

## Control of Insect Pests with Entomopathogenic Fungi

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### Abstract

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Diseases of silkworms and honeybees were recorded by the Chinese as early as 2700 B.C. In 1835 Agostino Basi was the first scientist to publish results showing that a fungus actually caused a disease in insects. The use of entomopathogenic fungi for insect control is not new. One of the first cases of their effective use was by Metchnikoff who showed that an insect-killing fungus could infect insects living in soil. Two primary factors have prevented the rapid development of entomopathogenic fungi. These are the lack of an effective mass production method and inadequate formulations for their field application. In recent years many of these barriers have been overcome. This manuscript will document how fungi infect insects, the steps necessary for the development of entomopathogenic fungi for IPM and progress made with these microorganisms for control of Sunn Pest, *Eurygaster integriceps* Puton.

In this new century we are faced with many challenges to improve agricultural productivity while preserving the delicate balance of nature and human wellbeing. Agriculture remains the foundation of the world's societies, but many of its practices threaten the very earth on which it depends. We must explore bold new strategies for managing our planet to ensure that it can sustain humankind for the future.

For decades agricultural pesticides have helped protect crops from the ravages of pests. These chemicals have been responsible for maintaining and increasing production. They have also served to increase the quality of our food and fiber. Now many pests have developed resistance to these chemicals and super strains of insects and pathogens can tolerate even the most toxic compounds (22). In some areas secondary pests have now assumed a primary status. Issues of environmental contamination and human safety are becoming more and more predominant. Scientists, pest managers and farmers are recognizing the need for better and safer crop management strategies. These factors have led to a renewed interest in biological control.

Entomopathogenic fungi are poised to become valuable tools in integrated pest management (IPM) because of significant advances in our ability to mass-produce and formulate large quantities of stable inoculum, and in the understanding of molecular events involved in the fungal infection process. More is now known about the genes controlling insect infection, and factors that enhance and inhibit spore germination and penetration into the insect host (3, 11, 24).

### The Infection Process

There are four basic phases in the infection process (Fig. 1). These are (a) attachment of the spore on the host, (b) germination of the spore, (c) penetration of the insect's

body and (d) proliferation within. External sporulation will occur if ambient conditions are suitable.

**Attachment.** The basic infective unit is the spore, or conidium, and once it lands on a host, it must adhere to the cuticle and germinate. Attachment is mediated by chemical components in the outer layers of the spore and the host's cuticle. Some fungal species, such as *Verticillium lecanii* and *Entomophaga maimaiga*, produce hydrophilic spores that are covered with mucus, which helps spores adhere to the insect, appears to provide protection from sunlight and desiccation and may modify the cuticular waxes to facilitate invasion (10, 12). In contrast, a rodlet layer, consisting of proteins, lipoproteins, glycoproteins or polysaccharides, covers dry hydrophobic conidia, such as those of *Beauveria bassiana* or *Metarhizium anisopliae* (7). This rodlet layer binds the conidia to the host cuticle by a combination of hydrophobic and electrostatic forces (4).

**Germination.** Following attachment, for successful infection, germination must take place. If conditions are favorable, it occurs within a few hours. This process may be mediated by several factors including abiotic conditions (e.g. temperature, humidity), and biotic factors such as fatty acids within the cuticle that can terminate fungal development, or lipids in the insect cuticle that can stimulate infection (7, 11). Information on temperature optima is important in the selection of candidate strains because they must be able to germinate and grow at temperatures prevailing when pest populations are present (20). It is commonly thought high ambient humidity is a pre-requisite for fungal infection to occur, but in fact this is not strictly true. Great inter- and intra-specific variation exists among different fungi in terms of their ability to germinate at low humidities (18); furthermore, a measure of ambient humidity often does not accurately reflect conditions at the leaf

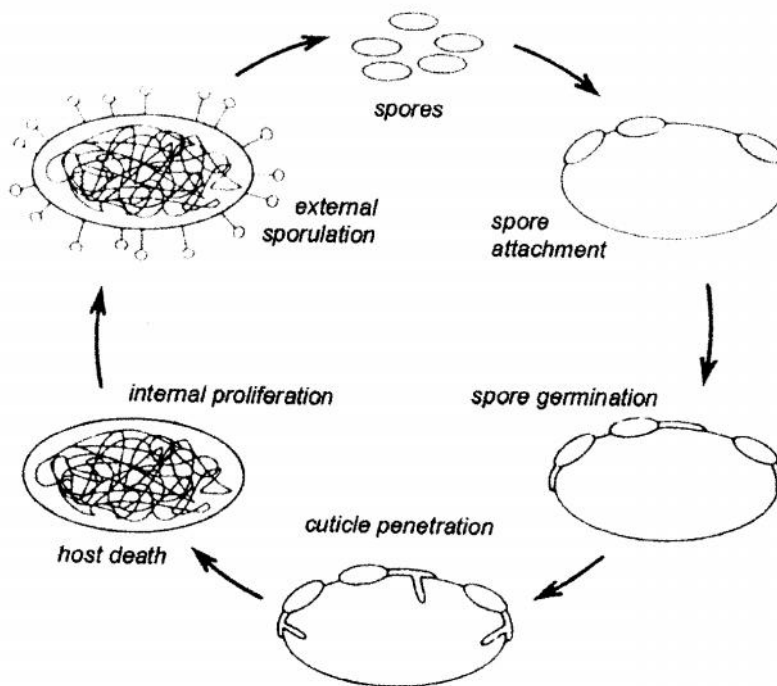
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surface, in the soil, or even on the insect itself, where the microclimate is usually sufficiently humid for infection to occur. For example, fungi often preferentially infect a susceptible host at jointed areas (e.g., the mouthparts), where the cuticle is less sclerotised and conditions are more humid (7).

**Penetration.** After germination, the resulting germ tubes actively produce mucus that enables them to adhere to the cuticle (11). The hyphae then usually differentiate to form specialized infection structures such as appressoria. Infection pegs are generally formed from these to penetrate the first layers of the cuticle. The fungi not only have to

survive the fungistatic and fungicidal compounds present in the insect cuticle, but must produce the right combination of enzymes and mechanical forces to degrade and pass through the different cuticular layers to the hemocoel (7).

Once in the hemocoel, fungi encounter the host's immune system. A fundamental basis for host-specificity is not only the ability to penetrate the insect cuticle, but also to survive within the host and escape host defense responses (8). Not all fungal cells that successfully penetrate the cuticle grow and reproduce in the hemocoel. Humoral and cellular defense responses, and in some cases behavioral responses, can cure fungal infections (2, 6, 11, 14).



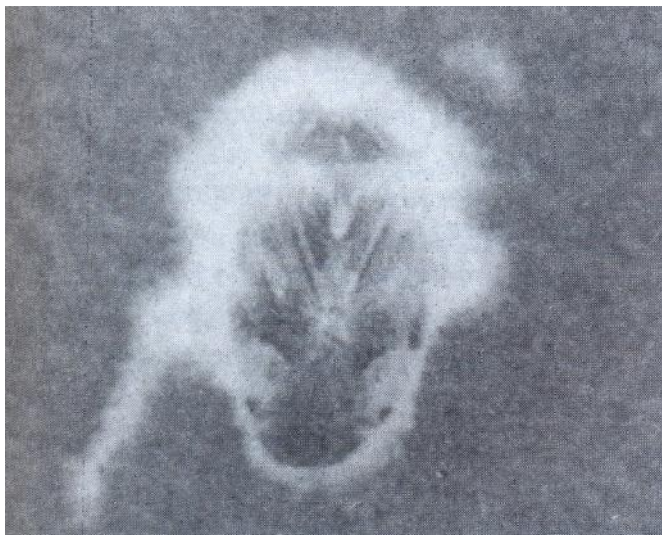
**Figure 1.** The infection cycle for entomopathogenic fungi.

**Proliferation.** If fungi can overcome the host's defenses, they will proliferate vegetatively within the insect hemocoel as yeastlike structures (blastospores) or hyphal bodies. For many fungi, host death is attributed to nutrient depletion following fungal growth throughout the hemocoel. Other fungi produce mycotoxins, a common feature among strains of *B. bassiana* and *M. anisopliae* (11). Hosts infected by toxin-producing fungi are often killed fairly rapidly and only a limited amount of vegetative growth occurs prior to insect death. After host death, though, such fungi will completely colonize the cadaver.

Outgrowth and sporulation on the cadaver surface will only happen if temperature and humidity levels are satisfactory (Fig. 2). High ambient humidity is especially important for spore production to occur. Once formed, spores may be dispersed in a variety of ways, both active and passive (10). Entomophthorean fungi have an active mechanism for conidial ejection. Sporulating cadavers are often found in exposed locations, and ejected conidia may be widely dispersed on air currents. Conidia of other species are passively released from sporulating cadavers by the wind

or rain splash, or through direct contact between infected and non-infected hosts. Spores may contact a suitable host immediately, potentially beginning the whole infection cycle once again, spreading the disease within a susceptible population; or, they may be deposited on a variety of other substrates such as soil or foliage. Survival of these conidia can range from hours to days to years, and varies among species, and is affected by temperature, solar radiation, and a host of other abiotic and biotic influences (10). Survival is a key issue when using fungi for insect control. It will determine when fungi must be applied so sufficient viable conidia are available when pests are present to effect control.

In summary, the processes involved in insect infection are attachment, germination, penetration, growth in the host, death of the host due to physiological and/or biochemical disruption, outgrowth, sporulation and dispersal.



**Figure 2.** *Lygus lineolaris* infected with *Beauveria bassiana*.

### Development of Entomopathogenic Fungi

The development of entomopathogenic fungi as a component of IPM is a multiphase process, the major ones being exploratory activities, pathogenicity trials, characterization, mass production and pilot testing (Fig. 3).

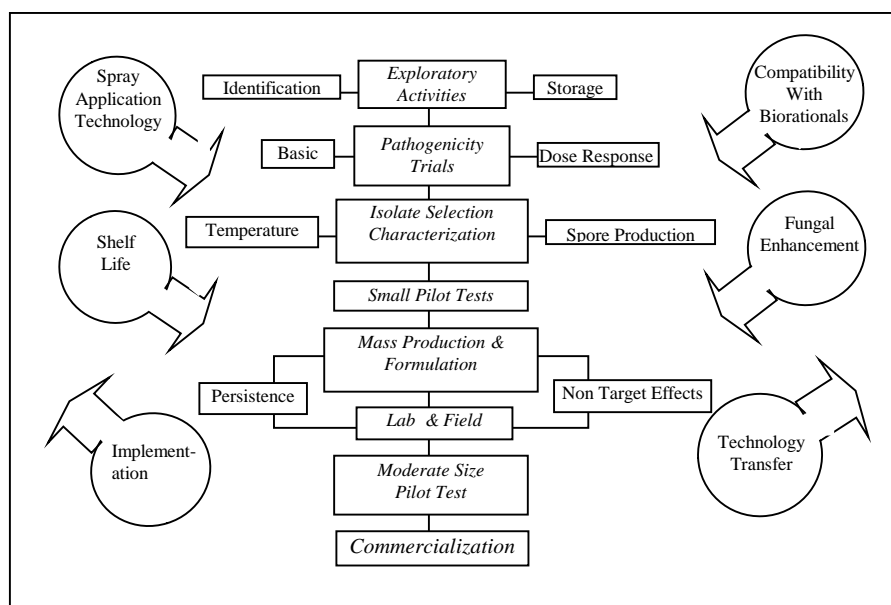
**Exploration.** Locating fungi for development as a biological control agent is arduous and time-consuming. Usually it requires searching sites where the target pest is active on the crop, or resting in aestivating or diapausing areas. There are several ways to obtain entomopathogenic fungi. The easiest is to search for insects showing symptoms of fungal infection. Early signs of infection are difficult to distinguish though some may show necrotic spots at infection sites. During later stages of infection, the insect may be off-color or mummified. They may also display unusual behavior, an uncharacteristic curl or shape to the body and/or a watery internal contents may be evident. The most obvious sign of infection is fungal outgrowth, which may appear over the entire body or at locations where the

body wall is thin, such as the intersegmental membranes or around the spiracles, anus, and joints of the legs.

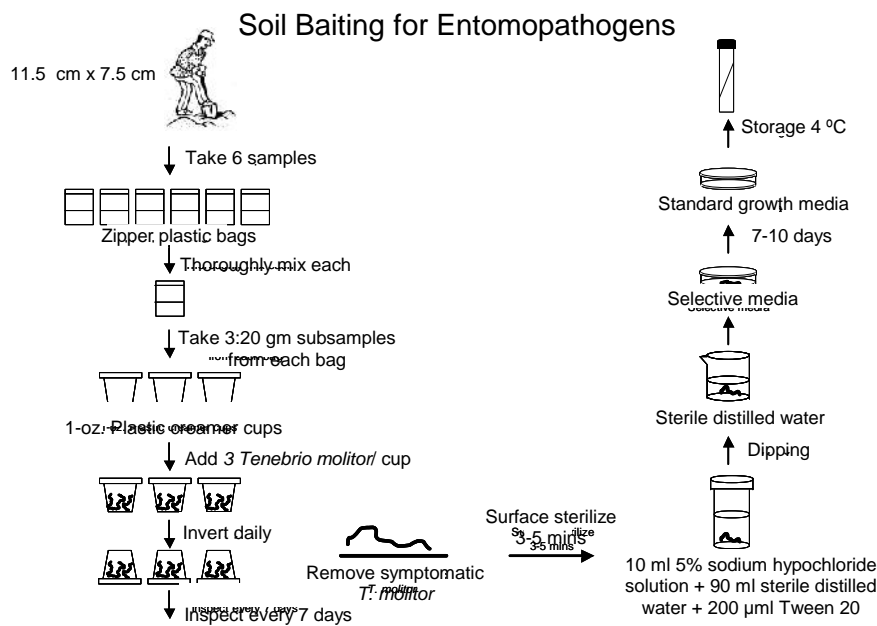
Another method of obtaining entomopathogenic fungi is by isolating them from soil. We successfully recovered many fungi from soils in Taiwan using a modified 'soil-baiting' technique (25) (Fig. 4). A soil sample is baited with susceptible larvae (e.g., *Galleria* spp., Tenebrionidae). Larvae move around the soil, come in contact with spores and become infected. Symptomatic larvae are then removed and processed. A description of how to isolate, culture and identify entomopathogenic fungi from cadavers is found in Lacey (17).

**Pathogenicity trials.** Once a collection of entomopathogenic fungi has been assembled, isolates must be tested against the target pest. Well designed bioassays are a critical first step in successfully identifying the most suitable isolates for further development. Test insects should be even-aged and as genetically homogenous as possible to decrease variability in results of the bioassays (23). Ideally, laboratory-reared individuals should be used rather than field-collected ones. Insects may be directly exposed to fungi by dipping in a spore suspension, or spraying it directly on the insects' body. Alternatively, a residual assay may be performed by treating filter paper and/or a leaf with a suspension and releasing the test insects onto the treated surface. For initial pathogenicity trials a single concentration (e.g.,  $5 \times 10^6$  spores/ml) is often used. Insects are held at a constant, fixed temperature, with infection/mortality being assessed after a pre-determined time, e.g., one week. Standardized bioassay methods appropriate to the target pest must be devised that minimize control mortality while maximizing treatment effect.

After promising isolates are selected, complex assays using multiple dosages run at a range of temperatures, with observations over the assay period are needed to determine  $LC_{50}$  and  $LT_{50}$  values. These assays identify those isolates that are most virulent at temperatures existing in field crops at the time of insect infestation, enabling elimination of all but the most promising isolates for further development.



**Figure 3.** Activities involved in the development of entomopathogenic fungi for IPM.



**Figure 4.** Steps used for collecting entomopathogenic fungi from soil.

**Characterization.** Fungi are an extremely diverse group of microorganisms, evident among different species and between isolates of the same species. For example, most fungi germinate most readily under relatively warm, humid conditions. Some require 100% rH and high temperatures to infect the pest, while others are effective over a wide range of environmental situations (18). Some have a natural capacity, because of their genetic makeup, to produce large numbers of spores, while others produce few—obviously a negative trait for mass production. Care must be taken to select strains that are not only pathogenic to the target pest but also possess the very best characteristics that will enhance their overall performance. A strain that works very well in the hot, humid environments of the tropics may not be suitable for the more arid regions of Syria. The characterization process requires extensive bioassays in the laboratory varying such things as type of media, temperature, humidity and time (13).

**Mass production.** There are numerous methods described in the literature for mass-producing entomopathogenic fungi with a range of complexity (1, 5, 16). Highly specialized and often proprietary methods have been developed for commercial production, but very simple methods requiring minimal equipment have also been described (9). Care must be taken to use equipment and methods appropriate for the setting in which they are intended to be used. Simplicity, ease of construction, availability of equipment and materials, and cost must be considered when designing efficient and appropriate mass-production techniques. For example, rice, a grain commonly used as a growth medium, may be readily available in some locations but relatively rare or expensive in others, where rural people prefer to eat it rather than use it to grow fungi.

Because of complex genetic and metabolic interactions, entomopathogenic fungi may lose virulence after growing and reproducing for several generations on artificial media, without exposure to hosts (21). Therefore, it is essential to

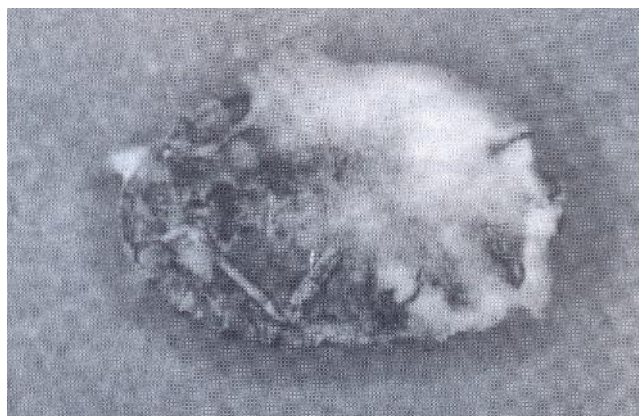
establish the virulence-generation relationship for the mass production methods developed. This indicates how long fungi can be continually produced on an inert medium before there is attenuation of virulence. Virulence may be maintained by periodically passaging and re-isolating a fungus through an insect host. Randomly selected spores from different production batches must be bioassayed routinely to ensure that pathogenicity has been maintained; this, together with an assessment of spore viability, must be a standard quality control procedure.

**Pilot testing.** One of the most important stages in the development of entomopathogenic fungi is pilot testing. Selected isolates need to be mass-produced in sufficient quantity for preparation of inoculum, e.g., spore suspensions for spraying, mycelial granules for incorporation in the soil, and tested in small plots against the target pest. Efficacy (assessed as either mortality, feeding cessation, reduced damage or increased yield) should be determined after reasonable lengths of time, that will vary according to the pest (e.g., multi-generation vs. single generation per cropping cycle), ambient conditions and the method of treatment. Results from small-scale pilot tests permit refinement of application times and dosages. Only after successful results are achieved in these tests should larger scale tests be considered. These tests usually precede what is termed operational testing, where farmers are furnished materials to apply. Care must be taken not to release fungal materials to farmers or commercial operations before testing is complete and efficacy data are available. Failure of a biological control agent as a result of inappropriate use or before efficacy can be assured can destroy farmers' confidence in this novel approach to pest management.

### Progress with IPM of Sunn Pests

In 1998 a cooperative research program between ICARDA and the University of Vermont, USA was initiated to develop the potential of entomopathogenic fungi for

management of Sunn Pest. Chemical insecticides are widely used against this pest, but their efficacy is declining (19). Because of the high cost, they are unavailable to large segments of the agriculture community, particularly in Central Asia, where the severity of Sunn Pest damage is increasing. Development of alternative management strategies is critically needed. Entomopathogenic fungi offer a previously untapped biological control agent against Sunn Pest. For years researchers and managers have observed mortality by fungi among overwintering Sunn Pest (Fig. 5) (15). However, few attempts have been made to exploit the potential of these microorganisms for Sunn Pest. As described above, the first step in the development of fungi for biological control is obtaining isolates from infected Sunn Pest, on which pathogenicity trials can be conducted.



**Figure 5.** Overwintering Sunn Pest infected with *Beauveria bassiana*.

**Exploratory activities.** Since the initiation of this cooperative project, we have searched Sunn Pest overwintering sites for infected cadavers throughout much of the range of this pest. This has been a challenging task. Though Sunn Pest are easy to find in wheat fields during their active feeding period, over the winter they are highly dispersed in the foothills surrounding the fields and are well camouflaged under litter beneath shrubs and trees.

*Syria and Turkey.* The overwintering habitats of Sunn Pests varied depending upon geographic location. If mountains were present surrounding wheat fields, the insect was found on the steep slopes beneath litter at the bases of thorny bushes. Commonly they were resting under leaves, moss or soil clumps. Sometimes they were at the edge or under rocks. At lower elevations without mountains nearby, Sunn Pest was found under the litter of low lying scrubs or at the bases of Eucalyptus and Mediterranean pine trees. Over 1,000 Sunn Pest were collected in 1998, from which 104 fungal accessions were made (Fig. 5). Isolates in the genus *Beauveria* were most commonly recovered, and less frequently *Paecilomyces* and *Fusarium*. The latter is considered a moderate entomopathogen, but some strains have been found to be highly pathogenic to some insects.

*Central Asia.* In January 1999 we collected over 400 symptomatic Sunn Pests from overwintering sites in northeastern and southeastern Uzbekistan, from which 35 isolates were recovered. Most were *B. bassiana* (85%), though also represented was *Paecilomyces*. In 2000 about 500 Sunn Pests were collected from southeastern Kazakhstan and northern areas of the Kyrgyz Republic. About 50 fungi were recovered, Over 50% of them *B. bassiana*, but also found were *Paecilomyces* sp., *P. farinosus*, *P. lilacinus*, *Hirsutella* sp., *Fusarium* sp. and *Verticillium lecanii*.

## Summary

About 200 strains of entomopathogenic fungi have been isolated from material collected from Sunn Pest overwintering sites in Central and West Asia as a result of our exploratory activities in the past 3 years. Though several genera of recognized entomopathogenic fungi were found infecting overwintering Sunn Pest, most were *B. bassiana*, suggesting that this pathogen may hold the most promise for management of this pest. These isolates have now been placed in permanent storage. They represent a significant block of new material for test purposes from a host species never previously examined for fungi. The next step in the development process, now underway, is to conduct assays to identify those strains that are most pathogenic against Sunn Pest.

## Acknowledgments

This research would not be possible without the dedicated expertise of Dr. Svetlana Gouli and William Reid who patiently recovered and purified the isolates found infecting Sunn Pest. The identification of isolates was verified by Dr. Richard A. Humber USDA, ARS, Ithaca, N.Y. We also appreciate the ICARDA personnel and local scientists in Central and West Asia, too numerous to mention by name, who assisted with in-country arrangements. Without their help we would never have been able to locate the elusive Sunn Pest or their fungi. Support for this work was given by the Conservation, Food and Health Foundation, and US Agency of International Development.

## المخلص

باركر، بروس، ماركاريت اسكندر، ميشيل براون بردج ومصطفى البوحسيني. 2000. مكافحة الآفات الحشرية باستخدام الممرضات الفطرية. مجلة وقاية النبات العربية. 18: 133-138.

سجلت أمراض دودة الحرير ونحل العسل في أوائل 2700 قبل الميلاد. يعتبر أوغستينو بازي أول باحث نشر نتائج تبين أن الفطور تسبب فعلياً أمراضاً للحشرات في عام 1835. إن استخدام الممرضات الفطرية في مكافحة الحشرات ليس جديداً. أحد استخداماتها الفعالة الأولى كانت من قبل ميتشنيك أوف الذي أظهر أن الفطور القاتلة للحشرات يمكن أن تصيب الحشرات التي تعيش في التربة. إن العاملين الأوليين اللذين حدا من التطور السريع للممرضات الفطرية للحشرات هما؛ الحاجة لطريقة فعالة في تربيتها الكمية وعدم وجود الصيغة التطبيقية الملائمة لاستخدامها في الحقل. تم التغلب في السنوات الحالية على العديد من هذه العقبات. يعرض هذا البحث كيفية إصابة الفطور للحشرات، الخطوات الضرورية لتطوير الممرضات الفطرية للحشرات لإدخالها ضمن برنامج الإدارة المتكاملة للآفة والنجاح الذي أمكن تحقيقه باستخدام هذه الأحياء الدقيقة في مكافحة آفة السونة (*Eurygaster integriceps* Puton).

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