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Review

Behavioral studies, molecular approaches, and modeling: Methodological contributions to biological control success

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ABSTRACT

Modern biological control practitioners must increasingly demonstrate a level of rigor that can only be achieved through use of effective methodological tools such as modeling, behavioral studies and molecular approaches. The use of these technologies is maturing rapidly in biological control and makes tangible contributions to its success. Behavioral studies often uncover important aspects of biology that would otherwise be overlooked, such as the influence of pre-release handling on establishment success and the response of natural enemies to host-induced plant volatiles. Molecular approaches allow the identification and detection of genetically distinct populations of invasive pest species and their natural enemies, tracing the origin of invasive pest populations and compatible natural enemies, and development of improved recombinant natural enemies. Modeling enables theory and empirical observation to optimize agent selection and release, and to predict quantitative impacts on target and non-target populations. All three methodologies, particularly in combination, contribute to our understanding of the reasons for success and failure in biological control, and together with post-release validation studies build the foundations to improve the success of future biological control releases.

1. Introduction

From its earliest examples, such as the domestication of cats for control of rodents in grain stores in the Mediterranean basin or the manipulation of weaver ant colonies for the protection of citrus orchards in China, the goal of biological control has been management and the approach largely driven by empirical observation. While numerous successes have been achieved, particularly in the context of insect pests and weeds, there remains some skepticism regarding the predictability of control by natural enemies and the scope of its application to a broad range of pest taxa in managed ecosystems. With such spectacular successes in biological control as the control of cottony cushion scale (*Icerya purchasi* Maskell) by the vedalia beetle (*Rodolia cardinalis* [Mulsant]), of prickly pear cacti (*Opuntia vulgaris* Mill.) by the cactus moth (*Cactoblastis cactorum* [Berg.]), of rabbits (*Oryctolagus cuniculus* L.) by rabbit hemorrhagic disease virus (*Lagovirus* sp.), and of take-all disease (*Gaeumannomyces graminis* [Sacc.] Arx & Oliv. var. *tritici* Walker) by suppressive antagonists (DAPG-producing *Pseudomonas fluorescens* [Flügge] Migula) there can be no doubt as to the potential that selected natural enemies can have to suppress the abundance or activity of target pests. However, there have also been numerous failures as well as successes in biological control, and in some instances, unintended consequences such as impacts on non-target organisms in the environment (Louda et al., 2003; van Lenteren et al., 2006).

At least some of the failures in biological control can be attributed to the 'shotgun' approach to biological control that began in the 1940s and 1950s as synthetic pesticides gained popularity in pest management (Gurr et al., 2000). The notion that either the best natural enemy species would prevail or that multiple natural enemies would provide an additive impact on target pests prevailed for several decades, and lead to biological control being characterized as an art rather than a science (van Lenteren, 1980). More recently, however, it has become clear that the interaction of a natural enemy with its host is far from the simple trophic or competitive linkage initially envisaged by many biological control practitioners. The manipulation of natural enemy populations can have complex ramifications throughout the food web that may or may not result in the suppression of the target pest or in unintended consequences (Holt and Hochberg, 2001; Lynch et al., 2002). There also remains uncertainty regarding the factors that lead to success in biological control (Gurr and Wratten, 2000), and that define what makes a good natural enemy or manipulation strategy whether for insect pests (Murdoch et al., 2003), weeds (Myers and Bazely, 2003), vertebrate pests (McLeod et al., 2007) or soil-borne diseases (Mazzola, 2007). Thus there is an increasing need for greater scientific rigor in biological control research, and a variety of current and evolving methodologies are

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now widely available and can be applied to future implementation projects.

Several recent research developments provide valuable opportunities to enhance the potential for success of biological control programs. Arguably the most important of these is securing sufficient funding to support the development of a new program to its conclusion. In this context, an *ex ante* economic evaluation can be particularly helpful in ensuring that the selected problem is of sufficient magnitude to justify to the necessary investment and continued support of a program (Jetter, 2005; Jarvis et al., 2006). However, three other areas of advancement are behavioral studies, molecular approaches, and modeling. Rather than providing an in depth review of these methodologies, our aim here is to highlight and provide key references to some of these more recent findings, and to indicate how they can be employed to facilitate and improve our understanding of the manipulation of natural enemies and provide new insights into the development of biological controls.

2. The importance of behavioral studies in biological control

Behavior has been a neglected component of biological control that few practitioners have taken into consideration in the development of new programs. However, both host and natural enemy behavior can have an important influence on the impact of biological control agents. For example, a much neglected aspect of behavior in the context of biological control introductions concerns the conditions that arthropod natural enemies experience before being released. Natural enemies are frequently held in captivity for several days prior to field release and can either be provided with hosts to attack which will result in a steady depletion of lifetime fecundity, or deprived of hosts to maintain future reproductive potential. Using *Mastrus ridibundus* (Grav.), an ichneumonid cocoon parasitoid of the codling moth (*Cydia pomonella* [L.]), as an example, Hougardy and Mills (2006), were able to show that four or more days of host deprivation lead to very high rates of dispersal from the field release site in comparison to individuals that either experienced lower levels of deprivation or various levels of oviposition before release. In contrast, oviposition before release lead to a progressive decline in foraging success, whereas all levels of host deprivation resulted in the same high level of foraging success in field cages (Hougardy and Mills, 2007).

While there have been numerous studies of the foraging behavior of insect parasitoids (Mills and Wajnberg, 2008), there have been surprisingly few on the foraging behavior of arthropod predators (Nakashima and Hirose, 2003; Vanas et al., 2006) and herbivores (Shea et al., 2000; Newman, 2007). Despite this anomaly, there is no reason to believe that the responses of predators and herbivores to pre-release conditions would be any different to that of parasitoids and thus optimizing the life stage and condition of natural enemies that are destined for field release could lead to greater success in both their establishment and impact.

Another aspect of the pre-release behavior of natural enemies that needs to be given sufficient consideration is mating success. Although not a common cause for concern, the frequency of mating is much lower for monandrous species (Gadenne et al., 2001), and normal mating behavior can be greatly influenced by environmental conditions, chemical and visual cues, substrates, and sex ratios (e.g. Quicke, 1997; Joyce et al., 2008). Combined with a short lived adult stage, compromised mating success can lead to poor success in the introduction of novel biological control agents. A good example of this is the crambid moth *Austromusotima camptozonale* (Hampson) that is currently being released in Florida for control of the climbing fern *Lygodium microphyllum* (Cav.) R. Br. (Boughton et al., 2007). Adult females have a short adult lifespan with a mean of 5.7 days, and lay most of their eggs on days 1–3. As is typical for a monandrous species, both males and virgin females mate only once per night, with mating success most effective with male biased sex ratios (1:2) in colony cages. Under such circumstances, releases of juvenile stages may be more effective in achieving establishment than releases of adults.

Foraging behavior can also inform the likelihood of non-target attacks by introduced natural enemies (Duan and Messing, 1997; Babendreier et al., 2005). For example, diurnally active non-target weevil hosts would be expected to be more susceptible to attack by an introduced weevil parasitoid that searches for hosts during the day using visual cues to than non-target weevil hosts that are nocturnal in their activity (Couch et al., 1997). Perhaps more importantly, however, the environmental conditions experienced by a natural enemy during host range testing can influence their foraging behavior and the degree of specificity observed. Thus the sessiid moth *Tinthia myrmosaeformis* (Herrich–Schäffer) showed little host specificity in contact bioassays with test plants, greater specificity in multiple-choice cage tests, and the highest level of specificity in open field experiments (Turanli and Schaffner, 2004). While host range assessment of natural enemies continues to make use of naïve test insects and endpoint measurements such as oviposition (weed agents) or parasitism (insect agents), behavioral observations can provide important insights into the process of host acceptance and rejection and deserves greater attention in future studies (Duan and Messing, 2000; Babendreier et al., 2003).

In the context of augmentative biological control an interesting phenomenon that occurs among ectotherms is behavioral fever, whereby pathogen-infected individuals seek out thermal environments in which they can elevate their body temperature by 4–6 °C, sufficient to increase their chances of survival or reproduction (Moore, 2002; Thomas and Blanford, 2003; Adamo, 2006). This has been particularly well studied among grasshoppers and locusts, where the use of biopesticides based on fungal pathogens has resulted in unexplained variation in the speed of kill following application. It is now clear that substantial delays in pathogen growth within hosts can occur when body temperature is raised to levels that exceed the upper threshold for development of the pathogen (Inglis et al., 1996; Blanford and Thomas, 2000), and this can be sufficient to allow reproduction before death (Elliot et al., 2002). Thus at least for augmentative biological control of acridids it is important to understand the role of host behavioral fever in developing effective biopesticide applications.

It is also well known from the biological control of vertebrate pests that indirect effects of fertility control and population density on behavior in wild populations can have important consequences for the spread of directly transmitted pathogens. Two different approaches to fertility control in possums can lead to opposite indirect influences on the frequency of contacts among individuals and thus the horizontal transmission of the directly transmitted pathogen *Leptospira interrogans* [Stimson] servovar *balcanica*. Sterilization of females by tubal ligation can lead to an increased rate of transmission due to an increased frequency of estrous cycles (Caley and Ramsey, 2001), whereas disruption of endocrine control by gonadectomy can lead to a significant reduction in the rate of transmission (Ramsey, 2007). Thus at least for directly transmitted pathogens, a thorough understanding of the behavior of the target pest can be an essential prerequisite for effective deployment of pathogens in the biological control of vertebrates.

Behavior can also be important in conservation biological control, as exemplified by recent developments in our understanding of the role that host-plant induced volatiles (HIPVs) play in both the recruitment and retention natural enemies in crop ecosystems (James, 2005; Turlings and Ton, 2006; Khan et al., 2008). Whether acting as direct attractants or indirectly as signals for plants to emit a broader range of natural volatiles that mimic those released