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Evaluation of pre-harvest *Bacillus licheniformis* sprays to control mango fruit diseases

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Abstract

Bacillus licheniformis was evaluated as a pre-harvest spray treatment either on its own or alternated with copper oxychloride to control mango fruit diseases. Prior to initiating the spray trials, *in vitro* and *in vivo* studies were done to determine the effect of stickers, spreaders, a biostimulant and a copper fungicide on the biocontrol agent's ability to effectively attach to and colonise the mango leaf surface. Bioboost, Nufilm-P, Biofilm and Agral 90 did not affect antagonist growth *in vitro*. However, copper oxychloride and Supafilm inhibited the *in vitro* growth of *B. licheniformis*, more pronouncedly after 8 h. The *in vivo* study showed that stickers and spreaders did not improve the ability of *B. licheniformis* to attach to and colonise the leaf surface. Pre-harvest *B. licheniformis* applications alone and alternated with copper sprays applied at 3-weekly intervals from flowering until harvest controlled moderate levels of anthracnose, bacterial black spot and soft rot.

Keywords: Biocontrol; Antagonists; Anthracnose; Bacterial black spot; Soft rot

1. Introduction

Mango (*Mangifera indica* L.) is a highly prized exotic fruit on European markets, and one of the most important fruit crops grown in tropical and subtropical regions (Nakasone and Paull, 1998; Nofal and Haggag, 2006). With its origin in India, mango has been grown for around 6000 years, and is currently cultivated in many parts of the world (Vivekananthan et al., 2004). South Africa exports the bulk of its mangoes to Central Europe (Finnemore, 1999; PPECB Export Directory, 2005) and is competing with India, Pakistan, Mexico, Brazil, Peru, Venezuela, Jamaica, Australia, Egypt, Ivory Coast and Mali on international markets (Nakasone and Paull, 1998; Nofal and Haggag, 2006). In order to remain competitive, the South African mango industry has to ensure that export consignments adhere to consistent high levels of quality, product safety, absence of diseases or insect damage and reduction of chemical residues.

One of the most important pre-harvest diseases of mango is bacterial black spot (BBS), caused by *Xanthomonas campestris* pv. *mangiferaeindicae*. The estimated pre-harvest yield loss due to this disease is more than 80% on chemically untreated young orchards, especially with susceptible cultivars (Boshoff et al., 1998). Post-harvest diseases affect fruit quality, limiting the product's marketability and causing further economic losses. Anthracnose, caused by *Colletotrichum gloeosporioides* Penz. (Vivekananthan et al., 2004), and soft rot (SR) including stem-end rot and body rot, caused by the *Fusicoccum* anamorph of *Botryosphaeria* (Jacobs, 2002; Slippers et al., 2004), are the most common post-harvest diseases of mango (Johnson et al., 1991; Bugante et al., 1997).

Current control measures for mango fruit diseases include extensive pre-harvest spraying with copper oxychloride. However, the build-up of copper levels in soils has become an area of concern for the local industry due to new restrictive requirements by major retailers regarding allowable residues in agricultural soils. Furthermore, stricter maximum residue levels set by the European Union (EU) and the reduction of available, registered fungicides,

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particularly for niche crops, have negatively impacted on the availability of effective chemical control products. Together with the EU requiring all pesticides to be re-registered, this resulted in a move towards the development of alternative disease control measures. One such alternative is biological control, which has been evaluated successfully on various crops, including mango (Gerhardson, 2002; Vivekananthan et al., 2004; Govender et al., 2005). Pre-harvest mango field sprays with the antagonist *Bacillus licheniformis* proved effective in controlling both pre- and post-harvest diseases (Korsten et al., 1991; De Villiers and Korsten, 1994). Furthermore, preliminary integration of chemical and biological control proved to be a viable alternative for control of mango fruit diseases (De Villiers and Korsten, 1996).

In order to initiate a successful biological control programme, fundamental data concerning the relationship between antagonist, pathogen and the environment are required. Further, the effect of commercial fungicides or additives on the viability and performance potential of the antagonist need to be determined (Korsten et al., 1992). This is of importance since different additives (e.g. stickers, spreaders and wetting agents) are commercially applied with fungicides to enhance their adherence to the leaf surface (Hassal, 1990; Harvey, 1992). With field sprays, adequate coverage of the foliage and fruit and effective attachment of the product to the surface are necessary to optimise crop protection. Different stickers and spreaders including Biofilm, Nufilm-P and Agral 90 were previously evaluated with no negative effect on the attachment and survival of *B. licheniformis* observed (Korsten et al., 1992). The ability of bacteria to attach effectively to plant surfaces for prolonged periods of time is crucial to ensure that the antagonists colonise and survive on the surface (Marques et al., 2002). In order to enhance biocontrol product performance, nutrient additives are often added during applications to facilitate effective antagonist growth and optimum colonisation.

The aim of this study was therefore to: (a) evaluate semi-commercial pre-harvest *B. licheniformis* sprays applied on their own or alternated with copper oxychloride on mango trees at 3-weekly intervals from flowering until commercial harvest for the control of fruit diseases; (b) evaluate the efficacy of different additives (Biofilm, Supafilm, Nufilm-P and Agral 90) in terms of enhanced attachment and colonisation of the leaves/fruit by *B. licheniformis*; (c) evaluate the fungicide, copper oxychloride, for its compatibility with the antagonists when used in integrated applications; and (d) evaluate a biostimulant, Bioboost, for its potential growth stimulatory activity on the antagonist.

2. Materials and methods

2.1. Effect of additives on B. licheniformis

Six different compounds were evaluated *in vitro* to determine their effects on the antagonist's growth. These included a fungicide, copper oxychloride (UCP Universal, Johannesburg, South Africa; 2.5 g active ingredient (a.i.) l^{-1}); a biostimulant, Bioboost (Plaaskem, Johannesburg, SA; 0.2 g a.i. l^{-1}) and four different adjuvants: Biofilm (spreader-sticker, Plaaskem; 2.5 g a.i. l^{-1}), Supafilm (sticker-spreader, Plaaskem; 2.5 g a.i. l^{-1}), Nufilm-P (wetter-sticker, Hygrotech, Tzaneen, SA; 2.6 g a.i. l^{-1}) and Agral 90 (wetter-spreader, Kynoch Agrochemicals, Durban, SA; 1.8 g a.i. l^{-1}). The a.i.s of the agents were as follows: Bioboost: vitamins, enzymes, plant growth stimulants; Biofilm and Supafilm: wetting agents, fatty acids, glycol ethers, spreading agents; Nufilm-P: pine resin, fatty acids, glycol ethers; and Agral 90: alkylated phenyl–ethylene oxide condensate. The diffusion test (Du Toit and Rautenbach, 2000) was adopted as follows: Standard 1 nutrient agar (STD 1) (Biolab, Johannesburg) was prepared according to manufacturers' instructions and allowed to cool to $\pm 40\,^{\circ}C$ before adding the commercial biocontrol product, *B. licheniformis*, at 10^9 cfu ml^{-1} (Stimuplant cc., Pretoria, SA) to each litre of medium. The flasks were shaken gently before pouring the medium into Petri dishes, allowing them to solidify for 24 h before use. Three agar plates were selected for each test compound, adopting a complete randomised design. Each plate was divided into halves. In the centre of each side, a well was made using a sterile cork borer (6 mm diameter). On the one side of the plate, the well was filled with 10 µl of sterilised water as control and the other with 10 µl test compound. Plates were incubated at 37 °C for 24 h. Observations were made onwards, taking three diameter measurements of each inhibition zone, if present. The experiment was performed in triplicate. Data were statistically analysed using the statistical program GenStat (2000). One-way analysis of variance (ANOVA) was used to test for differences in average mean values between the test compounds. Treatment means were separated using Fisher's protected *t*-test with least significant difference (LSD) at the 5% level of significance ($P = 0.05$).

2.2. Adherence of B. licheniformis to mango leaves

Three-year-old cv. Kent mango trees, grown in the greenhouse at the University of Pretoria's experimental farm, were selected for this trial. Four test compounds, i.e. Biofilm, Supafilm, Nufilm-P and Agral 90, were prepared at the recommended rate to evaluate enhancement of antagonist attachment and colonisation. Three trees were selected randomly for each test compound. Trees were sprayed with a hand-held one-litre sprayer containing a suspension of commercial *B. licheniformis* (10^9 cfu ml^{-1}) at a final concentration of 5 ml l^{-1} of tap water with one of the test compounds added at the recommended rate. Control trees were sprayed with a suspension of commercial *B. licheniformis* in tap water. Two leaves were picked on four sides (northern, southern, western and eastern) of each tree. Leaves were picked 1, 5, 15, 30, 60 and 120 min after spraying the different treatments. Five discs were cut from