

# Entomopathogenic Fungi as an Important Natural Regulator of Insect Outbreaks in Forests (Review)

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## 1. Introduction

With over 1 million species insects are not only the largest group of animals, but also a group that causes the most damage in forest management. Hence it is important to understanding the biology of their natural enemies. Among them are entomopathogenic fungi. Entomopathogenic fungi are a very heterogeneous group. Belong to different systematic groups and even their biology is often very different. However, all of them are pathogenic in relation to insects, and actually all arthropods, and their effectiveness in infecting their hosts is so large that it can become a factor regulating the abundance of insects. Importantly, the harmful insects (from the human point of view) include of course the forest pests.

In this paper we would like to introduce biology, systematics, geographical distribution, and give examples of natural and man-stimulated biocontrol of forest pests by entomopathogenic fungi.

## 2. Geographical and ecological distribution of entomopathogenic fungi

Entomopathogenic fungi are an important and widespread component of most terrestrial ecosystems. It seems they are not only in places where there are no victims – insects nor other arthropods. Of course spread of individual species of entomopathogenic fungi are different. However some of them can be found practically throughout the world. An example of such species may be *Beauveria bassiana* which is reported from tropical rainforest (Aung et al., 2008), and has been found in Canada as far north as latitude 75° (Widden & Parkinson, 1979). Entomopathogenic fungi have been also recorded north of the Arctic Circle. They have been *Tolyposcladium cylindrosporium*, *B. bassiana* and *Metarhizium anisopliae* in Norway (Klingen et al., 2002), and *B. bassiana*, *M. anisopliae* and *Isaria farinosa* (= *Paecilomyces farinosus*) in Finland (Vänninen, 1995). What more, entomopathogenic fungi have been reported also from Arctic Greenland (Eilenberg et al., 2007) and Antarctica. In the latter location including endemic Antarctic species *Paecilomyces antarctica* isolated from the Antarctic springtail *Cryptopygus antarcticus* in the peninsular Antarctic (Bridge et al., 2005).

Also cosmopolitan fungi belonging to the genus *Beauveria*, *Lecanicillium*, *Conidiobolus* and *Neozygites* have been found on Antarctic sites, but without their arthropod hosts (Bridge et al., 2005).

Studies of Quesada-Moraga showed that altitude has no influence on presence of entomopathogenic fungi in range up to 1608 m, what more altitude was found to be predictive for the occurrence of *B. bassiana* (Quesada-Moraga et al., 2007). However, studies made on wider range of altitudes (up to > 5200 m) made by Sun & Liu showed great importance of this factor on the species diversity of insect-associated fungi (Sun & Liu, 2008).

There are different groups of entomopathogenic fungi in different habitats. Different insect pathogenic mycofloras could be found in the soil and different in the overground environment. Sosnowska found in Poland that in the Białowieża Forest soil litter and soil surface layer dominated *Hypocreales*, but in understory trees and in the canopy – *Entomophthorales*, and in the meadow and rush communities species of spider pathogenic fungi of the genus *Gibellula* (Sosnowska et al., 2004). The *Entomophthorales* are commonly reported as pathogens of forest pests in temperate forest habitats (Burgess, 1981), but are rare in tropical forests (Evans, 1982). Humid tropical forests had a rich and varied insect pathogenic fungal species and the great majority of species belong in the genus *Cordyceps* (*Ascomycota*: *Hypocreales*) (Evans, 1982; Aung et al., 2008). While other species of *Hypocreales* such as *Beauveria*, *Metarhizium* and *Isaria* were the dominant fungi found on soil insects (Samson et al., 1988; Keller & Zimmerman, 1989).

Despite the fact that both *B. bassiana* and *M. anisopliae* are common everywhere there is known that *B. bassiana* seems to be very sensitive to the disturbance effects of cultivation and thus restricted to natural habitats. The ability of *M. anisopliae* to persist in cultivated soils is well established. Therefore the first is more frequent in forest, and second in arable soils (Rath et al., 1992; Vänninen, 1995; Quesada-Moraga et al., 2007; Sánchez-Peña et al., 2011).

Most reports show that frequency of entomopathogenic fungi in intensively cultivated soils is lower than in forest soils (Vänninen et al., 1989; Miętkiewski et al., 1991; Vänninen, 1995; Chandler et al., 1997; Bałazy, 2004). However, there were some exceptions from this rule, e.g. higher frequency of bait insect infections in pasture soils than in soils from either forest or cropland (Baker & Baker, 1998).

Entomopathogenic fungi are commonly found in soil and leaf litter of worldwide forests, however in temperate forests the diversity of entomopathogenic fungi is relatively low in comparison with tropical habitats (Evans, 1982; Grunde-Cimerman et al., 1998; Aung et al., 2008). However, compared to agricultural areas the diversity of entomopathogenic fungi in the temperate forests is quite high (Sosnowska et al., 2004). The differences in their prevalence and diversity of species were also found between the different types of forests (Miętkiewski et al., 1991; Chandler et al., 1997).

### 3. Systematics and biology of entomopathogenic fungi

#### 3.1 Systematics of entomopathogenic fungi

Entomopathogenic fungi are a very heterogeneous group of insect pathogens. It is known nearly 700 species belonging to approximately 100 orders. Although only a few of them have been studied well. Most of them belong to the order *Entomophthorales* of the phylum

*Glomeromycota* and to *Hypocreales* of the phylum *Ascomycota* (Hibbett et al., 2007; Sung et al., 2007). Recent phylogenetic studies within entomopathogenic fungi resulted in significant revision of many species of entomopathogenic fungi. For example, such species as *Paecilomyces farinosus* and *P. fumosoroseus* currently belong to the genus *Isaria* (Luangsa-ard et al., 2004), and species *Verticillium lecanii* to the genus *Lecanicillium* (Zare & Gams, 2001). There are many species in the *Ascomycota* in which the sexual phase (teleomorph) is not known and which reproduce entirely asexually (anamorphic fungi). Sexually reproducing hypocrealean fungi occur in the genera *Cordyceps* and *Torrubiella*. These fungi are important natural control agents of many insects in tropical forests. Genus *Cordyceps* has many anamorphs, of which *Beauveria*, *Lecanicillium* and *Isaria* are the best known and described (Blackwell, 2010). Recent phylogenetic studies have demonstrated that the genus *Beauveria* (so far known to be only anamorphic fungus) is monophyletic within the *Cordycipitaceae* (*Hypocreales*), and has been linked developmentally and phylogenetically to *Cordyceps* species (Rehner et al., 2011). Despite recent interest in the genetic diversity of many groups of entomopathogens, the genus *Beauveria*, in contrast to other species, has not received critical taxonomic review (Rehner et al., 2011).

### 3.2 The life cycles of *Hypocreales* and *Entomophthorales*

The life cycles of *Hypocreales* and *Entomophthorales* are slightly different. Nevertheless, the survival and spread in the environment of both groups is dependent on the infection of the host that invariably leads to its death. The life cycle of entomopathogenic fungus consists of a parasitic phase (from host infection to its death) and a saprophytic phase (after host death) (Fig. 1 and Fig. 2).

In contrast to other entomopathogens (bacteria and viruses), which enters the insects with food, entomopathogenic fungi infect their host through the external cuticle. The process of infection involves: adhesion of the spore on the insect cuticle, penetration of the cuticle by the germ tube, development of the fungus inside the insect body and colonization of the hemocoel by fungal hyphae. The spores of the entomopathogenic fungi are usually covered with a layer of mucus composed of proteins and glucans, which facilitates their attachment to the insect cuticle. Germinating spores of several entomopathogenic fungi produce specialized structures called appressoria. The appressorium is responsible for attachment of germinating spore to the epicuticular surface. The process of penetration of the insect cuticle is a result of mechanical pressure and enzymatic activity of the germ tube. The major role in the penetration plays the secretion of sequential lipases, proteases and chitinases. Inside the insect body most entomopathogenic fungi grow as yeast-like propagules (blastospores), hyphal bodies or protoplasts lacking a cell wall. These structures are spread through the hemocoel. Death of an insect is usually a result of mechanical damage caused by growing mycelia inside the insect (mummification), or toxins produced and released by the pathogen. *Beauveria*, *Metarhizium*, and *Tolyposcladium* are known that secrete a whole range of toxins. Some of them like destruxin, bavericin, and efrapeptins are fully described chemically, and is known their action and contribution in the process of pathogenesis (Roberts, 1981; Hajek & St. Leger, 1994). For *Entomophthorales* there are limited data about the release of toxins (Bogus & Scheller, 2002). In this case, death is the result of the total colonization of host tissues by the fungus.

After host death, the fungus colonizes the cadaver and during 2-3 days forms aerial hyphae and then sporulates (Fig. 1 and Fig. 2). Whereas *Hypocreales* produce only asexual spores, species of *Entomophthorales* produce two types of spores: asexual (primary conidia) and sexual (zygo- or azygospores) called resting spores (Fig. 1 and Fig. 2). Conidia of *Hypocreales* and primary conidia of *Entomophthorales* are produced externally on the surface of an insect after its colonization and death. *Entomophthorales* and *Hypocreales* differ in the way dispersal of spores. The first of these are actively discharged from cadavers by hydrostatic pressure, while the latter are spread by wind. If primary conidium from cadavers does not land on a new host, it germinates and forms secondary conidia (some species can also produce tertiary and quaternary conidia). The majority of *Entomophthorales* produce resting spores (internally within cadavers). Cadavers containing resting spores (azygospores) initially attach to the branches of trees, and then fall to the ground and then azygospores are leached into the soil. Under favourable conditions, azygospores begin to germinate to form germ conidia and infect new hosts. Resting spores allow entomophthorales species to survive unfavourable periods or the temporary lack of hosts. In this way many species of *Entomophthorales* synchronize their development with the development of insects. Hypocrealean fungi can also survive in the environment (if do not land on a new host), as mummified cadavers or as conidia in soil (Hajek & St. Leger, 1994; Hajek & Shimazu, 1996).

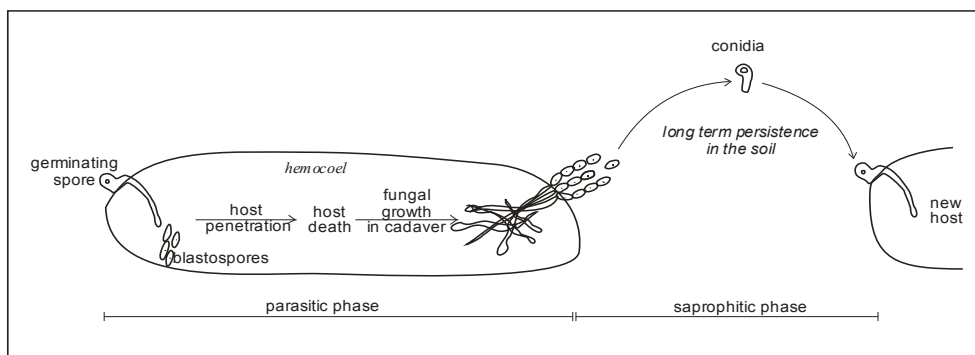


Fig. 1. The life cycle of entomopathogenic fungi from the order *Hypocreales*

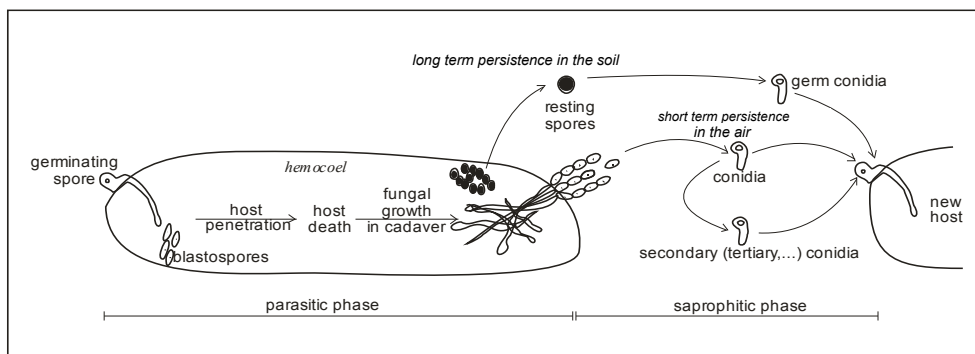


Fig. 2. The life cycle of entomopathogenic fungi from the order *Entomophthorales*

### 3.3 Intraspecific variation of entomopathogenic fungi

Entomopathogenic fungi can attack insects from different orders: Lepidoptera, Coleoptera, Hemiptera, Diptera, Orthoptera, Hymenoptera, as well as non-insect arthropods. But while some species of fungi (who belongs mainly to the *Hypocreales*) have a very wide spectrum of potential victims, others (mainly *Entomophthorales*) are pathogens only one particular species of insect.

The question arises whether the specialization of the pathogen, and thus its virulence towards particular hosts, is associated with genetic diversity of the pathogen. In the case of entomopathogenic fungi with a wide range of hosts such as *Beauveria bassiana* numerous studies have confirmed that strains of diverse geographical origin but isolated from the same host species showed a greater similarity in the genetic structure than strains from the same area but isolated from different host species (Poprawski et al., 1988; Neuvéglise et al., 1994; Cravanzola et al., 1997; Couteaudier & Viaud, 1997; Maurer et al., 1997; Castrillo & Brooks, 1998). For example, Maurer et al. (1997) by analysing the RFLPs (restriction fragment length polymorphism) and RAPDs (random amplification of polymorphic DNA) of 38 strains of *Beauveria bassiana* isolated from various geographical sites and from diverse species of Lepidoptera (*Ostrinia nubilalis*, *Diatraea saccharalis*, *Maliarpha separatella*) and Coleoptera (*Sitona humeralis*, *S. discoideus*), found a few homogenous groups of strains with similar genetic structure. The first group consisted of all strains isolated from *O. nubilalis*, and the second one included strains from *D. saccharalis*. Strains isolated from various species of *Sitona* formed the third group of strains with similar genetic structure. Furthermore, in laboratory tests, strains from *O. nubilalis* were highly virulent towards this host and less (or not) virulent against the other hosts. Similarly, Neuvéglise et al. (1994) have shown the relationship between the genetic structure of *Beauveria brongniartii* strains and their biological origins (host species). The results of PCR-RFLP revealed a perfectly homogenous group of strains isolated from *Hoplochelus marginalis*. All the strains isolated from *H. marginalis* were more virulent against this host (30-100% mortality) than the strains isolated from different insects (10% mortality). Castrillo & Brooks (1998) also found a high similarity between *B. bassiana* isolates obtained from *Alphitobius diaperinus*.

Literature data on *Metarhizium anisopliae* do not confirm such a clear relationship between genotype and specialization in relation to a particular host (Tigano-Milani et al., 1995; Fungaro et al., 1996; Bridge et al., 1997). A lot of studies indicate the crucial importance of the geographical origin of isolates (St. Leger et al., 1992; Cobb & Clarkson, 1993; Leal et al., 1994; Leal et al., 1997) and habitat type (Bidochka et al., 2001). Studies of Leal et al. (1994; 1997) showed that among 40 isolates of *M. anisopliae*, strains from the same country were more similar in the genetic structure than those from different countries despite the same host. Interesting observations to support a relationship between the genetic structure of *M. anisopliae* isolates and habitat type (agricultural and forested areas) along with abiotic factors (temperature and exposure to UV radiation) provided Bidochka et al. (2001). On the basis of various genetic markers (allozymes, RAPD, RFLP), Bidochka et al. (2001) divided 83 strains of *M. anisopliae* into two distinct groups, each associated with different habitat type. The group from forested areas showed ability for growth at low temperatures (at 8°C), while the group from the agricultural areas showed ability for growth at high temperatures (37°C) and resilience to UV exposure. The association of habitat and thermal preferences was also found for *B. bassiana* (Bidochka et al., 2002). Recently, more sensitive and reliable molecular

methods also indicate a certain association between *B. bassiana* isolates and their geographical origins and not between the genetic structure of the fungus and host systematic position (Wang et al., 2005; Fernandes et al., 2009). There is a hypothesis that the saprophytic phase has an evolutionary impact on genetic structure of many species of entomopathogenic fungi including *B. bassiana* (Bidochka et al., 2002; Ghikas et al., 2010; Ormond et al., 2010; Garrido-Jurado et al., 2011). In studies conducted by Ormond et al. (2010) in a conifer forest, molecular analyses (ISSR-PCR) indicate that below-ground and above-ground isolates of *B. bassiana* are genetically diverse.

Relatively little is known about genetic diversity of *Entomophthorales*. The host range within *Entomophthorales* is generally narrow. Therefore, it would seem that the genetic diversity of isolates from the same host may be small. One of the better-studied species in this respect is *Entomophaga maimaiga*. In North America where it produces numerous epizootics in a population of *Lymantria dispar*, *E. maimaiga* characterized by relatively low genetic diversity. *E. maimaiga* was introduced to the USA from Japan, and such low genetic diversity proves that it spread from a small number of individuals (Hajek et al., 1995). Nielsen et al. (2005a) comparing by AFLPs 30 *E. maimaiga* isolates originating from the USA, Japan, China and Russia found that native populations from Asia were more diverse than the USA populations. The authors hypothesize that the population now present in the USA came from Japan of a result of accidental introduction rather than the deliberate release. In contrast to the Asian isolates, no correlation between geographical location and clade was found among the US isolates. The authors explain this by the fact that *E. maimaiga* was introduced into the USA relatively recently; therefore genetically distinct subpopulations may not have evolved yet.

Another specialized entomophthoralean species *Erynia neoaphidis* – major aphid pathogen – shows greater genetic diversity. Rohel et al. (1997) using PCR-ITS and RAPD methods, identified four separate groups with high genetic variability among 30 isolates originating from diverse countries and hosts. Only in some cases RAPD groupings could be related with geographical origin and there was no apparent relationship between host and ITS or RAPD pattern. Similar results have received Tymon & Pell (2005) using ISSR, ERIC and RAPD techniques.

Very little intraspecific variation has been demonstrated for *Entomophthora muscae*, a common pathogen of flies (Jensen et al., 2001; Jensen & Eilenberg, 2001). These studies show low genetic variation within isolates from the same host taxon. In a study conducted by Jensen et al. (2001) several different genotypes within *E. muscae* s. str. have been documented, and each of the genotypes was restricted to a single host taxon, suggesting high host specificity.

Molecular studies of entomopathogenic fungi are very important in the context of choosing the appropriate species or strains for biological control, as well as distinguish wild strains from those introduced artificially in order to monitor and to track isolates after field application.

## 4. Epizootiology of insect disease

### 4.1 Fungal epizootic as a limiting factor of insect outbreaks

Insects are essential part of forest ecosystems and at low density have negligible impact on tree growth. Although occasionally, some insect species quickly increase their numbers

giving catastrophic impacts on trees and, in some cases this can lead to the complete destruction of large areas of natural or planted forests. Insect outbreaks are often the result of disturbance in biocenotic balance caused for example by sudden events such as fire or hurricane, or as a result of human activities, such as changes in the planting structure (monocultures), but also by more global processes like climate change (Hunter, 2002). In many specific cases, the initiation of insect outbreaks is the result of many factors, and mechanisms of their occurrence are not fully understood and explained. Similarly, there are many factors causing the collapse of outbreaks: depletion of food resources, natural enemies, and unfavourable weather.

Among the natural enemies of insect, infections (epizootics) caused by entomopathogenic fungi are one of the frequently observed causes of collapse of outbreaks. In insect pathology epizootic is defined as an unusually large number of cases of disease in a host population. Epizootic diseases are sporadic, limited in time, and in a given area, and characterized by a sudden change in prevalence (Fuxa & Tanada, 1987). Entomopathogenic fungi are constantly present in populations of insect hosts but when density of the host population is normal infections occur sporadically (enzootic phase of insect diseases). However, during insects outbreaks fungi that infect insects can increase their numbers enough to spread in the environment and contribute to the reduction of insect's population (epizootic phase) (Fuxa & Tanada, 1987). It is very difficult to predict the occurrence of epizootic and not always in different pathogen-host systems the same factors initiate its development in pest population. Furthermore, epizootics caused by entomopathogenic fungi in forested habitats are less numerous than those in other habitats, particularly in agricultural areas because the forest ecosystem is more complex and more stable compared with "agroecosystem". Forest ecosystems also have many different mechanisms for regulating the number of pests.

Spatial and temporal spread of the epizootic depends on the effective transmission of the pathogen in the population of the target insect and insect susceptibility to infection. These two main factors are closely related to climatic factors (mainly temperature and humidity) and biotic environments (other pathogens, parasitoids and predators). All these factors act simultaneously on both sides of pathogen-host system by modifying the growth and development of both pathogen and insect population. Transmission is the transfer of infective propagules between individuals through direct contact. Entomopathogenic fungi in the insect population can be transmitted in three ways: horizontally (from infected insects on healthy individuals within a single generation), vertically (between generations), and be moved by vectors. This third method plays an important role in the transmission of fungi to new habitats (Fuxa & Tanada, 1987). It is supposed that natural epizootic in a population of insects do not have to be initiated by a highly virulent strain of the fungus. In the laboratory, the virulence of the strain can be increased with passaging (repeated in-vitro transmission of the tested strains of fungi from infected to healthy individuals of target insect) (Hyden et al., 1992; Hughes & Boomsma, 2006). Most likely, under natural conditions there is a natural passage during which the pathogen increases its virulence.

Fungal epizootics occur naturally in many insect populations during outbreaks and are frequently the primary cause of the collapse of many insect populations (Table 1). Hicks & Watt (2000) reported that in 1998 outbreak of the pine beauty moth (*Panolis flammea*) in lodgepole plantations in Scotland collapsed by a fungal epizootic. *Entomophaga aulicae*, *Nomuraea rileyi* and *Beauveria bassiana* were recorded from infected larvae. Together they caused 88% mortality in the population of *P. flammea*.

Epizootics *Lymantria dispar* are found in areas of natural occurrence of the pest (from Europe to Asia), but also in new areas where *L. dispar* is an invasive species (North America). Shimazu & Takatsuka (2010) in Japan, during outbreak of *L. dispar* found many dead and living larvae on the surface of boles of the host trees. Laboratory investigation of the gypsy moth cadavers revealed that most of them were infected with the nuclear polyhedrosis virus (NPV), the fungal pathogen *Entomophaga maimaiga*, or a mixed infection of those two pathogens. They also found larvae infected by *Isaria javanica*. Earlier Aoki (1974) reported *Paecilomyces canadensis* and *Entomophthora aulicae* (= *Entomophaga maimaiga*) from *L. dispar* larvae in the same Prefecture as the study of Shimazu & Takatsuka (2010). Aoki (1974) found that 80% of collected larvae were killed by *Entomophthora aulicae* and 19% by a mixed infection with *P. canadensis*.

In North America *Lymantria dispar* is an invasive species. It was imported into the USA from France that was accidentally introduced in Massachusetts in 1869, while *E. maimaiga* – natural enemy causing widespread epizootics in the areas of natural occurrence – was first recorded in North America in 1989. *E. maimaiga* probably comes from the introductions that took place in 1910 and 1911 when diseased gypsy moth cadavers collected in the Tokyo area were released near Boston, Massachusetts. However, no fungal infections were recovered and establishment was presumed to have failed. And until 1989, despite several attempts of introduction, neither *E. maimaiga* nor any other entomophthoralean fungus has ever been observed in the North American gypsy moth populations (Andreadis & Weseloh, 1990; Hajek et al., 1995). Since 1989 almost every year epizootics caused by *E. maimaiga* in the USA gypsy moth populations have been observed and *E. maimaiga* is considered the main factor suppressed outbreaks of *L. dispar* (Hajek et al., 1995; Hajek, 1997). During outbreaks of *L. dispar*, epizootics of nuclear polyhedrosis virus (NPV) are as common as fungal infections caused by *E. maimaiga* (Table 1). However, NPV requires high-density populations for development of epizootics, whereas *E. maimaiga* can cause high level of infection in low-density as well as high-density populations (Hajek et al., 1995). Furthermore, it was found that climatic conditions especially moisture is critical in the development of epizootics of *E. maimaiga*. Hajek (1997) found that in the year of low rainfall prevailed viral infections, whereas in year with more rainfall dominated fungal infections. In other surveys it was found that the greatest production of conidia of *E. maimaiga* by cadavers took place on the day of rainfall, whereas maximum germination of resting spores occurred 1-2 days after significant precipitation (Weseloh & Andreadis, 1992).

Mixed infections of entomopathogenic fungi and viruses are also common in other pathogen-host systems (Table 1). Ziemnicka (2008a) has confirmed that in the population of *Leucoma salicis* nuclear polyhedrosis virus acts as a density-dependent mortality factor and most epizootics were recorded at temperature above 15°C and low humidity. Some laboratory studies and field observations also indicated that fungi and viruses that occur within one insect's population might act in a synergistic manner (Malakar et al., 1999; van Frankenhuyzen et al., 2002; Ziemnicka, 2008b). For example, artificially induced viral epizootic in the population of *L. salicis* not interfere with the occurrence of the epizootic caused by *Beauveria bassiana*, which extended the population decline phase of 6 to 8 years (Ziemnicka, 2008b).

In Japan, outbreaks of *Syntypistis punctatela* in beech stands are known to occur synchronously among different areas at intervals of 8–11 years. Kamata (2000) has found that this periodicity is the result of several factors (predators, parasitoids, pathogens,



delayed induced defensive response), which act as time-delayed and density-dependent factors. Among them fungal disease caused by predominantly *Cordyceps militaris*, was considered to be the most plausible factor for generating cycles of the beech caterpillar population. Kamata (1998, 2000) observed the most severe infections caused by *C. militaris* at the population peak and in the period of its decreasing phase. His field observations also showed that infections caused by *C. militaris* affected the beech caterpillar population both in outbreak and non-outbreak areas. During outbreaks mortality of *S. punctatella* caused by *C. militaris*, *B. bassiana* and *P. farinosus* (= *I. farinosa*) ranged from 96 to 100% suggesting important roles of these parasites in natural control of this pest (Kamata, 1998).

Quite unique epizootic loci in the summer-autumn complex of the multispecies communities of forest lepidopterans and sawflies in Siberia had been discovered by Kryukov et al. (Kryukov et al., 2011). In this survey at least 30 species from 7 families were found to be *C. militaris* hosts. Field observations were carried out from 2007 to 2009 in three localities where the number of insects infected by *C. militaris* in the period of mass appearance of stromata ranged, depending on the location, an average from 0.5 to 1.5 specimens/m<sup>2</sup> (in some places even up to 20 specimens/m<sup>2</sup>). They also observed that with reduction in the number of caterpillars in the tree crowns also decreased the level of defoliation of trees (5-25%). Moreover, they did not find any living pupa in leaf litter on the trial sites.

Similarly, widespread epizootic caused by entomopathogenic fungi have been found in the population of the pine sawfly *Diprion pini* in Poland (Sierpińska, 1998). During outbreak of 1794 collected larvae 1826 isolates of fungi were isolated. The most frequently isolated species were *I. farinosa* (603 isolates), *C. militaris* (542), *B. bassiana* (311), and *L. lecanii* (179). These species have caused 30-80% mortality of overwintering larvae.

There are also examples (though very rare) epizootic caused by fungi of not fully proven insecticidal properties. Marcelino et al. (2009) discovered in the population of *Fiorinia externa* epizootic caused by *Colleotrichum* sp. - a fungus widely known as phytopathogen. Poprawski & Yule (1991) also determined in natural populations of *Phylophaga* spp. such phytopathogenic fungi as *Aspergillus*, *Penicillium* and *Fusarium*, but only occasionally, not as in the case of *Colleotrichum* on a large scale. Some authors consider that phytopathogenic fungi, under some circumstances, may infect and kill insects (Teetor-Barsch & Roberts, 1983; Poprawski & Yule, 1991).

Relatively few studies have investigated the influence of insects' behaviour on fungal infection and development of epizootics. For example, Hajek (2001) studied the unusual behaviour of late stage larvae of *L. dispar* to the risk of infection by *E. maimaiga*. Late instars larvae of *L. dispar* move down from the tree canopy and wander during daylight hours under leaf litter or in cryptic locations on tree boles. Hajek (2001) has found in field experiment that larvae caged over the soil were at much greater risk of *E. maimaiga* infection compared with larvae caged in the understory vegetation or on tree trunks. She also found that infections occurred with even brief exposures to the soil. The author assumes that infection of all larvae caged on the ground where initiated by germ conidia from germinating resting spores. The density of resting spores at the base of the tree is usually the highest. During epizootics, cadavers of late instars fall from tree trunks and *E. maimaiga* resting spores are leached into the soil. Therefore, such behaviour of larvae, rather uncommon in other Lepidoptera, to remain near tree base exposes them to the areas with highest titers of *E. maimaiga* resting spores.

Outbreak pest	Tree host or type of forest	Country	Epizootics/limiting factors	References
<i>Cinara pinea</i>	Pine forests	China	<i>Erynia canadensis</i>	Li et al., 1989
<i>Dendrolimus pini</i>	<i>Pinus silvestris</i>	Poland	<i>Isaria farinosa</i> <i>Cordyceps militaris</i> <i>Beauveria bassiana</i> <i>Lecanicillium lecanii</i>	Sierpińska, 1998
<i>Dendrolimus punctatus</i>	Masson pine	China	<i>Beauveria bassiana</i>	Ge et al., 2009,
<i>Fiorinia externa</i>	<i>Tsuga canadensis</i>	USA	<i>Colletotrichum</i> sp. <i>Lecanicillium lecanii</i> , <i>Beauveria bassiana</i>	Marcelino et al., 2009
<i>Hyblaea puera</i>	<i>Tectona grandis</i>	India	parasites, Buculovirus HpNPV and <i>Beauveria bassiana</i>	Gowda & Naik, 2008
<i>Leucoma salicis</i>	<i>Populus nigra</i>	Poland	nuclear polyhedrosis virus <i>Beauveria bassiana</i>	Ziemnicka, 2008a, 2008b Ziemnicka & Sosnowska, 1996
<i>Lymantria dispar</i>	<i>Larix leptolepis</i>	Japan	<i>Entomophthora aulicae</i> mixed infection with <i>Paecilomyces canadensis</i>	Aoki, 1974
" – "	Mixed hardwood forest	USA	<i>Entomophaga maimaiga</i> and nuclear polyhedrosis virus	Andreadis & Weseloh, 1990 Elkinton et al., 1991 Hajek, 1997
" – "	<i>Larix leptolepis</i> and <i>Alnus japonica</i>	Japan	nuclear polyhedrosis virus and mixed infection with <i>Entomophaga maimaiga</i> , and <i>Isaria javanica</i>	Shimazu & Takatsuka, 2010
<i>Malacosoma disstria</i>		New York and Maryland, USA	<i>Furia gastropachae</i> (= <i>Furia crustosa</i> )	Filotas et al., 2003
Multispecies communities of lepidopterans and sawflies	Different coniferous and deciduous forests	Siberia, Russia	<i>Cordyceps militaris</i> <i>Cordyceps</i> sp.	Kryukov et al., 2011
<i>Orgyia leucostigma</i>	<i>Abies balsamea</i>	Nova Scotia, Canada	<i>Entomophaga aulicae</i> , nucleopolyhedrovirus	van Frankenhuyzen et al., 2002
<i>Panolis flammea</i>	<i>Pinus contorta</i>	Scotland	<i>Isaria farinosa</i> , nuclear polyhedrosis virus <i>Entomophaga aulicae</i> , <i>Nomuraea rileyi</i> , <i>Beauveria bassiana</i>	Hicks et al., 2001 Hicks & Watt, 2000 Watt & Leather, 1988
<i>Syntypistis</i> (= <i>Quadricarifera</i> ) <i>punctatella</i>	<i>Fagus crenata</i>	Japan	<i>Cordyceps militaris</i> <i>Isaria farinosa</i> <i>Beauveria bassiana</i> ,	Kamata, 1998, 2000

Table 1. Examples of outbreaks of forest pests and epizootics caused by entomopathogenic fungi

Many insects spend at least part of his life in the soil. Soil is also a natural environment for entomopathogenic fungi. Therefore, such natural behaviours of insects related to their biology, such as accumulation in the soil or leaf litter to wintering or pupation, conducive to fungal infections and natural reduction of many insect pests. Tkaczuk and Miętkiewski (1998) determined the natural reduction of *Dendrolimus pini* population caused by entomopathogenic fungi during hibernation period. Microbiological analysis of the pine sawfly cocoons showed that entomopathogenic fungi were responsible for the mortality of

overwintering larvae, depending on location from 12 to 52%. Epizootics described by Kryukov et al. (2011) and Sierpińska (1998) also occurred during the overwintering of insects in the form of pupae and larvae in leaf litter.

On the other hand, many forest pests occupy cryptic habitats, where they are protected from direct contact with fungi, and where thermal and moisture conditions are not conducive to the development of fungal infection. For example, such group of insects are bark beetles (Curculionidae: *Scolitynae*). In the available literature we did not find studies that describe extensive epizootics in this group of insects, although there are numerous reports of entomopathogenic fungi isolated from the bark beetles (Bałazy, 1968; Glare et al., 2008; Brownbridge et al., 2010; Draganova et al., 2010; Tanyeli et al., 2010) and tested in the context of biological control this group of insects (Kreutz et al., 2004a; Draganova et al., 2007; Sevim et al., 2010; Tanyeli et al., 2010, Zhang et al., 2011)

## 4.2 Insect resistance to fungal infections

In the population of a particular host, individuals are not equally susceptible to infection. Different species of hosts are also not equally susceptible to infection from a particular species or strain of the pathogen. On the one hand, different species or even strains of the fungus may display different levels of virulence and parasitic specialization against a specific host. But on the other hand, susceptibility of the host may change with its development (larvae are usually more sensitive than adults) or may depend on its behaviour and individual resistance to infection. Insects have relatively primitive immunological system, although they can react to the entrance of the fungal pathogen inside their body. Cellular and humoral defence mechanisms, such as antimicrobial proteins, phagocytosis and multihemocytic encapsulation of fungal structures, have been observed. Encapsulation is always associated with production of melanin. After infection, the fungal propagules are encapsulated within melanin (melanization process). Melanins act antagonistic to fungi, inhibiting their growth (Butt, 1987; Hajek & St. Leger, 1994; Boguś et al., 2007).

They are also known examples of non-specific: morphological (Smith & Gula, 1982; Saito & Aoki, 1983) behavioural (Viliani et al., 1994; Myles, 2002) and physiological (Serebrov et al., 2006; Rohlf & Churchill, 2011) defence mechanisms to avoid fungal pathogens. Viliani et al., (1994) found in laboratory experiments that the application of mycelial particles in soil affected the behaviour of both larval and adult Japanese beetles, *Popillia japonica*. Grubs avoided soil that contained high concentrations of *Metarhizium anisopliae* mycelium for up to 20 days after application. Some insects have the ability to detect and alert the presence of an infected individual in the population. Very interesting behaviour in this regard have social insects such as termites. The presence of conidia-dusted termites in colony caused the alarm manifested by rapid bursts of longitudinal oscillatory movement by workers. The intensity of alarm peaked about 15 minutes after introduction of the conidia-dusted termites, at which time 80% of the termites were aggregated near the treated individual. Alarm and aggregation significantly subsided after 24 minutes and were then followed by grooming, biting, defecation, and burial of the infected termite (Myles, 2002). In defence against infection, insect may produce on the surface of its body fungistatic compounds that inhibit spore germination and growth. It was found that certain fatty acids on the surface of *Heliothis zea* larvae inhibit the germination of spores of

*Beauveria bassiana* and *Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus*). Non-pathogenic (saprophytic) fungi and bacteria occurring naturally on the insect cuticle could inhibit germination of spores of entomopathogenic fungi (Smith & Grula, 1982; Saito & Aoki, 1983).

## 5. Entomopathogenic fungi in biological control of insects

### 5.1 Strategies of biological control

Chemical insecticides are commonly used in plant protection. The consequence of this is to increase the resistance of insects to various chemical substances contained in plant protection products. Over 500 arthropod species now show resistance to one or more types of chemicals (Mota-Sanchez et al., 2002). Other serious problem is invasive species that are accidentally introduced to a new country or continent and which escape their coevolved natural pathogens or predators. This forces to seek new, alternative and more environmentally safe, methods of reducing outbreaks of pests. In recent years more attention paid to the possibility of using natural enemies, including entomopathogenic fungi, in control of insect pests. Aside from playing a crucial role in natural ecosystems, entomopathogenic fungi are being developed as environmentally friendly alternatives in agriculture and forestry. They can be increasingly exploited for forest pest management as biological control agents and in the attempts to improve the sustainability of forest ecosystem.

Biological control is defined as the use of living organisms to suppress the population density, or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Eilenberg et al., 2001). Thus, the aim of biological control is not a complete elimination of target species, but reducing its population below the economic threshold of harmfulness.

There are four strategies for biological control: classical, inoculation, inundation and conservation biological control. However, in forestry, only classical and inundation biological control strategies are widely used. Classical biological control is the intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control (Eilenberg et al., 2001). In the case of microorganisms, widely distributed in nature, the term exotic means the use of a particular strain or biotype, which is not native to the area where the pest is controlled. Introduced species to induce long-term effect has to acclimate to the area under certain climatic conditions, multiply and spread. So it is important to understand of the biology of species "exotic" and target, as well as the ability to monitoring its presence in the area. Inoculation biological control is also the intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently (Eilenberg et al., 2001). Inoculation involves releasing small numbers of natural enemies at prescribed intervals throughout the pest period, starting when the density of pest is low. The natural enemies are expected to reproduce themselves to provide more long-term control. Inundation biological control is the use of living organisms to control pests when control is achieved exclusively by the released organisms themselves (Eilenberg et al., 2001). In practice this means the release of large numbers of mass-produced biological control agents (so-called biopesticides) to reduce a pest population without

necessarily achieving continuing impact or establishment. Pest population is treated such a quantity of biopesticide to get immediate results (on the pattern of use of chemical insecticides). Conservation biological control is such modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests (Eilenberg et al., 2001). Conservation techniques involve the identification and manipulation of factors that limit or enhance the abundance and effectiveness of natural enemies.

## 5.2 Field application of entomopathogenic fungi

Attempts for practical application of entomopathogenic fungi, in classical or inundation biological control strategy, are always preceded by laboratory tests. Laboratory tests are mainly aimed to select for highly virulent strains, determine the optimal dose of inoculum, to examine the impact of biotic and abiotic factors on the fungus used and testing different methods of application (Lingg & Donaldson, 1981; Markova, 2000; Wegensteiner, 2000; Kreutz et al., 2004a; Dubois et al., 2008; Shanley et al., 2009; Augustyniuk-Kram, 2010; Zhang et al., 2011). Laboratory tests do not always coincide later with their practical use, but provide valuable information on the activity of entomopathogenic fungi and their potential role in biological control of many dangerous pests. In laboratory conditions, Markova (2000) studying the susceptibility of *Lymantria dispar* larvae, *Ips typographus*, *Hylobius abietis* beetles and diapausing *Cephaelia abietis* larvae on various strains of *Beauveria bassiana*, *Isaria farinosa* (= *Paecilomyces farinosus*), *Metarhizium anisopliae* and *Lecanicillium lecanii* (= *Verticillium lecanii*) obtained very different results. Larvae of *L. dispar* were susceptible to only one strain of *B. bassiana*, but this strain acted too slowly. Beetles of *Ips typographus* were susceptible to all tested strains, while the beetles of *H. abietis* were only susceptible to one strain of *M. anisopliae* and one strain of *B. bassiana*. Similarly *Cephaelia abietis* larvae were susceptible to infections by one strain of *B. bassiana* and one strain of *I. farinosa* (Markova, 2000). From other studies it is known that good results in the control of *L. dispar* were obtained using the entomophthoralean fungus *Entomophaga maimaiga* that is a major natural enemy in endemic Asian gypsy moth population. Nielsen et al. (2005b) assessed virulence and fitness of the six strains of *E. maimaiga* originating from Japan, Russia, China and North America in different gypsy moth population (originating from Japan, Russia, Greece and USA). They found that all *E. maimaiga* isolates tested were pathogenic to all populations of *L. dispar*, regardless of the geographical origin of the fungal isolates, with at least 86% mortality. However, fungal isolates differed significantly in virulence (measured as time to death) and fitness (measured as fungal reproduction) (Nielsen et al., 2005b).

The use of a microorganism in practice is not easy. The biggest problem is that it is very difficult to predict the effects of biological control agents before their release. The success of field trials depends on many factors that must be taken into consideration. Quite often observed is the phenomenon of lower efficiency of biological control agents applied in the field compared with laboratory tests. Among many factors such features of entomopathogenic fungi as high virulence against target insect, harmless for beneficial organisms (non-target species), warm-blooded animals and humans, high resistance to biotic and abiotic environmental conditions are critical in achieving satisfactory results in field trials (van Lenteren et al., 2003; Jackson et al., 2010). Impact on non-target organisms

Pest	Pathogen	Country	Methods	Effect	References
<i>Agrilus planipennis</i>	<i>Beauveria bassiana</i>	USA	Pre-emergent trunk spray (10 or 100 x 10 <sup>13</sup> conidia/ha) and polyester fiber bands impregnated with a sporulating culture of <i>B. bassiana</i> (6.4 x 10 <sup>8</sup> conidia/cm <sup>2</sup> )	Infection rates ranged from 58.5 and 83% at two application rates, longevity of females and males was significantly reduced, females laid fewer eggs, prolonged larval development. Fiber bands method - 32% mortality adult on treated trees vs. 1% on control trees.	Liu & Bauer, 2008
<i>Anoplophora glabripennis</i>	<i>Beauveria bassiana</i> , <i>B. brongniartii</i>	China	Two methods were compared: fiber bands fastened around tree trunks was compared with trunk sprays	Longevity was decreased by both strains compared with controls, with females killed earlier by <i>B. brongniartii</i> than by <i>B. bassiana</i> . This decrease in longevity was independent of the application method used. Daily oviposition rate per female were also reduced by both strains.	Dubois et al., 2004
<i>Ips typographus</i>	<i>Beauveria bassiana</i>	Germany	Introduction of <i>B. bassiana</i> -inoculated beetles into untreated population or natural population of beetles were lured into a pheromone traps and treated there with conidia of <i>B. bassiana</i>	Significant reduction in the length of maternal galleries and the number of larvae and pupae were observed. In experiment with pheromone traps significant reduction in the number of bore holes were observed. Additionally, no larvae, pupae and juveniles were found under the bark of trunks of treated trees.	Kreutz et al., 2004b

Pest	Pathogen	Country	Methods	Effect	References
<i>Lymantria dispar</i>	<i>Entomophaga maimaiga</i>	USA	Resting spores were released around the bases of oaks in 41 plots (2-years trials).	In the second year of study <i>E. maimaiga</i> infections were detected in 40 of the 41 release plots. Infection levels in release plots averaged 72.4 % and were associated with declining egg mass densities. The next year after the first application <i>E. maimaiga</i> was abundant 1,000 m from release plots.	Hajek et al., 1996
" - "	" - "	Bulgaria	Cadavers of <i>L. dispar</i> containing <i>E. maimaiga</i> resting spores	Only 6.3% larvae collected in experimental plots contained spores and resting spores of <i>E. maimaiga</i>	Pilarska et al., 2000
<i>Melolontha melolontha</i>	<i>Beauveria brongniartii</i>	Denmark	Barley kernels colonized by fungus were placed in holes of 10 cm depth around trees or barley kernels were mixed with the soil and placed around the new trees during re-plantation	30% more trees without damages	Eilenberg et al., 2006
" - "	" - "	Denmark	10 or 30 g barley kernels per tree were thrown directly into the planting hole before the tree was inserted into the hole	Significant effect was achieved 1 and 1/2 year after the application. Almost 13% of the untreated trees had been killed, 25% showed substantial discoloration and needle trees. None of the treated trees died and only single trees showed discoloration.	Eilenberg et al., 2006

Pest	Pathogen	Country	Methods	Effect	References
" – "	" – "	Switzerland	Blastospores were applied by helicopter at the swarming places. The mean dose $2.0-3.7 \times 10^{14}$ spores ha <sup>-1</sup>	The infection rates of the treated adults ranged between 30 to 99 %. The treatment reduced the average reproduction rate. The population density 6 and 9 years decrease 50-80%	Keller et al., 1997
<i>Strophosoma capitatum</i> and <i>S. melanogrammum</i> larvae	<i>Metarhizium anisopliae</i>	Denmark	Conidial suspension – $1.0 \times 10^{14}$ per ha; procedure was repeated twice, year after year	In the same year after the first application were not achieved satisfactory results. One year and two years after the first application the accumulated density of <i>S. melanogrammum</i> weevils was reduced by 60 and 40%. For <i>S. capitatum</i> only in the first year after the first application achieved a reduction of up to 38% of beetles.	Nielsen et al., 2007

Table 2. Examples of biocontrol of forest pests by using entomopathogenic fungi

must always be considered as a side effect during field applications of entomopathogenic fungi. The results of numerous works suggest little impact of entomopathogenic fungi on non-target organisms (James et al., 1995; Parker et al., 1997; Traugot et al., 2005; Nielsen et al., 2007). And finally, the use of entomopathogenic fungi on a large scale depends above all on the possibility of cheaper mass rearing on artificial media. Unfortunately, most fungal biopesticides are produced on the basis of hypocrealean fungi, among which the majority belongs to polyphagous species that is a broad spectrum of potential hosts. Entomophthoralean fungi amongst many species are more specialized (monophagous), therefore not of interest among potential producers of mycoinsecticides because of difficulties in their cultivation on artificial substrates and the multiplication of infective material on a mass scale (Pell et al., 2001).

Examples of practical application of entomopathogenic fungi can be found in Table 2. Each example briefly describes the method of application and the final effect measured in the reduction of the target insect populations, or health status of trees. In the described examples, depending on the target insect and fungus, different methods of application were used. In the case of soil-dwelling pests the most appropriate and most common method is introduction into the soil barley kernels colonized by fungus. In European countries, this method is widely used against larvae of *Melolontha melolontha* in various crops (Keller et al., 1997; Fröschle & Glas, 2000; Bajan et al., 2001; Vestergaard et al., 2002). Recently, a



commercial product based on barley kernels colonised by *Beauveria brongniartii* (Melocont®-Pilzgerste) was tested under EU-funded project BIPESCO which aim was to study and develop entomopathogenic fungi for the control of subterranean insect pests like scarabs and weevils. Another fairly common method is the use of fiber bands (=fungal bands) impregnated with entomopathogenic fungi and placed around tree trunks or branches. This method was first developed as a biological control method to control adults of Japanese pine sawyer, *Monochamus alternatus*, the main vector of pine wilt disease caused by the pinewood nematode, *Bursaphelenchus xylophilus* (Shimazu, 2004). Currently, this method gives satisfactory results in biological control of invasive species *Anoplophora glabripennis* and *Agrilus planipennis* (Table 2). Despite rare reports of natural epizootics among bark beetles, attempt to biological control, particularly laboratory tests, are very promising. In laboratory studies efficacy of entomopathogenic fungi against bark beetles is very high, reaching up to 80-100% for some strains (Draganova et al., 2007; Sevim et al., 2010; Tanyeli et al., 2010). In field trials Kreutz et al. (2004b) also achieved significant reduction in number of larvae, pupae and juveniles. According to Hunt, as high mortality of bark beetles may be associated with the lack in their cuticle certain lipids that inhibit germination of fungal spores (Hunt, 1986).

### 5.3 Conservation biological control and Integrated Pest Management (IPM strategy)

Recently more attention is focused on the study of entomopathogenic fungi in natural environments, and hence in conservation biological control strategy. Conservation biological control is a strategy in which forest management and environmental manipulations are adopted to enhance conditions for the development of different groups of natural enemies of pests. In forestry conservation biological control requires actions on a large scale, not only in wooded areas, but also beyond, in adjacent areas. In Europe, an example of pest that control is not limited to one environment is the previously mentioned *Melolontha melolontha*. Adults are pests of deciduous trees. Eggs are laid in the soil usually in the areas adjacent to large forest complexes such as arable fields, nurseries, orchards, where the larvae feed on roots.

The use of entomopathogenic fungi, particularly in this strategy, requires a thorough knowledge of the biology and ecology of both pests and their natural enemies. It also requires recognition of factors that may interfere with their effectiveness. Conservation biological control also needs long-term and large-scale researches on multitrophic relationships between natural enemy and their hosts and their impact on natural regulation of serious insect pests (Tschardt et al., 2008).

Natural enemies and their potential hosts always exist within a particular biocenosis, where are affected by abiotic and biotic factors. One of them are pesticides that kill natural enemies as well as their potential hosts (Miętkiewski et al., 1997; Chandler et al., 1998; Meyling & Eilenberg, 2007). For this reason, many countries prohibited the use of chemical insecticides to control forest pests (e.g. Denmark). Therefore the only alternative is biological or integrated pest control. Integrated Pest Management in forestry is defined as “a combination of prevention, observation and suppression measures that can be ecologically and economically efficient and socially acceptable, in order to maintain pest populations at a

suitable level” (FAO, 2011). Biological control of pests through the use of natural enemies and other methods like mechanical control, planting proper trees in proper sites during reforestation is preferred over synthetic pesticides. Such actions favour sustainable control and efficiency of natural enemies (FAO, 2011). The previously mentioned project BIPESCO was intended to help replace or reduce the input of chemical pesticides in European agriculture, forestry and horticulture.

Numerous studies indicate that forest communities display greater diversity of entomogenous species than arable fields. Bałazy (2004) compared species richness of entomopathogenic fungi in forest areas (mainly in protected areas like national parks) with areas used for agriculture found that out of all 210 species over 60% was collected in the forest protected areas. Only 20 species were found in annual crops. Significantly more infected host cadavers were found in other habitats such as extensively utilized meadows, mid-field, shelterbelts, woodlots and swamps. Evans (1974) also found that even in forests with very high biodiversity as tropical rain forests, the diversity of entomopathogenic fungi in damaged forests is less than undisturbed, and that the incidence of natural epizootic decreased. Such natural and semi-natural habitats are often refuges for insects and entomopathogenic fungi. Thus, the protection of this group of pathogens and their natural habitats is also important question in the context of conservation of biodiversity. Improving our understanding of the ecology of entomopathogenic fungi is also essential to further develop these organisms in biological control of many serious insect pests.

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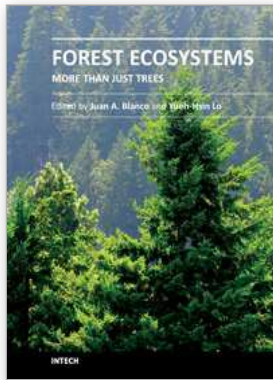
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## **Forest Ecosystems - More than Just Trees**

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The common idea for many people is that forests are just a collection of trees. However, they are much more than that. They are a complex, functional system of interacting and often interdependent biological, physical, and chemical components, the biological part of which has evolved to perpetuate itself. This complexity produces combinations of climate, soils, trees and plant species unique to each site, resulting in hundreds of different forest types around the world. Logically, trees are an important component for the research in forest ecosystems, but the wide variety of other life forms and abiotic components in most forests means that other elements, such as wildlife or soil nutrients, should also be the focal point in ecological studies and management plans to be carried out in forest ecosystems. In this book, the readers can find the latest research related to forest ecosystems but with a different twist. The research described here is not just on trees and is focused on the other components, structures and functions that are usually overshadowed by the focus on trees, but are equally important to maintain the diversity, function and services provided by forests. The first section of this book explores the structure and biodiversity of forest ecosystems, whereas the second section reviews the research done on ecosystem structure and functioning. The third and last section explores the issues related to forest management as an ecosystem-level activity, all of them from the perspective of the other parts of a forest.

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