Available online at www.sciencedirect.com
ScienceDirect
Biological Control 46 (2008) 242–255
Biological Control
www.elsevier.com/locate/ybcon

The development and multiple uses of a standardised bioassay method to select hypocrealean fungi for biological control of aphids

Helen Hesketh [a,b,*], Peter G. Alderson [b], Barry J. Pye [c], Judith K. Pell [a]

[a] Plant & Invertebrate Ecology Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
[b] School of Biosciences, University of Nottingham, Loughborough, Leicestershire LE12 5RD, UK
[c] Biological Chemistry Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

Received 4 December 2007; accepted 7 March 2008
Available online 15 March 2008

Abstract

A technically standardised bioassay method was designed, evaluated and used to assess virulence and host range of hypocrealean fungi against aphids. A track mounted sprayer was used to apply conidia because hand held versions of the same sprayer can be used for field applications, thereby allowing the outcome from laboratory experiments to predict activity in the field accurately. Eighteen fungal isolates were assessed in single concentration bioassays against the black bean aphid *Aphis fabae* Scopoli. Isolates comprised commercially available mycoinsecticides (based on *Beauveria bassiana* and *Lecanicillium longisporum*) and isolates of *B. bassiana*, *Lecanicillium* spp., *Paecilomyces fumosoroseus* and *Metarhizium anisopliae*. Aphid mortality was in excess of 80% for 15 isolates, and HRI 1.72 (*L. longipsorum*), Z11 (*P. fumosoroseus*), Mycotech strain GHA (*B. bassiana*) and ARSEF 2879 (*B. bassiana*) were studied further. Multiple concentration bioassays identified HRI 1.72 as the most virulent isolate against *A. fabae* with significantly smaller LC_{50} and LT_{50} values compared to other isolates. A precise LC_{50} value (2.95×10^2 conidia ml^{-1}) was calculated for HRI 1.72 using a second multiple concentration assay with smaller concentrations of conidia. The four isolates were applied at a single concentration (1×10^8 conidia ml^{-1}) against *Myzus persicae*, *A. fabae*, *Acyrthosiphon pisum*, *Metopolophium dirhodum*, *Sitobion avenae* and *Rhopalosiphum padi*. A ranking of aphid susceptibility was obtained, such that *S. avenae* > *M. persicae*, *A. pisum*, *A. fabae* > *R. padi*. Results indicate the importance of standardising bioassay methods to reduce bioassay variability without compromising the ability to use the bioassay to investigate fungus–host interactions under varying abiotic and biotic conditions.

Keywords: Entomopathogenic fungi; Bioassay; *Beauveria bassiana*; *Lecanicillium longisporum*; *Paecilomyces fumosoroseus*; *Metarhizium anisopliae*; *Aphis fabae*; *Acyrthosiphon pisum*; *Myzus persicae*; *Rhopalosiphum padi*; *Sitobion avenae*; *Metopolophium dirhodum*; Virulence; Host–pathogen interactions

1. Introduction

Aphids are serious pests in agricultural and horticultural crops through direct feeding damage and the transmission of plant viruses (Harrington and van Emden, 2007). Increasing aphid resistance to common insecticides (Foster and Devonshire, 1996; Harrington and van Emden, 2007) has stimulated interest in developing alternative methods of control in an effort to move towards sustainable cropping practices (Carter, 1989; Gurr et al., 2004; Pell, 2007; Thomas, 1999). Mitosporic entomopathogenic fungi in the order Hypocreales, such as *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium longisporum* (Petch) Zare & Gams (formerly known as *Verticillium lecanii* (Zimmermann) Viégas) and *Paecilomyces fumosoroseus* (Wize) Brown and Smith, have been successfully developed as biological control agents against a number of different pests, including aphids (e.g. Copping, 2004; Faria and Wraight, 2007; Powell and Pell, 2007; Shah and Goettel, 1999; Shah and Pell, 2003). Mitosporic fungi are ideal pest control agents as they are generally host specific (biocontrol strains

* Corresponding author. Present address: NERC Centre for Ecology & Hydrology, Mansfield Road, Oxford OX1 3SR, UK. Fax: +44 0 1865 281696.
E-mail address: hhesketh@ceh.ac.uk (H. Hesketh).

1049-9644/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2008.03.006

may be selected specifically for a narrow host range), infective conidia can be formulated for conventional application, they have low mammalian toxicity and do not leave harmful residues (Copping, 2004).

Using entomopathogenic fungi as mycoinsecticides against aphids has been successful (Faria and Wraight, 2007; Milner, 1997; Shah and Pell, 2003). The key biological attributes for a mycoinsecticide are generally considered to be virulence toward the target insect(s) and limited pathogenicity to non-target organisms (Goettel et al., 1990; Wraight and Carruthers, 1999). The definitions of virulence and pathogenicity vary widely between scientific disciplines and have been the source of much recent debate within invertebrate pathology (see Shapiro-Ilan et al., 2005 for discussion in response to Thomas and Elkinton, 2004). We adhere to the definitions of Steinhaus and Martignoni (1970) who define pathogenicity as the state of being pathogenic and hence the potential ability to produce disease and virulence as the disease producing power of an organism, i.e. the degree of pathogenicity within a group or species.

Bioassays must be repeatable and reliable in order to determine accurately pathogenicity or virulence, but particularly virulence. Variability in results may be caused by the cumulative variation in the components of an assay and therefore detailed technical standardisation of every component of an assay is required, particularly during bioassay development (Burges and Thompson, 1971). Nevertheless, a standardised bioassay should be adaptable and allow the influence of multiple ecological or biological factors on pathogen virulence to be tested. Abiotic conditions for example may alter the virulence of a pathogen, reducing a highly virulent pathogen to a less virulent pathogen under particular conditions (Casadevall and Pirofski, 2001). Alterations in host–pathogen relationships, specifically between fungi and aphids, can be related to factors such as differences in host genotype (Ferrari et al., 2001; Stacey et al., 2003), differences in host association with facultative symbionts (Scarborough et al., 2005), temperature (e.g. Blanford et al., 2003; Feng et al., 1999; Yeo et al., 2003) and humidity (e.g. Andersen et al., 2006; Hsiao et al., 1992). Consequently, bioassays designed to assess virulence of entomopathogenic fungi within the context of a complex of abiotic and biotic factors are likely to select isolates of fungi that are better adapted both physiologically and practically to the environmental niche in which they are to operate as biological control agents (e.g. Chandler et al., 2001).

The work we report here is part of a larger study to evaluate the virulence of hypocrealean fungi to different aphid species (specifically those that attack cereal and legume crops in the UK) in a pragmatic framework incorporating not only fungal efficacy but also the effects of important abiotic and biotic factors on aphid–fungus interactions. At initiation of this project, the development of a simple standardised bioassay method was required to screen fungal isolates against a representative target aphid but the method had to be versatile enough to answer other hypotheses relating to the ecology of host–fungus interactions within and between different aphid species. The black bean aphid *Aphis fabae* Scopoli was our representative target aphid; it is a major pest on legumes in the UK (Harrington and van Emden, 2007) but there have been few studies to investigate aspects of the pathogenicity and virulence of entomopathogenic fungi against this species (Shah et al., 2004; Yeo et al., 2003; Zayed and Zebitz, 1997). A restricted number of isolates selected in these initial stages of our study were to be assessed in field trials by application of fungal conidia to arable crops using an ultra low volume electrostatically charged rotary atomiser, the APE 80 (Arnold and Pye, 1981). The sprayer can be hand held for field applications or mounted on a track system for laboratory use (Yeo et al., 2003) allowing results from laboratory experiments to predict accurately efficacy under field conditions. Within this remit a number of considerations and compromises were made to optimise the bioassay method, the basis of which was bedded in standard bioassay techniques and designs exemplified in reviews by Butt and Goettel (2000), Navon and Ascher (2000), Hatting and Wraight (2007). Whilst standard bioassay techniques must be adhered to, we feel that too few reports detail the methods used to develop standardised bioassays for a specific purpose and that, without these details, it is difficult for the reader to develop bioassay methodologies suitable to answer the hypotheses they wish to test. Therefore, we report on the pertinent technical issues that were evaluated during development of this bioassay method and then show how the method was used in both single and multiple concentration bioassays to evaluate the impact of several entomopathogenic fungi against *A. fabae* in the laboratory. In addition, we report on the adaptability of the standardised bioassay method for evaluating the impact of selected entomopathogenic fungi against other aphid species, explicitly the pea aphid, *Acyrthosiphon pisum* (Harris), the English grain aphid, *Sitobion avenae* (Fabricius), the rose-grain aphid, *Metopolophium dirhodum* Walker, the bird-cherry oat aphid, *Rhopalosiphum padi* (L.) and the peach-potato aphid, *Myzus persicae* (Sulzer).

2. Materials and methods

2.1. Fungus cultures

The fungi selected were isolated from a range of insect species across several orders (Table 1). Isolates were selected based on their reported activity against aphids or other arthropod hosts and included some active ingredients of commercial products. The isolate identified as Mycotech GHA *B. bassiana* was isolated by the authors from Mycotrol WP® (Emerald BioAgriculture Corp., USA (previously: Mycotech Corp., USA)). To reduce variability between bioassays, an established method was adopted for producing conidia. Isolates were never