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MICROBIAL CONTROL OF AGRICULTURAL PESTS IN TAIWAN

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FOREWORD

Integrated pest management is currently accepted as one of the key strategies in sustainable agriculture. It considers all the possible control options and chooses those that maximize profit in both the short and the long term. One option which is receiving increasing attention is the use of microbial pesticides containing organisms which are entomopathogenic (i.e. pathogenic to insects).

The most famous of these, *Bacillus thuringiensis (Bt)* is already widely used in many Asian countries, and is the only microbial insecticide registered for agricultural pest control in Taiwan. This important Bulletin discusses the use of *Bt* to control diamondback moth and other serious pests. Hundreds of *Bt* isolates have been tested in Taiwan, and *Bt* genes are also being used in programs to breed crop plants resistant to insect attack. It also presents recent research on other promising biological control agents, including green muscardine fungus, and virulent baculoviruses. Finally, the direction of future studies is discussed.

Microbial products are ideal for use in integrated pest management because of their selectivity and environmental safety. We hope that this Bulletin will be of value to all those who are working to develop pest control strategies which make less use of chemicals and more use of biological resources, to control pests in ways which are not harmful to human health or the environment. It is based on a paper first presented by its author at an international seminar on "*Use of Biological Control Agents under Integrated Pest Management*", held in October 1993 in Fukuoka, Japan, and co-sponsored by the University of Kyushu and the University of Saga.

MICROBIAL CONTROL OF AGRICULTURAL PESTS IN TAIWAN

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(Chinese Abstract) 摘　　要

微生物防治策略爲害物綜合防治體系中的重要一環，已證實在某些情況下有駕凌化學防治的優點，臺灣在本世紀初即有利用蟲生病原菌防治有害昆蟲的實例，日本則在1910年著手蟲生眞菌防治害蟲的研究。1980年代在臺灣由具有不同背景的大學與機關的研究人員組成了一組工作團隊，致力於微生物防治各方面計劃的執行，因此目前正有良好成效的防治方案付諸實行。

蘇力菌（*Bacillus thuringiensis*）爲臺灣唯一登記於防治農業害蟲之微生物殺蟲劑，在田間試驗中對小菜蛾（*plutella xylostella*），白粉蝶（*Artogeia rapae*）及玉米螟（*Ostrinia furnacalis*）有頗佳之防治效果；添加取食促進劑或輔助劑則增強其功效。目前還發展出許多控制品質的分析方法，最近幾年，部份專家學者尚致力於蘇力菌的調查及遺傳工程方面的研究。

黑殭菌（*Metarhizium anisopliae*）對甜菜葉蛾（*Spodoptera exigua*），紅胸葉蟲（*Brontispa longissima*）及斑飛蝨（*Laodelphax striatellus*）的防治效果已經評估。品系改良則主要是培育出抗殺菌劑之突變體。白殭菌（*Beauveria bassiana*）對甘藷蟻象（*Gylas formicarius*），大豆細緣椿象（*Riptortus clavatus*）和玉米螟之致病力亦經田間測試。同時也研究這些眞菌的生產過程。許多測試品系之昆蟲會被蟲草屬（*Cordceps* spp.）所感染；蠟蚧幹枝孢菌（*Verticellium lecanii*）對蚜蟲有致病性；紅頭菌（*Sphaerostible auranticola*）則對山茶圓介殼蟲（*Pseudaonidia duplex*）有致病力。

毒性桿狀病毒如小菜蛾及白粉蝶之顆粒體病毒（*PxGV*及*ArGV*），斜紋夜盜（*S. litura*），甜菜葉蛾和玉米穗蟲（*Heliothis* sp.）之核多角體病毒（*SlNPV, SeNPV*及*HsNPV*）亦被檢定及田間寄主測試。同時也評估各種輔助劑之效力。目前則欲改良加卅苜蓿尺蠖蛾核多角體病毒（*Autographa californica* NPV）活性之遺傳性。

害蟲微生物防治之未來研究應加強本土蟲生菌的分離，篩選和系統改良，應用上的策略，大量的生產與製劑技術的改進；同時有關微生物殺蟲劑的分子和遺傳工程上的研究亦屬重點之一。

(Japanese Abstract) 摘　　要

微生物防除は総合的害虫管理の重要な構成要素であるばかりか、農薬に勝る長所もある。台湾では昆虫寄生性微生物は今世紀始めから利用され、1910年は日本人研究者が糸状菌を害虫防除に初めて使った。1980年代には学際的研究班が組織され各種の微生物防除が試みられた。台湾で登録されている唯一の微生物剤は*Bacillus thuringiensis*であるが、コナガ、アワノメイガ、*Artogeia rapae*に対する野外試験でも効果が見られ、摂食刺激物質の添加でその効果が増進する。品質管理のための検定法もいくつか作られ、新系統の検索や遺伝子工学

による改良なども考えられている

緑きょう病菌はシロイチモンジヨトウ、コロナツハムシ、ヒメトビウンカへの適用が試みられ、突然変異を利用した殺菌剤抵抗性の系統の作出もされている。白きょう病菌はアリモドキゾウムシ、ホソヘリカメムシ、アリノメイガで野外実験がなされている。*Cordceps spp.*は各種の昆虫に病原性を示す。*Verticellium lecanii*はアブラムシに、*Sphaerostible auranticola*はミカンマルカイガラムシに有効性を示す。

バクロウイルスのうち顆粒病ウイルスではコナガ(PxGV), *A. rapae* (ArGA), 核多角体ウイルスではハスモンヨトウ(SINPV), シロイチモンジヨトウ(SeNPV), ツメクサの1種(HsNPV)が分離され、それぞれの害虫に野外試験が行われている。同時に各種の補助剤の評価も行われている。ウリハのNPVについては遺伝子工学的手法による改善が試みられている。

(Korean Abstract)

초 록

병해충종합관리(IPM)에서 중요한 요소인 미생물학적 방제전략은 농업해충방제에 있어서 화학제품보다 우수한 점들이 부분적으로 증명되어졌다. 대만에서는 20세기 초부터 곤충병원균이 해충방제를 위해 이용되었다. 일본의 연구진들이 1910년에 해충방제를 위한 곤충병원성 곰팡이를 최초로 이용하였다. 학문간 재휴 훈련사업과 더불어 여러 대학과 연구소의 연구진들이 1980년대에 모임을 형성하여 여러가지 미생물학적 방제사업을 수행하였다. 그 이후로 많은 활발한 프로그램들을 통하여 훌륭한 결과들을 얻었다.

대만에서 농업해충 방제를 위한 미생물 살충제로 유일하게 등록되어 있는 BT제(*Bacillus thuringiensis*)는 배추좀나방(*Plutella xylostella*), 배추흰나비(*Artogeia rapae*), 조명나방(*Ostrinia furnacalis*) 등을 방제하기 위한 포장시험에서 만족할만한 결과를 얻었다. 그들의 효과는 섭식자극제나 보조제의 첨가로 인하여 증진되었다. 품질관리를 개발하기 위한 여러 시험들도 행해졌다. 최근에는, 여러 연구팀들이 B. t.의 탐사와 유전공학 방면으로 관심을 집중시키고 있다.

녹강균(*Metarhizium anisopliae*)을 파밤나방(*Spodoptera exigua*), *Brontispa longissima*, 애멸구(*Laodelphax striatellus*)에 살포하여 그 효과를 평가하였으며, 살균제 저항성 돌연변이를 개량하기 위한 strain개발이 수행되었다. *Cylas formicarius*, *Riptortus clavatus*, 조명나방(*O. furnacalis*) 등에 병원성인 백강균(*Beauveria bassiana*)의 포장시험이 행해졌으며, 이 진균의 생산과정 역시 연구되었다. *Cordceps* spp.는 실험된 수많은 종류의 곤충에 대해 감염성이다. *Verticellium lecanii*는 진딧물에 병원성이다. *Sphaerostible auranticola*는 조개깍지벌레(*Pseudaonidia duplex*)에 병원성으로 밝혀졌다.

P. xylostella(*PxGV*), *A. rapae*(*ArGV*) 등의 과립형 바이러스와 *S. litura*(*SINPV*), *S. exigua* (*SeNPV*), 그리고 *Heliothis* sp.(*HsNPV*)와 같은 핵다각체형 바이러스 등의 전염성이 강한 baculoviruses 등이 확인되었고 자신의 숙주에 대한 포장시험이 실시되었다. 여러 부가제에 대한 효과를 평가하기 위한 시험도 아울러 수행되었다. *Autographa californica NPV*의 활성을 유전적으로 증진하기 위한 시도가 행해졌다.

해충에 대한 미생물학적 방제연구는 앞으로 고유한 곤충병원균의 분리, screening과 strain개발; 적용전략; 대량생산과 제제기술; 그리고 미생물 살충제에 대한 분자적, 유전공학적 연구 등을 강조해야 한다.

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ABSTRACT

Microbial control strategy, a valuable component in integrated pest management, has partially demonstrated its advantages over chemicals in agricultural pest control. In Taiwan, entomopathogens have been used to control insect pests since the beginning of this century. Japanese researchers first utilized entomofungi for insect control in 1910. A research team consisting of members from different universities and institutes with interdisciplinary training projects was formed in the late 1980s to conduct various microbial control projects. Since then, many active programs have been carried out that have achieved good results.

Bacillus thuringiensis, the only microbial insecticide registered for agricultural pest control in Taiwan, gave satisfactory results in field trials in the control of Plutella xylostella, Artogeia rapae and Ostrinia furnacalis. Their efficacy has been enhanced by the addition of feeding stimulants or adjuvants. Several assays have also been developed for quality control. In recent years, several academic groups have focused their attention on the exploration and genetic engineering of B. thuringiensis.

Green muscardine fungus, Metarhizium anisopliae, was applied to Spodoptera exigua, Brontispa longissima and Laodelphax striatellus, and its effect was evaulated. Strain improvements were carried out to breed fungicide-resistant mutants. Beauveria bassiana pathogenic to Cylas formicarius, Riptortus clavatus and O. furnacalis was field tested. The production process of this fungus was also studied. Cordyceps spp. are infective to a number of insect species tested. Verticellium lecanii is pathogenic to aphids. Sphaerostible auranticola was found to be pathogenic to Pseudaonidia duplex.

Virulent baculoviruses such as granulosis of P. xylostella (PxGV), A. rapae (ArGV), nuclear polyhedrosis virus of S. litura (SlNPV), S. exigua (SeNPV), and Heliothis sp. (HsNPV) were identified and field tested against their own host. Tests were also conducted to evaluate the effectiveness of various adjuvants. Attempts were made to genetically improve the activity of Autographa californica NPV.

Future studies of the microbial control of insect pests should emphasize the isolation, screening and strain improvement of indigenous entomopathogens; application strategies; mass production and formulation technology; and molecular and genetic engineering research for microbial insecticides.

INTRODUCTION

Simply stated, the microbial control of insects is the use of entomopathogenic microorganisms to regulate insect population levels. The procedures used to achieve microbial control include the intentional creation of epizootics or the utilization of naturally occurring epizootics (Harper, 1987). Entomopathogenic microorganisms, including bacteria, fungi, viruses and protozoa, have

Keywords: Agricultural pests, *Bacillus thuringiensis*, baculoviruses, fungi, microbial control, Taiwan,

already been developed as microbial insecticides. Although microbial insecticides account for <1% of the total insecticide market, this form of pest control has become the focus of attention over recent years. This is due to the improved performance and cost competitiveness of microbials, in addition to the increasing resistance of arthropods to chemical insecticides, environmental concerns, and the lack of development of new insecticides (Starnes *et al.* 1993).

Growing public consciousness of the potential hazards of chemical insecticides used to control insect pests has urged scientists from Taiwan, ROC, to develop microbial control technology as an alternative or complimentary strategy to chemical control. In fact, microbial control offers a number of advantages over chemical control. Microbial control creates no pesticide residues and pollutants, provides a high level of safety for human beings and non-target organisms (Kao and Tzeng 1992) and has a low likelihood of pest resistance.

In Taiwan, the use of entomopathogenic microorganisms to control insect pests has been recognized as having great potential since the turn of the century (Yen 1977). However, information on the microbial control of insects was scarce before the 1980s (Yen 1977, Hou 1986, 1987, 1991, Kao 1993). Among the different microbial control agents developed and tested, *Bacillus thuringiensis*, baculoviruses and muscardine fungi are considered to be the most promising candidates to control insect pests. This article reviews the current status of the microbial control of agricultural pests in Taiwan since 1983. The prospects of the exploration, development and utilization of microbial control of agricultural pests are also briefly discussed.

BACILLUS THURINGIENSIS

B. thuringiensis (*Bt*) is a gram-positive, rod-shaped bacterium which has been registered for use against several lepidopterous vegetable pests in Taiwan. *Plutella xylostella*, (diamondback moth), the most destructive insect of cruciferous crops, has developed resistance to a number of synthetic insecticides in Taiwan (Sun 1992). This has resulted in farmers' interest in the use of *Bt* to control *P. xylostella*. Academically, research has been undertaken to discover novel strains, genetic engineering, enhancement of efficacy field trials and quality control of *Bt*.

1. Isolation and Characterization of Local *Bt* Isolates

Hundreds of *Bt* isolates have been isolated from different areas in Taiwan. In addition to plasmid profile and western blotting, polymerase chain reaction (PCR) was also used for the characterization of *Bt* isolates (Chak and Young 1990, Li 1991, Chen 1992, Chao 1992, Feng 1993). Using a set of specific oligoprimers for PCR amplification, Chen (1992) found that the *Bt* YMB 96 strain contained *cry*IA(a) and *cry*IA(c) genes. Chao (1992) detected six kinds of *cry*I gene types residing in his *Bt* isolates. Feng (1993) observed that the distribution of *cry* gene profiles in local isolates was geographically dependent. *Bt* isolated from the soils of plain areas contained *cry*IA(a) + *cry*IA(c) (53%), *cry*IC + *cry*ID (32%), and *cry*IV (15%). Samples collected from high mountain areas tended to contain multiple *cry*-type genes: *cry*IA(b) (13.6%), *cry*IA(c) (0.59%), *cry*IA(a) + *cry*IA(b) (0.59%), *cry*IA(a) + *cry*IA(c) (10.6%), *cry*IA(a) + *cry*IA(b) + *cry*IA(c) (69.2%), *cry*IA(a) + *cry*IA(c) + *cry*IC + *cry*ID (3.55%), and *cry*IV (1.8%). In 1993, Chak and Jen determined the sequence of *cry*IA(b) gene from *Bt aizawai* HD 133. It is believed that more and more high-potency and broad-spectrum novel *Bt* strains will be discovered in the near future.

2. Genetic Engineering of *Bt*

A collaborative research program, financially supported by the National Science Council, ROC, on breeding crop plants resistant to insect attack by incorporation of genes encoding *Bt* δ– endotoxins has been conducted by several institutions. The preliminary results were satisfactory. PCR amplified *Bt*-icp genes that expressed the best insecticidal activity in *Escherichia coli* were cloned into plant expression vector pB1121 to obtain p*Bt*121N and p*Bt*121NR. Through the *Agrobacteria* transformation system, the *Bt*-icp gene was successfully transferred to tobacco and Brassica plants. The existence of the *Bt*-

icp gene in kanamycin resistant plants was analyzed by southern blotting and PCR techniques. The results from both assays positively indicated the existence of a *Bt*-icp gene in the plants. Among 42 transgenic tobacco plants tested, three transgenic tobacco plants were resistant to tobacco cutworm, *Spodoptera litura* (Chen 1992, Chen *et al.* 1993a,b). In order to improve residual activity on foliage, Huang (1993) expressed *Bt* Toxins into the bacteria *Erwinia herbicola* colonizing the leaves of Brassica plants. After transforming *cry* genes, carried by a plasmid vector into *E. herbicola*, the transformed *E. herbicola* expressed insecticidal crystal protein and showed insecticidal activity to *P. xylostella*. These transformed *E. herbicola* were able to stay on cabbage leaves under natural environmental conditions for about seven days. To test the stability of the *cry* gene containing plasmid in *E. herbicola*, a series of tests on the subcultures in ampicillin-free LB medium was performed. The results indicated that the plasmid would be excluded from *E. herbicola* within a few days under ampicillin-free culture conditions (Huang 1993).

3. Enhancement of *Bt* efficacy

Addition of 0.1% octyl methoxy cinnamate to the *Bt* preparations was found to prolong by 1.62 times the persistence of their toxicity to *P. xylostella*. Starch, sugar and 0.05% methyl paraben mixed with the *Bt* preparations slowed down the rainfall wash-off from rape leaves and increased *Bt* efficacy by 4.2 times. Incorporation of over 0.1 ppm simigrin into low dosage *Bt* preparations doubled the insecticidal efficiency, which then increased 8 times to the 4th instar larvae if simigrin was added at 1 ppm. Some metal ions were found to slightly enhance *Bt* toxicity to *P. xylostella* (Chung, 1991).

4. Field control of *P. xylostella*, *Artogeia rapae* and *Ostrinia furnacalis* with *Bt*

Results obtained from field trials for pest control were satisfactory. Use of *Bt* (biobit 120 bp/ml FP, 800 and 1500 x; bag-a-bug 120 bp/ml FP, 1500 x; thuricide 300 bp/g WP, 1000x; and 1.76% 4L EC, 800x and 1600x) was effective in controlling both *P. xylostella* and *A. rapae* within seven days of application (Su 1991b). Results of two field experiments indicated that the newly commercialized SAN 415-SC and other experimental *Bt* products such as florbac-FC, florbac-XLV, TC0918 and CGA237218, provided better control against *P. xylostella* than other brands. All *Bt* products provided fair control of *A. rapae* (Kao *et al.* 1990). Field trials were conducted on a *Bt* product, thuricide and a pyrethroid insecticide, permasect, alone or in combinations to control *P. xylostella*, in common cabbage. When two cabbage plots were sprayed with thuricide mixed with adjuvant there was a significant result (Hsiao 1983). It was also found that with adjuvants, *Bt* was more efficient than chemical insecticides in the control of *P. xylostella* when applied to crucifers in the winter season (Chou 1987). Chiou (1990) showed that a combination of *Bt kurstaki* with *Beauveria bassiana* enhanced its effectiveness against *O. furnacalis*.

5. Quality control of *Bt* products

A standardized bioassay protocol developed for the evaluation of preparations derived from *Bt* against *P. xylostella*, was adopted by the Council of Agriculture (COA) as a guideline for *Bt* product registration (Kao and Tuan 1992). The enzyme-linked immunosorbent assay (ELISA) detecting systems established by monoclonal and polyclonal antibodies were developed for *Bt* δ-endotoxin (Hsieh 1987, Tuan *et al.* 1993). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was also developed to quantify δ-endotoxins (Kao *et al.* 1993). Application of the ELISA, SDS-PAGE to monitor the δ-entoxins in conjunction with bioassay were also examined. Those assays were useful, not only in fundamental research and product discovery, but also in the regulatory control of *Bt* (Tuan *et al.* 1993, Kao *et al.* 1993). An *in vitro* assay devised with cultured *P. xylostella* cells in agar plate and trypan blue layered on the top surface rapidly detected the toxicity of *Bt* δ-endotoxins (Lee 1993).

ENTOMOPATHOGENIC FUNGI

An investigation of local entomopathogenic fungi was conducted by the Department of Plant Pathology and Entomology, National Taiwan University. Nearly 20 fungi genera were isolated from hundreds of diseased insects, and several new species were discovered and recorded (S.S. Tzean, personal communication).

Metarhizium anisopliae

Attempts to control beet armyworm, *Spodoptera exigua*, with entomopathogenic fungi has been carried out since 1985. Three fungi, *Metarhizium anisopliae*, *Beauveria bassiana* and *Nomuraea rileyi*, were used to control beet armyworm on *Gypophila* and green onion in field trials. The *M. anisopliae* and *B. bassiana* treatments resulted in better control of the beet armyworm on *Gypsophila* than the insecticide treatment. *M. anisopliae* and *B. bassiana* were both applied twice weekly for eight weeks on a green onion field. Results showed that the insect damage to green onion was reduced from 34% to 11% after fungal treatment. Yields of green onion increased by about 50% (Kao and Tsai, 1989).

Two field trials of fungal treatment with *M. anisopliae* var. *anisopliae* against coconut leaf beetle, *Brontispa longissima*, were conducted in southern Taiwan in 1986 and 1987. After three applications of *M. anisopliae* var. *anisopliae* formulated as homogenous biomass, in granules or in a conidial suspension, *B. longissima* could not be detected (Liu *et al.* 1989).

M. anisopliae var. *anisopliae* MA-805, isolated from the smaller brown planthopper, *Laodelphax striatellus*, is highly pathogenic to its host. Enhancement of virulence was obtained from serial passages through *L. striatellus* five times and one passage through the brown planthopper, *Nilaparvata lugens* (Lee and Hou 1989 a,b). Of 12 pesticides tested, hymexazol 30L, benomyl 50WP, tricyclazole 75 WP, pendimethalin 34 E.C., paraquate 24 E.C., and alachlor 45.1E.C. suppressed the mycelial growth of *M. anisopliae* var. *anisopliae* NA-805 (Lee and Hou 1989c).

Insecticides such as EPN, bifenthrin and chlorpyrifos reduced the leaf area of *Gypsophilla paniculata* damaged by larvae of the beet armyworm, *S. exigua*. This reduction was 10 fold when *Gypsophilla paniculata* was treated by inoculation with *M. anisopliae* var. *anisopliae* Ma2 for 24 to 48hr, but it did not affect Ma2 sporulation on cadavers. Mixing methomyl at concentrations recommended for field applications with Ma2 spore suspensions had a synergistic effect on *S. exigua* larvae, reducing the damage rate to leaves and the time the Ma2 proved lethal to larvae (Tsai *et al.* 1993).

Benomyl- and carbendazim-resistant isolates (UV-Be, VU-Ca, Mu-Be, Mu-Ca) of *M. anisopliae* var. *anisopliae* were obtained when the fungal conidia were treated with ultraviolet light or mutagenic agents. These isolates had cross resistance and showed stable fungicide resistance. Their virulence to the larvae of the beet armyworm was different. The isolate with the highest virulence, UV-Be, showed the higher lipolytic enzyme activity. A progressive increase in benomyl and carbendzim concentrations on culture media could also induce the appearance of fungicide resistant Fe-Be and Fe-Ca (Tsai *et al.* 1993).

Mass production of *M. anisopliae* var. *anisopliae* MA-805 was carried out using grain seeds and plant residual substrates. It was found that coarse rice, corn cob and bagasse were effective for sporulation of this fungus (Lee and Hou 1989c). Kao and Tsai (1989) and Kao *et al.* (1989) found that conidia could be mass produced with steamed, polished rice at 28°C, with a 24hr photoperiod.

Beauveria bassiana

B. bassiana was isolated from Taiwanese soils infested with sweet potato weevil, *Cylas formicarius* (Su *et al.* 1988, Su 1991 a,c). The effect of pesticides on this fungus was studied in the laboratory. Results showed that mycelial growth was completely inhibited by the fungicide Sporatak (Su 1988a). Field trials showed that spraying with 1.6×10^8 conidia/ml, at planting and rootstock formation time or broadcasting of soybean seeds containing 10^7 conidia/g at planting time effectively controlled the weevils (Su 1992 a).

Strains of *B. bassiana* isolated from Hemoptera, Coleoptera and Lepidoptera were most virulent to bean bug, *Riptortus clavatus*

The order of susceptibility of various stages of *R. clavatus* to inoculation by *B. bassiana* was as follows: fifth instar, fourth instar, adult and third instar. In the field experiments, *B. bassiana* coated on rice grains was the best carrier compared with spraying *B. bassiana* after insect release, or spraying *B. bassiana* on *R. clavatus* before release (Hu 1992).

Under open-air conditions, solid-state soybean substrate inoculated with blastospores, a granulated form of the *B. bassiana*-soybean mixture (BSM) and a powdered form of *B. bassiana*-soybean powder (BSP) could be produced, their spore concentrations being ca. 1×10^{8}–10^{9} and 1×10^{8}–10^{9} conidia/g, respectively. The pathogenicity of BSP and BRP (*B. bassiana* rice powder) was different, their LC50 to *O. furnacalis* being 4.8×10^{8} and 9.5×10^{8}, respectively (Chiu 1989).

During the late whorl stage, from September to October, each corn plant was infested with 10 2nd-instar larvae of *O. furnacalis* and was then treated with 2 to 3 g of BSM 2 days after larval release. Only 0.19 and 0.36 larvae/plant and 13.0 and 27.8% infestation were found in treated blocks surveyed at 2 and 4 weeks post-treatment, while 4.58 and 4.26 larvae/plant and 100% infestation were recorded in untreated blocks (Chiu 1989).

Cordyceps spp.

Three entomogenous fungi, *Paecilomyces cicadae*, *P. farinosus* and *P. javanicus*, were isolated from the parasitized insects collected in mountains from July 1986 to June 1988. Inoculation tests showed that *P. cicadae* parasitized and killed the larval stage of *O. furnacalis*, *Heliothis armigera*, *Argyrophoce schistaceana*, *Sesamia inferens*, *Corcyra cephalonica*, *P. xylostella*, *Bombyx mori*, *A. rapae crucivora*, *Daphnis nerii*, *Theretra silhetensis*, *S. litura* and *Andraca bipunctata*. *P. farinosus* parasitized the larvae of *O. furnacalis*, *H. armigera*, *A. schistaceana*, *S. inferens*, *C. cephalonica*, *P. xylostella*, *B. mori*, *A. rapae crucivora*, *A. bipunctata*, *Homona coffearia* and *A. bipunctata*. *P. javanicus* parasitized the larve of *O. furnacalis*, *H. armigera*, *A. schistaceana*, *S. inferens*, *C. cephalonica*, *B. mori*, *A. rapae crucivora* and *A. bipunctata*. These three *Paecilomyces* spp. are imperfect states of *Cordyceps* fungi, and were first recorded in Taiwan in 1988 (Wang 1988).

Seven *Cordyceps* fungi; i.e. *C. aphodii*, *C. gracilis*, *C. memorabilis*, *C. militaris*, *C. sobolifera*, *C. sphingum* and *Cordyceps* sp., were introduced from foreign countries between July 1986 and June 1988. The larval stage of 19 lepidopteran species were inoculated with each of the imported *Cordyceps*. Results showed that *C. sphingum* parasitized nine species of insects; *C. militaris* and *Cordyceps* sp. parasitized eight species; *C. gracilis* parasitized seven species; *C. aphodii* and *C. memorabilis* parasitized six species; while *C. sobolifera* parasitized none of them (Wang 1989).

Verticellium lecanii

V. lecanii was isolated from *Rhopalosiphum padi* and was reported to be highly pathogenic to *Myzus persicae*, *Macrosiphonella sanborni*, *Toxoptera aurantii*, *Liphis erysimi*, *Aphis gossypii*, and *Saissetia oleae*. The cultural characteristics and pathogenicity of the fungus were very stable after 13 subcultures on Sabouraud's medium (Peng 1985), but no field trials have been reported.

Sphaerostible auranticola

The red-headed scale fungus *S. auranticola* (B. & Br.) Petch was neither an obligate parasite nor obligate. The infectivity of the fungi inoculated in the female of the camellia scale, *Pseudaonidia duplex*, on pumpkin was 20% after 42 days. The infectivity of the fungus inoculated in the camellia scale on tea was 25% after 57 days. The influence of the pesticides on spore germination and mycelial growth varied with the type and concentration of the pesticide. In general, the higher the concentration tested, the more the germination rate was inhibited. The application of this fungus in the field needs further study (Hsiao and Hsu 1985).

BACULOVIRUSES

Virulent baculoviruses have been isolated from lepidopterous insects. Both laboratory pathogenicity tests and field efficacy trials were conducted with encouraging results.

Artogeia rapae granulosis virus (ArGV) and *Plutella xylostella* granulosis virus (PxGV):

The efficacy of mixtures of 50% tokuthion E.C., and the ArGV to control *A. rapae* was evaluated in cabbage fields. Treatment of plots with 2, 3, 4 or 5 applications of ArGV at 1 LE/L was effective against *A. rapae* (Su 1985). Bactospeine (*Bt*), 50% tokuthion E.C., ArGV, Ar GV mixed with 50% tokuthion E.C. or with bactospeine was found to be effective in controlling *A. rapae* in the cabbage field. GV alone or mixed with spreader-sticker, with spreader-sticker + yeast-extract, with spreader-sticker + skim milk powder or spreader-sticker + active carbon, were effective in the control of *A. rapae* (Su 1986).

PxGV or tokuthion E.C. alone, a mixture of PxGV and sticker (CS-7) or a combination of PxGV and sticker with skim milk powder was highly effective against *P. xylostella* in the field (Su 1987a).

Application of a combination of PxGV, CS-7 with active carbon (five weekly applications until the crop was harvested) provided effective control of *P. xylostella* (Su 1988b).

Application of ArGV provided highly effective control of *A. rapae*. The following combinations of e.g. ArGV + PxGV + CS-7, ArGV + bactospeine + CS-7, ArGV + PxGV + Bactospeine + CS-7 and tokuthion, were observed to be efficient against both *A. rapae* and *P. xylostella*. Combination of two GVs with any of the following protectants such as yeast, skim milk powder, active carbon, yeast + CS-7, skim milk powder + CS-7, and active carbon + CS-7, could effectively control those two species, but GVs with skim milk powder or yeast induced black rot, soft rot and alternaria leaf spot diseases on the treated plants (Su 1987b).

The treatment of plots with mixtures of PxGV and ArGV at 5,7, 10, or 14 day intervals provides effective control against both *P. xylostella* and *A. rapae*, but the treatments at 5 or 7 day intervals were better than the others (Su 1987c).

Application of AxGV, PxGV virus, or *Spodoptera litura* nuclear polyhedrosis virus (SINPV) alone were significantly effective in the control of *A. rapae*, *P. xylostella* or *S. litura* respectively. The use of mixtures of ArGV and PxGV, ArGV and SINPV or PxGV and SINPV resulted in the effective control of *A. rapae* and *P. xylostella*, *A. rapae* and *S. litura* or *P. xylostella* and *S. litura* respectively. The combination of two GVs and one NPV was also effective in controlling *P. xylostella*, *A. rapae* and *S. litura* (Su 1989).

Spodoptera litura nuclear polyhedrosis virus (SINPV)

Third instar larvae treated with 10 x 10^6 PIBs/mL after 4-year, 2-year, 1/2-year storage, and a fresh virus caused 83.3%, 96.7%, 100.0% and 100.0% mortality, respectively. The control rates when *S. litura* was treated by SINPV combinations were: India ink 91.3%, red ink 86.2%, active carbon powder 88.5%, skim milk powder 90.3% or SINPV 88.1% alone. The control rates for *S. litura* treated by the following SINPV combinations were: green 90.5%, red 86.7%, white 69.7%, yellow dye 82.7% or SINPV alone 84.9%. Control rates for *S. litura* applied with a combination of SINPV with palcrasol As-29 were 87.1%, strong spread 83.2%, and CS-7 70.6% or SINPV alone 75.8%. (Su 1992).

Heliothis nuclear Polyhedrosis Virus (HsNPV):

Application of HsNPV to *H. armigera* gave better control of 1-day-old larvae than 4-day-old larvae. *H. armigera* densities were significantly reduced 14 days post application with HsNPV, and control was better than that achieved with carbofuran. Application of the virus increased from 18.7% to 36.1% the proportion of ears of corn free of larval damage. The injection of HsNPV preparations into corn ears was more effective than spraying the ears (Tuan *et al.* 1989a). HSNPV preparations were more stable and persistent in the period January to February and April to May than in the period June to July and October to November when spayed on corn silks under field conditions. The HsNPV was inactivated by weak alkaline dew (pH 8.1) collected from soybean leaves, but it remained active on corn, tomato and asparagus with a pH of 7.2-7.3. Artificial heavy rainfall of 242 mm/h for 30 min did not wash off the

HsNPV preparations sprayed on the corn silks (Tuan *et al.* 1989b). Lecithin added to the NPV commercial product reduced the LT50 values by 2 to 3 days compared to inoculation with NPV only. This suggests the possibility of using lecithin to enhance the insecticidal activity of viral insecticides (Tuan and Hou 1988).

Spodoptera exigua Nulcear Polyhedrosis Virus (SeNPV)

A nuclear polhedrosis virus (NPV) was isolated from diseased *S. exigua* on baby's breath, *Gypsophila paniculata*. This NPV was found to be highly pathogenic to *S. exigua* (Kao *et al.* 1991). On the basis of food consumption, virus yield, larval mortality and microbial contamination, it has been suggested that SeNPV is best produced by an artificial diet of 1.54 ± 0.23g (diameter 1.0 cm, height 0.5 cm), moculum rate of 2 x 10^7 PIBs/cm^2 diet surface, at 30 C and harvested 5 to 6 days after inoculation (Huang and Kao, unpublished data).

SeNPV preparation made from the infected larvae of *S. exigua* at a rate of 3.6 x 10^{12} PIB/ha alone or mixed with 2.8% bifenthrin 3500x, demonstrated successful results when applied on green onion (Kao *et al.*, unpublished data).

Laboratory tests were conducted with leaves of *G. paniculata* to determine whether the 18 candidate adjuvants would increase the effectiveness of NPV against the early 4th-instar larvae of *S. exigua*. Bioassay demonstrated that of the materials tested, 15 adjuvants increased the effectiveness. Bivert, agral 90 and nu-film 17 were the most effective (Kao *et al.* 1991).

One hundred and one substances were tested in the laboratory as protectants for SeNPV against ultraviolet UV inactivation. Absorbance of these substances was measured using a diode array spectrophotometer at wavelengths ranging from 280 to 330 nm. Twenty-six candidates which showed higher UV absorbance were incorporated in the adjuvant measurements. Leaves of *G. paniculata* were dipped with virus-bivert UV protectant-mixtures, air-dried, exposed to UV radiation (λ = 312nm, 1200μW / cm^2, 10 min) and then fed to 4th-instar larvae for bioassay. Results showed that the addition of 1% uric acid, 1% activated carbon, 1% folic acid or 1% xanthine to the viral suspension provided significant UV protection for SeNPV (Kao and Huang 1992).

Genetic Improvement of the Cytotoxicity of *Autographa californica* Nuclear Polyhedrosis Virus (AcNPV)

The δ -endotoxin gene from *Bt aizawai* 7.29 was inserted into AcNPV. The recombinant viruses, Acendo-17, Acendo-UW1 and Acendo-1993, were purified by end point dilution and identified by dotblot hybridization, PCR method and southern blotting. Results showed that the expression of the δ-endotoxin gene by the AcNPV expression system improved the cytotoxicity of this virus. The cytotoxicity of this virus is worthy of further study, particularly with regard to its insecticidal activity (Hu 1993).

CONCLUSION

It is likely that insect management by the use of synthetic organic chemicals will still dominate the pesticide market worldwide in the year 2000. However, major thrusts are underway in industrial, academic, and government research laboratories to discover and develop more selective methods of insect control which are compatible with biological, toxicological, environmental, and societal requirements (Altstein *et al.* 1993). In Taiwan, environmental concerns and insecticide resistance have recently led many farmers to consider the use of microbial control for agricultural pests. It is suggested that more manpower and research should be given to this.

Isolation, Screening and Strain Improvement of Indigenous Entomopathogens

Taiwan's tropical and subtropical climate, diversified topography and mosaic cropping system, provide rich microbial resources for discovering novel agents with high pathogenicity and broad spectra. The selection of effective strains of entomopathogens, and microbial breeding and genetic manipulation for strain improvement, are essential to the development of microbial insecticides (Aizawa 1987).

Development and Integration of Useful Microbial Control Strategies

Microbials are ideal for use in integrated pest management because of their selectivity and environmental safety (Starnes *et al.* 1993). It is imperative that microbial control strategies be combined with integrated pest management systems. To maximize the efficiency and ecological compatibility of microbial control, specific methods of application should be designed for the use.

Improvement of Mass Production and Formulation Technology

The success or failure of any microbial insecticide depends mainly upon the technology of its mass production. It is essential that a stable strain be selected with high pathogenicity and productivity. This will optimize culture conditions and cut down production costs during the process.

Opportunities exist for enhancing field effectiveness and prolonging shelf life through improved formulation. Suggested areas for advancement include microencapsulation, biotechnology and the use of various additives such as stickers, spreaders, and feeding stimulants as well as UV protectants.

Encouragement of Molecular Biology and Genetic Engineering Studies

Research efforts should be directed towards biotechnology. Studies such as plasmid curing and conjugation, development of transgenic microorganisms and transgenic crops provide new insights in the continuing progress of research into microbial control.

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DISCUSSION

Dr. Sastrosiswojo asked what factors affect the efficacy of *Metarhizium anisopliae*, and how these were overcome. Dr. Kao explained that the green fungus *M. anisopliae* was used as a biocide in a green onion field. The area is very humid with plentiful irrigation water, which is probably one reason for the satisfactory results. High light intensity should be avoided: the fungus was applied in the late afternoon, not during a period of bright sunshine.