IMPACT OF THE RELEASE OF ENTOMOPATHOGENS IN THE ENVIRONMENT[1]

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ABSTRACT - The environmental impact of entomopathogens depends on two factors: the fate of the pathogen after its release, and the effects of the pathogen on various components of the environment. Three factors affect the fate of a released entomopathogen: its persistence, population growth, and dispersal. Environmental fate after release varies with the pathogen species and environmental factors. Data concerning environmental effects after release are scanty but somewhat similar for viruses, bacteria and fungi. The major demonstrated effects are the increase in numbers of pathogen units in the environment and decrease in host-insect numbers and damage to crop plants. Viruses and bacteria have been demonstrated to harm invertebrate parasitoid and predator populations, primarily by reducing their common resource, the host insect population. However, they rarely have as severe an effect as chemical insecticides. The potential environmental impact of recombinant-DNA entompathogens has become a primary consideration in their release for microbial control. There are three major concerns about releasing such organisms: they might have unexpected, deleterious properties after release; they could cause ecological disruptions as have other biological introductions; and they could transfer genetic material to other organisms, causing the first two areas of concern to resurface. Overall, R-DNA organisms are perceived as having a low probability of causing environmental harm but potentially severe consequences if harm occurs.

Index terms: environmental impact, microbial control, biopesticides, recombinant-DNA, entomopathogens.

IMPACTO DA LIBERAÇÃO DE ENTOMOPATÓGENOS NO AMBIENTE

RESUMO – O impacto dos entomopatógenos sobre o ambiente depende de dois fatores: o destino do patógeno depois da sua liberação e os efeitos do patógeno sobre os vários componentes do meio. Três são os fatores que afetam o destino do entomopatógeno liberado: a sua persistência, crescimento populacional e dispersão. O destino no meio ambiente depois da liberação varia de acordo com a espécie do patógeno e com fatores ambientais. Dados referentes aos efeitos sobre o meio ambiente são escassos, mas são semelhantes para vírus, bactérias e fungos. Os maiores efeitos demonstrados são o aumento em número dos patógenos no meio, a diminuição dos insetos-hospedeiros e diminuição de danos nas plantas cultivadas. Foi demonstrado que os vírus e bactérias danificam os grupos de parasitóides e de predadores principalmente ao diminuírem a sua fonte comum, a população dos insetos-hospedeiros. Contudo, eles raramente provocam efeitos tão fortes quanto os inseticidas químicos. O impacto potencial de entomopatógenos DNA-Recombinantes sobre o meio tornou-se uma consideração primordial na sua liberação para controle microbiano. Há três aspectos importantes sobre a liberação desses organismos: após a liberação eles podem provocar rupturas ecológicas e outras introduções biológicas; e podem transferir material genético para outros organismos, levando ao ressurgimento dos dois primeiros aspectos. Finalmente, organismos R-DNA têm poucas probabilidades de causarem danos ao meio, mas podem levar a conseqüências severas se isto acontecer.

Termos para indexação: impacto sobre o meio ambiente, controle microbiano, biopesticidas, DNA-recombinante, entomopatógenos.

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INTRODUCTION

The environmental impact of releases of entomopathogens has been an area of research interest for at least 20 years, and its importance has not diminished. The reason for the current interest is that risk assessment of imminent releases of genetically engineered entomopathogens requires knowledge of previous impacts of the parental organisms. Also, for various reasons, risk, assessment of genetically engineered microorganisms has raised new environmental concerns about releasing natural strains of entomopathogens (Fuxa 1990). Entomopathogens have had only relatively minor environmental impacts in the past (Fuxa 1989, Laird et al. 1990), and their relative safety has become the primary rationale for their development and usage in developed nations. However, increased concerns about environmental effects and food safety of pesticides in these nations has assured that environmental releases of all agents, whether biological or chemical, will be intensively scrutinized.

Environmental impact of a biological release depends on two factors, the fate of the organism and its effect on various components of the environment. Fate is an important consideration because the release of a harmful agent can be acceptable if that agent does not persist or spread in the environment. Thus, the public in developed nations was not overly concerned about harmful effects of chemical pesticides as long as it was thought that these effects were largely confined, for example, to the top few centimeters of soil in an agricultural field. However, if these same chemicals can contaminate groundwater that supplies drinking water, then the potential impact and public perception change. Since there is some concern about the environmental effects of biological releases, the environmental fate of entomopathogens is critical to their development for biological control because they are living, replicating agents.

There will be three purposes to the current paper. The first will be to review the fate of previous releases of natural strains of entomopathogens. The second will be to review environmental effects of such releases. These reviews will include the entomopathogenic bacteria, viruses, and fungi, the three groups for which releases of recombinant-DNA organisms is the most likely in the near future. The third purpose will be to evaluate the concerns about releasing genetically engineered entomopathogens.

FATE OF ENVIRONMENTAL RELEASES

The environmental fate of a biological release or introduction depends on three factors: the organism's persistence, dispersal, and population growth. The environmental fate of released entomopathogens has varied with the pathogen species and environmental factors. Environmental fate determines how widespread and persistent will be the effects, whether positive or negative, of a biological release.

Persistence of released entomopathogens

Persistence of entomopathogens in the environment has been the subject of far more research than the other two components that determine an organism's fate after release. Generally, persistence has been greater in host populations or soil than on vegetative surfaces.

Viruses - Virtually all the research of viral persistence has concerned the baculoviruses; the long-term persistence of viruses is primarily in soil and in primary, secondary, and alternative hosts.

There is little doubt that the soil reservoir contributes to long-term persistence of baculoviruses. Nuclear polyhedrosis viruses (NPVs) of *Lymantria dispar* and *Orgyia*

pseudotsugata in forests persisted at least one year in soil after release (Podgwaite et al. 1979, Thompson & Scott 1979). Evaluations of persistence in soil after release in row crops indicated that these NPVs persist at least over winter or one year; examples include the NPVs of *Heliothis armigera, Mamestra brassicae, Pseudoplusia includens,* and *Trichoplusia ni* (Thomas et al. 1972, Roome & Daoust 1976, Young & Yearian 1979, Evans 1982). NPVs have been detected in soil up to 2-5 years after release for control of *P. includens* and *T. ni,* though in at least one case this was assisted by recycling of the virus in the insect population (Jaques 1967, 1969, McLeod et al. 1982). A *Pieris brassicae* granulosis virus (GV) persisted 2 years in soil (David & Gardiner 1967), and a *Pieris rapae* GV had a half-life in soil of 8-9 weeks after release (Payne 1982).

The host insect population is the other reservoir in which insect viruses have persisted for long time periods after release, though there also are examples of lack of persistence in this fashion. The NPVs of *Choristoneura fumiferana* and *Neodiprion lecontii* persisted 1-2 years in host populations after their releases into forests (Morris et al. 1974, deGroot et al. 1979). The *Anticarsia gemmatalis* NPV persisted in the host population for 3 years after one release in a row crop but did not persist after another (Beach et al. 1984, Richter & Fuxa 1984); similarly, the *Neodiprion sertifer* NPV persisted from year to year in host insects in one case but did not persist in another (Bird 1961). The *Adoxophyes orana* GV persisted 3 months in host insects (Shiga et al. 1973). The *Oryctes* nonoccluded baculovirus is well known for its persistence. This virus persisted in host populations 0-38 weeks at different sites in one study with *O. rhinoceros* (Gorick 1980), and in other research it survived 17 months to 7 years in populations of *O. monoceros* and *O. rhinoceros,* respectively (Young & Longworth 1981, Lomer 1986).

Insect viruses survive only a matter of days on vegetative surfaces. The GVs of *Laspeyresia pomonella* and *P. rapae* and the NPVs of *A. gemmatalis, Heliothis armigera, Heliothis* spp., *Neodiprion taedae linearis,* and *T. ni* persisted over a range of 1-32 days after release on foliage or fruit surfaces (Jaques 1967, Ignoffo et al. 1972, 1974, 1976b, Young & Yearian 1974, 1986a, Roome & Daoust 1976, Moscardi et al. 1981, Tatchell & Payne 1984). The NPVs of *M. brassicae* and *T. ni* persisted 80 days and 12 weeks, respectively, when the virus recycled in the host population (Ignoffo et al. 1980, Evans & Allaway 1983).

One study has examined the persistence of NPV in bird feces, which is important to biotic transport of these viruses. The NPV of *Gilpinia hercyniae* survived passage through bird guts for 3 days and was still viable in the feces (Entwistle et al. 1978).

Bacteria - Bacterial persistence after environmental release is similar to that of the viruses in that the bacteria retain activity for relatively long periods in soil or host populations but not on vegetative surfaces. Additionally, bacterial persistence in water and air has received some attention from researchers.

Entomopathogenic bacteria have persisted for months or years in soil after various releases. *Bacillus thuringiensis* has retained viability in soil for periods ranging from 8 weeks to almost 3 years (Saleh et al. 1970, West et al. 1984, Petras & Casida 1985, Benz 1987). Also, *Bacillus popilliae* var. *melolonthae* persisted 2 years (Hurpin & Robert 1976), and *Bacillus sphaericus,* a pathogen of mosquito larvae, was still viable in soil of a roadside ditch 9 months after its release (Hertlein et al. 1979). *B. popilliae* also has been known to persist 45 years in soil, though this included recycling in host insect populations (Hutton & Burbutis 1974).

Virtually all the research of persistence of entomopathogenic bacteria on vegetative surfaces has involved *B. thuringiensis.* In both trees and row crops, this bacterium has remained viable 30 days or less on foliage

(Smirnoff et al. 1973, Ignoffo et al. 1974, Morris 1977b, Frye et al. 1983, Sneh et al. 1983); the half-life generally has been measured at less than 1 day, though it can be as long as 2.5 - 8 days (Ignoffo et al. 1974, Pinnock et al. 1975, 1978, Lynch et al. 1980, Sorensen & Falcon 1980). The *B. thuringiensis* δ-endotoxin and β-exotoxin have retained activity for 20 and 12 days, respectively. *B. thuringiensis* has been known to persist for 2 years on bark (Benz 1987), perhaps due to the increased possibility of being protected from sunlight in cracks and crevices.

Bacterial persistence in water and air has been studied with *B. thuringiensis* and *B. sphaericus*. *B. thuringiensis* was detected by spore count (i.e., viability was not determined) up to 30 days after release into a river and for 17 days in the air after high-altitude spraying (Smirnoff et al. 1973, Buckner et al. 1974). Two mosquito pathogens, *B. thuringiensis* var. *israelensis* and *B. sphaericus*, usually are detected and remain viable in water for less than 7 days (Mulligan et al. 1978, 1980), though the latter has remained viable for at least 28 days (Mulligan et al. 1980). However, if *B. sphaericus* recycles in the host population, then this bacterium can persist in water for at least 20 - 60 days (DesRochers & Garcia 1981, 1984).

Fungi - Most studies of fungal persistence after release have been in row crops, though there have been a few in other ecosystems. As with the viruses and bacteria, long-term persistence of entomopathogenic fungi has been in soil or in host populations.

The fungi generally have persisted in soil or leaf litter for 1 year or less, though persistence of 1 - 2 years has been observed. *Beauveria bassiana* has persisted in soil for at least 15 days to at least 1 year after release (Watt & LeBrun 1984, Müller-Kögler & Zimmerman 1986), and *Metarhizium anisopliae* has remained viable at least 2 years in compost heaps (Latch & Falloon 1976). The other studies have been with *Nomuraea rileyi*; this fungus has remained viable in soil in row crops for 93 - 450 days (Sprenkel & Brooks 1977, Ignoffo et al. 1978a, Thorvilson et al. 1985).

Several fungal species have persisted for long periods in host populations. *B. bassiana* and *N. rileyi* have persisted for 32 weeks and 105 days, respectively, in host cadavers (Thorvilson et al. 1985, Daoust & Pereira 1986b). Persistence in living host populations has been studied in a variety of ecosystems and has ranged from 2 months to 22 years. The fungal species included *Coelomomyces indicus*, *Coelomomyces punctatus*, *Coelomomyces stegomyiae*, *M. anisopliae*, and *Myiophagus ucrainicus* (Gad & Sadek 1968, Chapman 1974, Young 1974, Weiser 1982, Hänel & Watson 1983, Latch & Kain 1983, Laird 1985).

Though there are exceptions, the entomopathogenic fungi persist on plant parts only a few days. *B. bassiana* remained viable 5-21 days on plant surfaces (Gardner et al. 1977, Ignoffo et al. 1979, Daoust & Pereira 1986a), though it was able to survive inside corn stalks throughout a growing season (Lewis & Cossentine 1986). *M. anisopliae* survived 3 days on plant parts (Daoust and Pereira 1986b), and *N. rileyi* conidia had a half-life of 2-10 days on vegetation (Ignoffo et al. 1976a, Gardner et al. 1977). *Triplosporium fresenii* survived over winter in an orchard (Bitton et al. 1979).

There have been two studies of fungal persistence in aquatic habitats. *Lagenidium giganteum* persisted at least 96 days in flooded woodland (Jaronski & Axtell 1983) and 85 days in a rice field (Kerwin & Washino 1986). In both cases the fungus was recycling in the host population.

Dispersal of released entomopathogens

Dispersal of an entomopathogen after release is one of the most important factors in a biological release and is a particular concern in risk assessment of genetically engineered microorganisms. The reason for this is that, if

dispersal of an organism can be predicted and controlled, then that organism can be tested in small field plots even if it is potentially persistent and harmful. If it can be contained, the harmful effects will be very localized, and the organism can probably be destroyed eventually.

Dispersal of entomopathogenic viruses has been the subject of several studies, but dispersal of the bacteria and fungi is only poorly understood. The methods of transport and dispersal of entomopathogenic viruses, bacteria, and fungi have been reviewed previously (Fuxa 1991). Dispersal of all three groups is a major need for further work.

Viruses - As with persistence, virtually all the research of dispersal of entomopathogenic viruses has been with releases of baculoviruses. The transport mechanisms in these cases have not always been identified, but in other cases these viruses have been dispersed by infected host insects, parasitic or predatory arthropods, birds, spray drift, or wind-blown leaves or larvae.

Dispersal distances have been estimated for a number of NPV releases. In row crops, dispersal has ranged from the *Heliothis zea* NPV being transported throughout a soybean plant (Ignoffo et al. 1978b) to the same virus being moved 240 m in 45 days (Gard 1975). Other NPVs released in row crops have dispersed intermediate distances: *A. gemmatalis* NPV, 69 m in 1 year (Richter and Fuxa 1984); *H. zea* NPV, 180 m (Smith et al. 1984); and *M. brassicae* NPV, 2.5 m (Evans & Allaway 1983). Dispersal in forests has been even more variable. The NPVs of *C. fumiferana* and *L. dispar* did not spread after release (Cunningham 1982), and dispersal of *Malacosoma disstria* NPV and *N. sertifer* NPV ranged from 0-32 km and 0-40 ha, respectively (Bird 1961, Stairs 1965). Other releases in forests (NPVs of *Diprion* (= *Gilpinia*) *hercyniae, Hyphantria cunea, L. dispar, Lymantria monacha, N. lecontii,* and *N. sertifer*) resulted in viral transport ranging from 30-850 m or over 290 ha (Bird 1961, Hukuhara 1973, deGroot et al. 1979, Burges 1981c, Cunningham & Entwistle 1981, Cunningham 1982, Entwistle et al. 1983). Birds have been estimated to have transported NPVs 805 m (Burges 1981c) or up to 7 km/day (Entwistle et al. 1978). In one case NPV transport through trees and open ground was estimated at 1-188 m (Suzuki & Kunimi 1981).

There are fewer data regarding transport distances for GVs and nonoccluded baculoviruses. The dispersal of the *A. orana* GV was estimated as "low" after release in an orchard (Shiga et al. 1973). On the other hand, the *Oryctes* baculovirus is noted for its dispersal. This virus has been estimated to have a rate of spread of 1-3 km/month (Young 1974, Gorick 1980, Lomer 1986).

Bacteria - Dispersal of bacteria has seldom been studied, perhaps because the best-known entomopathogen, *B. thuringiensis*, seems to have little capability for spread in the environment. Estimates of dispersal of this bacterium include zero spread, 15 km due to spray drift (Dulmage & Aizawa 1982), and worldwide dispersal in grain due to human trade (Burges & Hurst 1977). *B. thuringiensis* var. *israelensis* has spread over areas of 3.4 - 7.3 ha or distances of 250 m due to water flow (McLaughlin & Vidrine 1985, Finch et al. 1986, Gibbs et al. 1986). The only other bacterium whose dispersal has been estimated is *B. popilliae*. This bacterium has been observed to spread into nearby pastures and fields after its release (Klein 1981).

Fungi - Dispersal of entomopathogenic fungi is a major unknown in the epizootiology of insect diseases. The fungi have the potential for long-range dispersal due to the possibility that conidia or other spore-like stages can be carried great distances by wind. However, there have been few studies of fungal dispersal and almost no studies of wind as a transport mechanism for entomopathogenic fungi.

Perhaps the most accurate estimates of

fungal spread have been in studies in which the transport mechanism involved insect hosts or spray drift. Insect hosts have transported *N. rileyi* throughout a soybean plant (Ignoffo et al. 1977), *M. anisopliae* up to 300-400 m (Latch & Falloon 1976), and *Massospora cicadina* at least 132 m (Lloyd et al. 1982), though the latter did not involve a release. Other studies have included more general observations. *Aschersonia* spp. did not spread "readily" in citrus (Burges & Hussey 1971b); Entomophthoraceae did not spread in adverse conditions (Wilding 1981); *Hirsutella thompsonii* did spread to untreated areas (McCoy & Selhime 1977); *N. rileyi* did not spread to untreated plots at a distance of 700 ft in one study (Ignoffo et al. 1976a) but might have spread over 0.8 ha in another (Fuxa 1984); and *Verticillium lecanii* spread to adjacent plots (Hall 1980). In an aquatic study, *L. giganteum* spread throughout 800 m^2 due to water current (Jaronski & Axtell 1983).

Population growth of released entomopathogens

Population growth or decline of a released entomopathogen is heavily interdependent with persistence and dispersal and certainly is important to risk assessment. For example, if a pathogen population persists and grows after a release, it almost certainly will be transported outside the release site, even if only at a slow rate or over short distances. Population growth has been included in only a few studies of entomopathogen releases.

Viruses - Viruses have received the most research attention among entomopathogens as far as population growth after release is concerned. There probably have been a large number of releases in which the virus did not persist in the environment, and in which viral population density was not estimated or reported.

Increases as well as decreases in viral population density have been observed after releases in row crops and forests. Viral populations have increased in row crops by 12-84X after 6 wk for *Phthorimaea operculella* GV (Matthiessen et al. 1978), by >800X after 1 year for *P. includens* NPV (McLeod et al. 1982), and by >6X after 4.5 months for *T. ni* NPV (Thomas et al. 1972). However, populations have decreased by 73-99% after 95 days for *A. gemmatalis* NPV (Young & Yearian 1986b), by 99.8% after 2 weeks for *P. rapae* GV (Tatchell & Payne 1984), by 22% after 1 season and 96% after 2 seasons for *P. includens* NPV (Young & Yearian 1979), and by 32% over 4.5 months and 85% over 318 weeks for *T. ni* NPV (Thomas et al. 1972, Evans 1986). In forests, the population of *Lymantria dispar* NPV did not increase over natural levels after 1 year (Podgwaite et al. 1979), the *Neodiprion sertifer* NPV produced 0.95-8.35 polyhedral inclusion bodies (PIB) per PIB released after 25 days (Mohamed et al. 1983), and the *O. pseudotsugata* NPV population grew by 100% after one release and decreased by >99.9% over 1 year after another (Thompson & Scott 1979).

Bacteria - Bacterial population growth after release has been estimated only once, perhaps because the best studied bacterium, *Bacillus thuringiensis*, has so little persistent activity. *B. popilliae* reached levels of 100 billion spores/kg of soil in the upper inch after release (Faust & Bulla 1982), though this bacterium does not replicate in soil, water, or food preparations (Burges 1979). *B. thuriengiensis* does not grow saprophytically in the environment except in very limited niches (Burges 1982). However, *B. sphaericus* can grow saprophytically in polluted water (Burges 1982), and it can produce 10^5 to 10^6 spores per mosquito cadaver in laboratory and in the field (Davidson et al. 1984, DesRochers & Garcia 1984).

Fungi - There have been no direct estimates of population density resulting from field releases of fungi, though in certain cases

rough estimates can be made from the literature. Like some bacteria, certain fungi can grow saprophytically in the environment. It has been estimated in the laboratory that pathogen units of *B. bassiana* increase 2-10X in soil (Gottwald & Tedders 1984, Storey Gardner 1987), and *Hirsutella thompsonii* has been formulated to grow saprophytically after application to foliage (McCoy 1978). Percentage change in fungal population density after release can be estimated from data for % mortality over time and host population density. For exemple, *Lagenidium giganteum* introduced into a rice field controlled mosquitoes, but the number of resulting dead insects (and, presumably, the number of pathogen units, assuming short persistence) declined by ca. 90% in 62 days (Kerwin & Washino 1986). *N. rileyi,* which is typically released at a rate of ca. 10^{13} conidia/ha., can in turn produce 2.5 x 10^{13} to 2.5 x 10^{16} conidia/ha. (Ignoffo 1981, 1985).

Conclusions about environmental fate

We have only a few data about certain aspects of environmental fate of entomopathogens after their release, such as dispersal of fungi and population growth of bacteria and fungi. Also, we do not have a good conceptual understanding of aspects of environmental fate in which there have been numerous studies, such as persistence of viruses. For example, we do not know why some viruses (e.g., the NPVs of *A. gemmatalis* and *N. sertifer*) have established in the environment after certain releases but not after others. This will make prediction of population dynamics for risk assessment somewhat difficult.

ENVIRONMENTAL EFFECTS OF ENTOMOPATHOGEN RELEASES

In addition to environmental fate, the other factor determining entomopathogen impact on the environment is the actual positive or negative effects on various environmental components. This paper will review only the effects observed after actual releases of entomopathogens, not laboratory or microcosm studies.

Environmental effects of viruses

Certain environmental effects of viruses released for insect control have been studied comprehensively, but others have received little attention. The effects studied comprehensively are the ones for which releases are intended: increasing the pathogen population, decreasing the insect population, and increasing vegetation or fruit growth. The literature is replete with examples of reductions in host insect population density and damage (Burges & Hussey 1971a, Burges 1981a, Kurstak 1982).

The viruses have the potential to cause an important side effect on the host insect population, the induction of resistance to disease. Resistance has never been demonstrated definitively after release of an entomopathogenic virus, perhaps because the viruses generally have not been applied as widely as chemical insecticides. However, resistance that results from the release of a virus might have caused reduced infection rates in one case (Briese & Podgwaite 1985). On the other hand, after 27 yr there was no indication of resistance in *Diprion* (= *Gilpinia*) *hercyniae* populations to an NPV that was released accidentally and established in North America (Cunningham 1978). Nevertheless, the possibility of resistance should be considered when release strategies are being designed.

The effects of entompathogens on non-target organisms have been the subject of much research, though the great bulk of this has been in the laboratory. Lautenschlager et al. (1978, 1979) found no adverse effects on mice, voles, or birds after field release of *L. dispar* NPV. Field application of *Choristoneura fumiferana* NPV did not harm small mammals (Buckner et al. 1975). Cabbage for human consumption can contain

4.6 x 10^6 to 3.3 x 10^{10} NPV PIB/kg, apparently resulting from natural epizootics, with no potential for causing ill effects (Thomas 1975).

Viruses released for insect control can indirectly harm nontarget organism (parasites and predators) by competitive displacement or by reducing a common resource, namely, the host insect population. Applications of *Adoxophyes orana* GV in orchards (Shiga et al. 1973) and *Heliothis armigera* NPV in sorghum (Teakle et al. 1985) apparently reduced insect parasitoid populations in this manner. Application of *C. fumiferana* NPV and entomopox virus in forests had no overall effect on parasitoid populations (Morris 1977a), and application of *S. frugiperda* NPV in corn reduced parasitoid populations in some tests but had no effect in others (Hamm & Ware 1982). Similarly, application of *Pseudoplusia includens* NPV reduced parasitism by the entomopathogenic fungi *Nomuraea rileyi* and *Entomophthora* sp. (Holloway 1971), and the *Anticarsia gemmatalis* NPV reduced parasitism by *N. rileyi* (Moscardi et al. 1981, Richter & Fuxa 1984, Moscardi & Ferreira 1985). On the other hand, aerial application of *L. dispar* NPV in forests had no effect on populations of mice, voles or birds (Lautenschlager et al. 1978, 1979). Populations of these animals probably were not affected as much as the parasitoids or pathogens in the previous examples because the latter were more host specific and thus dependent on the host insect for a food source.

Little is known about other effects of released viruses on nontarget organisms. Recombination can occur between closely related baculoviruses (Faulkner & Boucias 1985). Presumably, therefore, genes in a released baculovirus could be distributed into field populations of related viruses. Also, it has been demonstrated that the ingestion of one baculovirus can activate a presumably latent infection of another baculovirus in an insect (Longworth & Cunningham 1968, Jurkovícová 1979, McKinley et al. 1981), but this has not been demonstrated in field populations.

The only known effect on soil of viral application is the increase in the viral population; the virus cannot grow in the soil substrate thereby causing some adverse effect, and the viral proteins are not known to be toxic to or a food source of any soil organisms. Though a relatively heavy viral population can accumulate and persist in soil, little virus can be found below a depth of about 10 cm after either natural epizootics or artifical apllication (Jaques 1969, 1970; Hukuhara 1973; Thompson & Scott 1979; Entwistle 1986).

There are virtually no data concerning viral effects in water or air. Entomopathogenic viruses can be transported through the air by aerosols, insects, and other agents, but it is unlikely that they are transported or persist by themselves in the air (Bird 1961) because they are killed by sunlight. Naturally occurring entomopox virus populations were rapidly diluted by water currents in flood control channels (Harkrider & Hall 1978). Finally, because of their adhesion to particles in layers near the surface of soil, it is not likely that NPVs or GVs will contaminate groundwater (Jaques 1975).

One positive, indirect environmental effect of viruses for insect control is likely but has not been well demonstrated. These viruses would replace a proportion of chemical insecticides currently used and thereby alleviate to some degree their adverse environmental effects: pollution of air, soil, and water; hazards to nontarget organisms; insect resistance; and outbreaks of secondary pests. For example, it has been estimated that the *Heliothis* NPV, if it could be made practical for insect control, could reduce the use of chemical insecticides in cotton by 7.7 X 10^6 kg annually (Ignoffo & Anderson 1979).

Environmental effects of bacteria

Environmental effects of entomophathogenic bacteria, like those of the

viruses, are largely innocuous. The major effects are a short- or long-term increase in the pathogen population, a decrease in the target insect population, and a decrease in damage to host plants (Burges & Hussey 1971a, Burges 1981a, Kurstak 1982).

In addition to lowering population densitities of target insects, bacteria can have a qualitative effect on the host population by induction of resistance. Insect resistance to bacteria applied in the field has not yet been demonstrated, although resistance to *B. thuringiensis* in *Plodia interpunctella* has occurred in stored grain (McGaughey 1985). In contrast, *Spodoptera littoralis* populations in the field and laboratory became more susceptible after exposure to *B. thuringiensis* (Sneh & Schuster 1983). It is possible that scarab beetles could become resistant to *B. popilliae* after 12 yr in the field, but there are not yet enough data to support that conclusion (Dunbar & Beard 1975). Finally, there are several examples of sublethal effects, such as reduced vigor, size, fecundity, and pupation, in insects that survive exposure to *B. thuringiensis* (Morris 1982, Smirnoff 1983).

As with the viruses, most of the research of effects of entomopathogenic bacteria on nontarget organisms has been in the laboratory. Bacteria released in the field either have not affected or have adversely affected invertebrate predator and parasitoid populations, probably by reducing populations of the host insect. *B. thuringiensis* reduced beneficial arthropods in five experiments but did not affect them in eight others (Forsberg 1976, Morris 1977b, Reardon et al. 1979, Morris et al. 1980, Jaques & Morris 1981). *B. thuringiensis* released in the field did not affect populations of vertebrates or nontarget invertebrates in terrestrial and aquatic environments (Forsberg 1976, Faust & Bulla 1982). *B. thuringiensis* can successfully compete for survival against other bacterial species in some, but not all, types off soil (Dulmage & Aizawa 1982). *B. thuringiensis* var. *israelensis* did not affect populations of nontarget species from six orders of insect and one mite in two field experiments (Colbo & Undeen 1980), Gibbs et al. 1986), and *B. thuringiensis* var. *tenebrionis* had no adverse effect on two entomophagous insects (Krieg et al. 1984). Similarly, another field trial indicated no adverse effects by *B. sphaericus* on nontarget species of Crustacea, Ephemeroptera, or Diptera (Mulligan et al. 1978).

Little is known about other effects of released entomopathogenic bacteria on non-target organisms. Based on laboratory data, it is generally believed that the danger of genetic exchange by *B. thuringiensis* through transduction or transformation is very low (Faust & Bulla 1982, Morris 1982). Two varieties of *B. thuringiensis* proved stable after 9 mo in a field experiment (Dulmage & Aizawa 1982). On the other hand, Forsberg (1976) did not believe that there was enough evidence to support conclusions about genetic stability. The only other known effect induced by released bacteria is the increase in numbers of microorganisms. In laboratory tests, when *B. thuringiensis* was introduced into field soil that contained no insects, microbial populations increased, including other bacteria, Actinomycetes, fungi, and nematodes (Pruett et al. 1980), Petras & Casida 1985). Cadavers of mosquitoes killed by *B. thuringiensis* var. *israelensis* are fed on by protozoa and saprophytic fungi, and *B. thuringiesis* var. *israelensis* vegetative cells are directly used for food by protozoa in aquatic habitats (Aly et al. 1985).

The only demonstrated effects on soil, water, or air of bacterial application are an increase in the population of the bacterium applied and its toxins and occasionally increased populations of other microorganisms. The *B. thuringiensis* population in soil may not increase beyond a certain level in some situations, despite repeated applications (Dulmage & Aizawa 1982). The two bacteria used for aquatic applications, *B. thuringiensis* var. *israelensis* and *B. sphaericus*, settle to the bottom within

hours (Mullingan et al. 1980, Mulla et al. 1982, Aly et al. 1985, Apperson et al. 1986, Gibbs et al. 1986). Burges (1979) estimated that a typical application of *B. thuringiensis* to terrestrial plants increases the *Bacillus* population by only 5% and that this percentage drops in 3 mo. He concluded that the proportion of *B. thuringiensis* reaching public water supplies would be "very small in comparison with normal soil *Bacillus* spp".

Finally, the bacteria, particularly *B. thuringiensis*, replace a proportion of chemical insecticides and reduce their adverse environmental effects when they are used for insect control. Morris et al. (1986) estimated that *B. thuringiensis* was sprayed on about 870,000 ha of Canadian forest for spruce budworm control in 1985, replacing about half of the quantity of chemical pesticides used previously.

Environmental effects of fungi

The major effects of entomopathogenic fungi released into the environment again result mainly from their intended purpose. The fungi have caused short-and long-term increases in the released fungal population and decreases in the host-insect population and in damage to the host plant or other resources consumed by the insect (Burges & Hussey 1971a, Burges 1981a).

Certain fungal species have more potential than the viruses, bacteria, or other fungi to adversely affect non-target organisms; there has been little field evidence of such effects, and they would not be nearly as severe as those resulting from chemical insecticides. *Verticillium lecanii, Hirsutella thomponii, Nomuraea rileyi, Lagenidium giganteum,* and most Entomophthorales have fairly specific host ranges and have proven safe to date in laboratory tests (Burges 1982). *Metarhizium anisopliae* and *Beauveria bassiana* occasionally infect reptiles, perhaps as opportunistic pathogens (Burges 1981b); and certain fungi being researched for insect control, particularly *B. bassiana,* cause allergic reactions in humans (Burges 1981b). Certain fungi, such as *B. bassiana, M. anisopliae* (Hall & Papierok 1982), and *Tolypocladium cylindrosporum* (Garcia & Sweeney 1986), have broad host ranges among insects and thus could affect populations of beneficial insects. However, there are few data concerning direct or indirect effects of fungi on non-target organisms after field releases. In one experiment, *N. rileyi* had no deleterious effect on insect parasitoids (Hamm & Ware 1982). In another example, natural epizootics of several entomopathogenic fungi interfered with beneficial insects which in turn resulted in increases in populations of pestiferous Noctuidae in cotton (Falcon 1973). *Beauveria bassiana* is pathogenic to honeybees in the laboratory but is not known to infect them in nature (Laird 1973). It is likely that further research will indicate that fungi, like bacteria and viruses, indirectly and perhaps directly reduce populations of beneficial insects, though not to the same extent as many chemical insecticides. The only other effects of fungi on non-target organisms were demonstrated with soils returned to the laboratory; *B. bassiana* in soil apparently served as a food substrate or source for bacteria, amoebae, and soil animals such as Acarina and Collembola (Fargues et al. 1983) and thus could conceivably affect populations of those organisms.

The only demonstrated effect of released fungi on soil, water, or air is increased pathogen population density. Several fungi such as *B. bassiana* are often found to occur naturally at detectable levels in soil (Doberski & Tribe 1980, Quinn & Hower 1985). More than 95% of conidia of *N. rileyi* and *B. bassiana* stay in the upper 5 cm after application to various soils (Ignoffo 1981, Storey & Gardner 1987), and only small percentages of *B. bassiana* conidia are carried by water to depths of 10-15 cm. (Storey & Gardner 1987). Thus there is little chance that conidia of these two species will reach

groundwater after release. There have been no counts of numbers of conidia in the air after release of a fungus, but it is possible that such counts would indicate an increase. Natural epizootics have increased the number of conidia of *N. rileyi* and *Entomophthora gammae* in the air above soybean fields (Kish & Allen 1978, Harper et al. 1984). Heavy rains wash the air of wind-borne conidia of *N. rileyi* (Kish & Allen 1978).

ENVIRONMENTAL RISKS OF RECOMBINANT-DNA ENTOMOPATHOGENS

There are virtually no data in the literature concerning environmental effects of recombinant-DNA (r-DNA) entomopathogens, largely because regulatory agencies in developed nations have permitted very few releases. Though there are no data on environmental effects, the environmental risks of releases of r-DNA microorganisms in general have been a major topic of discussion. Before the viewpoints about these environmental risks are summarized, it is important to point out the potential environmental benefits of r-DNA entomopathogens. Genetic engineering is expected to greatly expand the use of microbial control of insects and thus reduce the array of hazards or harmful side effects associated with chemical insecticides.

There are three major, complex concerns about releasing r-DNA organisms into the environment (Fuxa 1990). The first is that they might have unexpected and deleterious properties after release. Some scientists believe that r-DNA is so different from other genetic manipulations that there will be unexpected problems, just as there were with DDT. Genetic exchange among genera, multiple mutations, and unusual evolutionary potential could cause problems such as unexpected pathogenicity in non-targets. Other scientists believe that r-DNA organisms will be more predictable. There has been no evidence of such problems in the laboratory, and genetic exchanges between distantly related organisms is much more likely to decrease the recombinant's environmental fitness rather than cause unexpected pathogenicity. They also argue that the addition of DNA with coding for specific functions is not the same as random disruption of DNA or random mutation.

A second category of concern is that the recombinant organisms could cause ecological disruptions. Some scientists argue that the organism could itself become a pest or some sort of "super species". They support this contention by the past history of accidental and intentional biological introductions; a disturbing proportion of them has been environmentally harmful. R-DNA organisms could have new survival modes and advantages even with changes in only one or a few genes; the influenza viral strains are often given as a pertinent example. One of the more likely problems is the induction of resistance to the agent in the insect populations, due partly to certain release strategies currently under consideration or development. Scientists in favor of release point out that the likely reduction of fitness of the recombinant and the danger of generalizing all past releases to draw conclusions about carefully designed r-DNA organisms invalidates most of these concerns. Examples such as the influenza virus represent very specific genetic changes that will differ from the ones being added by man. Additionally, most of the history of agriculture is based on biological introductions.

The third concern is the unintended transfer of the genetic material to other organisms after release. If this happens, then the other areas of concern can resurface. Such transfers certainly are possible according to laboratory experiments. However, they are less likely in nature, and, if they do occur, the recipient organism is much more likely to decline in environmental fitness and competitiveness than to cause any environmental problems.

These arguments have had an interesting

side effect. A committee of the U.S. National Academy of Sciences, as well as other prestigious groups of scientists, have concluded that the organism to be released and its target environment, not the method by which it was modified, should be the basis for risk assessment (National Academy of Sciences 1987, Tiedje et al. 1989). This conclusion, along with the sometime troublesome history of biological releases in agriculture, has led to a re-examination of policy in the USA for releasing natural strains, including entomopathogens. These complexities have made it difficult to do research in this area with natural strains in recent years, though there are indications that this problem is becoming less severe.

CONCLUSION

It is clear that the environmental impact of entomopathogen releases has been minimal to date, but it also is clear that this is a critical area for further research if microbial control, particularly with r-DNA entomopathogens, is to be widely implemented. Perhaps the only risks associated with the natural strains are the possibility of inducing resistance in host populations and the possibility of initiating disease epizootics in populations of arthropods that are in danger of extinction and that are closely related (depending on the host range of the entomopathogen) to the target species. These risks will certainly be outweighed by the benefits of reducing reliance on chemical insecticides. Concerning r-DNA microorganisms, these are generally perceived as having a low probability of causing environmental harm but potentially severe consequences if harm occurs (Florio 1985, Davis 1987). Zero risk will not be possible nor should it prevent releases; the potential benefits of release are likely to outweigh the risks. However, the potential benefits and risks must be carefully considered before releases, particularly in developing nations where regulation of the use of alternative control agents are poorly developed (NorAgric 1990). Thus, regulatory agencies and the general public probably should recognize that eventually some environmental problem will arise from a release. If such problems can be kept to a minimum and can be used as experience to avoid further mistakes, then society undoubtedly will benefit just as it has from many other biological introductions during the history of agriculture.

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