INSECT FUNGI FOR THE CONTROL OF BROWN PLANTHOPPER, *NILAPARVATA LUGENS*, AND MALAYAN RICE BUG, *SCOTINOPHARA COARCTATA*

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Proefschrift

ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
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STELLINGEN

1. Het aantal sleutelplagen op rijst in tropisch Azië is gering en hun belang vaak overschat.

2. Natuurlijke bestrijding van brown planthopper in tropisch Azië is zelfs op gevoelige rijstrassen effektief, mits het gebruik van breed spektrum insekticiden wordt vermeden.

3. Wanneer er een goede teeltbegeleiding gegeven wordt zal de overgang van brown planthopper bestrijding met breed spektrum insekticiden naar bestrijding van sleutelplagen met specifieke middelen leiden tot hogere opbrengsten, lagere uitgaven en een geringere belasting voor de mens en het milieu.

4. Spontane epidemieën van insektenschimmels in brown planthopper populaties worden bevorderd door verzwakking van de insekten door afname van de voedselwaarde van de rijstplant. Dit gebeurt meestal in situaties van schade door hoppers of vlak voor de oogst - en dus laat vanuit een ekonomisch oogpunt.

5. Infekties van insekten met schimmels worden gestimuleerd door milieufaktoren - de hoeveelheid inoculum is hierbij van relatief weinig belang. Voor de toepassing van schimmels in de insektenbestrijding is het daarom van meer belang in te spelen op faktoren, die infektie in het veld beïnvloeden of deze te manipuleren, dan te zoeken naar nieuwe isolaten of genetisch gemanipuleerde stammen met hogere virulentie.

6. Gebruik van droog mycelium van entomogene schimmels verdient aanbeveling voor die situaties waar het mikroklimaat sporulatie van het mycelium op het gewas toelaat.

7. Synanamorphic genera in the Fungi Imperfecti should be synonymized *only* if 1) careful study indicates that any consolidation will be strictly limited to a few synanamorphic genera, *and* 2) the resulting expanded generic concept (despite any phylogenetic advantages gained) does not destabilize the taxonomy of similar (morphologically defined) genera to create large and meaningless genera.
geciteerd uit: R.A.Humber and M.C.Rombach, *Mycologia* 79: 375-382, 1987.

8. Uit het aantal verzamelde nieuwe soorten ten opzichte van het aantal bekende soorten dat recentelijk werd herverzameld is af te leiden dat de meeste insektenschimmels reeds zijn beschreven.

9. Veel Filippijns tropisch regenwoud zou gespaard blijven als Filippino's het idee op zouden geven dat rijst, gekookt op houtskool, beter smaakt dan rijst gekookt op gas.

10. De tijdsduur van minuten en seconden zijn voor velen moeilijk te schatten; het verdient daarom aanbeveling oude tijdseenheden als "a rice cooking" (Madagascar), "a locust frying" (Mindanao, Filippijnen), "two swings of a donkey's tail" en "a pissing while" (Engeland) weer in te voeren.

11. In een dichotome sleutel voor identificatie van supermachten vormt de richting waarin de grenswachters staan gekeerd het eerste couplet.

Stellingen behorende bij het proefschrift van M.C.Rombach: "Insect fungi for control of brown planthopper, *Nilaparvata lugens*, and Malayan black bug, *Scotinophara coarctata*". Wageningen, 15 September 1987.

Promotor: dr. J.C. van Lenteren
hoogleraar in de entomologie,
in het bijzonder de oecologie der insecten

Aan mijn ouders
Aan Maureen

CONTENTS

Photograph on front: the fungus *Hirsutella citriformis* infecting the brown planthopper, *Nilaparvata lugens*.

Several chapters of this thesis have been or will be published :

CHAPTER 2: Rombach, M.C., G.M.Rombach, and D.W.Roberts, 1987. Pathogens of rice insect pests: a bibliography. Insect Sci. Applic. (in press).

CHAPTER 3: Rombach, M.C., R.A.Humber, and H.C.Evans, 1987. *Metarhizium album* a fungal pathogen of leaf and planthoppers of rice. Trans. Br. mycol. Soc. 87 (in press).

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CHAPTER 4: Rombach, M.C., R.M.Aguda, B.M.Shepard, and D.W.Roberts, 1986. Infection of rice brown planthopper, *Nilaparvata lugens* (Homoptera; Delphacidae) by field application of entomopathogenic Hyphomycetes (Deuteromycotina). Environm. Entomol. 15: 1070-1073.

Rombach, M.C., R.M.Aguda, and D.W.Roberts, 1986. Biological control of the brown planthopper, *Nilaparvata lugens* (Homoptera; Delphacidae) with dry mycelium applications of *Metarhizium anisopliae* (Deuteromycotina; Hyphomycetes). Philipp. Ent. 6; 613-619.

Rombach, M.C., R.M.Aguda, B.M.Shepard, and D.W.Roberts, 1986. Entomopathogenic fungi (Deuteromycotina) in the control of the black bug of rice, *Scotinophara coarctata* (Hemiptera; Pentatomidae). J. Invertebr. Pathol. 48: 174- 179.

CHAPTER 5: Rombach, M.C., R.M.Aguda, and D.W.Roberts, 1988. Production of *Beauveria bassiana* (Deuteromycotina; Hyphomycetes) in different liquid media and subsequent conidiation of dry mycelium. Entomophaga 33(3), in press.

CHAPTER 6: Rombach, M.C., 1987. Insect fungi for the control of rice insect pests. Neth. J. Agric. Sci. (in prep.).

CHAPTER 1
GENERAL INTRODUCTION

BIOLOGICAL CONTROL (1.1)

In tropical rice agrosystems there is a need for selective control measures for various insect pests. Several insect pathogens are promising candidates for development as microbial insecticides, and there are possibilities for use of entomogenous fungi in the biological control of the brown planthopper, *Nilaparvata lugens* (Stahl), BPH, and the Malayan rice bug, *Scotinophara coarctata* (F.), MRB. For understanding of biological control, integrated pest management, and the possible implementation of selective microbial insecticides in pest control programs, some principles of biological control are discussed below.

Introduction . Natural biological control, or natural control, is a common phenomenon - the regulation by natural enemies of living organism's population density at a lower average than would otherwise occur (DeBach 1964). The regulation of populations in all natural systems, being tropical, temperate, terrestrial or marine, is almost invariably influenced by one or another form of natural biological control. Also, most potential pest organisms are normally kept at densities below damage thresholds by naturally occurring enemies. More specifically, biological control of insect pests is "the reduction of insect pest numbers by the combined action of natural enemies including parasites, predators and pathogens to lower levels than would occur in their absence". This statement was often proved, either by removal of natural enemies with pesticides (DeBach 1964), or by the introduction of pests in new areas where only few natural enemies occur.

Applied biological control is one of the oldest known methods of insect pest control; for many centuries Chinese farmers control citrus pest by the introduction of a predatory ant, *Oecophylla sp.* in orange trees (Clausen 1956). In rice, Chinese farmers place bundles of rice straw in freshly harvested fields - the fields are flooded, making lycosid spiders take shelter in the straw bundles. These bundles, full with spiders, are transferred to growing crops where the spiders forage again on pest insects. This method is practiced since ancient times.

Disturbance of the relationships between potential pest and natural enemies populations (e.g. by pesticide applications) can lead to outbreaks - and phytophagous insects which normally occur at very low numbers become primary pest species (DeBach 1964; Huffaker and Messenger 1976). In rice, Kenmore (1980) shows that large populations of BPH, one of the target pests of this study, build up after insecticide applications. Populations of the same hopper also showed strong increase in cage exclusion experiments, in which hopper populations were kept in field cages after removal of natural enemies. These experiments show the immense importance of natural enemies in BPH regulation. If no residues of natural enemy populations are left, renewed introduction of the natural enemies and colonization of the infested area can often reverse this resurgence phenomenon and the pest species are again suppressed to low levels (see various examples by DeBach 1964).

Classical biological control. In classical biological control, a natural enemy of an introduced pest is usually collected from the original distribution site of the pest species, multiplied in the laboratory and released at the location where the pest was newly introduced. In some cases the natural enemy establishes itself in the pest population and long-term pest control is achieved; examples of this approach are given by Clausen (1978).

This approach of introducing parasites and predators is based on the fact that many pests are accidentally introduced to new areas and multiply rapidly, not regulated by natural enemies. Pest control by introduction of natural enemies can be stable and long term, and far less expensive compared to other control methods.

Classical biological control almost always costs only a fraction of other control strategies. Thorough economic analysis of biological control programs are few. For a cost-benefit analysis of control of the mealybug *Antonina graminis* (Maskell) see Dean et al. (1979); Hart et al. (1978) provide an analysis of biological control of *Aleurocanthus woglumi*, a citrus blackfly, in Florida. In general, benefits per invested unit of money are at least 30 times as high in biological control compared to chemical control (van Lenteren 1986) - this includes projects where introductions have to be repeated every crop or season.

In classical biological control of the inoculative introduction type returns are often one thousand to a million fold within the first few years after introduction. Examples of biological control of introduced pests are discussed by DeBach (1964, 1972), Hagen and Franz (1973), van den Bosch, Messenger, and Gutierrez (1982), and Huffaker and Messenger (1976).

Successful, "classical type" biological control is highly cost effective - and a logical strategy for introduced pests.

Classical biological control in rice. With regard to rice, in the early 1920's the rice stem borer *Chilo suppressalis* (Walker) was accidentally introduced on Hawaii with rice straw packing from Japan. The species multiplied at a high rate, not hampered by natural enemies. In a few years the rice industry of Hawaii was in serious danger. Subsequently, 2 hymenopteran egg parasites (*Trichogramma japonicum* and a *Telenomus sp.*) and 3 larval parasites (*Bracon chinensis*, *Eriborus sp.* and *Apanteles sp.*) were introduced from Japan, the area of origin of the stemborer pest. The parasites were mass produced, released, and became established (van Zwaluwenburg et al. 1928; Pemberton 1948). Within a few years the stem borer population was suppressed below damaging levels and rice harvests returned to normal. This is an example of a pest species which could rapidly increase in a newly colonized area and was subsequently controlled by introduction of its original parasites.

Introductions of natural enemies are not always as successfull as the example mentioned above. In the late 1920's, a parasite of stem borer larvae introduced to Japan from the Philippines failed to become established; this was probably due to adverse cold winters in Japan. Stemborer parasites transferred from Taiwan, Japan, and the United States to the Philippines became established, but proved of little value in stem borer control (Yasumatsu and Torii 1968).

Integrated Pest Management. Integrated pest management (IPM) was initially intended to combine biological control with other forms of pest control such as cultural, mechanical, and chemical methods. Therefore, IPM requires an understanding of various factors influencing insect pest populations. These factors include such diverse phenomena as the crop, insect population dynamics, and climatological factors. However, biological control by natural enemies should be the foundation of all IPM systems. If these enemies do not occur naturally they should be introduced. When insecticides are used, they should be as selective for the target pest as possible, numbers of applications and rates should be minimized and properly timed in order to maximize effectiveness of the natural enemies. Discussions on several aspects of IPM can be found in Knipling (1979).

IPM is regarded as the most advanced and sophisticated control strategy. IPM is based on extensive ecological knowledge - including knowledge on such diverse topics as economic injury

levels, pest-crop interaction, biology of natural enemies, distribution of pest species and pest forecasting. Decisions on pesticide applications, planting dates, and other agricultural practices can now be made aided by sophisticated computers. Computer models are fed with information on weather, crop age, and information on pest and natural enemy numbers (Adkisson and Frisbie 1979; Huffaker 1980). It is expected that the use of these models will increase in the future.

IPM research is a typical *"third wave"* (Toeffler 1979) activity: highly technical, with a large pool of background knowledge, highly dependent on information, and customized and finely tuned with regard to specific crops, insect pests, locations, and economical considerations. However, once various control parameters are identified and established the practicing of the control (e.g. sampling and decision making) by the farmer can be simple following "cookbook" procedures, e.g. as advocated in the illustrated guide to IPM in rice by Reissig et al. (1985).

INTEGRATED PEST MANAGEMENT OF RICE PESTS (1.2)

Key pests of rice. There are few key insect pests of rice - taking the numbers of insects feeding on the crop into account. Key pests, as normally defined, occur on a regular basis, cause substantial damage - and are not provoked directly through man's activities. Of course, every pest on cultivated crops can simply be regarded as a result from man's activities - he planted the crop as a monoculture in the first place. However, virtually all rice pests are brought about by crucial changes in agricultural practices. These practices include the injudicious use of chemical pesticides, cultural measures such as double cropping, wide spread planting of one or few genetically similar rice varieties, planting of long-duration rice varieties, ratooning (growth of a second- and third crop from stubbles of the first crop), and excessive use of fertilizers. For ease of reference we will regard only these factors as *"man's activities"*.

With very few exceptions all key pests of rice are all lepidopteran species. At present by far most widely distributed and most important key pests are stemborers (Pyralidae and Noctuidae), leaf folders (*Cnaphalocrocis medinalis* and *Marasmia spp.*), and, infrequently, armyworms such as *Spodoptera spp.* and *Mythimna separata* (Walker), semiloopers, and hairy caterpillars (Noctuidae).

In rice, secondary, or "provoked", pests can rarely occur as key pests. BPH is very rarely a key pest in the vast rice growing area near Cairns, Northern Australia. There, BPH occurred as a key pest, in a restricted area, and only once during the last 6 years. All other BPH outbreaks in the same area could be easily traced back to resurgence effects caused by pesticide applications.

In the temperate regions of Korea and Japan BPH was already an important pest long before chemical insecticides were developed. However, the ecology of BPH in these temperate areas is radically different from the ecology in more tropical areas - the seasonal massive immigration of BPH in the temperate zones upsets the natural balance in favor of the pest. Similarly, BPH can be a key pest on tropical rice after immigration from resurgence areas (e.g. presently in the Klaten area, near Yogyakarta, Java, Indonesia) ; however, this resurgence was caused by pesticides in the first place.

Some other key pests are of local importance, e.g. gall midge, *Orselia oryzae* (Wood-Mason) in India, whorl maggot *Hydrelia philippina* Ferino (Ephydridae) in the Philippines, rice hispa, *Dicladispa armigera* (Oliver), in parts of Bangladesh and India, and white grub, *Holotrichia spp.* in watershed projects in Indonesia and upland areas (e.g. Cagayan del Oro, Mindanao) in the Philippines. In the Philippines the MRB can be regarded as a key insect pest on the island of Palawan; the insect was introduced about one decade ago, and natural enemies have not yet given sufficient control. However, it should be noted that also in its native distribution area, which

includes Malaysia, this insect infrequently causes considerable damage (van Vreden and Ahmadzabidi 1986) - this elevates this insect to an occasional key pest in Malaysia.

Effectiveness of natural enemies. Walker (1962) lists more than 1400 insects which were reported as rice pests; this number has increased since. However, most of these insects are collected infrequently, occur in low numbers and cause negligible damage. Nearly all of these potential pests of rice are controlled by complexes of natural enemies and thus escape notice - except the few key pests.

The effectiveness of naturally occurring enemies can be improved by augmentation and conservation. There are many examples of pest control by augmentation of natural enemies (Ridgway and Vinson 1977).

The natural enemies of rice pests include large numbers of species of predators and parasites (Yasumatsu and Torii 1968; Chiu 1979; Nickel 1964; Kenmore 1980; van Vreden and Ahmadzabidi 1986), and insect pathogens (Rombach and Shepard 1987; Chapter 2, this thesis). For most of the natural enemies in the rice ecosystems their specific role is not understood - however, their combined action effects in a remarkable suppression of the pests. Singling out one naturally occurring parasite or predator, followed by mass production, and regular mass release could be an answer when pest problems arise. However, in an outdoor situation as rice such techniques can be applied only with great difficulties - one can foresee problems in relation with the immense areas to be treated, costs, and logistics. The mass production of most species of parasites and predators is cumbersome and expensive because insect hosts have to be reared in large numbers. Mass production of parasites and predators on a regular base for rice pest control is not likely to succeed, except for restricted "classical biological control" release efforts.

Conservation of natural enemies. In tropical rice ecosystems conservation of natural populations of natural enemies is more practical than augmentation, and of crucial interest to pest control. Of most importance is the judicious use of chemical pesticides, which is one of the keys to natural enemy conservation.

Paradoxically, insecticides are blamed for much of the pest problems in rice, including evoking BPH resurgence (Heinrichs et al. 1982; Reissig et al. 1982; Kenmore, 1980; Nishida, 1975). However, this claim has not been substantiated for all pests in all rice growing regions. In the more temperate areas of Korea and Japan several insect pests, including BPH, immigrate yearly (Kisimoto 1979). Therefore, the development of populations of pests and natural enemies differs substantially from tropical, year round rice cultivation. There populations of host and prey are nearly continually present which results in stable populations. In temperate areas epidemics of BPH are initiated by long-distance migration from mainland China - and the immigrating BPH populations can overwhelm populations of natural enemies, which are mainly spiders. Here serious crop loss can be prevented by applications of insecticides in late July and early August (Kisimoto 1971, 1976).

Proper choice of pesticide compound and timing of the insecticide application can enhance the impact on the pest population and minimize effects on natural enemies. However, applications with most broad spectrum compounds carry risks (resurgence is observed occasionally) and finely tuned treatments require insect monitoring, expensive spray equipment and relatively expensive insecticides - techniques farmers are not likely to follow.

There certainly is a place for chemical insecticides in IPM programs on rice - but only as emergency measures for correcting instability rather than as basic tools.

Problems with chemicals: Guadalcanal and Java. Despite new compounds and techniques, in tropical rice insecticides often trigger avalanches of pest problems rather than preventing them. Resurgence of BPH following pesticide use is the best known example (Heinrichs 1979; Heinrichs and Mochida 1984). We observed dramatic outbreaks of BPH on the island of Palawan, but only in those fields where insecticides were used to control the black bug.

Guadalcanal, Republic of the Solomon Islands. Over a decade of heavy insecticide use against cutworm, armyworm, and BPH (resurgence populations) decimated populations of natural enemies in the isolated rice growing area on Guadalcanal. Often a dozen insecticide applications are made per crop (equivalent to about 1 spray per week). The populations of natural enemies are now destroyed to such an extent that interruption of chemical treatments still results in almost total yield loss to hopperburn, even when BPH resistant rice varieties are planted. This is a situation where a one time small mass production effort might help to recuperate depleted numbers of the most important parasites and predators. However, the first step should be the development of a specific strategy to control lepidopteran key pests. This will preserve populations of natural enemies, and natural suppression of BPH will almost certainly follow. Specific projects for Guadalcanal, one on the use of a nuclear polyhedrosis virus (NPV) against the cutworm (*M. separata*) and armyworm larvae (P.F.Entwistle, pers. comm.), and on entomogenous fungi for initial BPH control are underway. Hopefully, over a few seasons insecticide usage will decrease, populations of natural enemies will be replenished and grow, and populations of pest insects will stabilize at low levels. The isolated rice growing area as Guadalcanal seems to be ideal for this approach because interference by immigrating insects is limited.

Java, Indonesia, BPH. In some tropical rice growing areas (e.g. Java and Sumatra: Indonesia) the point is reached that no new plant resistance for BPH is available, pesticide use is extreme - and, consequently, hopperburn severe. Additionally, plant viruses such as the ragged stunt- and grassy stunt virus become a problem, probably due to weakened plants and high transmission rates. At present, this situation dominates large rice growing areas on Java and Sumatra - now probably Asia's largest area afflicted by this insect.

The Indonesia brown planthopper outbreaks are largely caused by excessive insecticide usage for other insect pests, often applied when not needed, or applications on a calendar basis. Therefore, firstly, BPH management in Indonesia should focus on control of other insect pests with conservation of natural enemies.

The few examples above illustrate that chemical pesticides alone are not the panacea for rice pest problems. However, specific and effective insecticides, such as Dimilin(R) (a molting inhibitor for use against leaf feeding lepidoptera), and Applaud(R) (a contact action molting inhibitor for use against plant- and leafhoppers), are now available. These safe and specific insecticides can be included in IPM programs. Applaud was already applied in BPH infested areas in Indonesia, with favorable results on both BPH- and populations of natural enemies - the spider community was relatively unharmed while BPH populations were severely affected by the chemical.

We are all aware of the consequences of pesticide resistance in insects, environmental pollution on a vast scale and, last but not least, general costs increases of chemical pesticides. Therefore, the importance of biological control, within an IPM framework, cannot be overstated.

THE PROBLEM: BROWN PLANTHOPPER, BPH, AND MALAYAN RICE BUG, MRB (1.3)

BPH. Plant resistance and pesticides. The use of resistant rice varieties, pesticides, and other gross changes in agricultural practices has provoked BPH, once a minor pest, to become serious. For years BPH was the most destructive pest of rice in many Asian countries - and referred to as the "threat to asian rice production" (IRRI 1979). Now the pest status of BPH decreased in some areas, including the Philippines, for reasons not well understood; but a decrease in pesticide usage seems to be of major importance. In other areas, such as the Solomon Islands and Indonesia, BPH problems increased.

The BPH pest problem in tropical rice growing areas started during the sixties and seventies, as a direct result of "green revolution" practices. These practices include increase fertilizer evolution" practices. These practices include increase fertilizer use, double or even triple planting, improved irrigation, and the introduction of high yielding rice varieties. These varieties were introduced along with chemical pesticides - and offered to the farmers as a package.

In tropical rice, double or triple cropping can enhance BPH population growth (Mochida and Suryana 1979), and allows multiple generations of other pests, such as leaffolder, to develop. The green revolution with its irrigation projects and other cultural measures promoted planting of several crops per year - and insect pests, including BPH, increased. Synchronized planting and crop rotation were suggested as measure to control BPH (Oka 1979). This practice results in a fallow period over large areas - which will lead to unstable pest and natural enemy populations. It can be argued that pest species with a high biotic potential (e.g. BPH) benefit more from instability than do predators and parasites - and small scale experiments at IRRI and in Mindanao (Philippines) do suggest this effect. However, the minimum area for a synchronous planting experiments should be about 1200 ha, an estimation based on BPH dispersal data (Loevinsohn 1984). Clearly, research on this aspect of BPH population dynamics is needed to clarify the effectiveness of synchronous planting in BPH control. Also, increased nitrogen levels due to fertilizers, improved irrigation, and multiple crops have an influence on BPH populations, but can not explain the often explosive population increase.

The combination of high levels of plant resistance *and* chemicals can be unfortunate - it makes that the deleterious effects of the pesticides are not noticeable *as long as* the plants remain resistant. In practice farmers are, inadvertently, lured into the conviction that pesticides work. However, in effect, plant resistance overwhelms the deleterious effects of pesticides on populations of natural enemies. Over time, BPH populations adapt to the plant factors, and their numbers explode. The importance of natural enemies is not recognized by the farmer, because "the pesticides worked", while in reality, his BPH populations were low (*despite* the pesticides), through plant resistance. High levels of insect resistance in rice tend to break down (Gallagher 1987) due to selection of virulent "BPH-biotypes", which are merely populations which adapt to a specific plant gene One recent BPH resistant variety, IR54, was severely damaged by hopperburn within 3 years of its release in Mindanao, the Philippines (K.Gallagher, pers.comm. 1986) - and Cheng (1977) estimates that only 10 generations are necessary for a field population to adapt. In the greenhouse, Pathak and Heinrichs (1982) selected a virulent population which overcame resistance conferred by the bph2 gene within 7 generations. Field populations of BPH have few individuals with limited tolerance for plant resistance. The environment, in this case the host plant, strongly favors these genes and populations capable of feeding on previously resistant plants are selected (Hedrick 1983).

These virulent characteristics selected for are present in all BPH populations, albeit at different frequencies. Planting resistant rice varieties sets the selection procedure in motion. It should be realized that this threat of population selection hardly justifies expensive rice breeding programs which are *solely* focused on complete BPH resistance.

Up to the present, BPH resurgence resulted in increased plant breeding efforts to include new genes for resistance, and increased pesticide use. Introduction of rice varieties with new genes for resistance suppresses BPH populations, but populations adapt, and the cycle is repeated.

It should be noted that BPH population growth is slow on partial resistant rice varieties such as IR36, with has complementary resistance factors (probably a second gene for resistance), besides the major gene.for resistance. However, growth is not completely inhibited as on the complete resistant varieties, and new virulent populations are selected much slower on these partial resistant varieties because of this low selection pressure. Also, biological control is in general more successful when low, but relatively stable populations of the pest insect are present - as is the case on partial resistant varieties.

Even in stable tropical rice systems BPH might, occasionally, cause problems, due to immigration pulses, pesticide applications against key pests, or other factors. There is a need for selective control agents to suppress ensuing populations while not harming natural enemies. Therefore, the possibilities of utilizing mass-produced inocula of entomogenous fungi for BPH control were investigated (Chapter 4).

MRB. Introduction on Palawan. About a decade ago the MRB was introduced in the rice growing areas of Palawan, the Philippines (Barrion et al. 1982); the bug was probably introduced with shipments of rice from either mainland Malaysia or Sabah, Northern Kalimantan. Whatever the origin or route, within a few years the bug established itself in the Maasin region near Brooke's Point in the south of Palawan and multiplied rapidly. Over the last years large areas of rice were, and occasionally still are, completely devastated, or "bug burned". At present the whole Palawan Island is infested, up to the Northern Tay-Tay area. For the first few years, native natural enemies were unable to keep this newly introduced pest under control. The few natural enemies include a hymenopteran wasp (*Telenomus triptus* Nixon; Scelionidae) which parasitizes the egg masses, and the entomogenous fungi *Metarhizium anisopliae* and *Beauveria bassiana*. Additionally, the fungus *Paecilomyces lilacinus* (Thom) Samson, which naturally infects nymphal and adult black bugs in Malaysia, was introduced in a limited black bug infested area. About one year after its release, *P. lilacinus* was recollected at the original release sites and at several km distance suggesting establishment of this Malaysian fungus. However, as Smith and DeBach (1942) pointed out, establishment alone is not at all a sign of success. It does not indicate parasitization levels; the natural enemy can simply have replaced another mortality factor. Although *P. lilacinus* is collected regularly, no high infection levels in black bug populations were observed.

At present, black bug populations are declining in some areas of the island, including our experimental areas. Whether this decline is due to the action of native natural enemies, or to the newly introduced biocontrol agents has still to be evaluated.

Some effective insecticides for MRB control were identified (Rao 1977), but virtually all compounds have a broad spectrum activity, and applications often resurge BPH populations. Therefore, methods of selectively controlling MRB using entomogenous fungi were investigated (Chapter 4).

CHAPTER 2

PATHOGENS OF INSECT PESTS OF RICE: A BIBLIOGRAPHY

INTRODUCTION

The value of insect pathogens in insect pest control has long been recognized. The first pioneering attempts to control insects with pathogens date back to the last century (Steinhaus, 1956; Heimpel, 1972; Tinsley, 1979). Steinhaus (1947, 1949, 1963) laid the base for an organized study of insect pathology by compiling the widely scattered information on the subject. These monumental publications still serve as usefull guides to scientists training in the field of insect pathology.

The knowledge of pathogens of insect and mite pests of agricultural and medical importance has increased considerably during the last two decades (Burges and Hussey, 1971, Burges, 1981; Hoy and Herzog, 1985). This trend has been partly stimulated by the realization that chemical pesticides are not the panacea for all pest problems. The consequences of pesticide resistance in insects, environmental pollution on a vast scale, and the high costs of chemical pesticides are all well recognized.

In integrated pest management (IPM) all control tactics, including chemicals, cultural control, and biological agents are combined in a concerted effort to control pests.

Knipling (1979) reviews various IPM aspects; Brader (1979) discussed some of the specific problems of integrated pest control as it relates to the developing world. These and similar publications increased research efforts in basic and applied insect pathology. Modern technology, including genetic engineering of the microbes, is now being applied to some insect pathogens (e.g. Faulkner and Boucias, 1985).

Since the first registration of an insect pathogen (*Bacillus popilliae* Dutky, about 1950) various bacterial-, viral-, protozoan- and fungal pathogens have been registered or registration is being sought. With the current worldwide trend in insect pest control moving from a total reliance on chemical pesticides towards integrated pest management systems there seems to be a bright future for microbial insect control agents (Falcon, 1985).

Insect pathogens usually possess the special features required for implementation in integrated pest management systems: - host specificity, -high virulence, -harmlessness towards natural enemies of the target pest, and - environmental safety.

The pathogen faunas of important medical pests and insect pests of various agricultural crops such as soybean, citrus, potato and greenhouse crops are, in general, well documented (see various authors in Burges, 1981; Hoy and Herzog, 1985; Hussey and Scopes, 1985). In contrast, pathogens of rice insect pests are relatively unknown. This certainly is unfortunate, taking into account the sheer size of rice area and the massive amount of people which subsists on this single crop. Most insect microbials, in particular entomopathogenic bacteria and fungi, can be mass produced at relatively low technology levels (Tinsley and Hussey, 1981); therefore, they are well suited for use in the third world.

METHODS

The information presented in this bibliography was mainly extracted from the library of the International Rice Research Institute, the USDA-ARS reprint collection (Boyce Thompson Institute for Plant Research, Ithaca, NY) and from our personal reprint collections. In the IRRI library journals related to rice entomology were checked from 1960 (IRRI's founding year) up to the 1985 issues. Virtually all references mentioning any aspect of insect pathology in rice were included; material from IRRI which has not been officially published is available free of charge from the Rice Pest Information Retrieval Service (IRRI, Department of Entomology).

This bibliography is primarily organized by groups of insect pathogens which are of interest to rice entomologist and insect pathologist: -insect pathogenic bacteria, -fungi, -viruses , - protozoa, and -nematodes. Narratives give brief introductions to the groups and to some of the most pertinent literature in relation to rice. With each group a few references to general review articles are included; from these references clues to more specific literature can be extracted. Records of pathogens of rice insects are tabulated (Table 1). The same pathogen can be collected from the same host and same location repeatedly; in these cases, only the first or the most prominent collections are tabulated. In addition, the references are classified by subjects (Table 2). These subjects include pathogen groups and field and laboratory experiments. Though not insect pathogens *sensu stricto*, a list of symbiotic microorganisms of rice insects is included in these subject listings. We realize that a listing by insect host or host group rather than pathogens can be more relevant to the applied entomologist - therefore, this type of list is also provided. The numbers in the tables refer to the numbers in the lists of references.

INSECT FUNGI

Virtually all major groups of fungi contain entomopathogenic species; Roberts and Humber (1981) list various entomogenous fungi and discuss their classification and biology. Many fungi are effective in biological control of insects (Burges, 1981) - at present isolates from about 15 genera of fungi are being considered for further commercial development (Roberts and Humber, 1984).

Unlike other microorganisms such as protozoa, bacteria and viruses, most fungi infect the insect host by direct penetration through the cuticle rather than by oral ingestion; these fungi can infect insects with sucking mouthparts such as planthoppers, leafhoppers and pentatomid bugs. Therefore, entomopathogenic fungi have high potential for biological control of these groups of pests.

Fungus species considered for biological control are practically all Hyphomycetes (Deuteromycotina) or Entomophthorales (Zygomycotina). These groups differ considerably in their morphology and biology (Roberts and Humber, 1981). Both groups will be briefly discussed as they relate to rice pests.

Deuteromycotina. The subdivision Deuteromycotina contains a relatively large number of entomopathogens (about 150 spp.); a taxonomic review, key and illustrations of this group are provided by Samson (1981). Some entomopathogenic deuteromycetes have received much attention historically; the white- and green muscardine fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, are both well known. For example, *M. anisopliae* has been collected on various rice pests, but occurs most frequently on planthoppers, leafhoppers, and rice black bugs (*Scotinophora spp.*). *M.*

flavoviride W.Gams and Rozsypal was collected from plant- and leafhoppers in the Philippines and on the Solomon Islands (Rombach, Humber and Roberts, 1986) - the variety *M. flavoviride* var. *minus* was introduced to accomodate these small spored isolates.

M. anisopliae is used as a biological control agent in several crops (Ferron, 1981); however, only very few experiments were done in rice. Albornoz and Parada (1984) obtained a total mortality in a population of *Sogatodes oryzicolus* (Muir) 12 days after a single application with *M. anisopliae* conidia. Rombach et al. (1986a) infected the *Nilaparvata lugens* Stahl (brown planthopper, BPH) by application of *M. anisopliae* and *M. flavoviride* conidia. Morimoto (1957) reviewed his studies on the biological control of the pentatomid black bug *Scotinophara lurida* with *M. anisopliae* and *Paecilomyces lilacinus* (Thom) Samson in Japan. Mortality rates were 60-100% up to 46 days after treatment of the black bug population. Rombach et al. (1986b) controlled *Scotinophara coarctata*, another black bug of rice, with entomogenous fungi on the island of Palawan, Philippines. Single applications of *B. bassiana*, *M. anisopliae*, and *P. lilacinus* conidia significantly reduced the populations of the black bug over a period exceeding 2 months.

Hirsutella citriformis Speare is one of the most widely distributed fungus diseases of the brown planthopper (BPH) of rice in Asia; especially during periods of high insect density *H. citriformis* is often a major mortality factor (Rombach and Shepard, 1987).

Beauveria bassiana is a cosmopolitan insect fungus also reported from various rice pests (Table 1). Several isolates of *B. bassiana* were tested for their virulence against the BPH, green leafhopper (GLH), and the white backed planthopper (WBPH), *Sogatella furcifera* (Horvath), at IRRI, Los Banos, Philippines. These tests indicate that strain differences in pathogenicity exist (Aguda, 1983). Studies on the biological control of rice stemborers and other lepidopteran pests with *B. bassiana* were restricted to bioassay tests in the laboratory (Nayak et al., 1978; Nayak and Srivastava, 1979; Yadava et al., 1979). N'Doye (1976) observed reduced oviposition in survivors of a mycosis in a laboratory population of *Chilo suppressalis*. Recently, bioassay tests in the laboratory showed that larvae of the leaffolder *Marasmia patnalis* can easily be infected with *B. bassiana* (R.M.Aguda, unpubl., 1985).

Fusarium spp. were reported from several insect pests of rice (Table 1); these pests include stemborers and plant- and leafhoppers. It should be noted, however, that *Fusarium spp.* are, with few exceptions, opportunistic and saprophytic fungi (Teetor-Barsch and Roberts, 1983) - they are most likely of limited value in biological control.

Verticillium lecanii (Viegas) Zimm. was collected from plant and leafhoppers of rice in the Philippines. Narayanasamy and Baskaran (1979) reported results from tests on the control of a whorl maggot, a stem borer, and a leaffolder using *V. lecanii*, insecticides, and *Bacillus thuringiensis*. At present, *V. lecanii* is marketed as a microbial insecticide for pest control in greenhouses in Europe. Possible applications of this fungus against pests of rice should be further evaluated.

Lepidopteran larvae on rice can be infected by various entomophtorelean and hyphomycetous entomogenous fungi. However, most of these fungi were collected only occasionally and epizootics in dense host populations in the field are rare. An exception is *Nomuraea rileyi*, a fungus which causes epizootics in populations of various lepidopteran larvae. In Luzon (Philippines) a natural and dramatic outbreak of *N. rileyi* prevented damage by an increasing population of a *Spodoptera sp.* on rice (F.Medrano, pers.comm., 1985). Also, there is strong circumstantial evidence that *N. rileyi* regularly prevents outbreaks of the second and most damaging generation of the hairy caterpillar, *Rivula atimeta* (Swinhoe), a common pest in South Cotabato (the Philippines).

Entomophthorales. Erynia (Entomophthora) delphacis (Hori) Humber is an obligate pathogen of plant- and leafhoppers; *E. delphacis* is distributed all over Asia. Growth and germination of *E. delphacis* was studied by Shimazu (1976, 1977) - the species sporulated best on Sabouraud dextrose agar (SDA) fortified by an extract of insect pupae or egg yolk. Relative humidity exceeding 93% was a requirement for germination. Field experiments with *E. delphacis* for the control of the BPH were initiated recently (R.S.Soper, pers.comm., 1985).

Erynia radicans (Brefeld) Humber, Ben-Ze'ev and Kenneth was collected as *Entomophthora sphaerosperma* Fres. from BPH by Shimazu (1979); the influence of the host stage and temperature on the formation of resting spores was studied.

Conidiobolus coronatus (Cost.) Batko is probably the most common pathogen of plant- and leafhoppers of rice (Table 1). Especially in insect cultures with dense insect populations *C. coronatus* can be an important mortality factor. BPH in rice can be controlled with a single application of *C. coronatus* conidia (R.M.Aguda, pers. comm., 1984). A suitable medium for the mass production of *C. coronatus* with coconut milk as main ingredient was developed by Padua and Gabriel (1978). However, *C. coronatus* can cause mycosis in horses and man (King, 1979); and its safety has to be studied in more detail before large scale production is initiated.

Entomophthora grylli (Fres.) Batko was collected on an unidentified species of grasshopper from Indonesia (fungus identified by Richard A. Humber, pers.comm., 1985). Weiser et al. (1985) reported *E. grylli* from the locust *Oxyahyla intricata*, a serious pest of rice in Viatnam. The pathogen infected 84-90% of the population. However, its application in control programs is currently limited by inability to grow the mycelial stage of the fungus in artificial culture.

INSECT BACTERIA

Virtually all bacteria in microbial insecticides are of *Bacillus spp.*; these bacteria produce spores for survival in the environment and greatly prolong shelf life of the products. Additionally, several *Bacillus* species produce proteinaceous endotoxins on which their entomotoxicity is based. Commercial preparations of *B. thuringiensis* Berliner (further referred to as B.t.) have been on the market for over two decades. Not surprisingly, most tests on microbial control of rice pests with bacteria involved B.t. products. Srivastava and Nayak (1978) report on control in the field of the rice leaffolder (*Cnaphalocrocis medinalis*) by four different commercial products containing B.t. spores; the larvae were highly susceptible to the bacterium. Nayak et al. (1978) report on infection of different species of stemborers with B.t. products. All larval stages of the borers can be infected by a cut stem technique, by feeding small pieces of rice stem soaked in suspensions of the products to larvae.

The internal parts of the rice stem are in general free of pathogens. Therefore, under natural conditions, only neonate larvae of stemborers are exposed to pathogens during the short time period of first feeding prior to penetration of the stem. In a greenhouse experiment, Nayak et al. (1978) found that a 1% Thuricide suspension sprayed at the time of hatching reduced the incidence of dead hearts and the number of living larvae in the tillers by 76% and white heads by 67% . The activity of the product persisted for 15 days under the greenhouse conditions; this period might be considerably shorter under field conditions, due to UV-light sensitivity of the spores. Weekly sprays with the product may be necessary to consistently infect hatching larvae before the stems are invaded. The adverse effect of sunlight on a B.t. preparation was tested by the Hunan Institute of Microbiology (Anonymous, 1981) in China. The preparation was applied early in the morning and late in the afternoon to a rice field infested with second instar larvae of the leaffolder

of rice (*C. medinalis*). The morning treatment resulted in 22-39% mortality while the afternoon treatment resulted in 58-72% mortality of the larvae. Therefore, application of B.t. late in the afternoon is recommended to prevent loss of infective material through exposure to sunlight and high temperatures. Bounias and Guennelon (1974) tested a preparation of B.t. on larvae of the stemborer *C. suppressalis*. Larval populations which were fed on an artificial diet incorporating different concentrations of bacterium spores showed high mortality rates. The authors conclude that the bacterium could only be used for control of *C. suppressalis* if sufficient plant coverage could be ensured for the two-months hatching period. This implies almost weekly application with a B.t. product.

In China, there are various studies on B.t. as a biological control agent for rice insect pests (Chiu, 1984). High control levels of rice skipper larvae (*Parnara sp.*) were claimed after only one application of the pathogen. In combination with low dosages of insecticides (e.g. trichlofon, at 1/5-1/10 of the recommended rate) were effective (cited in Chiu, 1984). It was claimed that B.t. was also used successfully against rice pests including stem borers, army worms and leaffolders (cited in Hussey and Tinsley, 1981).

Although B.t. is often collected as a natural infection in insects (Thomas and Poinar, 1973), no such infections of rice insects have been reported. However, other bacteria have been collected from rice pests (Table 1); the pathogenicity of most of these bacteria has still to be confirmed in bioassay tests as well as in the field.

INSECT VIRUSES

Virus diseases of insects, mites and ticks were catalogued by Martignoni and Iwai (1981), including over 1200 virus-host records. The major groups of insect viruses and their pertinent characteristics were tabulated by Payne (1982); of these viruses, isolates of the baculoviruses and the cytoplasmic polyhedrosis viruses are promising biological control agents - both of these groups form stable inclusion bodies.

Mythimna (Pseudaletia) separata was reported with a NPV infection by Neelgund and Mathad, (1972). This isolate was highly effective in the field for controlling *M. separata* (Neelgund, 1975). The larvae died in the typical position for NPV-killed larvae, viz. hanging down with the last prolegs clinging to the host plant. Neelgund (1975) concludes that the armyworm can be infected by this nuclear polyhydrosis virus in the laboratory as well as in the field. More collections of NPV's are given in Table 1.

Due to their secluded habitat, rice stemborer larvae are rarely found infected with insect viruses. The *Chilo* iridescent virus (CIV, iridescent virus type 6) was collected and described by Fukaya and Nasu (1966). An artificial diet contaminated with CIV was fed to the *Chilo* larvae to multiply the virus for identification purposes. Ishikawa and Muroga (1976) tested the influence of the CIV on the development of *C. suppressalis* larvae and the greater wax moth, *Galleria mellonella* L. There are various studies on the replication of the virus (Kelley and Tinsley, 1974a, 1974b) and its biochemical structures (Tinsley, 1972, 1973). Possibilities of using this virus in biological control of stemborers are probably limited because the virus cannot be produced in vitro and protective structures such as inclusion bodies are not produced.

INSECT PATHOGENIC MICROSPORA (PROTOZOA)

Few pathogenic microspora of rice insects were reported. Toquebaye and Bouix (1983) collected and described *Nosema manierae* infecting the stemborer *Chilo zacconius* Blezenski. However, no information on its effectivity in the field and practical application is available. An unidentified microspora parasite was observed in rice mites in Malaysia (Lo and Ho, 1982). Research on insect pathogenic microspora in relation to rice pests should be encouraged because these pathogens have shown to be effective on other crops (see various authors in Burges, 1981).

INSECT NEMATODES

Several insect pathogenic nematodes are promising biological control agents. Poinar (1979) provides keys to the common species of insect pathogenic nematodes and discusses their biology and ecology.

Commercial products of nematodes are available, such as *Heterorhabditis sp.* for control of the black vine weevil, *Otiorhynchus sulcatus* F. in greenhouses (W.R.Simons, pers. comm., 1985) and *Romanomermis culicivorax* Ross and Smith for control of mosquito larvae. For steinernematid and heterorhabditid nematodes a mass rearing method has been developed (Bedding, 1984); this method can be applied profitably for high valued crops and should be evaluated for rice.

There is little information regarding the interaction of insect nematodes and rice pests. Various species of nematodes and their insect hosts collected in rice are listed in Table 1. Recently, Pena and Shepard (1985) reported on the natural incidence of nematodes on the brown planthopper, the whitebacked planthopper and the green leafhopper in Laguna Province, Luzon, Philippines. The highest incidence of parasitism, 36-50%, occurred during the wet season; this was probably due to high relative humidity which enhanced the activity of the nematodes.

The strain DD-136 of *Steinernema feltiae* Filipjev (= *Neoaplectana carpocapsae* Weiser) is the only nematode reportedly tested in the field for the control of different species of stemborers and the rice water weevil (Ramahrishnan and Kumar, 1981; Meneses-Carbonell, 1983). Because *S. feltiae* can infect a wide range of lepidopteran hosts (Poinar, 1979), more lepidopteran pests of rice should be tested as possible targets for this nematode.

Table 1. Records of insect pathogens classified by pathogen species, host, location, and reference. Numbers refer to the list of references.

PATHOGEN	HOST	LOCATION	REF.
FUNGI: DEUTEROMYCOTINA			
Metarhizium anisopliae	*Chilo suppressalis* (SSB)	Philippines	68
	Sesamia inferens (PSB)	Philippines	68
	Recilia dorsalis (ZLH)	Philippines	21
	plant- and leafhoppers	China	79
	Scotinophara lurida	Japan	149
	S. coarctata	Philippines	218
Metarhizium album	*Cofana spectra*	Sri Lanka	192
	" "	Indonesia	21
	Nephotettix virescens (GLH)	Philippines	214
Metarhizium flavoviride var. *minus*	BPH	Philippines	215
		Rep. Solomon Islands	215
Beauveria bassiana	GLH	Taiwan	47
		Thailand	77
		India	202
		China	116
	BPH	Taiwan	47
		India	238
		China	116
		Philippines	21
	SBPH	China	117
	WBPH	China	117
	ZLH	Philippines	21
	plant- and leafhoppers	China	79
	Chilo auricilius (SSB)	India	162
		Iran	211
		Philippines	68
	Chilo sp.	India	138, 202
		China	117, 118
	Cnaphalocrocis medinalis (LF)	India	202
		China	117
	Pelopidas mathias (RS)	India	164, 202
	Gryllotalpa sp.	China	117
	S. coarctata	Philippines	218
	Scotinophara sp.	China	117

(Table 1, cont.)

PATHOGEN	HOST	LOCATION	REF.
Nomuraea rileyi	*Mythimna separata*	Philippines	38
	plant- and leafhoppers	China	79
Hirsutella citriformis	BPH	Rep. Solomon Islands	25
	BPH	Philippines	14
	WBPH	Rep. Solomon Islands	125
Hirsutella barberi	*Chilo sp.*	Indonesia	253
Hirsutella sp.	BPH, GLH	Philippines	68, 69
Paecilomyces farinosus	GLH	Thailand	77
	Chilo simplex	Japan	96
	BPH	Japan	23
P. lilacinus	*S. lurida*	Japan	149
	S. coarctata	Philippines	218
		Malaysia	218
Fusarium sp.	*Chilo sp.*	China	78
	BPH, WBPH	India	189, 260, 24
	GLH	India	24
	Melanitis ledaismene	India	159
	Scirpophaga incertulas (RYSB)	Indonesia	253
Cephalosporium sp.	BPH, GLH	India	24
Aspergillus flavus	RS	India	261
Penicillium sp.	SSB	Philippines	68
FUNGI: CHYTRIDIOMYCOTINA			
Myiophagus ucrainicus	*Chilo sp.*	Japan	245
FUNGI: ZYGOMYCOTINA			
Entomophthora grylli	grasshoppers	Thailand	219, 220
	Oxyahyla intricata	Vietnam	268
E. sphaerosperma	BPH	Japan	231
E. fumosa	BPH	India	221
E. muscae	*Hydrellia philippina*	Philippines	63, 68

(Table 1, cont.)

PATHOGEN	HOST	LOCATION	REF.
Entomophthora sp.	*Mythimna separata*	New Zealand	253
	" "	Philippines	38
	Chilo sp.	S. Africa	253
	Nephotettix cincticeps	Taiwan	278
	WBPH, BPH	Fiji Islands	76
	BPH	India	131, 132
	"	Philippines	68
	GLH	China	82
	"	India	132
Erynia delphacis	BPH	Japan	80, 221, 58, 59, 228
Conidiobolus coronatus	SBPH, BPH	Japan	183
	BPH	Philippines	14, 56, 68
	Nephotettix impicticeps	Philippines	68
Syncephalastrum racemosum	*Cofana spectra*	India	137
BACTERIA: EUBACTERIALES			
Bacillus cereus	LF	India	193
Bacillus subtilis	*Melanitis ledaismene*	India	160
Serratia marcescens	*M. ledaismene*	India	237
unident. bacterium	*Mythimna separata*	Philippines	38
VIRUSES			
granulosis virus (GV)	*Mythimna separata*	not stated	135
	SSB	not stated	135
	LF	not stated	135
	"	India	91
	"	Fiji Islands	245
	"	not stated	124
nuclear polyhedrosis virus (NPV)	*Mythimna separata*	not stated	135
	" "	India	52, 168
	SSB	not stated	135
	Chilo sp.	Japan	245
	PSB	India	72
	Spodoptera mauritia	India	92

(Table 1, cont.)

PATHOGEN	HOST	LOCATION	REF.
	RS	India	161
	Nymphula depunctalis	India	50, 93
non-occluded virus	*Mythimna separata*	not stated	135
Chilo iridescent virus (CIV)	SSB	not stated	135
	"	Japan	10, 67, 142
iridescent virus (IV)	LF	not stated	135
NEMATODA			
Mermis sp.	WBPH	Japan	145
	BPH	India	130
Amphimermis zuimushi	GLH	Taiwan	47
Agamermis unka	BPH	Japan	85, 95, 58
Epigonatopus sakai	GLH	Taiwan	47
Hexamermis cathetospiculae	RYSB	Malaysia	196
Hexamermis sp.	BPH	India	130
nematode (unident.)	GLH, BPH, WBPH	Philippines	191
	WBPH	India	189
	Chilo simplex	Japan	85
	Chilo sp.	Bangladesh	88
	RYSB	" "	88
	"	Malaysia	188
	GLH, WBPH, BPH	Indonesia	263
	BPH	Indonesia	185
	"	Sri Lanka	185
Parasitorhabditis sp.	RYSB	India	89, 158
MICROSPORA (SPOROZOA)			
Nosema manierae	*Chilo zacconius*	Africa	257b
unident.Sporozoa	*Steneotarsonemus spinki* (Tarsonemidae, Acari)	Malaysia	122

Table 2. References classified by subject. Numbers refer to the list of references.

INSECT HOST

Brown planthopper, BPH, *Nilaparvata lugens* (Delphacidae) and smaller brown planthopper, SBPH, *Laodelphax striatellus* (Delphacidae) : 1, 2, 14, 18, 19, 21, 23, 24, 40, 41, 42, 44, 47, 56, 57, 58, 59, 60, 68, 69, 71, 75, 76, 79, 85, 95, 108, 109, 111, 112, 113, 116, 120, 125, 130, 131, 132, 139, 148, 157, 183, 184, 185, 187, 191, 194, 200, 201, 215, 217, 221, 222, 228, 229, 231, 237, 238, 239, 253, 263.

Whitebacked planthopper, WBPH, *Sogatella furcifera* (Delphacidae) : 1, 2, 76, 79, 80, 117, 125, 145, 157, 189, 253, 260, 263.

Green leafhopper, GLH, *Nephotettix virescens* (Cicadellidae): 1, 2, 24, 44, 47, 51, 77, 82, 106, 116, 118, 132, 139, 144, 148, 155, 162, 191, 202, 213, 214, 224, 229, 263, 278.

Zig-zag leafhopper, ZLH, *Recilia dorsalis* (Cicadellidae) : 3, 21, 68.

Stemborers, SB, Pyralidae; Noctuidae : 10, 15, 16, 17, 28, 39, 43, 48, 49, 65, 66, 67, 68, 69, 72, 78, 85, 86, 88, 89, 95, 96, 97, 98, 99, 100, 101, 102, 117, 118, 121, 134, 138, 141, 142, 151, 154, 158, 161, 162, 163, 165, 166, 173, 174, 179, 180, 181, 188, 196, 199, 202, 205, 206, 207, 209, 211, 233, 235, 237, 245, 246, 248, 249, 250, 253, 256, 257b, 258, 259, 262, 266, 269, 270, 271, 272, 273, 275, 277.

Leaffolder, LF, *Cnaphalocrocis medinalis* (Pyralidae) : 13, 76, 91, 110, 115, 117, 118, 124, 135, 152, 154, 193, 199, 202, 236, 245.

Army- and cutworms,(Noctuidae), and caseworms,(Pyralidae) : 12, 34, 37, 38, 50, 52, 53, 54, 55, 69, 90, 92, 93, 107, 110, 123, 126, 127, 128, 129, 133, 135, 136, 159, 160, 167, 168, 169, 170, 171, 172, 225, 226, 232, 240, 253, 257b, 264, 265.

Rice skipper, RS, *Pelopidas mathias* (Hesperiidae) : 7, 11, 115, 161, 164, 202, 261.

Black bugs, *Scotinophara spp.* (Pentatomidae) : 117, 149, 150, 218, 276.

LABORATORY EVALUATION OF PATHOGENICITY

Insect fungi : 2, 5, 13, 39, 51, 109, 111, 112, 133, 143, 149, 150, 159, 164, 165, 166, 175, 177, 178, 183, 194, 217, 229, 231, 235, 238, 261, 271, 278.

Insect bacteria : 5, 15, 28, 69, 89, 163, 199, 201, 237, 263, 269.

Insect viruses : 26, 53, 54, 55, 86, 107, 126, 127, 128, 129, 167, 169, 170, 171, 172, 179, 180, 225, 232, 264, 265.

Insect Microspora : 257b.

Insect nematodes : 89, 90, 203, 207, 208.

FIELD EVALUATION

Insect fungi : 5, 17, 19, 21, 56, 71, 84, 140, 149, 150, 154, 187, 217, 218.

Insect bacteria, *Bacillus thuringiensis* : 7, 11, 12, 17, 22, 84, 140, 154

Insect viruses: 167.

Insect nematodes : 140, 203, 209, 258, 270.

MISCELLANEOUS

Symbiotic (non-pathogenic) relationships : 41, 42, 75, 114, 143, 144, 155, 157, 175, 176, 177, 178, 184.

Pathogen-insecticide compatability : 1, 3, 4, 39, 139, 149, 150, 154, 201, 203, 225, 226.

Mass-production of pathogens : 7, 17, 27, 65, 70, 84, 113, 134, 149, 186, 234.

Insect viruses (biochemistry; ultrastructure) : 25, 37, 48, 67, 98, 99, 100, 101, 102, 103, 136, 142, 180, 181, 256, 257.

General : 6, 8, 9, 17, 20, 23, 30, 31, 32, 33, 43, 45, 46, 65, 66, 73, 84, 87, 94, 104, 119, 121, 147, 156, 198, 204, 208, 216, 227, 273, 274, 275.

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CHAPTER 3

NEW AND RARE *METARHIZIUM* SPP.

During the course of field experimentation numerous rice insects infected by insect fungi were collected. These collections contained the taxon *Metarhizium flavoviride* var. *minus* var.nov., and the rare insect fungus *M. album* Petch.

Section 3.1. *Metarhizium flavoviride* var. *minus* var. nov., a pathogen of plant- and leafhoppers on rice in the Philippines and Solomon Islands.

Section 3.2. *Metarhizium album* a fungal pathogen of leaf- and planthoppers of rice.

Section 3.1

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METARHIZIUM FLAVOVIRIDE VAR. *MINUS*, VAR. NOV., A PATHOGEN OF PLANT- AND LEAFHOPPERS ON RICE IN THE PHILIPPINES AND SOLOMON ISLANDS

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Metarhizium flavoviride Gams & Rozsypal var. ***minus*** var. nov. is described from the brown planthopper, *Nilaparvata lugens* Stål (Homoptera, Delphacidae), on rice in the Philippines and the Solomon Islands and from the zig-zag leafhopper, *Recilia dorsalis* (Motschulsky) (Homoptera, Cicadellidae), in the Philippines. The new variety has also been collected on a grasshopper in the Galápagos Islands.

The conidia of the new variety are smaller (mostly 4.5-7 x 2-3 µm) and more consistently ellipsoidal to ovoidal than those of *M. flavoviride* var. *flavoviride* [(6.5-)7-9[-11] x 4.5-5.5 µm]. The new variety may form synnemata in culture.

At present, three species of *Metarhizium* are recognized: Most authors have followed Tulloch (1976) in accepting *M. anisopliae* (Metschn.) Sorok. (with cylindrical phialides bearing chains of cylindrical conidia that usually adhere laterally to form columnar conidial columns) and *M. flavo - viride* Gams & Rozsypal (with narrowly clavate phialides bearing chains

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of ovoid or ellipsoidal conidia that typically adhere in parallel masses). Rombach et al. (1986b) are restoring the name *Metarhizium album* Petch for a pathogen of Asiatic homopterans that forms clavate phialides with nonadherent chains of white to brown, ellipsoid to ovoid conidia.

Metarhizium flavoviride is primarily an entomogenous species reported from curculionid beetles (Gams & Rozsypal 1973; Marchal 1977), from a grasshopper (Evans & Samson 1982), and from agricultural soils in Germany and the Netherlands (Gams & Rozsypal 1973). This species has been studied as a specific pathogen of black vine weevils, *Otiorrhynchus sulcatus* F. (Marchal 1977; Soares et al. 1983; Poprawski et al. 1985a) and of the onion root maggot, *Delia antiqua* (Meigen) (Poprawski et al. 1985b). The small-spored variety described below causes natural epizootics of the brown planthopper, *Nilaparvata lugens* Stål, and of the zig-zag leafhopper, *Recilia dorsalis* (Motschulsky), on rice in the Philippines and Solomon Islands. Field populations of brown planthoppers can be infected by sprayed applications of the small-spored variety (Rombach et al. 1986a).

Metarhizium flavoviride Gams & Rozsypal (1973) is characterized by the formation of sporodochia bearing clavate phialides (9-14 x 3-4.5 µm) and chains of large ellipsoidal conidia (Fig. 1a); the conidial masses of this species are pale green to distinctly yellow-green. Cultures of *Metarhizium flavoviride* are slow growing, flat, and slow to produce conidia. The ovoid to ellipsoid conidia and clavate phialides are the major diagnostic characters which separate *M. flavoviride* from *M. anisopliae* with its distinctly cylindrical conidia and cylindrical phialides.

In recent years, numerous isolations identified as *Metarhizium flavoviride* were made from plant- and leafhoppers on rice in Asia (Fig. 2a). Most of these isolates are preserved in liquid nitrogen at the Collection of Entomopathogenic Fungi (ARSEF), USDA-ARS Plant Protection Research Unit (Boyce Thompson Institute, Ithaca, New York).

On the plant- and leafhoppers (Fig. 1b), *Metarhizium flavoviride* shows a slightly different morphology from that originally described for *M. flavoviride* by Gams & Rozsypal (1973), most notably in the smaller dimensions (Table 1) and more consistently ovoid shape of the conidia. In artificial culture (Fig. 1c), the characteristics are slightly more variable. Tulloch (1976) separated the varieties *M. anisopliae* var. *anisopliae* and *M. anisopliae* var. *majus* (Johnston) Tulloch* mainly by the shape and dimensions of their conidia. It is appropriate, therefore, to expand the concept of *M. flavoviride* defined by Gams & Rozsypal (1973) to accommodate these Asiatic isolates from plant- and leafhoppers on rice in a new

* This is an obligatory orthographic correction of the varietal name usually cited as *M. anisopliae* var. *major*.

TABLE 1. Conidial measurements of *Metarhizium flavoviride*. Isolates (listed by ARSEF numbers) were grown on Sabouraud dextrose agar + 1% yeast extract at room temperature. Sizes expressed as mean ± standard deviation for 75 conidia measured in lactophenol/aniline blue.

ARSEF	HOST/SOURCE	COUNTRY	SIZE (µm)
	M. flavoviride var. *flavoviride*		
2133[a]	*Ceutorrhynchus macula-alba*	Czechoslovakia	7-9(-11) x 4.5-5.5[b]
1184	*Otiorrhynchus sulcatus*	France	8.5 ± 1.1 x 3.8 ± 0.5
2025	agricultural soil	Fed. Rep. Germany	8.7 ± 2.9 x 4.5 ± 0.6
2026	agricultural soil	Netherlands	9.2 ± 0.9 x 5.4 ± 0.6
	M. flavoviride var. *minus*		
2037[a]	*Nilaparvata lugens*	Philippines	4.6 ± 0.7 x 2.7 ± 0.3
1099	*Nilaparvata lugens*	Philippines	4.6 ± 0.6 x 2.3 ± 0.4
1547	*Recilia dorsalis*	Philippines	5.2 ± 0.8 x 2.6 ± 0.4
1764	*Nilaparvata lugens*	Solomon Islands	5.4 ± 0.7 x 2.6 ± 0.4
1768	*Nilaparvata lugens*	Solomon Islands	5.9 ± 0.8 x 2.5 ± 0.4
2023	Acridid grasshopper	Galápagos Islands	4.9 ± 0.7 x 2.6 ± 0.4

[a] Type strain of variety.
[b] From Gams & Rozsypal (1973); the type strain subculture examined here, as noted by Gams and Rozsypal, was unpigmented and produced few typical phialides but many extremely variable clavate to cylindrical conidia, 8.6 ± 1.7 x 3.0 ± 0.5 µm.

variety of this species; these isolates consistently produce smaller conidia and sometimes form fasciculate masses of hyphae (synnemata) in artificial culture (Fig. 2b). Varieties of this species are characterized primarily by conidial dimensions.

Metarhizium flavoviride Gams & Rozsypal var. ***flavoviride***, *Acta Bot. Neerl.* 22, 518-521 (1973). **FIG. 1A**

Cultures Examined: ARSEF 2133 (ATCC 32969 < CBS 218.56), ex type culture, from larvae/pupae of *Ceutorrhynchus macula-alba*, Brno, Czechoslovakia, 1956. ARSEF 1184 (INRA MF-88 = CBS 700.74), from *Otiorrhynchus sulcatus*, Brittany (France), January 1974. ARSEF 2025 (CBS 125.65), from soil under *Brassica oleracea*, Kiel-Kitzeberg, Fed. Rep. Germany, 1963 or 1964. CBS 380.73, from soil under *Brassica oleracea*, Kiel-Kitzeberg, Fed. Rep. Germany, 1963 or 1964. ARSEF 2026 (CBS 473.73), from agricultural soil, coll. J. W. Veenbaas-Rijks, Wageningen, Netherlands, 1973.

Metarhizium flavoviride Gams & Rozsypal var. ***minus*** Rombach, Humber, & Roberts, **var. nov.** **FIGS. 1B, 2**

Morphologiae conidiophororum, phialidium clavatarum, conidiorum ellipsoideorum ovoideorum, et habitu in cultura Metarhizium flaviviride simulant, autem conidia viridi-grisea parviora, 4.5-7 x 2-3 µm, et (in cultura) synnemata albida laxe fasciculata sparsim fecunda formans.

Holotype: **CUP 61454,** on *Nilaparvata lugens* Stål, experimental rice field, International Rice Research Institute, Los Baños, Philippines, leg. M.C. Rombach, 5 November 1985.

Isotypes: **CBS, IMI,** and at the USDA-ARS Plant Protection Research Unit (Ithaca, New York).

Cultures Examined: ARSEF 2037, type culture isol. from holotype, leg. M.C. Rombach, November 1985. ARSEF 543, from greenhouse culture of *Recilia dorsalis* (Motschulsky), International Rice Research Institute, Low Baños, Laguna, Philippines, D. W. Roberts, August 1980. ARSEF 1547, from *Recilia dorsalis* (Motschulsky), Pangasinan, Luzon, Philippines, leg. R. M. Aguda, September 1982. ARSEF 1763 through 1773 (inclusive), from *Nilaparvata lugen* s Stål on rice, Guadalcanal Plains, Guadalcanal, Rep. Solomon Islands, coll. by D. T. Ho, isol. by M. C. Rombach, February 1985. ARSEF 2023 (CBS 544.81, as *M. flavoviride*) from Acrididae (Orthoptera), Santa Fé Island, Galápagos Islands, coll. H. C. Evans, 1981. Numerous cultures from *Nilaparvata lugens* on rice from the International Rice Research Institute, Los Baños, Philippines.

The branched conidiophores, clavate conidiogenous cells, and long chains of ellipsoidal conidia of *M. flavoviride* var. *minus* generally resemble those of *M. flavoviride* Gams & Rozsypal (1973) but differ in their dimensions. The conidial chains of both varieties aggregate into columnar masses similar to those of *M. anisopliae.* The conidiogenous cells of var. *minus* are 8.4 ± 1.2 x 2.8 ± 0.3 µm (on Sabouraud dextrose

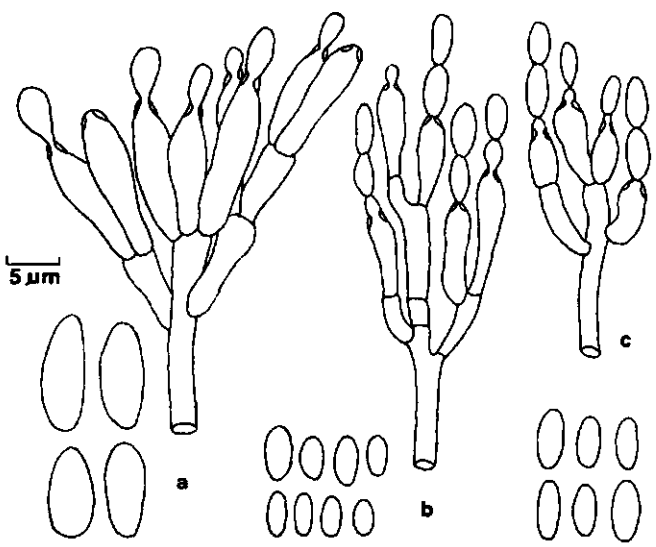

Fig. 1. Conidiophore and conidia of (a) *Metarhizium flavoviride* var. *flavoviride* (isolate CBS 380.73a), (b) *M. flavoviride* var. *minus* on the brown planthopper, and (c) in culture, ARSEF 1547.

agar + 1% yeast extract) whereas those of var. *flavoviride* are 9-14 x 3–4.5 μm (from malt extract and oatmeal agars). The conidia of var. *minus* are 4.5-7 x 2-3 μm and dull grey-green in mass (with no significant hint of yellow pigments); the larger (6.5-11 x 3.5-5 μm) conidia of var. *flavoviride* are usually yellow-green. Conidial sizes in several strains of these varieties are listed in Table 1. Because conidia of both barieties tend to become slightly narrower after repeated transfers on artificial media; isolates should be frozen in liquid nitrogen or lyophilized at the earliest chance. In culture, *M. flavoviride* var. *minus* may produce synnemata up to 10 mm high (Fig. 2b) rather than the more usual flat, stroma-like sporodochia. These synnemata often remain white and sterile or may show fertile patches.

Metarhizium flavoviride var. *minus* resembles *M. album* Petch in the morphologies of the conidiophores, clavate phialides, and conidia. These species differ in their conidial colors since *M. album* is whitish-brown (with no hint of green pigmentation). Also, the flat, smooth hymenial surface with conidial chains adhering in large columns produced by *M.flavoviride* var. *minus* differs markedly from the raised sporodochial masses of hyphal bodies produced by *M. album*. The recognized species of *Metarhizium* are also distinguished unambiguously by comparisons of the electrophoretic mobility of their various isoenzyme systems.

Fig. 2. *M. flavoviride* var. *minus*. (a) Infecting brown planthopper; insect on right is an early stage of postmortem development with protruding white mycelium; specimen on the left exhibits a darker fertile patch. (b) Synnemata of ARSEF 1547 in culture; note apical fertile patches (fp). Both micrographs, x 7.

To date, the small-spored variety of *M. flavoviride* has been collected from plant- and leafhoppers on rice in the Philippines and Solomon Islands; it was absent from extensive collections of fungi from auchenorrhynchid hosts from rice in South Korea, India, and Sri Lanka. A collection of *Metarhizium flavoviride* on a grasshopper from the Galápagos Islands (Evans & Samson 1982) also belongs to var. *minus* (see Table 1). Clearly, this variety may prove to be a much more widely distributed and important pathogen of insects than is now apparent.

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Section 3.2

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METARHIZIUM ALBUM A FUNGAL PATHOGEN OF LEAF- AND PLANTHOPPERS OF RICE

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Metarhizium album, a name widely regarded as a synonym of *M. anisopliae*, is restored for a fungus collected on plant- and leafhoppers (Homoptera: Auchenorrhyncha) from rice. In the Philippines and Indonesia it caused epizootics in populations of *Nephotettix virescens* and/or *Cofana spectra*, respectively. A single collection on an unidentified leafhopper from mango is reported. Comparison of types revealed that *M. brunneum* is a synonym of *M. album*; the species is characterized by the pale brown colour of its conidial masses, clavate phialides (10–12·5 × 2–3·5 μm), ovoid to ellipsoidal conidia ((3–4)4–6 × 1·5–2·5 μm) and growth of bulging masses of hyphal bodies rather than mycelium prior to sporulation.

The primary taxonomic criteria for delimiting species of *Metarhizium* are the shapes of conidia and conidiogenous cells, presence or absence of a subhymenial zone of swollen hyphal bodies, and whether conidial chains adhere laterally to form prismatic columns. The occurrence of many natural and artificial colour variants of *Metarhizium* species suggests that colours of conidial masses and mycelium have only secondary taxonomic value. Conidial size is useful in delimiting varieties.

A synoptic key to the taxa of *Metarhizium* is provided.

Species of *Metarhizium* Sorokin are regularly reported to attack insects (Veen, 1968; Ferron, 1981) and are occasionally isolated from soil (Domsch & Gams, 1970; Domsch, Gams & Anderson, 1980). In recent years, *Metarhizium* species were applied successfully against insect pests of several crops (Ferron, 1981), including pests of rice in the tropics (Rombach, Shepard & Roberts, 1986).

Petch (1931) reviewed the older literature on the taxonomy of *Metarhizium anisopliae* (Metschnikoff) Sorokin. In her monograph, Tulloch (1976) recognized *M. anisopliae* and *M. flavoviride* Gams & Rozsypal as the only acceptable species in the genus. Brady (1979*a*, *b*) provided descriptions, illustrations and pertinent references for these two species.

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† The original varietal name, *major*, is a comparative adjective with the incorrect inflection for a neuter noun.

Tulloch (1976) followed Johnston (1915) in distinguishing *M. anisopliae* var. *anisopliae* (Fig. 1*a*) and *M. anisopliae* var. *majus*† (Johnston) Tulloch (Fig. 1*b*) by their conidial dimensions. *Metarhizium anisopliae* var. *anisopliae* is a cosmopolitan pathogen of innumerable insects (Veen, 1968); *M. anisopliae* var. *majus* is largely restricted to *Oryctes* (Coleoptera: Scarabaeidae: Dynastinae) and has been widely tested for the control of *Oryctes rhinoceros* (L.) in coconut palms (Latch & Falloon, 1976; Marschall, 1978).

Metarhizium flavoviride Gams & Rozsypal is known from curculionid beetles (Gams & Rozsypal, 1973; Marchal, 1977), from agricultural soils in Europe (Gams & Rozsypal, 1973), and is being studied for use against black vine weevil, *Otiorrhynchus sulcatus* F. (Marchal 1977; Soares, Marchal & Ferron, 1983; Poprawski, Marchal & Robert, 1985) and the onion maggot, *Delia antiqua* (Meigen) (Poprawski, Robert, Majchrowicz & Boivin, 1985). A new variety of this species, *M. flavoviride* var. *minus* (Rombach, Humber &

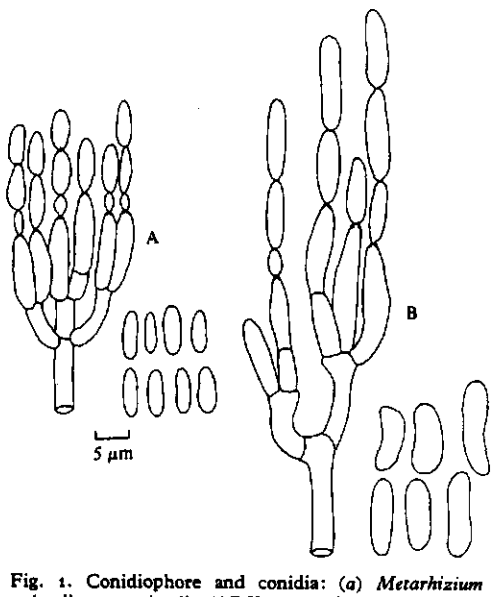

Fig. 1. Conidiophore and conidia: (*a*) *Metarhizium anisopliae* var. *anisopliae* (ARSEF 1548, from *Scotinophora coarctata*, Palawan, Philippine) and (*b*) *Metarhizium anisopliae* var. *majus* (ARSEF 1946, from *Oryctes rhinoceros*, Luzon, Philippines).

Roberts, 1986), with smaller and more consistently ellipsoidal conidia, causes epizootics on brown planthoppers, *Nilaparvata lugens* Stål, and zig-zag leafhoppers, *Recilia dorsalis* (Motschulsky), on rice in the Philippines and Solomon Islands; it has also been found on a grasshopper from the Galápagos Islands (Evans & Samson, 1982).

Recently, a series of collections of a *Metarhizium* species producing chains of small, brown (or rarely white), ellipsoid conidia on clavate phialides were made from various leafhoppers (Homoptera: Cicadellidae) on rice in Asia. These fungi were referable to neither *M. anisopliae* nor *M. flavoviride* but immediately recalled *M. album* Petch (1931) and *M. brunneum* Petch (1935), two species which have been almost universally regarded as synonyms of *M. anisopliae*.

The taxonomic fates of *Metarhizium album* and *M. brunneum* suffered from incomplete examinations of their types and misinterpretations of a brown-spored strain of *Metarhizium* (NRRL 1944 = IMI 14746) isolated from wireworm larvae (Coleoptera; Elateridae) by Rockwood (1950). Latch (1965) examined NRRl 1944 (but not the type of *M. brunneum*) and concluded that *M. brunneum* was synonymous with *M. anisopliae*. In his classic study on *M. anisopliae*, Veen (1968) accepted Latch's (1965) synonymy without himself examining the type of *M. brunneum*. Gams & Rozsypal (1973) apparently examined no material of *M. brunneum* before suggesting that *M. brunneum* might be identical to *M. anisopliae*. Tulloch (1976) interpreted the type of *M. album* to be an immature specimen of *Metarhizium anisopliae* without noting either its relatively small, ellipsoid conidia (3–4 × 1·75 μm) or the presence of hyphal bodies in the convoluted stroma. She also examined the type of *M. brunneum* without noting its small, ellipsoid conidia or clavate phialides. Further, Tulloch (1976) treated Rockwood's brown-spored culture, IMI 14746, as if it were authentic material of *M. brunneum*, since it was identified as this species by Petch in a letter to Dr W. L. White (cited Rockwood, 1950). In a separate letter enclosed in the packet of dry cultures in IMI 14746 now in the Herb. IMI, Petch stated, 'the conidia are rather larger, 5–8 × 2–2·5 μm, than in the type [of *M. brunneum*], but I don't think the difference is large enough to constitute a new species. I should put it as *M. brunneum*.' We examined both this herbarium specimen and cultures of IMI 14746, and found the conidia to be typical of *M. anisopliae* (5·5–8 × 2–2·5 μm, cylindrical, with a slight central constriction); few cylindrical phialides were found. The cultures grew slowly and produced little aerial mycelium. We conclude, therefore, that IMI 14746 is a brown colour variant of *M. anisopliae*. Rockwood (1950) mentioned *Isaria*-like strands ('synnemata') spreading from the subterraneous host; similar structures have been noted on other collections of *M. anisopliae* from hidden insects (Evans, 1982).

Clearly, there is no sound basis for regarding *M. album* and *M. brunneum* as synonyms of *M. anisopliae*. The diverse *Metarhizium* collections from homopteran pests of Asian rice reported by Rombach, Humber & Robert, (1986), by Rombach, Shepard & Roberts (1986), and here indicate the need for a general revision of taxa within this genus.

Metarhizium album Petch (1931) was described from Sri Lankan specimens of *Cofana* (*Tettigoniella*) *spectra* (Distant), a large, common leafhopper (Homoptera: Cicadellidae) on Southeast Asian rice. Petch distinguished *M. album* from *M. anisopliae* by its small, ovoid, white conidia, its cerebriform, convoluted stroma, and clavate conidiogenous cells. *M. brunneum* Petch (1935) was described from a cicadellid host collected at Laguna, Luzon, Philippines. Petch distinguished *M. brunneum* from *M. anisopliae* by its pale brown colour and clavate phialides and from *M. album* by conidial colour and by the absence of a convoluted

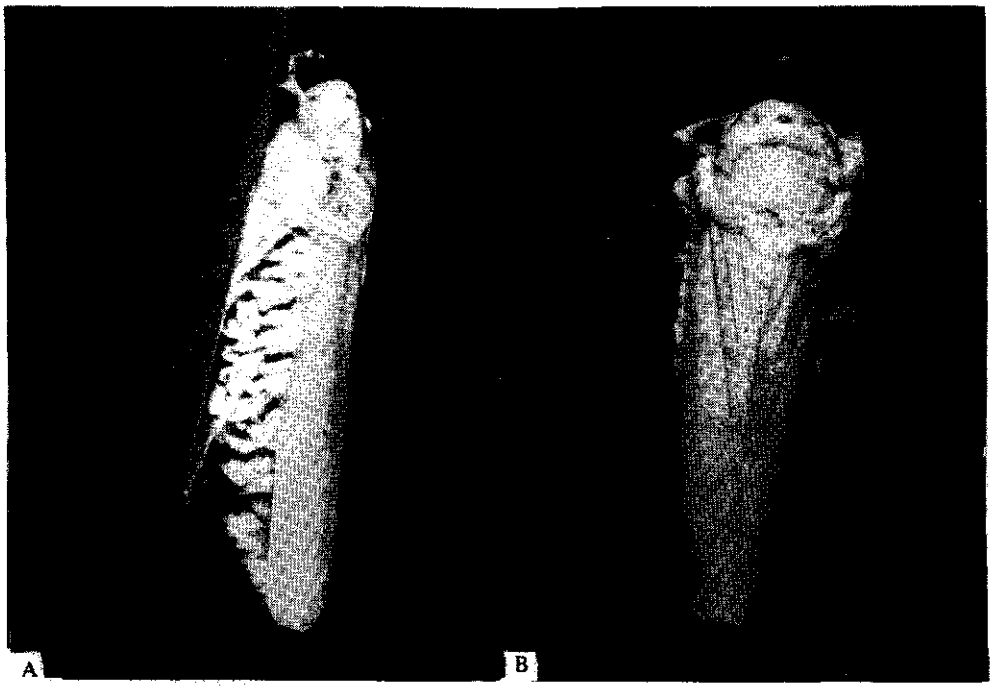

Fig. 2. *Metarhizium album* infecting *Cofana spectra* from Indonesia, (A) side view, and (B) top view of the same insect.

stroma; he did not mention the strong similarities between *M. album* and *M. brunneum* in their ovoid conidia, clavate conidiogenous cells, verticillate conidiophores, and hosts from the Cicadellidae.

More recently, specimens and cultures of a *Metarhizium* species with pale brown conidia were collected from green leafhoppers (GLH), *Nephotettix virescens* (Dist.) (Homoptera: Cicadellidae), on rice from several sites in the Philippines and Indonesia. One isolate, hereafter designated as MLAG, was made from *N. virescens* in rearing cages at the International Rice Research Institute, Philippines (Figs 2A, 3A); this culture is deposited as ARSEF 1942 in the Collection of Entomopathogenic Fungi (USDA-ARS Plant Protection Research Unit, Ithaca, NY); this collection was from the same host family and the same area as the type of *M. brunneum*. Relatively few GLH adults were infected, and the infection never reached epizootic proportions, even at high host densities. Another isolate, MROX (ARSEF 1941), with white to brownish conidia, was made from GLH near Roxas on the southern Philippine island of Palawan. A third isolate, MTUG (ARSEF 2081), was made from an unidentified leafhopper on a mango tree, at Tuguerarao on northern Luzon. Collections on the Philippine island of Leyte were made from both zig-zag leafhoppers, *Recilia dorsalis* (Motsch.), and GLH on rice with relatively low host population densities (1–5 insects/hill); the brown-spored *Metarhizium* was the only fungal pathogen collected from these Leyte sites. A large collection of this same fungus (Fig. 2B) was also made from an epizootic on *Cofana spectra* (the type host of *M. album*) near Kotamobagu, on Sulawesi Utara, Indonesia; the culture, IMI 300150 (= ARSEF 2082), is hereafter designated as MSUL.

Cultures of the above strains on potato dextrose agar (PDA), malt agar, Sabouraud dextrose agar + 1% yeast extract (SDAY), and Emerson's YpSs produce white colonies which almost without exception become pale brown after the initiation of sporulation; only a very small proportion of sporulating cultures remained pure white. Both in culture and on the host, the convoluted basal

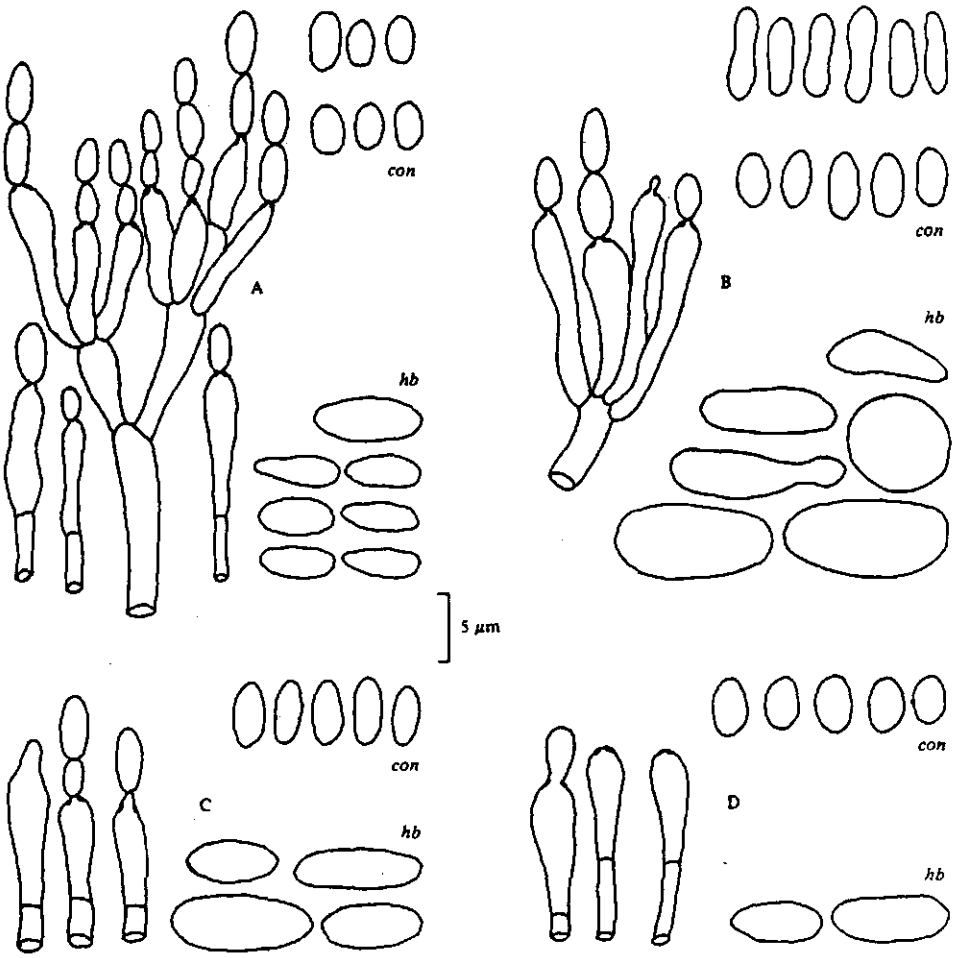

Fig. 3. *M. album* (A) on the green leafhopper (MLAG) and (B) in culture; (C) *M. brunneum*, type specimen; (D) *M. album*, type specimen; (*con* = conidia; *hb* = hyphal bodies)

stroma (Fig. 4) of the strains from rice insects is almost totally replaced by the coherent masses of brown conidia. A few mature colonies of MLAG were greyish white rather than pale brown, but were also found to be sterile.

Because the specimens and cultures from rice insects strongly indicated that *M. album* and *M. brunneum* are identical, we examined their type specimens. The host in the holotype of *M. brunneum* is a large cicadellid (Homoptera) attached to a leaf of a plant other than rice. The fungal infection is too advanced to allow further identification of the host; phialides, conidia and hyphal bodies but no complete conidiophores were observed (Fig. 3C). A convoluted mass of hyphal bodies underlying the stroma was almost totally obscured by large masses of whitish-brown conidia; a few patches of white mycelium were apparent. Petch (1935) mentioned no study of a culture, so his description of *M. brunneum* is based wholly on this single specimen. On the holotype of *M. album*, very few hyphal bodies were found underlying the yellowish-white conidial mass; the phialides are distinctly clavate (Fig. 3D). *M.*

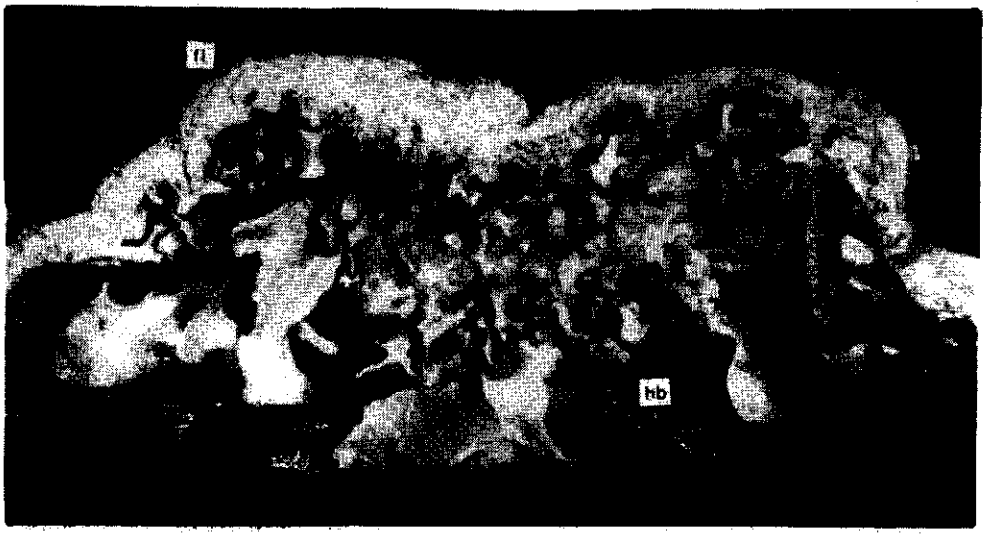

Fig. 4. *M. album*, cross-section through culture showing stroma of hyphal bodies (*hb*) and fertile layer of conidiophores (*ff*).

brunneum is based on a later developmental stage than that described earlier as *M. album* and is, therefore, a synonym of *M. album*. Petch (1931) studied *M. album* in culture, but the strain was not preserved.

Microscopical examination of collections from Indonesia and five areas in the Philippines indicated that these fungi correspond in all basic morphological characters to the fungus described by Petch as both *M. album* and *M. brunneum*. The following description is based on our examinations of cultures and all pertinent specimens, includings holotypes.

METARHIZIUM ALBUM Petch, *Trans. Brit. Mycol. Soc.* **16**: 71 (1931).
Metarhizium brunneum Petch, *Trans. Brit. Mycol. Soc.* **19**, 189 (1935).

Stroma-like covering on insect host cerebriform, convoluted, consisting of a basal mass of bulging hyphal bodies covered by a felt-like hymenium; surface of conidial mass irregular due to variable size of conidiophores and convoluted character of the basal stroma. *Hyphal bodies* hyaline to slightly yellowish white, 5–12 × 2·5–5 μm, cylindrical to ellipsoidal, often bilaterally flattened, with rounded ends or 1 or 2 small terminal scars due to yeast-like budding by which new hyphal bodies arise; in host body, hyphal bodies similarly shaped, ellipsoidal (7·5–12 × 8 μm) or globose (up to 17·5 μm diam); masses of hyphal bodies protruding through intersegmental membranes or soft areas of host exoskeleton and becoming pure white, loosely interwoven hyphae which later give rise to a closely interwoven stroma-like layer of conidiophores, conidiogenous cells, and conidial chains. *Colonies* on SDAY or Emerson's YpSs agar growingly slowly, attaining diameter of 1–1·5 cm after 2 weeks; pure white to yellowish white, or greyish white becoming pinkish to fawn to pale brown upon sporulation; stromatic masses of hyphal bodies common on PDA; reverse slightly yellow or uncoloured. *Conidiophores* variable in length with basal stalk 15–80 × 2–3·5 μm; apical branching loosely verticillate, with each branch bearing 2–5 conidiogenous cells (Fig. 3) frequently arranged on metulae, 7·5–10 × 2–3 μm. *Conidiogenous cells* phialidic, clavate, 10–12·5 × 2–3·5 μm, strongly tapered toward apex, with necks greatly reduced or absent; often slightly more swollen in culture. *Conidia* on the host broadly to narrowly ellipsoid or ovoid, (3–)4–6 × 1·5–2·5 μm (in culture, 5–7·5 × 1·5–2 μm and more variable in shape or elongated ellipsoidal to cylindrical, up to 8–11 μm in length), produced in basipetal chains; white, whitish-brown or pale brown in mass. *Teleomorph* not known.

Specimens and cultures examined. Metarhizium album: Type (K), on *Tettigoniella* (= *Cofana*) *spectra* (L.) (Homoptera: Cicadellidae), Sri Lanka, J. C. Hutson,

Jan. 1928. On *Nephotettix virescens* (Dist.) (Homoptera: Cicadellidae) on rice, greenhouse of International Rice Research Institute, Laguna, Philippines, Coll. R. M. Aguda, Nov. 1984 (cultured by M. C. Rombach as MLAG, ARSEF 1942). On *Nephotettix virescens* on rice, near Roxas, northern Palawan, Philippines, D. J. Im, July 1985 (cultured by MC Rombach as MROX, ARSEF 1941). Culture, ARSEF 2081 (MTUG), from leafhopper (Homoptera: Cicadellidae) on mango, Tuguerarao, Cagayan, northern Luzon, Philippines, M. J.Cock, Dec. 1985. Culture (MSUL), IMI 300150 (= ARSEF 2082), from *Cofana spectra* (Homoptera: Cicadellidae) on rice, near Kotamobagu, Sulawesi Utara, Indonesia, Mar 1985, H. C. Evans. Cultures, ARSEF 2176 and 2178, from *Nephotettix virescens* on rice, Baybay, Leyte, Philippines, M. C. Rombach, May 1986. Cultures, ARSEF 2177 and 2179, from *Recilia dorsalis* (Motsch.) (Homoptera: Cicadellidae) on rice, Baybay, Leyte, Philippines, M. C. Rombach, May 1986. Culture, ARSEF 2229, from *Nephotettix virescens* on rice, near Villanueva, Misamis Occidental, Mindanao, Philippines, M. C. Rombach.

Metarhizium brunneum: Type (K), on homopterous insect (Cicadellidae), Agricultural College, Laguna, Philippines, G. O.Ocfemia, Dec 1931.

Additional material examined: IMI 14746, as *M. brunneum*, dry culture in Herb. IMI and living culture (= ARSEF 2107), from *Limonius* sp. (Coleoptera: Elateridae), Forest Grove, Oregon, USA, L. P. Rockwood, 1933. Culture, IMI 113863 (= ARSEF 2042), as *M. brunneum*, from peat soil in cedar bog, Guelph, Ontario, Canada, G. L. Barron 10277, 1960.

Development of *M. album* in culture begins with the formation of irregularly shaped, swollen, and easily disarticulated hyphal bodies, 5–7·5 μm wide, which are later displaced by interwoven, septate hyphae. Swollen hyphae may occasionally form fascicles up to 5 mm long on an agar surface. Conidiogenesis proceeds by formation of bulging, stroma-like masses of interwoven, hyaline hyphae and conidiophores rising up to 10 mm above the agar surface (Fig. 4). These fertile zones are surrounded at first by vein-like zones of pure white, sterile hyphae, but as the diameter of these zones increases they tend to coalesce into large fertile surfaces. Sparse, cottony, white aerial masses of hyphae sometimes break through the hymenial surface to establish loose networks over portions of the hymenium. Isolates MSUL and MTUG produce cylindrical conidia (8–11 × 1·5–3 μm) in culture; these conidia are different from the small ellipsoidal conidia produced on the insect.

The grey-green isolates of *M. flavoviride* var. *minus* from plant- and leafhoppers on rice (Rombach, Humber & Roberts, 1986) produce conidiophores, clavate phialides, and ellipsoidal conidia like those of *M. album*. However, *M. flavoviride* produces a smooth hymenial surface of laterally adherent chains of greenish conidia whereas *M. album* produces a raised sporodochial mass of hyphal bodies bearing white or brownish conidial chains; the conidial mass of *M. album* is powdery or floccose because the conidial chains do not adhere in prismatic columns.

COLOUR VARIANTS OF *METARHIZIUM* SPECIES

Naturally occurring conidial colour variants. Several brown isolates of *M. anisopliae* are known. The brown-spored strain (NRRL 1944 = IMI 14746 = ARSEF 2107) from wireworms (Coleoptera: Elateridae) isolated by Rockwood (1950) is discussed above. He remarked on the roughened surface of the colonies and on round-headed bodies developing under the sporiferous layer, but mentioned no mass of hyphal bodies. This development of round-headed masses of white, sterile mycelium is a common phenomenon in sporulating cultures of all *Metarhizium* spp. (M. C. Rombach, unpubl.). IMI 113863 (= ARSEF 2042), listed in the CAB International Mycological Institute catalogue as *M. brunneum*, was isolated by G. L. Barron from soil in Canada; colonies of this strain are yellow-brown, but produce complex conidiophores, phialides and conidia typical of *M. anisopliae*.

Latch (1965) reported on *Metarhizium* species infecting pasture-inhabiting grubs and beetles in New Zealand. He found that each individual host species is infected by a distinct strain differing from the strains affecting the other insect species. One isolate from *Costelytra zealandica* White (Coleoptera: Scarabaeidae) was dark green on the host but dark brown in culture. Based on this fact and on extensive conidial measurements of New Zealand strains, Latch concluded that all his isolates were *M. anisopliae*.

Pure white, sporulating specimens of *M. flavoviride* infecting the brown planthopper of rice have been collected in the Philippines. These isolates develop pigmentation only after prolonged incubation periods in culture (M. C. Rombach, unpubl.).

Kawakami & Naka (1979) isolated a pale-green variant of *M. anisopliae* from soil in Japanese mulberry fields. This strain produced typical *M. anisopliae* conidia.

Induced conidial colour variants. Changes in conidial colour can be artificially induced in isolates of *Metarhizium* species. As mentioned above, Latch (1965) observed a colour change from dark green on the host to dark brown in culture. Roberts (1967) produced brown-coloured mutants of *M. anisopliae* by radioactive irradiation of green strains; the mycelium of several of these irradiated isolates was yellow rather than white. Repeated

subculturing of these strains can induce further colour changes: after a year of growth in culture, one irradiated strain (ARSEF 1278), which originally produced drab brown conidia, spontaneously began to produce strikingly pink conidia and to excrete bright red droplets (M. C. Rombach, unpubl.).

Al-Aidroos (1980) and Messias & Azevedo (1980) produced various colour mutants by ultraviolet irradiation of green-spored strains. Al-Aidroos (1980) stated that the spore sizes of diploid phenotypes did not differ significantly from the wild-type haploid parents. Riba, de Azevedo, Messias, Dias da Silveira & Tuveson (1985) studied auxotrophic conidial colour mutants of *M. anisopliae* and showed that diploid strains are always green while (haploid) segregants from them are white or yellow.

Colour changes in *Metarhizium* can also be induced chemically. One green-spored strain of *M. anisopliae* produced brown conidia if grown in the presence of 100–1000 p.p.m. of the pesticide carbaryl. Similarly, an isolate of *M. flavoviride* var. *minus* produced unpigmented rather than grey-green conidia when grown in the presence of 100 p.p.m. carbaryl (M. C. Rombach, unpubl.). Cocoa pod extracts suppressed the formation of green conidial pigments and resulted in greyish-brown colonies of *M. anisopliae* var. *majus* (H. C. Evans, unpubl.).

TAXONOMIC CRITERIA FOR *METARHIZIUM*

Tulloch (1976) used only the shape, size and colour of conidia to delimit the species and varieties of *Metarhizium;* she seems to have placed particular emphasis on variability of conidial colour when placing *M. album* and *M. brunneum* in synonymy with *M. anisopliae;* a species whose conidial masses are usually green but may be any of a very diverse range of colours. The above discussion of colour variants together with Tulloch's (1976) comments on conidial pigmentation suggests that the colours of mycelium and conidia in *Metarhizium* species merit only secondary taxonomic value.

The arrangement of conidial chains has a profound effect on the gross appearance of a culture or specimen; the hymenium of *M. anisopliae* or *M. flavoviride* has a distinctly waxy appearance because of the densely packed adherent chains, whereas that of *M. album* is dusty or powdery because the chains have no such pronounced tendency to aggregate in columns.

Conidial size may be more important for separating varieties (Tulloch, 1976; Rombach, Humber & Roberts, 1986) than species.

The importance of host and geographical ranges is difficult to evaluate in view of the vast number and taxonomic diversity of recorded hosts for *M. anisopliae* from all corners of the globe. That *M. album* seems to be restricted to cicadellids (or related homopterans) in Asiatic rice paddies may be artifactual; a similar conclusion about the host and geographical ranges of *M. flavoviride* var. *minus* might have been made except for collection of this small-spored variety on a grasshopper from the Galápagos Islands (Rombach, Humber & Roberts, 1986).

Electrophoretic studies of isoenzyme systems in many strains of all recognized taxa of *Metarhizium* (B. May & D. W. Roberts, unpubl.) support the validity of all taxa accepted here (although *M. anisopliae* var. *majus* differs less from *M. a.* var. *anisopliae* than do some groups of strains currently assignable to that species and variety).

Primary taxonomic value in separating the species should be placed on the clavate or cylindric shape of the conidiogenous cells; on the oval or cylindric shape of the conidia; on whether the conidial chains adhere laterally into prismatic columns (or, eventually, into solid plates) or remain relatively separate; and on the presence or absence of a subhymenial zone of inflated hyphal bodies.

SYNOPTIC KEY TO THE SPECIES OF *METARHIZIUM*

See Korf (1972) for a detailed explanation of the use of this sort of key.

1. *M. album* Petch
2. *M. anisopliae* (Metschnikoff) Sorokin var. *anisopliae*
3. *M. anisopliae* (Metschnikoff) Sorokin var. *majus* (Johnston) Tulloch
4. *M. flavoviride* Gams & Rozsypal var. *flavoviride*
5. *M. flavoviride* Gams & Rozsypal var. *minus* Rombach, Humber & Roberts

CONIDIOGENOUS CELL

cylindrical to narrowly ellipsoid – *2, 3*
clavate to broadly ellipsoid – *1, 4, 5*

CONIDIA

Shape

cylindrical (often narrowed centrally) – *2, 3*
ovoid, ellipsoid – *1, 4, 5*

Conidial length (average; from host)

$\leqslant 7$ μm – *1, 2, 5*
7–9 μm – *2, 4*
9–11 μm – *4*
$\geqslant 11$ μm – *3*

Colour of conidial mass

grass-, dark- or olive-green – *2, 3*
grey-green – *2, 5* (pale)
yellow-green – *2, 4* (pale)
olive-buff – *4* (pale)
fawns to brown – *1* (pale), *2*
white – *1*

COLONY GROWTH CHARACTERISTICS

Subhymenium with inflated hyphal bodies
Present (at least during early sporulation) – *1*
Absent – *2, 3, 4, 5*

Conidial chains and texture of conidial mass
Chains adhere laterally in columns or plates; mass is smooth-surfaced – *2, 3, 4, 5*
Chains weakly adherent or non-adherent; mass with powdery surface – *1*

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CHAPTER 4

INSECT PATHOGENS AGAINST RICE PESTS

Insect pathogens, general. The value of insect pathogens in insect pest control has long been recognized (Heimpel 1972) - and attempts to control insects with pathogens started in the last century (Steinhaus 1956). Insect pathogens, particularly insect bacteria and -fungi are safe, can be propagated outside their natural hosts at low technology levels and on cheap, local, substratums. These microbial agents are ideal for third world use on tropical crops such as rice. In China (Hussey and Tinsley 1981) and in East European countries (Ferron 1978; Lappa and Goral 1986) significant progress was made in the use of insect pathogens, particularly *Bacillus thuringiensis*, insect viruses, and entomogenous fungi. However, the progress in microbial control in China and Eastern European countries can possibly be partly attributed to the absence of modern pesticide technology and less strict commercial requirements and standards - consistent superiority of the microbial products over pesticides has still to be proven in the field.

Fungi for BPH and MRB control. Plant- and leafhoppers, and pentatomid bugs such as the MRB are prone to fungus infection. Because they suck from plant vessels they escape pathogens which require oral uptake. Infection of these plant sucking insects by bacteria, viruses and protozoa are not common, and possibly of little significance. The apparent prevalence of entomogenous fungi among these insects triggered interest in possible use of fungi for biological control of BPH and MRB.

BPH, natural epizootics. In insect rearing cages fungi are prevalent at all BPH population densities and practically year round. However, in the field spectacular natural epizootics of entomogenous fungi invariably seem to occur in BPH populations when insect densities peak, at grain filling, about 6-4 weeks prior to rice harvest. These epizootics are often so extensive that no living BPH can be found between masses of sporulating cadavers sticking to rice stems and leaves. The factors governing the epizootic development in the field are not at all clear. Often different fungus species can be found causing epizootics in the same field and even on the same rice hill, in other fields only one particular fungus is present. Reasons for these peculiar distributions of fungal species are not known. However, insect density, insect stress, environmental factors such as temperature and humidity, and the presence of sufficient inoculum of the fungus disease are probably main factors governing epizootics. Also, the genetic make-up of insect populations can change at increasing insect densities - and thus the inherent susceptibility to fungus disease may increase. Unfortunately, natural fungus epizootics often occur when insect feeding and transmission of plant pathogenic viruses already caused damage.

MRB, natural epizootics. Natural fungus epizootics affecting high percentages of MRB populations were never observed. However, few fungus infected individuals can mostly be collected - over the years *M. anisopliae* was most prevalent on Palawan, and *P. lilacinus* in Malaysia.

Reasons for this difference in epizootology of fungi in BPH- and in MRB populations are not known.

Induced epizootics. This thesis reports on the induction of fungus epizootics in BPH populations (section 4.1); long term epizootics with various fungi were induced by single applications with several insect fungi. A second test shows that BPH populations can be significantly suppressed by *M. anisopliae* dry mycelium (section 4.2). It should be noted that *B. bassiana* became the agent of first choice after the production process was studied in more detail in the laboratory (Chapter 5).

Recent tests in Korea (Aguda et al. 1987), show that *B. bassiana* mycelium can also be used for BPH suppression.

Field tests on Palawan showed that MRB populations can be significantly suppressed by artificial dissemination of the fungi *B. bassiana*, *M. anisopliae*, and *P. lilacinus* (section 4.3).

Possible implications of this field research for microbial control of BPH and MRB are discussed (Chapter 6).

Section 4.1. Infection of the rice brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) by field application of entomogenous Hyphomycetes (Deuteromycotina).

Section 4.2 . Biological control of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) with dry mycelium applications of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes).

Section 4.3 . Entomopathogenic fungi (Deuteromycotina) in the control of the black bug of rice, *Scotinophara coarctata* (Hemiptera; Pentatomidae).

Section 4.1

Infection of Rice Brown Planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), by Field Application of Entomopathogenic Hyphomycetes (Deuteromycotina)

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ABSTRACT Five entomopathogenic Hyphomycetes were tested under field conditions for biological control of brown planthopper, *Nilaparvata lugens* (Stål), in rice. Suspensions of conidia of *Metarhizium anisopliae* (Metsch.) Sorokin, *M. flavoviride* Gams & Roszypal, *Beauveria bassiana* (Bals.) Vuill., and *Hirsutella citriformis* Speare were applied at a rate of $4–5.10^{12}$ conidia per ha. In addition, *M. anisopliae* and *Paecilomyces lilacinus* (Thom) Samson were applied as preparations of dry mycelium at a rate of 1.5–2 kg/ha. Mortality due to fungus infection ranged from 63 to 98% 3 weeks after application. There were no consistent differences between fungus species. The mycelium preparation sporulated on the plant and was as effective as the conidia suspension in infecting brown planthopper. Hyphomycetous fungi should be evaluated further for control of brown planthopper in rice.

KEY WORDS *Nilaparvata lugens*, rice, fungi, biological control, mycelium application

DURING RECENT decades, brown planthopper (BPH), *Nilaparvata lugens* (Stål), has risen from a sporadic occurrence to the status of a major pest of rice in many areas of tropical Asia. There are several reports of the effectiveness of natural biological control of BPH (e.g., Rao 1983, Greathead 1983, Chiu 1979). However, present control tactics focus mainly on the introduction of resistant rice varieties and on application of chemical pesticides (Heinrichs 1979). Unfortunately, in some areas new biotypes of BPH have developed that overcome resistance in the host plant (Pathak 1975). Also, serious resurgence of the pest resulting from treatments with various chemical pesticides continues to be a common phenomenon (Heinrichs & Mochida 1984, Kenmore et al. 1985). Therefore, to help ensure long-term control of BPH, more ecologically sound integrated pest management programs should be developed and implemented.

A complex of fungal pathogens, including various hyphomycetous and entomophthoralean fungi, has been identified from BPH populations (unpublished data). Previously published studies on the use of entomogenous fungi for BPH control are limited to experiments using small containers in the laboratory (Srivastava & Nayak 1975). In our study, tests with several hyphomycetous pathogens for the control of BPH were conducted in field plots. Treatments included applications of conidial suspensions of *Metarhizium anisopliae* (Metsch.) Sorokin, *M. flavoviride* Gams & Roszypal, *Beauveria bassiana* (Bals.) Vuill., and *Hirsutella citriformis* Speare. In addition, mass-produced preparations of dry mycelium of *M. anisopliae* and *Paecilomyces lilacinus* (Thom) Samson were tested.

Materials and Methods

Field Plots. A variety of a Korean (BPH-susceptible) glutinous rice was transplanted to a field near Victoria (Laguna Province, Luzon, Philippines). The experiment was carried out during the rainy season with medium to heavy rains mostly in the afternoon. About 3 weeks before the start of the experiment, the pyrethroid insecticide Cypermethrin was applied at half the recommended rate to eliminate natural enemies and induce resurgence of the pest (Heinrichs & Mochida 1984). The field was divided into 25 plots, each 4 m^2 and separated by 2-m intervals. A few days after insecticide application, potted rice plants infested with large numbers of BPH were introduced at the center of each plot; this ensured development of BPH populations. BPH from the potted plants were allowed to develop to the next generation; mixed BPH populations of different instars and adults were present at the start of the experiment. Each of seven fungal treatments was applied in three plots; four control plots were treated with water. Treatments were arranged in a randomized complete block design. A gauze cage enclosing 12

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Table 1. Origin of fungal isolates

ARSEF	Fungus species	Host	Country
1548	*M. anisopliae*	*Scotinophara coarctata* (F.)	Philippines
714	*B. bassiana*	*N. lugens*	China
1547	*M. flavoviride*	*N. lugens*	Philippines
1550	*H. citriformis*	*N. lugens*	Philippines
252	*B. bassiana*	*Leptinotarsa decemlineata* (Say)	United States
1552	*P. lilacinus*	*Scotinophara coarctata*	Malaysia

ARSEF numbers refer to the Collection of Entomopathogenic Fungi, USDA-ARS Plant Protection Unit, Boyce Thompson Institute for Plant Research at Cornell, Ithaca, New York.

hills of rice (ca. 1 m^2) was erected in the center of each plot after application of the pathogens.

Preparation of Fungal Materials. *M. anisopliae* (Ma), *M. flavoviride* (Mf), two isolates of *B. bassiana* (Bb and Bb252), and *H. citriformis* (Hc) were applied as suspensions of conidia. *M. anisopliae* (Mam) and *P. lilacinus* (Plm) were also applied as dry mycelium. Origins of the fungal isolates are given in Table 1.

Conidia of the fungi were produced on standard mycological media: Ma and Mf on Emerson's YpSs agar and Bb and Hc on Sabouraud dextrose agar enriched with 1% yeast extract. Conidia were washed from the surface of the plates by 75–100 ml of a solution of 0.02% Tween80. Concentrations of the conidia were determined with standard hemocytometer techniques. Over 98% of the conidia germinated in Sabouraud dextrose broth after 24 h incubation on a rotary shaker in viability tests just before field application.

Conidia of *H. citriformis* are difficult to produce due to low sporulation rates, slime production of the mycelium, and irregular growth patterns. Therefore, a mixture of conidia and mycelium particles of *H. citriformis* was applied in this experiment.

Conidia of strain Bb252 were produced on wheat bran by a small mass-production unit using a diphasic fermentation process. The material used in these experiments was produced in 1982 and stored at 4°C for >3 years. Over 95% of the conidia germinated in viability tests in a liquid medium.

Mycelium (Mam and Plm) was produced in 12-l bubbler-type fermentors in a molasses (1.5%) and yeast extract (1.5%) broth. The mycelium was dried, milled, and stored at −20°C for >1 month. This production method is similar to the marcescent process described in detail by McCabe & Soper (1985) for entomophthoralean fungi. Before application, samples of the mycelium were rehydrated and incubated on moist filter paper in a petri dish for 4 days; >95% of the mycelial particles sporulated.

Application of Fungal Materials. Before application in the field, the conidial suspensions were diluted to the appropriate concentrations with water. Two liters of conidial suspension (treatments Mf, Ma, Bb, Bb252) were applied to each plot by knapsack sprayer at a rate equivalent to $4–5.10^{12}$ conidia per ha. A similar rate of combined conidia and mycelium particles of Hc was applied. The dry mycelium preparations were soaked in water for several hours before application. The moist cake was crumbled by hand onto the plants in the center of the hills within each plot at a rate of 0.5–1 g per plot. Because of the inefficient application method, this rate was estimated to be equivalent to ca. 1.5–2.0 kg of dry mycelium per ha.

Table 2. Range of the percentage mortality due to fungus infection at 7, 14, and 21 days after treatment

	Days after treatment		
	7	14	21
Inside cages			
M. flavoviride (Mf; conidia)	0.3–1.4	7.2–34.5	60.3–100
M. anisopliae (Ma; conidia)	0.2–5.5	12.6–60.4	91.1–100
H. citriformis (Hc; conidia)	0.0–3.2	15.9–27.5	46.9–94.1
B. bassiana (BbE; conidia)	0.0–0.4	9.3–24.2	50.9–75.0
B. bassiana (Bb252; conidia)	0.2–0.4	13.1–58.2	65.0–95.2
P. lilacinus (Plm; mycelium)	2.5–6.8	19.1–24.7	31.3–100
M. anisopliae (Mam; mycelium)	0.0–36.4	22.5–82.5	82.0–100
Control	0.0–0.3	0.0–0.7	0.0–0.3
Outside cages			
M. flavoviride (Mf; conidia)	0.5–1.5	25.4–43.4	93.3–100
M. anisopliae (Ma; conidia)	2.0–4.1	23.6–65.8	88.9–100
H. citriformis (Hc; conidia)	0.0–5.1	11.1–34.4	80.0–100
B. bassiana (Bb; conidia)	0.0–0.0	21.6–32.1	90.0–100
B. bassiana (Bb252; conidia)	0.0–0.6	10.1–66.6	85.2–93.5
P. lilacinus (Plm; mycelium)	0.0–1.8	16.7–30.3	72.7–100
M. anisopliae (Mam; mycelium)	0.7–1.3	35.5–42.3	90.3–100
Control	0.0–0.0	0.0–16.3	0.0–8.3

Evaluation of Effects. Counts of live and infected BPH were made from 12 hills within each cage and from 12 hills outside each cage from each plot. Counts were taken 1 day before the application of the pathogens and at 7, 14, and 21 days thereafter. The last count was done a few days before harvest of the rice. Infections of BPH by entomogenous Hyphomycetes are typical and can be recognized easily in the field; the fungi affix the dead insects to the plant. Field samples, which were regularly brought back to the laboratory, confirmed these identifications. At week 0 there were differences in starting numbers of BPH be-

Table 3. Results of ANOVA on mean percentages mortality (arcsine transformed) data from three fungus-treated replicates

	7 days			14 days			21 days		
	SS	df	*P*	SS	df	*P*	SS	df	*P*
Inside cages									
Between treatments	400.13	6	0.47	863.60	6	0.45	2,058.24	6	0.58
Within treatments	952.88	14		1,965.26	14		5,923.87	14	
Total	1,353.01			2,828.86			7,982.11		
Outside cages									
Between treatments	257.45	6	0.04	367.67	6	0.70	1,269.16	6	0.61
Within treatments	195.54	14		1,352.97	14		3,876.53	14	
Total	452.99			1,720.63			5,145.68		

tween the replicates. For this reason, comparison of numbers of live insects in the different treatments was not possible. Comparison of infection levels avoided these differences in insect densities because percentages are compared.

Differences in mortality levels between the treatments were analyzed on a weekly basis. Analysis of variance (ANOVA) was applied to the mean percentage mortality (arcsine transformed) data from the three fungus treatments and four control replicate cages. Significance was accepted at $P = 0.05$.

Results and Discussion

The ranges of the percentage mortality due to fungus infection are given in Table 2, the results of the ANOVA in Table 3. Outside the cages, significant differences between fungal treatments and the control were present 7 days after treatment; however, general infection rates were low, and these differences are not of practical value. Inside the cages, no differences were observed at that time. Fourteen and 21 days after treatment, the infection rates of BPH inside and outside the cages were obviously higher in all fungus-treated plots compared with the control.

There were no consistent differences in infection rates between the fungal treatments at 14 or 21 days after treatment. This included the treatments with fungi isolated from hosts other than BPH, Mam, Plm, and Bb252, and treatments with dry mycelium, Mam and Plm. By 21 days, 100% mortality had occurred in many replicates. There was a slightly larger variation between the fungal treatments inside compared with outside the cages. The reason for this is not known. A low (<10%) mortality of BPH in the control plots was caused by *M. flavoviride* from a natural inoculum.

Cameron (1969) proposed application of mycelial particles as field inoculum. Using this method, the fungus *H. thompsonii* Fisher was successfully applied in the field by McCoy et al. (1971, 1975) against the citrus rust mite, *Phyllocoptruta oleivora* (Ashmead). In general, mycelia of entomogenous fungi do not infect the host, but under suitable conditions of temperature and relative humidity, infective conidia are produced by the mycelium in the field. This use of mycelium greatly simplifies the mass-production process, because the solid phase (the production of conidia by mycelium on a solid substrate) is eliminated. Therefore, the present results might provide a basis for future mass production and application. In our experiments, sporulating mycelium fragments were observed sticking to the rice plants over periods <1 week after application. The effect of this continuous supply of infective conidia on the development of mycosis in the BPH populations should be further investigated.

The dosage equivalent to $4–5.10^{12}$ conidia per ha applied in the treatments Ma, Mf, and Bb was sufficient to cause infection. It may be that lower dosages than used in this experiment are also effective. The rate of 1.5–2.0 kg of dry mycelium was probably an overdose. Significant control levels can be achieved using rates as low as 700 g/ha (unpublished data). With better formulations and more efficient application methods, much lower effective dosages are expected.

It is not certain whether all fungi and preparations will be similarly effective in controlling the BPH population under other conditions of relative humidity, temperature, insect density, and insect stress since these factors are known to be important in infection and subsequent development of epidemics in insect populations (see various authors in Burges [1981]). The possible role of these organisms in the biological control of BPH should be further investigated.

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BIOLOGICAL CONTROL OF THE BROWN PLANTHOPPER, *NILAPARVATA LUGENS* (HOMOPTERA; DELPHACIDAE) WITH DRY MYCELIUM APPLICATIONS OF *METARHIZIUM ANISOPLIAE* (DEUTEROMYCOTINA; HYPHOMYCETES).[1]

Michiel C. Rombach[2], Remedios M. Aguda[3] and Donald W. Roberts[2]

ABSTRACT

Different rates of dry mycelium and a suspension of conidia of the entomogenous fungus *Metarhizium anisopliae* (Metsch.) Sorokin were evaluated for the suppression of populations of the brown planthopper, *Nilaparvata lugens* Stal on rice. The mycelium was applied at rates equivalent to 700, 3500, and 7000 g/ha; the conidia at a rate equivalent to 2.5×10^{12} conidia/ha.

Significant control of the populations was achieved at two weeks after application and up to harvest of the rice crop; differences between treatments were present but not consistent. Dry mycelium preparations of *M. anisopliae* should be further evaluated for use in control of the brown planthopper.

INTRODUCTION

The brown planthopper (BPH, *Nilaparvata lugens* Stal) has risen to the status of a major pest or rice in some areas of tropical Asia (Anonymous 1979). The status of BPH as a rice pest and the importance of entomogenous fungi as natural enemies of BPH have been discussed elsewhere (Rombach and Shepard 1987) a bibliography on pathogens of rice pests, including BPH, is provided by Rombach, Rombach and Roberts (1987). Entomogenous fungi are promising control agents because they can be relatively easily produced — and BPH can be infected in the field by application of conidia and by dry, viable mycelium of several entomogenous Hyphomycetes (Rombach *et al.* 1986a). This method of pest control has a large potential for developing countries because mycelium can be produced relatively simple in large volumes.

Conidia suspensions at a rate equivalent to $4\text{-}5 \times 10^{12}$ conidia per ha and preparations of dry mycelium of the green muscardine fungus, *Metarhizium anisopliae* (1.5-2 kg/ha), were tested in previous studies (Rombach *et al.*

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1986a). In this paper, the effects of one conidial suspension and 3 different rates of a preparation of dry mycelium were compared on BPH field populations.

MATERIALS AND METHODS

Location

A BPH-susceptible glutinous rice variety was transplanted in the experimental field at Victoria, Laguna. To ensure development of BPH populations, large numbers of BPH adults and nymphs were introduced at several locations in the field on potted rice plants from the greenhouses of the International Rice Research Institute. The second generation of the introduced BPH population was used in the experiment. Each plot was 4 square meters interspaced by 2 m. A gauze cage enclosing 12 hills of rice was erected in the center of each plot prior to the application of the pathogens. Each of four treatments was performed in 3 cages along with 3 control treaments treated with water only. The 15 plots were arranged following a complete randomized design.

Preparation of the fungal materials

The isolate of *M. anisopliae* (ARSEF 1548, Collection of Entomopathogenic Fungi, USDA-ARS Protection Unit, Boyce Thompson Institute for Plant Research, Ithaca, NY, U.S.A.) originated from the black bug (*Scotinophara coarctata* F.) collected from rice in Palawan. The isolate was preserved in liquid nitrogen; for 4 months prior to the experiment, the fungus was maintained by regular transfer on Emerson's YpSs agar, followed by about 10 transfers in shaker cultures in Sabouraud dextrose broth.

Conidia of the fungus were produced and harvested as described previously (Rombach *et al.* 1986a). Dry mycelium was produced in 12 liters "bubbler"-type airlift fermentors in a molasses (1.5%)/yeast extract (1.5%) broth similar to the marcescent method as described by McCabe and Soper (1985) for entomophthorelean fungi and briefly discussed by Rombach *et al.* (1986a; 1986b). The mycelium was stabilized with a maltose solution, dried, and stored in a formulation of starch (15% of the mycelium dry weight) for several weeks at -20^{0} C in a freezer. A few hours before application in the field, samples for the different treatments were weighed and soaked in a small amount of water. In viability tests 70% of this formulated mycelium showed growth and sporulation after 4 days of incubation under moist conditions in petri dishes. The viability and sporulation of this particular batch of mycelium was low compared to batches used in previous experiments (Rombach *et al.* 1986a, 1986b). In our experience with the marcescent production, sporulation of mycelium of *M. anisopliae* (isolate ARSEF 1548) decreases after prolonged maintenance on artificial media, in particular in liquid shaker cultures — therefore, viability tests should be carried out before using dry mycelium so application rates can be corrected accordingly.

The conidia were applied at a rate equivalent to $2.5x10^{12}$ conidia per hectare; this treatment will be further referred to as Mc. The mycelium was applied at a rate of 0.5 (Ma1), 2.5 (Ma2) and 5 (Ma3) g mycelium preparation per plot of 4 sq. m. These rates are equivalent to 700, 3500 and 7000 g dry, viable and sporulating mycelium per hectare, respectively.

Application of the fungal materials

Five hundred ml was applied per plot by ultra low volume spinning disc applicator. The nozzle of the applicator was adapted for the relatively large swollen mycelium particles (up to about 0.4 mm diameter) of the soaked mycelium.

Evaluation

Counts of live and infected BPH were made from 12 hills within each cage and from 20 hills outside each cage. Counts were taken 1 day before application and 1, 2, 3, 4 and 5 weeks thereafter; the last count was one day before harvest of the crop. At each evaluation, the live and infected insects were counted. Due to heavy rainfall and winds, fungus-infected insects were difficult to recover from the 3rd week onwards. Therefore, total numbers of insects per replicate were analyzed instead of mortality rates . The numbers of insects in the treatments were subjected to analysis of variance (ANOVA) for each week separately; significance was accepted at the P=0.10 level.

High numbers of predators of BPH were present inside and outside the cages during the experiment. Outside the cages these predators, particularly lycosid spiders, had reduced BPH populations to about less than 1 insect per 5 hills after 3 weeks. Therefore, these data were not further analyzed. Inside the cages BPH numbers were also reduced, however not as drastically as outside the cages; the reason for this difference is not known. One of the M1 cages showed, in contrast with all other cages, unusual large numbers of 1st instar nymphs at the 3 week interval. This single replicate was removed from analysis from the 3rd week onwards.

RESULTS

The mean numbers of insects per treatment and standard deviations are given in Fig. 1. At the time of application and after the 1st week, there were no significant differences between the treatments and the control. At week 2, 3, 4 and 5, there was a significant difference between the conidia treatment (Mc) and the control. All the mycelium treatments (M1, M2, and M3) differed significantly from the control at 2, 3 4 and 5 weeks, except for M1 at 2 and 3 weeks and M3 at 4 weeks. M1 had significantly higher numbers of insects than Mc and M2 in the 3rd week. Similarly, higher numbers of insects were present in M1 compared to Mc in the 4th week.

Large variation in insect numbers in the control were observed (see

standard deviation bars, Fig. 1). This variation is probably due to differential settling of BPH from the potted plants, creating an irregular distribution.

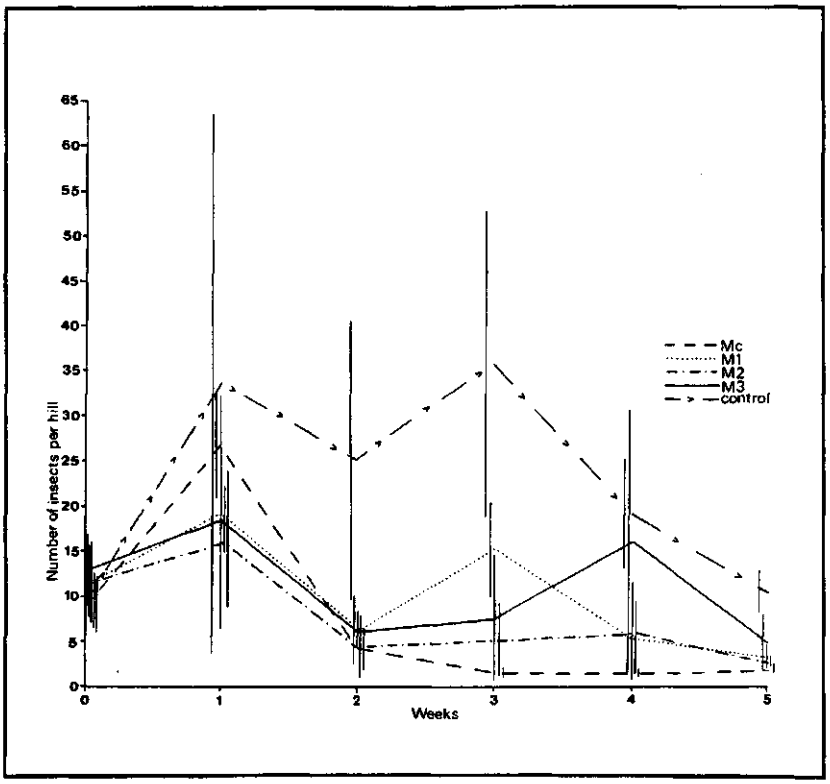

Fig. 1. Numbers of BPH per hill of rice in the different treatments (Mc=*Metarhizium anisopliae*, conidia suspension; M1=dry mycelium, 700 g/ha; M2= 3500 g/ha; M3=7000 g/ha). Bars represent standard deviations.

DISCUSSION

The dosage equivalent to 2.5×10^{12} conidia per hectare (Mc) was sufficient to infect the BPH population and to suppress the insect numbers up to harvest of the crop. This rate of conidia is well within the range of 10^{12}-10^{13} of dosages generally applied in the control of insect populations in the field with fungi. These field tests include the use of *M. anisopliae* for biological control of spittlebug *Deios (Mahanarva) postica* (Wlk.) on sugarcane in Brazil (Ferron 1980) and tests with other fungi to control various insect species (Ferron 1981; Farques, Cugier and Weghe 1980; Ramakers and Sam-

son 1984). This rate of conidia is about half the rate applied in a previous experiment with BPH (Rombach *et al.* 1986a). About similar rates of infection of BPH are achieved; however, the high infection levels seemed to be achieved faster with the higher dosage. In the previous experiment mortality rates of 40% were present at two weeks after application compared to estimated infection rates of about 15-20% in this experiment (Fig. 1.: treatment Mc, 2nd week). This observation agrees with experiments with *Verticillium lecanii* (Viegas) infecting aphids under greenhouse conditions (Hall 1980). In these experiments higher concentrations of the fungus also tended to reach optimum control levels faster compared to lower concentrations. However, comparison of results of these different field experiments is difficult because environmental conditions change. Lower dosages of conidia, although not tested in this study, may infect equally high percentages of the BPH populations.

The use of mycelium of entomogenous fungi as field inoculum was already proposed by Cameron (1969); however, the idea of using dry mycelium is relatively new. In general, the mycelium of entomogenous Hyphomycetes does not infect the insect directly, but under suitable conditions of temperature and relative humidity infective conidia are produced on the surface of the mycelium particles. In our field experiments, small sporulating clumps of mycelium were observed sticking to the rice plants for a period of one week after the application. Apparently sterile clumps show a fertile rim near the plant surface when examined under the microscope.

The numbers of insects in the different treatments with the fragmented mycelium showed a similar course over the 5 week period (Fig. 1). Differences between the treatments with mycelium were present; however, these differences are inconsistent over time and no conclusions on dosage response of mortality can be drawn. The phenomenon of a slow dosage response of insects to fungal pathogens has often been shown for different insect-fungus relationships in laboratory bioassay studies (see various authors in: Burges and Hussey 1971; Burges 1981). Experiments in which different rates of fungi were applied for the control of field populations of insect pests are rare. Our present results agree with those of Hall (1980) and Hall and Burges (1979). In their experiments a 100- and even 1000-fold increase in the dosage of conidia of *Verticillium lecanii* (Viegas) did not significantly increase mortality levels in aphid populations in greenhouse experiments. Furthermore, Ignoffo *et al.* (1978) reported no differences in reduction of populations of *Heliothis zea* (Boddie), even at 100-fold different rates of conidia of *Nomuraea rileyi* (Farlow) Samson. Also, a three-fold increase in rate of the mycoacaricide Mycar® against mites on citrus in Florida did not result in significantly lower mite infestation (McCoy and Couch 1982). Lower dosages of dry mycelium, which might be equally effective in suppressing the BPH population, were not tested in this experiment.

This experiment demonstrated that suspensions of conidia (at a rate equivalent to 2.5×10^{12} conidia/ha) and of dry mycelium (rate equivalent to 700, 3500 and 7000 g/ha) of *M. anisopliae* can significantly suppress

populations of BPH from two weeks after application onwards. Whether biological control of BPH with dry mycelium produced by the marcescent method is economically feasible has to be further investigated; the necessary data on production, formulation and application costs are not available yet.

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Entomopathogenic Fungi (Deuteromycotina) in the Control of the Black Bug of Rice, *Scotinophara coarctata* (Hemiptera; Pentatomidae)

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The effects of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces lilacinus* on populations of the black bug (*Scotinophara coarctata*) were studied. Adult black bugs were kept on rice plants in screen cages in the field. Cages were placed in four different plots in two different rice fields. Two different fungal materials were tested, suspensions of conidia and mass-produced dry mycelium. The numbers of bugs were significantly reduced in all fungal treatments compared to the control over a period up to 9 weeks, except in one of the plots where severe drought hampered control. In addition, numbers of nymphs were suppressed by the fungi in the well-irrigated plots. The overall performance of the different species of fungi and different materials was similar. Implementation of dry mycelium preparations of the fungi in integrated control programs for the black bug should be considered.

KEY WORDS: *Scotinophara coarctata;* Pentatomidae; rice; entomogenous fungi; control; *Metarhizium anisopliae; Beauveria bassiana; Paecilomyces lilacinus;* mycelium.

INTRODUCTION

Stinkbugs of the genus *Scotinophara* are common pests of rice in several Asian countries (Miyamoto et al., 1983). Of the six *Scotinophara* spp. present on rice in the Philippines only *S. coarctata* (F.) was reported as a serious pest (Barrion et al., 1982). At present, damage by *S. coarctata* is serious on Palawan Island, the Philippines.

Effective insecticides have been identified for the chemical control of *S. coarctata* (Rao, 1977). However, on Palawan Island insecticides are prohibitively expensive for most rice farmers. In addition, various chemical insecticides cause serious resurgence of the brown planthopper, *Nilaparvata lugens* (Stål) (Heinrichs and Mochida, 1984). Indeed, outbreaks of the brown planthopper have been observed following applications of broad-spectrum pyrethroid insecticides against the black bug (M. C. Rombach and B. M. Shepard, unpubl. observ., 1984).

Tolerance toward damage by the black bug was detected in certain breeding lines of rice (Heinrichs et al., 1986). Still, there is an urgent need for selective control agents to be utilized in integrated pest management programs.

Hymenopteran parasites have been reported from eggs of black bugs including *S. coarctata* (Grist and Lever, 1969). In contrast to the egg stage, the nymphal and adult stages seem to be relatively free of predators and parasites. However, nymphs and adults of *Scotinophara* sp. are host to several deuteromycetous fungal species (Morimoto, 1957; Rombach et al., 1986a). *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Bals.) Vuill. were recently collected infecting *S. coarctata* on the island of Palawan. *S. coarctata* adults infected by *Paecilomyces lilacinus* (Thom.) Samson were collected in Malaysia.

Biological control of the black bug using natural fungal enemies might prove to be

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useful in the future. In contrast to other natural enemies, insect pathogenic fungi can be mass produced relatively easily on cheap media; the newly developed method of producing dry mycelium (McCabe and Soper, 1985) which sporulates in the field might further facilitate production of field inoculum. Therefore, the entomopathogenic fungi *Metarhizium anisopliae, Beauveria bassiana,* and *Paecilomyces lilacinus* were tested in the field for biological control of *S. coarctata.*

MATERIAL AND METHODS

Field plots. Two experimental areas (each about 1 ha) were used. One was situated near the Palawan National Agricultural College (PNAC) at Arborlan, Palawan. The second experimental field of a similar size was located several miles south of PNAC in the Mabini area (MAB). The PNAC field was more exposed and drier than the MAB field. In the course of the experiment the PNAC field suffered from inadequate moisture because of a lack of proper irrigation.

Half of each field was transplanted to the black bug-tolerant breeding line IR-10781-3-2-2 (plots PNAC-T and MAB-T), the other half to IR-20, a black bug-susceptible variety (plots PNAC-S and MAB-S).

Eighteen nylon screen cages (1 m^2 by 1.5 m high, 0.5 mm mesh) were placed on each plot. Each cage covered 12 hills of rice. A hill was composed of about three to five plants (approximately 20 tillers). Forty days after transplanting, 180 adult black bugs were introduced into each cage. Each of the five fungal treatments were applied in three cages along with three controls treated with water. The 18 treatments were arranged following a randomized complete block design.

Production of fungal materials. Two different fungal materials were used in the experiment, suspensions of conidia and mass-produced dry mycelium. The fungi applied as suspensions of conidia were *M. anisopliae* (Ma), *B. bassiana* (Bb) and *P. lilacinus* (Pl); fungi applied as mass-produced dry mycelium were *M. anisopliae* (Mam) and *P. lilacinus* (Plm).

Ma and Pl are recent isolates from the black bug (deposited under ARSEF 1548 and ARSEF 1552 in the Collection of Entomopathogenic Fungi, USDA–ARS Plant Protection Research Unit, Ithaca, New York, formerly the USDA–ARS Insect Pathology Research Unit). The strain Bb (ARSEF 714) was isolated from the brown planthopper from China.

Conidia of Ma and Pl were produced in Petri dishes on Emerson's YpSs agar; conidia of Bb were produced on Sabouraud dextrose agar enriched with 1% yeast extract. Conidia were washed from the surface of the colonies with a 0.025% Tween-80 solution. Concentrations of the different suspensions were determined by standard hemocytometer methods. In viability tests in Sabouraud dextrose broth, over 95% of the conidia germinated within 20 h.

Suspensions were diluted to 5×10^5 conidia/ml; 500 ml of this suspension was applied per cage. This rate is equivalent to 2.5×10^{12} conidia/ha.

Mycelium of *M. anisopliae* (Mam) and *P. lilacinus* (Plm) was produced by a process similar to the "marcescent process" described in detail by McCabe and Soper (1985). Briefly, mycelium was grown in 12-liter "bubbler"-type airlift fermentors in a molasses (1.5%) and yeast extract (1.5%) broth. Mycelium, harvested from the bubblers, was stabilized with a maltose (10%) solution and dried on racks in a sterile hood. The dried mycelium mats were ground in a hand-operated coffee grinder to small particles (within the range 0.1–0.3 mm). These particles were stored at −20°C for several weeks prior to the field application. In viability tests over 95% of the particles produced conidia after several days of incubation on moist filter paper. The dried material was applied at 0.15–0.2 g per cage, equivalent to 1.5–2 kg/ha.

Application and evaluation. Two liters of suspension of conidia (Ma, Bb, and Pl) and small particles (<0.1 mm) of Mam and Plm were applied with a conventional knapsack sprayer. The dry mycelium was soaked in water for several hours before application. Large swollen mycelium particles (up to 0.5 mm) of Mam and Plm were distributed by hand onto the centers of the hills.

Counts of live and fungus-infected black bug adults were made at 2, 5, 7, and 9 weeks after treatment. Nymphs appeared in noticeable numbers after 5 weeks at the PNAC and MAB sites. Therefore, counts of nymphs were recorded at 7 and 9 weeks. However, high nymphal mortality occurred at the PNAC site because of drought. No fungal infection was observed in these nymphs and consequently these data for the PNAC site were omitted from analysis.

During the experiment it appeared that a large number of the infected insects fell off the plants and disappeared in the mud. For this reason only data on live adults at both sites and nymphs at the MAB site were analyzed.

Numbers of adults at all intervals were analyzed by analysis of variance (ANOVA); significance was accepted at the $P = 0.05$ level. The numbers of live nymphs were analyzed by ANOVA for both plots at MAB at the 9-week interval.

RESULTS

Mean numbers per treatment of adult black bugs in the four plots are depicted in Fig. 1. The numbers of nymphs for plots MAB-R and MAB-S are given in Fig. 2.

Differences between the control and the fungus treatments were significant at all intervals in all plots, except for the 2- and 9-week intervals in plot PNAC-T; at the 9-week interval in the plot MAB-S treatment Bb did not differ from the control.

At the 9-week interval, there were no significant differences among the fungal treatments in the plot MAB-T. In plot PNAC-T treatment, Mam had significantly higher numbers compared to Pl and Ma at this interval. In plots MAB-S and PNAC-S, differences among the fungal treatments were found at the 9-week interval. In plot PNAC-S, the Pl, Ma, and Mam treatments had significantly lower numbers of adults compared to Bb. Nevertheless, numbers in the latter treatment were still significantly lower than the control. In the MAB-S plot Plm and Ma had significantly lower insect numbers compared to Mam. However, Mam still differed from the control.

There were no significant differences in numbers of adult black bugs from the plots transplanted to susceptible and those with the tolerant rice variety.

At the 9-week interval significantly high numbers of nymphs were present in the untreated cages compared to cages treated with fungi at the MAB-S and MAB-T sites, except for treatment Bb in MAB-S (Fig. 2). In MAB-S treatment Mam had higher numbers of nymphs compared to Ma.

DISCUSSION

Morimoto (1957) reported on the microbial control of *Scotinophara lurida* (Burmeister) by *M. anisopliae* and *P. lilacinus* in Japan. In these experiments conidia of both fungi, applied as either a dust or a spray, caused significant mortalities in the populations of *S. lurida*. Applications with conidia of *M. anisopliae* were most effective, initiating epizootics which caused 40–100% mortality of the insects for periods exceeding 2 months (Morimoto, 1957). Although in our present studies the numbers of adult and nymphal bugs were significantly suppressed, epizootics infecting total populations did not develop in any of the cages. Estimated infection levels were 15–80% (as derived from numbers of live adult black bugs, Fig. 1). These results agree with previous observations which showed that, even under conditions of high insect density, only low (5–10%) natural infection levels with *M. anisopliae* occur. Infections with *B. bassiana* were rare in

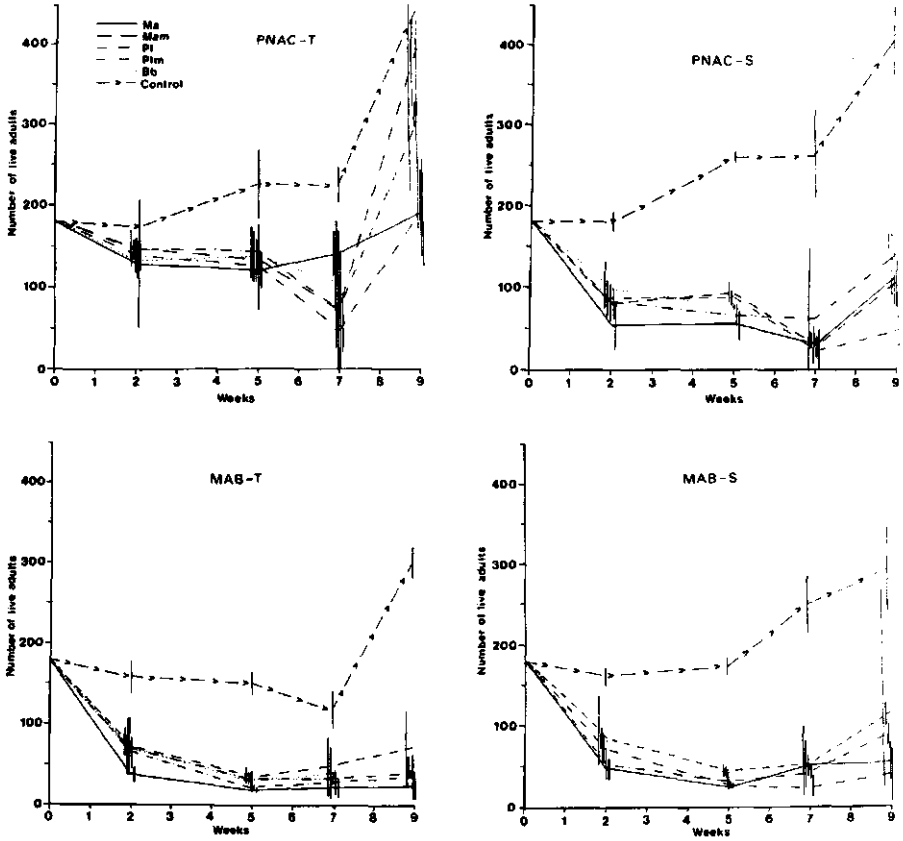

FIG. 1. Mean numbers of adult black bugs for different fungal treatments over a period of 9 weeks in four plots. Bars represent standard deviation. Bb = *B. bassiana;* Ma = *M. anisopliae;* Pl = *P. lilacinus;* Mam = *M. anisopliae*, mycelium; Plm = *P. lilacinus*, mycelium.

natural black bug populations and *P. lilacinus* was never collected infecting the black bug on Palawan.

Morimoto (1957) claimed that, in general, *P. lilacinus* was significantly less virulent toward the black bug than was *M. anisopliae*. In our present experiments no significant differences in the performance of Pl and Ma could be detected; however, bioassay studies under more carefully controlled conditions should be carried out to detect such differences.

Whether the lower numbers of nymphs in the treatments resulted from fungal infection or from decreased oviposition due to infection in the adult population can not be concluded from these present studies. Molting of nymphal black bugs might protect them from fungal infection, just as it is an effective defense mechanism for larvae of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) for infection with *B. bassiana* (Vey and Farques, 1977). However, because high numbers of infected

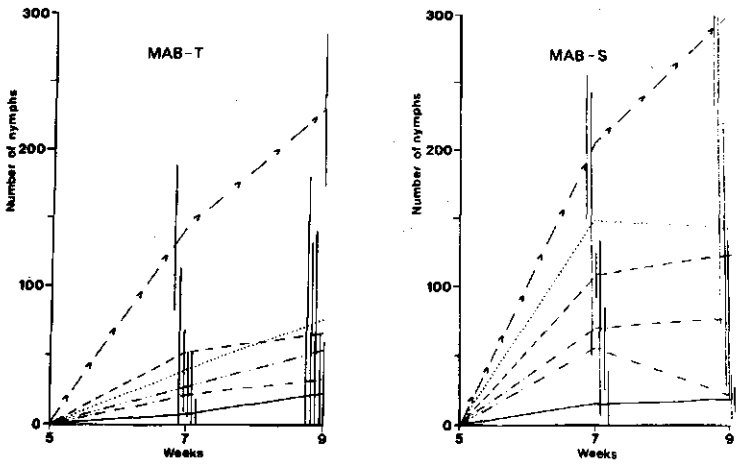

FIG. 2. Mean numbers of black bug nymphs in the different fungal treatments and the control. Bars represent standard deviation. For abbreviations, see Fig. 1.

nymphs were observed in the fungus-treated cages there is little doubt that low populations of nymphs were, at least partly, due to fungal infection. In this respect, the susceptibility of nymphs and adults of the black bug toward fungal infection should be further studied to optimize control efforts.

The dosage equivalent to 2.5×10^{12} conidia/ha as applied in the treatments Ma, Pl, and Bb was sufficient to significantly suppress the adult black bug populations. Rates of conidia within the range 10^{12}–10^{13} conidia/ha have been widely applied in tests with entomogenous fungi. This includes the use of *B. bassiana* against *L. decemlineata* (Farques et al., 1980) and the use of *M. anisopliae* for the biological control of spittlebugs on sugarcane in Brazil (Ferron, 1981). Yet, it may be that rates lower than those applied in this experiment are also effective in suppressing the black bug populations.

Besides conidia, particles of mycelium can also be used as field inoculum. In general, mycelium of entomopathogenic fungi does not directly infect the insect. However, under suitable conditions of temperature and relative humidity, infective conidia are produced on the surface of the mycelium clump. This method greatly simplifies mass production because the "solid production phase" (production of conidia on a solid substrate) is eliminated. In this respect, McCoy et al. (1971, 1975) reported on the mass production, formulation, and application of moist mycelium of *Hirsutella thompsonii* Fisher against the citrus rust mite *Phyllocoptruta oleivora* Ashm. However, losses in viability of the moist mycelium were found during storage (McCoy et al., 1975). Using the recently introduced production technique of the marcescent process (McCabe and Soper, 1985) problems related to losses of viability of the mycelium during storage are virtually eliminated. Mycelial material of *M. anisopliae* was applied in previous field experiments on the biological control of the brown planthopper. In this experiment sporulating mycelium fragments sticking to the rice plants were observed over periods up to 1 week after the application (Rombach et al., 1986b). Little is known about the formula-

tion and application of the dry mycelium preparations; therefore, lower rates of dry mycelium, formulated and applied more efficiently, may also be effective in suppressing the black bug population.

In this experiment, fungal treatments differed at several intervals in different plots; however, these differences were not consistent and no conclusions about the performances of the different materials can be drawn.

Further field experimentation and bioassay tests are necessary to substantiate any conclusions on the relative effectiveness of the different species of fungi. Also, more detailed experiments should be undertaken to elucidate the influence of the important environmental factor of irrigation levels on the infection of black bug populations.

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CHAPTER 5

MASS PRODUCTION OF ENTOMOGENOUS FUNGI

A novel method of fungus production was recently introduced (McCabe and Soper 1985) for the production of entomophthoralean fungi. With this "marcescent process" dry mycelium is produced by submerged fermentation, the mycelium harvested, dried, and milled. The product can be stored for some time and applied in the field with conventional spray equipment - the mycelium particles sporulate on the plant in the field, and these conidia subsequently infect the insects.

We modified the marcescent production techniques for use with the muscardine fungi *Beauveria bassiana* and *Metarhizium anisopliae*, and with *Paecilomyces lilacinus*. The mycelium products of these fungi can be used against rice pests (see Chapter 4, this thesis).

Optimalization of mycelium growth and conidia production is important for efficient fungus production. In the next section experiments on the conidia production by *B. bassiana* mycelium grown in different liquid media are reported.

Section 5.1. Production of *Beauveria bassiana* (Deuteromycotina; Hyphomycetes) mycelium in different liquid media and subsequent sporulation of dry mycelium.

Section 5.1

PRODUCTION OF *BEAUVERIA BASSIANA* (DEUTEROMYCOTINA; HYPHOMYCETES) IN DIFFERENT LIQUID MEDIA AND SUBSEQUENT CONIDIATION OF DRY MYCELIUM

INTRODUCTION

Various species of entomogenous fungi are presently being tested for use against important agricultural and medical insect pests (Burges & Hussey, 1971; Burges, 1981; Hoy & Herzog, 1985). For large scale field experimentation and commercialization of the fungi development of efficient mass production techniques is necessary.

Several techniques for the mass production of entomo- pathogenic Hyphomycetes are available, mostly designed to yield infective conidia; the conidia are harvested and formulated for storage and field use. These methods are mostly variations on production techniques using solid substrates - e.g. production of *Metarhizium anisopliae* (Metsch.) Sorok. (Aquino et al., 1977), *B. bassiana* (Bajan et al., 1975) and other entomopathogenic Hyphomycetes (Goettel, 1984). In techniques using the relatively labor intensive di-phasic fermentation the mycelium is grown in liquid media followed by inoculation of a solid medium or an inert carrier on which conidia are produced (Ferron, 1981; Soper & Ward, 1981). Mycelium can also be incubated in shallow, aerated vessels to produce conidia directly on the surface of the mycelium pellets (Kybal & Vlcek, 1976; Samsinakova et al., 1981).

However, for less expensive mass production a single phase, liquid fermentation process is desirable, allowing existing engineering, fermentation, and production routines to be utilized. With this technology a product containing dry blastospores of *Verticillium lecanii* Viegas (Hall, 1981) was produced. Also, techniques were developed to produce *B. brongniartii* (Delacr.) dry blastospores (Blachère et al., 1973) and, recently, *B. bassiana* conidia which are produced submerged (Thomas et al., 1987). The latter production method might revolutionize *B. bassiana* mass production, submerged production of conidia being the most desirable single phase process. However, the drying-, storage-, field application properties and virulence of submerged produced *B. bassiana* conidia are not known yet.

Production of *B. bassiana* dry mycelium is a single phase fermentation process, not unlike production of Entomophthoralean mycelium as patented by McCabe and Soper (1985). The mycelium is produced by liquid fermentation, stabilized with additives, dried, and milled. This mycelium can be formulated and stored. The material can be applied in the field with conventional spray equipment; however, when large mycelium particles (>0.3 mm) are produced use of a spinning disc applicator is advisable (Rombach et al., 1986c). After field application the mycelium sporulates on the plant - and these conidia infect the insects.

Effect of media. Media composition is of key importance for growth and sporulation of *B. bassiana* (e.g. Ferron, 1981) - but, when grown on agar media, nutrients from the medium sustain growth *and* sporulation. However, after the marcescent process is applied virtually no media remains in the product to sustain conidiation - and sporulation depends totally on inherent qualities of the mycelium.

In this study the results of tests on growth of *B. bassiana* mycelium in liquid media of different composition, and subsequent sporulation of dry mycelium are reported.

MATERIALS AND METHODS

Origin of the isolate and inoculate preparation. The isolate of *B. bassiana* (ARSEF 714) originated from the brown planthopper, *Nilaparvata lugens* (Stahl) (Homoptera; Delphacidae) from China. It is a multi-spore isolate, which was stored in liquid nitrogen up to 3 months prior to the experiment. Before the experiment the isolate was maintained on SDY-agar (Sabouraud dextrose agar with 1% yeast extract) on which it was routinely transferred every 2 weeks.

Inoculum for the experiment was grown in 1 l Sabouraud dextrose broth in 2 l erlenmeyer flasks on a rotary shaker (150 rpm) for 3 days at room temperature (24-28 °C). The liquid culture consisted mainly of mycelium pellets and blastospores. The culture was harvested by vacuum suction in a sterile Buchner funnel and washed 3 times in a solution of basic salts, i.e. KH_2PO_4 (1.5 mg/ml), $MgSO_4$ (0.5 mg/ml) and $CaCl_2$ (0.01 mg/ml), to prevent transfer of nutrients to the media to be tested. The washed mycelium was suspended in 500 ml sterile basic salts solution to which antibiotics (300 units/ml of penicillin / streptomycin) were added. The suspension was blended in a sterile Waring blender for about 20 s at medium speed (about 50 rpm) to break up mycelium pellets. The final inoculat was a smooth, homogenized suspension. The density of the mycelium in the inoculum suspension was adjusted to 2 mg/ml.

The media . *Preparation of the media.* All media were prepared with a basic salt solution and trace elements; components of the media were reagent quality. The basic salts were (in g/l): KH_2PO_4, 1.5; $MgSO_4.7H_2O$, 0.5; $CaCl_2$, 0.01; the trace elements (in mg/l): H_3BO_3, 0.03; $MnSO_4.4H_2O$, 0.04; $Na_2MoO_4.2H_2O$, 0.025; $CuSO_4.5H_2O$, 0.08; $ZnSO_4.7H_2O$, 0.4; $FeCl_2.6H_2O$, 0.5; $CoCl_2.6H_2O$, 0.4. Of each medium 8 flasks were prepared and sterilized (at 110 °C for 20 min.). After cooling the pH was adjusted with a sterile 2 N NaOH solution to pH 6.25-6.50. Each flask contained 27 ml of medium to which 2.5 ml of inoculate was added, an inoculation rate equivalent to 5mg/replicate. To counteract the dilution effects of the inoculate the media were prepared at increased (10%) concentrations of nutrients.

The media. The media are grouped in 2 main groups, i.e. the "carbohydrate concentration groups" (CS and CM groups) and "yeast extract concentration groups" (YS and YM groups).

Carbohydrate concentration group. Preliminary experiments showed that mycelium grown in sucrose and maltose broth produced more conidia compared to mycelium grown in similar media with starch, and dextrose as carbohydrate sources. Moreover, starch often precipitates during fermentation, and dextrose can caramelize during heat sterilization, which complicates experimentation and mass production. Therefore, sucrose and maltose were tested in these experiments. In the concentration groups the influence of different concentrations (0, 0.5, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, and 6%) of the same ratio (1:1) of sucrose (the CS group) and maltose (the CM group) and yeast extract were tested. Preliminary experiments showed maximum conidiation of dried mycelium when grown at 3 to 4% of these media - therefore treatments were grouped around these values in this experiment.

Yeast extract concentration group. In this group the influence of different concentrations (0, 0.1, 0.25, 0.50, 0.75, 1, 1.5, 2, 2.5, 3, 4, and 6%) of yeast extract on growth and subsequent conidiation was tested. These quantities were added to a sucrose (2%, in the YS group) and maltose (2%, in the YM group) broth. Preliminary experiments showed that *B. bassiana* mycelium produced most

conidia per mg dry mycelium when grown in a broth containing carbohydrates and yeast extract in about a 3:1 ratio. Therefore, the treatments were grouped around this ratio.

Inoculation, incubation, and evaluation . *Inoculation.* The growing culture vessels used for the experiment were cylindrical, glass bottles (length 9 cm, diameter 3 cm), closed with autoclavable plastic screw caps. After filling with the media (27.5 ml) the flasks were closed with the plastic caps. After inoculation (2.5 ml) the cap was loosened, to ensure oxygen exchange. For each media 8 flasks were inoculated - i.e. 3 replicates for dry weight measurements, 5 replicates for determination of sporulation. Immediately after inoculation the 348 flasks were placed on a rotary action flask shaker (150 rpm).

Incubation and dry weight measurements. The shaker was kept in a constant temperature room at 23-25 °C under weak fluorescent illumination for 8-10 hours per day. After 72 h on the shaker 3 replicates of each medium were removed, the mycelium collected by vacuum suction on pre-weighed filter paper, washed, dried (4 h at 75 °C), and weighed. At the same time the other replicates (5 for each medium) were removed and placed in a refrigerator (3 °C).

Sporulation. After weighing of the mycelium of the 3 replicates the contents of the 5 remaining replicates were pooled, and blended in a Waring blender for 10 s at medium speed (50 rpm). The blending procedure was standardized, as mycelium can be killed by excessive chopping. Using the data on dry weight obtained above, aliquots of liquid containing the equivalent of 160 mg mycelium were taken from the bottles with a pipet. These were placed on sterile filter papers (Whatman #1, diameter 5cm) and the broth removed by vacuum suction in a sterile Buchner funnel. The mycelium mat was washed 3 times with sterile water to remove nutrients. After the mycelium mat was dry, the filter paper with mat were placed on filter paper in a Petri dish (9 cm diameter). One ml of sterile water was added and the dish closed with Parafilm to prevent desiccation.

For each treatment 5 to 8 replicates were prepared; this number depended on mycelium growth and thus volume which carried the equivalent of 160 mg dry weight mycelium.

The Petri dishes were incubated on a laboratory bench under subdued fluorescent illumination (8 h/day) and room temperature (25-28 °C). During the conidiation period of 8 days the Petri dishes were randomized several times as to equalize experimental conditions. After this conidiation period the lids were inverted and the dishes placed for 24 hours in a stove (75 °C) to dry. To evaluate conidial production conidia were sampled from the surface of the dry mycelium mat by transfer of the filter paper carrying the mycelium and conidia to a test tube with 10 ml Tween80(R) . The contents were vigorously mixed for about one minute. The filter paper was removed and the remaining conidia removed by washing with a further 10 ml Tween80 solution. Close examination of the remaining mycelium mass on the filter paper showed that virtually no conidia were left. The conidium suspension was adjusted to 39 ml and 1 ml formaldehyde(15%) was added.

The concentrations of conidia were determined with an Improved Neubauer haemocytometer counting 5 squares of 16 cells in 2 drops of suspension for each replicate. The actual number of conidia produced by the 160 mg mycelium was calculated from these numbers.

Analysis. Data on growth rate (dry weight of mycelium/30 ml media), and conidiation (conidia/160 mg dry weight mycelium) were analyzed, and data on conidial production/ml broth calculated. Differences in mycelium growth, production of conidia per mg mycelium, and conidial production per ml broth were tested on significance by Student's t-test. Differences were accepted as significant at the P=5% level.

RESULTS AND DISCUSSION

Mycelium- and conidial yields . *Mycelium yield (mg/ml, Fig.1).* Optimal yields were harvested from the CS(3.5%) media (12.31 mg/ml), the CM(4%) media, the YS(1.5%) media, and the YM(2.5%) media. In the CS and the CM groups the yields decline with increasing concentration, after a peak has been obtained, but the yields are not significantly different from the peak value, except for media CM(6%), which yielded significantly less compared to CM(4%). These trends of decreasing mycelium yields after the peak value agree with findings of Samsinakova (1966); she found that increasing concentrations of dextrose and starch above 2.5%, in combination with 1 and 2% corn steep liquor resulted in decreasing yields of mycelium. In the same studies generally higher yields were obtained with maltose compared to sucrose media. This is in contrast with our findings, and might be caused by nutritional preferences of the different *B. bassiana* strains.

Conidial yield (conidia/mg, Fig.2). Optimal conidiation ($4.6x10^6$ conidia/mg) was detected in mycelium from YM(0.75%), YS(0.75%), CS(3.5%), and CM(3%) media - and all these peaks differed significantly from the other media in the same groups, except for CM(1%), which did not differ from CM(3%). Significant differences were present between groups, with the YM(0.75%) producing more conidia ($4.62x10^6$ conidia/mg) compared to YS(0.75%), followed by CS(3.5%), and CM(1%).

Conidial yield (conidia/ml broth, Fig. 3). While the YM(0.75%) medium produces maximum numbers of conidia/mg dry mycelium, the CS(3.5%) medium produces a larger equivalent of conidia/ml broth - because of superposition of the significantly higher mycelium yields (mg/ml) on conidial production (conidia/mg). The conidial production is $3.72x10^7$ conidia/ml. This yield is significantly higher compared to all other media, although the difference with YM(0.75%) is of no practical value.

Production estimates . Production of conidia on the mycelium particles in the field can be maximized in different ways, i.e. by maximizing *mycelium* production (McCoy et al., 1972, 1975, 1978), or maximizing *subsequent conidial* production by the mycelium on the plant. We found that most conidia were produced by mycelium grown in a yeast extract (0.75%)/maltose (0.75%) broth. It should be noted, however, that virulence of conidia produced on different media can differ (Kmitowa, 1979) - this aspect was not tested in these experiments.

B. bassiana grows in cheap liquid media - and, therefore, cost of fermenter space is probably the production limitation rather than costs of media. Thus, for large scale fermentation, yields should be optimized for volume fermenter space (ml) necessary to produce the mycelium with maximum conidium production rather than for mg dry weight. The yield expressed per ml fermenter space can be calculated by superimposing mycelium yield (mg/ml) on subsequent conidium production (conidia/mg). We found that the largest equivalent of conidia /ml broth was produced by the sucrose (3.5%)/ yeast extract (3.5%) broth.

It should be noted that the data on absolute mycelium yield, from shaker flasks can not be extrapolated directly to large scale fermenters - aeration and slurry turnover in the bottles on the shaker is poor compared to laboratory and industrial fermenters. However, relative differences between media are likely to remain the same.

In fermenters *B. bassiana* can be produced at rates up to 25 mg/ml dry weight. In rice, brown planthopper and black bug can be successfully controlled with doses of conidia of about 2.5-$5x10^{12}$ conidia/ha (Rombach et al., 1986a, 1986b, 1987; Aguda et al., 1987). These data, combined with our findings of a maximum conidium production of about $5x10^6$ conidia/mg suggest 20-40 l

fermenter space to produce the equivalent conidia for treatment of 1 ha of rice for these insects. These findings suggest that the production of dry mycelium might be a practical solution for *B. bassiana* mass production for use on rice.

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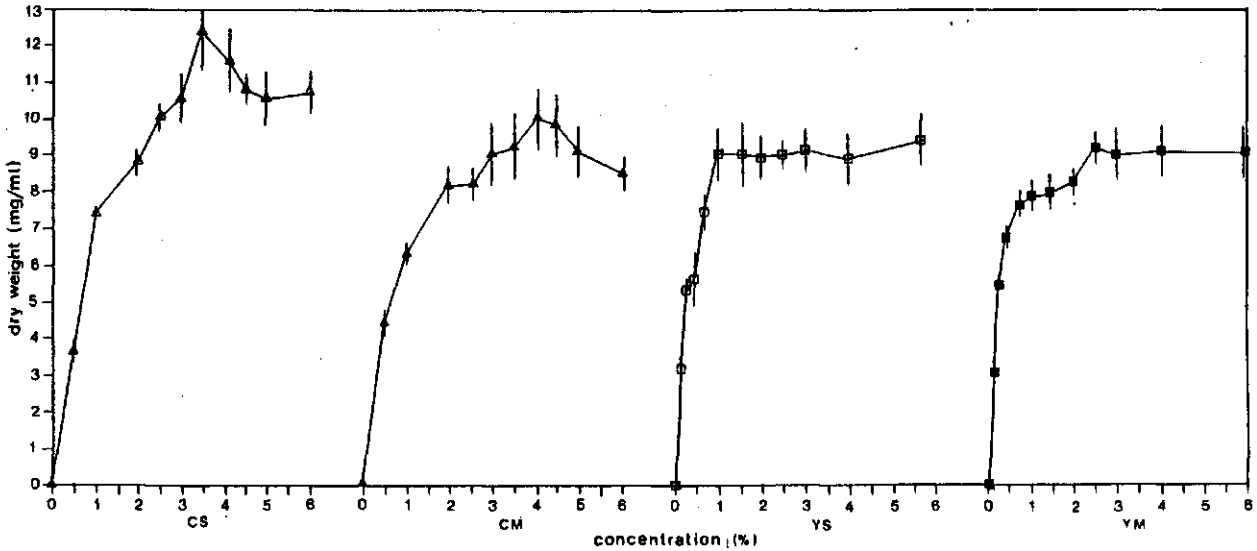

Fig.1. Dry weight (mg/ml) of *B. bassiana* mycelium harvested from media of different composition. Bars represent standard deviation.

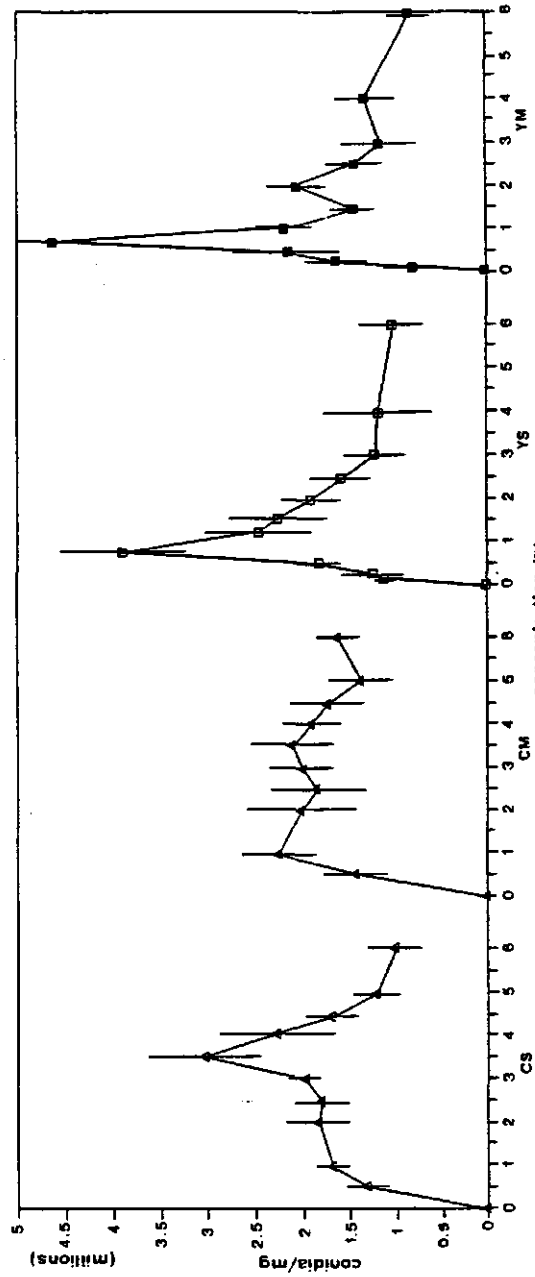

Fig.2. Numbers of *B. bassiana* conidia produced per mg dry and washed mycelium harvested from media of different composition. Bars represent standard deviation.

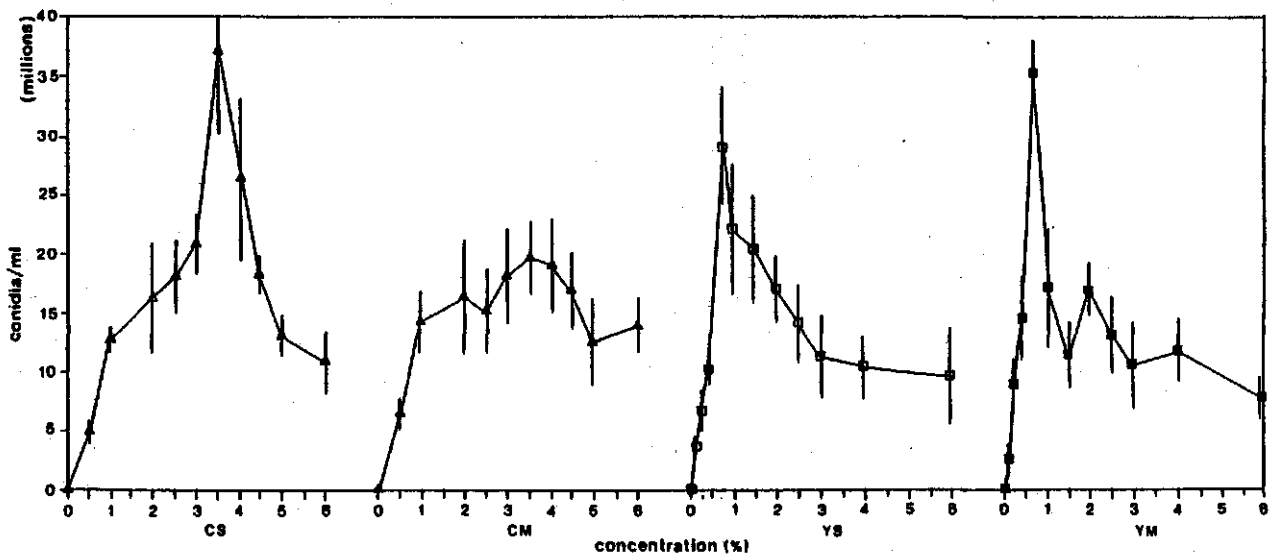

Fig.3. Calculated numbers of *B. bassiana* conidia produced per ml media of different composition. For details see text. Bars represent standard deviation.

CHAPTER 6

GENERAL CONCLUSION

Microbial control of BPH and MRB. The current trend in rice insect pest control is moving from a total reliance on chemical pesticides and total plant resistance toward more complex IPM systems. These IPM systems should be based on as many control tactics as possible. This not only increases stability, but specific strategies can be custom tailored for each specific rice growing situation. In rice IPM schemes there might be a future for microbial insect control agents; insect pathogens possess the special features required for implementation in such systems. Of these insect pathogens the entomogenous fungi deserve special research emphasis for application in the warm and humid tropical rice ecosystems - they can be utilized against leaf-, and planthoppers and pentatomid bugs.

Fungus epizootics can be induced in BPH populations by application of artificial inoculate of different fungi. Artificially introduction of *M. anisopliae* dry mycelium in increasing brown planthopper (BPH) populations caused infection and significant suppression. Also, populations of the Malayan rice bug (MRB) can be suppressed by fungus applications. Effects of the fungus treatments were apparent after 1-2 weeks in the BPH and MRB populations; although statistically significantly lower populations were obtained in treated plots, no complete control was achieved.

Mass production - Economic feasibility. Media for liquid fermentation of *B. bassiana* for maximum conidia production in the marcescent process were determined. Maximum media were defined as media in which fungus growth resulted in a maximum production of conidia after drying of the mycelium. *B. bassiana* was selected because the mycelium produces conidia after repeated washing, thereby greatly facilitating production experimentation. Mycelium of *Metarhizium spp.* and *Nomureae rileyi* tend to loose viability after washing and drying without protecting agents. Also, more conidia per gram dry weight were produced by *B. bassiana* compared to the *Metarhizium spp.*, which is of great practical importance. It was calculated that sufficient mycelium to produce conidia for treatment of 1 ha of rice for BPH or MRB can be produced in an estimated 20-40 liter fermentor space.

Costs of such a production effort are difficult to estimate; many -field, -production and marketing variables are not known. However, a speculative breakdown of production costs of a small unit, producing material to treat 17.500 ha/yr, over a 5 year period, resulted in about Us$15.-/ha - at current Philippine salaries and pricing. Taking a safety- and profit margin into account this amount probably exceeds US$30.-/ha at selling to the farmer. This is more than the price for which many pyrethroid and carbamate insecticides are now sold in the Philippines and in several other Asian countries.

However, the fungi are selective control agents, and safe to man and environment. The fungi do not cause resurgence of secondary insect pests. Their development and use should therefore be stimulated by government grants and international funds.

Political and sociological considerations. Political and economic changes occurred with development of rice technology in the Philippines and other Asian countries. What once was a village, or at most a provincial level agricultural industry, has now become embedded in world trade and markets, regulated by central governments, and foreign aid structures. As Kenmore (1980) states: *"Peasant rice farmers in the present day societies are marginal participants in the economy; they provide food (for*

fixed prices) to keep urban and rural society functioning, but they have minimal input into the distribution of society's surplus production and receive few benefits from the dominant urban society they support, since the urban sector is more tightly linked to an international economic system than to the rural economy".

Recently, under the new US Food Security Act, US farmers will start to pour top quality grains, mainly rice, into the world markets - and at very low prices. This, in combination with Thailand's heavily subsidized rice export tremendously suppresses world rice prices. Consequently, for several Asian countries, rice has become less expensive on the world market than at home. Paradoxically, for some governments, such as the Korean and the Solomon Islands, rice became a rich source of income. Government agencies obtain cheap rice on the world market, which they sell through a monopolizing agency in their own country at artificially high local prices. Also, nearly all Asian countries have known a glut as never before - their rice production has more than doubled since 1964. Also the Philippines, after a few years of drought and devastating typhoons, should produce a rice surplus by the next year. This is due to IRRI's "miracle" rice varieties, improved technologies, and grossly increased agricultural inputs.

By the end of 1986 the world farmers will have accumulated a 300 million ton grain surplus - rather different from the 35 million ton shortage as forecasted earlier by the Asian Development Bank. Several countries, including Indonesia, are therefore changing their agricultural priorities from rice to other crops, including soybean and other legumes, cocoa, rubber, and coffee. To make matters worse, governments started to support farmers with price cuts, tax breaks and other incentives - and the Reagan administration will continue to subsidize farm exports. This will drive prices lower and will hit hardest where it should not hit - at the smaller farms in poor Asian countries. As M.Meyer & B.Eads put it in Newsweek (March 31, 1986): *"For every ton of surplus grain - and there are 300 million tons - there is one starving person.... and that's the tragedy, and the failure, of the decade...."*

Economics vs. pest control. At present pesticide and fertilizer inputs put a heavy burden on the Asian rice farmer. Although often applied in excess, fertilizers can not be simply disconnected from high yields - agronomic laws can only be bend to a limit. In contrast, many pesticide applications, at least on the Philippines, are not necessary, and even make matters worse. To lower the farmers burden pest control strategies with as low inputs as possible should be developed - resulting on optimum use of natural biological control in combination with planting of partial resistant cultivars and other cultural practices. These measures should be based on solid threshold estimates, of which most have not yet been developed. The use of insect fungi for combatting key pests such as MRB and the occasional BPH outbreak should be encouraged. In the rice IPM systems application of microbial- and selective chemical pesticides should be sparingly used and merely be regarded as purely corrective measures for insect populations which escaped biological control - and certainly not as cornerstones on which to base the pest control system. These applications are relatively expensive, and with the present trends in world rice prices profit margins become very narrow - which dictate the farmer to minimize his inputs.

APPENDIX A

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APPENDIX B

SUMMARY

Introduction: Many potential pest organisms are normally kept at densities below damage thresholds by naturally occurring natural enemies in virtually all agricultural crops. This natural control can be enhanced by introduction of new biological agents ("classical biological control") or manipulation of indigenous organisms (Chapter 1). Integrated pest management (IPM) combines biological control with other forms of pest control, such as cultural, mechanical, and chemical methods. However, natural biological control should be the basis for all integrated pest management systems, and certainly in rice - which is, at present, a low input crop.

Key pests. There are very few key pests in tropical rice. Key pests occur on a regular basis, and can cause substantial damage without being provoked by man's activities, e.g. excessive pesticide usage. For example, in tropical rice Malayan rice bug is a key pest, brown planthopper a secondary pest. Control programs should focus on specific control of these key pests without damaging natural enemy populations and provoking secondary pests.

Pesticide use. In several rice growing areas, e.g. on Guadalcanal (Solomon Islands), and on Java and Sumatra (Indonesia) disastrous pest problems are being created through excessive pesticide usage. Programs aimed at minimizing pesticide use and restauration of natural enemy complexes have been initiated in these areas.

Brown planthopper and Malayan rice bug

Nilaparvata lugens, brown planthopper (BPH). Introduction of rice varieties resistant to BPH, the use of broad spectrum insecticides on a large scale, and other agricultural measures provoked BPH to become a major insect pest of rice in tropical Asia. In this area, BPH is an example of an induced, or secondary, pest.

In rice growing areas in Korea and Japan BPH outbreaks are caused by immigration waves from China. After the fallow period of the winter the relatively low populations of natural enemies are overwhelmed by the immigrating BPH populations. However, also in these temperate areas the massive population growth is stimulated by insecticide usage. Broad spectrum insecticides kill natural enemies and thereby render biological control less efficient.

Scotinophara coarctata, Malayan rice bug (MRB). This shield bug was probably inadvertently introduced in Palawan (Philippines) about one decade ago. Now, damage can be found in large areas of rice all over Palawan, although its relative occurrence can differ between different areas on the island. Effective insecticides were identified, but chemicals are expensive for the Palawan subsistence rice farmer, and some of the effective insecticides can resurge BPH populations. Introduction of parasitic wasps from Australia, Malaysia, and the USA were not successful; the wasps established in the MRB populations, but parasitization levels are low. MRB is a key pest on Palawan - damaging populations build up regularly without provocation by man's activities, such as insecticide applications.

Specific control. Conventional insecticides are, in general, expensive, can cause secondary pests, and insects develop resistance to the compounds. Therefore, development of specific and inexpensive control methods for BPH as well as for MRB are needed.

Insect pathogens. Insect pathogens have been used for pest control. Several of the organisms (insect bacteria, -fungi, -nematodes) can be produced on artificial media, and applied in the field.

A literature review on insect diseases, i.e. insect fungi, -viruses, -bacteria, -microsporidia, and -nematodes, of rice pests is presented (Chapter 2). The bibliography contains 278 references, and the major groups of insect pathogens as they relate to rice pests are briefly discussed. Available records on diseases, insect hosts and locations of collections are tabulated. Also, the references are grouped by subject, e.g. by insect pathogen, host, laboratory vs. field studies, etc.

It can be concluded that there is much information on various diseases of rice insects in the literature. However, only few publications report biological control experiments with insect pathogens in the field.

Insect fungi. Insect fungi infect the insect host by penetration through the cuticle rather than by oral ingestion; the fungi can infect sucking insects such as plant- and leafhoppers and pentatomid bugs. These insects do normally not ingest microbes from the internal parts of the plant; natural epizootics of insect fungi are often observed in BPH populations, and also the MRB can regularly be collected infected by fungal pathogens.

The insect fungi are safe for important groups of natural enemies such as predators and parasites as well as to wildlife and man, they can be isolated and grown on artificial media, and various production methods are available. Fungus products can be stored, and applied in the field using conventional spray equipment.

These fungi might be of use in the biological control of rice pests, in particular of sucking pests such as BPH and MRB.

Field tests. Field experimentation with various insect fungi for BPH and MRB control is discussed in Chapter 4. The fungi are applied as suspensions of conidia and as suspensions of a dry mycelium product . The mycelium is grown in fermentors, dried, and milled. Conidia are produced on the mycelium clumps sticking to the plants in the field - and these conidia can infect the insects.

BPH. Five entomopathogenic Hyphomycetes were tested under field conditions for biological control of BPH. Suspensions of conidia of *Metarhizium anisopliae*, *M. flavoviride* var. *minus*, *Beauveria bassiana*, and *Hirsutella citriformis* were applied equivalent to a rate of 4.5×10^{12} conidia/ha. In addition, *M. anisopliae* and *Paecilomyces lilacinus* were applied as preparations of marcescent mycelium at a rate of 1.5-2 kg/ha. Mortality due to fungus infection ranged from 63 to 98% 3 weeks after application. There were no consistent differences between the fungi. The mycelium sporulated on the plant and was as effective as the conidia suspensions in causing high levels of fungus infection.

In a second experiment different rates of dry mycelium and suspensions of *M anisopliae* conidia were evaluated for BPH biological control. The mycelium was applied at an equivalent rate of 700, 3500, and 7000 g/ha.; the conidia were applied at a rate equivalent to 2.5×10^{12} conidia/ha. Significant control of the BPH populations was achieved at 2 weeks after application and up to harvest of the rice; differences between treatments were present but not consistent.

These field experiments show that a) the insect fungi can infect BPH in the field, b) *M. anisopliae* infective materials can significantly suppress BPH populations, and c) dry mycelium can be equally effective for infection as well as suppression compared to conidia of the same fungus. These results justify research on maximizing the marcescent process (Chapter 5).

MRB: In this wet season experiment the effects of the entomopathogenic fungi *B. bassiana*, *M. anisopliae*, and *P. lilacinus* on MRB populations were studied. All fungi occur naturally in MRB field populations, although natural infection levels are low (5-10%). *B. bassiana* and *M. anisopliae*

were collected from Philippine MRB populations, and *P. lilacinus* from a Malaysian population. The latter species was introduced in the Philippines in the course of these studies; the species established and could be isolated from MRB collected near the original experimentation site 1 year after the field studies.

In the experiment adult MRB were kept on rice plants in cages in the field. Cages were placed in 4 different plots in 2 different fields. Mass produced marcescent mycelium, as well as suspensions of conidia of the fungi were tested. MRB numbers were significantly reduced in all fungal treatments compared to the control over a period up to 9 weeks, except in one of the plots where severe drought occurred. In addition, numbers of nymphs were suppressed in the irrigated plots. The overall performance of the different species of fungi and different materials was similar.

Mass-production experiments. Possibilities for large scale application of entomogenous fungi for rice pest control would be greatly enhanced if dry mycelium can be used rather than conventional products containing insect fungus conidia. In the marcescent process dry mycelium materials are produced by liquid fermentation. The slurry is filtrated, stabilized with additives, dried, and milled. The product is thus a dry mycelium powder. This material can be formulated, stored, and applied in the field with conventional spray equipment.

In preliminary experiments it was found that *B. bassiana* mycelium remains viable after washing and drying without protectants. This is in contrast to mycelium of *M. anisopliae* and *M. flavoviride* var. *minus*, which die after washing and drying without protectants. Also, *B. bassiana* mycelium produces about 5-10 times as much conidia per mg dry mycelium compared to *M. anisopliae*. Therefore, *B. bassiana* was selected for these growth experiments.

The constituents of the liquid fermentation medium is of key importance for mycelium growth in the fermentor and subsequent sporulation on the plant in the field. In this chapter the effects of the composition of liquid media on *B. bassiana* growth and conidiation are reported. Two carbohydrate sources (sucrose and maltose), and one nitrogen/vitamin source (yeast extract) were tested for growth and conidiation. Maximum mycelium growth (12.31 mg/ml) was in the sucrose(3.5%)/ yeast extract(3.5%) medium, but washed mycelium from a maltose(2%)/yeast extract(0.75%) medium produced the maximum of $4.6x10^6$ conidia/mg.

In commercial production yields per fermentor volume, rather than yields per mg dry mycelium are important, especially when relatively cheap media such as sucrose and yeast extract are used. With the data the yield per volume was calculated. The sucrose(3.5%)/ yeast extract(3.5%) and the maltose (2%)/ yeast extract(0.75%) media produce most conidia (equivalent to $3.52\text{-}3.72x10^7$ conidia/ml) per fermenter volume.

Production estimates. Industry can grow *B. bassiana* in large scale fermenters at an efficiency rate up to 25 mg dry mycelium /ml. MRB and BPH can be controlled, in the wet season, with a dosage of about $2.5\text{-}5x10^{12}$ conidia/ha. It can thus be estimated that about 20-40 l fermenter space can produce material for treatment of 1 ha of rice for these pests.

Conclusions. In this chapter the economic feasibility of large scale production of *B. bassiana* for rice pest control is discussed. It is estimated that a small production unit, producing material for treatment of 17.500 ha/yr, and functioning over a 5 year period can produce the mycelium at a cost of about US$ 15.-/ha. This basic price dictates that the final market price will certainly be more than US$ 30.-per ha, which is more than for which most carbamate insecticides sold in tropical Asia.

However, the fungal products are specific, do not cause resurgence, are safe for humans and wildlife - and their use should be stimulated with government subsidies or international funds.

Finally, it is concluded that, in rice, microbial and other selective pesticides should be sparingly used. They should be merely regarded as purely corrective measures for the occasional key pest. The applications are relatively expensive, and with the present trends in world rice prices the profit margins become very narrow - which dictates farmers to minimize pest control inputs and rely on natural biological control as much as possible.

Taxonomy . During the course of field experimentation numerous rice insects infected by insect fungi were collected. These collections contained the new taxon *Metarhizium flavoviride* var. *minus* var.nov., and the rare insect fungus *M. album* Petch (Chapter 3).

M. flavoviride Gams and Rozsypal can be divided in the variety *flavoviride* from curculionid beetles and soil from temperate areas, and in the new variety *minus* which was isolated from homopteran insects from the tropics. The new variety was found on BPH in the Philippines and Solomon Islands, *Recilia dorsalis* (Cicadellidae) in the Philippines, and a grasshopper in the Galapagos Islands; its conidia are smaller (mostly 4.5-7 x 2-3 µm) and more consistently ellipsoidal to ovoidal than those of var. *flavoviride*. The new variety may form synnemata in culture. The varieties differ in the morphology and dimensions of the conidia and phialides, and in characteristics of the colonies on agar media.

In a second taxonomy study the species *M. album* Petch is restored for a species from plant- and leafhoppers of rice. In the Philippines and Indonesia *M. album* caused epizootics in populations of *Nephotettix virescens* and *Cofana spectra* respectively. *M. brunneum* Petch is a synonym of *M. album*; the species is characterized by the pale brown color of the conidial masses, clavate phialides, 10-12.5 x 2-3.5 µm, ovoid to ellipsoidal conidia, (3)4-6 x 1.5-2.5 µm, and growth of bulging masses of hyphal bodies rather than mycelium prior to sporulation. It is suggested that the primary criteria for delimiting species of *Metarhizium* are the -shapes of conidia and conidiogenous cells, -presence or absence of a subhymenial zone of swollen hyphal bodies, and -whether the conidia adhere laterally to form prismatic columns. The occurrence of many natural and artificial color variants of *Metarhizium* species suggests that colors of conidial masses and mycelium have only secondary taxonomic value. Conidial size is useful in delimiting species. A synoptic key to the taxa of *Metarhizium* is provided.

SAMENVATTING

Inleiding. In vrijwel alle landbouwgewassen worden potentiële plagen dikwijls onderdrukt door natuurlijk voorkomende organismen. Deze natuurlijke bestrijding kan worden versterkt door introductie van nieuwe organismen ("klassieke biologische bestrijding") of door manipulatie van lokale populaties natuurlijke vijanden: hoofdstuk 1. Geïntegreerde bestrijding ("integrated pest management", IPM) combineert biologische bestrijding met andere methoden, zoals cultuurtechnische en chemische bestrijdingsmethoden. Biologische bestrijding is echter de basis van alle IPM programma's.

Sleutelplagen. Sleutelplagen zijn insectensoorten die regelmatig schade aanrichten zonder direkt gestimuleerd te zijn door landbouwkundige activiteiten zoals overmatig insecticidengebruik. Er komen slechts enkele sleutelplagen op rijst voor. In de tropen is de Malayan rice bug (MRB, *Scotinophara coarctata*) een sleutelplaag, en de brown planthopper (BPH, *Nilaparvata lugens*) een secundaire plaag op rijst. IPM programma's moeten gericht zijn op specifieke bestrijding van de sleutelplagen zonder natuurlijke vijanden van potentiële secundaire plagen aan te tasten.

In een aantal rijstgebieden zoals Guadalcanal (Solomon Eilanden) Java en Sumatra (Indonesie) zijn door overmatig insecticiden- gebruik ernstige plaagproblemen ontstaan. In deze gebieden is men begonnen de bestrijdingsprogramma's te richten op minimalisering van pesticidengebruik en op het herstel van populaties van natuurlijke vijanden.

Brown planthopper en Malayan rice bug

Brown planthopper, BPH. In tropisch Azië hebben het planten van insectenresistente rijstvariëteiten, het grootschalig gebruik van chemische breedspectrum insecticiden en andere landbouwkundige maatregelen geleid tot BPH-explosies. In dit gebied is BPH een duidelijk voorbeeld van een geïnduceerde of secundaire plaag. In rijstgebieden in gematigde streken, zoals Korea en Japan, worden de explosies echter voornamelijk veroorzaakt door BPH-immigranten vanuit China. Deze immigratiedruk kan niet worden weerstaan door de relatief lage populaties natuurlijke vijanden, die na de winter aanwezig zijn. Ook hier wordt massale vermeerdering gestimuleerd door insecticiden-gebruik omdat veel van deze middelen de natuurlijke vijanden - voornamlijk predatoren - doden en de biologische balans verstoren.

Malayan rice bug, MRB. Deze wants is waarschijnlijk ongeveer 10 jaar geleden in Palawan (Filippijnen) ingevoerd. Nu worden grote oppervlakten rijstbouw door de wants aangetast, waarbij de schade van gebied tot gebied verschilt. Sommige insecticiden onderdrukken MRB populaties, maar de middelen zijn duur en leiden bovendien tot problemen met secundaire plagen, bijvoorbeeld van BPH. Introducties van sluipwespen uit Maleisie, Australie en de Verenigde Staten boekten tot op heden weinig succes. Na introduktie kunnen de wespen wel uit in het veld verzamelde eieren worden gekweekt, maar de frequentie van parasitering blijft in het algemeen laag. Op Palawan is deze wants nu een sleutelplaag geworden: populaties van de wants richten schade aan ook zonder dat natuurlijke vijanden zijn onderdrukt door landbouwkundige maatregelen zoals overmatig pesticidengebruik.

Specifieke bestrijding. De ontwikkeling van specifieke bestrijdingsmethoden voor BPH en MRB is belangrijk omdat conventionele insecticiden op enkele uitzonderingen na duur zijn, en vaak secundaire plagen veroorzaken. Ook ontwikkelen insecten vaak resistentie tegen de middelen.

Insectenpathogenen. Verscheidene insectenpathogenen zijn al in gebruik in de insectenbestrijding. Sommige van deze organismen zoals insecten bacteriën en insectenschimmels kunnen buiten de gastheer worden geproduceerd en daarna in het veld worden verspreid.

Insectenschimmels. Insectenschimmels infecteren het insect door penetratie van de cuticula, en niet - zoals de meeste andere insectenziekten - door opname met het voedsel. Deze schimmels zijn daarom belangrijke natuurlijke vijanden van de BPH en de MRB.

In BPH veldpopulaties worden vaak epidemieën van insectenschimmels gevonden en ook de MRB wordt regelmatig met schimmelinfecties aangetroffen. De betreffende schimmels zijn veilig voor belangrijke groepen natuurlijke vijanden zoals spinnen en sluipwespen, als ook voor de mens. Deze schimmels kunnen op kunstmatige media worden geïsoleerd, en verschillende massa-productiemethoden zijn beschikbaar. Schimmelconidia en droog mycelium kunnen worden bewaard, en in het veld met conventionele spuitapparatuur worden verspoten. Deze schimmels kunnen gebruikt worden voor de biologische bestrijding van insectenplagen op rijst, in het bijzonder bij de bestrijding van BPH en MRB.

Literatuuroverzicht. In hoofdstuk 2 wordt een overzicht van insectenziekten bij rijstinsecten: insecten bacteriën, -schimmels, -virussen en -aaltjes. De literatuurlijst bevat 278 referenties naar publikaties betreffende dit onderwerp. De insectenziektes worden in een tabel vermeld, gegroepeerd

per onderwerp, zoals ziektesoort, gastheer, laboratorium-, of veldexperiment. De conclusie is dat er wel veel informatie over ziekten van rijstinsecten beschikbaar is, maar dat er slechts enkele veldexperimenten met insectenziektes zijn uitgevoerd.

Veldexperimenten . Over een mogelijk gebruik van de schimmels in de biologische bestrijding van BPH en MRB werden een aantal veldexperimenten uitgevoerd: hoofdstuk 4. In de experimenten worden schimmels gebruikt in de vorm van suspensies van conidia en suspensies van een droog myceliumproduct. Dit mycelium werd in fermentoren gekweekt, gedroogd en vermalen. In het veld produceert dit mycelium conidia en deze conidia infecteren weer het insect.

BPH. In een eerste veldexperiment werden epidemieën van 5 verschillende schimmelpreparaten in BPH populaties geïnduceerd. Suspensies van conidia van *M. anisopliae*, *M. flavoviride* var. *minus*, *B. bassiana* en *Hirsutella citriformis* werden toegepast in een dosering van $4.5x10^{12}$ conidia/hectare. Ook droge mycelium preparaten van de schimmels *M. anisopliae* en *Paecilomyces lilacinus* werden verspoten in een dosering van 1.5-2 kg/hectare. Na 3 weken bleek 63 tot 98% van de BPH met schimmel geïnfecteerd te zijn. Er konden geen verschillen worden vastgesteld tussen de verschillende behandelingen. Het mycelium van *M. anisopliae* en *P. lilacinus* sporuleerde op de planten en had evenveel effekt als de suspensies van conidia.

In een tweede experiment van de biologische bestrijding van de BPH werden verschillende doseringen van droog mycelium en 1 dosering van conidia van *M. anisopliae* met elkaar vergeleken. De mycelium doseringen bedroegen 700, 3500 en 7000 g/hectare, de conidia suspensie $2.5x10^{12}$ conidia/ hectare. In de behandelde gazen kooien bleken, gerekend van 2 weken na de bespuiting tot aan de oogst, de BPH populaties significant kleiner te zijn vergeleken met de kontrole behandeling. De behandelingen vertoonden onderling wel enig verschil, maar de verschillen waren niet konsistent.

Deze experimenten laten zien dat: a) bespuitingen met materiaal van de insectenschimmels in BPH populaties epidemieën kunnen veroorzaken, b) *M. anisopliae* conidia en mycelium de BPH aantallen kunnen reduceren, en c) droog mycelium even doeltreffend infectie kan veroorzaken en populaties kan reduceren als conidia .

MRB. Als experiment werd gedurende de regentijd op het eiland Palawan getracht de MRB te bestrijden met materiaal van de schimmel *B. bassiana*, *M. anisopliae* en *P.lilacinus*. Deze schimmels worden alle op MRB gevonden, maar percentages natuurlijke infectie zijn over het algemeen erg laag: maximaal 5-10%.

In het experiment werden volwassen insecten in gazen kooien op rijstplanten geplaatst. Het experiment werd in 4 velden op 2 verschillende locaties herhaald. Droog mycelium en conidia werden getest in doseringen van resp. 1.5-2 kg/hectare en $2.5x10^{12}$ conidia/hectare. De aantallen wantsen werden bij alle schimmelbehandelingen, vergeleken met de kontrole behandeling, significant verminderd, behalve in een veld waar het irrigatiesysteem onklaar raakte en de rijst van 2 weken na de bespuiting tot aan de oogst droog kwam te staan. Eveneens werden nymphen in de geïrrigeerde velden geïnfecteerd. De infectiepercentages varieerden van 15 tot 80%. Hierbij werd weinig verschil gevonden tussen de schimmelsoorten en de verschillende preparaten, d.i. mycelium en conidia.

Massaproductie . De toepassing van schimmels in de bestrijding van BPH en MRB zou aanzienlijk worden vereenvoudigd als in plaats van conventionele conidia-producten droog mycelium kan worden gebruikt. In het marcescent proces wordt het mycelium gekweekt in een vloeibaar medium in fermentoren. Na de groei worden mycelium en medium gescheiden door filtratie, de vochtige

myceliumkoek gewassen, beschermingsmiddelen tegen schade bij het drogen toegevoegd, en tenslotte het mycelium gedroogd en gemalen. Dit droog myceliumpoeder kan worden bewaard en in het veld worden verspoten.

In eerdere experimenten bleek, dat het *B. bassiana* mycelium in tegenstelling tot dat van *M. anisopliae* en *P. lilacinus* zonder beschermingsmiddelen en zonder verlies aan levensvatbaarheid kan worden gedroogd. *M. flavoviride* var. *minus* groeit in eerste instantie erg langzaam, en *M. album* groeit vrijwel niet in vloeibare media. Verder produceert *B. bassiana* ongeveer 5-10 maal zoveel conidia/ mg droog mycelium als *M. anisopliae*. Daarom werd *B. bassiana* voor deze groeiproeven uitgekozen.

De invloed van 2 koolhydraatbronnen (sucrose en maltose), en 1 stikstof/vitamine bron (gist extract) op groei en sporulatie van *B. bassiana* mycelium werd bestudeerd. Maximale myceliumgroei (12.31 mg/ml droog gewicht) werd in een sucrose(3.5%)/ gist extrakt(3.5%) medium gevonden, terwijl mycelium uit een maltose(2%)/ gist extract(0.75%) medium het maximum aantal conidia ($4.6x10^6$ conidia/mg) produceerde.

In de commerciele productie van schimmels is het gebruikelijk om de productie per fermentor volume te optimaliseren, vooral als de ingrediënten voor de media relatief goedkoop zijn. Met behulp van de groei- en sporulatie uitkomsten kunnen opbrengsten per fermentorvolume berekend worden. De sucrose(3.5%)/ gist extract(3.5%)- en de maltose(2%)/ gist extract(0.75%) combinaties produceerden het meeste conidia/ mycelium per ml equivalent ($3.52-3.72x10^7$ conidia/ml).

Deze resultaten zijn gebaseerd op kleine volumina: in flessen op de schudmachine. Deze opbrengsten zijn over het algemeen minder dan in grotere industriële fermentoren. In de eenvoudige "bubble"-fermentoren op IRRI worden reeds opbrengsten van 15-20 mg droog mycelium/ml verkregen. In de industrie kan *B. bassiana* worden geproduceerd met een efficiency van ongeveer 25 mg droog mycelium/ ml. BPH en MRB populaties kunnen significant worden onderdrukt met doseringen van $2.5-5x10^{12}$ conidia/hectare. Voor behandeling van 1 ha kan een evenredige hoeveelheid mycelium voor de productie van deze hoeveelheid conidia waarschijnlijk worden geproduceerd in 20-40 liter fermentor volume.

Conclusies. Naar schatting kan een kleine productie-eenheid die materiaal voor 17.500 hectare per jaar produceerd en met een afschrijftijd van 5 jaar een hectare equivalent schimmelmateriaal leveren voor US$ 15.- aan productiekosten. De schatting is gebaseerd op huidige Filippijnse salarissen, bouw-, en grondstofkosten. Dit betekent dat het uiteindelijke product ongeveer US$30.- per hectare zal gaan kosten, na toevoeging van transport- en handelskosten. Dit is meer dan betaald wordt voor de meeste carbamaat insecticides in een groot gedeelte van tropisch Azië.

Echter, door hun specificiteit en veiligheid voor milieu en de mens is het gebruik van insectenschimmels te verkiezen boven het gebruik van de chemische middelen. Misschien kan daarom het gebruik van schimmels met fondsen van overheden of internationale hulp worden gestimuleerd.

Algemene conclusie. Door de huidige lage rijstprijzen op de wereldmarkt zijn de winstmarges van de rijstboer in tropisch Azië zeer gering. De boer is aangewezen op natuurlijke biologische bestrijding. Chemische- en microbiële insecticiden zijn relatief duur. Ze zullen spaarzaam moeten worden gebruikt als laatste redmiddelen voor een enkele sleutelplaag.

Taxonomische studies . Gedurende de over de biologische bestrijdingsexperimenten van BPH en MRB in het veld werden vele met insectenschimmels geïnfecteerde rijstinsecten verzameld. Deze verzamelingen leverden het nieuwe taxon *Metarhizium flavoviride* var. *minus* var.nov., en de zeldzame schimmelsoort *M. album* Petch op. Deze verzamelingen werden in dit proefschrift in hoofdstuk 3 beschreven en besproken.

M. flavoviride Gams en Rozsypal kan worden onderverdeeld in de variëteit *flavoviride* die onder andere op snuitkevers en in de bodem voorkomt, en de nieuwe variëteit *minus* die uitsluitend op tropische homoptere insecten voorkomt. De varieteit *minus* werd geïsoleerd van BPH (Filippijnen en Solomon Eilanden), *Recilia dorsalis* (Filippijnen), en een sprinkhaan (Galapagos Eilanden). De conidia van de variëteit *minus* zijn vergeleken met de variëteit *flavoviride* kleiner (meestal 4.5-7x2-3 µm) en meer consistent ellipsvormig tot eivormig. De variëteit *minus* kan op agar media synnemata produceren.

M. album. In een tweede taxonomische studie wordt *M. album* Petch hersteld voor een soort die, ten onrechte, was verworpen en beschouwd werd als een synoniem van *M. anisopliae*. *M. album* werd onder andere aangetroffen op de homoptere insecten *Nephotettix virescens* (Filippijnen) en *Cofana spectra* (Indonesië). Bestudering van de type specimens leidde tot de conclusie dat *M. brunneum* Petch een synoniem is van *M. album*. *M. album* verschilt van de andere *Metarhizium* soorten in kleur van de conidia (wittig bruin), de knotsvormige phialiden, 10-12.5 x 2-3.5 µm, de ellipsvormige tot eivormige conidia, (3)4-6 x 1.5-2.5 µm, en de groei van kolonies door vermeerdering van "hyphal bodies", en niet van mycelium tot aan de sporulatie fase.

De belangrijkste criteria voor onderscheiding van soorten binnen het genus *Metarhizium* zijn de vorm van conidia en phialiden, de aan- of afwezigheid van groei door "hyphal bodies" voor de sporulatie, en de formatie van prismatische kolommen van conidia. Het voorkomen van natuurlijke en kunstmatige kleurvariaties van de *Metarhizium* soorten maakt dat kleur van de conidia moet worden beschouwd als een secundair kenmerk. De maten van conidia kunnen worden gebruikt voor onderscheid in variëteiten. Een synoptische sleutel voor *Metarhizium* soorten wordt gegeven.

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 17 Maart 1955 te Barneveld geboren. In 1974 werd het diploma Atheneum B behaald aan de Scholengemeenschap "de Amersfoortse Berg" te Amersfoort. Het doctoraalexamen biologie werd behaald aan de Rijksuniversiteit te Utrecht in 1981. Van 1981-1983 werd er gewerkt voor Koppert B.V. (Berkel en Rodenrijs) aan integratie van insectenschimmelproducten in bestrijdingsschema's van witte vlieg in kassen in Nederland en Spanje.

In de periode 1984-1987 werd, gesteund door fondsen van de US Agency for International Development, het Boyce Thompson Institute for Plant Research at Cornell University en het International Rice Research Institute onderzoek verricht aan biologische bestrijding van enkele rijstplagen met insectenschimmels. Op dit onderzoek is dit proefschrift gebaseerd.

Vanaf Maart 1987 zal, gesteund door de Jessie Smith Noyes Foundation, het Boyce Thompson Institute en het International Rice Research Institute, gedurende 3 jaar gewerkt worden aan geïntegreerde bestrijding van rijstinsecten in Azie.

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