ENVIRONMENTAL FACTORS AFFECTING PERSISTENCE OF ENTOMOPATHOGENS

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ABSTRACT

This symposium review will focus on the impact of environmental factors (primarily sunlight, temperature, humidity-water) on the field persistence of selected representative species of entomopathogens (bacteria, fungi, protozoans, viruses). Sunlight is probably the most destructive environmental factor affecting the persistence of entomopathogens and commercial microbial pesticides. Temperatures prevalent in most agro-ecosystems (ca. 10 to 40°C) generally do not adversely affect entomopathogens. Effects at temperature extremes, however, might be obtained when the entomopathogens are stressed by other factors (e.g., light, water, chemicals). The effect of humidity or water on entomopathogens also is difficult to separate from its combination with other environmental factors. Humidity or water *per se* generally does not directly affect the activity or viability of viral or bacterial entomopathogens, respectively. In contrast, lack of moisture reduces the infectivity of many protozoan spores and moisture generally is a primary requirement for germination of conidia and survival of entomopathogenic fungi.

RESUMEN

Esta revision se concentra en el impacto de los factores ambientales (primordialmente luz solar, temperatura, agua-humedad) en la resistencia en el campo de varias especies de entomopatogenos (bacterias, hongos, protozoarios y virus). La luz solar es probablemente el factor mas destructivo del medio ambiente para la persistencia de entomopatogenos y pesticidas comerciales microbiales. Las temperaturas que prevalecen en la mayoria de los agroecosistemas (entre 10 y 40 C) generalmente no afectan adversamente a los entomopatogenos. Los efectos de las temperaturas extremas, sinembargo, pueden afectar cuando los entomopatogenos han sido afectados por otros factores (e.g., luz, agua, quimicos). El efecto de la humedad o agua en los entomopatogenos es algo dificil de separar de su combinacion con otros factores del medio ambiente. La humedad o agua por si solas generalmente no afectan directamente la actividad y viabilidad de entomopatogenos bacteriales y virales, respectivamente. En contraste, la falta de humedad reduce la virulencia de muchas esporas de protozooarios y la humedad generalmente es un requerimiento primordial para la germinacion de las conidias y la sobrevivencia de los hongos entomopatogenos.

In this review I will try to provide information that may be helpful in developing guidelines that might apply to particular situations. One should keep in mind, however, that this information is based upon relatively few examples. Whenever possible, I have selected examples that directly relate to field situations. When this was not possible, and in many cases it was not possible, laboratory results were used. Thus, generalizations of pathogen survival once presumed in an experimental design, must be verified or refuted by field experimentation.

Two major technical problems are associated with the effective field use of microbials: their proper *application* i.e., their placement where and when they will exert the

most control; and their *persistence* i.e., keeping them active as long as the destructive stage of the pest is present. Although both *application* and persistence are of utmost importance for effective insect control of pests, my review today will focus on the effects of abiotic factors (mainly temperature, water, and sunlight) on persistence of entomopathogens. Selected species of each group of entomopathogen (protozoa, fungi, bacteria, viruses) will be used as representative models.

Effects of Temperature

In most agroecosystems ambient temperature during the growing season ranges from about 10 to 40°C; however, the optimum range for infection, growth and development for most entomopathogens lies between 10 and 30°C. In general, temperature within the range of 10 to 30°C for less than 30 days (the period within which most crops would be vulnerable) does not effect the stability of many entomopathogens (Table 1). Deleterious effects at temperatures less than 10°C or greater than 30°C, however, can occur when entomopathogens are stressed by interactions with water, sunlight, foliage or soil chemicals and/or other biotic or abiotic factors. Temperatures above 35°C generally inhibit growth and development of entomopathogens, and viability or insecticidal activity of inoculum is markedly decreased as temperatures approach 50°C (Table 1). Temperatures less than 10°C also inhibit growth and development of entomopathogens, but may increase persistence of the inoculum. As an example, the *Heliothis* nucleopolyhedrosis virus was still active after more than 25 years storage at 5°C, but, all insecticidal activity was lost after only 100 days at 50°C (Table 1).

The temperature stability of representative species of bacteria, fungi, protozoa and viruses is summarized in Table 1. The upper extreme of field temperature is most detrimental to species of entomopathogenic protozoa and fungi. The half-life of *Nosema* spores exposed at 50°C is less than 8 hours but can be greater than 300 days at 10°C. Spores of *Beauveria bassiana* and *Metarhizium anisopliae* have a thermal death point of less than 15 min at 50°C. The half-life of dry spores of *Bacillus thuringensis* exposed at 50°C is greater than 100 days while wet spores have a half-life of less than 60 days (Table 2). Spores of *Bacillus popilliae* exposed at 60°C are inactivated after an exposure of only 15 minutes. Although occluded insect viruses are not inactivated at normal field temperatures, the inactivation profile at 50°C (e.g., *Heliothis* NPV) suggest that longer exposures at 30°C may reduce viral activity (Table 1).

The following are some temperature-stability guidelines, based upon this review, that might be used to design and conduct field experiments with entomopathogens in the Caribbean: (1), short term (less than 30 days), exposure at temperatures greater than 5°C and less than 20°C generally should have little or no effect on persistence of entomopathogens; (2) exposures higher than 30°C (interpolated from longevity studies at 45-50°C) could affect persistence of protozoa and fungi, have some effect on bacterial spores, but little or no effect on viruses; and (3) short term exposure (less than 30 days) at temperatures greater than 20°C but less than 30°C should not adversely affect the persistence of entomopathogens.

Effects of Water

Water, other than as a dispersal and diluting vehicle or in combinations with other environmental factors, may or may not limit persistence and subsequent field effectiveness of entomopathogens. As an example, inclusion bodies of the *Heliothis* NPV are very stable in water at 30°C (>365 days), however, when exposed to sunlight, viral activity was significantly reduced about 3-fold (Table 2). The lack of water can limit germination and subsequent infection of the host by many species of some fungi and

TABLE 1. ESTIMATED LONGEVITY FOR REPRESENTATIVE SPECIES OF ENTOMOPATHOGENIC BACTERIA, FUNGI, PROTOZOA AND VIRUSES EXPOSED AT VARIOUS TEMPERATURE REGIMES.[1]

Entomopathogen	Inoculum	Longevity in Days at Various Temperatures 5°-10°C	20°-30°C	45°-50°C
	BACTERIA			
Bacillus thuringensis	spores	>5,000	>300	100
Bacillus thuringensis	toxin	>5,000	90	>10
Bacillus popilliae	spores	>9,001		<1
	FUNGI			
Entomophthora destruens	conidia	1,000	30	<1
Beauveria bassiana	conidia	900	85	<1
Metarhizium anisopliae	conidia	455	85	<1
Nomuraea rileyi	conidia	120	30	<1
	PROTOZOA			
Nosema necatrix	spores		300	<1
Nosema plodiae	spores	120	270	
Nosema spp.	spores	0-300	90-300	<1
	VIRUSES			
Heliothis NPV	inclusions	>10,000	>10,000	<100
Trichoplusia NPV	inclusions	>1,500	>1,000	<100
Pieris GV	granules	>1,500	>365	<50

[1]From: Brooks et al. 1988, Clerk & Madelin 1965, Hall & Bell 1960, Ignoffo 1964, 1985, Ignoffo & Garcia (*unpub*), Ignoffo et al. 1977, Jacques 1977, Krejzova 1971 a,b, 1973, Maddox 1977, McCoy et al. 1988, Muller-Kogler 1964, Pinnock et al. 1977, Roberts & Campbell 1977, Steinhaus 1960, Teetor & Kramer 1979, Walstad et al. 1970, West et al. 1984, Young & Yearian 1986.

protozoa, and moisture, generally as free water, is needed for sporulation of many species of fungi. Spores of some species of *Nosema* survive best in water while other species must have dry conditions to survive storage for several months. Drying completely destroys the infectivity of spores of *Nosema algerae*.

The effects of water, in combination with a temperature of 30°C, on the stability of representative entomopathogens is summarized in Table 2. In general, spores of *Bacillus thuringensis*, conidia of *Nomuraea rileyi* and inclusion bodies of the *Heliothis* NPV

TABLE 2. EFFECTS OF WATER AT 30°C ON STABILITY OF REPRESENTATIVE SPECIES OF ENTOMOPATHOGENS.[1]

Species	Inoculum	Estimated Loss in Stability (% in days) WET	DRY
Bacillus thuringensis	spores	18% 200	0% 200
Nomuraea rileyi	conidia	100% <7	0% 100
Heliothis NPV	inclusions	0% 365	0% 9125
Nosema plodiae	spores	50% 270	90% 30
Vairimorpha necatrix	spores	0% 90	90% 6

[1]From: Brooks 1988, Ignoffo 1964, 1981, Ignoffo & Couch 1981, Kaya 1977, Kellen & Lindegren 1968, Maddox 1977, Thomson 1958.

survive longer if they are dry. In contrast, water is necessary and may enhance the survival of spores of *Nosema plodiae* and *Vairimorpha necatrix*. Short-term exposures (less than 30 days) should not adversely effect the spores of *B. thuringensis*, spores of *N. plodiae* and *V. necatrix*, and the inclusions bodies of the *Heliothis* NPV. Spores of *N. rileyi*, however, once committed to germination by the presence of water, quickly lose viability if they do not infect a host.

Some generalizations one can make from the available data are that short-term exposure of entomopathogens to water (greater than 7 but less than 30 days): (1) can reduce the viability of fungal conidia; (2) may reduce insecticidal activity of protozoan spores; and (3) will essentially have little or no effect on bacterial spores or crystals and viral inclusion bodies.

Effects of Solar Irradiation

Natural sunlight (the active spectrum is between 290 and 400 nm) is the most destructive environmental factor affecting the persistence of entomopathogens. The half-life of different types of inoculum (conidia, spores, occluded virions, toxins) exposed to natural sunlight is estimated at about one hour for the most sensitive entomopathogen to about 96 h for the most resistant entomopathogen.

Sunlight may directly or indirectly inactivate entomopathogens. The direct effect may be deletions, cross-linking, strand breakage, and/or formation of labile sites on DNA. Indirect effects may be due to generation of highly reactive radicals that in turn inactivate entomopathogens. It has been my working hypothesis that highly reactive radicals (e.g. peroxides, hydroxyls, singlet oxygen) produced by near-ultraviolet irradiation (UV) are primarily responsible for reducing the field persistence of entomopathogens and microbial pesticides. Examples of the relative sensitivity of representative species of entomopathogens, to both natural and simulated sunlight, are presented and discussed for each pathogen type (Tables 3, 4, 5, 6).

TABLE 3. Estimated half-life of selected entomopathogenic viruses exposed to simulated and natural sunlight.[1]

Virus[2]	Application site	Half-life (hours)
	SIMULATED SUNLIGHT	
CPV *Heliothis*	glass	2.2
NPV *Heliothis*	glass	2.2
NPV *Trichoplusia*	filter	<1.0
GV *Pieris*	glass	2.0
EPV *Euxoa*	glass	2.4
NPV *Lambdina*	glass	10.0
	NATURAL SUNLIGHT[3]	
NPV *Trichoplusia*	cabbage leaves	48
NPV *Heliothis*	cotton leaves	3-36
	corn silk	16
	soybean leaves	53
NPV *Epiphyas*	apple leaves	<48
GV *Pieris*	cabbage leaves	48

[1]From Bullock 1967, Cantwell & Franklin 1966, David 1969, David & Gardiner 1966, David et al. 1968, Ignoffo & Batzer 1973, Ignoffo et al. 1972, 1973, 1974, Jacques 1967, 1972, 1977, MacCollum & Reed 1971, Morris 1971.

[2]CPV-cytoplasmic polyhedrosis virus; NPV-nucleopolyhedrosis; EPV-entomopox virus; GV-granulosis virus.

[3]Days of exposure were transposed to hours.

Effects on Viruses

All viruses i.e. cytoplasmic polyhedrosis virus (CPV), nucleopolyhedrosis virus (NPV), granulosis virus (GV) and entomopox virus (EPV) were sensitive (except for the *Lambdina* NPV) when exposed to simulated sunlight (Table 3). Viruses exposed to natural sunlight on foliage appear to be more stable than those exposed to simulated sunlight. This difference, however, is probably due to shielding from sunlight provided by plant parts and/or also sampling time. Field stability studies with entomopathogens commonly include sampling on a 24 h basis. Optimum exposure to the degradative effects of sunlight-UV, however, is about 4 h/day. Thus, the first day of sampling (24 h post exposure) would transpose to about 4 h of actual optimum exposure to sunlight.

Effects on Protozoa

Spores of entomopathogenic protozoans are as sensitive as other entomopathogens to sunlight whether exposed on foliage, glass or in water (Table 4). The half-life of most protozoan spores exposed to sunlight ranged from about 2 to 6 h. The high value of 78 h for *Vairimorpha necatrix* on bean leaves probably reflects, as with virus samples, UV-shielding and time of sampling rather than a true estimate of half-life.

Effects on Fungi

The half-life of conidia of most species of entomopathogenic fungi exposed to simulated sunlight ranged from about 1 to 4 h (Table 5). A highest half-life (14.8 h) for spores of *Aspergillus niger* may be due to UV-shielding provided by black-pigmented conidia. The half-life of fungal spores exposed to natural sunlight on foliage range from about 4 to 200 h (Table 5). Differences in half-life between exposure to natural or simulated sunlight are again probably due to shielding, provided by the plant, and sampling time. As an example, the half-life of conidia of *N. rileyi* directly exposed on glass to simulated sunlight or on cabbage leaves to natural sunlight was 2.4 h and 3.6 h, respectively, a difference of only 1.2 h.

TABLE 4. ESTIMATED HALF-LIFE OF SPORES OF SELECTED SPECIES OF ENTOMOPATHOGENIC PROTOZOANS EXPOSED TO SUNLIGHT.[1]

Species	Application Site	Half-Life (hours)
Nosema fumiferanae	cherry leaves	4-5
Nosema heliothidis	cotton leaves	2-4
Nosema algerae	water	<4
Nosema trichoplusiae	glass	<6
Octosporae muscadomesticae	water	<3
Vairimorpha necatrix	water	2.1
	corn leaves	<3
	bean leaves	<78
Vavraia culicis	water	3

[1]From: Ignoffo et al. 1977, Kaya 1977, Kelly & Anthony 1979, Kelly et al. 1981, Kramer 1970, Maddox 1973, 1977, McCoy et al. 1988, Sikorowski & Lashomb 1977, Tettor & Kramer 1979, Wilson 1974.

TABLE 5. ESTIMATED HALF-LIFE OF SPORES OF SELECTED SPECIES OF ENTOMOPATHOGENIC FUNGI EXPOSED TO SIMULATED AND NATURAL SUNLIGHT.[1]

Species[2]	Application Site	Half-life (hours)
	SIMULATED SUNLIGHT	
Aspergillus niger	glass	14.8
Beauveria bassiana	glass	1.9-2.0
Metarhizium anisopliae	glass	1.3-4.0
Nomuraea rileyi	glass	2.4
	NATURAL SUNLIGHT	
Beauveria bassiana	soybean leaves	48-72[3]
Nomuraea rileyi	soybean leaves	48-192[3]
	cabbage leaves	3.6

[1]From: Fargues et al. 1988, Gardner et al. 1977, Ignoffo 1977, Ignoffo et al. 1976, 1977, Ignoffo & Garcia (*unpub*), Muller-Kogler 1964, 1965, Roberts & Campbell 1977, Zimmerman 1981.
[2]Exposed as conidia.
[3]Days of exposure were transposed to hours.

Effects on Bacteria

The pattern of persistence of bacterial toxins and spores exposed to simulated or natural sunlight generally followed that of the other entomopathogens (Table 6). This in spite of the fact that toxin(s) are non-living entities while spores, conidia, vegetative cells or virions, are living entities. The fact that both living and non-living entities are similarly affected by exposure to sunlight support the hypothesis of indirect inactivation of entomopathogens by highly reactive radicals.

TABLE 6. ESTIMATED PERCENT-LOSS IN SPORE VIABILITY OR INSECTICIDAL ACTIVITY OF *BACILLUS THURINGENSIS* EXPOSED TO SIMULATED OR NATURAL SUNLIGHT.[1]

Inoculum	Application Site	Viability or Activity (%-loss: time)
	SIMULATED SUNLIGHT	
Spore viability	filter	50%:30 min
Endotoxin activity	glass	50%:3.8 hrs
	NATURAL SUNLIGHT	
Spore viability	red cedar leaves	80%:1 day
	soybean leaves	8%:1 day
	redbud leaves	50%:1-22 days
Endotoxin activity	red cedar leaves	20%:1 day
	soybean leaves	65%:1 day
	cabbage leaves	22%:5 days
	tobacco leaves	50%:4-5 days
	cotton leaves	50%:2-4 days
Endotoxin (pure) activity	cotton leaves	50%:4 days

[1]From: Beegle et al. 1981, Cantwell & Franklin 1966, Garcia & Desrochers 1979, Hostetter et al. 1975, Ignoffo et al. 1974, 1977, Leong et al. 1980, Mulligan et al. 1980, Pinnock et al. 1971, 1974, 1975, Toumanoff & Lapied 1954, Wolfenbarger et al. 1972.

Spore viability of *B. thuringensis var. kurstaki* was reduced 50% after 30 minutes exposure to simulated sunlight (Table 6). Endotoxin activity also was reduced, however, it required about 8-times more sunlight exposure (3.8 h) to obtain a 50% loss in insecticidal activity. The extent of the persistence after exposure to natural sunlight again may depend on the nature of the foliage and the extent of sunlight shielding provided by the plant. The viable spore half-life of *B. thuringensis* on foliage varied from about 1 to 22 days. Insecticidal activity of *B. thuringensis* preparations ranged from 1 to 5 days. A purified preparation of *B. thuringensis* endotoxin had an insecticidal half-life of about 4 days. In general, spores of *B. thuringensis* are more sensitive than endotoxins(s) to sunlight.

Summary and Conclusion

As previously stated, most current knowledge of persistence of entomopathogens is based on a few representative species using data summarized from their use in different ecosystems with differing experimental designs. Data on bacteria are predominately from studies on *Bacillus thuringensis* and *Bacillus popilliae*. Most generalizations with fungi are based on results from species of *Beauveria*, *Entomophthora*, *Metarhizium*, and *Nomuraea*. Microsporidians, more specifically species of *Nosema*, are the basis for generalizations developed for entomopathogenic protozoa. Generalizations for entomopathogenic viruses are mainly based on results with the baculoviruses of *Heliothis*, *Trichoplusia*, *Pieris*, *Orgyia*, and *Lymantria*.

In conclusion, this review hopefully provides guidelines that can be used in planning research with entomopathogens in the Caribbean. These are two points I again wish to emphasize: *first*, environmental problems associated with field applied entomopathogens are primarily technical and/or strategic and thus are experimentally solvable and; *second*, generalizations on environmental effects can be used as guidelines for designing experiments but these generalizations must eventually be confirmed or refuted by actually conducting the experiments.

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POTENTIAL FOR BIOLOGICAL CONTROL OF SOIL INSECTS IN THE CARIBBEAN BASIN USING ENTOMOPATHOGENIC NEMATODES

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ABSTRACT

The numerous species and geographic isolates of entomopathogenic nematodes have a diversity of biological attributes which make them adaptable to many uses, especially management of soil pests. Entomopathogenic nematodes are viable substitutes for soil insecticides due to wide host range, persistence, mobility, safety, ease of application,