Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *84,* Supl. III, 81-88, 1989

IMPORTANCE OF EPIZOOTIOLOGY TO BIOLOGICAL CONTROL OF INSECTS WITH VIRUSES

JAMES R. FUXA

Department of Entomology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803, USA

Almost 90 years passed before scientists interested in entomopathogens realized that a lack of understanding of their ecology or epizootiology was a major stumbling block in developing them for microbial control of insects. Ecology and epizootiology similarly were ignored with the advent of genetic engineering of these agents. Recently it has been increasingly recognized that ecology-epizootiology is a central issue to biological control with natural isolates of viruses and other pathogens for three reasons: first, epizootiology is closely allied to ecology, and both fields are basic to the use of viruses in integrated pest management (IPM); second, viruses are disease agents, so increasing the prevalence of viral disease (i. e., microbial control) is basically a problem in epizootiology; and third, epizootiology is critical to genetic engineering of viruses both in their design and in environmental risk assessment.

Epizootiology can be briefly defined as the study of animal disease at the population level. It encompasses the total environment as well as the host and pathogen populations, so it is heavily allied with ecology. If viruses are to be used for insect population suppression, they must be incorporated into IPM (Fuxa, 1987a). Perhaps the most accepted definition of IPM (see Flint & van den Bosch, 1981) is that it is "a pest management system that, in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest populations at levels below those causing economic injury". This, and virtually all definitions of IPM, states or implies the basic importance of ecology. Thus, understanding how viruses work in an epizootiological framework is critical to their being accepted for use in IPM. Additionally, microbial control with viruses is essentially applied epizootiology. Just as a primary purpose of medical epidemiology is to decrease disease levels in human populations, a prime purpose of microbial control is to increase disease levels in insect pest populations.

In order to discuss epizootiology of baculoviruses in insect control, it is important to understand the approaches to using baculoviruses for insect pest management (Fuxa, 1987a). Viruses used for inundative augmentation are applied for immediate control of the pest population and are not intended to recycle. In inoculative augmentation, releases are required on some recurring basis yet result in recycling (for control of more than one pest generation), usually limited to a single season. Introduction-establishment is the establishment of a virus species or strain where it does not usually occur, which results in permanent suppression of the pest. Environmental manipulation involves the enhancement of naturally-occurring pest suppression by means, usually cultural manipulations, other than addition of pathogen units into the environment.

The worldwide numbers of successes of microbial control with viruses has differed according to the types of approach and ecosystem (Table I). Most of the successes have been through introduction-establishment, including a few in row crops. This is partly because the definition of "successful" control is different for introductions than it is for inundative augmentation; in the latter the pest population must always be reduced below the economic injury level, whereas an introduction is successful if it does this only part of the time. Three of the four successful introductions in row crops did not permanently reduce the pest population below the economic injury level. All the successes have been by means of the "ecological" approaches to control (introduction, inoculative augmentation, environmental manipulation), though in five cases the type of augmentation could not be determined from

TABLE I

Worldwide number of successful cases of microbial control of insects with viruses (i. e., viruses actually in use), classified according to approach and ecosystem[a]

Type of approach	Ecosystem	
	row crops, fruit	trees, pastures
Inundative augmentation	0	0
Unknown augmentation	5	0
Inoculative augmentation	2	5
Introduction-establishment	4	8
Environmental manipulation	0	1

[a] Derived from: Burges & Hussey, 1971; Chin, 1979; Burges, 1981; Richter & Fuxa, 1984a; Huber, 1986; Harper, 1987; Cunningham, 1988.

the literature. Inundative augmentation has not been successful.

The reasons for success or failure of viruses in insect control are due largely to factors that can be considered epizootiological or ecological, though economics also is important. In some of the cases the reasons for success or failure of attempts at insect control with baculoviruses have been documented. The *Heliothis* nuclear polyhedrosis virus (NPV) in the USA has been considered technically but not economically successful (Huber, 1986), though it certainly was not even technically successful in some areas, such as Louisiana (Clower, D.F.). This NPV, intended for inundative augmentation, failed because it was too expensive, slow, and host-specific (Huber, 1986; Jutsum, 1988). The *Orgyia pseudotsugata* NPV, intended for inoculative augmentation, is not used partly because of its specificity, high cost, and the long time periods between pest outbreaks (Podgwaite, 1985; Huber, 1986). The *Cydia pomonella* granulosis virus (GV), intended for inundative augmentation, is not yet being used for control of codling moth primarily because it is too slow, too host-specific, and less persistent than chemical insecticides (Jaques et al., 1987; Crook, N. E.). The *Lymantria dispar* NPV has had only limited success in inoculative augmentation because its use is advantageous over that of chemicals only in very limited ecological situations: when gypsy moth is the primary pest, when environmental considerations are paramount, and when immediate control is not required (Lewis, 1981). The *Pseudoplusia includens* and *Anticarsia gemmatalis* NPVs were introduced for permanent pest suppression in the USA, an important example of persistent control in a row crop. However, the introductions were only partially successful (i. e., did not permanently suppress the insect populations below the economic injury level) because of the unstable environment and highly variable pest populations (Harper, 1987). The use of the *Anticarsia gemmatalis* NPV has been successful for inoculative augmentation in Brazil because there the host insect is a key pest, the cost is low since the virus is field-grown, and the NPV can be applied as crude suspensions (Moscardi, F.). Finally, the *Gilpinia hercyniae* NPV and *Oryctes rhinoceros* baculovirus have been successful through introduction-establishment and inoculative augmentation, respectively, for similar reasons: in both systems there are stable habitats and host populations, as well as good horizontal and vertical transmission (Harper, 1987).

Examples from research of two insect-virus systems in Louisiana can be used to illustrate the array of epizootiological parameters that can affect the prevalence of viral infections and the use of viruses for insect control. These parameters include speed of kill and action thresholds; effects of the ecosystem and environment; delivery of the virus, including timing and transmission; and host resistance.

Viruses kill their hosts relatively slowly; this is likely to eliminate their use in some crop-pest situations and affect action thresholds (the population density at which treatment is implemented in order to maintain a positive cost-benefit relationship in crop production) in others. The slowness in killing the insect, for example, > 7 days in the case of *A. gemmatalis* NPV (Richter & Fuxa, 1984b), allows further damage after the insect becomes infected. In some situations, as in the case of pests of foliage in soybean, this might be acceptable because the plant can compensate for damage with no loss in yield. In others, such as *C. pomonella* in apples, even slight damage greatly reduces the value of the crop.

The use of a slow agent like a virus will likely require different action thresholds than those in current use, which have been developed almost exclusively for chemical insecticides. The same number of insects eating at the same rate but for a longer time period will simply do more damage and therefore must be treated earlier. For example, infestations of *A. gemmatalis* in Louisiana were sprayed with NPV at different times during two different infestations. The second spray date in both cases was at approximately the time at which VBC reached insecticide action thresholds. The virus was significantly less effective in reducing pest numbers and damage when it was sprayed at insecticide action thresholds that it was when sprayed earlier (Richter & Fuxa, 1984a). Thus viruses cannot simply be substituted for chemicals with the same application technology, as is usually the case, and be expected to perform as well as chemical insecticides. When repeated applications normally are used against a key pest, such as *C. pomonella* in apples, then timing, particularly after the initial treatment, might not be so critical. However, the situation can be more confusing, and require more research, in forests and other situations, where economic injury levels are more difficult to quantify than in agroecosystems (Morris, 1980). Also, maturation immunity, which makes it beneficial to treat the youngest larvae possible, confuses the threshold and timing issues.

The ecosystem and environment affect microbial control with baculoviruses. Worldwide, the stability of the ecosystem has affected the success of the ecological approaches to control (Table I). Introduction-establishment has been more successful in forest ecosystems, with their biotic complexity and ecological stability, than in row crops. Also, different economic injury levels in various agroecosystems affect the success or even the feasibility of microbial control, as discussed above.

A multitude of environmental factors affects insect viruses, and these factors can interact in complex ways (Fuxa & Tanada, 1987). Comparative ecology of factors affecting prevalence of NPV in *Spodoptera frugiperda* has illustrated some of these complexities. In cornfields at Hammond, Louisiana, where NPV naturally occurs in large amounts in the soil, abiotic factors were most correlated with prevalence of the viral disease. Rainfall and solar radiation had negative effects, probably because they affected persistence of virus on the insect's feeding substrate. On the other hand, at a nearby site where there is little virus in the soil (St. Gabriel, Louisiana), biotic variables including host density dependence and size of the host plant had the greatest correlation with disease prevalence (Mitchell & Fuxa, in press). Furthermore, in a study of the same insect and virus at the same locations but in a different agroecosystem (pastures), the amount of virus in the soil, rainfall, presence of cattle, and tilling of soil all affected the disease prevalence (Fuxa & Geaghan, 1983). In contrast to corn, rainfall in pastures was positively correlated with prevalence, perhaps because it helped move the virus from soil to leaf surface in this much shorter host plant. The NPV was not host-density dependent within one growing season in pastures (Fuxa & Geaghan, 1983). There also can be interactions among variables. For example, the presence of cattle in pastures had a greater effect on prevalence of NPV in FAW as the amount of virus in the soil increased, probably because the cattle helped spread the virus or transport it from the soil to the plant surface where the insect feeds (Fuxa & Geaghan, 1983).

Not only can a particular variable, such as rainfall, have different effects in different crops or at different sites of the same crop, but also a particular variable can have different effects depending on the approach to microbial control. For example, *S. frugiperda* on different host plants growing at the same site at the same time had different levels of nuclear polyhedrosis. Epizootics developed more quickly in signalgrass than in corn, probably due to the structure of the two different plants (Fuxa, 1982). Thus, in this respect, corn would be at a disadvantage for the ecological approaches to insect control. But if the effect of transmission or movement of the NPV in the field is bypassed, as in a laboratory experiment (Richter et al., 1987) or as might be expected by spraying virus for inundative augmentation, then the susceptibility of the insect is greater on corn than on signalgrass; and based on this factor alone better results from inundative augmentation would be expected in corn.

Delivery of the pathogen to the insect, including natural transmission, is an epizootiological factor important to microbial control. With respect to inundative augmentation, the delivery system (i. e., spray technology, adjuvants, equipment, etc.) must replace or simulate natural transmission. Delivery in turn can affect economics, reliability, efficacy, and adverse effects of the environment on the virus

(Young & Yearian, 1986). Delivery to the insect in the sense of non-manipulated, natural transmission is critical to the ecological approaches to control. Effective transmission, preferably vertical transmission, has been shown by modeling studies to be virtually essential to the introduction-establishment approach to control. It also is important to inoculative augmentation and environmental manipulation, since both approaches depend on recycling of the pathogen (see Fuxa, 1987a). The fact that efficient transmission was observed as an important factor in some of the successful introductions discussed above supports the modelers' conclusions. Novel delivery such as autodissemination of viruses has proved not only feasible but also effective in inoculative augmentation (e. g., *Oryctes* baculovirus) and introduction-establishment (e. g., *Gilpinia hercyniae* NPV), perhaps because it simulates natural transmission (Bird, 1961; Herper, 1987). Vertical transmission of NPV is being found in an increasing number of systems, sometimes at extremely low levels as in *S. frugiperda,* and thus may be a fairly widespread phenomenon (Smits & Vlak, 1988; Fuxa & Richter, unpublished data).

Another epizootiological factor that will affect use of viruses is resistance. The experience with chemical insecticides in conjunction with the early experimental results with viruses indicate that resistance to viruses is likely to be a widespread phenomonen. Four of six insects selected for resistance in the laboratory have exhibited decreased susceptibility (Briese, 1986; Fuxa et al., 1988). *Spodoptera frugiperda* developed a 3-5X resistance ratio to its NPV in selection experiments, with a possible theoretical ratio of 20X. The range of LD_{50}s and ld-p slopes in the laboratory selection experiment were almost identical to those observed in several years of field experiments (Fuxa, 1987b, Fuxa et al., 1988). Interestingly, the resistant insects reverted almost to their original level of susceptibility when selection pressure was removed (Fuxa & Richter, 1989). Reversion is a common phenomenon with chemical insecticides; but susceptibility does not revert completely to the original level, and reversion lessens after repeated application and relaxation of selection pressure. Thus it will be interesting to learn how insect-baculovirus systems behave, because they have been experiencing natural selection pressure and relaxation cycles for millions of years, though perhaps not to the same level of mortality as would occur with viral insecticides. Resistance will occur to viruses in many cases, but there are not enough data in various systems to say whether it will be as severe a problem as with chemical insecticides.

The relative importance of these and other epizootiological and IPM considerations vary considerably depending on the approach to biological control with viruses (Table II). For viruses to be used successfully, consideration must be given the approach to control and the particular ecosystem, not just the activity of a particular virus against a particular pest. Inundative augmentation is at a severe disadvantage to the three ecological approaches due to three characteristics of baculoviruses. It is the only approach in which the insecticide must have quick action, unless more appropriate action thresholds alleviate its remedial nature. Host specificity and the necessity of costly production in live cells also are less disadvantageous with the ecological approaches, in which more than one generation of insects are controlled by zero or one application. Slow action and costly production are not as disadvantageous to biological control in developing nations, due to less stringent consumer attitudes and greater availability of hand labor, respectively.

Some other characteristics of baculoviruses are not as disadvantageous to inundative augmentation. Persistence in storage is helpful in augmentation but unimportant to the other two approaches, in which the virus is not applied by the grower. Persistence in the insect's habitat is unimportant to inundative augmentation but critically important to the other three. Overall persistence in the ecosystem is essential for recycling, and persistence outside the host can help compensate for a lack of efficient transmission (Fuxa, 1987a). Persistence on the feeding surface is desirable in all approaches to control, because it enhances horizontal transmission. High virulence enhances inundative augmentation, in which quick and extensive mortality are advantageous; and moderate virulence is best for the approaches that depend on recycling, partly because it contributes to a more stable host-pathogen relationship. The observations of resistance to viruses help demonstrate why moderate virulence is better for relatively permanent control: release of a virulent strain

TABLE II

Relative importance of various epizootiological and IPM considerations to the four approaches to biological control with viruses

Epizootiological or IPM consideration	Approach to Biological Control			
	inundative augmentation	inoculative augmentation	introduction-establishment	environmental manipulation
Quick mortality	usually necessary	unimportant	unimportant	unimportant
Specificity in a pest complex	usually severe disadvantage	moderate disadvantage	slight disadvantage	moderate disadvantage
Low-cost production	highly desirable	desirable	unimportant	unimportant
Persistence in or on:				
storage	helpful	helpful	unimportant	unimportant
feeding surface	desirable	desirable	desirable	desirable
general habitat	unimportant	essential	essential	essential
Ideal Virulence	high	moderate-to-high	moderate	moderate
Efficient transmission	unimportant	desirable	very desirable	desirable
Natural movement from reservoir	unimportant	very desirable	very desirable	possible target of manipulation
Host density-dependence	unimportant	somewhat important	important	somewhat important
Ability to cause natural epizootics	unimportant	essential	essential	essential
Pest's economic injury level (EIL)	must always suppress below EIL	must usually suppress below EIL	must suppress below EIL only part of time	must always suppress below EIL

is likely to induce resistance and an inconsistent level of pest control. Natural transmission and movement of the virus are among the most important attributes for the ecological approaches, because if there is no host-to-host transfer of the disease, there will be no epizootics. Inundative augmentation bypasses natural transmission, and environmental manipulation is likely in many cases to be used to improve it. The ecological approaches have a disadvantage in that density-dependence is important to epizootics, which is not the case with inundative augmentation. Also, it is essential to these approaches for the virus to cause natural epizootics; these in turn depend on several of the factors already mentioned, such as transmission, virulence, and persistence in the environment. Finally, introduction-establishment has an important advantage over the other approaches in that it does not necessarily have to always reduce the pest population below the economic injury level. A permanent introduction, due to its low long-term cost, can be economical even if it reduces the pest population below the EIL only a small percentage of the time. In the other three approaches, where the user is performing some sort of manipulation to combat or prevent economic injury on a short-term basis, he must have tangible results or he will not continue that manipulation in the future.

The final of the three ways in which epizootiology is important to microbial control with viruses is its pertinence to genetic engineering research. There are two reasons for this: design of engineered viruses and environmental risk assessment. First, the design of viruses genetically engineered for insect control should be based on epizootiology. The manipulations likely to be accomplished in the near future are amenable to single-gene manipulations and are coincidentally the ones most likely to contribute to inundative augmentation, not to the three ecological approaches. These manipulations include decreased persistence with polyhedrin deletion mutants in order to alleviate environmental risks (Vlak, J. M.), increased virulence and speed of kill, and improved production. Virulence and speed of kill are being improved by engineering genes for toxins, hormones, or enzymes into the genome (Payne, 1988; Vlak,

J. M.). Production has been improved by adapting the virus to grow in different production host insects (Payne, 1988).

In the more distant future, extended host range and persistence are the potential targets that could improve the chances for inundative augmentation. Tailoring the host range to fit the pest situation, in particular, would be a dream-come-true for IPM specialists. Unfortunately, this is a multigenic trait that is not likely to be manipulated in the near future.

Inoculative augmentation presents an intermediate situation for genetic engineering. Some immediate, perhaps even knockdown, effect is desirable in the treated generation of pest insect, but some persistent activity also is required. So perhaps this is the approach that has the most to benefit by genetic manipulations, but also the one in which the greatest "balancing-act" will be necessary. For example, virulence would be beneficial in treating the initial generation but might be detrimental to persistent activity.

Genetic engineering is unlikely to make any impact on introduction-establishment in the near future for two reasons. First, as mentioned above, the changes that would increase the enzootic level of virus in a population are ones not likely to be made in the near future, primarily because they are complex at the molecular level. These include vertical transmission, persistence, and host range. Second, the regulatory environment in developed nations is not conducive for the time being to permanent release of a genetically engineered organism. There are legitimate concerns (Fuxa, in press-b) that should prevent such releases in any situation for the time being, though it is likely that eventually they will be found environmentally safe.

For the more distant future, the opportunities for genetic engineering to impact this approach are almost limitless. If virulence, transmission, persistence, and host range all prove amenable to genetic manipulation, then virtually any pest situation should have the potential to be at least partially alleviated with this approach, due to the fact that such control will fit in unobtrusively into other pest management and growing practices and will not have to compete with other means of pest control.

The second reason that epizootiology is important to genetic engineering of viruses for improved biological control is that epizootiology and ecology recently have become recognized as a major bottleneck in research and development of genetically engineered microorganisms (GEMs) for field release because so little is known about the environmental risks. The possible hazards associated with release of GEMs boil down to three major, very complex, issues (Fuxa, in press-a, -b). First, some scientists believe that GEMs might have unexpected properties, like pathogenicity for the wrong hosts, or unusual evolutionary potential. Second, they would be unpredictable in the environment and could themselves become pest species, as have many other introductions. And third, there could be unintended transfer of genetic material to other organisms, causing the first two areas of concern to resurface.

No single technology will suffice for risk assessment of GEMs. There are four types of studies that will be used to assess risk of GEMs, but only three can be done prior to release (Fuxa, in press-a, in press-b). These include laboratory experiments with the GEM, release of the GEM into microcosms, and the biology and ecology of the parental (wild-type) organisms. The fourth type of study is the monitoring of population dynamics and environmental effects after the actual release of a GEM. Of the three studies that can be done prior to release, in no case can the results be extrapolated to draw conclusions or make predictions about the actual releases of a GEM; therefore it will be important to have all three types of study to minimize the danger of extrapolation (Fuxa, in press-a, in press-b).

The probability of environmental harm is a product of six probabilities: release, survival, multiplication, dissemination, transfer of genetic information, and harm (Levin, 1982). Several of these (survival, multiplication, dissemination) are ecologically and epizootiologically important characteristics of an organism.

Some of these factors, such as persistence, are fairly well understood for insect viruses, though even persistence can vary greatly among or within species (see Fuxa, in press-a). The long-term persistence of these viruses is in soil or litter and in primary, secondary, and alternate hosts. Viruses released for biological control of insects are known to persist up to 7 years in the host population and more than 5 years in soil, but only 1-32 days on foliage or 3 days in bird guts.

Dispersal of viruses after release has not been studied as well as persistence. Released viruses have been known to be dispersed distances of zero to many kilometers in one year (see Fuxa, in press-a). In certain cases, such as that of the *Gilpinia hercyniae* NPV, the virus can be expected to spread throughout the range of the host population. Certain viruses, such as *Lymantria dispar* NPV, have been known to be dispersed after some releases but not after others, even though it successfully established itself in the host population.

There have been few estimates of viral population density subsequent to release in a host population (see Fuxa, in press-a). At various times after release, populations of some viruses have been known to decline virtually to zero after infecting the targeted generation of host insects, whereas others have increased to > 100X the number of virions that was released.

Adverse environmental effects caused by releases of insect viruses likewise have been little studied (see Fuxa, in press-a). Development of resistance to virus after release for control of insects has never yet been demonstrated, perhaps because they generally have not been applied extensively as have chemical insecticides. However, resistance due to a release of a virus may have caused reduced infection rates in one case (Briese & Podgwaite, 1985). Viruses released for insect control have indirectly harmed non-target organisms, particularly parasitic and predatory arthropods, by competitive displacement or reducing a common resource, namely the host insect population. Recombination can occur between closely related baculoviruses; presumably, therefore, genes in a released baculovirus could be distributed into field populations of related viruses. The only known environmental effect of viral release on soil, water, or air is the increase in the viral population in soil.

Thus, we do not have a very good understanding of the population dynamics of natural strains of viruses. It would be difficult to estimate the probabilities of survival, multiplication, and dissemination of even these parental organisms, much less extrapolate to predict what would happen after release of a GEM. It is clear that epizootiology will be a major area of research if risk assessment is to be based on more than just the "gut feelings" of a few experts.

Epizootiology must play a major role in the future of microbial control with viruses because such control will be in the context of IPM with its heavy basis in ecology, because viruses have their own ecological niches which must be throughly understood for the manipulation of epizootics, and because of environmental concerns regarding the release of genetically engineered viruses. Just one environmental disaster would likely result in severe, adverse public reaction to the entire concept or releasing a GEM for insect control. Epizootiology will have a central role regardless of whether released viruses are based on natural strains or are genetically manipulated and regardless of whether they are used in augmentation or introduction-establishment. If such is not the case, we can expect all the mistakes of the past to be repeated. Scientists and funding agencies must be certain that ecological information is available when the IPM specialists evaluate components to include in interactive research or control programs, and when the genetic engineers are designing a virus for insect control.

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