The Importance of Biological Control in Agriculture

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Introduction

Michael Grodowitz – Research Entomologist

The Biological Control of Pests Research Unit (BCPRU); whose main mission is the development of biological and biorational (i.e., having a minimal disruptive influence upon the environment and its inhabitants) components for sustainable and environmentally compatible pest management; is comprised of 10 scientists and 16 support personnel. The unit is housed within the USDA-ARS National Biological Control Laboratory (NBCL) located at the Jamie Whitten Delta States Research Center (JWDSRC) in Stoneville, Mississippi. The NBCL was officially formed in 2002. This laboratory is the first facility in the world to have both the infrastructure and the scientific specialisations needed to fully investigate integrated research on the use of biocontrol technologies. The BCPRU researchers develop practical methods of mass propagation, storage, delivery of beneficial organisms, targeted release strategies for integrated pest management, and application of classical biocontrol for the management of invasive insects and weeds. Current research activities include mass-rearing of economically important insect species, molecular biology of both insects and plant pathogens, fermentation, invasive ant management, and biocontrol of invasive weeds. In 2015, the BCPRU produced 17 publications; 1 patent; developed or modified artificial diets for three species; identified potential biorational compounds for invasive ant control; completed host-specificity studies on egg parasitoids of the kudzu and bagrada bugs; demonstrated cost effective strategies to reduce aflatoxin contamination; and furthered the use of pathogens for invasive plant management.

Following are some prime examples of current and ongoing research activities at the BCPRU:

Production of Predatory Mites as Biocontrol Agents

Juan A. Morales-Ramos – Research Entomologists
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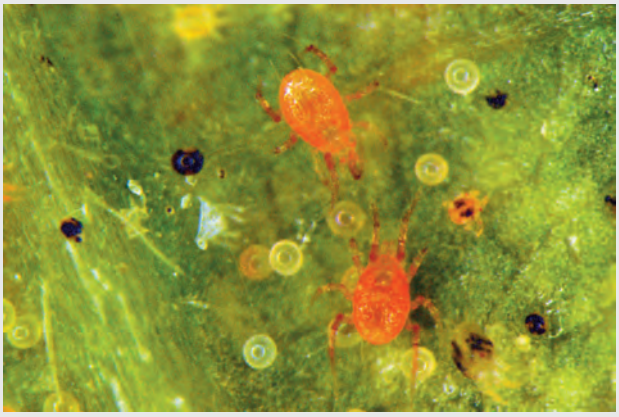

Figure 1. *Phytoseiulus persimilis* feeding on spider mite eggs

Most predatory mites belong to the family Phytoseiidae and are highly effective predators used mainly to control the two-spotted spider mite *(Tetranychus urticae)* in many different crops. However, phytoseiid mites have been shown to provide effective control of other mite pests as well as some insects including thrips and white flies (Bolckmans 2007). It is estimated that at least 20 species of phytoseiid mites are produced commercially and sold around the world for biological control of mite pests in different cultivars (Zhang 2003). Among the most important predatory mites produced commercially include *Phytoseiulus persimilis* (Figure 1), *Amblyseius swirskii, Neoseiulus californicus, N. cucumeris, N. fallacis, Iphiseius degenerans, Galendromus helveolus, G. occidentalis, and Mesoseiulus longipes* (Zhang 2003, Leppla 2014). Production methods range from open systems in greenhouses to fully enclosed using climate controlled rooms (Bolckmans 2007). Some predatory mites can be reared on alternative prey eliminating the need to culture plants for spider mite production. Others, like *I. degenerans*, can be reared solely on pollen. However some of the most important phytoseiid predators, like *P. persimilis*, must be produced on their natural prey (spider mites). This requires the use of multiple isolated greenhouses to culture bean plants, infest them with spider mites and then use them to feed the predators. A pure culture of spider mites (free of predators) must be maintained for inoculating the main production greenhouse, where predators are later introduced (Gilkeson 1992). A section of the bench is harvested when it has reached the maximum predator density. Introduction of *P. persimilis* into the infested plants requires perfect timing to allow maximum spider mite reproduction without losing the plants to the mite infestation. Predator harvesting often exposes the predators to stressful conditions of starvation and many are lost to inefficient collection methods. Enclosed rearing systems offer the potential of greater control of environmental conditions and better containment preventing excessive losses. Several enclosed methods have been proposed to rear *P. persimilis* by introducing its natural prey into different types of enclosure (Theaker and Tonks 1977, Fournier 1985, Overmeer 1985, McMurtry et al. 1989, Shih 2001, Morales-Ramos and Rojas 2014). But, modifications to scale up those systems in order to achieve the required level of predator production have proven difficult. One promising example of an enclosed and continuous rearing system for phytoseiid mites has been developed in the National Biological Control Laboratory. This method relays on bean plants grown using trays and infested by spider mites. Plants are later cut from the soil base to release the trays, which are introduced into large cages where predatory mites are later introduced (Morales-Ramos et al. 2012) (Figure 2). Trays with freshly infested plants are introduced through a door to the upper part of the cage and after being exposed to the predatory mites for a week, they are removed from the bottom of the cage using a lower door. Trays are

Figure 2. Enclosed and continuous system to produce predatory mites

moved down using a simple patented mechanism. Gravid predatory mite females tend to remain close to the prey, while young adult predatory mites tend to move and migrate to the upper parts of the cage. Mites accumulate in a collection cup located at the top of the cage from where mites are collected daily. A small prototype occupying approximately ½ m^2 of space was capable of a continuous production of up to 14,000 predators per week. Dimensions of the cage may be increased to achieve higher production levels and using hydroponic methods may simplify procedures to place the infested bean plants in the trays. This enclosed system holds promise for the future of predatory mite production.

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Importance of Ladybird Beetles as Biocontrol Agents in Greenhouses and High Tunnels

Eric W. Riddick – Research Entomologist

Ladybird beetles (a.k.a., lady beetles, or ladybugs) are well-documented as important biological control agents (predators) of plant pests in urban and agricultural landscapes throughout the world (Dixon 2000, Hodek et al. 2012). Ladybird are less well-known for their role as predators of pests in semi-enclosed systems, such as in greenhouses, plantscapes, or high tunnels (Yang et al. 2014). One of the research projects conducted involves the assessment of ladybird beetles as predators of aphids in high tunnels in the southern USA. Aphids are pests with a long history of infesting crop plants in greenhouses and other protective structures (Blümel 2004). The capacity of ladybirds to suppress aphid populations in greenhouses and high tunnels is equivocal. Successful suppression apparently depends on a number of biotic factors including host plant defenses (trichomes), predator/prey densities, and intraguild interactions of ladybirds with other biocontrol agents (predators and parasitoids) that could increase or even decrease suppression by ladybird beetles. At the National Biological Control Laboratory, scientists are developing techniques to mass-produce ladybird beetles, e.g., the pink spotted lady beetle, *Coleomegilla maculata* (Figure 3) for release into high tunnels to suppress aphid populations on plants, such as strawberry. Some of the research conducted has centered on the development of cost-effective diets to use as alternative food for *C. maculata*. Also, preliminary releases of

Photo: E. W. Riddick, D. T. Johnson, and E. García, 2015

Figure 3. Pink spotted ladybird beetle *(Coleomegilla maculata)*

C. maculata immature and adult stages on strawberry plants, infested with aphids, suggest that this particular species could serve as a great ally in biocontrol of aphids in greenhouses and high tunnels in the U.S. However, more research is necessary to determine predation efficiency. More information is needed on survival rates of immature and adult stages, post release, and on the interaction of released ladybirds with predators (from the neighboring landscape), which find their way into high tunnels each growing season. Of particular concern is preventing aphid-tending ants from entering high tunnels and interfering with the predation potential of ladybirds.

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Biological Control of the Imported Fire Ant

Jian Chen – Research Entomologist

The red imported fire ant, *Solenopsis invicta*, is one of the most successful invasive ants in the world, and is regarded as one of world's worst invasive exotic species. Native to South America, *S. invicta* has been introduced into many countries and regions, including the United States, Australia, China, the Philippines, Thailand, Taiwan, Hong Kong, Macau, etc. (Ascunce et al., 2011). A tremendous effort has been made over the past few decades to mitigate fire ant problems using biological control agents. These agents include parasitoids, such as phorid flies, fungi, bacteria, microsporidia, viruses, and nematodes. *Beauveria bassiana*, an EPA-approved insect biological control agent, has also been investigated for fire ant control. Unfortunately, although it has shown great promise in the laboratory, it has been less successful under field conditions. Environment conditions in an ant nest are constantly being regulated by the ant workers for the survival and development of the colony and this is believed to make them amenable for the growth of microbial pathogens (Hölldobler and Wilson 1990). Furthermore, high genetic relatedness among individuals in a colony may also make social insects prone to diseases (Tarpy 2003). Therefore, for ants to thrive, they must depend on their successful evolution of effective defense strategies against diseases. A range of defense strategies have been evolved for combating infectious diseases in social insects (Schlüns and Crozier 2009). Defenses against pathogens at group level in social insects have been described as "social immunity" (Cremer et al. 2007; Cotter and Kilner 2010). Antimicrobial agents are a crucial component in social immunity. Volatility of antimicrobial agents may have tremendous effect on their efficacy against pathogens in an ant colony. Nonvolatile compounds may work more passively since direct contact by pathogens is required, whereas volatile compounds can function more actively because they can effectively reach the targets by fumigating the whole nest. Researchers at the National Biological Control Laboratory demonstrated the antimicrobial property of nest volatiles produced by red imported fire ants against *B. bassiana*. The germination rate of *B. bassiana* spores were significantly reduced after they were exposed to nest volatiles within an artificial ant nest. This was determined by simulating in an artificial fire ant nest the levels and fluctuations of O_2 and CO_2 as those detected in fire ant nests. The germination rate of *B. bassiana* was not suppressed in the artificial nest due to the changes of O_2 and CO_2; but the toxicity of nest volatiles. Nest fumigation may be an important component of the social immune system in *S. invicta*. This information is important not only in understanding social immunity in fire ants, but also in developing biological control strategies using pathogenic microorganisms, since any successful biological control agents must be able to overcome the toxicity of fire ant nest volatiles.

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Trichoderma as a Biological Control Agent of Insect Pests

Brad Elliot – Microbiologist

Trichoderma spp., a cosmopolitan, filamentous fungi that is commonly isolated from soil, have been shown to enhance nutrient uptake, stabilise soil nutrients, promote root development and increase root hair formation (Harman, 2006) in plants. These attributes identify *Trichoderma* spp. as a promising biological control agent ideal for further research and product development for the control of insect pests. The drying of *Trichoderma* spp. on a large scale is a major constraint due to the loss of conidia viability at elevated temperatures, and can hinder the development of new products. Research conducted at the National Biological Control Laboratory has shown that the microencapsulation of the aerial conidia of *Trichoderma harzianum* through spray drying at elevated temperatures offers several advantages for formulation development and downstream processing of this organism (Jin, Custis, Biological Control 2011). Microencapsulation is generally defined as a process that encases one substance within another on a small scale. This process will normally produce encased materials ranging from less than one micron to several hundred microns in size. It is preferred that formulations of *Trichoderma* exhibit a level of 5×10^9 cfu/g to be useful in a variety of applications (Harman and Custis, 2006). In order to achieve this density level the finished dried *Trichoderma* conidia must be greater than 90% pure conidia and be in the form of a flowable powder. Utilising different sugars as our microencapsulating agent we were able to develop a method for spray drying the conidia of *Trichoderma harzianum*. A two phase solid production system was used to produce the conidia needed for our research, utilising the liquid phase to produce our initial inoculums for the solid phase. Following liquid phase production in shake flasks solid media consisting of 50% Rice Chaff and 50% Extra Long Grain Enriched Rice was inoculated and incubated in growth rooms at 28^0C for 10 days. Resulting conidia were washed from the solid media with DI water and poured through a 100 mesh screen. This conidial suspension was centrifuged to produce a paste which was then used for the subsequent microencapsulation and spray drying experiments. Varying levels of sucrose, molasses and glycerol solutions were analysed for their efficacy as microencapsulating agents, along with varying spray drying temperatures. Results indicated that *Trichoderma* conidia encapsulated with sugar solutions ranging from 0.5% to 8% resulted in higher survival rates (cfu/g) than the non-microencapsulated controls. Temperature studies indicated that optimum inlet drying temperatures are between 50^0C and 80^0C respectively. Our results demonstrated a spray drying process that could produce a flowable (particle size around 10-25µm) technical powder containing over 99% conidia ideal for use in formulation development.

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Augmentative Use of Plant Pathogens as Bioherbicides

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Weeds cost growers billions of dollars each season for management and crop yield losses. The use of fungi and bacteria as augmentative biological control agents (bioherbicides) is recognised as a significant technological innovation for the control of weeds (Charudattan 2005, Hallett 2005, Rosskopf et al. 1999, Weaver et al. 2007). Worldwide interest in this field exists, and because of real and perceived health and environmental concerns, the need to develop non-chemical weed management tools and strategies is more acute than ever. Weeds present an enormous problem, but the development of herbicide resistance, on a worldwide basis has greatly intensified weed control issues. Currently, about 250 species of weeds have become resistant to various herbicides, with over 30 weeds documented as resistant to the herbicide, glyphosate (Heap 2015). Glyphosate use in glyphosate-resistant crops has exacerbated the development of weed resistance. *Amaranthus palmeri* (serious problem in the southern U.S.) and *Conyza canadensis* (widespread in North America) are examples of two problematic weeds of several major crops.

Relatively recently, biotypes of these species have developed resistance to some herbicides (e.g. glyphosate, paraquat, triazines, etc.); chemicals that once controlled them. Research at NBCL and elsewhere has demonstrated that the fungus, *Myrothecium verrucaria* (MV) can control weeds from various families (Figure 4) (Anderson and Hallett 2004, Boyette et al. 2014b, Hoagland et al. 2011b, Hoagland et al. 2007, Walker and Tilley 1997). Under greenhouse and laboratory conditions, MV controlled glyphosate-resistant and –susceptible *A. palmeri* seedlings, suggesting this fungus as a potential bioherbicidal candidate against this onerous weed (Hoagland et al. 2013). Another fungal pathogen, *Colletotrichum truncatum*, exhibited potent bioherbicidal activity on the weed, *Sesbania exaltata* (Figure 5.) (Boyette et al. 2008a). Additionally, a bacterial pathogen, *Xanthomonas campestris* was evaluated as a bioherbicide against *Xanthium strumarium* (Boyette and Hoagland 2013a; 2013b) and also found to infect and kill *C. canadensis* (Boyette and Hoagland 2015). Furthermore, both glyphosate-resistant and -susceptible *C. canadensis* populations

Figure 4. The bioherbicidal fungus, Myrothecium verrucaria (MV), can control kudzu in the field. MV-treated plot (right); untreated control plot (left)

Figure 5. Hemp sesbania, infected and killed by application of the bioherbicidal fungus, Colletotrichum truncatum in the field. Arrowheads depict infected, necrotic and/or dead seedlings

of these weeds were controlled by *X. campestris* (Boyette and Hoagland 2015). We have also discovered important positive interactions (additive and/or synergistic effects) when some bioherbicides were applied in formulations containing low levels of certain herbicides (Boyette et al. 2014a, 2008a, 2008b, 2006, Hoagland et al. 2011a). We are currently investigating bioherbicide formulation improvements and synergistic interactions with certain chemicals to enhance pathogen (bioherbicide) efficacy on herbicide resistant and other economically important weeds.

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