BIOLOGICAL CONTROL: AN INDUSTRIAL PERSPECTIVE

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ABSTRACT

The CIBA-GEIGY Corporation is the U.S. subsidiary of CIBA-GEIGY Limited, Basel, Switzerland, one of the largest agricultural chemical companies in the world. The focus of the Insect Control Sector is toward newer, safer technologies that fit into IPM programs and are socially and environmentally acceptable. As part of this strategy CIBA-GEIGY has major efforts in biological control, with priorities in *Bacillus thuringiensis*, *Beauveria bassiana* and *Trichogramma* spp. research. CIBA-GEIGY's first *Bacillus thuringiensis* product, coded CGA-237218, is a transconjugate from *Bacillus thuringiensis* subsp. *aizawai* and *Bacillus thuringiensis* subsp. *kurstaki* parents. This transconjugate strain shows increased lepidopteran activity over that of the parental strains, especially against pests in the genera *Heliothis*, *Spodoptera*, *Pieris*, and *Mamestra*. EPA approval is expected in 1992. A cooperative effort between CIBA-GEIGY and the University of Florida is in progress to develop a commercial *Beauveria bassiana* product for control of the citrus root weevil complex in citrus. CIBA-GEIGY also has cooperative projects underway with Canadian institutions and the USDA to develop the parasitic wasp, *Trichogramma minutum*, for control of the eastern spruce budworm, *Choristoneura fumiferana*, an economically important pest of Canadian forests.

RESUMEN

La corporacion CIBA-GEIGY es una subsidiaria de CIBA-GEIGY Ltd., de Basel, Suiza, la cual es una de las companias agroquimicas mas grandes del mundo. La filosofia de la compania con respecto al control de insectos, se basa en el desarrollo de tecnologias nuevas que ante todo sean inofensivas, que se puedan implementar en programs de control integrado de plagas, y que sean aceptadas tanto social como ambientalmente. Como parte de esta estrategia, la CIBA-GEIGY ha colocado sus mayores esfuerzos en el area del control biologico con prioridad en *Bacillus thuringiensis*, *Beauveria bassiana* y *Trichogramma* spp. el primer producto de la CIBA-GEIGY con *B. thuringiensis*, codigo CGA-237218, es una bacteria que ha sido transformada geneticamente y que tiene como padres *B. thuringiensis* subsp. *aizawai* y *B. thuringiensis* subsp. *kurstaki*. Esta bacteria es mas activa que sus padres en el control de lepidopteros, especialmente plagas del genero *Heliothis*, *Spodoptera*, *Pieris*, y *Mamestra*. Se espera que el EPA la apruebe en 1992. La CIBA-GEIGY con la cooperacion de la Universidad de Florida estan desarrollando un producto comercial de *Beauveria bassiana* para el control de los gorgojos de las raices de citricos. La CIBA-GEIGY tambien esta cooperando con instituciones canadienses y el USDA para desarrollar una avispa, *Trichogramma minutum*, que parasite el gusano oriental de los apices del pino, *Choristoneura fumiferana*, el cual es una plaga muy importante en los bosques canadienses.

CIBA-GEIGY Corporation is the U.S. subsidiary of one of the largest chemical companies in the world—CIBA-GEIGY Limited, headquartered in Basel, Switzerland. CIBA (an acronym for *C*hemical *I*ndustry of *BA*sel) Limited began in 1884 as a manufacturer of synthetic dyes for the silk industry and soon ventured into the field of medicines. The company later extended its research and production activities to other branches of chemistry. Geigy was formed in 1758 and was also primarily concerned with dyestuffs but branched out in the 20th century into agricultural chemicals and pharmaceuticals. In 1970 CIBA Limited and Geigy merged to form CIBA-GEIGY, which today has affiliated companies in 50 countries. The Agricultural Division of the U.S. company is headquartered in Greensboro, North Carolina, and has been an important member of the agricultural industry for more than 40 years, manufacturing and distributing insecticides, herbicides, fungicides, plant growth regulators and, since 1974, seeds (with the acquisition of Funk Seeds International). In 1983 CIBA-GEIGY opened its Agricultural Biotechnology Research Unit which is dedicated to creating, in the laboratory, new plants that cannot be developed through traditional breeding, including the incorporation of resistance to insects.

Over the years many successful and useful insecticides have been developed by CIBA-GEIGY: DZN^R (diazinon), Curacron^R (profenfos) and Supracide^R (methidathion), for example. In the 1970's new research resulted in the development of the insect growth inhibitor cyromazine (Trigard^R), that is highly selective for dipterous pests (especially agromyzid leafminers) and effective at the very low rate (for the 70's) of 140 grams active ingredient per hectare (g ai/ha). Since then, newer insect growth inhibitors have been found by CIBA-GEIGY that are active at rates as low as 1.0-2.0 g ai/ha.

As an industry leader in innovative technology, CIBA-GEIGY has focused its research efforts in insect control toward newer and safer technologies. These technologies include new chemical leads, behavior modifying chemicals, chemosterilants and growth inhibitors, expertise in integrated pest management and the development of biological control agents. To date the development of biological controls has focused on 1) *Bacillus thuringiensis*, 2) the entomopathogenic fungi *Beauveria brongniartii* and *B. bassiana*, and 3) parasites belonging to the genera *Trichogramma* and *Encarsia*. The following is a discussion of CIBA-GEIGY's efforts in biological control.

BACILLUS THURINGIENSIS

CIBA-GEIGY's efforts with *Bacillus thuringiensis* (Bt) were initiated about 10 years ago, with major efforts in our Basel, Switzerland, laboratories. However, CIBA-GEIGY Research and Development personnel worldwide are involved in collecting samples for isolation of new, potentially more active strains and in field testing of strains that are in development.

The first strain of commercial development by CIBA-GEIGY is coded CGA-237218 (U.S. Patent 4,935,353; NCTC accession number 11821), with the suggested trademark in the USA of Agree^{TM}. CGA-237218 is a transconjugate from *Bt* subsp. *aizawai* and *Bt* subsp. *kurstaki* parents, with the HD-135 mutant serving as the receptor of genetic material from the HD-191 mutant. The lepidopteran activity of this transconjugate is increased over that of the parents, especially against pests in the genera *Heliothis*, *Spodoptera*, *Pieris* and *Mamestra* (Table 1).

In the USA, field tests with CGA-237218 have been conducted primarily on vegetable crops, especially cole crops, tomatoes and leafy vegetables by CIBA-GEIGY, university and private contract researchers. The effective use rate is 1120-2240 g/ha. Figure 1 is a summary of lepidopterous pest control in cole crops from 10 field trials across the USA, demonstrating the activity of CGA-237218 on 3 major pests—*Pieris rapae*, *Trichoplusia ni*, and *Plutella xylostella*. *P. rapae*, which is quite sensitive to Bt's, was easily controlled by all rates of CGA-237218. *T. ni* required higher rates for effective control while *P. xylostella* was intermediate in this respect.

CGA-237218 is in full development within CIBA-GEIGY, with full submission for registration expected by mid-1991 and anticipated sales under the Agree^{TM} trademark in late 1992. Initial marketing of a "50%" wettable powder product containing 2.5-5.0% delta endotoxin (as determined by chromatography) is planned. Additional strains are in development.

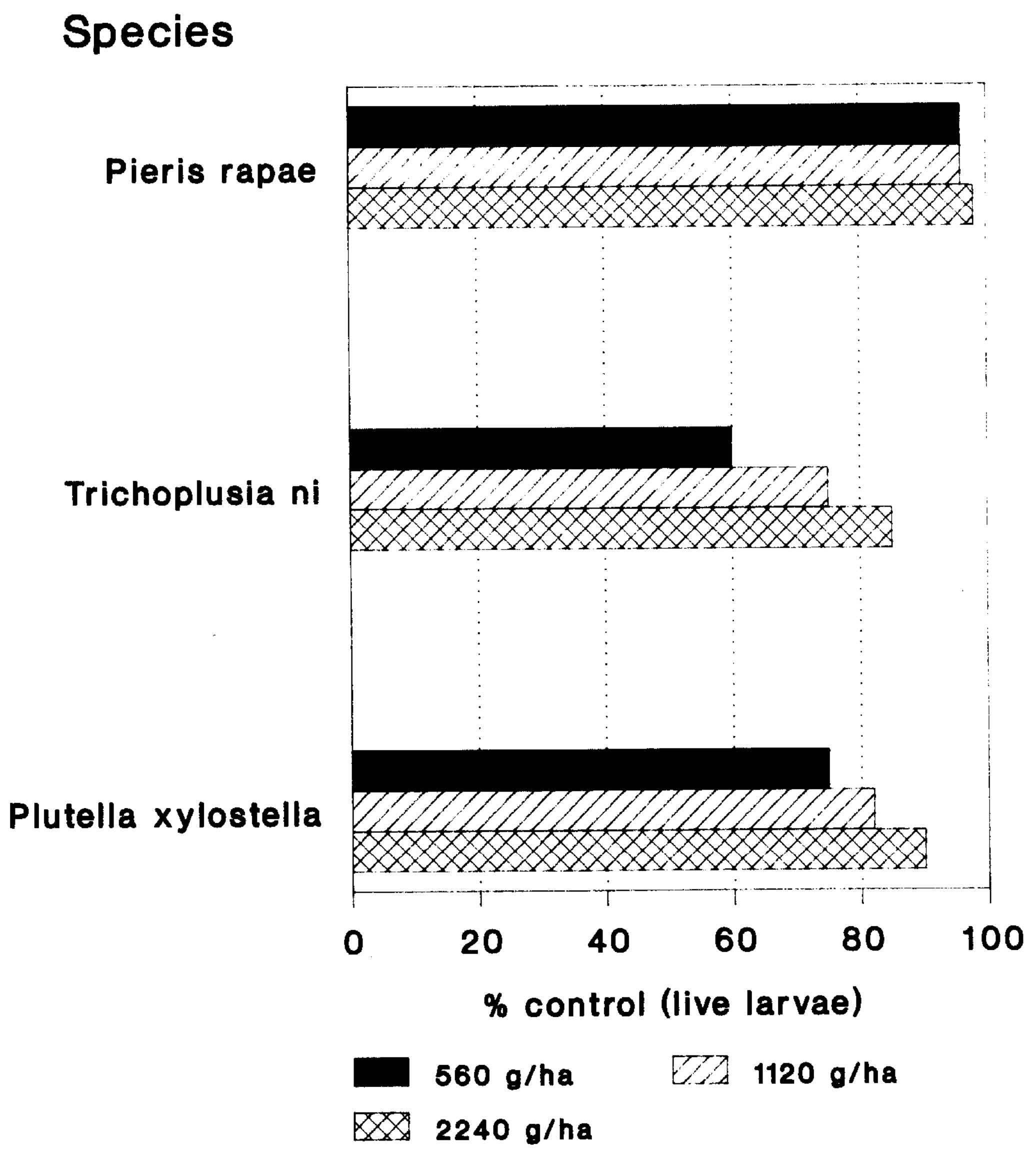

Fig. 1. Summary of lepidopterous pest control in cole crops with CGA-237218.

TABLE 1. BIOLOGICAL ACTIVITY OF CGA-237218 VERSUS ITS PARENTAL STRAINS.

	LC_{50} (ppm) in Diet Bioassay		
Insect Species	HD 191	HD 135	CGA-237218
Heliothis armigera	48.0	228.0	44.0
Heliothis virescens	5.8	205.0	4.8
Spodoptera littoralis	10,000	445.0	330.0
Pieris brassicae	0.98	1.2	0.72
Mamestra brassicae	>10,000	185.0	162.0

Entomopathogenic Fungi

Insects are attacked in nature by fungi representing all classes. The 2 most important groups of entomopathogenic fungi are the Deuteromycetes and Phycomycetes (Ferron 1978). The genus *Beauveria* (Deuteromycetes: Moniliales) is the best known and contains 2 species, *B. bassiana* and *B. brongniartii* (MacLeod 1954).

Entomopathogenic fungi differ from bacteria (e.g., *Bacillus thuringiensis*) and viruses in that the most common route of infection is through the surface of the integument, although infection can occur through the spiracles and possibly via ingestion (Gardner & Noblet 1978). This advantage allows for the infection of insects independently of their feeding activity. With *Beauvaria*, the generalized infection cycle proceeds as follows: conidia contact the integument, germinate, penetrate (by mechanical pressure and/or enzymatic activity) via production of a germ tube, enter the haemolymph, where hyphal growth continues, and spread throughout the body resulting in death due to organ destruction and/or toxin production (Madelin 1966, Roberts 1981). In the haemolymph the fungus produces hyphal bodies, also called blastospores, which spread the infection throughout the body by giving rise to more hyphae. In nature the blastospore stage is found only inside the insect body. After host death, hyphae emerge from the cadaver to produce conidia for further disease spread.

Although *Beauvaria* has the advantage of not having to be ingested, there are also some disadvantages, the most notable of which are 1) the inactivation of infective units by ultraviolet light (UV) and heat and 2) the problem of mass production and formulation of infective units required for commercialization. To date there has been no successful mass production and formulation of a *Beauveria* preparation on a commercial scale. *Beauvaria* conidia and blastospores cannot be spray dried because temperatures of 50-70°C kill them (Roberts & Campbell 1977). As a comparison, *B. thuringiensis* is spray dried at over 100°C. However, *Beauvaria* conidia can be frozen for months without detrimental effects (Muller-Kogler 1967). UV light is also detrimental to conidia and may be the key reason for the disappointing results from foliar applications to control the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera:Chrysomelidae) (Gaugler et al. 1989). Roberts and Campbell (1977) suggest that insect control with entomopathogenic fungi in soil habitats may be more rewarding (than foliar habitats) due to higher moisture content, moderate temperatures and protection from UV light.

One of the first pests successfully controlled by the application of an entomopathogenic fungus was the May beetle or European cockchafer, *Melolontha melolontha* (Coleoptera:Scarabaeidae), by the fungus *Beauveria brongniartii* (Keller & Zimmerman 1989). Adult cockchafers swarm between the end of April and the beginning of June, with most swarming concentrated along borders of forests (Keller et al. 1989). A feeding, mating and egg maturation period of about 7-10 days is followed by a return of the female to breeding sites, where eggs are deposited at a depth of approximately 10-20 cm. Larvae eclose after 4-6 weeks, passing through 3 larval instars during their 2 year development (Keller et al. 1989). Larvae feed on roots of a variety of orchard, vineyard and pasture crops, where sufficient numbers can cause heavy damage. In addition, such high value crops as strawberries are also attacked.

B. brongniartii has been observed as a pathogen of cockchafers for over 100 years. A major problem in using *B. brongniartii* to control cockchafers in the field has been introducing the inoculum into the soil where it can come into contact with larvae. There are 2 possible methods of contaminating the breeding sites: 1) introduction of inocula by mechanical methods and 2) introduction using the egg-depositing females as vectors (Keller et al. 1989). In the cockchafer control program in Switzerland and Italy, swarming adults are sprayed (by helicopter) with a blastospore suspension, with the treated adult female bringing the infective unit into the soil when she returns to oviposit (Keller 1986). For several seasons *B. brongniartii* blastospore suspension was produced by

CIBA-GEIGY Pharma Division, Basel, Switzerland, in a 4000 liter fermenter. The goal of the cockchafer control program is to reduce populations over time, as the fungus acts slowly and long-term observations are necessary to evaluate efficacy (Keller & Zimmerman 1989).

Although blastospores are not infective units in nature, they are as infective as conidia in bioassays (S. Keller, personal communication). However, because conidia are the infective units in nature and are more resistant to adverse environmental conditions, therefore probably more tolerant to formulating, a commercial *Beauveria* product would be most feasible if conidia could be produced in culture and formulated to enhance stability and ease of application. For these reasons, CIBA-GEIGY has conducted research in liquid production of *Beauveria* conidia. In the USA a cooperative effort between CIBA-GEIGY and the University of Florida is in progress to develop *B. bassiana* for control of the citrus root weevil complex. This complex is made up of 5 weevil species: *Artipus floridanus*, *Diaprepes abbreviatus*, *Pachnaeus litus*, *P. opalus* and *Pantomorus cervinus* (Coleoptera:Curculionidae). Citrus root weevils appear to be an excellent candidate for control by *B. bassiana* for several reasons: 1) a highly virulent citrus root weevil strain of *B. bassiana* exists; 2) eggs are laid in the citrus tree canopy and, upon eclosion, neonate larvae drop to the soil to burrow to the roots, thus a susceptible stage is present in the soil habitat where conidial survival is more likely (as discussed by Roberts and Campbell, 1977); and 3) no effective larval controls are currently available.

To date, the infectivity of liquid-produced *B. bassiana* conidia versus aerial conidia (produced on Sabourad Dextrose Agar [SDA]) has been compared in laboratory bioassays on larvae of 3 insect species—Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera:Chrysomelidae), *Diabrotica balteata* (Coleoptera:Chrysomelidae), and the little leaf notcher, *Artipus floridanus*. In addition, the infectivity of liquid-produced *B. brongniartii* conidia versus the CIBA-GEIGY blastospore suspension was compared in a laboratory bioassay on third instar *M. melolontha* (Coleoptera:Scarabaeidae) larvae by Dr. S. Keller, Swiss Federal Research Institute, Reckenholz, Switzerland. In all 4 cases liquid-produced conidia were as infective as SDA-produced conidia and the blastospore suspension (Figs. 2 & 3).

Research is in progress to optimize conidial yields in liquid culture and develop a commercially acceptable formulation of *Beauveria bassiana*.

TRICHOGRAMMA

Another area of biological control that CIBA-GEIGY is pursuing is the use of the parasitic wasp species, *Trichogramma minutum* and *T. pretiosum* (Hymenoptera: Trichogrammatidae), for insect control. *Trichogramma* spp. wasps parasitize the eggs of over 200 lepidopteran species, including such economically important pests as the tobacco budworm, *Heliothis virescens* (Noctuidae); the bollworm/corn earworm/tomato fruitworm, *Helicoverpa zea* (Noctuidae); the eastern spruce budworm, *Choristoneura fumiferana* (Tortricidae) and the European corn borer, *Ostrinia nubilalis* (Pyralidae). Effective pest control with *Trichogramma* spp. has been demonstrated in Europe where the European corn borer is controlled by *T. evanescens* on approximately 20,000 ha of corn.

There are 2 major obstacles to overcome in the commercial development of *Trichogramma* spp. as insect control agents: 1) production costs and scale up and 2) unreliable quality. Lesser problems include method of application and effective rates of release. However, there are opportunities as well: one production process could be utilized to control over 100 lepidopteran pests, cost effective control with *Trichogramma* would increase the volume of use, and the social and environmental acceptability of biological control.

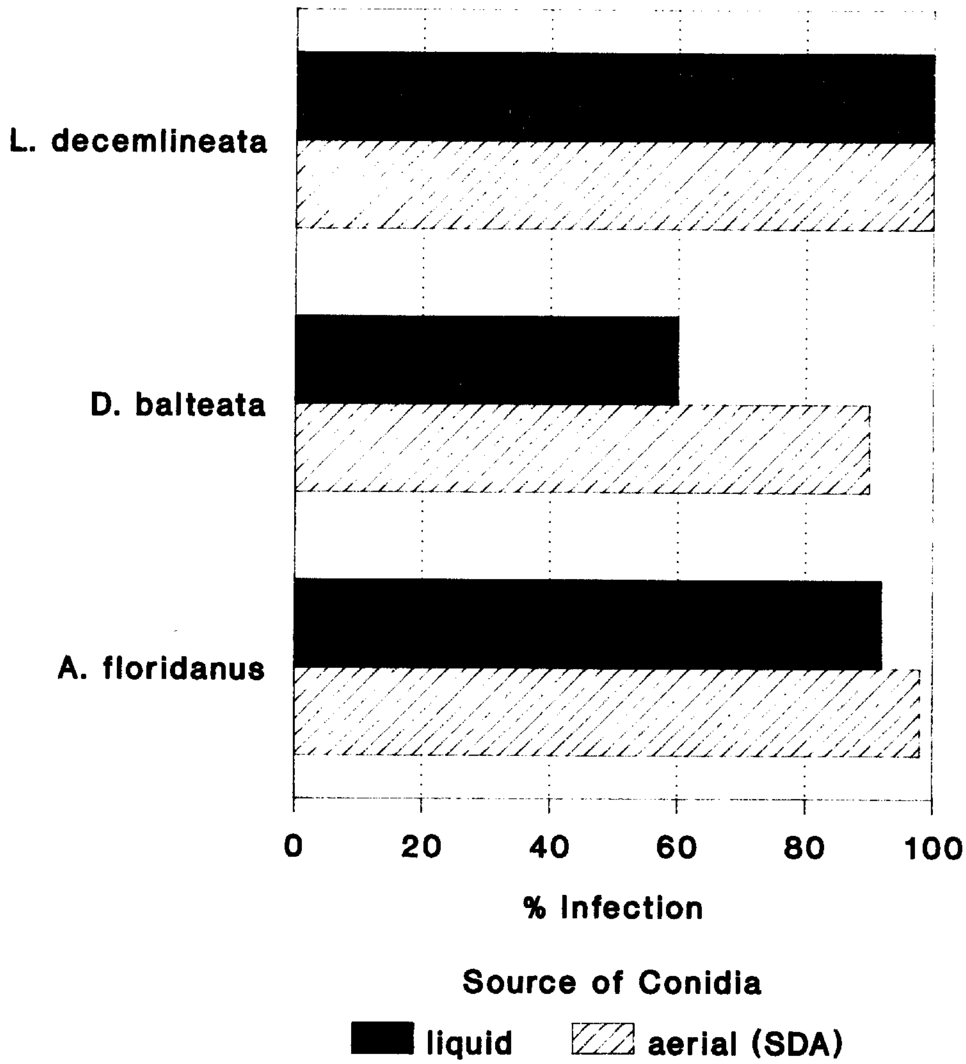

Fig. 2. Comparison of *Beauveria bassiana* infectivity with liquid-produced versus aerial-produced (on SDA) conidia.

CIBA-GEIGY has 2 cooperative *Trichogramma* projects in progress in North America. The largest, most visible and furthest advanced is the Spruce Budworm Control Project in cooperation with the University of Toronto, University of Guelph and the Ontario Ministry of Natural Resources. The eastern spruce budworm, *Choristoneura fumiferana*, is a major forestry pest in Canada. From 1977 to 1981 the average annual loss (defoliation) due to this pest was $44 \times 10^6 m^3$, equivalent to 66% of the volume harvested in the same period (Gross 1985). Five Canadian provinces (Ontario, British Columbia, Manitoba, Quebec and Nova Scotia) have adopted a non-chemical approach to forest insect pest control (Smith et al. 1990). In 1979, 1% of the forest area treated with insecticides was treated with *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) and 99% with chemical insecticides while in 1988, of the 750,000 ha treated, 64% was treated with *B.t.k.* and 36% with chemical insecticides (Smith et al. 1990). The long-term goals of the CIBA-GEIGY Canada project are the eventual replacement of current chemical controls with *Trichogramma minutum* and enhancing the growth and development of

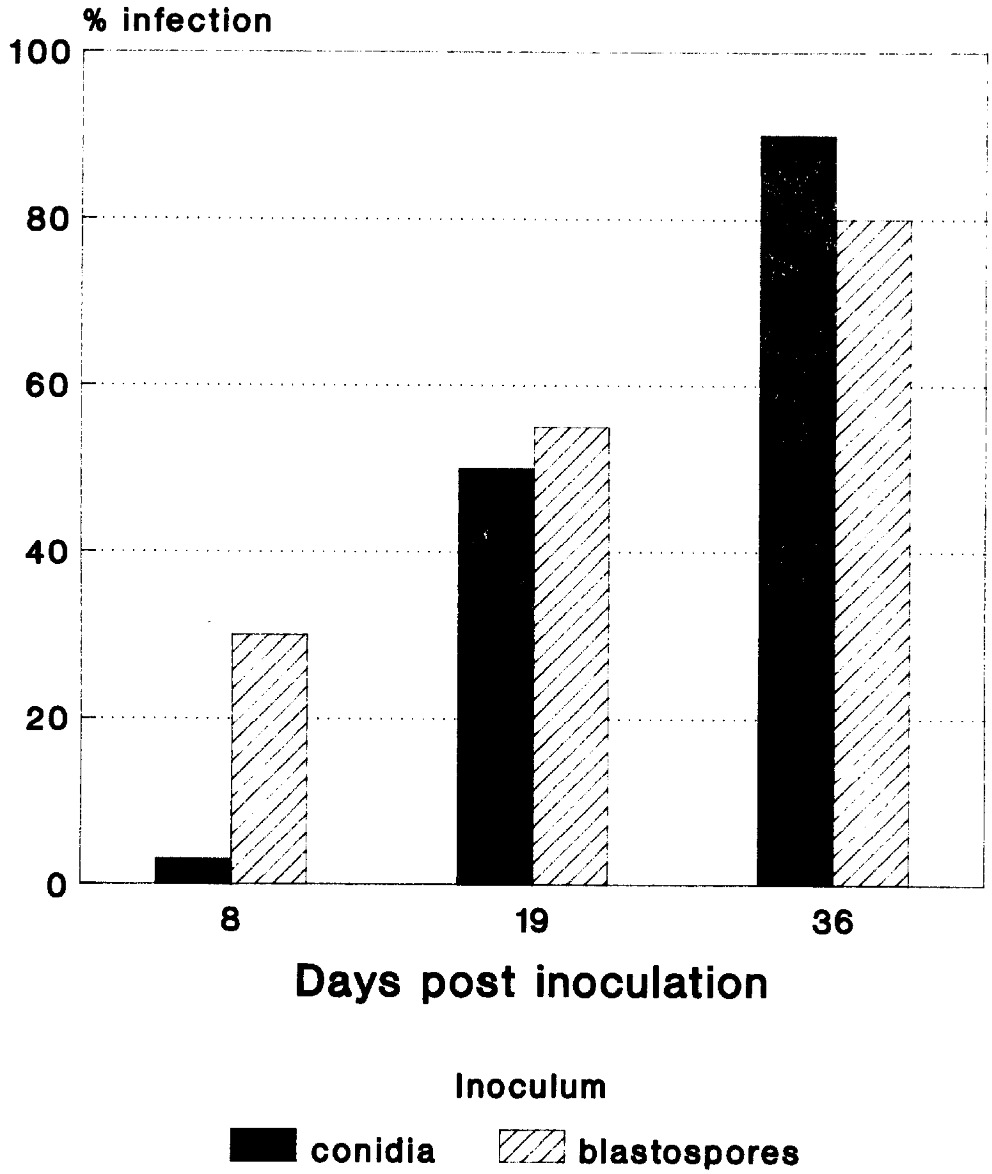

Fig. 3. Comparison of *Beauveria brongniartii* infectivity with liquid-produced conidia versus liquid-produced blastospore suspension on L3 *Melolontha melolontha.*

our knowledge in the area of biological control. The 3 step research project will investigate 1) rearing *in vivo*, 2) species selection and 3) *Trichogramma minutum* releases into areas where eastern spruce budworm is a problem. To this end, CIBA-GEIGY is in the process of building a *Trichogramma* rearing facility near Toronto. In tests conducted by Smith et al. (1990) rates of 8-23 × 10^6 females/ha resulted in 42-83% reduction in larval densities the following year while a release of 23 × 10^6 females/ha, followed by one application of *B.t.k.* the following spring, reduced spruce budworm populations by 93%.

The second cooperative project with *Trichogramma* is with the USDA to develop an *in vitro* production method for *T. minutum* and *T. pretiosum.*

Additionally, another CIBA-GEIGY biological control project is in progress in Europe to develop another parasitic wasp species, *Encarsia formosa*, for biological control of whiteflies in protected crops (i.e., glasshouse vegetable production).

NEMATODES AND VIRUSES

CIBA-GEIGY has exclusive marketing and development rights for professional turf and ornamental uses of entomogenous nematodes produced by the Biosys Corporation. The first labeled product will be Exhibit (*Steinernema carpocapsae* strain 27) for control of various turf and ornamental pests, including larvae of: black vine weevil, *Otiorhyncus sulcatus* (Coleoptera:Curculionidae); strawberry root weevil, *O. ovatus* (Coleoptera: Curculionidae); the Japanese beetle, *Popillia japonica* (Coleoptera:Scarabaeidae); fungus gnats, *Bradysia* spp. (Diptera:Mycetophilidae); and various armyworms, cutworms, sod webworms and billbugs in turf.

Additionally, CIBA-GEIGY has tests in progress to evaluate insect pathogenic viruses as insect control agents.

SUMMARY

In summary, CIBA-GEIGY has a large effort in biological insect controls, with major priorities in *Bacillus thuringiensis, Beauveria bassiana* and *Trichogramma* spp. research and development. These efforts are in harmony with CIBA-GEIGY's strategy to focus on newer, safer technologies that fit into IPM programs and are socially and environmentally acceptable.

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ENHANCING BIOLOGICAL CONTROL'S CONTRIBUTIONS TO INTEGRATED PEST MANAGEMENT THROUGH APPROPRIATE LEVELS OF FARMER PARTICIPATION

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ABSTRACT

Most recent development literature calls for greater farmer participation in agricultural research and technology transfer. Interestingly, biological control specialists do not seem to be involved in the trend. Four methodological models for developing and implementing biological control are proposed and then analyzed for their applicability to the Caribbean and Central America. Profit-generating biological inputs can be developed without farmer involvement, but grower involvement is required in the implementation phase. Inoculative releases and classical biological control do not require farmer involvement in the implementation phase, but may benefit from farmers' support and participation in the research and development phase. Alternatively, conservation and manipulation techniques require extensive farmer involvement in both the research and implementation phases. Unfortunately, biological control researchers generally ignore farmers as collaborators, even when their participation is key for implementation in heterogeneous agroecological and socioeconomic environments. Biological control in developing neotropical countries is seriously limited by financial and personnel constraints; a series of difficult strategic and operational decisions must be made if biological control is to contribute significantly to IPM in the area.

RESUMEN

La literatura de desarrollo reciente hace un fuerte llamado para una mayor participacion de agricultores en la investigacion agricola y transferencia de tecnologias. Sorpren-