Progress in Biological Control

Antonieta De Cal
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How Research Can Stimulate the Development of Commercial Biological Control Against Plant Diseases

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Biological control of pests, weeds, and plant and animal diseases utilising their natural antagonists is a well-established but rapidly evolving field of applied ecology. Despite its documented applications and systematic development efforts for longer than a century, biological control still remains a grossly underexploited method of pest management. Its untapped potential represents the best hope to providing lasting, environmentally sound, and socially acceptable control of most problem pests in agriculture, and of invasive alien organisms threatening global biodiversity. Based on the overwhelmingly positive features of biological control, it is the prime candidate in the search for reducing dependency on chemical pesticides. Public demand for finding solutions based on biological control is the main driving force in the rapid developments in the various strategies of utilising natural enemies for controlling noxious organisms. This book series is intended to accelerate these developments through exploring the progress made within the various aspects of biological control, and via documenting these advances to the benefit of fellow scientists, students, public officials, and the public at large. Each of the books in this series is expected to provide a comprehensive, authoritative synthesis of the topic, likely to stand the test of time.

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Chapter 1
Importance of Ecological Windows for Efficacy of Biocontrol Agents

Naresh Magan

1.1 Introduction

There has been significant focus for many years on the development and use of biocontrol agents (BCAs) for the control of plant pathogens of a range of durable and horticultural crops, pre-harvest for soil-borne and foliar diseases and post-harvest pathogens. The mechanism of action has often been suggested to include direct antagonism between the BCA and fungal pathogen, competitive occupation by niche exclusion of the pathogen, or combined with production of secondary metabolites, hyperparasitism, or the production of volatile organic compounds (VOCs). In almost all these cases the target has been to reduce plant disease symptoms and minimise impacts on yield and quality loss in the context of the food security agenda and more sustainable systems less reliant on chemical control measures as inputs. An additional driver has been the removal of almost 50% of chemical crop protection compounds for agricultural and horticultural production systems by the EU which has led to many of the major chemical companies to examine more seriously the integration of management of pests and diseases including the use of BCAs. Indeed, the question of whether BCAs had come of age was posed in 2012 (Glare et al. 2012) although the bottlenecks in relation abiotic factors in many cases still remain.

Many potential candidate BCAs have thus been developed for pre-harvest, soil-based and post-harvest pathogens of both perishables and durables (Spandaro and Droby 2016; Köhl et al. 2019a). However, this has often not been translated into effective formulated products for commercial applications. The most successful area where biocontrol has been achieved is during post-harvest phases where both

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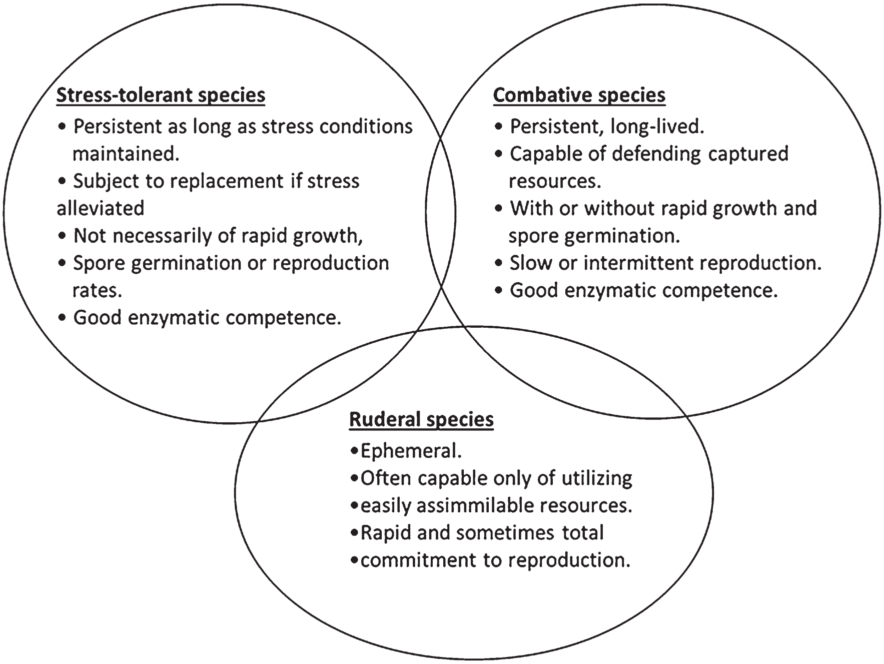
temperature and high relative humidity (Relative Humidity, RH; >95%) are maintained to conserve perishable quality thus allowing the BCA to effectively compete. This has been especially with post-harvest wound pathogens and in glasshouse systems where RH can be maintained at very high levels by misting prior to BCA applications for control of both pathogen and pest control.

The ecological parameters of the BCA must match those of the target pathogen to ensure that the former can compete effectively with the latter and reduce disease. Often this has not received enough attention, and thus limited the development of more generic BCAs for commercial disease control applications. The key abiotic parameters which influence both potential BCAs and the target pathogen include the water availability (water activity or water potential) in the rhizosphere/phyllosphere, atmospheric RH, temperature and sometimes pH of the ecological niche where efficacy is required. In addition, maintaining the available water at levels which allow the BCA to effectively germinate and become established has been challenging but is critical for efficacy. This is also partially dependent on the type of microorganism (bacteria/actinomycetes, yeast or filamentous fungus), the production method, and the formulation for crop application. Indeed, the viability of formulated BCAs needs to be considered in terms of the ecological window for growth/establishment to compete effectively with the pathogen in the specific niche. Indeed, the application of BCAs to foliar plant surfaces represents a challenge because of the variability of the phyllosphere microclimate and the physiological and morphological nature of foliar surfaces. Unlike chemical formulations, BCA viability in formulations needs to be conserved and effective adjuvants need to be included for effective adherence to the plant surfaces and subsequently compete effectively with a fungal pathogen or be able to attach and infect pests.

The strategies employed by fungi also differ from an ecological perspective with some having so-called Combative (C-selective), Stress-related (S-selective) or Ruderal (R-selective) strategies for primary resource capture, and to survive and prosper in a particular ecological niche. Sometimes these can merge resulting in merged secondary strategies for nutrient capture such as C-S, S-R, C-R, C-S-R. Of course, both BCAs and pathogens have these characteristics to try and remain dominant in particular niches. Figure 1.1 shows the relative profiles and attributes of fungi which may be classified based on their strategies. Thus it is important to understand the specific strategy utilized by a microbial pathogen for plant infection. The BCA must thus be able to have a strategy which allows competition with the pathogen for efficacy to be achieved.

1.2 Environmental Screening for BCAs

When examining candidate BCAs and plant pathogens we need to consider the water availability tolerances of microorganisms. Figure 1.2 shows the different ranges of water stress tolerances exhibited by bacteria, actinomycetes and fungi (yeasts and filamentous species) for germination and growth. It is important to note



Stress-tolerant species

- Persistent as long as stress conditions maintained.
- Subject to replacement if stress alleviated
- Not necessarily of rapid growth,
- Spore germination or reproduction rates.
- Good enzymatic competence.

Combative species

- Persistent, long-lived.
- Capable of defending captured resources.
- With or without rapid growth and spore germination.
- Slow or intermittent reproduction.
- Good enzymatic competence.

Ruderal species

- Ephemeral.
- Often capable only of utilizing
- easily assimmilable resources.
- Rapid and sometimes total
- commitment to reproduction.

Fig. 1.1 Characteristics of strategies used by microorganisms for effective establishment in an ecosystem (from Magan, 2007)

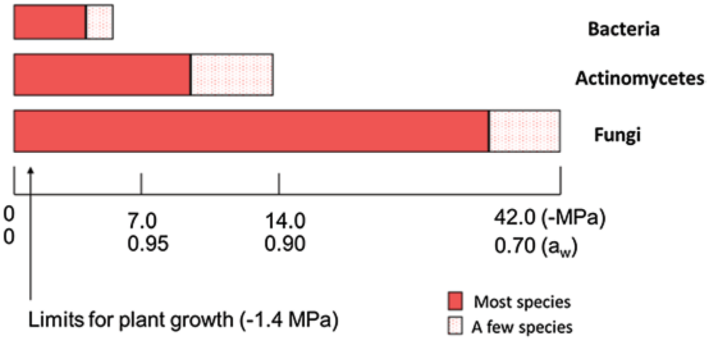


Fig. 1.2 Diagrammatic representation of water availability ranges for bacteria, actinomycetes and fungi in raw commodities (water activity, a_w) or in soil (water potential). The wilting point of plants is included for reference

these ranges, in the context of the wilting point of most plants, as activity often occurs under conditions where plant growth might not occur by microbial activity does occur. This clearly shows that when considering the control of plant pathogens, we need to identify the tolerance range of the potential BCAs and that of the pathogen. Thus, if one is trying to control a xerotolerant of xerophilic species that is able to tolerate a wide range of water stress conditions, then the potential BCA should be able to effectively compete and remain active over a similar range of environmental conditions. Otherwise, *in vitro* efficacy will not be translated into *in planta* control of the target pathogen.

Indeed, often *in vitro* screening is made in many studies on rich media that completely ignores the ecosystem in which control of a pathogen may be required. Often the natural fluxes in environmental factors, which occur in nature, are totally ignored. This often results in choosing potential BCAs that are not suitable for the control of the target pathogen because the ecological windows do not match. Studies by Rungjindamai et al. (2013) showed that screening microorganisms for control of *Monilinia laxa*, responsible for brown rot of stone fruits, showed a number of isolates of interest *in vitro*. However, subsequent trials on detached cherries and plums showed that only 2 of 12 strains were able to effectively have potential for control (a *Bacillus* and *Aureobasidium* strains). Previously, Köhl et al. (2011, 2019b) have eloquently examined aspects of a step-wise approach to screening BCAs. In addition, I believe, this systematic approach should include an environmental screening process to ensure that the interacting environmental conditions (e.g. water availability x temperature x pH) and interactions with the biotic niche in which efficacy is required needs to be simulated in laboratory-based assays. Indeed, potential BCAs should not normally be able to grow at the body temperature (37 °C) as this will make development and commercial exploitation difficult because of potential impacts on the users.

Of course, the life cycle of the targeted pathogen must be examined in detail to enable the identification of the weak spots where potential biocontrol may be effective. For example, often foliar pathogens have a component of their life cycle where they survive as saprophytes on crop debris/residues in soil. This helps in choosing the component of the life cycle that may be vulnerable to competitive antagonists to reduce inoculum potential of the target pathogen. In soil, the ability to tolerate matric potential stress is important for effective competition with pathogens. Pathogens are exception, and thus are very resilient and often have ruderal characteristics ecologically. Often BCAs may not have the same characteristics and thus must be examined for their ability to grow in soil into the rhizosphere zone from inoculum formulated with a relevant food source. Establishment needs to be optimized so that effective colonization can occur in the rhizosphere or phyllosphere to compete effectively with the pathogen.

1.3 Niche Occupation and Exclusion: Impact of Environmental Conditions

In different ecosystems, the modes of action for inhibiting pathogens by BCAs are wide ranging (e.g., competition for nutrients, synthesis of cell-wall-degrading extracellular enzymes/antibiotics/secondary metabolites, induction of plant resistance

(Benitez et al. 2004; Magan and Aldred 2007; Medina et al. 2017). It has been suggested that a key characteristic of a BCA should be the ability to compete effectively for the available nutrients in the ecological niche, and in this way facilitate niche exclusion of the pathogen. It is thus useful to have knowledge of the relative range of nutrients which can be utilised by a BCA and compare this with that of the target pathogen. This may provide information on the competitive advantage of a BCA and perhaps result in exclusion from a specific ecological niche. A number of studies have now suggested that nutritional partitioning of resources in terrestrial ecosystems (e.g., phyllosphere, rhizosphere) may influence the ability of both the BCA and the pathogens to either co-exist or dominate in a particular niche (Wilson and Lindow 1994; Arroyo et al. 2008; Cray et al. 2015; Di Francesco et al. 2017). Recently, studies with *Aureobasidium pullulans* strains for control of *Monilinia laxa*, the cause of peach brown rot, examined the competition for nutrients and space. This suggested that in peach-based juice media *M. laxa* could grow rapidly by utilising asparagine. However, in the presence of the *A. pullulans* strains this C-source was rapidly converted to aspartic acid resulting in a reduction in germination of *M. laxa*. It was thus suggested that the potential BCAs were able to compete for space and nutrients effectively. However, these studies did not consider environmental conditions that may impact on the relative niche occupation.

It is important to overlay interactions with the relevant abiotic factors (e.g., temperature, water availability, pH) which may further influence the interactions between a BCA and a pathogen (Magan and Aldred 2007; Medina et al. 2017). It is thus critical to examine the interaction between abiotic factors and utilisation of relevant nutritional sources by a BCA and the pathogen to obtain useful information on possible niche exclusion and relative competitiveness (Lee and Magan 1999; Mohale et al. 2013; Samsudin et al. 2016).

Wilson and Lindow (1994) and Wilson et al. (1999) originally suggested the Niche Overlap Index (NOI) approach for the development of biocontrol of bacterial pathogens. This involved the identification of the utilisation pattern of relevant carbon sources (CSs) utilised by a BCA and a pathogen, and those utilised in common. This is translated into a NOI that in turn reflects whether the antagonist:pathogen co-exist and share an ecological niche (NOI values >0.9) or occupy separate ecological niches (NOI values <0.9). The NOI thus helps to explain the co-existence or niche exclusion by different microbial species and is a measure of relative secondary resource capture. This has been used as a method for identifying and choosing appropriate potential BCAs. The way this has been quantified is by examining the relative utilization of appropriate CSs where biocontrol is targeted. For example, in maize or wheat the major amino acids, carbohydrates and lipids present in the target matrices are used. The NOI can then be calculated:

$$\mathrm{NOI} = \frac{\text{Common carbon sources } \left(\text{utilized by both antagonist and pathogen}\right)}{\text{Total carbon sources utilized by antagonist}}$$

The NOI has been shown to be significantly modified by water availability and temperature (Marin et al. 1998). Thus, it is possible to use this *in vitro* approach to enhance the understanding and the potential for effective biocontrol of a target pathogen over a range of interacting environmental conditions (Mohale et al. 2013; Samsudin et al. 2016). Figure 1.3 shows an example of the relative utilization of C-sources by a pathogen and an antagonist based on the key nutrient sources in maize under interacting abiotic factors. This shows how the utilization patterns may change due to such stress. This was subsequently used to compare the NOI between the pathogen and the antagonist. The relative NOI values for the antagonist and the pathogen at different water availability x temperature conditions changed. At 25 °C + 0.98 a_w, and 30 °C + 0.95 a_w both antagonist and pathogen occupied similar niches (co-existed) with NOIs of 0.90 respectively. However, under the other conditions tested, they occupied different niches with NOIs of 0.88 (25 °C + 0.95 a_w) and 0.80 (30 °C+ 0.98 a_w). Thus, the ability for niche exclusion by a BCA will be significantly influenced by the relative capability for utilisation of relevant CSs in which control of a pathogen is required. It is important to use the right C-source range relevant to the pathogen:antagonist:plant system and the appropriate range of interacting environmental conditions (Magan and Aldred 2007; Mohale et al. 2013; Samsudin et al. 2016).

The use of the NOI approach can also be complimented by more detailed studies on the relevant rates of utilisation of key CSs by the pathogen and BCA. Recently,

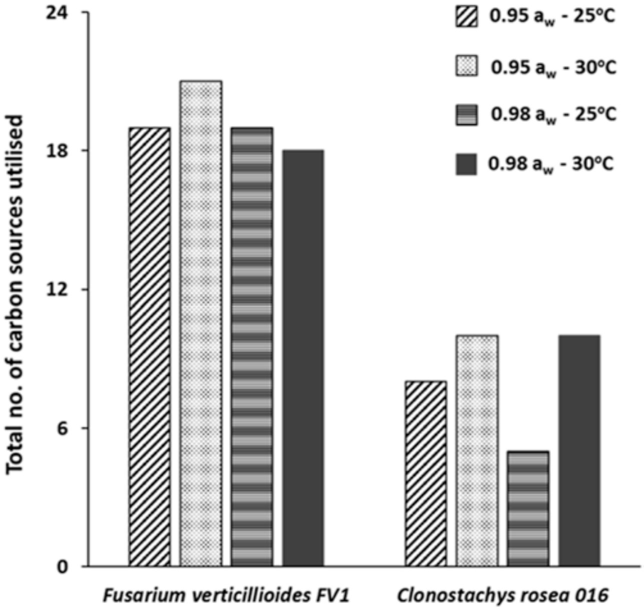


Fig. 1.3 Example of the effect of water activity/temperature regimes on the total number of carbon sources (n = 24) relevant to maize utilized by the pathogen *Fusarium verticillioides* and the potential antagonist *Clanostachys rosea* after 7 days incubation (from Samsudin et al. 2016)

the use of the Bioscreen® to monitor the temporal rates of CSs utilisation patterns by specific BCAs and pathogens has been used to try and understand the kinetics of the mechanism of action for control of mycotoxigenic fungi by BCAs (Mohale et al. 2013; Samsudin et al. 2016). Such information may help in understanding the reasons why specific BCA strains are able to compete effectively with the pathogen, and better understand the nutritional resource partitioning under different environmental conditions in utilising relevant C-sources. For example, utilisation of carbohydrates, amino acids and lipids may differ for a pathogen and the potential BCA as interacting abiotic factors are imposed (Medina et al. 2017). Such studies have shown that rates of nutritional utilisation changes under different environmental conditions and can determine whether competition of nutrients is the major characteristic whereby a BCA is able to effectively exclude a pathogen in a particular niche under different water x temperature stresses.

1.4 Optimum and Boundary Environmental Conditions for Growth of BCAs and Pathogens

The optimum and marginal conditions for growth of both the pathogen and the potential BCA must be examined to understand whether the competitiveness may occur over the range of conditions over which control is required. This is critical in both the rhizosphere, the phyllosphere and on edible plant surfaces, especially where BCAs may be applied pre-harvest for subsequent post-harvest efficacy (Teixido et al. 2006). For fungal BCAs an example is given in Fig. 1.4 where the optimum and marginal conditions for water availability x temperature range is considered for a pathogen (*Botrytis cinerea*) and two BCAs, *Candida sake* and *Ulocladium atrum*. This clearly shows that there is overlap between the conditions over which active growth and establishment may occur between the pathogen and the two BCAs (Fig. 1.4).

Bacteria such as *Bacillus subtilis* and *Pantoea agglomerans* have received significant attention as biocontrol agents of *B. cinerea* pre-harvest, and for post-harvest pathogens of pome and citrus fruit respectively. *Pantoea agglomerans* was found to be very effective against *Penicillium digitatum* and *P. italicum*, pathogens which cause significant post-harvest rots in citrus fruit resulting in significant economic losses. The water relations of this bacterial strain was examined and it was found to grow over a wide range of temperatures from 3–4 °C to 35 °C with a_w limits of around 0.95 (Costa et al. 2002). In contrast when we examine the water relations of the two *Penicillium* pathogens, they are able to grow over a range of temperatures of 5 °C to 30–35 °C and over the range of 0.99–0.87 a_w (Plaza et al. 2003; Fig. 1.5). Thus, while the pathogens have a wider range of a_w tolerances, under low temperature storage conditions (5 °C) and high humidity, *P. agglomerans* has an advantage as it is able to compete effectively in the ecological window where the *Penicillia* are significantly less competitive (Canamas et al. 2008a, b).

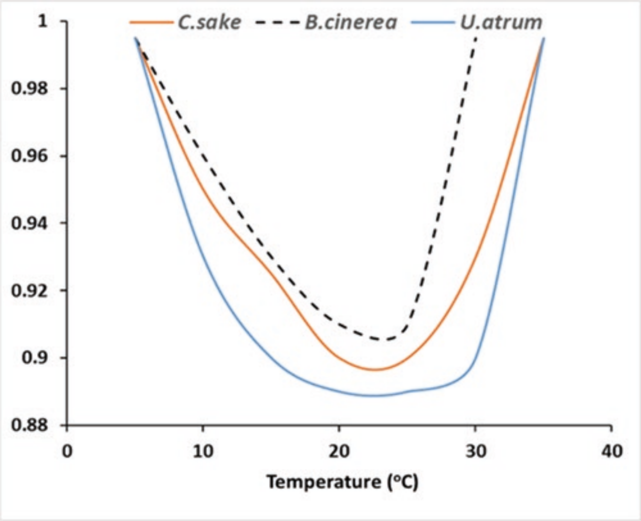


Fig. 1.4 Diagrammatic representation of the two-dimensional range of interacting water activity x temperature conditions over which growth of the pathogen *Botrytis cinerea*, and two biocontrol agents, a yeast (*Candida sake*) and a filamentous fungal species (*Ulocaldium atrum*) are able to grow. (Data adapted from Teixido et al. 1998; Sancisi-Frey 2000; Bulgar et al. 1987; Celibertio et al. 2015)

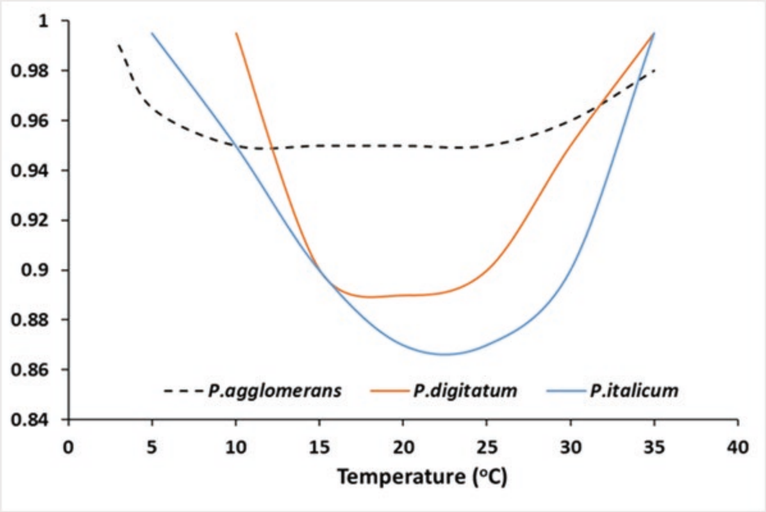


Fig. 1.5 Comparison of the water activity x temperature boundaries for growth of the biocontrol agent *P. agglomerans* and two post-harvest pathogens, *P. digitatum* and *P. italicum.* (Adapted from Costa et al. 2002; Plaza et al. 2003)

Of course the optimum and boundary conditions for growth of pathogens and BCAs will depend on whether they are relatively sensitive to interacting abiotic factors or whether they are xerotolerant or xerophiles, as the window would change. Thus, control of mycotoxigenic fungi such as *Aspergillus flavus* in soil and on ripening maize silks is based on the use of non-toxigenic strains of the same species to outcompete the toxigenic ones, especially under drought stress conditions (Bandyopadhyay et al. 2016). It has been suggested that this involves the better competiveness of the non-toxigenic strains when applied to soil on a nutritional carrier, such as sorghum grain, from which the BCA is able to colonise soil and outcompete the pathogen (toxigenic strain) in soil under relatively dry conditions reducing contamination of maize with the carcinogenic mycotoxin aflatoxin B_1. The possible effect of environmental conditions on entomogenous fungi has also been shown to vary with water x temperature conditions, and thus the ecological windows for germination and growth for individual strains of *Beauveria bassiana*, *Metarhizium anispoliae* and *Isaria farinosa* can help in determining which strains would be more appropriate for pest control in specific climatic conditions (Borisade and Magan 2015).

There has been interest in the using ecophysiological approaches to improve the quality of BCA inoculum for better establishment in the target niche and try to widen the abiotic window for efficacy against a pathogen. This has often been achieved by manipulation of growth conditions of the BCA to physiologically stress the BCA and facilitate the synthesis of higher amounts of endogenous stress related reserves, especially of compatible solutes, which have been shown to be beneficial for control of both pathogens and pests using BCAs (Pascual et al. 2000; Teixido et al. 1998; Canamas et al. 2007; Ypsilos and Magan 2005). Enhanced endogenous amounts of trehalose (desiccation protectant), and compatible solutes, especially low molecular weight sugar alcohols (glycerol, erythritol) in fungal inocula and increases in glyceine betaine and ectoines in bacterial BCAs have been found to enhance tolerance of a_w x temperature conditions and sometimes heat shock, especially in marginal abiotic conditions (Teixidó et al. 2005; Canamas et al. 2007; Andersen et al. 2006; Mokiou and Magan 2008).

Indeed such ecophysiological manipulation has also been shown to influence the competence of the BCA and its potential for biocontrol. For example, changing the production conditions by growing under water stress (0.995 vs 0.98, 0.95 a_w) modified with the non-ionic solute (glycerol) to produce conidia of *U.atrum* with enhanced compatible solute and trehalose content. These conidia had better competence under water stress conditions and the NOI for the modified inocula were different from that of the unmodified inocula. Thus, *U. atrum* could co-exist and compete effectively with *B. cinerea* in the same niche for nutrients and influence the activity of the pathogen. The pathogen appeared to occupy a separate niche from the antagonist at 0.98 and 0.95 a_w and only co-existed with the antagonist with freely available water (Fig. 1.6). Thus, by modifying the BCA the potential for control was enhanced.

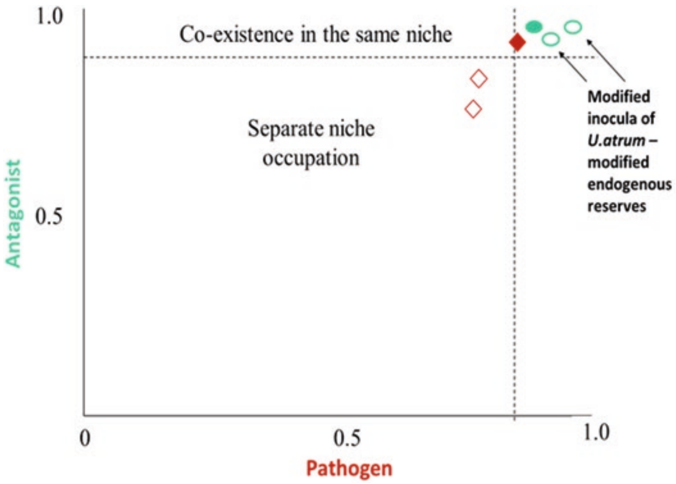


Fig. 1.6 Comparison of the Niche Overlap Indices between for *Botrytis cinerea* (Pathogen) and *Ulocladium atrum* (Antagonist) based on the number of C-sources utilized individually and those used in common. The modified conidia of *U.atrum* were grown on oat-based media modified with solute modification with glycerol to 0.98 and 0.95 a_w (Sancisi-Frey and Magan, unpublished data)

1.4.1 Formulations and Ecological Windows

It is critical to have formulations of BCA inocula of the right quality which then formulated such that viability can be conserved for long enough for competitive exclusion of plant pathogens or infection of insect pests can be successfully initiated. In glasshouse conditions where control of all abiotic and indeed biotic parameters can be achieved, misting prior to application of a BCA has often given successful results. However, in the phyllosphere and to some extent rhizosphere where the fluxes of water x temperature are more extreme this is still a significant challenge.

Since the major hurdle in the development of BCAs for control of both pathogens and pests has been the abiotic window, attempts have been made to combine ecophysiological manipulation of production to enhance the resilience of BCA inocula and then combine this with different formulations either via adjuvants for protecting the cells/conidia, including milk-based protectants, surfactants, stickers and water entrapment using nanospheres/nanocages. For potential efficacy in the phyllosphere this is crucial. A good example is that of *P.agglomerans* which was applied to citrus fruit pre-harvest for control of post-harvest pathogens. The establishment on the citrus fruit surfaces was very poor for non-physiologically modified cells. However, osmotically-adapted cells with higher endogenous contents of compatible solutes had better resilience to environmental fluxes, and with the addition of a sticker (fungicover, 5%) resulted in better establishment on the citrus fruit surface. This improved subsequent control of post-harvest pathogens (Teixido et al. 2006; Canamas et al. 2008a, b). Recently, Carbó et al. (2018a, b, 2019) used the BioScreen rapid bioassay system for screening of different formulations of *C.sake* to identify the best formulations under different environmental conditions over which efficacy against *B.cinerea* might be potentially optimized. This showed that there were differences between an existing commercial formulation and two new ones based on potato starch and maltodextran, produced using fluidized-bed spray drying for conserving viability and efficacy (Carbó et al. 2017). Assays for control of *B.cinerea* using these formulations at different temperatures (0, 20 and 30 °C) and RH values (40 and 85%) on pears, apples and tomatoes were positive with the new formulations (Carbó et al. 2019).

Studies with the BCA *E.nigrum*, formulated for pre-harvest application for post-harvest control of brown rot of peaches was investigated in extensive trials in two seasons in France, Italy and Spain. These compared fresh and formulated conidia of *E.nigrum* with and without fungicides (integrated control) applied prior to harvest (Larena et al. 2005). While fungicide applications were effective in Italy and France, integrated control was effective in Spain. This certainly suggests that BCAs alone or as part of an integrated control strategy may be important in wider applications for successful use of BCAs, especially in the field to control pathogens in the phyllosphere.

1.5 Resilience of BCAs Under Climate Change Conditions

There has been significant interest in the last few years of the impact that climate change scenarios may have on control of plant diseases in staple crops (Magan et al. 2011; Gasperini et al. 2019). It has been suggested that both pests and pathogen diversity and movement from the equator towards the poles may be occurring at between 5 and 10 Km/year (Bebber et al. 2013, 2014). Thus both screening and choice of the strain of a BCA may become important as tolerance/resilience in climate change conditions which includes an increase in temperature (=2–4 °C), elevated CO_2 conditions (400 ppm today vs 1000 ppm in the future) and fluxes of extreme drought stress and wet periods which may significantly impact control strategies. Certainly, studies of entomogenous fungi suggests that only a few strains of *B. bassiana*, *M. anisoliae* and *I. farinosa* would grow effectively under changes in temperature and water stress (Borisade and Magan 2014). Indeed, the virulence of such BCAs of pests was significantly reduced under climate change factors (Borisade and Magan 2015). It may be very important when screening and choosing potential strains for pathogen control to include such extreme abiotic factors as this may influence efficacy and in some case may require modifications of formulations to improve their resilience, especially in the phyllosphere and rhizosphere.

Recent studies with different formulations of the BCA *C. sake* showed that the three-way interacting climate change conditions influenced the viability of the yeast cells in different formulations. Thus, some formulations are more resilient than others under such abiotic stress factors (Carbó et al. 2018a, b). Studies with other BCAs such as *Clanostachys rosea* and non-toxigenic strains of *A. flavus* for aflatoxin control in maize have also shown that biocontrol efficacy could be reduced or have no effect on the pathogen or toxin production (Medina et al. 2017). Thus, it is critical that there is more focus on formulating BCAs such that the resilience can be improved under the expected environmental changes that will affect both crop production and pest/disease control strategies.

1.6 Conclusions

In summary, it is very important to develop robust environmentally related screening systems for the identification of candidate BCAs which will have the ecological competence required for control of a target pathogen (see Chaps. 3, 4 and 11). The environmental window, including boundary conditions for pathogens need to be understood and this compared with that of the candidate antagonists for evaluating potential efficacy. The NOI concept can be adapted to include abiotic regimes for helping to understand the mechanism of action of the candidate BCA and on whether niche exclusion or nutrient competition are the major characteristics employed. The cophysiological manipulation of the production of specific BCAs can be utilized to try and expand the environmental window over which pathogen control can be achieved. BCA identification and formulation may need to change to ensure that resilience and biocontrol can be achieved under the expected climate change scenarios which are becoming more common in many regions of the world.

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Chapter 2
More Ecological Research Needed for Effective Biocontrol of Plant Pathogens

Xiangming Xu and Mike Jeger

2.1 Introduction

The biological control of plant pathogens involves the use of non-pathogenic (or weakly pathogenic) microbes to control pathogens. Antagonists may directly parasitize pathogens, such as mycoviruses against fungal pathogens and *Ampelomyces quisqualis* against powdery mildews. Antagonists may function by occupying and using resources in a non-pathogenic manner and in so doing exclude pathogens from colonising plant tissues. Other important biocontrol mechanisms include antibiosis and induced resistance. Because of the great diversity of plant pathogens, host species, tissue types (leaves, stems, fruits, flowers, seeds and roots) and associated environmental niches, antagonist species are also quite varied. Nevertheless, much of the research and development in biocontrol of plant diseases have been focused on species from a limited number of genus, including *Trichoderma* and *Bacillus.* In biocontrol of plant diseases, the focus is usually on the augmented introduction of antagonists to control diseases, aiming to increase the numbers or modify the distribution of the antagonists. Unlike biocontrol of insects, limited attention has been paid to developing conservation biocontrol measures that enhance conditions for survival and reproduction of resident antagonists at the expense of pathogens.

Despite extensive research and development in biocontrol of plant diseases, current crop disease management still relies on conventional fungicides. Only a limited number of biocontrol products have been commercialised; success in biocontrol of

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plant diseases in field crops has been limited (Cunniffe and Gilligan 2011), most successes being achieved under more controlled conditions, e.g. greenhouse crops and produce in post-harvest stores. To improve control efficacies, there has recently been increasing interest in studying efficacies arising from combined use of biocontrol agents (BCAs) to exploit the often claimed synergies between multiple BCAs. However, most published experimental data instead showed antagonistic (rather synergistic) interactions among BCAs (Xu et al. 2011a).

The complex ecological processes involved in biocontrol of crop diseases have been often cited to explain the lack of biocontrol success as well as variable biocontrol efficacies that are usually achieved. Tritrophic interactions are often complex, particularly under both temporally and spatially variable conditions. Despite the recognition of the importance of ecological knowledge in biocontrol (Juroszek and von Tiedemann 2011), there is limited information on the fate of biocontrol organisms under natural conditions. Newton et al. (2010) introduced the concept of ecological tolerance in order to better understand interacting microbial populations on the phyllosphere. Furthermore, biocontrol outcome under a given environmental condition may also depend on other resident microbiota in the pathogen-antagonist interacting zone. For a given biocontrol organism we need to understand how: (1) the population dynamics depends on external conditions, (2) antagonists are dispersed, and (3) the biocontrol efficacy is influenced by the biocontrol population dynamics and external conditions? Without such knowledge, it would be difficult to predict biocontrol outcome and hence to optimise the deployment of BCAs in practice.

In this chapter, we aim to highlight the importance of ecological knowledge of biocontrol systems in order to predict biocontrol outcomes and to optimise deployment of BCAs, focusing on biocontrol in the phyllosphere. The environmental effects on biocontrol outcomes are expected to be greater on foliar diseases than on soilborne diseases because of larger variabilities in weather conditions in phyllosphere. For biocontrol of soilborne diseases, research has been focussing on understanding the nature of suppressive soils (Termorshuizen and Jeger 2008) and more recently indigenous microbiome on disease suppression (Schlatter et al. 2017). Recent advances in next-generation sequencing of microbiomes have provided new tools to characterise microbial communities and their functions. We first summarise key modelling results on the effects of external conditions on biocontrol, then review experimental evidences about the effects of external conditions on biocontrol organisms and the resulting biocontrol outcomes, and finally give our own thoughts on topics in biocontrol ecology that need to be addressed enabling prediction of biocontrol outcome with confidence.

2.2 Theoretical Modelling of Biocontrol

Management of plant diseases involves an assessment of disease risks and the impact of different control measures on disease development. Forecasting systems are developed to predict disease risks to facilitate more efficient use of management options. These models usually consider the dynamic effects of external factors on pathogen/disease development without explicitly considering dynamics of specific control measures. This omission may not have much impact for control measures involving conventional fungicides and host resistance. However, it could have large impact for control measures based on living organisms (i.e., biocontrol) for which the control activity is usually directly related to its population dynamics under a given set of conditions.

Disease control is dependent on the BCA population size. An exponential dose-response relationship fitted well with the observed biocontrol relationships for *Pseudomonas fluorescens:*take-all, *Sporidesmium sclerolivorum:Sclerotinia minor*, and *Trichoderma harzianum:*damping-off systems (Johnson 1994). This diminishing return in biocontrol efficacy relative to increasing BCA inoculum dose was also observed for biocontrol of crown gall on tomato and cherry (Johnson and DiLeone 1999). However, the precise nature of the dose-response relationship may depend on biocontrol mechanisms (Larkin and Fravel 1999) and specific biocontrol systems (Montesinos and Bonaterra 1996). The relationship between BCA population size and pathogen inoculum/disease development was modelled as a negative exponential type (Paulitz 2000), which suggested that disease control is reduced when the pathogen inoculum is very high.

A spatially explicit simulation study suggested that biocontrol efficacy of *Ulocladium atrum* against *Botrytis cinerea* is largely affected by their spatial expansion abilities (Kessel et al. 2005). Similarly, the importance of spatial host genetic heterogeneity in affecting biocontrol of fungal pathogens with mycoviruses was demonstrated in a simulation study (Liu et al. 2000).

A theoretical model for biocontrol of foliar diseases was developed to assess the efficacy of different biocontrol mechanisms (Jeger et al. 2009), using a standard susceptible-infected-removed epidemiological framework where host-pathogen dynamics was coupled with one for the pathogen-BCA dynamics via a specific biocontrol mechanism. This mean-field deterministic model was further expanded to study biocontrol efficacies in relation to combining two mechanisms in one BCA and to the combined use of two BCAs each with a different mechanism. Combining mechanisms within a single BCA were generally more effective than combining multiple BCAs each with a single but different biocontrol mechanism (Xu et al. 2010). Two BCAs with a single but different mechanism would rarely combined synergistically (Xu et al. 2011b), agreeing with most published experimental results (Xu et al. 2011a). Two BCAs would rarely combine synergistically under temporally fluctuating conditions (Xu and Jeger 2013a); however, synergy between two BCAs under spatial heterogeneity was possible especially when two BCAs differ greatly in their development between two niches (Xu and Jeger 2013b).

The model of Jeger et al. (2009) provided a general modelling framework for biocontrol of foliar plant disease to assess the efficacy of different biocontrol mechanisms. This model was further extended to evaluate induced resistance as a mechanism of biocontrol (Abdul Latif et al. 2014). The original model (Jeger et al. 2009) did not distinguish plant parts that were affected by the disease, nor any variation in biocontrol efficacy depending on the plant part infected. This is an important niche issue, as pathogens such as *B. cinerea* can infect and cause symptoms on leaves, flowers and fruits. Following the previous modelling framework, biocontrol of a plant pathogen affecting both leaves and flowers was developed, taking into account the transfer of both the plant pathogen and BCA from the leaves to flowers (Jeger and Xu 2015). Differential rates of transfer of the pathogen and the BCA, varying with their time-dependent population densities on leaves, were found to have a major impact on eventual biocontrol outcomes.

A similar generic model was developed to study biocontrol of soilborne pathogens (Cunniffe and Gilligan 2011). Modelling results showed that small changes in biocontrol parameters and pathogen initial inoculum could lead to large differences in biocontrol efficacies and that efficient bulking-up efficiency is needed for antagonists to achieve good control. In addition, the spatial distribution of propagules in soil is an important factor in determining the ability of mycoparasites to control soil-borne plant pathogens (Jeger et al. 2004).

Modelling biocontrol of both foliar and soil borne diseases indicated the importance for antagonists to be able to colonise effectively susceptible healthy host tissues (Jeger et al. 2009; Cunniffe and Gilligan 2011). Yet, this specific ecological feature of biocontrol organisms is often neglected, making predicting and hence optimising biocontrol difficult.

2.3 Effects of Environmental Conditions on Biocontrol Systems

To predict population dynamics of bacterial and fungal microbes in the phyllosphere, we need to understand better their behaviour on the leaf-surface, their interactions with other microbes and the host plants under the influence of external conditions (e.g. climatic conditions and nutrients).

2.3.1 Population Dynamics

Current experimental research has been focusing on pathogen/disease dynamics. However, modelling studies have shown that predicting biocontrol outcomes needs knowledge of the dynamics of biocontrol organisms as well. Thus, as for pathogens, we need to understand how biocontrol organisms survive/reproduce, spread and colonise both diseased and healthy host tissues under the influence of external factors.

The proportion of viable cells of *Pseudomonas syringae* was high on bean leaves under humid conditions irrespective of their aggregated state, but decreased when periodically exposed to desiccation stress (Monier and Lindow 2003). The survival of cells in aggregates promoted a highly clustered spatial distribution of bacteria on leaf surfaces. Bacteria can form biofilm in response to external environmental conditions, which may affect biocontrol outcome significantly in terms of microbial survival and the extent of contact between antagonists and pathogens on host tissue surface (Morris and Monier 2003).

The impact of bacterial endophytes on *Fusarium graminearum* in wheat cannot be easily inferred from simplified *in vitro* assays (Whitaker and Bakker 2019). Furthermore, biocontrol outcomes were environment-dependent and such a dependency varied with specific endophytes, suggesting that environmental gradients should be incorporated into testing for microbial interactions in plant hosts. Limitation in the use of *in vitro* biocontrol studies was similarly demonstrated for the inhibition of *F. guttiforme* by yeast strains (Korres et al. 2011).

Biocontrol organisms need to occupy and survive in the same niches as pathogens in order to achieve biocontrol success. The dynamics of BCA *Aureobasidium pullulans* when applied to organically grown strawberries differed between two seasons (Sylla et al. 2013c). Repeated application of *B. amyloliquefaciens* and *T. harzianum* led to increased presence of the biocontrol organisms on strawberry leaves, but not for *Beauveria bassiana*, suggesting that *B. bassiana* could not survive on the leaf surface for long (Sylla et al. 2013a). A *B. licheniformis* strain colonised strawberry tissues, persisted for 3–5 days, and formed aggregates on plant surfaces, resulting in a biofilm to resist fluctuating conditions (Kong et al. 2010). This strain persisted on strawberry plants for a long time under a controlled condition but failed to persist for more than 7 days on plant surfaces under fluctuating conditions in a polythene tunnel.

The population size of a *P. fluorescens* strain decreased progressively within 2–3 weeks after application and then remained stable thereafter (Pujol et al. 2005). An introduced *P. fluorescens* strain A506 successfully colonised recently opened flowers, and its population size remained high for 5–10 days before decreasing sharply (Lindow and Suslow 2003). However, it is not clear what factors led to observed population reductions.

The population sizes of pathogenic *P. syringae* strains were greater than those leaf-associated populations of non-pathogenic strains on bean plants under dry conditions but not under moist conditions (Wilson et al. 1999). Recoverable *P. fluorescens* populations from leaf surfaces of red pine were extremely sensitive to leaf wetness but relatively insensitive to temperature (Knudsen and Hudler 1987). Establishment of several antagonists (*P. fluorescens* and *Pantoea agglomerans*) of *Erwinia amylovora* (causing fireblight diseases) was inhibited by increasing temperature and stigma age on detached crab apple flowers (Pusey and Curry 2004). The inability of these biocontrol strains to colonise relatively old stigmas that are conducive to *E. amylovora* is likely to reduce their biocontrol efficacy under field