A predictive model for biological control of cercospora spot: Effect of nutrient availability

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ABSTRACT

In South Africa, uncertainty over the ability of the biological control agent Bacillus subtilis *(Avogreen®) to control anthracnose (*Colletotrichum gloeosporioides*) and cercospora spot (*Pseudocercospora purpurea*) under variable environmental conditions of avocado has restricted its commercial use. Field application of Avogreen® with or without added nutrients during the 2004/05 season resulted in no cercospora control. Avogreen on its own provided control of anthracnose for fruit destined for the local market but not for export fruit. Adding nutrients improved control of anthracnose for simulated export consignments. Ineffectiveness might be contributed by unavailability of nutrients necessary for the specific biological control mechanism on the phylloplane. Ammonium chloride and D-(+)-trehalose supported antagonism without supporting pathogenic growth. All variables affecting antagonist performance will subsequently be integrated into a predictive modelling program for* B. subtilis *that will consist of a combination of climatological information and antagonist behaviour as influenced by agricultural practices and the physical environment.*

INTRODUCTION

In South Africa avocado (*Persea americana* Mill.) is susceptible to various fruit diseases with cercospora spot (*Pseudocercospora purpurea*) regarded as one of the most important pre-harvest diseases and anthracnose (*Colletotrichum gloeosporioides*) as one of the most important post-harvest diseases (Darvas & Kotzé, 1987). Currently, the use of synthetic fungicides is the primary means of controlling these diseases (Lonsdale & Kotzé, 1989). Following the need to reduce chemical residue levels on fruit after harvest, potential environmental contamination and the occurrence of fungicide tolerant strains of pathogens, biocontrol has emerged as one of the most promising alternatives (Spadaro & Gullino, 2003; Ippolito & Nigro, 2000). The ability of Avogreen® to control cercospora and anthracnose has been demonstrated successfully on both commercial and semi-commercial scale (Van Eeden & Korsten, 2004; Van Eeden *et al*., 2003; Korsten *et al*., 1995; Korsten *et al*., 1993; Van Dyk *et al*., 1997). Uncertainty over the ability of the biocontrol agent to perform under variable environmental conditions has, however, restricted the use of this organism in disease control programs.

Effective suppression of plant pathogens by biological control organisms is largely based on their ability to survive in the environment. If parameters affecting survival can be correlated with disease control, it may be possible to manipulate or promote these parameters in order to improve control of the disease (Collins & Jacobsen, 2003; Collins *et al*., 2003). For microbial colonization to occur on leaves, a carbon source for energy and growth and a nitrogen source must be present (Mercier & Lindow, 2000). These growth substrates may be derived from leakage from the waxy cuticle (Wrona & Gleason, 2005). Sources of nutrients are usually available in the form of pollen, honeydew, dust, air pollution and microbial debris (Leveau & Lindow, 2001; Brock *et al.*, 1994; Atlas & Bartha, 1993). A variety of volatile and semi volatile organic compounds such as flower scents, pheromones, attractants and deterrents, plant sap leakage from wounds (inflicted from frost or insect damage) further provides nutritional sites for a large range of micro-organisms (Lindow & Brandl, 2003; Riederer *et al.*, 2002; Leveau & Lindow, 2001; Mercier & Lindow, 1998). Upon arrival on a new leaf surface, microbes usually find themselves in an environment with sufficient nutrients to carry the indigenous population and enough nutrients to allow a short adaptation period. The sugar levels are depleted and growth of the population slows down and stabilise in equilibrium with the nutrients available to the indigenous microbial population (Leveau & Lindow, 2001). However, as microbes differ in their ability to colonise different surfaces, so might their ability to metabolise different compounds (Leveau & Lindow, 2001). The composition and quantity of nutrients in the phylloplane is largely influenced by plant species, leaf age, leaf physiological status and tissue damage, and it is therefore suggested that these factors also determine the species in the phylloplane and the ability of micro-organisms to survive in this environment (Yang, 2000; De Jager *et al.*, 2001; Jacques *et al.*, 1995).

Competition for these nutrients is often suggested as a potential mode of action in biological control systems (Everett *et al.*, 2005; Janisiewicz & Bors, 1995). For this, both the pathogen and the antagonist must have the same requirement for a specific nutrient (Everett *et al*., 2005; Janisiewicz & Bors, 1995). If the growth limiting nutrient for the competing microbes differ, the addition of the nutrient that favours the antagonist may lead to an increased population size and therefore improved biocontrol (Janisiewicz *et al*., 2000). Presence of certain nutrients may also result in the production of antifungal volatiles (Fidaman & Rossall, 1994) and antibiotics (Milner *et al*., 1995). Incorporation of these nutrients during biological control may therefore have an effect on the success of disease control (Everett *et al*., 2005; Havenga, 2004).

The main objective of this study was to investigate the effect of nutrients on the ability of *B. subtilis* to successfully control cercospora and anthracnose of avocado.

MATERIALS AND METHODS

The percentage disease free fruit was monitored in a commercially sprayed orchard in order to determine the effect of added

nutrients on *B. subtilis*. Ten trees per treatment were randomly selected from Orchard 12 (28-year-old 'Fuerte' trees) on the farm Sterkstroom in the Limpopo Province. Avogreen (10^8 cells/ml Stimuplant cc, Pretoria) was applied to treatment 2, 3 and 4 at the time intervals indicated in **Table 1**. A nutrient mixture was obtained from Stimuplant (Pretoria). This mixture consisted of a minimal medium containing low levels of tri-ammonium citrate and L-aspartic acid as carbon and nitrogen sources (Havenga, 2004).

The mixture was added at 5 ml/ℓ to the Avogreen spray tank in treatment two and at 2 g/ℓ to the Avogreen spray tank in treatment three. Coprox Super (Copper Oxychloride, Saadchem) (powder formulation) and Knowin 500 SC (Carbendazim, Plaaschem) (systemic fungicide) (0.5 ml/ℓ) were sprayed alternately in treatment four (chemical control). Treatment one was left unsprayed and served as the control. All spray schedules corresponded with the dates for the commercial programme for the area (**Table 1**). Applications were made using handguns at 30 ℓ/tree. Fruit were harvested during the commercial harvest season.

Pre- and post-harvest disease assessment took place at Springfield packhouse. Fruit were rated for cercospora spot on a 0 – 6 scale, where 0 represented uninfected fruit and 6 severe infection before subjecting it to a Gezogerm (25 ml/ℓ) bath. Fruit from each treatment were commercially packed before the treatments were split into two batches. One batch was kept at room temperature for immediate ripening to simulate local marketing conditions and the other was first commercially stored at 5°C at the packhouse for 28 days prior to ripening at room temperature to simulate export conditions. Once ready to eat all fruit was assessed for anthracnose on a 0 – 10 scale (0 = uninfected fruit; 10 = severely infected fruit). Data from all experiments were analysed using the GenStat statistical program (2000).

Table 1. Spray program for pre-harvest applications of Coprox Super, Knowin 500SC, Avogreen® and Avogreen® combined with nutrient mixtures in the 2004/05 growing season.

Treatment number	Treatment program	Rate	Month of application			
			Oct	Nov	Dec	Jan
1	Control					
2	Avogreen®	5 ml / ℓ	✓	✓	✓	✓
3	Combination A					
	Avogreen®	5 ml / ℓ	✓	✓	✓	✓
	Nutrient mixture	5 ml / ℓ	✓	✓	✓	✓
4	Combination B					
	Avogreen®	5 ml / ℓ	✓	✓	✓	✓
	Nutrient mixture	2 ml / ℓ	✓	✓	✓	✓
5	Chemical control					
	Coprox Super	3 g / ℓ		✓		✓
	Knowin 500SC	0.5 ml / ℓ	✓		✓	

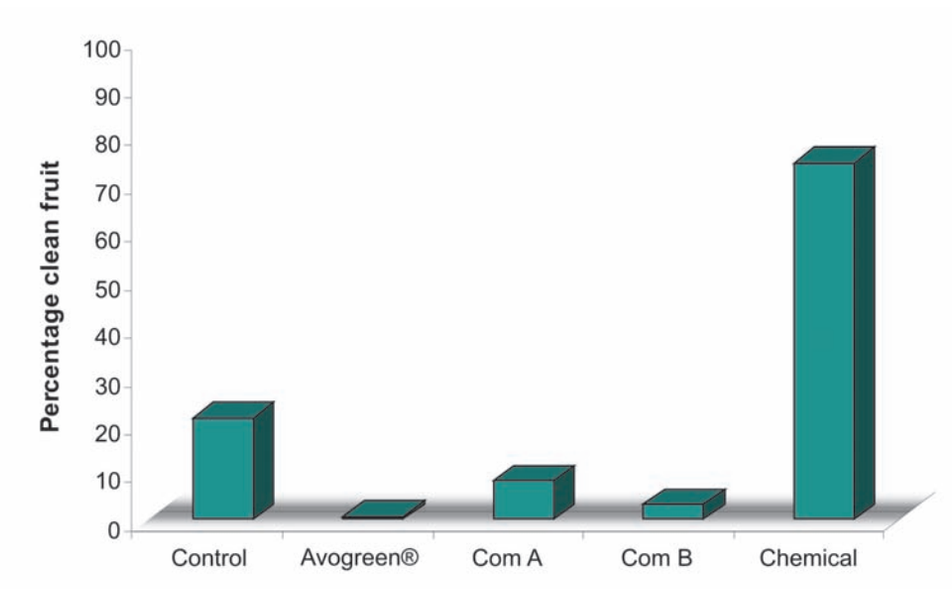

Figure 1. Effect of nutrients on biocontrol efficiency on fruit rated for cercospora.

RESULTS AND DISCUSSION

When a sample of individuals is classified, according to two attributes, results are presented in a two-way frequency table known as an r x c contingency (Snedecor & Cochran, 1980). In this study, individual values were classified according to each treatment and to percentage clean or export fruit. This information was required to determine if the distribution of clean fruit is the same for each treatment. The Chi-square row-by column test is useful to determine if there are significant differences between the two independent attributes. This test has certain limitations (Siegel, 1956), namely, no category may have

an expected frequency of less than three.

Several authors have reported the complexity of the metabolism of *B. subtilis* (Sung & Yasbin, 1998). Bacteria, in general, are renowned for their metabolic efficiency and adaptability. In order to respond quickly and appropriately, they measure extracellular nutrient concentrations, intracellular nutrient pools and fluxes in the concentration of these pools (Fisher & Sonenshein, 1991).

Both nutrient treatments, although not significantly, seemed to increase the ability of *B. subtilis* to control cercospora spot, with combination A (higher concentration) slightly more effective than combination B (**Figure 1**). The chemical disease control program was significantly more successful than any of the other treatments for both cercospora spot and anthracnose rated for local and export markets (**Figure 1** to **3**). All three the biological control treatments showed lower percentage clean fruit than the control (**Figure 1**).

This might be contributed to nutrient depletion on the plant surface. On arrival on a leaf surface, a micro-organism finds itself in an environment with sufficient nutrients to allow short and transient adaptation to the new environment. The organism multiplies and starts colonization of the local area. As the nutrient source is depleted, cells cease multiplication and the total population slows down and eventually halts (Leveau & Lindow, 2000). *B. subtilis* are known to sporulate under nutrient poor conditions (Atlas & Bartha, 1993).

Because the entire leaf population responds to the lowering in nutrient levels, the total microbial population on the leaf surface also decrease (Leveau & Lindow, 2000). This may result in colonisation of other microbes including pathogens on the plant surface. In the control treatment, the normal phylloplane population still exist and offers slightly more efficient disease control.

The Avogreen® treatment showed cleaner fruit than the control for fruit rated for anthracnose on the local market (**Figure 2**). The nutrient treatments showed reduced control ability in **Figure 2**. This might be contributed to depletion of the added nutrients over time, resulting in a lower population level (therefore reduced control) that is in equilibrium with the nutrients left on the fruit surface (Leveau & Lindow, 2001).

There was no statistical difference between any of the treatments in fruit rated for anthracnose after export simulation (**Figure 3**). Results showed slightly better control in the two nutrient treatments than in the Avogreen® treatment. It is possible that more *Bacillus* spores were present on the fruit in the nutrient treatments than in the Avogreen® treatment due to the nutrient addition.

The spores of *B. subtilis* are known for their resistance and survival potential at extreme temperatures (Atlas & Bartha, 1993), which may become viable at more favourable environmental conditions, favouring early colonisation (before other micro-organisms) on the plant surface and therefore more optimal control (Atlas & Bartha, 1993).

Insufficient control might therefore be contributed to nutrient unavailability. Havenga (2004) examined the effect of different glucose and nitrogen based nutrients on control of *C. gloeosporiodes*. In *in vitro* studies, a medium containing citric acid, D-(+)-galactose, pyruvate and benzoate provided a basis for sustained inhibition by *B. subtilis* against *C. gloeosporioides* (Havenga *et al*., 1999). Havenga (2004) suggested that improved control can be established through more selective nutrient selection.

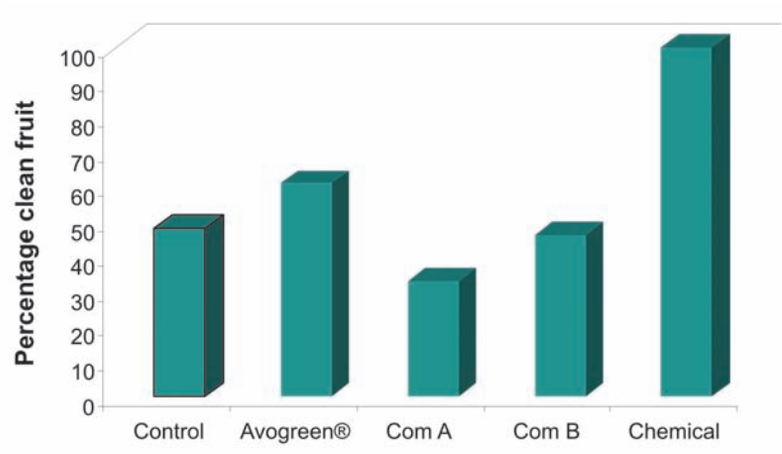

Figure 2. Effect of nutrients on biocontrol efficiency on fruit simulated for the local market and rated for anthracnose.

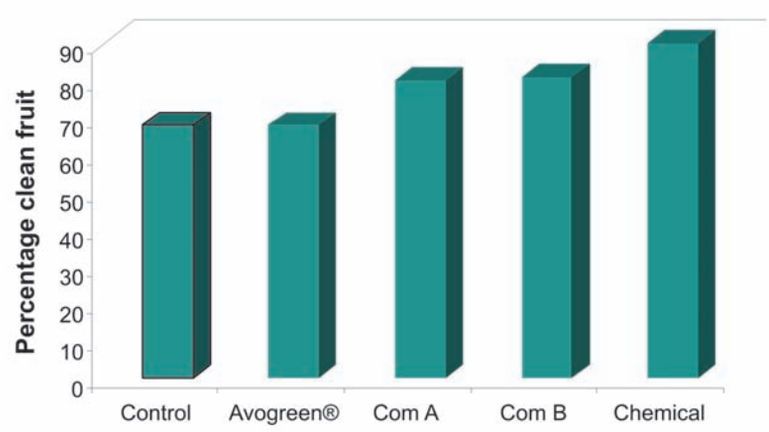

Figure 3. Effect of nutrients on biocontrol efficiency on fruit simulated for the export market and rated for anthracnose.

They found that the amino acid that supported antagonism most optimally was ammonium chloride, while L-arginine, L-glutamic acid, L-(+)-asparagine, L-glutamine and L-alanine also supported antagonism without increasing the growth of the pathogen (**Table 3**). It was further found that the carbon source most effective for enhancing antagonistic potential of the biocontrol product without favouring the pathogen is D-(+)-trehalose (**Table 4**). L-(-)-arabinose, pyruvate, D-(+)-mannitol, D-(-)-sorbitol and starch also promoted antagonism and not pathogen growth. Biocontrol might therefore be enhanced through addition of specific carbon and nitrogen sources (Collins & Jacobsen, 2003; Collins *et al*., 2003; Janisiewicz *et al*., 1992).

Certain biocontrol mechanisms may, however, also be repressed through the addition of specific nutrients. According to Fiddaman & Rossall (1993), the addition of sugars such as D-glucose, D-galactose and sucrose suppress ammonia production by the bacterium.

They found *B. subtilis* only formed antifungal volatiles during low sugar concentrations. A range of activity was detected on different media, and most probably reflected the metabolic activity of the organism in the presence of different substrates. These results were confirmed by Collins *et al*. (2003), who found that disease control was not necessarily correlated with *Bacillus* cell density during biological control of cercospora spot on sugar beet.

The specific mode of action can therefore be targeted through selective nutrient selection (Collins & Jacobsen, 2003; Wisniewski *et al*., 1991; Slininger &

Jackson, 1992; Krebs *et al*., 1993; Fiddaman & Rossall, 1994; Milner *et al*., 1995; Duffy & Défago, 1999). It is therefore very important to target the specific mechanism involved during biocontrol when nutrient addition is considered.

Insignificant control of cercospora and anthracnose in the current study is in clear contrast with previous results. Cercospora control during the past two years was between 80% and 100% in commercial and semi-commercial trials. Anthracnose control varied between 87% and 97% for local market fruit and 60% to 97% for fruit simulated for export conditions (Van Eeden & Korsten, 2004; Van Eeden *et al*., 2003). The current study suggests that one of the reasons for this phenomenon might be insufficient supply of specific nutrients in the phyllosphere. Future studies will focus on the effect of tree age, tree health, leaf health, leaf age, leaf location, ripening stage, cultivar and environmental conditions on naturally occurring nutrients in the phyllosphere. Further field trials on the effect of nutrient addition during biocontrol programmes, and the timing of their application, will also be conducted.

Increased economic and environmental pressure has led to a need for more accurate and safe crop protection measures (Levitan, 2000). Although existing cercospora spot forecasting models currently contribute to targeted disease control programs for avocados (Darvas, 1982), integration of such a model with a biocontrol predictive system may provide growers with a disease control program that adheres to the requirements of organic programmes and EurepGap. This model will also focus on eliminating variable parameters that may influence product performance. These parameters include climatic variables and agricultural practices. The final aim of this study will be to incorporate all variables investigated during the past four years into a predictive modelling system for survival of *B. subtilis* on avocado fruit.

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