Journal of Industrial Microbiology & Biotechnology (1999) 22, 407–417

http://www.stockton-press.co.uk/jim

CHAPTER 9

The First Viral Pesticide: Past, Present, and Future*

CARLO M. IGNOFFO

Biological Control of Insects Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Columbia, Missouri 65201

The review describes the development and commercialization of a rod-shaped, double-stranded, DNA insect virus, *Baculovirus heliothis*, into a viral insecticide. This virus, which attacks only caterpillars of a major pest genus (*Heliothis*) is currently available as a product called Elcar® . The technology associated with the production, evaluation of specificity, and efficacy is discussed and related to possible future developments. An evaluation of the benefits and impact of the development and commercialization of *B. heliothis* is also made.

INTRODUCTION

About 15 yr after its initial isolation from a cotton field, the nucleopolyhedrosis virus of *Heliothis* species was formally registered as a commercial viral pesticide (Ignoffo 1973a,b,c). An exemption from tolerance, granted on May 1973 (Anon. 1973), was followed by label approval in December 1975. Currently, the virus is marketed by Sandoz Inc. under the tradename ELCAR. Another company, Nutralite Products Inc., a subsidiary of Amway Inc., has an experimental product called Biotrol-VHZ.

DISCUSSION

Selection of Candidate

The question is often asked . . . Of all the potential viral candidates, why concentrate on the *Heliothis* virus? There were several major reasons for this decision (Ignoffo 1973a). Species of *Heliothis,* cotton bollworm, tobacco budworm (Fig. 1) are major pests throughout the world, and damage about 30 different commodities. Annual losses and cost to control *Heliothis* on major crops in the United States alone are estimated to be greater than one billion dollars (Anon. 1965, 1966; Ignoffo 1973a). As incentives for selecting this virus, *Heliothis* spp. are resistant to organochlorine, organophosphorus, and carbamate insecticides; resistance is increasing; and at times improper uses of chemical insecticides, especially on cotton, have created problems with toxic residues and environmental pollution.

There are four viruses reported from species of the *Heliothis* complex: two DNA viruses (granulosis virus, nuclear polyhedrosis virus) belonging to the genus *Baculovirus,* an RNA cytoplasmic virus in the genus *Reovirus,* and a recently discovered noninclusion, DNA virus which is probably in the genus *Iridovirus.* The nuclear polyhedrosis virus was selected for development above the others because preliminary tests had indicated that: (1) it was highly

*Mention of commercial or proprietary products in this paper does not constitute endorsement by the USDA.

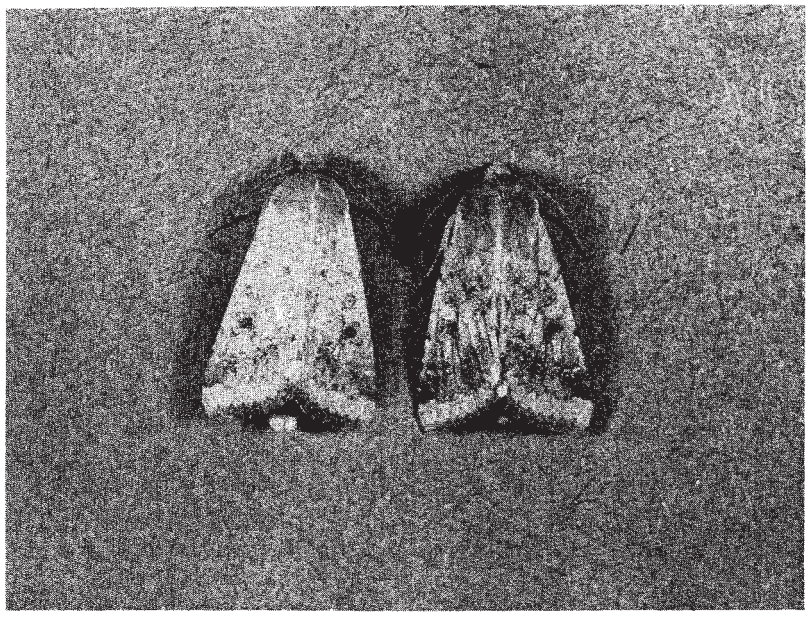


FIG. 1. Adult moths of the cotton bollworm *(Heliothis zea)* alias the corn earworm, soybean podworm, and tomato fruitworm.

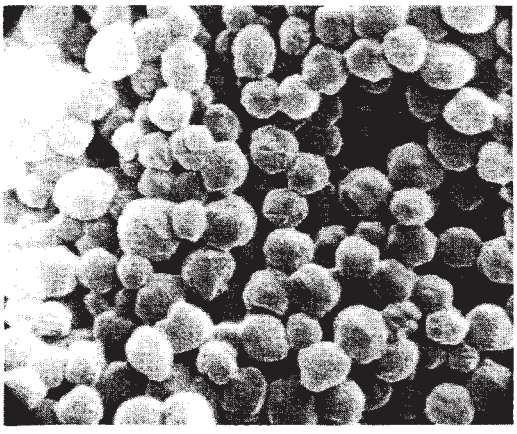
specific; (2) could be continuously produced; (3) was effective against *Heliothis* larvae damaging several commodities; and (4) was not toxic or pathogenic to nontarget organisms. These facts make it easy to see why an effort was made to develop this virus into a viral insecticide. Successful completion of a viral-insecticide program could: (1) significantly reduce environmental pollution; (2) establish necessary guidelines and protocols for evaluating efficacy and safety; (3) provide a functioning industrial technology for production; and (4) possibly stimulate active industrial participation in the development of other potentially useful microbial insecticides.

Candidate Identity

This virus is called a nuclear polyhedrosis virus (NPV) because it produces crystal-like, proteinaceous, irregular polyhedral-shaped inclusion bodies (diameter, 916.4 ± 0.5 mμ) in the nuclei of infected cells (Fig. 2). The infectious virions (Fig. 3), randomly occluded and imbedded singly in the cubic-lattice structure of the inclusion body, are rod-shaped (336 ± 22 mμ × 62 ± 4 mμ) and contain double-stranded DNA (Gregory et al. 1969) (Fig. 3). All nuclear polyhedrosis viruses are placed in the genus *Baculovirus* (Vago et al. 1974); the scientific name of the *Heliothis* NPV is *Baculovirus heliothis. B. heliothis* is very specific and only species belonging to the genus *Heliothis* are known to be susceptible, e.g. *H. zea, H. virescens, H. armigera, H. phloxiphaga, H. obtectus* (Ignoffo et al. 1965).

Mode of Action

Inclusion bodies of *B. heliothis* must be ingested to be infective. Shortly after ingestion, the polyhedral inclusion bodies dissolve within the midgut. The released infectious virion or the nucleic acid pass through the epithelial cells lining the midgut into the hemocoel and



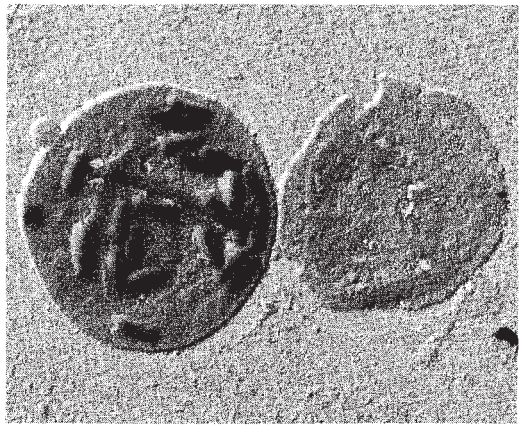


FIG. 2. Polyhedral-shaped inclusion bodies of *Baculovirus heliothis* as viewed under SEM (ca. 10,000X).
FIG. 3. Virions in an inclusion body partially dissolved by alkalai (ca. 30,000X).

subsequently invade the nuclei of susceptible cells. Within a day after ingestion, the usually clear hemolymph becomes increasingly cloudy. Another 1 to 2 days later polyhedral-shaped bodies are detected in the hemolymph. Virtually all of the cells are susceptible. Assembly of virions and formation of inclusion bodies occur only within the nucleus. Lysis of cells with disintegration of larvalt issue begins shortly thereafter. Death of young larvae can occur within 2 days of ingestion of virus; however, older larvae generally die 4 to 9 days after ingestion of virus. Shortly after death, the larvae become flaccid and the integument ruptures, releasing billions of inclusion bodies.

Critical Developmental Pathway

Development of the *B. heliothis* began with a concept in the laboratory in 1961, progressed through various phases, and eventually attained technical realization as a proto-product in 1971. A program-evaluation-review technique was used to facilitate this development (Fig. 4). The program encompassed a study of the virus per se, as well as techniques and data on its production feasibility: safety to man, vertebrates, beneficial insects, and plants; and effectiveness against target pests. These three objectives of production, safety, and effectiveness, which were broad generalizations during the laboratory phase, were more critically defined at each successive phase as more data were accumulated. The questions asked at each phase were: Can *technical* success be attained? And can *technical* success be translated into *commercial* success? The criterion of technical success would be attained if the virus could be continuously produced, was safe, and would effectively suppress the target pest. Commercial success would be achieved if the product could be produced, was effective, and could be used at costs that would return a reasonable profit to the producer.

Program Cost

Cost directly attributed to developing the virus product during phases 0 to 3 inclusive (Fig. 4) was estimated at about two million dollars, with a total involvement of about 33 research team years (Ignoffo 1975a). Approximately 80% of the involvement, both in dollars and

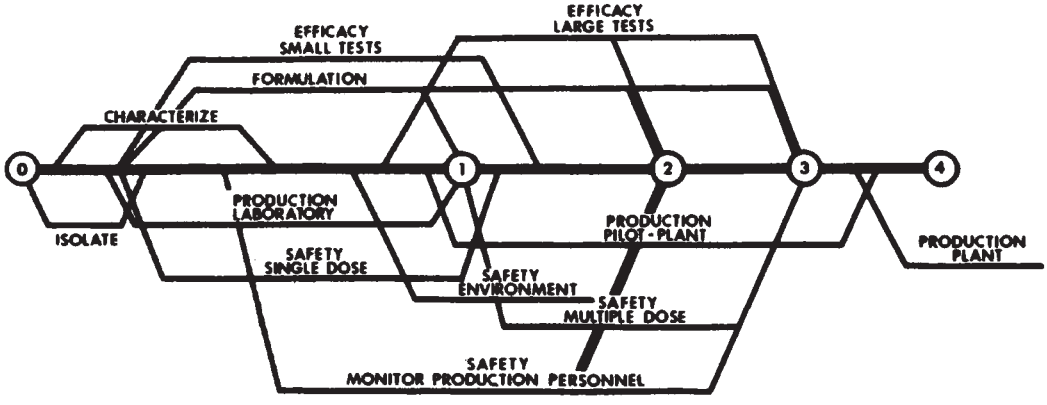


FIG. 4. Phases in the development of *Baculovirus heliothis* into a commercial product.

personnel, was borne by industry, the remainder, by federal and state agencies. During the initial characterization and testing phases, about 6% of the total cost was to bring the virus to the point of being considered for commercialization by industry. Costs in developing a viral insecticide (or other safe entomopathogens) are considerably less (generally 2 to 5 times less) than that for developing a chemical pesticide. Two of the largest costs in developing a chemical insecticide are the initial screening for active compounds and the intermediary metabolism and fate of metabolite studies which are required for all toxic chemical pesticides. Neither is required for nontoxic microbial insecticides.

Production of Baculovirus heliothis

Potential product demand. An average of 12.8 million acres of cotton was planted in the United States during the period 1971 to 1974 inclusive (Anon. 1975). Assuming a conservative average of three applications/acre/season, and about 5% of the cotton is treated for bollworms, then about 1.9 million acre-treatments of virus-product would be required each year. The recommended average rate for use of virus on cotton is 240 billion inclusion bodies/acre which is equivalent to the yield from about 25 larvae. Thus, a minimal production capacity of about 50 million larvae/year would be needed to satisfy the demand for cotton alone.

Current technology. Currently, production of *B. heliothis* is in *Heliothis zea* larvae (Fig. 5). A semi-synthetic diet containing essential nutrients and a gelling agent is dispensed into individual cubicles of a plastic tray. Newly hatched larvae, individually placed in each cubicle and incubated at a constant temp for 5–7 days are then exposed to the virus. During the next 6–8 days, the virus infection-replication process produces 5,000–10,000 times more virus than that originally used. Virus-killed larvae are collected, triturated, screened, and processed into a dry, technical product. This preparation, standardized as to activity and purity, and formulated with various additives to increase field stability and efficacy, is then packaged for sales. The current cost for ELCAR is $3.12/acre-treatment.

The conservative estimated production level for controlling *Heliothis* on cotton alone of about 2 million acre treatments annually translates to about 1/8 million larvae/day. The technology for this production level, resulting in a virus product competitive with chemical

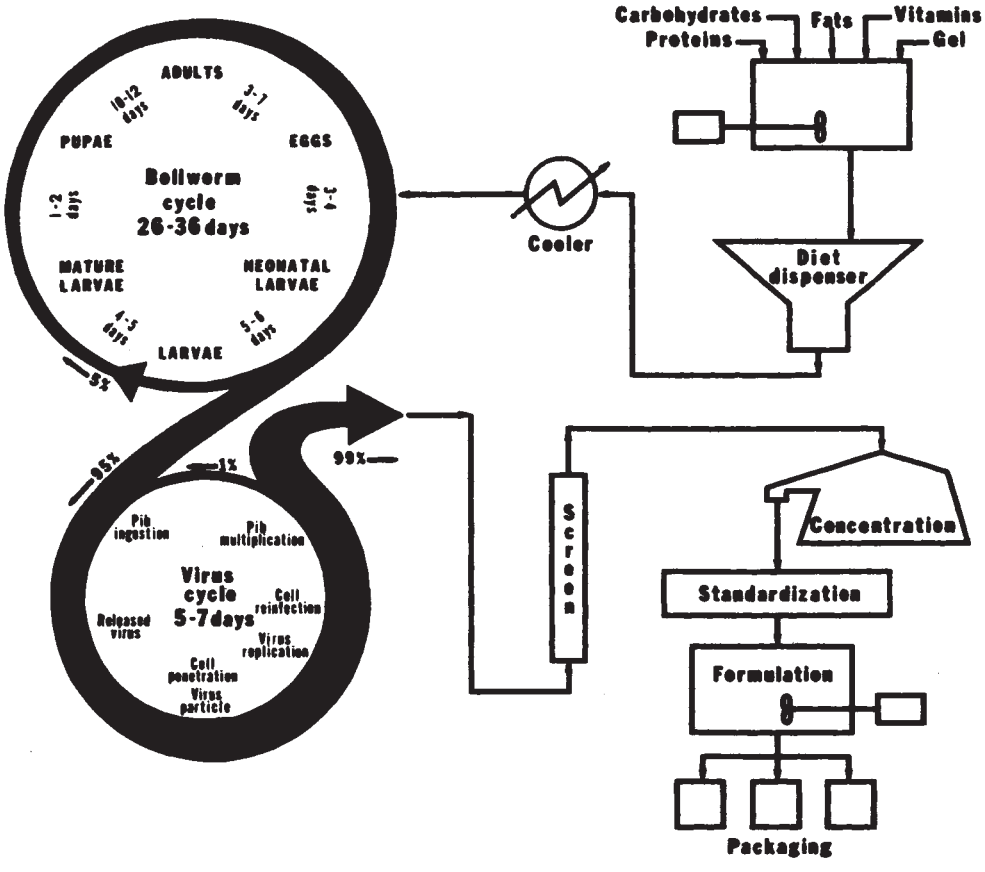


FIG. 5. Diagram of the process used to produce *Baculovirus heliothis.*

insecticides, is available. Further modification of the technology for infesting, rearing, infecting, and harvesting larvae and further improvements in automation with the resultant reduction in production personnel could significantly reduce production cost even further. An estimated 50 to 90% reduction in present production cost could be anticipated in the immediate future.

Future technology. Production of *B. heliothis* in tissue culture will not be commercially possible for a long time. Although a *Heliothis* cell line is available (Hink and Ignoffo 1970), no one is currently evaluating the feasibility of using this line to produce *B. heliothis.* Replication of *B. heliothis* in this line is not complete (Ignoffo et al. 1971), nor is the technology sufficiently developed to even demonstrate the feasibility of this approach. Current work on other cell lines and viruses could, of course, be translated to production of *B. heliothis.*

An important advancement would be to establish criteria to evaluate the feasibility of the cell-system approach *vis-à-vis* production in larvae. The establishment of meaningful criteria and acquisition of pertinent data (media cost, personnel costs, quality control, and potential contamination, virus yield, equipment capital investment, etc.) should permit an objective evaluation and comparison of both techniques.

The most significant development in production of insect viruses probably will come from a quantum jump in tissue-cell technology or some radically new, exotic technique, perhaps something similar to production in a nonhomologous host, possibly by use of the recombinant-DNA approach. There are many reports of replication of plant, vertebrate, or invertebrate viruses in nonhomologuos hosts such as bacterial or yeast cells (Ignoffo 1967; Ignoffo and Hink 1971; Ignoffo 1973b). Although replication in these systems is yet to be corroborated, the basic assumption still appears valid.

Safety of Baculovirus heliothis

Current status. Results to date using current modes of administration and techniques for demonstrating the presence or absence of *B. heliothis* indicate that the virus is safe for its intended use on food crops (Ignoffo 1973b, 1975b). An exemption from the requirement of a tolerance was published by the EPA in May 1973 (Anon. 1973), and a label for sale and use was issued in December 1975. To achieve this status, *B. heliothis* was tested against 24 plant species, 36 invertebrate species, and about 18 vertebrate species with no adverse reactions being attributed to the administration of the virus.

Although all present information indicates that *B. heliothis* is very specific, absolute specificity or safety cannot be guaranteed in all living systems for all time. Consequently, we must be constantly vigilant and continue our research efforts to re-evaluate past tests of safety as more knowledge on replication of insect viruses is obtained and more sensitive techniques are developed for detecting the presence of an insect virus. Continued re-evaluation after safety is once established is an important phase in the development and use of an insect virus, and the use of any virus should be carefully monitored to insure that deleterious aftereffects will not develop. In the final analysis, the risks of field use of *B. heliothis* or any virus must be balanced against the benefits obtained. We must remember that all currently registered chemical insecticides are known to be toxic to man, other animals, and plants at some level. Some are teratogenic and others mutagenic. Insect viruses evaluated to date are not toxic, teratogenic, or mutagenic.

Future developments. In order to continue our assurance that *B. heliothis* is safe, future research and development should provide a better understanding of the nature of specificity and the manner in which the virus infects, replicates, and develops in homologous and nonhomologous hosts. In addition, future research should develop procedures for identifying and characterizing virus isolates, and most importantly, develop sensitive techniques which will detect low levels of virus or virus components in organisms or in the environment.

Efficacy of Baculovirus heliothis

General comments. Registration and labeling of a viral pesticide, although an important *technical* achievement, does not ensure that the virus will be a *commercial* success. We often forget that the transition of a proto-product from a *technical* to a *commercial* success is difficult, consumes both time and money, and can be extremely frustrating. Many viral pesticides are technical successes; not all will be commercial successes. What can be done to facilitate and expedite more commercial successes? One way is through the nontechnical or administrative route; that is, to defray costs of development and production by private or public agencies primarily interested in their development (Ignoffo 1972b). Developers could

be assisted through public loans, loan guarantees, shared-cost grants, procurement incentives; permitting joint ventures and cooperative research; and, most of all, permitting patenting of the microbial agent.

The other way is through technological developments, the area wherein researchers can make significant contributions. There are two major research approaches to improving the efficacy of viral insecticides. The first is by increasing or preserving the inherent activity and the second by dispersal, i.e., increasing the probability of contact with the pest. It is easy to demonstrate experimentally that an agent is capable of suppressing or controlling a pest. However, it is more difficult, especially during the early development phases to achieve consistently a high level of performance. Even the most toxic, highly active chemical insecticides are not 100% consistent in performance.

Activity increase. Efficacy via the inherent activity approach may be increased by selection for more active isolates or by increasing the activity per viral unit through production or formulation technology. Both have been successfully used with *B. heliothis* and conceivably could be used successfully to further increase activity. For example, activity was increased initially at least 50-fold by choosing the most active of all available natural isolates, and activity also was increased about twofold through selection (Shapiro and Ignoffo 1970). Feeding attractants or gustatory stimulants also have notably increased effective activity through inducing the insect to selectively feed on baited virus (Ignoffo et al. 1976a).

Preservation of activity. Formulation efforts through use of adjuvants can significantly preserve the inherent activity and thereby increase consistency of performance. The addition of a sunlight-UV protectant or use of microencapsulation, for example, increased persistence two- to three-fold over that of unprotected virus. The goal for a UV-protectant should be to attain a half-life of at least five days following field application of the virus. If this is achieved, then inactivation thereafter will mean that about 30% of the original activity will still be present 3-4 wk post-application. Published data also demonstrated that changes in standard operating procedures during virus propagation, harvesting, and preparation of the technical viral product (prior to formulation) could increase inherent activity about two- to five-fold (Ignoffo et al. 1976b; Ignoffo and Shapiro 1978).

Dispersal of activity. One of the least researched and understood phases of microbial insecticides is how best to apply them. The *B. heliothis* virus, as well as many microbial insecticides, has sufficient activity to control effectively damaging populations of pests. The biggest problem to be solved now is how to increase the probability of contact between the target pest and the agent (Ignoffo 1972a). We have assumed, for want of specific information, that application systems developed for chemical insecticides will work. Experience has indeed shown that they will work, but are they ideal? We have assumed that a system designed to deliver fast-acting soluble or emulsifiable, volatile, small molecular weight, chemical insecticides is ideal for delivering high molecular weight, nonvolatile, particulate, slower-acting biologicals. This approach should be challenged. If research indicates that the current application technology is best, then good; at least, this has been experimentally determined. If current systems are not ideal, then we should define those factors which are ideal, establish desired performance specifications for those factors, and design equipment especially suited for delivering microbial insecticides. Research on in-

creasing or preserving activity should not be discontinued in favor of application technology. Both should and must be conducted concurrently. I am emphasizing, however, that additional research on ways to increase the probability of contact between the target pest and the infective agent will probably be the most productive and thus *must be conducted.*

There are some beginning efforts on optimizing the dispersal of microbial insecticides using *B. heliothis* and *Bacillus thuringiensis* as models (Smith et al. 1977a,b). These current efforts and those of the recent past have indeed demonstrated that increasing coverage by increasing volume per acre or decreasing droplet size can result in more effective control of *Heliothis* species by *B. heliothis* (Chapman and Ignoffo 1972; Falcon et al. 1974; Ignoffo 1972a).

Is spraying or dusting the only way to disperse *B. heliothis?* (Ignoffo 1978). For example, can *B. heliothis* be introduced into an agro-ecosystem in sufficient quantities to initiate an epizootic (especially for crop systems other than cotton)? Quite recently, Gard (1974) sought to use moths attracted to traps baited with virus dust as a means of dispersal. This field study demonstrated that the approach was feasible. Another approach to establishing efficacy over a longer period would be an evaluation of what happens to the pest population, population of beneficial insects, and levels of economic damage during an extended 5-year use of only *B. heliothis.* This approach could be compared to areas of minimal chemical insecticidal use as well as heavy chemical use. The basic assumption herein is that pest populations and beneficial insect populations will resurge and normalize during continual use of *B. heliothis* and that yields will correspondingly increase steadily because of the selected, combined control by natural parasites, predators, and the application of *B. heliothis.*

Expansion of label to other pests. One of the most obvious future developments for *B. heliothis* is the extension of the current label from use on only cotton to use on other crops (Ignoffo et al. 1965). Recent field results indicate that *B. heliothis* can be used effectively to control *Heliothis* on soybeans at rates lower than that used for cotton (Ignoffo et al. 1978). Extension of efficacy of *B. heliothis* to soybeans could be followed by use on sorghum, vegetables, tobacco, and peanuts. Use on corn, especially sweet corn, will probably be limited because of the feeding behavior of *Heliothis* larvae on corn. The selection of this commodity priority is based upon previous experience (Ignoffo et al. 1965) which indicated that the feeding behavior of *Heliothis* species on a particular crop can significantly influence field effectiveness and consistency, even though the inherent activity of the virus is the same. For example, eggs of *Heliothis* are laid directly on the corn silks and neonatal larvae penetrate the corn ear soon after hatching (Table 1). About 90% of the total larval life is

TABLE 1. *Impact of oviposition site, larvae feeding behavior, and plant response to anticipated control of* Heliothis *species by use of* Baculovirus heliothis

Commodity	Oviposition Site	Feeding Site	Percent Larval Stage Exposed at Site	Relative Plant Rebound to Damage
Corn	silk	in ear	10	Negligible
Cotton	terminal leaves	in bolls	40	Average
Soybean	terminal leaves	on pods	90	Excellent

in the protective confines of the ear and the corn plant does not readily rebound from *Heliothis* damage by developing extra new ears or larger ears or kernels. Consistent, economic control with *B. heliothis* on corn is therefore very difficult.

On cotton, the *Heliothis* eggs are laid on or in the vicinity of terminal buds, and larvae may feed on exposed leaves and buds for about 40% of their larval life. The rest of the larval stage is in the protective confines of cotton bolls. Cotton plants are able to compensate for some of this feeding damage; certainly more so than for corn. These factors permit a higher probability of effective, consistent control of *Heliothis* on cotton than on corn.

Soybeans can be used as an example of another extreme. Larvae of *Heliothis* are exposed for nearly 90% of their larval life and freely move over the entire plant. In addition, soybeans have a remarkable ability to compensate for *Heliothis* damage by either producing more pods or larger seeds. Thus, a higher probability of effective, consistent control of *Heliothis* species can be anticipated on soybeans than on either cotton or corn.

Benefits and Impact of the First Viral Pesticide Program

Development of a safe, nonpolluting product. One of the most obvious achievements and benefits of the *B. heliothis* program was the development of a safe, viral product. This was a unique achievement. It demonstrated that industrial, federal, and state scientists working together could develop a naturally occurring entomopathogen into a safe, biological control product.

Establishment and development of an industry. Prior to 1965, industrial facilities, technology, and personnel for developing an insect virus were essentially nonexistent. As the results of development of *B. heliothis,* a functioning production technology, industrial facilities, and trained personnel are now available. Semi-automated systems and inexpensive artificial diets for producing *Heliothis* spp. have been developed which, with slight modification, could be used to produce other insect viruses and indeed other obligate entomopathogens. Pilot plants which produce a million caterpillars per month are operational, and larger plants can produce more than a million caterpillars per week.

Establishment of guidelines and protocols for evaluation of safety and efficacy. The establishment of protocols and guidelines for evaluating viral insecticides, made possible by the development of *B. heliothis,* was another highly significant achievement. No guidelines or protocols for microbial insecticides existed prior to 1968 when petitions were first submitted to FDA by industry. In fact, the lack of a clear-cut regulatory policy for evaluating the risks of use of an insect virus was a major obstacle and at several junctures almost resulted in industrial withdrawal from research and development of *B. heliothis.*

Presently, guidelines and protocols for evaluating risks have been established for the first time for baculoviruses, i.e., NPV and granuloses viruses. The time to complete a safety-hazard evaluation program also has been clearly defined. Machinery for the review of microbial insecticides, both personnel and procedure, is now functional. In addition, guidelines developed for the baculoviruses now may be used in initial negotiations with regulatory agencies for registration of other potential microbial insecticides.

Application of acquired knowledge to other programs. The technology developed for *B. heliothis* and the problems and their solutions have and still are being directly and in-

directly applied to the development of other microbial insecticides. Specifically, major biological and pathological problems concerned with the continuous production of insects for virus propagation are defined and have been resolved. Major technical problems of formulation, stability, and application are defined; some have been resolved; others are currently under investigation. To conclude, we have come a long way in a little over a decade. It has taken a lot of hard work and commitment. It frequently has been frustrating but also interesting and certainly exciting. Hopefully, these efforts will eventually benefit all of us to lead a fuller life in our environment.

LITERATURE CITED

Anonymous. 1965. *Losses in Agriculture.* Agricultural Handbook 291, Agricultural Research Service, United States Department of Agriculture.

Anonymous. 1966. *Crop Production,* 1966 Annual Summary. Agricultural Research Service, United States Department of Agriculture.

Anonymous. 1973. Code of Federal Regulations 38:10643.

Anonymous. 1975. Agricultural Statistics, 1975. U.S. Dept. of Agric., U.S. Govt. Printing Office.

Chapman, A. J., and C. M. Ignoffo. 1972. Influence of rate and spray volume of a nucleopolyhedrosis virus on control of *Heliothis* in cotton. *J. Invertebr. Pathol.* 20:183–186.

Falcon, L. A., A. Sorensen, and N. B. Akesson. 1974. Pioneering research on aerosol application of insect pathogens. *Calif. Agric.,* 28:11–13.

Gard, I. E. 1974. Autodissemination of insect viruses. *Entomol. Soc. Am.* Minneapolis, Minn., 1974 (paper presented).

Gregory, B. G., C. M. Ignoffo, and M. Shapiro. 1969. Nucleopolyhedrosis of *Heliothis:* Morphological description of inclusion bodies and virions. *J. Invertebr. Pathol.* 14:186–193.

Hink, W. F., and C. M. Ignoffo. 1970. Establishment of a new cell line (IMC-HZ-1) from ovaries of cotton bollworm moths, *Heliothis zea* (Boddie). *Exp. Cell Res.* 60:307–309.

Ignoffo, C. M. 1967. Possibilities of mass-producing insect pathogens. Pages 91–117 *in Proc. Int. Colloq. Insect Pathol. Microbial Control,* Wageningen, The Netherlands, Sept. 5-10, 1966. North-Holland Publ. Co., Amsterdam.

_____. 1972a. An appeal for research. *J. Invertebr. Pathol.* 19:i-ii.

_____. 1972b. Principles and pathogens: Use of pathogens. *Proc. N. Centr. Br. ESA* 27:39–40.

_____. 1973a. Development of a viral insecticide: concept to commercialization. *Exp. Parasitol.* 33:380–406.

_____. 1973b. Effects of entomopathogens on vertebrates. *N.Y. Acad. Sci.* 217:141–172.

_____. 1973c. Toward registration of a viral insecticide. *Misc. Publ. Entomol. Soc. Am.* 9:57–61.

_____. 1975a. Entomopathogens as insecticides. *Environ. Lett.* 8:23–40.

_____. 1975b. Evaluation of in vivo specificity of insect viruses. Pages 52–57 *in* Max Summers, Reto Engler, Louis A. Falcon, and Patrick V. Vail, eds. *Baculoviruses for Insect Pest Control: Safety Considerations.* Am. Soc. Microbiol., Washington, D.C.

_____. 1978. Strategies to increase the use of entomopathogens. *J. Invertebr. Path.* 31.

Ignoffo, C. M., and W. F. Hink. 1971. Propagation of arthropod pathogens in living systems. Pages 541–580 *in* H. D. Burges and N. W. Hussey, eds. *Microbial Control of Insects and Mites.* Academic Press, London.

Ignoffo, C. M., and M. Shapiro. 1978. Characteristics of baculovirus preparations processed from living and dead larvae. *J. Econ. Entomol.* 71:186–188.

Ignoffo, C. M., A. J. Chapman, and D. F. Martin. 1965. The nuclear-polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virescens* (Fabricius). III. Effectiveness of the virus against field populations of *Heliothis* on cotton, corn, and grain sorghum. *J. Invertebr. Pathol.* 7:227–235.

Ignoffo, C. M., M. Shapiro, and W. F. Hink. 1971. Replication and serial passage of infectious *Heliothis* nucleopolyhedrosis virus in an established line of *Heliothis zea* cells. *J. Invertebr. Pathol.* 18:131(134.

Ignoffo, C. M., D. L. Hostetter, and D. B. Smith. 1976b. Gustatory stimulant, sunlight protectant, evaporation retardant; three characteristics of a microbial insecticidal adjuvant. *J. Econ. Entomol.* 69:207–210.

Ignoffo, C. M., D. L. Hostetter, K. D. Biever, C. Garcia, G. D. Thomas, W. A. Dickerson, and R. Pinnell. 1978. Evaluation of an entomopathogenic bacterium, fungus, and virus for control of *Heliothis zea* on soybeans. *J. Econ. Entomol.* In press.

Ignoffo, C. M., W. C. Yearian, S. Y. Young, D. L. Hostetter, and D. L. Bull. 1976a. Laboratory and field persistence of new commercial formulations of the *Heliothis* nucleopolyhedrosis virus, *Baculovirus heliothis. J. Econ. Entomol.* 69:233–236.

Shapiro, M., and C. M. Ignoffo. 1970. Nucleopolyhedrosis of *Heliothis:* activity of isolates from *Heliothis zea. J. Invertebr. Pathol.* 16:107–111.

Smith, D. B., D. L. Hostetter, and C. M. Ignoffo. 1977a. Laboratory performance specifications for a bacterial *(Bacillus thuringiensis* var. *kurstaki)* and a viral *(Baculovirus heliothis)* insecticide. *J. Econ. Entomol.* 70:437–441.

_____. 1977b. Ground spray equipment for applying a microbial insecticide *(Bacillus thuringiensis)* on soybeans. *J. Econ. Entomol.* 70:633–637.

Vago, C., K. Aizawa, C. Ignoffo, M. E. Martignoni, L. Tarasevitch, and T. W. Tinsley. 1974. Present status of the nomenclature and classification of invertebrate viruses. *J. Invertebr. Pathol.* 23:133–134.