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Host specificity in biological control: insights from opportunistic pathogens

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Abstract

Host/prey specificity is a significant concern in biological control. It influences the effectiveness of a natural enemy and the risks it might have on non-target organisms. Furthermore, narrow host specificity can be a limiting factor for the commercialization of natural enemies. Given the great diversity in taxonomy and mode of action of natural enemies, host specificity is a highly variable biological trait. This variability can be illustrated by opportunist fungi from the genus *Lecanicillium*, which have the capacity to exploit a wide range of hosts – from arthropod pests to fungi causing plant diseases – through different modes of action. Processes determining evolutionary trajectories in host specificity are closely linked to the modes of action of the natural enemy. This hypothesis is supported by advances in fungal genomics concerning the identity of genes and biological traits that are required for the evolution of life history strategies and host range. Despite the significance of specificity, we still need to develop a conceptual framework for better understanding of the relationship between specialization and successful biological control. The emergence of opportunistic pathogens and the development of ‘omic’ technologies offer new opportunities to investigate evolutionary principles and applications of the specificity of biocontrol agents.

Introduction

Biological control of arthropod pests, weeds and plant diseases has been practiced for centuries. It is a cost-effective, environmentally friendly approach for resolving pest problems in terrestrial and aquatic ecosystems. The two main categories of biological control are the classical and augmentative strategies. Classical biological control involves selecting natural enemies of invasive species in their native range and releasing them in the recently invaded environment. Introduced natural enemies are expected to establish, spread and have a self-sustaining effect on the target pest. At least 2700 arthropod biological control agents have been introduced worldwide (Cock et al. 2010). Augmentative biological control refers to the production and release of indigenous or exotic natural enemies into various environments. They are expected to rapidly control pest populations, but not to persist over the growing season. More than 230 species of arthropod biological control agents are commercially available today (van Lenteren 2012).

Prey/host specificity of natural enemies has long been a critical issue in biological control. The spectrum of action of a biological control agent has evolutionary, environmental and economic implications. Specificity first establishes the intrinsic potential of a given species to become an efficient natural enemy of a target pest. It also allows the prediction, to a large extent, of the detrimental effects that released biological control agents might have on indigenous flora and fauna. Furthermore, narrow prey/host specificity can be a limiting factor for the commercialization of natural enemies by the biological control industry.

Despite the recognition of specificity as a central topic of biological control, we still need to develop a conceptual framework for understanding the relationship between specialization and successful biological control. The literature is filled with excellent descriptive studies and manipulative experiments that complement theoretical and phylogenetic considerations to assess the host range of biocontrol agents. Additionally, an increasing number of publications focus on the potential ecological impacts of natural enemies with

different degree of specificity (see below). However, there is no common theory to link host specificity and successful biological control by natural enemies, especially in the context of the increasing use of pathogens as biopesticides.

In this article, my aim is to illustrate that host/prey specificity is a highly variable biological trait of biological control agents. As a consequence, it has a major impact on nearly all important aspects of biological control, from the selection of a candidate agent to its effectiveness in reducing pest populations and overall economical and environmental impact once released. I will first review the major issues linked to host/prey specificity of biological control agents. Next, I will focus on pathogens used as biopesticides and discuss the question of extensive host range and multiple modes of action in the fungus *Lecanicillium*. I will then briefly examine how specificity in opportunistic pathogens may determine the aspects of their effectiveness and use in biological control. I focus my attention on the intrinsic host specificity of biological control agents prior to their release in the environment. Processes of evolutionary change (e.g. host shift) and local adaptation to physical and biotic conditions encountered by natural enemies once released have been discussed by Roderick and Navajas (2003), Hufbauer and Roderick (2005) and Hopper et al. (2006).

Specificity of biological control agents

Specialization has a long tradition of study in evolutionary biology because it is central to most of the relationships between species. This concept is relevant to the aspects of the evolution (speciation, radiation, life history) and ecology (distribution and abundance) of a given species and has consequences for the dynamics of plant and animal communities (Thompson 1994). Specialization has also important implications to the management of endangered species, establishment of exotic invasive species and biological control.

The specificity of a natural enemy is established by the number and taxonomic diversity of the host or prey it exploits, that is, the host/prey range. Generalist species are capable of exploiting various resources and switching from one species to another as competition increases or host/prey condition deteriorates while specialist species establish fewer but stronger links with their host/prey. The evolutionary mechanisms and processes responsible for the patterns of host specificity have been intensely debated in the literature. Mode of development, phylogeny and shared ecology are the most important determinants for the evolution of an organism's specificity. For example, parasitoids are strongly constrained by the physiological suitability and nutritional value of the host (Vinson 1975). Their specificity can be determined by the phylogenetic relationship of

the hosts (e.g. Braconidae; Wharton 1993). The host range of a parasitoid is also shaped by the spatial and temporal availability of potential hosts as well as by their detectability by foraging females (Strand and Obrycki 1996). Finally, a variety of other determinants, such as the effectiveness of behavioural or physiological defences of the host and the aspects of the physiological state of the parasitoid (experience, host deprivation, age and life expectancy, mating status), could also modulate the expression of host use.

Prey/host specificity appears to be one of the most variable biological trait of biocontrol agents. Parasitoids, predators, nematodes and microbials currently used in biological control programmes show various degrees of specificity, from organisms having a narrow host range restricted to a species or a genus to those with a wide spectrum of potential hosts covering several orders, classes and even kingdoms. Several factors make inferences about the relative host specificity among the types of biological control agents very challenging. First, past and present host/prey associations remain mostly or partially unexplored for pathogens, parasitoids and predators. Furthermore, the literature is peppered with unreliable host/prey lists or misidentified specimens. Second, most information about host range of biocontrol agents is based on laboratory experiments, and it is well established that the ecological host range (i.e. specificity of an organism in nature) can differ greatly from laboratory host range, the latter being usually broader. Third, within-species variability in host specificity is common. A generalist species may infect and develop successfully in hosts belonging to several taxa, while populations or strains are highly host-specific. For example, *Beauveria bassiana*, an entomopathogenic fungus, exploits over 200 species of insects in nine orders, but some isolates show a high degree of specificity (Feng et al. 1994). This is a common problem for most types of natural enemies. Fourth, as we will see below for fungi, some pathogens' use in biological control has the capacity to exploit a wide range of hosts through very different modes of action. This versatility makes host specificity an exceptionally difficult biological trait to measure.

As identified above, host specificity has three main practical applications in biological control. Specialization (i) influences the effectiveness of a natural enemy, (ii) largely profiles the environmental risks associated with released biocontrol agents and (iii) can be a limiting factor for the commercialization of natural enemies by the biological control industry.

Specificity versus effectiveness

A primary goal of biological control research has always been the identification of the specific attributes of natural enemies that are likely to enhance success. Models and case

studies bear out that the degree of success may depend on the ability of natural enemies to act through density-dependent processes, exploit (search, find and attack) pests, tolerate climatic conditions, persist at low pest densities, be synchronized in time and space with pest populations and inflict high mortality on specific pest stages. Furthermore, a central and recurrent principle (but see below) of biological control is that host/prey specificity is a required biological trait for natural enemies.

As reported by Chang and Kareiva (1999), in one of the first publications on insect control, Wardle and Buckle (1923) identify specificity as one of the four keys to effective control. This idea made its way to the contemporary literature. For example, Waage (2001) argued that ecologists and biological control practitioners rapidly realized that the use of relatively specific natural enemies was fundamental to the success of several well-known biological programmes conducted during the nineteenth and twentieth centuries (e.g. *Rodalia cardinalis* against the cottony cushion scale in California citrus orchards, *Cactoblastis cactorum* against prickly pear cactus in Australia, *Apoanagyrus* (= *Epidinocarsis*) *lopezi* against the cassava mealybug in Africa). Historically, the relevance of host/prey specificity to the effectiveness of biological control agents and the success of introductions gradually made it the logical first step in the selection of candidates (Waage 2001). This perspective was shared by a number of prominent scientists and led Greathead (1995) to conclude for classical biological control that 'on the whole, the types of organisms used as biological control agents are now limited to species belonging to groups of organisms that experience has shown to tend to have very restricted host ranges and do not readily exhibit switches in host preferences'.

However, although specialization has routinely been intertwined with an enhancement in the efficiency of a biological control agent, this question remains largely open. The link between specificity and effectiveness of a biocontrol agent is questionable and needs to be refined and tested. On one hand, co-evolution and specialization of natural enemies should enhance their effectiveness in exploiting hosts/prey and reducing pest populations through density-dependent processes (Hassell 1978; May and Hassell 1988). On the other hand, specificity may actually impair the effectiveness of a biocontrol agent via the evolution of reduced virulence, host counter-adaptation through an arm race, lack of persistence in the absence of the host/prey and other ecological and evolutionary processes. For example, Fagan et al. (2002) theoretically examined the capacity of specialist and generalist natural enemies in reducing the spread of a pest population showing different levels of Allee effect. They concluded that, while generalists can reduce the spread of the pest population regardless of the dynamics of the pest, because they

can persist at high density in the absence of the pest, specialists can only be effective for pest populations showing a strong Allee effect. This pattern suggests that high specificity may be a disadvantageous trait in biological control when the objective is to spatially confine the distribution of a pest species.

The level of specificity needed for a biocontrol agent to be successful is context-dependent. For example, the extent to which correlations between specificity and the capacity to reduce pest populations is likely to vary among and within taxa of biocontrol agents. Is specificity an equally important life history trait in the context of inoculative or mass release of a biocontrol agent to maximize effectiveness? In classical biological control, does specificity impede establishment, dispersal and effectiveness of an exotic natural enemy? The challenge is to adapt the ecological and evolutionary theory to biological control and to develop a better understanding of the role of host specificity relative to the types of natural enemies and the tactics used in biological control. Curiously, predictions about specificity of biocontrol agents have become more obvious and common for non-target effects than effectiveness.

Specificity versus non-target effects

The capacity of a biological control agent to establish in a new ecosystem and to spread in the environment is a key advantage for a sustainable and recurrent pest control strategy. However, it constitutes a risk to non-target species and local communities through often complex direct and indirect effects. Several studies have shown that the introduction of biological control agents to new countries may cause a decline in – and on the occasion extinction of populations or species that are not the target pest (Howarth 1991; Simberloff and Stiling 1996; Lynch et al. 2002). Most negative effects concern early introductions of vertebrate predators in an era when vertebrates were being spread without regulation worldwide (e.g. rabbits and foxes in Australia for hunting). The classical and most stunning example occurred in Hawaii in 1883 when a few farmers introduced the small Indian mongoose to control rats in sugarcane. This generalist predator had a strongly detrimental impact on native and endemic birds (Pimentel 1986). Nevertheless, modern biological control has been generally successful and safe.

The nature, probability of incidence and environmental risks associated with a biological control candidate are difficult to estimate. In principle, the risk of hazardous effects on non-target organisms can be greatly minimized by the selection of specific biological control agents. Typically, one of the first steps in the risk assessment process is to gather information about the prey or host range of the candidate to assess the type and magnitude of the ecological

effects that might occur following a release in the targeted environment. The community of scientists and practitioners involved in biological control have therefore established guidelines and standard procedures for host/prey range testing for predators, parasitoids, nematodes and pathogens (van Lenteren et al. 2006; Kuhlmann et al. 2006).

The degree of specificity that needs to be demonstrated and the level of risk that is acceptable depend on the importance of the pest problem and the presence of ecologically and economically important non-target species (e.g. endangered species, crop plants and pollinators) in the environment where the biological control agent is to be released. These parameters are also contingent upon the different biological control strategies. Classical biological control is of primary concern because it entails the release of exotic natural enemies with dispersal and permanent establishment as an objective. If successful, the introduction leads to an irreversible situation. In contrast, the release of native biological control agents in augmentative biological control programs, either in large (inundative) or small (inoculative) numbers, is less likely to interfere with native flora or fauna over a long period of time, although there is a risk of significant temporary non-target effects soon after the application of large numbers of natural enemies within a restricted area (Hajek et al. 2003).

Different national and international guidelines have been developed and implemented for assessing potential risks associated with the release of biological control agents in a new region (e.g. FAO Code of Conduct for the Import and Release of Exotic Biological Control Agents). Ecological risk assessment is now mandatory in a growing number of countries and, regardless of their effectiveness to control pest populations, only natural enemies that are safe for the environment should receive approval for release. As a result, according to Culliney (2005) for weed control, 'there have been no reported cases, in which introduced agents have had significant, negative impacts on non-target plant populations or on native ecosystems' in regions and countries where rigorous protocols have been developed.

Specificity versus commercialization

The commercial production and sale of biological control agents have been a successful and environmentally safe alternative to chemical pesticides for more than 40 years. Of the 230 species of natural enemies on the market today, the majority belongs to the Arthropoda (95.2%) (van Lenteren 2012). Within this group, parasitoids, which are typically more specific than predators, are the most common products (52.2%). In 2003, van Lenteren and Tommasini estimated that 45% of the species used in augmentative biological control were of alien origin. However, because of

the concerns about the release of exotic organisms and additional levels of regulation, there is a current trend in the biological control industry to promote the use of indigenous natural enemies, even for exotic pests (van Lenteren 2012).

Host/prey specificity constitutes a particular issue for the biological control industry. While specialized biological control agents provide the advantages of restricted host range, their narrow spectrum of action can also be a limiting factor for commercial use (Lynch 1995). This specificity can make biological control less attractive than broad spectrum chemical pesticides when an assortment of pests and diseases threatens a crop. The costs of production and commercialization of a multitude of biological control agents with a narrow range of action can be prohibitive for the industry. In addition, this situation adds to the complexity of conducting biological control programs for growers who have to deal with a number of different natural enemies, each having its own properties. To my knowledge, very few pest species can justify the commercialization of their own natural enemy within the context of augmentative biological control. One of the latest examples would be the predatory mite *Amblyseius swirski* for the control of the western flower thrip *Frankliniella occidentalis* in Europe (Messelink et al. 2006).

Lecanicillium spp.: a case study

In recent years, the use of pathogens has been increasing, mainly as formulated biopesticides for applications in augmentative biological control, primarily against arthropod pests and plant diseases. Microbial biopesticides are developed, commercialized and used like chemical pesticides. The candidates are selected under laboratory conditions for their virulence as well as the ease of production, formulation and application (Waage 1995). *Bacillus thuringiensis* (or Bt), a soil-dwelling bacterium, is by far the most commonly used biopesticide worldwide in both terrestrial and aquatic environments. Many other products have attained only modest success, in part because of relatively low virulence (high inoculum loads have to be applied) and overall poor and unpredictable performance under field conditions (Thomas 1999). The first pathogens commercialized as biopesticides showed a relatively high degree of host specificity (Federici and Maddox 1996). However, new biopesticides with increasing host range are emerging as useful components of biological control. These products, through the criteria used for their selection, the processes developed to modify their virulence and host range, and the conditions under which they are applied, should inspire us to revisit the host specificity paradigm in biological control.

In this section, using the fungus *Lecanicillium* spp. as a model system, I will examine the aspects of the role of fungi

as biological control agents, their intra- and interspecific variability in host specificity and the relationship between specificity and the mode of action. Specificity being a highly variable trait in *Lecanicillium* spp., I will next explore how it can be managed to enhance biological control.

Fungi as biocontrol agents

Parasitic fungi are used for the control of arthropod pests (Hajek et al. 2003; Goettel et al. 2005), plant diseases (Punja and Utkhede 2003; Jeger et al. 2009) and weeds (Charudattan 2001). In general, the release of microbes in classical biological control has been limited compared to the introduction of predators and parasitoids (Waage 1995) because of the concerns about non-target effects, fear about microbes being released near human populations and difficulties in the past with identifying, producing and releasing pathogens. Nevertheless, according to Hajek and Delalibera (2010), a total of 59 introductions of exotic fungal pathogens, from an estimated total of 20 species, have been conducted in new areas to control insects and mites. When all programs before 2000 are considered, 32.1% of introductions of fungi resulted in the establishment (Hajek and Delalibera 2010).

Most currently, commercialized fungi can be considered generalist natural enemies because they possess a relatively large spectrum of action. Some may therefore represent a higher risk to non-target organisms than fungi introduced in the context of classical biological control. Examples from mycoinsecticides include species or strains of *Beauveria*, *Metarhizium* and *Lecanicillium*, which can infect hundreds of insect hosts from different genera, families or orders. A similar pattern can be observed for mycofungicides, but at a somewhat higher level of host specificity (Jeger et al. 2009). In contrast, mycoherbicides typically have a very narrow host range to prevent the infection of endangered or beneficial plants, such as crops (Charudattan 2001).

Lecanicillium spp. (formerly *Verticillium lecanii*) (Zimmermann) Zare & W. Gams are opportunistic and widely distributed ascomycete fungi of the order Hypocreales. The species concept, phylogeny and taxonomic status as well as the genetics of *Lecanicillium* spp. remain somewhat unclear. Strains isolated from different hosts and various locations show very high levels of polymorphisms based on nuclear ribosomal RNA and mtDNA analyses (Kouvelis et al. 2004). The genus recently received a critical taxonomic review using rDNA sequencing to assess diversity within the taxon (Zare et al. 2000; Zare and Gams 2001). As a result, the species formerly known as *V. lecanii* has been divided into a number of new taxonomic entities, including *L. lecanii*, *L. longisporum*, *L. attenuatum*, *L. nodulosum* and *L. muscarium*. In this article, following Goettel et al. 2008, I will use *Lecanicillium* spp., unless rDNA sequencing from the new

nomenclature was used to identify the species, because of uncertain identification in the literature. As we will see below, this problem does not overly impair the analysis of host specificity in *Lecanicillium* spp.

The *Lecanicillium* spp. group includes a number of important species that are used for the control of pests and diseases in agriculture. *Lecanicillium* spp. is currently used as a bioinsecticide, with a minimum of 15 products being – or in the process of being – commercialized worldwide (Goettel et al. 2005; Faria and Wraight 2007). For example, Vertalec[®] (Koppert BV, Berkel en Rodenrijs, The Netherlands) (*L. longisporum*) against aphids; Mycotal[®] (Idem: Koppert BV, Berkel en Rodenrijs, The Netherlands) (*L. muscarium*) against whiteflies and thrips; Verticilin[®] (Idem: Koppert BV, Berkel en Rodenrijs, The Netherlands) (*L. muscarium*) against whiteflies, aphids and mites; and Vertiril[®] (Idem: Koppert BV, Berkel en Rodenrijs, The Netherlands) (*L. longisporum*) against whiteflies and thrips have been commercialized by Koppert BV (Goettel et al. 2008; Ravensberg 2011).

Host specificity

Over the last two decades, the genus *Lecanicillium* has become a fruitful model for studying questions in host specificity and action mode of biological control agents. A first attempt to synthesize our knowledge about this group of fungi and suggest perspectives for further research was published by Goettel et al. (2008). In this section, my aim is to show that *Lecanicillium* spp. specificity is complex and characterized by an exceptionally wide range of hosts.

At present, it is impossible to provide an exhaustive analysis of the host range of *Lecanicillium* spp. because (i) their identity to the species level remains unclear in most published studies, (ii) their host relationships have mainly been established for the organisms of economic importance (agricultural pests and plant diseases), and (iii) all studies on host suitability and pathogenicity have been conducted under laboratory and greenhouse conditions. Nevertheless, some general inferences can be drawn from the literature.

First, host range is an exceptionally variable biological trait in *Lecanicillium* spp. This species complex exhibits a very wide host range, including insects, mites, nematodes and phytopathogenic fungi (Askary et al. 1998; Goettel et al. 2008 and references therein). Within the Insecta, *Lecanicillium* spp. have been shown to affect Orthoptera, Thysanoptera, Homoptera, Coleoptera, Diptera, Lepidoptera and Hymenoptera; this list is not exhaustive. Similarly, potential plant pathogen hosts include fungi from a minimum of four classes: Leotiomycetes, Urediniomycetes, Agaricomycetes and Oomycetes.

Second, this broad host range can also be observed at the species level in *Lecanicillium*. For example, both

Table 1. Host range of *Lecanicillium* sp., strain DAOM 198499*.

Host species	Host taxa	Source
Codling moth (<i>Cydia pomonella</i>)	Insecta (Lepidoptera)	Agriculture and Agri-Food Canada
Potato aphid (<i>Macrosiphum euphorbiae</i>)	Insecta (Homoptera)	Askary et al. (1998) Kim et al. (2007)
Green peach aphid (<i>Myzus persicae</i>)	Insecta (Homoptera)	Kim et al. (2007)
Fox glove aphid (<i>Aulacorthum solani</i>)	Insecta (Homoptera)	Kim et al. (2007)
Cucumber powdery mildew <i>Sphaerotheca fuliginea</i>	Fungi (Leotiomycetes)	Askary et al. (1998)
Green mold (<i>Penicillium digitatum</i>)	Fungi (Eurotiomycetes)	Benhamou and Brodeur (2000)
Root rot (<i>Pythium ultimum</i>)	Fungi (Oomycetes)	Benhamou and Brodeur (2001)

**Lecanicillium* spp., DAOM 198499, was originally isolated from a codling moth in Québec, Canada.

L. longisporum and *L. attenuatum* have the ability to exploit either insect or fungus hosts (Askary et al. 1998; Fournier and Brodeur 2000; Kim et al. 2007, 2010). The most convincing example of such a polyphagy is illustrated by *Lecanicillium* sp. DAOM 198499 (Table 1). The isolate was originally isolated from codling moth larvae, *Cydia pomonella* (Lepidoptera), collected in Québec, Canada. Strain 198499 does not appear to be associated with any *Lecanicillium* species described by Zare and Gams (2001) based on rDNA sequences (Goettel et al. 2008). The fungus has been shown in the laboratory to exploit both insects and pathogenic fungi. It has the capacity to develop and reproduce on one moth, four aphid species and three fungus species that belong to three different classes: Leotiomycetes (powdery mildew), Agaricomycetes (green mould) and Oomycetes (root rot) (Table 1). A similar pattern has been reported for *L. longisporum* and *L. attenuatum*, which also have activity against aphids and cucumber powdery mildew (Askary et al. 1998; Kim et al. 2007). From an applied perspective, Askary et al. (1998) suggested that such *Lecanicillium* species or strains could be used as biopesticides with a dual role (see below).

These observations, although most are from laboratory conditions, provide convincing experimental evidence that *Lecanicillium*, both at the genus and species levels, is opportunistic and biologically active against arthropods and fungi.

Mode of action

Lecanicillium sp. 198499 has evolved a diverse array of biochemical mechanisms to exploit a very wide range of hosts from different kingdoms. It possesses general traits

of establishing infections and overcoming fungal pathogen and arthropod defensive barriers. In our laboratory, we investigated and compared some of the modes of action by which *Lecanicillium* sp. 198499 exerts its parasitic and saprophytic activity against aphids and causal agents of powdery mildew, green mould and root rot.

The interaction between *Lecanicillium* sp. 198499 and the potato aphid *Macrosiphum euphorbiae* was explored by light, scanning and transmission electron microscopy (Askary et al. 1999). As with other entomopathogenic fungi, the process of aphid colonization involves chronological events including (i) spore attachment to the host cuticle through a mucilaginous matrix, (ii) spore germination and colonization of the cuticle surface, (iii) cuticle penetration by germ tubes, (iv) active multiplication of blastospores and invasion of host tissues and (v) release of the fungus from aphid cadavers through the production of conidiospores. Typically, the fungi's extracellular enzymes are involved in the degradation of the host insects' proteins, lipids and chitin (St. Leger et al. 1996a). Pathogenesis would also involve production and diffusion of toxic metabolites with insecticidal activity, as shown for other *Lecanicillium* spp. (Claydon and Grove 1982; Gidin et al. 1994).

Askary et al. (1997) described the inter- and intracellular interactions between *Lecanicillium* sp. 198499 and the fungus *Sphaerotheca fuliginea*, the causal agent of cucumber powdery mildew. They first observed that, following the inoculation of *Lecanicillium*, the rate and extent of *S. fuliginea* colonization on the leaf surface were reduced, probably through the release at a distance of antifungal metabolites. Based on ultrastructure and cytochemical observations, the pattern of host colonization involves the following chronological events: (i) following attachment to the pathogen's conidia or hyphae, the antagonist enters its host through mechanical pressure and release of cell wall-degrading enzymes (chitinases); (ii) penetration is followed by a saprophytic phase characterized by an extensive multiplication of *Lecanicillium* inside the pathogen hyphae; (iii) following depletion of protoplasm because of the digestion of host tissues, *Lecanicillium* is released from dead *S. fuliginea* cells. These results clearly demonstrate that *Lecanicillium* sp. 198499 acts through a strong antagonistic activity that adversely affects the structural integrity of its host. Benhamou and Brodeur (2000) further investigated the mechanisms by which *Lecanicillium* sp. 198499 could parasitize another fungal pathogen, *Penicillium digitatum*, the causal agent of green mould, and confirmed the importance of antibiosis in the mycoparasitic process. They also observed that antibiosis acts prior to parasitism and the subsequent colonization of *Penicillium*, as suspected for *S. fuliginea*.

The biological control of plant diseases can be increased by selecting microbial control agents that have the capacity to function not only as antagonists but also by stimulating

the plant's defence system (Cook 1993; Yedidia et al. 1999). Benhamou and Brodeur (2001) investigated the interactions between cucumber roots, a causal agent of root rot disease (*Pythium ultimum*), and *Lecanicillium* sp. 198499. Using an *in vitro* cucumber root system, cytochemical observations showed that *Lecanicillium* can develop at the root surface and have an antagonist effect on *Pythium*. Furthermore, *Lecanicillium* can colonize some epidermal and cortical cells without inducing extensive root damage and evoke biochemical events characteristic of the natural cucumber disease resistance process. Although the mechanism is not yet fully elucidated, these results suggest an induction of resistance through antifungal activity and the production of structural and biochemical barriers in the root tissues (Benhamou and Brodeur 2001).

These observational data do not provide a comprehensive understanding of the modes of action of *Lecanicillium* sp. 198499, nor do they provide enough information for an inclusive comparison among potential host types. However, they indicate that this fungus has the capacity to exploit a wide range of insect and fungus hosts through different modes of action. The mechanisms include parasitism, antibiosis, competition, induced resistance (probably) and saprophytism. Furthermore, the relative importance of these mechanisms appears to vary according to the host species involved. For example, while *Lecanicillium* mainly relies on hyphal interactions and hydrolytic enzymes such as chitinases to invade the cucumber powdery mildew pathogen, antibiosis through the release of toxins is a key determinant operating against the root rot pathogen.

The processes determining evolutionary trajectories in host specificity are likely linked to the modes of action of the natural enemy. Druzhinina et al. (2011) suggested that complex interactions of parasitic fungi with plants, microbes and animals may have evolved from 'saprophyagy on fungal biomass (mycotrophy) and various forms of parasitism on other fungi (mycoparasitism), combined with broad environmental opportunism'. This hypothesis is supported by recent advances in fungal genomics related to the identity of genes and the evolution of biological traits that are required for development as parasite, antagonist, endophyte or saprophyte (Druzhinina et al. 2001; St. Leger and Wang 2010). Gao et al. (2011) published a comparative analysis of the genome sequences of *Metarhizium anisopliae* and *Metarhizium acridum*; these two entomopathogenic fungi have been thoroughly studied at the molecular, biochemical and ecological levels and have been used as biological control agents. *Metarhizium anisopliae* has a very broad host range, with more than 200 insect host species (Driver et al. 2000). It may also colonize plant roots and act as a biopesticide (St. Leger 2008), similar to what was shown for *Lecanicillium* sp. 198499 (Benhamou and Brodeur 2001). In contrast, *M. acridum* is specific to some

locusts and grasshoppers (Driver et al. 2000). Concerning *Metarhizium* host specificity and infectivity, Gao et al. (2011) showed that (i) both species have a relatively larger number of genes encoding secreted proteins involved in fungus–insect interactions than other plant pathogens and non-pathogenic fungi, (ii) the generalist *M. anisopliae* possesses more genes encoding extracellular enzymes and toxins than *M. acridum*, and (iii) *M. anisopliae* has the capacity to up-regulate different genes in the presence of plants and insects. This functional genomic analysis provides the molecular basis for better characterizing the evolution of host specificity and virulence in pathogenic fungi.

Opportunistic pathogens in biological control

The science of biological control needs to partially revise its understanding of the role of host specificity with the emergence and commercialization of opportunistic pathogens used as biopesticides. They come from taxa that are opportunistic (e.g. *Lecanicillium*, *Metarhizium* and *Trichoderma*) and possess multiple modes of action. They typically have broader host range than pathogens introduced in classical biological control. These candidates have a commercial advantage because they can be used against various arthropod pests and plant diseases. This situation does not follow the tradition of modern biological control, which has always promoted great specificity for natural enemies to be released in both classical and augmentative biological control programs.

To fulfil requirements in terms of environmental risk and successful control of pest populations, specificity of opportunistic pathogens can be managed through different approaches. These relate to the strategies of application and the fate of microorganisms in the environment, and the 'management' of their virulence and modes of action.

Opportunistic pathogens, like most biopesticides developed and commercialized in the past, are designed for augmentative biological control. The strategy is characterized by single or multiple applications of large quantities of pathogens in targeted habitats when climatic conditions facilitate the interaction with arthropod pests, plant diseases or weeds. The level of specificity needed for a biocontrol agent to be successful under these specific circumstances is likely to be reduced because high levels of 'inoculum' are applied. Such an approach also helps to overcome detrimental abiotic conditions and host demographic stochasticity.

In general, there has been less concern about the long-term persistence of microbial biopesticides in the environment compared to those used in classical biological control, probably because biopesticides have been regarded to be like chemical pesticides, that is, short-acting products with few residual effects (Waage 1995). The first two types of organisms commercialized as biopesticides, Bt and

nematodes, have weak natural associations with pests on plants and were considered to have low persistence in the environment, and hence have to be reapplied frequently. Under this reasoning, broad host range of microbials used as biopesticides is not as problematic as for organisms that have the capacity to reproduce, establish and disperse in the environment. However, other types of biopesticides, including opportunistic fungi, may have the capacity to persist in the environment and have non-target effects. Biodegradation of biopesticides needs special attention because of their potential accumulation in the environment. While detailed protocols have been developed to assess risks posed by macroorganisms, we have a poor understanding of the fate in the environment of virus, bacteria and fungi applied as biocontrol agents. For one thing, to my knowledge, we know nothing about the relationship between specificity and persistence of microbial agents.

Modes of action of opportunistic pathogens are another important aspect to consider for the management of their specificity. The ultimate goal being to increase effectiveness while reducing both detrimental side effects and costs to the industry and users. As I described for *Lecanicillium* sp. 198499, modes of action evolved by generalist fungi are complex, involving more or less specific molecular events during the host recognition phase and differential production of enzymes and biologically active secondary metabolites during a given interaction. The mechanisms are not mutually exclusive, and their sequential contribution in the host exploitation process – as well as the possibility that they operate additively or synergistically – needs to be carefully investigated at the molecular and cellular levels. Furthermore, we can expect that opportunist pathogens may follow different strategies when exploiting a given host species. Some of these traits and processes could possibly be exploited when developing candidates for biological control.

Genetic engineering enables the development of microbial biological control agents that can be more virulent (Gressel et al. 2007; Federici et al. 2008; St. Leger and Wang 2010). Recombinant DNA techniques allow increases in the expression of pathogenicity genes [e.g. cuticle-degrading enzymes (Fan et al. 2007); toxic protease (St. Leger et al. 1996b)] and the use of transgenes that encode toxins to increase host mortality [e.g. scorpion toxin expressed in an entomopathogenic fungus (Pava-Ripoll et al. 2008)]. Of interest, recent research has focused on *Lecanicillium* spp. as potential biocontrol agents of the widely distributed soybean cyst nematode, *Heterodera glycines* (see review by Koike et al. 2012). Protoplast fusion in genomic DNA was performed to produce new *Lecanicillium* strains with increased pathogenicity and viability. For example, the hybrid strain AaF42 (*L. longisporum* × *L. muscarium*) reduced *H. glycines* egg density by 93% as

compared with the control (Shinya et al. (2008)). Genetic improvement using protoplast fusion might also be used to produce *Lecanicillium* hybrid strains with targeted host specificity.

To make this approach more effective, other candidate genes for virulence need to be identified by genome sequencing and comparative transcriptomics of additional viral, bacterial and fungal pathogens. The production of more virulent pathogens will also contribute to reduce the doses of pathogens to be released in the environment, thereby decreasing the risks of non-target effects. A better knowledge of the genetic basis of microbial host specificity should also lead to the development of recombinant strains of microbials showing narrow specificity (St. Leger and Wang 2010). Finally, knowledge of the genomic sequences of an opportunistic pathogens should facilitate identifying candidate genes for manipulation to target a specific or a combination of modes of action, which are better adapted to control a given pest species under a particular circumstance.

In 20–30 years, the biopesticide market could be dominated by fungi such as *Lecanicillium* spp. and *M. anisopliae*, which will be released against most pest organisms such as arthropods, plant pathogens and weeds. The ‘silver bullet’ could be available à la carte: genetic engineering will produce a multitude of highly virulent strains that are specific to a single or a combination of targeted organisms. Genes involved in the production of conidiophores, the specialized structure in the Ascomycetes responsible for the formation of conidia, could be knocked-down, thereby preventing asexual reproduction of the fungus once released in the environment. Such a scenario could not only prevent the long-term establishment of the fungus, thereby reducing potential non-target effects, but also favour the development of a renewable market for the industry, similar to what we observe for chemical pesticides.

Conclusion

In biological control, ‘issues concerning host specificity are always situation specific’ (Bellows and Headrick 1999), and their consequences for pest control effectiveness and the environment may need to be studied on a case-by-case basis. Specificity of a biological control agent can influence its effectiveness, the probability it could attack a non-target species, and its likelihood to be developed and commercialized by the industry. Host specificity has different implications depending on the origin (exotic versus indigenous or naturalized) and the type of biological control approach (classical versus augmentative).

Biological control was first established on the common assumption that specialist natural enemies are better adapted to find and exploit their prey/host. More recently,

the scientific and environmental communities have raised critical issues concerning the risks posed by introduced biocontrol agents on non-target organisms. Since then, most ecologists, biological control practitioners and regulators tend to focus on specificity not primarily because of its link to effectiveness but because it lessens the possible ecological risks associated with the introduction of natural enemies, often non-native, in the environment. Questions about non-target effects have become broader as methods for studying host range and approaches to risk assessment have become more refined.

One of my aims has been to demonstrate that the study of host specificity in biological control and its link to effectiveness is a crucial but, in some ways, neglected area of research. For one thing, the claim that host specificity is a highly desired biological attribute of biocontrol agents needs to be reassessed. This question provides an example in which an ecological principle (i.e. host specificity increases effectiveness) has been adopted based on a potential theoretical explanation (co-evolution and specialization enhance effectiveness) but, to my knowledge, this has rarely been confirmed by experimental evidence. Our field knowledge of the 'high' killing efficiency of specialist natural enemies and the consequences on pest populations remains rudimentary. The complexity of biological control and the simplified approaches to assess host specificity under laboratory or controlled conditions add to the uncertain role of host specificity in successful biological control. Three main caveats can be identified. First, the effectiveness of a biocontrol agent depends not only on its ability to find and kill pests but also on its capacity to reproduce on pests and persist in the habitat. Second, selected biocontrol agents do not usually have an evolutionary history with the targeted pest and the habitat into which they have to perform. Third, once introduced in new habitats, biocontrol agents are expected to retain a degree of specialization and effectiveness that was similar to the one observed in their native habitats. However, biocontrol agents and their pests act as important selective agents on each other and evolutionary processes may operate rapidly following the introduction.

To make biological control more effective and safer for the environment, we need to develop a nuanced understanding of the role of prey/host specificity. Potential benefits relate to the selection of the most appropriate candidates and the development of optimum release and management strategies. There are therefore advantages of integrating questions on patterns and processes of both effectiveness and environmental issues when studying host specificity and screening for potential biocontrol agents. The way forward calls for an integration of behavioural, ecological and evolutionary studies.

Highly opportunistic pathogens are increasingly finding their use in biological control as biopesticides. Their versa-

tile modes of action suggest an evolutionary capacity for adaptation to new hosts. They are therefore promising models to further explore the relationship between host specificity and biological control. Their genomes are easier to engineer than those of parasitoids and predators. Progress in 'omic' technologies and genetic engineering now enables the construction of microbial agents that can be more virulent, specific and safe for the environment. Furthermore, the production of pathogens that express different levels of virulence, produce specific anti-pest molecules or trigger the activation of a particular mode of action would provide powerful tool to investigate evolutionary principles and applications of the specificity of biocontrol agents. However, it is imperative that scientists developing opportunistic pathogens continue to rigorously weigh the advantages and hazards posed by these new biocontrol agents and help regulatory authorities to make decisions.

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