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Short Communication

Chemical compatibility testing of the entomopathogenic fungus *Lecanicillium muscarium* to control *Bemisia tabaci* in glasshouse environment

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ABSTRACT: The potential for using the entomopathogenic fungus *Lecanicillium muscarium* to control the sweetpotato whitefly, *Bemisia tabaci* has been well established in previous studies under both laboratory and glasshouse conditions. In the current study, five chemicals were assessed for their compatibility with *L. muscarium* for control of *B. tabaci* under glasshouse conditions. On treatments following the sequential application of chemical product and fungus high mortality of second instar larvae was obtained (the known most susceptible *B. tabaci* life-stage to fungal infection). Sequential treatment of Savona and Certis spraying oil with *L. muscarium* produced 95 % and 96 % larval mortality, respectively. Commercially, unacceptable poinsettia foliage damage was recorded seven days post application of Agri-50E. Other plant foliage may prove more tolerant to this product. Incorporation of these chemicals with *L. muscarium* into integrated control programmes for *B. tabaci* control in glasshouses is discussed. Further information has been added to the knowledge base for the combined use of chemicals and fungi for the control of *B. tabaci*.

Keywords: *Insecticides; Integrated pest management; Plant; Quarantine; Whitefly*

INTRODUCTION

The sweetpotato whitefly, *Bemisa tabaci* Gennadius (Hemiptera: Aleyrodidae), is a major pest of economically important crops worldwide (Gerling *et al*., 1980; Nomikou *et al*., 2000). Damage can be caused directly by feeding on phloem sap or indirectly by the large amounts of honeydew produced lowering photosynthesis. *Bemisia tabaci* is also a vector of many plant viruses (Alegbejo, 2000: Simón *et al*., 2003). Within the United Kingdom (UK), *B. tabaci* continues to remain a notifiable pest subject to a policy of eradication if found on propagators' premises or on plants moving in trade, and of containment/eradication if outbreaks occur at nurseries (Cuthbertson, 2005). The UK has Protected Zone status against *B. tabaci* and eradication generally involves use of chemical insecticides. There are several active ingredients currently used in the UK for treating *B. tabaci* outbreaks (Sharaf, 1986; Buxton and Clarke, 1994; Cheek and Macdonald, 1994; Cannon *et al*., 2005), but with chemical resistance being shown by *B. tabaci* populations (Prabhaker *et al*., 1985; Osborne and Landa, 1992; Cahill *et al*., 1994, 1996; Ahmad *et al*., 2002) an integrated strategy using both biological and chemical agents is required. The entomopathogenic fungus *Lecanicillium muscarium* has shown significant potential for incorporation into integrated pest management (IPM) programmes for the control of *B. tabaci*, where, second instar larvae have proven most susceptible to fungal infection (Cuthbertson *et al*., 2005a). *Lecanicillum muscarium* has also shown potential for control of *B. tabaci* larvae in glasshouses on a range of plant hosts, including poinsettia (Cuthbertson and Walters, 2005a; Cuthbertson *et al*., 2008a; Down *et al*., 2009). Previous investigations into chemical insecticide compatibility with *L. muscarium* have found varying results. Hall (1981) showed that chemicals such as pirimicarb and white oil could be 'tank mixed' with the fungus for the control of aphids, and similarly Cuthbertson *et al*. (2005b) proved that the fungus could be applied simultaneously with

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buprofezin, and also, when used sequentially with imidacloprid gave a high percentage mortality of *B. tabaci* second instar larvae.

The development of control strategies for non-indigenous insects within the UK is limited by legislation which precludes the intentional release of quarantine pests into ordinary experimental glasshouses (Williams and Walters, 2000; Cuthbertson *et al*., 2009a). As no outbreak site was identified for testing the efficacy of *L. muscarium* against *B. tabaci*, a designated quarantine glasshouse at The Food and Environment Research Agency, York, was used for experimental purposes. This study therefore, further investigates the compatibility of *L. muscarium* with a range of insecticidal products for control of second instar *B. tabaci* on poinsettia plants in UK glasshouses.

MATERIALS AND METHODS

Bemisia tabaci were cultured under quarantine conditions in perspex cages (60 × 60 × 80 cm) on poinsettia (*Euphorbia pulcherrima* c.v. Lilo Pink) plants at 23 ± 1 °C following the method of Cuthbertson *et al*. (2005a,b, 2008a,b). The entomopathogenic fungus *Lecanicillium muscarium* was supplied as Mycotal from Koppert Biological Systems Ltd., UK. The selected insecticidal products to test were as follows: Majestik (natural plant extract, 2.5 mL/100 mL water, Certis UK); Agri-50E (alginate/polysaccharide, 300 µL/100 mL, Fargo Ltd, UK); Certis Spraying Oil (petroleum oil, 1 mL/100 mL water, Certis UK); Savona (fatty acids, 2 mL/100 mL water, Koppert Biological Systems Ltd, UK); Oberon (spiromesifen, 0.05 g/100 mL water, Bayer CropScience). Following the method of Cuthbertson and Walters (2005a) three separate glasshouse trials were undertaken. Each trial tested two different chemicals, both on their own and in combination with *L. muscarium* in sequential treatment. Designated quarantine glasshouse cubicles were used for experimental purposes.

For each trial thirty plants were infested with *B. tabaci* following the methods of Cuthbertson *et al.* (2003, 2005a). After egg laying had occurred and the adults had been removed, the infested plants were transferred in sealed boxes to the designated glasshouse cubicle. The plants (treatments) were arranged randomly throughout the cubicle and conditions maintained at 25 °C for a further twelve days to allow the second instar to develop. After twelve days, the plants were divided into six groups, each containing five plants. Ten plants received a treatment of Savona, 10 plants a treatment of Certis Spraying oil and 10 a treatment of water as control. These were then left for 24 h after which: five of the Savona treated plants received an application of *L. muscarium* as did five of the Certis Spraying oil treated plants. Five of the water treated plants also received an application of *L. muscarium*. The remaining fifteen plants received an application of water. Both the pesticides and the fungus were applied using a hand held Hozelock® Polyspray hand held sprayer with cone nozzles. The procedure was repeated for each glasshouse trial to test the other chemical products. The data gained underwent analysis of variance.

RESULTS AND DISCUSSION

None of the treatments of chemical followed by fungus gave any significantly better control of whitefly compared to the chemical being used on its own (Fig. 1). Higher mortality was recorded following application of *L. muscarium* on foliage previously treated with Certis Spraying oil but it was not significantly better than the chemical control (d.f.= 1,19, F=3.13, P=0.09), the same phenomena was recorded for Oberon (d.f.= 1,19, F= 2.14, P = 0.15) and Agri-50E (d.f. =1,19, F=1.46, P=0.24). However, on assessment of plants seven days post application of Agri-50E commercially unacceptable foliage damage was recorded (Fig. 2). Some Agri-50E treated leaves subsequently died and fell off the plants.

For the successful introduction of an IPM programme information is not only needed on the biology of the control agent in question (Cuthbertson and Murchie, 2004, 2007) but also on its' compatibility with other control agents, namely chemicals (Cuthbertson and Murchie, 2006). Clarification of the effects of chemical insecticides on the wide variety of biological control agents, including entomopathogenic fungi is necessary. However, to date there have been few *in vitro* tests. Different biopesticides based on *L. muscarium* are utilised on greenhouse crops to manage pests such as greenhouse whitefly, aphids and thrips in various European countries (Osborne and Landa, 1992). Also, timing of the control agent application for effective pest control has been shown to be critical, since the target instars as well as the ambient temperature/humidity can influence efficacy (Williams and Walters, 1994; Cuthbertson and Walters, 2005b; Cuthbertson *et al*., 2003, 2005a).

The implementation of an IPM scheme may require

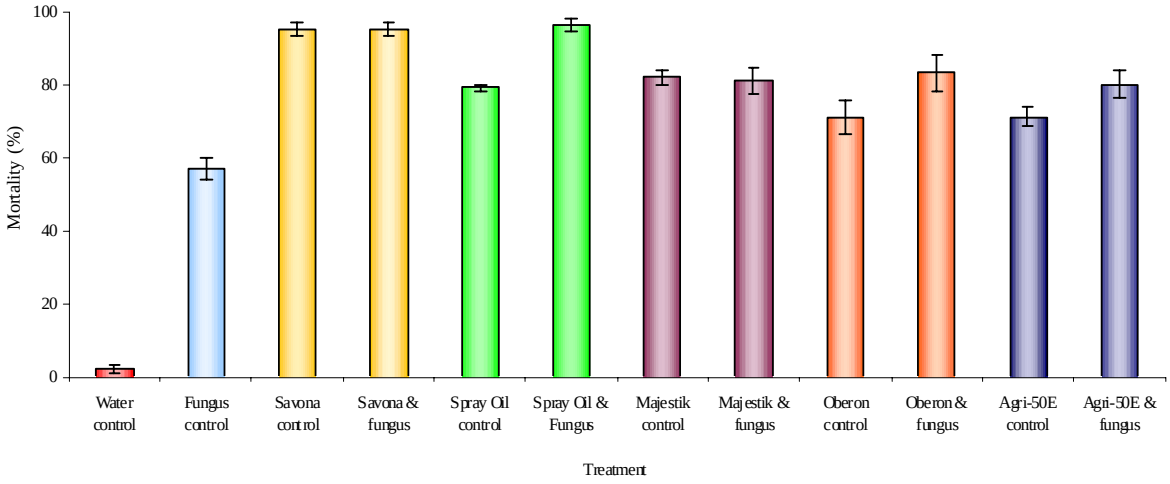

Fig. 1: Efficacy of *Lecanicillium muscarium* (10^7 conidia/mL) against second instar *Bemisia tabaci* on tomato and verbena foliage under glasshouse conditions, 20 ºC, 85 % relative humidity. Mortality recorded after 7 days. Bars are standard errors of the means (±SEM).

Fig. 2: Foliage damage recorded on poinsettia plants 7 days after spray application of Agri-50E (alginate/polysaccharide; 300 µL/100 mL water) within the glasshouse environment.

sequential rather than simultaneous applications of insecticides and entomopathogenic fungi. Apart from the study of Cuthbertson *et al*. (2005b) few previous studies have tested the effect of dry insecticide residue on fungal activity. In the current study, when *L. muscarium* was applied to plants sprayed 24 h earlier with a standard application of one of five contact insecticides, no significant reduction in infectivity (mycelial growth) was detected in any cases. Therefore, *L. muscarium* could be applied sequentially with Oberon, Savona, Certis spraying oil, Agri-50E and Majestik for the control of *B. tabaci* with second instars

again proving highly susceptible to fungal attack, as found by Cuthbertson *et al.* (2005a, 2009b); Cuthbertson and Brown (2009). However, after application of Agri-50E commercially unacceptable foliage damage was recorded (Cuthbertson, A.G.S., personal observation). It is, therefore, unlikely that this product would be a candidate for further research on poinsettia plants. Other plant species may, however, prove to be more tolerant of this product. Further work could involve applying the fungus followed by insecticides at increasing time intervals to investigate potential protection of the fungus with increasing time before application of an insecticide. Testing for Agri-50E, which did produce a high level of *B. tabaci* mortality and which also has shown previously a high potential for direct mixing with *L. muscarium* (Cuthbertson *et al.*, 2008a), phytotoxic effects on other plant species could prove useful in the development of IPM strategies.

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