POTENTIAL FOR BIOLOGICAL CONTROL OF SOIL INSECTS USING MICROBIAL PESTICIDES IN THE CARIBBEAN

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ABSTRACT

The negative environmental impact of chlorinated hydrocarbons and the potential for ground water contamination by the more soluble organophosphate pesticides have increased governmental and industrial support to development of microbial insecticides for soil insect management. There are many factors that must be considered when developing a pathogen for use in agricultural systems to control insect pests. Major factors include cropping system, target insect, soil type, and the pathogen itself. This paper addresses each of these factors and the influence they have on the success of an applied microorganism.

RESUMEN

El impacto negativo de los hidrocarburos clorinados y el potencial de contaminacion de aguas a traves de la aplicacion de los organofosforados ha dado como resultado un respaldo del gobierno y la industria a el control microbiologico de los organismos del suelo. Muchos factores deben tenerse en cuenta si se va a utilizar un patogeno en el control de insectos plaga; Estos factores incluyen el sistema de cultivo, el insecto plaga, factores el tipo de suelo, y el mismo patogeno. Se hace referencia en este manuscrito a la influencia de estos factores en el exito de la aplicacion de un microorganismo.

Historically, soil insects have been managed by applying long-residual pesticides such as chlorinated hydrocarbon, organophosphate or pyrethroid insecticides. The negative environmental impact of chlorinated hydrocarbons and the potential for ground water contamination by the more soluble organophosphate pesticides have increased governmental and industrial support of the development of microbial insecticides for soil insect management (McCoy 1990). This paper is an overview of the pathogens present in the soil and discussion of the feasibility for their use as microbial control agents of soil insects in agricultural systems in the Caribbean. Particular attention is given to abiotic and biotic factors associated with the agroecosystem, the target insect and the pathogen.

All major groups of entomopathogens (viruses, protozoa, bacteria and fungi) can be found in agricultural soils. However, the mere presence of a pathogen in the soil is not indicative of its ability to infect soil-inhabiting insects. In surveys measuring disease incidence in soil insect populations, fungi are the predominant group of microorganisms infecting all stages of insects. In Cuba, Montes et al. (1981) found that fungi, bacteria and nematodes were the predominant causes of mortality in adult, larval and pupal blue-green weevils (*Pachnaeus litus*) with over 50% of the insects killed by the fungus *Metarhizium anisopliae* (Metsch.) Sorokin. In a Florida survey, the fungi *Beauveria bassiana* and *M. anisopliae* were the most commonly recovered diseases among four species of citrus root weevil (Beavers et al. 1972, Beavers et al. 1983).

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For the majority of soil-borne pathogens, particularly viruses and protozoa, the soil functions primarily as a reservoir. Dead infected insects killed on foliage fall or are forced from the foliage during crop harvest or heavy irrigation, rainfall or by the wind. In the soil, pathogens may be protected from detrimental effects of desiccation, ultraviolet radiation and temperature extremes.

A key factor influencing the pathogenicity of these organisms to soil insects is mode of infection. Protozoa, viruses and bacteria generally infect following ingestion of infectious propagules by a susceptible insect host. Inoculum from the soil is introduced onto foliage either by wind, splashed rainfall or irrigation or it is carried onto foliage by insects that have contacted the soil. Propagules are consumed by insects feeding on the inoculated foliage. Because of their *per os* mode of infection, viruses and protozoa are primarily being developed as microbial insecticides of foliar feeding insects.

Although bacteria are more common as foliar-applied pathogens, two species have been successfully used to manage soil insects. *Bacillus popilliae*, the causal agent of milky spore disease, was first isolated from the Japanese beetle (*Popillia japonica*) in New Jersey in 1933 (Fleming 1968). Since then, the bacterium has been successfully produced *in vivo* in beetle grubs by 2 U.S. companies for control of the Japanese beetle (Klein 1980). Difficulties in developing economical *in vitro* production methodologies has limited further commercial success of this microbial pesticide. A strain of the facultative pathogen, *Serratia marcescens*, is currently being produced commercially under the trade name Invade by Monsanto NZ Ltd. in New Zealand for control of the grass grub, *Costelytra zealandica* (Jackson 1990). Since the shelf-life is less than 2 weeks, application of the organism to pastures is being made only by contracted, licensed applicators in order to insure product quality and proper application and timing (Jackson 1990). Pastures provide a very low profit margin for owners so the use of this microbial pesticide to control the grass grub suggests that the treatment is both effective and economical.

Fungi infect insects via penetration of the host cuticle. This contact mode of infection makes fungi more suitable for infecting soil insects than the *per os* mode of viruses, protozoa and bacteria. Movement of susceptible insects within or through inoculated soil is sufficient to initiate infection. For this reason, fungi are more amenable to development as microbial insecticides of soil insects.

There are many factors that must be considered when developing a pathogen for use in agricultural systems to control insect pests. The major factors include cropping system, target insect, soil type, and the pathogen itself. Each of these factors has within it, many parameters that can influence the success of an applied microorganism. The remainder of this paper will address the effects of these parameters on microbial control.

Cropping System

The type of cropping system can be important in the success or failure of a microbial insecticide since crop characteristics and production strategies vary tremendously between crops (Carruthers & Soper 1987). Canopies of many crops, particularly perennial crops, provide more uniform microenvironmental conditions which are more conducive to disease development than those found in some row crops (Carruthers et al. 1985). For example, in Florida citrus groves soil temperatures at 15 cm are 5-10°C cooler under the canopy than in unshaded soil (DuCharme 1967). The microenvironmental stability afforded by a dense canopy is not present during the early stages of both annual and tree crop development or following crop harvest.

Annual crop cultural practices such as plowing and harvesting also may be favorable to the implementation of microbials. For example, harvesting of alfalfa in southern California stimulated development of *B. bassiana* epizootics in Egyptian alfalfa weevil populations because the foliar feeding weevils were knocked to the ground and contacted the fungal inoculum in the soil (Johnson et al. 1984). The method and timing of application of a microbial pesticide should be integrated with the cultural practices in order to maximize potential contact between the inoculum and the host.

Also the ability of the crop to withstand injury sustained during the time it takes for the pathogen to initiate disease development is critical. Low damage thresholds of some crops, particularly annuals, combined with a slow mode of action of the applied pathogen may prevent the use of microbial pesticides in some crops (McCoy 1990).

Agrichemical Inputs

Agrichemicals, including pesticides, fertilizers and spray adjuvants can affect the efficacy of microbial pesticides. In some instances, pesticides function synergistically with pathogens to increase mortality of a target insect. Fonofos insecticide application increases the susceptibility of *Melolontha melolontha* to *B. brogniartii* (Ferron 1971). More often, however, there is a deleterious affect of the chemical on the applied pathogen. For example, in a study measuring the effects of 44 pesticides on the *in vitro* development of *Nomuraea rileyi*, the fungus was inhibited by 7 of 8 fungicides, 13 of 25 insecticides and 4 of 11 herbicides (Ignoffo et al. 1975). Likewise, in laboratory tests measuring germination and growth, 20 of 21 herbicides inhibited *B. bassiana* development to some degree (Gardner and Storey 1985). In the same study, *B. bassiana* infection of *Spodoptera frugiperda* in the soil was significantly inhibited by application of the label rates of both alachlor and oryzalin. In a similar study, 3 of 4 plant growth regulators and 1 of 8 spray adjuvants also significantly reduced *in vitro* growth and germination of the *B. bassiana* (Storey and Gardner 1986). These studies indicate that proper selection and scheduling of chemical and microbial pesticides is necessary to minimize the deleterious effects of chemicals on pathogens.

Insect Pest Life Cycle

Many economically important agricultural pests spend a large portion of their life cycle in the soil and damage the root systems of a wide variety of crops. The location of these pests within the soil profile differs according to species, developmental stage and host plant. Insect biology and soil movement should be known for each pest so that applications of microbial pesticides can be timed to maximize the probability of the insect contacting a reasonably high inoculum.

Commercial formulations of *B. bassiana*, and *M. anisopliae* are being evaluated as potential control agents of citrus root weevils in Florida (McCoy et al. 1984). The current management strategy being employed takes advantage of the fact that the weevils oviposit in the canopy of the citrus trees and upon egg hatch, the susceptible neonates fall to the ground and begin burrowing into the soil. Equally important is the fact that the majority of fungal conidia can remain in the upper 5 cm of the soil. As a neonate burrows, conidia passively attach to the cuticle, germinate, and kill the neonate before it can begin feeding on the roots. Fungal application after neonates have entered the soil decreases the potential for infection since the larvae have already moved through the surface layer of soil which contains the highest inoculum density.

Pathogen

Fourteen fungi are currently being developed commercially for management of foliar, aquatic and soil insects (McCoy 1990). Because of their suitability for infecting soil-inhabiting insects, the fungi *Beauveria bassiana, Metarhizium anisopliae* and

Paecilomyces fumosoroseus are being developed as microbial insecticides for control of soil insects.

Penetration, Persistence and Inoculum Density

The efficacy of microbial pesticides depends largely upon the location, persistence and density of the inoculum within the soil profile, because infectivity is directly related to the probability of a susceptible insect contacting infectious inoculum. If the inoculum remains only on the surface of the soil, only insects moving on or coming in contact with the surface will be exposed to the microbial pesticide. Similarly, if the inoculum leaches below the zone of soil inhabited by the pest, infection may not occur (Storey & Gardner 1987). Greenhouse and field trials indicate that 95% of commercially-formulated *B. bassiana* conidia remain in the upper 5 cm of the soil profile of 4 different Georgia soils (Storey and Gardner 1988). Unformulated *N. rileyi* conidia were also retained by a silt-loam soil (Ignoffo et al. 1977). Control strategies using microbial pesticides should take into consideration the limited vertical movement of the inoculum relative to target pest location within the soil.

Persistence of microbial pesticides is also important because it increases the window of opportunity for target pest infection and reduces the cost of re-application. *B. bassiana* conidia can persist for up to 2 yr (Wojciechowska et al. 1977, Lingg & Donaldson 1981) but persistence greater than 6 weeks would make microbial pesticides comparable to current chemical residual activity.

Positive correlations between soil inoculum density and disease incidence in soil insects is well established and generally accepted that mycosis develops in insects exposed to inoculum densities of 10^5 to 10^8 conidia per cc of soil (Ferron 1981). Insects that do not become infected when exposed to soil containing 10^8 conidia per cc are probably not amenable to control with the microbial pesticide tested due to the difficulty in achieving the required inoculum density under field conditions.

Production and Formulation

Production has been a limiting factor in the development of microbial control agents. Production of fungi has generally taken two routes: semisolid production of aerial conidia and submerged culture fermentation of mycelium (McCoy et al. 1988, McCoy 1990). Recently, Bayer AG released information on their BIO 1020 formulation of *M. anisopliae* (Andersch et al. 1990). The fungus is grown in liquid culture, and the mycelium formulated into uniform 5mm spheres (Andersch et al. 1990) that rehydrate in the soil and sporulate within 5 days (Storey et al. 1990). Submerged fermentation production of conidia is possible for a few fungal species and is considered a potential means of mass production (McCoy 1990). The commercialization of pathogens is a separate topic of discussion at this workshop and covered more thoroughly by Ferguson (1991).

Formulation of the microbial pesticide is very important to the success of the pathogen. Microbial pesticide formulation must consider two important factors that are not considered with chemical pesticides. First, the microbe is a living organism and therefore, the formulation must not be toxic to the organism. Second, the formulating material should not contain materials that support or enhance the development of antagonistic microorganisms present in the soil. Generally, soil bacteria and fungi present in non-sterile soil can outcompete the applied microorganism for utilization of the carbon source (Lingg & Donaldson 1981).

Field Evaluation of Microbials

In developing successful microbial control strategies, several key questions must be asked. First, is the target insect susceptible to the applied pathogen and to what degree is each stage susceptible. Initially, these questions must be answered in the laboratory under conditions similar to the actual field environment, including using nonsterile soil, comparable temperatures and moisture levels, and the same host that will be targeted in the field.

Second, is the susceptible stage accessible by the microbial pesticide? If the pathogen is applied to the soil surface and remains there but the host is located several inches deep in the soil profile, the probability of infection, and therefore control is minimized.

Third, what is the fate of the pathogen under the environmental conditions of the particular cropping system? In order to properly assess the activity, penetration and persistence of the applied microbial pesticide, extensive field monitoring must be conducted. Moisture levels in the upper 15 cm of soil can be monitored hourly using tensiometers fitted with current transducers which convert the soil suction reading of the tensiometer into an electrical impulse that is recorded with a data logger. Similarly, soil temperatures and rainfall should be recorded hourly.

Additionally, the applied microbial pesticide must be monitored by collecting and processing soil samples for propagule density and virulence prior to and after application until the inoculum density is undetectable. Continual monitoring of environmental parameters and the inoculum will provide a database useful for future assessment of microbial use in similar situations.

Feasibility of Microbials for the Caribbean

Microbials are feasible for use in the Caribbean given the history of soil pests in the region. Care must be taken in selecting isolates of pathogens that function best in the tropical climates. Because a pathogen is being mass-produced and may be commercially available does not mean it is suitable for use in all areas. For this reason, collection of new germplasm from the Caribbean countries must continue while testing available formulated material.

Once collected, the potential feasibility of the pathogen must be evaluated prior to application via bioassay. After field application, extensive monitoring of the system should be conducted so that when success is achieved, it can be repeated and when failures occur they will not be repeated.

The increased interest of industry in the production of microbials and the advances in large scale production methodologies make microbials a viable option for controlling many soil-inhabiting insects. Also, the ability to produce fungi using a "low-tech" production methodology may make them even more readily available to a larger number of growers who would not be able to pay for a product mass-produced using "high-tech" methods. Currently, there are several cottage production programs underway in Jamaica, Cuba, Venezuela and the Dominican Republic. Whatever method employed to produce the microbe, care must exercised so that the inoculum has the greatest potential for surviving and infecting the target host.

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EFFECT OF SOIL PHYSICAL FACTORS ON BIOLOGICAL CONTROL AGENTS OF SOIL INSECT PESTS

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ABSTRACT

An appreciation for the complexity of the soil environment is important when developing strategies for biological control of soilborne insect pests. Basic principles of soil physics and the effects of soil physical factors on entomopathogenic nematodes and fungi are reviewed, and areas for future research are suggested.

RESUMEN

Una consideración de la complejidad del ambiente del suelo es importante en el desarrollo de estrategias para la aplicación o mejoramiento del control biológico de plagas del suelo. Se revisan algunos principios básicos de la física del suelo y el efecto que los factores físicos tienen sobre los agentes de control biológico, y se sugieren futuras áreas de investigación.

Many economically important insects spend some portion of their life in the soil, and often these stages are destructive to crops (Villani & Wright 1990). Interest in biological control has increased because of concerns about the economic, environmental, and health costs of chemical crop protection practices (Hoy & Herzog 1985). Insect pathogens in particular show promise as biological control agents in soil (Fuxa 1987, Klein 1988). Soil can provide favorable physical conditions for survival of entomopathogens. For example, in comparison to the aerial environment, pathogens in soil are not subject to destruction by solar radiation, and humidity is relatively high and stable.

An appreciation for the complexity of the soil environment is important when developing strategies for biological control of soil insects. Many attempts at biological control have been made without adequate understanding of the ecology of the control and target organisms, resulting in inconsistent biological control (Ehler 1990). Soil is a dynamic system that responds to environmental changes and energy supply (Moore et al. 1988). In addition to physical factors (e.g., soil texture and structure, soil water status, gases, temperature, pH), numerous biotic factors (e.g., host population, host-plant of target insect, predators and antagonists of the biological control agent, alternate