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Interaction of *Colletotrichum gloeosporioides*, epiphytic microorganisms and nutrients on avocado leaves and fruit

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Abstract

The effect of nutrients on *Colletotrichum gloeosporioides*, the cause of avocado anthracnose, and on microorganisms that are antagonistic to the pathogen was studied in a series of laboratory and field experiments. Avocado (cv. Hass) leaves sprayed once with molasses, urea and yeast extract, either alone or in combination, were sampled after 7 and 28 days and populations of bacteria, yeasts and filamentous fungi on the leaf surface were determined. Urea had little effect on the number of microorganisms, but molasses increased microbial populations by 10- to 100-fold. When the antagonistic yeasts *Cryptococcus* sp. and *Aureobasidium* sp. were sprayed onto avocado leaves in the field with molasses, they maintained significantly higher populations than in the absence of molasses. However, molasses did not enhance populations of selected antagonistic bacteria (e.g. *Pseudomonas fluorescens*, *Pantoea* sp. and *Bacillus pumilus*) when sprayed on leaves. Molasses increased the saprophytic growth of *C. gloeosporioides* on detached avocado fruit and these fruit developed more disease than fruit treated with water alone. Detached avocado fruit treated with antagonistic yeasts, *Cryptococcus* sp., *Aureobasidium* sp. or pink yeast 734 in the presence or absence of molasses had fewer and smaller lesions than fruit without added yeasts. Regular molasses sprays applied to avocado trees in the field over an 8-month period did not increase anthracnose in fruit.

Additional keywords: antagonists, postharvest, yeasts, bacteria, molasses

Introduction

Nutrients are often a limiting factor to growth and survival of microorganisms on aerial plant surfaces (Andrews 1992). Consequently, the presence of extraneous nutrients from natural sources such as pollen and aphid honeydew or the artificial application of nutrients can temporarily elevate microbial populations (Fokkema 1973; Bashi and Fokkema 1977; Fokkema *et al.* 1979; Dik *et al.* 1992). In such situations, nutritionally versatile, fast growing organisms may proliferate, competing for nutrients and niche space with more fastidious species. Both these phenomena have been suggested as mechanisms by which one organism may suppress another on the phylloplane (Fokkema 1973; Fokkema *et al.* 1979; Lindow 1988; Wilson and Lindow 1994).

In an era where synthetic chemicals are becoming less acceptable for plant disease control, it may be possible to achieve naturally occurring biological control by using nutrients to stimulate the activity of competitors to the pathogen. One disadvantage of this concept is that nutrients may also indirectly or directly increase the inoculum potential of some pathogens (Grover 1971; Blakeman and Brodie 1977).

The response of *Colletotrichum* spp. to nutrients has been studied extensively. While exogenous nutrients are not essential for the germination of conidia of most species, their presence increases mycelial growth (Parbery 1981). In other instances, nutrients can reduce appressorium formation (Emmett and Parbery 1975). When nutrients are present along with other competitive microorganisms,

the interactions become complex. For instance, disease severity on cucumber leaves inoculated with *Colletotrichum orbicularae* (Berk. & Mont.) v. Arx was decreased when nutrients and antagonistic bacteria were present (Leben and Daft 1965; Leben *et al.* 1965). Appressorial formation by *C. acutatum* Simmonds (Blakeman and Parbery 1977), and *C. dermatium* f.sp. *spinaceae* (Pers.) Grove (Blakeman and Brodie 1977) was stimulated when the fungi were inoculated to sugar beet leaves in the presence of bacteria and nutrients. The authors suggested that nutrient stress due to competition enhanced appressorial formation in some *Colletotrichum* spp.

In order to examine some of these interactions on the avocado (*Persea americana* Mill.) leaf and fruit surface, a study was carried out to determine the effect of selected nutrients on naturally occurring microorganisms on the avocado phylloplane, the impact of molasses on the colonisation potential of selected antagonistic yeasts and bacteria, and the effect of molasses on *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in the presence or absence of these yeasts. In addition, a field trial was carried out to determine whether regular molasses sprays on avocado trees increased or decreased the incidence of anthracnose in fruit.

Methods

Effect of nutrients on naturally occurring microorganisms on leaves Four different nutrients were selected for field application after *in vitro* experiments (Stirling 1996) involving 46 bacteria and 23 yeasts confirmed their conduciveness to microbial growth. The nutrient treatments were applied separately to randomly selected small branches on each of four replicate, 7-year-old avocado trees (cv. Nabal) at Brookfield, Queensland. The nutrients (2.0% molasses; 1.0% urea; 2.0% molasses + 1.0% urea; 2.0% molasses + 0.5% yeast extract) and water were sprayed to run-off on 30–40 leaves per replicate with a hand-held atomiser. Four leaves per replicate were sampled from all treatments prior to spraying, and 7 and 28 days after spraying with nutrients. The numbers of filamentous fungi, yeasts and bacteria were estimated on individual leaves with a modified version (Stirling 1996) of a plate dilution frequency-most probable number (PD-MPN) technique described by Andrews and Kenerley (1978). Leaves collected aseptically were weighed and then blended in a Stomacher Lab-Blender 80 (Seward Medical, London) for 2 min in phosphate buffered saline (PBS; pH 7.2). After dilution, five replicate 20 µL aliquots were pipetted as spots onto three media: 1/3 strength tryptic-soy agar (1/3 TSA) + cycloheximide (75 µg/mL), potato-dextrose agar (PDA) + streptomycin (120 µg/mL) and yeast-extract, malt-extract agar (YMA; pH 3.7) and numbers of bacteria, filamentous fungi and yeasts, respectively, were estimated following incubation at 25°C for 3 days.

Effect of molasses on selected bacteria and yeasts The effect of molasses (2.0%) on the maintenance and survival of selected antagonistic bacteria and yeasts on the avocado phylloplane was studied in three experiments. The antagonists were selected by Stirling *et al.* (1995), but mutants were used in this study so that they could be monitored experimentally. Rifampicin-resistant bacterial mutants of *Bacillus pumilus* (isolates 359R1 and 480R2), *Pantoea* sp. (632R2) and *Pseudomonas fluorescens* (677R1), generated as detailed by Stirling *et al.* (1992), were grown in tryptic-soy broth (TSB) for 36 h at 26°C. *Aureobasidium* sp. 274C1, *Cryptococcus* sp. 772C1 and pink yeast 734C2, selected for carbendazim (Bavistin) resistance using methods described by (Fokkema *et al.* 1987), were cultured for 3 days in glucose-yeast-peptone broth.

Suspensions of each microorganism were diluted by adding 100 mL culture to 900 mL autoclaved tap water alone or water containing sufficient molasses to give a final concentration of 2.0%. The mixtures were sprayed onto avocado leaves to run-off with a hand-held atomiser. Diluted medium with or without molasses was sprayed on the controls. Once leaves were dry, initial numbers of microorganisms were determined by dilution plating. Individual leaves were blended for 2 min in a Stomacher Lab-Blender 80, the suspension serially diluted in PBS and aliquots (100 µL) spread on duplicate plates of PDA + carbendazim (40 µg/mL) for yeasts and 1/3TSA + rifampicin (100 µg/mL) for bacteria. Further samples were processed 7, 28 and 50 days after spraying. Whenever possible, dilution plates containing 30 to 300 colonies were used to estimate numbers of microorganisms.

A total of three colonisation experiments was carried out in the field. In the first experiment, the yeasts were tested by spraying 12-month-old avocado (cv. Hass) seedlings that had 35–40 leaves per plant. Four replicate plants were sprayed with each

yeast. The bacteria were tested in experiment 2 by selecting small branches carrying 30–40 leaves on mature Hass trees. Each isolate was sprayed on four replicate branches. In the third experiment, isolates 359R1, 480R2 (*B. pumilus*), 677R1 (*Pseudomonas fluorescens*), 772C1 (*Cryptococcus* sp.) and 274C1 (*Aureobasidium* sp.) were each tested as above on small branches carrying 40–45 leaves on mature trees (cv. Hass).

Effect of molasses on *C. gloeosporioides* A series of laboratory experiments was carried out to study the effect of molasses on growth and pathogenicity of *C. gloeosporioides* A111-2 (BRIP 19768) on avocado leaf disks and on detached fruit in the presence or absence of selected antagonists.

In the first of three experiments on spore germination, fungal spores (6×10^5/mL) were suspended in sterile molasses (autoclaved for 15 min at 121°C) at concentrations ranging from 0–2.0%. Aliquots (20 µL) of each mixture were then added to leaf disks which were placed on moistened filter paper in Petri dishes. Numbers of germinated spores and appressoria were estimated after 14 h at 25°C. A spore was considered to be germinated when the germ-tube length was at least equal to the width of a spore and/or an appressorium had formed. Molasses concentrations of 0–1.0% were used in the second and third experiments which followed the same procedure as above.

To examine the effect of molasses on disease development, 'cocktail' avocado fruit (seedless, finger-like fruit approximately 8–10 cm long) (cv. Fuerte) were washed, surface sterilised, air-dried, and inoculated with 20 µL of a spore suspension (7×10^5/mL) of *C. gloeosporioides* in sterile molasses with concentrations ranging from 0.02–2.0% (v/v). Fungal spores in sterile distilled water were used as a control. Each treatment was applied to ten replicate fruit, with each fruit having four marked areas for inoculation. Fruit were placed in a ripening box and maintained under 100% RH for 48 h at 25±1°C. They were then allowed to ripen at 24±1°C and 80–85% RH, and lesion development was noted. This experiment was repeated with the same experimental procedure.

In a third experiment, spores of *C. gloeosporioides* at four different concentrations (5×10^5, 5×10^4, 5×10^3 or 5×10^2 per mL) were prepared in 2.0% autoclaved molasses or sterile distilled water and inoculated onto fruit as above. Following incubation, fruit were assessed for disease as previously described.

The last two experiments on detached avocado fruit examined the interactions between the pathogen, antagonistic yeasts and molasses. 'Cocktail' fruit (cv. Fuerte) prepared as above were coated on one side with suspensions (10^9–10^{10} cfu/mL) of either *Aureobasidium* sp. 274, *Cryptococcus* sp. 772 or pink yeast 734 in 0.02% or 2.0% sterilised molasses containing methyl cellulose (0.3% w/v). Treatments with yeasts suspended in methyl cellulose were also included. Ten replicate fruit were treated with each yeast preparation and incubated at ambient temperature for 24 h. Spores (5×10^5/mL), of *C. gloeosporioides* were then inoculated to marked areas on these fruit and another ten replicate fruit that had received yeast treatments 1 h previously. Fruit used as controls received only methyl cellulose and the pathogen. Incubation conditions and disease assessment were as stated above. Additional replicates were also set up for estimating yeast populations on the fruit surface at the time of inoculation and 24 h and 48 h later. Microorganisms were extracted from disks of fruit peel with a Stomacher Lab-Blender 80 and their numbers determined by dilution plating.

To assess the effect of molasses on disease on fruit in the field, an avocado (cv. Hass) orchard that had not been sprayed with pesticides for several years at Maleny, Queensland was used as the experimental site. Eight trees of equal size were selected and molasses (2.0%) or water were sprayed to run-off with a knapsack sprayer onto randomly selected halves of each tree. Molasses or water treatments were applied in October and December 1994, and January, February, March and May 1995. Leaves were collected periodically to monitor populations of microorganisms with the PD-MPN technique. At each sampling, eight mature leaves were removed, four from the inner canopy and four from the outer canopy of four replicates of each treatment. Daily rainfall was measured with an automatic weather station (Monitor Sensor, Caboolture, Queensland) during the 7-month period. Once fruit were mature, 25 each were harvested from all replicates of the two treatments, incubated at 24±1°C and 80–85% RH and assessed for disease when they reached eating-ripe stage. An 11 point disease rating scale increasing in 10% increments was used, where 0 = no visible lesions and 10 = >90% of the fruit covered with lesions. Isolations were made from a random sample of lesions to confirm the presence of *C. gloeosporioides* and other pathogens. At the same time, five fruit from each replicate of the

two treatments were harvested to determine whether there was a difference in the range of fungi associated with mature unripe peel. Pieces of peel (4 mm square, 20 per fruit) were surface sterilised in 70% ethanol for 2 min, rinsed and plated on PDA + S. Following incubation at 25±1°C, fungi were identified to genus level.

Statistical analysis The error variances of $\log_{10}$ transformed data for each category of microorganism (on avocado leaves sprayed with nutrients) for each sampling date were found to be homogeneous using Bartlett's test (Snedecor and Cochran 1980). Therefore, a combined analysis of variance (ANOVA) across all three sampling times was performed using a split plot model from BALF (Biometry Statistical Software, Queensland Department of Primary Industries) and means compared using least significant difference (LSD). Data for the estimation of the colonisation potential of selected antagonistic yeasts and bacteria on avocado leaves (cv. Hass) in the presence or absence of molasses were treated in a similar manner to those above.

For the field trial at Maleny, $\log_{10}$ transformed data for populations of microorganisms on avocado leaves were subjected to ANOVA. Angular transformations of data (per cent lesion incidence) on the effect of yeasts, *C. gloeosporioides* and molasses on detached fruit were tested using a factorial model from BALF. Because lesion incidence in most control fruit in experiment 2 was 100% these data were not included in the analysis.

Results

Effect of molasses on naturally occurring microorganisms on leaves Population densities of filamentous fungi, yeast and bacteria on avocado leaves increased by 10- to 100-fold in all of the treatments containing molasses and these elevated populations remained for at least one month (Table 1). In many of the treatments containing molasses, microbial populations increased significantly with time, whereas they did not on leaves sprayed with water. Colonies of *Cladosporium* spp. that were visible to the naked eye and confirmed by microscopic examination often grew on the surface of nutrient-treated leaves but were absent from those treated with water. A detailed study on the species diversity of microorganisms on sprayed leaves was not carried out. However, populations of *Cladosporium* spp., *Aureobasidium* spp., *Cryptococcus* spp. and Gram-negative bacteria, which occurred on

Table 1 Populations of naturally occurring filamentous fungi, yeasts and bacteria on avocado leaves prior to spraying and 7 and 28 days after being sprayed with different nutrients or water

Microorganisms	Sampling time (days)	Mean number of microorganisms ($\log_{10}$ cfu/g)				
		Water	Urea	Molasses	Molasses + urea	Molasses + yeast extract
Filamentous fungi[A]	0	4.75	4.91	4.97	4.86	4.66
	7	4.74	4.92	6.04	5.93	6.84
	28	4.89	5.42	6.47	7.01	7.03
LSD^B ($P=0.05$) = 0.32						
LSD^C ($P=0.05$) = 0.33						
Yeasts	0	4.49	4.66	4.47	4.83	4.55
	7	4.79	4.65	6.37	5.84	6.94
	28	4.84	5.15	6.42	6.28	7.14
LSD^B ($P=0.05$) = 0.47						
LSD^C ($P=0.05$) = 0.49						
Bacteria	0	4.63	4.77	4.56	4.52	4.37
	7	4.93	4.87	5.41	5.82	6.77
	28	4.88	5.33	6.03	6.51	7.10
LSD^B ($P=0.05$) = 0.63						
LSD^C ($P=0.05$) = 0.61						

[A]For each category of microorganism there was a significant interaction of treatment × time.
LSD^B is for differences between sampling times; LSD^C is for differences between nutrients.

both water- and nutrient-treated leaves, were elevated in the presence of molasses on its own or when mixed with urea or yeast extract (Table 1). Urea on its own significantly increased populations of filamentous fungi at 28 days.

Effect of molasses on selected bacteria and yeasts In experiments 1 and 2, except for *Cryptococcus* sp. 772C1 and *Aureobasidium* sp. 274C1 (Figure 1), populations of most of the other microorganisms declined significantly with time, regardless of the treatment (Figures 1 and 2). *P. fluorescens* 677R1 was undetectable at 28 days and *Pantoea* sp. 632R2 after 50 days (data not shown). The yeasts 772C1 and 274C1 maintained significantly higher population densities in the presence of molasses than in its absence (Figure 1). There was a significant interactive effect of time × treatment for populations of 772C1 and 274C1. Population trends of *Aureobasidium* sp. 274C1, *Cryptococcus* sp. 772C1, *P. fluorescens* 677R1 and isolates 359R1 and 480R2 of *B. pumilus* in experiment 3 were similar to those in experiments 1 and 2 (data not shown).

Effect of molasses on *C. gloeosporioides* The presence of molasses on leaf disks increased the growth rate of *C. gloeosporioides* compared with water alone. Although similar numbers of spores germinated in both control and molasses treatments within 14 h (Table 2), only single, short (8–15 μm) germ tubes were produced in water. Germ tubes, greater than 20 μm long, often several from a conidium, or mycelial mats and secondary conidia, were produced in the presence of molasses. In all three experiments, numbers of appressoria decreased with increasing concentration of molasses (Table 2).

The results of the two experiments which examined disease development on detached fruit in the presence or absence of molasses were similar. Lesions developed on fruit in the inoculated areas regardless of the treatment. However, at the higher molasses concentrations (1 or 2%), lesions formed on unripe fruit 3–4 days after inoculation. The fruit treated with water or molasses (0.02 or 0.1%) did not develop lesions until they had ripened (data not shown).

In the experiment involving molasses (2.0%) and different spore concentrations of *C. gloeosporioides*, all fruit treated with molasses developed lesions at the four spore concentrations. Most untreated fruit inoculated with concentrations of 5×10^5 and 5×10^4 spores/mL (Figure 3) also developed lesions, but there were fewer fruit with lesions at the two lower concentrations (5×10^3 and 5×10^2/mL).

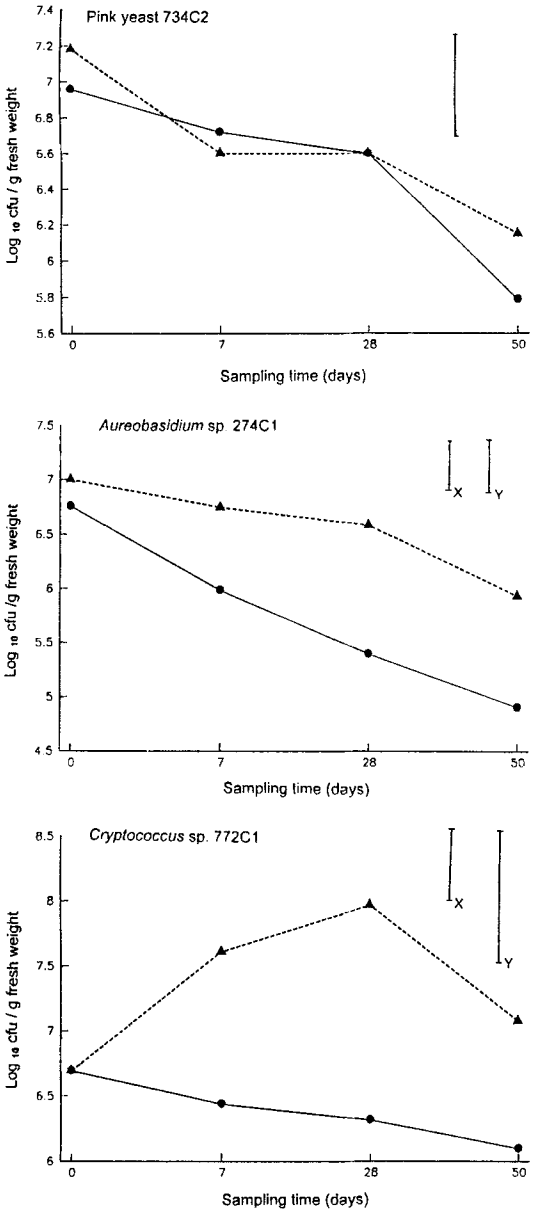

Figure 1 Populations of selected carbendazim-resistant yeasts when sprayed on avocado leaves with (▲) or without (●) molasses (experiment 1). Vertical bar represents LSD (P = 0.05) for mean populations at different times. With *Cryptococcus* sp. 772C1 and *Aureobasidium* sp. 274C1, the treatment × time interaction was significant and vertical bars represent LSD (P = 0.05) between times (X) and between treatments (Y).

In the experiments which examined interactions between antagonistic yeasts, the pathogen and molasses on the fruit surface, the number of yeasts on the surface at the time of application in all treatments was between 10^3 and 10^4 cfu/mm^2. After 24 and 48 h, this number was maintained on the fruit

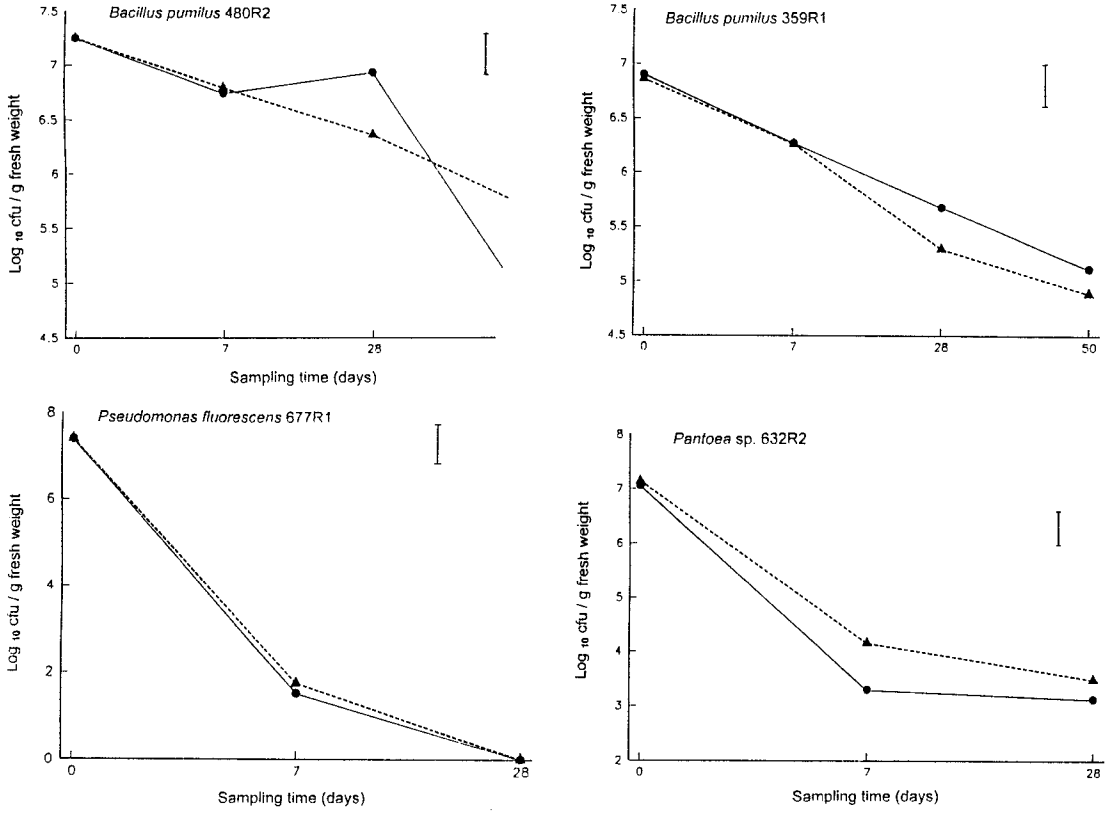

Figure 2 Populations of selected rifampicin-resistant bacterial isolates sprayed on avocado leaves with (▲) or without (●) molasses (experiment 2). Vertical bars represent LSD ($P = 0.05$) for mean populations at different times. The effect of molasses was not significant.

Table 2 The effect of molasses on germination of spores of *Colletotrichum gloeosporioides* on leaf disks after 14 h

Treatment	Mean % spores geminated ± SE	Mean % appressoria formed ± SE
Experiment 1		
Molasses 2.0%	100.0	0.0
Molasses 1.0%	100.0	3.3 ± 0.9
Molasses 0.1%	94.9 ± 1.2	18.8 ± 1.0
Control (water)	79.6 ± 3.2	44.8 ± 4.9
Experiment 2		
Molasses 1.0%	100.0	15.0 ± 2.9
Molasses 0.1%	100.0	21.5 ± 4.7
Control (water)	100.0	52.4 ± 3.1
Experiment 3		
Molasses 1.0%	100.0	9.0 ± 1.2
Molasses 0.1%	100.0	33.1 ± 9.7
Control (water)	98.1 ± 0.7	88.7 ± 5.3

treated with water or with 0.02% molasses. At the highest molasses concentration, there was a 4-fold increase in yeast numbers. In both experiments, there was a significant reduction in disease (per cent lesion incidence) in fruit treated with yeasts when compared to the fruit with no yeast treatments (Table 3).

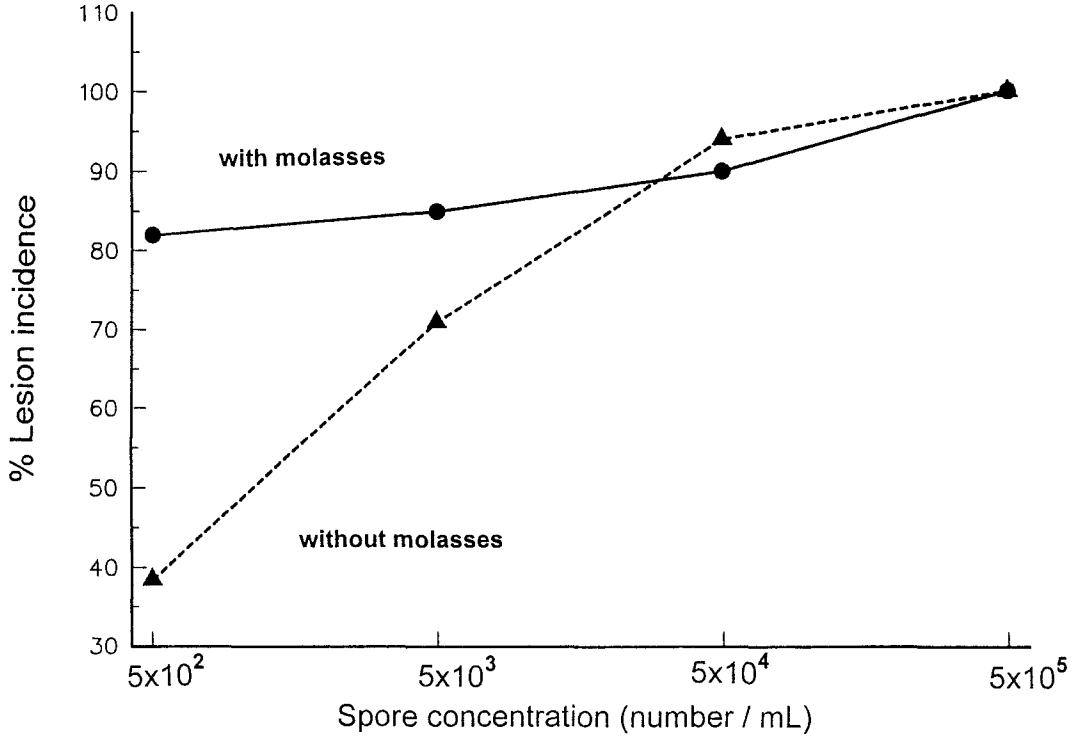

Figure 3 Incidence (%) of lesions on detached avocado fruit inoculated with different concentrations of spores of *Colletotrichum gloeosporioides* with or without molasses (2%).

Table 3 The effect of antagonistic yeasts in the presence or absence of molasses on the pathogenicity of *Colletotrichum gloeosporioides* to detached avocado fruit when inoculated at the same time as molasses and yeasts (Time 1) or 24 h later (Time 2)

	Mean lesion incidence (%)				
	Effect of yeasts				
	Aureobasidium sp. 274	Pink yeast 734	*Cryptococcus* sp. 772	No yeast (control)	LSD ($P=0.05$)
Experiment 1	0.65 (36.6)[A]	0.89 (60.4)	0.94 (65.2)	1.32 (93.8)	0.20
Experiment 2	0.71 (42.5)	1.19 (86.2)	1.29 (92.3)	1.54 (99.9)	0.18
	Effect of time				
	Time 1	Time 2	LSD ($P=0.05$)		
Experiment 1	0.88 (59.4)	1.03 (73.5)	0.14		
	Effect of molasses				
	No molasses[B]	0.02%	2.0%	LSD ($P=0.05$)	
Experiment 2	0.94 (65.2)	1.03 (73.5)	1.21 (87.5)	0.18	

[A]Data arcsine transformed; values in parentheses are equivalent means.
[B]Yeasts and/or pathogen suspended in water.

Aureobasidium sp. 274 gave the best control in the two experiments. In experiment 1, there was significantly less disease in fruit when the pathogen was inoculated soon after yeast application but this was not apparent in the second experiment. Lesion incidence increased as the molasses concentration increased in the second experiment but not in the first (Table 3). In both experiments, lesion diameter was significantly less in yeast-treated fruit when compared to the controls (data not shown).

In the field trial at Maleny, the total monthly rainfall (mm) from September 1994 to April 1995 was 11.6, 53.0, 60.4, 242.2, 122.4, 728.0, 151.2, and 145.2, respectively. Populations of filamentous fungi, yeasts and bacteria on avocado leaves in the pre-spray sample in October 1994 were similar in the half-trees selected for subsequent spraying with water or molasses (Table 4). In December 1994, 7 weeks after the first molasses spray, only yeast populations were significantly higher in the molasses treatment. On the other hand, all categories of microorganisms were significantly higher in the molasses treatment than the control in January 1995, 8 days after the second molasses spray (Table 4). The appearance of the fruit in the two treatments was similar throughout the season but colonies of *Cladosporium* spp. were sometimes visible on the underside of some leaves sprayed with molasses. There was no difference in the amount of *C. gloeosporioides* isolated from the peel of mature unripe fruit from the two treatments. The mean percentage incidence of isolation for the molasses- or water-sprayed fruit was 67.2 and 68.6, respectively. The range of other fungi isolated from the peel in the two treatments was also similar. There was no significant difference in the disease rating in ripened fruit in the two treatments, the means for molasses or water sprayed fruit being 3.3 and 3.4, respectively.

Discussion

If nutrients are to be sprayed onto avocado trees to stimulate beneficial microorganisms, important practical considerations are the cost and availability of the nutrient source and its conduciveness to microbial growth. The choice of urea was based on data presented by Crosse *et al.* (1968), and Burchill and Cook (1971) for the apple scab pathogen, *Venturia inaequalis* (Cke.) Wint. Urea sprayed on apple leaves had a stimulatory effect on naturally occurring fluorescent pseudomonads and other Gram-negative bacteria that were shown to be antagonistic to *V. inaequalis* in culture. Fluorescent pseudomonads are frequently isolated from the avocado phylloplane (Stirling 1996) but in this study, these and other bacteria were not stimulated by the addition of urea.

Table 4 Populations of filamentous fungi, yeasts and bacteria on avocado leaves unsprayed or sprayed with molasses

Sampling dates	Mean number of microorganisms (cfu/g)		
	Molasses	No molasses	LSD ($P=0.05$)
Filamentous fungi			
October 1994[A]	4.5	4.5	NS[D]
December 1994[B]	4.2	3.9	NS
January 1995[C]	4.8	4.2	0.20
Yeasts			
October 1994	5.1	5.1	NS
December 1994	4.9	4.5	0.26
January 1995	5.4	4.7	0.23
Bacteria			
October 1994	5.1	5.2	NS
December 1994	4.6	4.7	NS
January 1995	5.5	5.1	0.25

[A]Sample taken one day before first molasses spray.
[B]Sample taken 7 weeks after first molasses spray.
[C]Sample taken 8 days after the second molasses spray.
[D]NS = not significant.

Molasses is a relatively inexpensive and readily available source of nutrients and its suitability as a substrate for microbial growth was demonstrated by liquid culture studies (Stirling 1996). Therefore, the significant increase in populations of filamentous fungi, yeasts and bacteria after a single molasses spray at the Brookfield site was not unexpected. Molasses contains sucrose as the main carbon source and many other carbohydrates, amino acids, vitamins and minerals (Paturau 1989) which are conducive to microbial growth. Temporary enhancement of microorganisms by exogenous nutrients on aerial plant surfaces has been reported by several workers (Bashi and Fokkema 1977; Morris and Rouse 1985; Andrews 1992) but this report is the first for molasses. The positive response of avocado phylloplane microorganisms to molasses was confirmed in a long-term experiment at Maleny in which it was sprayed several times over a 7-month period. The increase in microbial populations in this experiment was not as pronounced as in the first, perhaps because it was carried out in a high rainfall environment and some of the molasses was most probably washed from leaves by rain. Molasses undoubtedly stimulates initial microbial activity on leaves, but may not have been solely responsible for the continuing elevated populations of microorganisms on the avocado phylloplane. The initial increase in microbial biomass with molasses would have increased the level of microbial metabolites and detritus on the leaf surface, providing a further source of nutrients for microorganisms. However, this nutrient cycling would not occur indefinitely and populations could be expected to gradually normalise once nutrients became limiting (Fokkema *et al.* 1979).

In some instances, selected antagonistic microorganisms may suppress disease on the phylloplane if present in sufficient numbers for sustained periods of time (Bashi and Fokkema 1977; Fokkema *et al.* 1979; Dik *et al.* 1992). One method of maintaining these microorganisms may be to apply them with a conducive nutrient source. In the present study, the beneficial effect of molasses on colonisation potential was more encouraging for yeasts than for bacteria. The increase in *Aureobasidium* sp. 274C1 and *Cryptococcus* sp. 772C1 after they were applied with a single spray of molasses was similar to that observed for natural populations of these organisms at the sites at Brookfield and Maleny. On the other hand, the rapid decline of *Pantoea* sp. 632R2 and *P. fluorescens* 677R1 was unexpected, as growth studies in liquid culture had indicated that molasses was not detrimental to these bacteria (Stirling 1996).

Relatively large numbers of microorganisms were sprayed onto leaves, but numbers recovered in leaf washings were low. There could be several reasons for this poor recovery. The physiological condition of cells subjected to rapid growth rates in liquid culture may have meant they were unsuitable for release into a hostile environment such as a leaf surface. Alternatively, established microflora on the phylloplane may have been too competitive for the introduced bacteria. However, failure to detect introduced bacteria may not necessarily mean lack of survival. Bacteria can be non-culturable but still remain viable as has been demonstrated for *Pseudomonas syringae* Van Hall on bean leaves, where 75% of the population was non-culturable within 80 h of spraying (Wilson and Lindow 1992).

Although nutrients could be useful in stimulating the activity of microbial antagonists, it is possible that those nutrients could also affect the pathogen. Our studies on the effect of molasses on *C. gloeosporioides* on avocado leaves and fruit showed that a similar number of spores of *C. gloeosporioides* germinated on leaf disks after 14 h in molasses or water but progressively fewer appressoria were produced with increasing concentrations of molasses. Such an increase in mycelial growth and a decrease in appressorium formation in the presence of exogenous nutrients has been observed by others for some *Colletotrichum* spp. (Emmett and Parbery 1975; Parbery 1981). When molasses was applied to detached avocado fruit at 1.0 and 2.0%, it stimulated disease development by *C. gloeosporioides* because lesions appeared on green fruit 3–4 days after inoculation. The enhancement of disease in the presence of molasses was confirmed by inoculating detached fruit with decreasing concentrations of fungal spores in the presence or absence of molasses. These results suggested that *C. gloeosporioides* grows saprophytically in the presence of exogenous nutrients. Although appressorium formation may initially be delayed, this appears to be a temporary effect. Eventually, appressoria are formed and rapid infection of green fruit occurs. A similar observation has been made for *Colletotrichum graminicola* (Ces.) G.W. Wils. on maize leaves (Williamson and Fokkema 1985). Exogenous nutrients initially delayed appressorium formation but eventually numbers doubled, resulting in more infection.

When individual microorganisms are introduced into a system involving nutrients and the pathogen, a three-way interaction is involved. Our study with selected yeasts on detached fruit with or without molasses showed that lesion incidence and lesion size due to *C. gloeosporioides* was less than when the antagonists were absent. In one experiment, disease was least severe when antagonistic yeasts were present without molasses suggesting that nutrients were not important for disease suppression. However, the interactions between yeasts, the pathogen and application times were obviously complex since in one experiment disease severity in fruit increased as the molasses concentration increased while there was no effect of molasses in the other. Yeast population densities also did not appear to have an effect on disease severity. Antagonists applied 1 h before application of the pathogen suppressed disease to a greater extent than when applied 24 h prior to the fungus and had increased in numbers on the fruit surface.

The field trial showed that regular molasses sprays did not increase the severity of anthracnose. Since this trial was carried out in an orchard which had not been sprayed with pesticides for several years and disease levels were naturally low, there was possibly a buffering effect of a well-developed, saprophytic microflora (Stirling 1996). Further evidence that nutrients do not necessarily increase anthracnose in avocado fruit was obtained in a series of field trials that studied the efficacy of biocontrol agents (L. Coates, personal communication). Nutrients applied with or without bacteria did not exacerbate disease.

The results of this study suggest that application of nutrients including molasses to avocado trees can enhance populations of naturally occurring microorganisms and certain introduced antagonists. Molasses applied to detached fruit enhanced disease but when sprayed in the field appeared to have little effect on anthracnose. Notwithstanding this, nutrients may be useful in building up depleted, useful saprophytic microbial populations in orchards subjected to frequent, long-term chemical spraying. Extensive field trials in a number of different environments over several seasons will be required to test such an hypothesis.

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