AMBER. **A**pplication and **M**anagement of **B**iopesticides for **E**fficacy & **R**eliability

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AMBER: Optimising the performance of biopesticides and improving grower practice

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As the number of marketed biopesticide products increases, there needs to be more emphasis on activities that enable growers to get the best out of new products. UK growers have reported a need to get better performance from existing biopesticides in commercial crop production. This echoes the findings of a review of the future of the biopesticides industry, written by leading experts in the field, which identified improving the delivery of biopesticides to the target, understanding the persistence of activity, and getting better information to growers as priorities for making biopesticides more effective (Glare *et al.,* 2012). Here, we take a look at some of the barriers to biopesticide adoption and consider different areas where biopesticide performance could be improved.

Risk aversion.

It is undoubtedly the case that growers need support for adopting biopesticides and implementing best practice. Because conventional chemical pesticides are used so widely, best practice advice is easy to access. Generally, the knowledge needed by farmers to get effective control with pesticides is lower than with biopesticides and other new technologies (Cowen, 1991; Cowen & Gunby, 1996). As a result, potential new users of biopesticides face large costs of adoption that will only decrease once the technology is used more widely, thereby disadvantaging early adopters. For many horticultural crops, cosmetic appearance of the plant or harvested product is critical when it comes to making a profit and there is a very small tolerance for pest or disease damage. Consequently, growers tend to be risk averse with respect to new, untested crop protection technologies. It is important that growers are given more confidence in using biopesticides, therefore. Workshops on farms or nurseries are considered to be one of the most effective ways demonstrating and explaining the use of biopesticides and passing on best practice guidance. Put simply, "proof of effect is the key to adoption" (Glare *et al*., 2012). On the positive side, wew practices are adopted more readily in horticulture than in other areas of agriculture because of the greater level of education and technical competence within the industry (Lohr & Park, 2002). For the AMBER project, the protected edibles, and ornamentals sectors include some of the most technologically growers in the UK, and so in principle we should be in a good position for rapid uptake of best practice guidelines for biopesticides.

Using biopesticides in IPM.

Biopesticides are not stand-alone products but work best as part of IPM, in which different individual crop protection tools work together as a portfolio. If a nursery wants to adopt IPM then they will have to decide which combination of tools to use. In practical terms, this has to be done incrementally, and the grower may have to face difficult choices about switching from one pest / disease control tool to another. At the moment, biopesticides are being used as replacements for one or more scheduled pesticide sprays in a calendar spray programme. Under this scheme, biopesticides are used either just before harvest to take advantage of their low harvest interval, or as a pesticide resistance management tool. This tactic can be useful, but it may not be appropriate in all cases. For example, biopesticides used for plant disease management are generally applied preventatively, as they work through multiple activities including elicitation of host plant resistance, which takes time to come into effect. This means that the biopesticide has to be applied early in the crop production cycle. For protected edible crops, biopesticides used against arthropod pests can be effective when used as a second line of defence to supplement biocontrol with natural enemies (Jacobson *et al*., 2001); in this case the decision to apply the biopesticide is dependent on close monitoring of pest populations and cannot be done on a calendar basis. Biopesticides fit very well into the second line of defence strategy on crops such as tomato, cucumber and pepper where only the fruit is harvested because we can accept lower levels of pest mortality, i.e. they are being used principally to slow down the rate of increase of the pest population while the macro-biological agents re-establish their control. Ideally, combinations of individual tools are needed that work synergistically together in IPM, such that one tactic in the portfolio results in an improved performance in others (Lacey *et al*., 2001; Morales-Rodriguez & Peck, 2009). The second line of defence strategy is one way in which this can be achieved.

Technical barriers to optimising biopesticide performance.
The technical challenges to improving biopesticide performance fall into three categories: (i) improvements to the intrinsic effectiveness of individual products, including higher potency, more consistent product quality, and longer persistence of effect (derived from better active ingredients, high throughput screening and improved formulation); (ii) novel delivery methods (e.g. new types of sprayers including robotics, encapsulation of microbial cells etc.); and (iii) improved implementation (Glare *et al.,* 2012; Martin *et al*., 2012; Ruocco *et al*., 2011; Vemmer & Patel, 2013).

<u>Improving the precision of delivery</u>. It is self-evident that the efficacy of microbial and botanical biopesticides is contingent upon delivering an effective amount of the agent to the target pest or disease. Changes to the way biopesticides are applied to the plant can give improved pest and disease control by ensuring better coverage and targeting the biopesticide to where it is needed. Changes to spray application method can provide significant improvements by optimizing water volumes and droplet zone, allowing the spray to be concentrated better in the target zone (Nuyttens *et al*., 2009; Peng & Wolf, 2011). There is also a need to understand the effective concentration of the biopesticide e.g. (for microbial biopesticides) the number of microbial cells per unit area of leaf surface, or per unit volume of growth substrate, required to give efficacy. Similar concepts apply to botanicals and semiochemicals. Unfortunately, there have been very few studies where the biopesticide application rate has been based on an understanding of the effective concentration (Jaronski, 2010). And despite the fact that the biopesticide delivery method can have a profound effect on efficacy, very little research has been done on identifying practical measures for optimising application (Gan-Mor & Matthews, 2003; Gwynn, 2011). The situation is most severe for foliar applications, where there is a poor understanding of how spray application techniques (including nozzle size, operating pressure, water volume, tank system etc.) affect efficacy.

<u>Timing and frequency of application</u>. Understanding how long a biopesticide remains active on the leaf or in the soil is important for determining when and how frequently it needs to be applied. This information should be combined with an understanding of (i) the effective concentration and dose of the biopesticide; and (ii) the biology of the pest or disease, and in particular its rate of reproduction (put simply, pests and diseases that reproduce quickly need to be controlled faster than those that reproduce slowly). Many of the published studies on the persistence of biopesticides relate to environmental fate and behaviour and have been done for environmental risk assessment rather than to find ways to improve efficacy (Mudgal *et al*., 2014). The persistenc of biopesticides is affected by a range of factors including UV radiation exposure, temperature, rainfall, humidity, and the microbiota and chemistry of the plant surface or soil (Leong *et al*., 1980; Collins *et al*., 2003). In general, microbial biopesticides do not persist for long on foliar surfaces. Therefore, providing growers with useful information about biopesticide persistence would appear to have a lot of potential for improving biopesticide performance. Microbial biopesticides tend to persist for significantly longer in soil, with

survival depending on the microbial species (including whether it has the ability to grow endophytically or in the rhizosphere) as well as factors such as organic matter content, pH, temperature, and soil biota (for further information see Scheepmaker & Butt (2010), Lo *et al*. (1996), Bae & Knudsen (2001, 2005), Bin, 1991, Savazzini *et al*., 2009; Feng *et al*., 2011).

Environmental factors affecting biopesticide use. Environmental factors that significantly affect the activity of biopesticides are UV radiation, temperature and humidity. Ambient temperatures directly determine the rate of activity of all microbial biopesticides (with the possible exception of Bt) and also have indirect effects through their influence on the activity of the target pest / disease and host plant (e.g. Thomas & Blanford, 2003). It is important to know not only what the optimum temperature is for a biopesticide, but also its thermal tolerance (i.e. the upper and lower temperatures at which the biopesticide will work). Almost all of the published work on biopesticide thermal biology has been done under constant temperatures, with very few researchers investigating fluctuating temperatures. This is important, given that temperatures can fluctuate to a considerable extent on plants grown outdoors. Humidity has a strong effect on the activity of fungal biopesticides, since most fungi require freely accessible water in order to germinate and grow (e.g. Andersen *et al*., 2006). Water availability is usually not a limiting factor to the performance of biopesticides used in the soil / plant growing media, with the possible exception of biopesticides being washed out of plant containers by irrigation water. For biopesticides used on foliage, the key factor is the environment within the plant canopy, specifically the microclimate humidity at the site of interaction of the biopesticide and the target pest or disease. For most pests and diseases, the microclimate is determined largely by the size of the leaf boundary layer, which varies according to plant species, leaf size and shape, canopy structure, wind speed, and temperature (Vesala, 1998). The microclimate humidity within the canopy can differ markedly from ambient humidity, and hence it is critical that any investigation of the influence of humidity on biopesticide performance is based on monitoring the conditions within the canopy (e.g. Boulard *et al*., 2002).

Compatibility between different P&D control agents. Understanding the compatibility of biopesticides with other crop protection agents is clearly important, as it will allow growers to plan IPDM programmes in a logical, evidence-based way. Some conventional chemical pesticides are antagonistic or lethal to microbial biopesticides. Hence it is important to determine which pesticides are compatible with biopesticides in IPM (Jaronski, 2010; Salman & Abuamsha, 2015). One obvious area of concern, for example, is whether chemical fungicides are compatible with fungal control agents. Manufacturers of microbial biopesticides provide technical information sheets to growers that list which chemical pesticides are compatible / incompatible with the microbial agent, but they often do not provide details of the methods used to test compatibility. Compatibility studies are often done using *in vitro* assays of microbial growth or survival during exposure to a test pesticide. These tests are often designed to mimic the types of exposure that will occur in the field. Different types of test can also give slightly different results, and hence it is usually a good idea to conduct more than one type of test (Chandler *et al*., 2015). In some cases there can be false-positive results in which inhibition observed in vitro does not translate to field scale effects. This can occur as a result of compartmentalization of the chemical pesticide within plant tissue, lower pesticide concentrations encountered under field conditions, or drying of pesticide residues on foliar surfaces (Jaronski, 2010; Inglis *et al*., 2001; Cuthbertson et al., 2005). The categorization of “compatible” and “incompatible” used by the biopesticide manufacturers tends to follow the IOBC scheme where a chemical pesticide is defined as “harmless” towards a biological control agent if it causes less than 25% reduction in control capacity. This may not provide the level of accuracy required for growers, particularly for ornamentals where even small amounts of visible disease symptoms or individual pest insects can make a plant unmarketable, and hence a reduction in efficacy of 25% of a biopesticide could impact seriously on its usefulness.

Some biopesticides could have negative effects on each other or on natural enemies (predators and parasitoids) and hence there is a need to understand their compatibility (e.g. Seiedy & Deyhim, 2015). Growers are likely to be concerned about: (i) the effect of botanicals on microbial biopesticides; (ii) the effect of mycoparasites on fungal pathogens of insects; (iii) interference between different microbial control agents of plant disease; (iv)the effect of bio-insecticides on natural enemies. Biopesticide companies provide recommendations about the compatibility of their products with other biopesticides and with natural enemies, but the list is not exhaustive. Growers want to use biopesticides that are compatible with other biopesticides and natural enemies and will know from practical experience and information from their advisors about which crop protection agents can be used together. A meta-analysis of experimental studies of the compatibility of microbial control agents of plant disease indicated that antagonistic interactions were more likely to occur than synergistic interactions (Xu *et al.,* 2011) which can result in suboptimal levels of control (e.g. Xu *et al*., 2010). In some cases problems can be avoided if two incompatible agents are separated in space or time. There have been a number of published studies demonstrating how biopesticides can be used together with natural enemies in IPM programmes, and this has been done to develop general principles for IPM, for example the use of biopesticides as a second line of defence to natural enemies on PE crops (e.g. Jacobson *et al*., 2001; Chandler *et al*., 2005).

References

Andersen, M., Magan, N., Mead, A. & Chandler, D. (2006). Development of a population-based threshold model of conidial germination for analysing the effects of physiological manipulation on the stress tolerance and infectivity of insect pathogenic fungi. Environmental Microbiology, 8, 1625 – 1634.

Bae, Y. & Knudsen, G.R., (2001). Influence of a fungus-feeding nematode on growth and biocontrol efficacy of *Trichoderma harzianum. Phytopathology*, 91, 301-306.

Bae, Y.S. & Knudsen, G.R., (2005). Soil microbial biomass influence on growth and biocontrol efficacy of *Trichoderma harzianum. Biological Control*, 32, 236-242.

Bin, L., Knudsen, G. R. & Eschen, D. J. (1991). Influence of an antagonistic strain of Pseudomonas fluorescens on growth and ability of *Trichoderma harzianum* to colonize sclerotia of *Sclerotinia sclerotiorum* in soil. *Phytopathology*, 81, 994-1000

Boulard, T., Mermier, M., Fargues, J., Smits, N., Rougier, M., Roy, J. C. (2002). Tomato leaf boundary layer climate: implications for microbiological whitefly control in greenhouses. *Agricultural and Forest Metereology,* 110, 159 - 176

Chandler, D., Davidson, G. & Jacobson, R. J. (2005). Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) on tomato, *Lycopersicon esculentum*. *Biocontrol Science and Technology*, 15, 37 – 54.

Collins, D.P., Jacobsen, B.J. & Maxwell, B. (2003). Spatial and temporal population dynamics of a phyllosphere colonizing *Bacillus subtilis* biological control agent of sugar beet cercospora leaf spot. *Biological Control*, 26, 224-232.

Cowen, R. & Gunby, P. (1996). Sprayed to death: Path dependence, lock-in and pest-control strategies. *Economics Journal*, 106, 521-542.

Cowen, R. (1991). Tortoises and Hares: choice among technologies of unknown merit. *Economics Journal,* 101, 801-814.

Cuthbertson, A.G.S., Walters, K.F.A., & Deppe, C. (2005). Compatibility of the entomopathogenic fungus Lecanicillium muscarium and insecticides for eradication of sweetpotato whitefly, Bemisia tabaci. Mycopathologia 160, 35-41.

Feng, X.M., Holmberg, A.-J., Sundh, I., Ricard, T. & Melin, P. (2011). Specific SCAR markers and multiplex real-time PCR for quantification of two *Trichoderma* biocontrol strains in environmental samples. *Biocontrol,* 56, 903-913.

Gan-Mor, S. & Matthews, G. A. (2003). Recent developments in sprayers for application of biopesticides – an overview. Biosystems Engineering, 84, 119 – 125

Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Kohl, J., Marrone, P., Morin, L. & Stewart, A. (2012). Have biopesticides come of age? *Trends in Biotechnology*, 30, 250 – 258.

Gwynn, R. (2011). Review of literature and existing state of the art with respect to the application of microbial biopesticides in agriculture. Final report of Defra project PS2027.

Inglis, G.D., Goettel, M.S., Butt, T.M. & Strasser, H. (2001). Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N (eds) Fungi as biocontrol agents: progress problems and potential. CABI Publishing, Wallingford, pp 23–70.

Jacobson, R. J., Chandler, D., Fenlon, J. & Russell, K. M. (2001). Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants. *Biocontrol Science and Technology*, 11, 381 - 400.

Jaronski, S. T. (2010). Ecological factors in the inundative use of fungal entomopathogens, *BioControl,* 55, 159 – 185.

Lacey, L.A., Frutos, R., Kaya, H.K. & Vail, P. (2001). Insect pathogens as biological control agents: do

Leong, K.L.H., Cano, R.J. & Kubinski, A.M. (1980). Factors affecting Bacillus thuringiensis total field persistence. *Environmental Entomology*, 9, 593-599.

Lohr L. & Park T.A. (2002). Choice of insect management portfolios by organic farmers: lessons and comparative analysis. *Ecological Economics*, 43, 87-99.

Martin, L., Marques, J.l., Gonzalez-Coloma, A., Mainar, A.M., Palavra, A.M.F & Urieta, J.S. (2012). Supercritical methodologies applied to the production of biopesticides: a review. *Phytochemistry Reviews*, 11, 413 – 431.

Morales-Rodriguez, A. & Peck, D.C. (2009). Synergies between biological and neonicotinoid insecticides for the curative control of the white grubs *Amphimallon majale* and *Popillia japonica*. *Biological Control* 51, 169 – 180.

Mudgal, S., De Toni , A., Tostivint, C., Hokkanen, H. & Chandler, D. (2014). Scientific support, literature review and data collection and analysis for risk assessment on microbial organisms used as active substance in plant protection products. *EFSA technical report*, 156 pp. http://www.efsa.europa.eu/en/supporting/doc/518e.pdf

Nuyttens, D., De Schampheleire, M., , Verboven, P., Brusselman, E. & Dekeyser, D. (2009). Droplet size and velocity characteristics of agricultural sprays. *Transactions of the American Society of Agricultural Engineers,* 52, 1471 – 1480.

Peng, G. & Wolf, T. M. (2011). Improving spray deposition on vertical structures: the role of nozzle angle, boom height, trave speed and spray quality. *Pesticide Technology*, 5, 67 – 72.

Ruocco,M., Woo, S., Vinale, F., Lanzuise S. & Lorito, M. (2011). Identified difficulties and conditions for field success of biocontrol. In, Classical and augmentative biological control against diseases and pests (Ed. Nicot, P. C.). IOBC, pp 45 – 57.

Salman, M. & Abuamsha, R. (2015). Potential for integrated biological and chemical control of damping-off disease caused by *Pythium ultimum* in tomato. *Biocontrol*, 57, 711 – 718.

Savazzini, F., Longa, C. M. O. & Pertot, I. (2009). Impact of the biocontrol agent *Trichoderma atroviride* SC1 on soil microbial communities of a vineyard in northern Italy. *Soil Biology & Biochemistry*, 41, 1457-1465

Scheepmaker, J. W. A. & Butt, T. M.T (2010). Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment and in accordance with EU regulations. *Biocontrol Science and Technology*, 20, 503-552

Seiedy, M., Tork, M. & Deyhim, F. (2015). Effect of the entomopathogenic fungus Beauveria bassiana on the predatory mite Amblyseius swirskii (Acari: Phytoseiidae) as a non-target organism. Systematic And Applied Acarology, 20, 2451- 250.

Thomas, M. B. & Blanford, S. (2003). Thermal biology in insect parasite interactions. *Trends in Ecology and Evolution*, 18, 344 – 350.

Vemmer, M. & Patel, A. (2013). Review of encapsulation methods suitable for microbial biological control agents . *Biological Control*, 67, 380 – 389.

Vesala, T. (1998). On the concept of leaf boundary layer resistance for forced convection. *Journal of Theoretical Biology,* 194, 91 – 100.

Xu, X-M., Jeffries, P., Pautosso, M. & Jeger, M. J. (2011). Combined use of biocontrol agents to manage plant disease in theory and practice. *Phytopathology*, 101, 1024 – 1031.

Xu, X-M., Robinson, J., Jeger, M. & Jeffries, P. (2010) . Using combinations of biocontrol agents to control Botrytis cinerea on strawberry leaves under fluctuating conditions. Biocontrol Science and Technology, 20, 359 – 373.