138	0,022b	0,036b	0,420b	0,058a	0,051a	0,382b
Control	96	0,010b	0,104c	0,9375b	0,292c	0,135b	0,469b
Nov : antagonist	126	0,103b	0,206c	1,040b	0,333c	0,246c	0,444b
Nov and Jan : antagonist	122	0,016b	0,057b	0,885b	0,320c	0,221c	0,352ab

Values followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05)

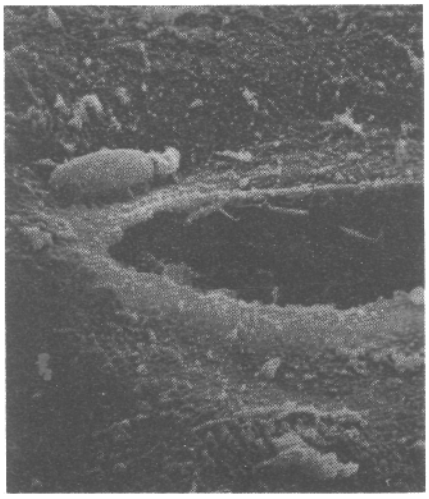
TABLE 4 Effect of dip treatments in *ca* 1 x 10^8 cells/mℓ of *B lichineformis* on pathological avocado fruit disorders

Treatment	No of fruit	Stem-end	Anthracnose	C/D complex	Lead	Vascular	Ripening
Control and wax	63	0,000a	0,032b	0,016b	0,635b	0,159b	
Control no wax	64	0,047b	0,000a	0,016b	0,406b	0,109b	
Antagonist and wax	61	0,000a	0,098c	0,000a	0,574b	0,148b	
Antagonist no wax	63	0,032b	0,016b	0,063c	0,254a	0,095a	
	251						

Values followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0,05)



(a)



(b)

Fig 3 Scanning electron micrographs of an antagonistic *B lichineformis* (a) on an avocado leaf (Mag: 9428 X), (b) attached to the fruit surface one hour after field application.

Copper oxychloride and Tag wax had no inhibition effect on the growth of *B lichineformis* at any of the concentrations tested.

Spraying twice with copper oxychloride proved to be the most effective treatment against anthracnose and the C/D complex (Table 3). None of the treatments significantly reduced stemend rot. The January copper spray proved to be the most effective treatment for reducing physiological disorders such as grey pulp and vascular browning. The November antagonist spray had the least effect on the incidence of anthracnose, the C/D complex, stem-end rot and physiological disorders. There was no significant differences in the ripening time of avocado fruit which had received the different treatments. Fruit dipping in *B lichineformis* followed by waxing proved to be the most effective treatment against the C/D complex (Table 4). The antagonist dip without

waxing proved to be the most effective treatment against physiological disorders, such as grey pulp and vascular browning. Waxed fruit exhibited less stem-end rot, while anthracnose was less evident in control fruit.

DISCUSSION

In this investigation, one of the prerequisites for effective biocontrol was met by selecting a naturally occurring avocado phylloplane inhabitant, which adapted to survival and growth under natural conditions. Its ability to attach and colonise after artificial application, was confirmed by re-isolations and SEM studies. However, other prerequisites for sustained biocontrol, viz the ability to multiply and spread (Blakeman and Fokkema, 1982), could not be achieved under natural field conditions.

Results obtained with the first pilot trial under pre-harvest field conditions revealed that biological control could not compete with standard copper oxychloride application. Although the *Bacillus* isolates were highly antagonistic to certain postharvest pathogens *in vitro,* they failed to give control under field conditions. This could possibly be ascribed to the general antibiotic action of the antagonist, 1e not only pathogens were inhibited but also certain saprophyte phylloplane organisms. The natural ecological balance could, therefore have been disturbed by a general decrease in microbial numbers, thus creating a vacuum favouring the latent pathogenic populations. Another explanation for the inability of the antagonist to compete with the copper oxychloride field applications, is the low retention rate of the antagonist on the leaf surfaces. According to Leben (1964), the main problem in biocontrol of postharvest diseases is to maintain high enough cell levels to give effective control, particularly under dry conditions in sunlight. This problem could be overcome by applying an initial concentration of antagonist cells that will not diminish beyond $10^5/cm^2$, the minimum effective concentration (Knudsen and Spurr, 1987).

Alternatively, a more effective spraying procedure could be used, such as more frequent sprays (Baker, Stavely and Mock, 1985), or by applying the antagonist as spores (Spurr, 1981). Ultimately integrated biological and chemical control could offer the most effective solution, since copper formulations do not affect the growth of the antagonist.

Future research will involve a detailed study of the avocado phylloplane and the influence of external factors on it, as well as the mechanism of antagonism. Such knowledge should be of great value in determining the optimum timing, formulation and quantity of application (Blakeman and Fokkema, 1982).

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