Current status of bio-pesticides development, farmer's acceptance and their utilization, and future perspective in Taiwan

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ABSTRACT

Taiwan has made significant progress in the development and application of biopesticides in the past two decades. Sex pheromones of *Cylas formicarius elegantulua, Eucosma notanthes, Spodoptera litura,* and *S. exgiua* have been synthesized, formulated, and utilized in many ways with satisfactory results, including monitoring, mass trapping, and mating disruption. Taiwan has made significant progress in the development and application of microbial insecticides in the past two decades. *Nomurea rileyi* was found to be pathogenic to *Heliocoverpa armigera* and *Spodoptera exigua.* Field applications gave effective control of *H. armigera*. *Metarhizium anisopliae* was applied to control *S. exigua*, *Brontispa longissima*, *Loadelphax striatellus*. *M. anisopliae* was used also for destruxin production. Destruxins showed high virulence to *S. exigua*. *Beauveria bassiana* was found to be a lethal factor in soil pernicious to *Cylas formicarius*. *B. bassiana* preparations were effective in controlling *C. formicarius*, *Ostrinia furnacalis*, and *Riptorus lineasis. B. bassiana* was also pathogenic to *Lipaphis erysimi.* The optimal growth and maximum sporulation condition of three isolates of *Verticillium lecanii* were investigated. *V. lecanii* was reported to be highly pathogenic to *Myzus persicae*, *Macrosiphoniella sanborni*, *Toxoptera aurantii*, *Liaphis erysimi*, *Aphis gossypii*, and *Saissetia oleae*. Many aspects relating to these above-mentioned fungi, e.g., characterization, breeding fungicide-resistant mutants, production process, recovery, formulation, factors affecting infectivity, and compatibility with pesticides, were also evaluated. Granulosis viruses of *Plutella xylostella, Artogeia rapae*, and nuclear polyhedrosis viruses of *Spodoptera litura*, and *S. exigua*, were identified and field tested against their own hosts. *Heliothis*

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NPV introduced from the United States was effective in controlling *H. armigera*. Tests were also conduced to evaluate the effectiveness of various adjuvants additive and UV protectants. The host spectra of *Autographa californica* nucleopolyhedrosis virus were examined. Attempts were also made to genetically improve the activity of *A. californica* nucleopolyhedrosis virus. Construction of recombinant AcMNPV containing EGFP (enhanced green fluorescence protein) gene or DsRed (red fluorescence protein) gene as tracer for environmental risk assessment was also attempted. *Bacillus thuringiensis* (*Bt*) is so far the only microbial insecticide registered for insect control against *P. xylostella*, *A. rapae*. etc. Isolation and characterization of local *Bt* isolates achieved fruitful results and obtained many novel *cry* genes. Transgenic *Bt*-bacterium expressed a good insecticidal effect against *P. xylostella*. The efficacy of *Bt* has been enhanced by the addition of feeding stimulants, adjuvants and UV protectants, or microencapsulation. Recently, the bioactivity of entomopathogenic-nematophilic bacterium, *Photorhabdus luminescens* was evaluated. Results showed that it exhibited high insecticidal and antimicrobial activities against local insect pests and plant pathogens. The plantform technology for mass production of microbial fungicides was established with the use of serial plot-scade liquid fermentors. High yield of durable biomass for targeted microbial groups (*Streptomyces* spp, *Bacillus subtilis*, and *Gliocladium virens*) was achieved. Their effectiveness in disease control was satisfactory.

(Key words: Biopesticides, Bioinsecticides, Biofungicides, Entomopathogenic fungi, *Bacillus thuringiensis*, baculoviruses, *Photorhabdus luminescens* Pheromones.

INTRODUCTION

Taiwan is located in tropic and subtropic area with high temperatures and humidity, which is suitable for infestations by many insect pests. In 1970s, several evidence indicated that the overused chemical insecticides to control insect pests caused many negative side-effects. For this reason, during 1980s and after, the Council of Agriculture has promoted the development of biopositicides. Since then, many active programs have carried out that have achieved good results.

Microbial insecticides include bacteria, fungi, protozoa or viruses. They may operate through mechanisms such as toxin production (e.g. *Bacillus thuringiensis* (*Bt*)), invasion parasitism (e.g. baculoviruses)（Matten *et al.* 1993）. Although microbial insecticides account for less than 1% of the market, this form of pest control has become the focus of

attention in recent years. This is because of the improved performance and cost competitiveness of microbials, the increasing resistance of arthropods to chemical insecticides, and the lack of development of new insecticides)（Starnes *et al.* 1993）.

In Taiwan, the use of entomopathogenic fungi to control insect pests has been recognized as having great potential since the turn of last century（Yen, 1997）. Among different microbial control agents developed and tested, *B. thuringiensis*, baculoviruses and entomopathogenic fungi are considered to be the most promising candidates to control insect pests. And, *Photorhabdus luminenscens* with multifunctional activities against plant pests is also worthwhile to be developed as biopesticide.

So, far, about 1000 insect pheromones have been isolated, identified and synthesized. Of these only a handful are used in Taiwan in IPM programs including sex pheromones of sweet potato weevil, carambola fruit borer, tobacco cutworm, and beet armyworm.

With the use of serial plot-seale liquid fermentors, technologies for the mass production of long durale biomass formulation of microbial fungicides established. The field frials exhibited satisfactory results against soil borne diseases.

This article reviews the recent progress on the development , application and future prospective of biopesticides in Taiwan.

DEVELOPMENT AND APPLI CATTON OF BIOINSECTICIDES

Entomopathgenic fungi

Isolation and Characterization

A total of 831 fungus-infested cadavers of insects, spiders, and mites were collected from varied habitats in different latitudinal zones. From these specimens, 24 genera consisting of 66 species were isolated and identified. Among them, four new species were found, plus one new combination and 37 newly recorded species from Taiwan itself （Tzean *et al.* 1997）A parasitic fungus, *Neozygites* cf. *adjarica* was obtained from the spotted spider mite （Shih and Shune, 1994）

Another investigation was carried out by Biopesticides Division, Taiwan Agritultural Chemicals and Toxic Substances Research Insititute. Results show that *Metarhizium*, *Beauveria*, *Nomuraea*, *Paecilomyces*, and *Aschersonia* are the most frequent genera of entomopathogenic fungi in Taiwan. The survey also showed the occasional occurrence of other entomopathogenic fungi, *Verticillium*, *Gibellula*, *Hirsutella*, and *Cordyceps*. The results of the API ZYM test showed that all isolates have broadly similar reactions, but that the test could be used to distinguish between different genera. There are difference in pathogenicity among isolates of *M. anisopliae*, *N. rileyi* and *B. bassiana* to the larvae of *Spodoptera exigua* and *Spodoptera litura* (Kao *et al.* 1998).

Random amplified polymorphic DNA (RAPD) was used to differentiate a total of 38

strains of entomopathogenic fungi isolated from 20 geographic regions of Taiwan and mainland China. Fungal isolates were obtained from 15 insect species. Banding patterns were generated from 3 selected primers (OPM 12, 18, and 20). Isolates were grouped into 10 clusters according to similarity, following cluster analysis using Jeffrey's coefficients. Three distinct genotypes were observed among the 38 isolates tested. On the basis of RAPD patterns, 2 genera and 1 species were recognized, namely *Beauveria*, *Metarhizium anisopliae* var. *anisopliae*, and *Nomuraea. Nomuraea* exhibited a more conservative banding pattern than each of the other genera. RAPD markers may be useful as identification biomarkers of specific biocontrol strains in a limited geographical area （Kao *et al.* 2002）.

The optimal temperature for mycelial growth of various *Verticillium lecanii* isolates was 24°C except 20°C for isolate F168. *V. lecanii* isolates were sensitive to higher temperature. Most isolates could not grow at 32°C, but they grew well between 16 - 28°C. On water agar, *V. lecanii* isolate F168 germinated significantly slower than other isolates which germination rate reached to 98% after 16 hr of incubation at 24°C. There was hundred-fold difference in the amount of spore production among tested isolates when they were incubated at 24°C for 9 days. API ZYM test revealed that most isolates had alkaline phosphatase, arylamidase, esterase/lipase, leucine N-acetyl-β-glucosaminidase, acid phosphatase, β-glucosidase, α-galactosidase, naphthol-AS-BI-phosphohydrolase activity. Each isolate showed different chitinase activity on the colloidal chitin-amended medium. Molecular techniques such as AFLP and RAPD of genomic DNA, RFLP of IGS and 18S rRNA and PCR of ITS were used to analyze the relationships among different *V. lecanii* isolates. The dendrogram produced from the RAPD data in this study divided the 10 tested isolates into 2 groups. Four local isolates were grouped in the same cluster. The AFLP analysis method provided a lot of information on polymorphism between isolates. AFLP and RAPD techniques could not differentiate between *Acremonium charticola* from *V. lecanii* isolates. The *V. lecanii* isolates could not be distinguished by IGS-RFLP and ITS sequence（Tsai, 2004）.

Nomuraea rileyi

N. rileyi was more virulent to *Heliocoverpa armigera* at 20℃ than at 30℃, and had a low LT_{50} value at all temperatures tested. The optimal temperatures for fungal development in diseased larvae were 20 and 25℃. High humidity and more than 12 hr illumination is necessary for conidial production on cadavers. Of the eight solid-stage culture materials tested, pig liver as a culture substrate had the highest spore production, i.e. 1.9 x 10^9 conidia/g（Tang, 1996）. *N. rileyi* caused 90.5 - 100% mortality in fourth-instar larvae of *H. armigera*, when applied at 10^7 conidia/mL to corn silks, and to leaves of soybean, tomato and chrysanthemum. Of the 22 pesticides tested, only two fungicides, maneb and propineb, were highly inhibitory to *N. rileyi*. Field applications of *N. rileyi* conidial suspension to neonate larvae were found to be as effective as 40.46%

carbofuran (EC) at 800-fold dilution in controlling *H. armigera* （Tang, 1996, Tang and Hou, 1998）. The pathogenicity of *N. rileyi* to *S. exigua* was studied. The LC_{50} values for 1st to 5th instars were determined to be 1.34 x10^6, 1.47 x 10^5, 4.97 x 10^5, 3.56 x 10^4 and 3.1 x 10^5 conidia/mL, respectively. Pupal mortality was 25% when 5th instar larvae were inoculated with a conidial suspension at 1 x 10^7 conidia/mL. The highest mortality of 4th instar larvae infected with *N. rileyi* conidia was 70%, when sprayed with inoculum from a distance of 2 m away and exposed to a windblow velocity of 6.0 kg/hr（Tang, 1998）.

Bioassays in laboratory showed that *N. rileyi*, was less virulent to younger instars of the corn earworm, *H. armigera*, than the older ones, the LC_{50} value to the 1st instars being ca. 625 fold higher than that to the 5th. Body surface (mm^2) was positively correlated to larval stage in days (r=0.982). Furthermore, determination of conidial numbers on the body surface of each stadium also revealed a positive correlation between the conidial loading quantity and the body surface (r=0.986). Therefore it is evident that higher virulence of *N. rileyi* to older instars is due to their larger body surfaces. This permits adherence of more conidia on their body, indicating that body surface is an important factor affecting virulence of *N. rileyi* to *H. armigera*. In addition, the higher virulence at the 5th stadium appears to be related to its longer duration, providing sufficient time for conidia to penetrate into the hemocoel to cause mortality. During the 4th stadium, a lower virulence was obtained for those larvae close to molting than newly emerged ones indicating that ecdysis is an important factor preventing conidial penetration into the hemocoel（Tang *et al*. 1999）.

The effects of environmental factors on infection of the entomopathogenic fungus, *N. rileyi*, isolated from the corn earworm, *H. armigera*, in Taiwan, to its host insect were studied in the laboratory. The fungus caused higher larval mortality at 20℃ than at 30℃ when 5 x 10^6 conidia/ml were sprayed on the fourth instar. However, mortality of the fifth instar injected with 1 x 10^3 conidia/larva was not significantly different when the inoculated larvae were incubated from 15 to 30℃ The fungal development in inoculated larvae was best at 20 and 25℃ after shifting from 20℃ to either lower or higher temperatures. The germination rate was higher at 20 and 25℃ than at 30 or 35℃. Conidial germination was better on the wash-off of insect cuticle than on Sabouraud maltose agar with yeast extract. Sporulation on chill-dried cadavers was maximal at 95 or 100% relative humidity than at lower levels of relative humidity. The time required for sporulation was 2 days less at 100% than at 95% relative humidity. Although photoperiod did not affect fifth instar mortality caused by *N. rileyi*, the median lethal time (LT_{50}) values were shorter upon incubating under light than in darkness. Incubation of infected cadavers under 12 or 24 h light resulted in 20-fold more conidial production than under full darkness. Therefore, illumination is necessary for development of this isolate on insect cadavers（Tang and Hou, 2001）.

Response Surface Methodology (RSM) was used to study the optimal media for *N. rileyi* in submerged cultural fermentation. In a shake flask culture, glucose was found to

be the best carbon source, and corn steep powder was found to be the best nitrogen source. The resulting equation from RSM was $Y=1.189+0.0958\ X_1,+0.0267\ X_2-0.0063\ X_3-0.1278\ X_1^2-0.1304\ X_2^2-0.149\ X_3^2$. The maximum point of cell production when the medium contained 3.2% glucose, 29% V8, and 0.5% corn steep powder, and when the dry weight of the cell was 12.1 g/L. When the optimal medium was applied to 5 L of fermentor at 2 vvm, 250 rpm, and 25℃, the harvest of cells was 16.4 g/L which was even higher than that in the shake flask culture（Lin *et al*. 2003）.

The formulation of *N*. *rileyi*, mainly works on the stability of conidia activity during preservation as well as on the mortality of the spore formulation under the exposure of UV. The fresh conidia of *N. rileyi* was heated at 35℃for 1 hr; then suspended into the soybean oil with 1% glucose. After the storage from 3 months to 6 months, the germination percentage of the formulated conidia was decreased from 63% to 46%. With 6 months old oil-based agent the mortality against 3th-instar larvae of *S. exigua* was remained 63.3% of mortality. Four percentage of conidia germination was found in the spore powder without formulation after 5 months storage. For the purpose of protection from UV, 0.5% zinc oxide was added to oil-based agent. The 74% of germination with 80% of mortality against 3th-instar larvae was found under the light of 30 Lux UV for 30 minutes. The spore powder germination with non-formulation closes to zero（Chien, 2004）.

A liquid inoculation system was designed and constructed for the mass production of *N*. *rileyi* spores. This system was made of aluminum and stainless steel combined with transport system, pressure supply system, inoculation system, and control system. Inoculation volume was controlled by adjusting spray time and pressure. And, the maximal inoculation number reached up to 3600 bottles per hour（Kao *et al*. 2006）. A solid-state fermenter also invented for the mass production of *N*. *rileyi* spores（Kao *et al*. 2004）. An oil-based recovery system was invented for separation and concentration of *N*. *rileyi*, *M. anisopliae* and *B. bassiana* spores with high efficiency （Kao *et al*. 2000, Kao *et al*. 2002）.

Metarhizium anisopliae

Field trials were carried out on the use of three fungi, *M. anisopliae*, *B. bassiana* and *N*. *rileyi*, to control *S. exigua* on *Gypophila paniculata* and green onion. The *M. anisopliae* and *B. bassiana* treatments gave better control of *S. exigua* on *G. paniculata* than insecticide. *M. anisopliae* and *B. bassiana* were both applied twice weekly for eight weeks to a field of green onion. Results showed that the insect damage to green onion fell from 34% to 11% after the fungal treatment. Yields of green onion increased by about 50%（Kao and Tsai, 1989）. After three applications of *M. anisopliae* var. *anisopliae*, formulated as a homogenous biomass in granules or in a conidial suspension, *Brontispa longissima* could not be detected（Lin *et al.* 1989）. The larval mortality of *P. xylostella* was 90%, three days after being inoculated with an *M. anisopliae* MA-126 suspension in

a concentration of 10^7conidia/mL. MA-126 wettable powder and liquid formulated preparations were applied with low dosages of chemical insecticide to control *P. xylostella* in 120 vegetable production net-houses in various parts of Taiwan（Lin, 1996）.

M. anisopliae var. *anisopliae* MA-805, isolated from the smaller brown planthopper *Laodelphax striatellus*, is highly pathogenic to its host. Virulence was enhanced from serial passages through *L. striatellus* five times and one passage through the brown planthopper *Nilaparvata lugens*（Lee and Hou, 1989a,b）. Six of 12 pesticides tested (hymexzel 30 L, benomyl 50 WP, tricyclazole 75 WP, pendimethalin 34 EC, paraquate 24 EC, and alachlor 45.1 EC), suppressed the mycelial growth of *M. anisopliae* var. *anisopliae* NA-805（Lee and Hou, 1989c）. Of the four insecticides tested, chlorpyrifos had the strongest inhibitory effects on *M. anisopliae* var. *anisopliae* Ma2. Mixing methomyl at concentrations recommended for field applications with Ma2 spore suspensions had a synergistic effect on *S. exigua* larvae, reducing the damage to leaves and the time it took larvae to die（Tsai, *et al.* 1993b）.

Benomyl- and carbendazin-resistant isolates (UV-Be, UV-Ca, Mu-Be, Mu-Ca) of *M. anisopliae* var. *anisopliae* were obtained when conidia were treated with ultraviolet light or mutagenic agents. These isolates had cross resistance, and showed stable fungicide resistance. A progressive increase in benomyl and carbendazin concentrations on culture media could also induce the appearance of fungicide-resistant (Fe-Be and Fe-Ca)（Tasi *et al*. 1993a）. Fungicides (benomyl, carbendazin, curzate, metalaxyl, and mancozeb) were highly toxic to *M. anisopliae* var. *anisopliae* Ma2 isolate. Although benomyl and carbendazin are chemically similar fungicides, the former is fungicidal to Ma2 while the latter is not（Tsai *et al.* 1994）.

Mass production of *M. anisopliae* var. *anisopliae* MA-805 was carried out using grain and plant residues as substrates. It was found that coarse rice, corn cob and bagasse were effective substrates 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℃, with a 24 hr photoperiod. Rice grain, soybean, and other agricultural by-products such as rice bran and rice steep were used as additives to formulate solid fermentation culture media for spore mass production. Yeast extract, dextrose and Sabourand broth were used in submerged fermentation for mycelia production (Liu, 1996)

M. anisopliae var. *anisopliae* (Ma2) was used for destruxin production. The results showed that when 30 g/L bactosacharose was used in submerged fermentation, the destruxin yield was 1.57 mg DB, 1.16 mg DMDB, 0.34 mg DA, and 0.14 mg DE. With solid-state fermentation, it was found that 100 g of rice (dry weight) yielded 4 mg DA, 3.03 mg DMDB, 2.29 mg DB, 0.95 mg DE and 0.21 mg DA2, and produced 2 x 10^9 spores per gram of rice（Hsieh *et al.* 1998a）. An extract of solid-state fermentation from Ma2 showed a high level of virulence to *S. exigua.* For third-instar larvae, the LC_{50} of the original extract was 1.41 x 10^5 ppm, 7.07 x 10^4 ppm, and 5.18 x 10^4 ppm, after 1 day 2

days and 3 days of infection, respectively. Among the fractions separated by a silica gel, the fractions containing 32% DA, 31% DB, and 4.5% DE had virulence equal to that of the original extract（Hsieh *et al.*1998b）.

Beauveria bassiana

B. bassiana was isolated from Taiwanese soils infested with *C. formicarius* （Su, 1991a,c, Su *et al*. 1988） Field trials showed that spraying with 1.6 x 10^4 conidia/mL at the time of planting or rootstock formation, or broadcasting soybean seeds containing 10^9conidia/g at planting time, effectively controlled the weevils（Su, 1991a） *B. bassiana* was pathogenic to 3rd, 4th and 5th stadium nymphs and adults of *Riptortus linearis*. At 25℃ and above, pathogenicity of *B. bassiana* decreased with an increase in temperature. Ultraviolet (UV) irradiation of conidia reduced the pathogenicity of *B. bassisna* to *R. linearis* （Hu et al. 1996） Under open-air conditions, three formulations 1) a solid-state soybean substrate inoculated with blastospores, 2) a granulated form of the *B. basiana*-soybean mixture (BSM) and 3) a powdered form of *B. bassiana*-soybean powder (BSP) -could be produced. Their spore concentrations were ca.1 x 10^8 -10^9 and 1 x - 10^9 -10^{10} conidia/g, respectively. The pathogenicity of BSP was different to that of *B. bassiana* rice powder. The LC_{50} to *O. furnacalis* of the two formulations was 4.8 x 10^6 and 9.5 x 10^7, respectively （Chiu, 1989）.

Corn plants at the late whorl stage were infested with ten 2nd-instar larvae of *O. furnacalis*, and then treated with 2-3 g of BSM two days after larval release. Only 0.19 larvae/plant and 13.0% infestation were found in treated blocks surveyed two weeks post-treatment. Four weeks after treatment, there were 0.36 larvae/plant and 27% infestation. This can be compared to the 4.58 and 4.26 larvae/plant and 100% infestation recorded in untreated blocks two and four weeks after treatment （Chiu,1989）. *B. bassiana* was able to infect the eggs, larvae and pupae of *O. furnacalis. B. bassiana* cultured on wine derivatives, and mixed with sand to form granules at 2 x 10^8 conidia/g, were as effective as carbofuran in screenhouse tests（Chiu and Hou, 1993）.

Liaphis erysimi Kalt treated with *B. bassiana* at a concentration of 10^7 conidia/mL showed almost 100% mortality. By Day 3, *B. bassiana* was able to grow at temperatures ranging from 10℃ to 30℃ in a yeast-peptone-dextrose medium（Hsiao and Lin 1995）. 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, 1988）. Zineb, iprodione, metalaxyl+mancozeb (MMC), metalaxyl+copper oxychloride (MCO), thiophenate methyl+streptomycin (TMS), propineb and imazalil were detrimental to *B. bassiana* at the concentrations tested. Bupirimate had the smallest inhibitory effect on the fungus. Imazalil showed complete inhibition in liquid culture, irrespective of the length of time the fungus was treated（Hsiao and Lin, 1995）.

The DNA sequence of PCR-amplified gene fragments from different *Beauveria* spp. isolates, using a PI-P3 primer set, was examined. The results showed more than 95%

homology among the isolates from Taiwan and one isolate from mainland China. However, the PCR amplification profiles of *Beauveria* spp. isolates using 18S rRNA primer set varied greatly. This can be used to characterize and identify isolates of *Beauveria* spp. (Shih *et al*. 1998)

Two local isolates of *B. bassiana* (Bb Col. 41 and 42) and one isolate of *V. lecanii* (HO 159) were bioassayed against turnip aphid (*L. erysimi)*. Seventy-five newly molted apterous admits from stock colony were randomly selected for each bioassay and exposed to the fungal pathogens place for 15 minutes. Either treated or non-treated aphids were then transferred individually into a 5.5 cm (dia.) petri disc and assigned each aphid as a replicate. Detached leaves from 4 weeks old and non-heading Chinese cabbage (*Brassicae chinensis)* plants were used as the food source. Treated and untreated aphids were placed at 20℃and 25℃ incubators with a photoperiod 12 L: 12 D. The mortality and fecundity of turnip aphids were recorded daily. The outgrowth and sporulation of these three fungal isolates on aphid cadavers after treatment were also recorded using a ranking system. The average fecundity in infected and control aphids differed significantly at 20℃ and 25℃after 3 days inoculation. The average fecundity of infected aphids for Bb-infected apterous female were 4.0 and 4.2, and Vl-infected was 4.4 and for control was 17.2 per aphid at 25℃, and corresponding fecundity was 1.2, 1.6, 2.3 and 14.3 at 20℃, respectively. Significantly more sporulation was found on VI when compared with other two fungal isolates at 20℃, while significantly greater aporulation on Bb Col.41 at 25 ℃. The liquefaction of cadavers infected by Bb Col. 42 was observed and might be responsible for the less sporulation of turnip aphid cadavers（Hsiao and Lin, 1995）.

Verticillium lecanii

V. lecanii could not grow at a temperature as high as 35℃. For *V. lecanii* F173, maximum growth and sporulation was shown at 25℃. The highest sporulation was shown when F173 was grown on yeast-peptone-dextrose medium. For both F159 and F113, optimal radial growth and maximum sporulation was shown at 25℃. The highest sporulation was shown when fungus was cultured on Sab (Sabouraud's dextrose agar), but in general, isolates of *V. lecanii* grown on yeast-peptone-dextrose medium showed better growth and sporulation（Hsaio and Yang, 1998）. Of the eight fungicides tested, propineb, imazalil, and matalaxyl+mancozeb, were fungicidal to *V. lecanii.* Buririmate, zineb, thiophenate methyl+dytrpyomyvin and iprodione were moderately inhibitory to mycelial growth and sporulation. Of the insecticides tested, insecticidal soap and abamectin had relatively little adverse effect on the mycelial growth and sporulation of *V. lecanii*（Lin *et al.* 1998）.

V. ecanii was isolated from *Rhopalosiphium padi* and was reported to be highly pathogenic to *Myzus persicae*, *Macrosiphoniella sanborni*, *Toxoptera aurantii*, *L. 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）. In other bioassay tests also showed that *V. lecanii* isolates at a concentration of 1x 10^7 conidia/mL were highly virulent to *L. erysimi*, *M. persicae*, and *Thrips palmi*. It caused 60% mortality of T. palmi after 5 days of treatment. The mortality of *L. erysimi* was 90%. In contrast, *V. lecanii* was not pathogenic to *A. gossypii*. Conidia of *V. lecanii* isolates germinated when the relative humidity was more than 94%. Conidial germination was affected by transient desiccation and high temperature. Conidia of *V. lecanii* isolates germinated and penetrated directly into *M. persicae* without producing appresorium. Most conidia could be found around setae（Tsai, 2004）.

A strong deleterious effect of UV irradiations on conidial germination of *V. lecanii* isolates was observed. Exposure to UV-B irradiation (310 nm, 540 μW cm^{-2}) or UV-C (254 nm, 120 μW cm^{-2}) for 2 minutes postponed the germination of three isolates tested (F096, VL159 and VL578). This delayed effect did not occur when conidia exposed under UV-A irradiation (360 nm, 540 μW cm^{-2}) for 90 minutes. A UV-induced dimer, cyclobutane pyrimidine dimer could be detected by using T4 endonuclease digestion method. Among 10 UV protectants tested, uric acid, folic acid, active carbon and xanthine provided significantly UV-protective effect for *V. lecanii.* Active carbon was the best UV protectant among them. Molasses or plumbago also possessed the UV-protective effect for the conidia of *V. lecanii*. Increasing the concentration of molasses in the conidial suspension enhanced the protective effect. The feasibility of *V. lecanii* isolates to control aphid on cabbage was investigated. Field trial showed that weekly spray of indigenous *V. lecanii* isolates could control aphid when the population density was below 20 aphids per leaf. (Tsai, 2004）

Baculoviruses

Virulent baculoviruses have been isolated from lepidopterous insects. Tests carried out in both the laboratory and the field gave encouraging results.

Five nucleopolyhedrovirus (NPV) isolates from Taiwan: *Spodoptera exigua* nucleopolyhedrovirus (SpeiNPV), *Spodoptera exigua* NPV (SpltNPV), *Perina nuda* NPV (Penu NPV), *Lymantria xylina* NPV (Lyxy NPV) and a isolate of *Autographa californica* NPV (AcMNPV-TWN_4) from *S. exigua* were used for this study. *EcoR* I profiles of genomic DNAs of five NPVs gave a clear pattern for identification, this result showed that *EcoR* I profiles were sufficient to identify these NPVs. A set of primers designated as primers 35 and 36（Chou *et al.* 1996）was used for amplification of polyhedrin gene from five NPVs by PCR and the size of amplicons was 680 bps. These polyhedrin gene fragments were sequenced and were them digested with *BsuR* I, *BspH* I, *BsiW* I, *Hpa* II, *Mse* I and *Taq* I DNA restriction endonuclease, respectively. It is suggested that PCR-RFLP technique is a feasible method for repid detection and identification of five NPVs isolated from Taiwan（Kao *et al*. 2000）.

Artogeia rapae Granulosis Virus (ArGV) and *Plutella xylostella* Granulosis Virus (PxGV)

The application of ArGV, PxGV virus, or *S. litura* nuclear polyhedrosis virus (SpltNPV) gave effective control of *A. rapae, P. xylostella* and *S. litura*, respectively. The use of mixtures of ArGV and PxGV, ArGV and SpltNPV, or PxGV and SpltNPV 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 (SpltNPV)

Third instar larvae treated with 10 x 10^6 PIBs/mL stored for four years showed a mortality of 83.3%. Larvae given the same treatment at the same concentration, but stored for only half a year, showed 96.7% mortality, while a fresh virus gave 100.0% mortality. *S. litura* applied with a combination of SpltNPV with palcrasol As-29 gave 87.1% mortality. A strong spread gave 83.2% mortality, and CS-7 70.6% or SpltNPV alone gave 75.8% mortality（Su, 1992）.

This SpltNPV isolate was highly pathogenic to *S. litura* larvae. The LC_{50} values were 5.47 x 10^5, 4.47 x 10^4, 6.16 x 10^5, 3.12 x 10^6, 1.4 x 10^7, and 7.28 x 10^8 PIBs./mL for the 1st to 6th instar, respectively, as assayed by inoculum-imbibing method. Mortality rates were higher, but were also slower to take effect, with older larvae（Tuan *et al*. 1995a）. The virus was more thermostable as a dry powder than in a suspension. The virus retained more than 80% of its original activity when exposed at 55℃ for 24 h. SpltNPV was not affected significantly by being in a suspension at 5, 7, and 9 pH for 24 h. However, exposure to a pH of 10 and 11, or to solutions containing chloride ions at high concentrations, greatly weakened its effect. Uric acid provided significant protection from sunlight for SpltNPV（Tuan *et al*. 1995b）. It is suggested that SpltNPV is well suited for mass production. An ideal method is to use early 5th-instar larvae, individually infected by incorporating the isolate into their diet (an inoculum of 3 x 10^6 PIBs/mL diet) and incubating them for 7 days at 30℃. The average yield was 1.4 x 10^9 PIBs/larva. Standardization and quality control of SpltNPV products can be achieved by visual counting, bioassay, SDS-PAGE, and ELISA. Application of SpltNPV at a low concentration on egg-masses immediately before hatching resulted in 77.4% larval mortality, compared with 50.4% on newly laid egg-masses. Applications of SpltNPV at high concentrations (10^8 PIBs/mL) resulted in 99.2% larval mortality, and a 94.25% reduction area in the area of leaves eaten. The control efficacy of SpltNPV at high concentrations was better than that of bifenthrin with *Bt* one week after application（Tuan *et al.* 1998）.

The activity of SpltNPV against *S. litura* larvae was enhanced by mixing the virus suspension with leucophor, fluorescent brightener 28, lecithin, phosphatidyl choline, and phosphatidyl ethanolamine. The mortality of the *S. litura* larvae increased with higher

concentrations of the chemicals in the virus suspension, with the exception of the fluorescent brightener 28. Of the six Granulosis viruses tested, the PxGV did most to enhance the effectiveness of SpltNPV（Lee, 1996）.

Heliothis nuclear Polyhedrosis Virus (HsNPV)

H. armigera densities were significantly reduced 14 days post application with HsNPV. Control was better than that achieved with carbofuran. The injection of HsNPV preparations into corn ears was more effective than spraying the ears（Tuan *et al.* 1989a）. The HsNPV was inactivated by the weakly alkaline dew (pH 8.1) collected from soybean leaves. Heavy artificial rainfall of 242 mm/h for 30 minutes failed to wash off HsNPV preparations sprayed onto corn silks（Tuan *et al*. 1989b）. Lecithin added to a NPV commercial product reduced LT_{50} values by 2 to 3 days, compared to inoculation with NPV alone （Tuan and Hou, 1998）.

Spodoptera exigua Nuclear Polyhedrosis Virus (SpeiNPV)

A SpeiNPV isolate was obtained from *S. exigua* larvae that originated from *G. paniculata* in Taiwan（Kao *et al*. 1991a,b）. This SpeiNPV isolate is highly pathogenic to *S. exigua* larvae. Its LC_{50} values are 1.6 x 10^6, 6.9 x 10^4, 4.1 x 10^4, 3.7 x 10^5, and 5.2 x 10^6, PIBs/mL for the first to fifth instar, respectively, as assayed according to an inoculum-imbibing method（Tuan *et al*. 1994）. Data on Food consumption, virus yield, larval mortality and microbial contamination suggest that SpeiNPV is well suited to mass production using an artificial diet of pellets each weighing 1.54 ± 0.23 g (diameter 1.0 cm, height 0.5 cm), inoculum rate of 2 x 10^5 PBs.cm^2 at 30℃ and harvested 5-6 days after inoculation（Huang and Kao, 1994）.

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.* 1991a）. 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 SpeiNPV （Kao and Huang, 1992）.

Field trials showed that good control of *S. exigua* in green onion was achieved at a rate of 1.5-2.0 x 10^{12}/ha, with Bivert as a spreader-sticker. Twenty hectares of green onion were sprayed with SpeiNPV to control *S. exigua* in 1996, with 67 farmers participating. The demonstration of this control strategy received favorable comments from participants. In 1997 the project was expanded to 200 ha with 213 farmers participating（Kao *et al.* 1997）.

Autographa California Nucleopolyhegrovirus (AcMNPV)

Trichoplusia ni, P. xylostella, and *S. exigua* were highly susceptible to AcMNPV. LC_{50} values of AcMNPV to 3rd instar larvae on the sixth day after inoculation were 1.24, 2.07 x 10^3, and 2.40 x 10^3 PIBs/mm^3, respectively. *Heliothis virescens, S. litura, O.*

fumacalis and *Galleria mellonella* were found to be moderately susceptible. *H. armigera* showed a very low mortality, while *Corcyra cephalonica* did not become infected with AcMNPV（Tuan *et al.* 1997）.

The δ-endotoxin gene from *Bt aizawai* 7.29 was inserted into AcMNPV. The recombinant viruses Acendo-T7, Acendo-Uwl and Acendo-1993 were purified by end point dilution, and identified by dotblot hybridization, the PCR method and southern blotting. Results showed that the expression of the δ-endotoxin gene by the AcMNPV expression system improved the cytotoxicity of this virus（Hu *et al.* 1994）.

For improving the UV resistance of AcMNPV, the melanin gene from *Streptomyces lividans* was cloned to the AcMNPV transfer vector pVL1393. The recombinant transfer vector DNA was cotransfected with parental viral DNA into a S17B cell line derived from *S. litura*. It showed that the recombinant virus, Actyr, contains a melanin gene. The expression of protein was checked by SDS-PAGE. The data level of protein expression increased over time after viral infection(Lin, 1995).

Jinn *et al*.（Jinn *et al*. 2005）employ the internal ribosome entry sites (IRES) element of encephalomyocarditis virus (EMCV) to generate a red and green dual fluorescence protein gene containing recombinant AcMNPV named vAcR-IR-G, to evaluate the efficacy of fluorescence proteins as recombinant AcMNPV tracer. vAcR-IR-G infected SF9 cells emitted red fluorescence as well as green fluorescence indicated the EMCV-IRES can mediate cap-independent translation in SF9 cells although the EMCV-IRES is not functional well in insect larvae infected with this recombinant AcMNPV. Interestingly, it is found that red fluorescence protein from *Discosoma sp*., DsRed, is an excellent marker as the recombinant AcMNPV tracer. Insect larvae, including *Trichoplusia ni*, *Spodoptera exigua*, and *Spodoptera litura*, infected with the recombinant AcMNPV containing the DsRed gene can emit red fluorescence under visible light. Thus, the red fluorescence protein from coral will facilitate the development of genetically modified as baculovirus insecticide.

An attempt to demonstrate the insecticide activity of boric acid on AcMNPV and SpeiMNPV was carried out in the laboratory. Results showed that boric acid significantly enhanced the insecticide activity of AcMNPV and SpeiMNPV and acted as an effective viral synergist（Jinn *et al.* 2004）.

Bacillus thuringiensis (*Bt*)

B. thuringiensis (*Bt*) has been registered for use against several lepidopterous pests of vegetables in Taiwan since 1960. *P. xylostella* (diamondback moth), the most destructive pest of cruciferous crops, has developed resistance to a number of synthetic insecticides in Taiwan(Sun, 1992). As a result, farmers became interested in the use of *Bt* to control the moth. Research into novel *Bt* strains, genetic engineering, enhancement of efficacy, optimization of the growth medium, field trials and quality control have been

carried out by a number of research institutes and universities in Taiwan.

Isolation and Characterization of Local *Bt* Isolates

Hundreds of *Bt* isolates have been isolated from different parts of Taiwan. In addition to plasmid profile and western blotting, polymerase chain reaction (PCR) was also used to characterize *Bt* isolates（Chak and Jen,1993, Chak and Young, 1990, Chak *et al.* 1994, Chak *et al.* 1995, Chao, 1992, Chen, 1992, Kao *et al.* 1994, Li, 1991）. Using a set of specific oligoprimers for PCR amplification, Chen（1992）found that the *Bt* YMB 96 strain contained *cry1Aa* and *cry1Ac* genes. Chao (1992) detected six kinds of *cry1* gene types residing in his *Bt* isolates.

Four different isolation techniques were employed to isolate *Bt* from stored materials collected from around Taiwan. Four hundred and eighty isolates were obtained. PCR analysis was applied to identify isolates harboring different *cry*-type genes. Thirty-eight distinct *cry*-type gene profiles were detected. The *cry*-genes included *cry1Aa*, *1Ab*, *1Ac*, *1B*, *1C*, *1D*, *1E*, *1F* and *cry1*. Agarose gel electrophoresis and SDS-PAGE were used to characterize plasmid and protein profiles for each isolate. Results showed significant differences in size among the isolates. Diet incorporation technique was undertaken to evaluate the bioactives of each isolate. *Bt* isolates Al-9, G2-1 and G3-3, containing *cry1Ab*, *1B*, and *cry1*, exerted the most insecticidal activity against *P. xylostella*（Kao *et al*. 1996）.

Two pairs of universal oligonucleotide primers designed by Kuo and Chak（1996）were used to probe the best conserved regions of all known *cry1*-type gene sequences, so that the amplified PCR fragments of the DNA template from *Bt* isolates would contain all possible *cry1l*-type gene sequences. The RFLP patterns of PCR-amplified revealed that 40 distinct *cry*-type genes have been identified from 146 *Bt* isolates from stored-grain warehouses in Taiwan. Of these, 29 *cry*-type genes were found to be possibly novel, including *cry1lAc**[1-5], *cry1C**[1], *cry1Cb**[1-4], *cry1D**[1-4], *cry1E**[1-7], *cry1F**[1-2], *cry1G**[1], *cry1Cb**[1] or *cry1D**[1] or *cry1F**[1], and unknown*[1-4]. However, the novelty of these *cry*-type genes needs to be confirmed（Kao *et al.* 2003）.

A new *cry* gene (*cry1Ca9*) was cloned and sequenced from a isolate (G10-01A), harboring possibly novel *cry1C* gene. The *cry1Ca9* gene consisted of an open reading frame of 3,567 bp encoding a protein of 1,189 amino acid residues. The polypeptide has the deduced amino acid sequences predicting molecular masses of 134.7 kDa. When the Cry1Ca9 toxin was expressed in *cry*B$^-$, a non-enterotoxigenic and non-cytotoxic plasmid negative *Bt* strain, elliptical crystals were produced. The recombinant strain exhibited high toxicity against *Plutella xylostella* （Kao *et al.* 2003）. A *cry1Ac* gene was cloned and sequenced from a isolate E9-11. This gene consisted of an open reading frame of 3.6 kb. The polypeptide has deduced amino acid sequences predicting molecular masses of 130 kDa. When the Cry1Ac toxin was expressed in *cry*B$^-$, spherical crystals were formed. The recombinant strain showed similar toxicity to that of Xentari, Delfin, and HD-73

against *P. xylostella*. However, it is more toxic against larvae of *Arotogeia rapae* than all other samples tested （Tzeng *et al*. 2002）.

Genetic Engineering of *Bt*

The epiphytic *Erwinia herbicola* has been transformed with *cry1Aa1* presented in plasmid pUN4. Protein extracts from this transformant exhibited strong toxicity against *P. xylostella*. The transformed *E. herbicola* also showed significantly antagonistic effects against *Xanthomonas campestris* pv. *campestris* and *E. carotovora* subsp. *carotovora*. This result firstly demonstrated the combinations of insecticidal activity, and the characteristics of antagonism and foliar colonization in the same transgenic epiphytic bacteria（Lin *et al.* 2003）.

Enhancement of *Bt* Efficacy

The addition of 0.1% octyl methoxy cinnamate to *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 sinigrin into low-dosage *Bt* preparations doubled the insecticidal efficiency, which then increased 8 times to the 4th instar larvae if sinigrin was added at 1 ppm（Chung, 1991）.

Bt formulations were prepared by: (a) Blending predetermined amounts of pregelatined starch, natural starch, *Bt* and water to form a mass; (b) Drying this mass by heating with a hot roller at temperatures between 50℃ and 100℃, (preferably between 60℃and 90℃), to form a dried mass; and (c) Crushing the dried mass to form the final product in the form of flakes, which should have a diameter of less than 900μ. Using this method, the processing time was reduced from 24 hours to less than 30 minutes, or even 3-15 minutes (Yang *et al.* 1994).

A biocidal formulation with improved resistance to ultraviolet rays is made up of: (a) *Bt*; (b) An anti-ultraviolet agent selected from the group consisting of 2-(2-H-benzotriazole-2-yl)-phenol and their derivatives; (c) Pre-gelatinized starch; and (d) Natural starch. The anti-ultraviolet agent, the pregelatinized starch and the natural starch are mixed then baked at 60 - 90℃ to form the *Bt* formulation（Yang *et al*. 1995）.

Optimization of Growth Medium

Response surface methodology (RSM) was used to assess the impact of the composition of various cultivation media, including tapioca, fishmeal, $CaCO_3$ and $(NH_4)_2SO_4$, on the growth of *Bt* YMB 96-1988. Estimated optimum compositions for the production of spores by *Bt* are as follows: Tapioca, 5.01%; fishmeal 5.86%; $(NH4)_2SO_4$ 0.06%. This mixture gave a maximum spore count of 8.56 x 10^8/mL. This value is close to the 8.35 x 10^8/mL spore density as counted from experimental observations （Liu and Tzeng, 1998）.

Characterization of the sporulation of *B. thuringiensis*

A wild-type and an rDNA sstrain of *B. thuringiensis* were cultured in a net-draft-tube modified 20-L airlift bioreactor. A comparison of the sporulation patterns suggests that the early sporulation strain has a lower final spore count. Results from off-gas analysis suggests that the CO_2, profile could be an alternative indication to spore counts for the examination of fermentation performance or even the mortality in bioassay of the cultivation product. The difference in mortality tests exthibited by the microorganism was attributed to different patterns of sporulation as well as different levels of gene control inside the cell itself. The sporulation kinetics of *Bt* was simulated by a simple modified Hill equation, where the initial glucose concentration could affect the timing of the onset of sporulation. The equation matches well with the experimental sporulation data for *Bt* in both wild-type and rDNA strains（Lin and Tzeng, 2000）.

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* was effective in controlling both *P. xylostella* and *A. rapae* within seven days of application （Su, 1991c）. Results of two field experiments indicated that newer commercial *Bt* products gave better control against *P. xylostella* than older ones. All *Bt* products gave fair control of *A. rapae*（Kao *et al.* 1990）. Chiuo（1990）showed that a ombination of *Bt kurstaki* with *B. bassiana* enhanced its effectiveness against *O. furnacalis*.

Quality Control of *Bt* Products

A standardized bioassay protocol has been developed to evaluate preparations derived from *Bt* against *P. xylostella*. It has now been adopted by the government as a guideline for *Bt* product registration（Kao and Tuan, 1992）. The ELISA detecting systems established by polyclonal antibodies was developed for *Bt* δ-endotoxin（Tuan *et al.* 1993）. SDS-PAGE was also developed to quantify δ-endotoxins（Tuan *et al.* 1993）. Application of the ELISA, SDS-PAGE to monitor the δ-endotoxins in conjunction with bioassay was also examined. Those assays were useful, not only in fundamental research and product analysis, but also in the regulatory control of *Bt* （Kao and Tuan 1995, Tuan *et al*. 1993）. An in vitro assay devised with cultured *P. xylostella* cells in agar plate, with trypan blue layered on the upper surface, rapidly detected the toxicity of *Bt* δ-endotoxins（Lee, 1993）.

Photorhabdus luminescens

Supernatant fluid of *Photorhabdus luminescens* culture was centrifuged, filtered, and bioassayed against 6 species of insect larvae. The LC_{50} values of protein preparations against the 3rd instar larvae of the lepidopteran *Plutella xylostella* amd *Galleria mellonella* were 56 and 200 ppm, repectively. Antagonistic effects of bacterial culture and

protein preparations on 10 species of fungi and 9 species of bacteria were screened by means of dual or concomitant culture methods. High antimicrobial activities were observed against *Botrytis cinerea*, *Glomerella cingulata*, *Alternaria mali*, *Phyophthora capsici*, *B. thuringiensis*, *B. subtilis*, *B. cereus*, and *Erwinia carotovora* subsp. *carotovora*. In conclusion, *P. luminescens* ATCC 29999 has high insecticidal and antimicrobial activities against local pests and pathogens（Hsieh *et al.* 2004）.

An entomopathogenic nematode, *Heterorhabditis brevicaudis* TG01, was isolated from sampled soils for the first time in Taiwan using the *Galleria*-bait method. Identification of the nematode was mainly based on observations under scanning electron microscopy (SEM) and nucleotide sequence of the internal transcribed spacer 1 (ITS1). *P. luminescens* subsp. *akhurstii* was isolated from nematodes and identified by phenotypic, biochemical tests, 16S rRNA and Biolog identification system. In this study, supernatant fluid of the bacterial culture was centrifuged, filtered, and bioassayed against 5 key pests of vegetables. It exhibited insecticidal activity against *Plutella xylostella*. Antagonistic effects of bacterial culture and protein preparations on 18 species of fungi and 12 species of bacteria were examined by means of dual or concomitant culture methods. High antimicrobial activities against *Glomerella cingulata*, *Colletotrichum musae, Xanthomonas spp.* and *Erwinia spp.* were observed. Studies were also carried out to test the preventability of mango anthracnose during storage with local *P. luminescens* and showed satisfactory results.（Hsieh *et al*. 2007）

Pheromones and attractants

The beet armyworm, tobacco cutworm, sweet potato weevil, citrus leaf miner moth, litchi fruit borer, green peach aphid, sugarcane wireworm, carambola fruit borer, oriental fruit fly and medlon fly are serious insect pests in Taiwan. Improved new synthesis methods and results of the isolation and identification of the above-mentioned 10 serious pests have subsequently unveiled. Yields of synthesized pheromones can he efficiently increased, and the costs for producing those pheromones can also be drastically reduced by the novel, simple and convenient methods developed.

The synthesized sex pheromones for the beet armyworm. the tobacco cutworm, the sweet potato weevil and the sugarcane wireworm have proven to be powerful attractants for each respective species, and were widely applied in the fields to control them. Synthesis of the sex pheromone of the litchi fruit borer was complicated, but it can be used not only for species identification but also for monitoring to schedule the proper time of application. Large-scale application of the sex pheromone of the carambola fruit borer was used in different ways for monitoring, mass trapping, and mating disruption. The unique property of the aphid alarm pheromone can be used to

enhance the effectiveness of insecticides against the aphid.（Yen *et al.* 2004）. Examples of the utilization of sex Pheromones are given below.

Sex Pheromone of Sweet Potato Weevil, *Cylas formicarius elegantulus*（Summers）

A modified and reliable method of synthesizing （Z）-3-dodecen-1-01 （E）-2-butenoate, the sex pheromone of *C. formicarius elegantulus*（Summers）, has been reported. The effective bioactivity of the pheromone synthesized by this new method has also been demonstrated（Yen and Hwang 1990）. An alternative method of synthesizing this sex pheromone without using carcinogenic etyhene oxide and HMPA has also been described. *C. formicarius* was strongly attracted to the synthetic sex pheromone （Lo *et al.* 1992）. Results showed that the funnel type polyethylene terephalate （PET） bottle trap, baited with 1mg of synthetic sex pheromone dispensed in polyethylene, was the most effective, cheapest and easiest way to mass trap male weevils （Hwang *et al*. 1989, Hwang *et al.* 1991）.

Sex Pheromone of the Carambola Fruit Borer, *Eucosma notanthes* Meyrick

The results showed that the use of this pheromone in double plexiglass boxes appeared to be a successful method of bioassay（Hwang *et al.* 1996）.Results showed that 0.5-1 mg Z-8-dodeceny1 acetate dispensed in a rubber septum was the most effective and long-lasting formulation for capturing male moths, and was also easy to prepare, A funnel-type triple PET bottle trap with 16 opening of 0.6-0.8 cm, and with the lure placed 5 cm above the openings, was an effective trap for male moths（Hwang and Hung 1994）. Results showed that when disruptant was used in a rubber septum, its potency lasted for five months. The reduction in damaged fruit was 10.8-71.6％. These results revealed that the permeation of synthetic sex pheromone in the field could be an effective and safe technology for the control of *E. notanthes*（Hwang and Hung 1997a,b.）.

Sex Pheromone of Tobacco Cutworm, *Spodoptera litura*（F.）

A new route to synthesize the major component of the sex pheromone of （Z, E）-9, 11-tetradecadien-1-yl acetate [（Z, E）-9, 11-TDDA] proposed. The final production (Z, E）-9, 11-TDDA）was 12.8g. The product was 86.0％ isomerically pure（Lo *et al.* 1988）.Partially purified synthetic [(Z, E)-9, 11-TDDA, 86％ active isomer)] mixed with the purchased 9.12-isomer （95％ active）in a ratio of 10:1 showed a promising attractiveness to male moths of S. litura in field tests. The

PET bottle trap was good for trapping the male moths （Lo *et al.* 1989, Lo *et al.* 1998）.

Sex Pheromone of Beet Armyworm *Spodoptera exigua* Hubner

A method has been developed of synthesizing two major components of the sex pheromone of *S. exigua* Hubner, namely, （Z, E）-9, 12-tetradecadien-1-yl acetate [（Z, E）-9, 12-TDDA] and （Z）-9-tetradecenol [(Z)-9-TDOL]. The results showed that combination of the synthesized components was effective, and its effectiveness could persist up to two months in the field（Yen *et al*. 1998）

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DEVELOPMENT OF MICROBIAL FUNGICIDES（Tzeng and Yeh, 2003；Tzeng *et al*. 2004）.

For the establishment of mass production techniques, a plant equipped with 5L to 750L series liquid fermentors and the necessary peripheral facilities was constructed. By use of *Streptomyces saraceticus* SS31 isolate and *Bacillus subtilis* BS1 isolate, each respectively as the model isolate, liquid fermentation protocols for the mass production of biomass formulation were established.

For *S. saraceticus* SS31, the established protocol yielded approximately a 4 x 10^{10} cfu/ml culture broth that contained mainly mature bacterial spores. The liquid formulation produced appeared to remain viable for more than 10 months when stored at 6℃ and was shown to be effective in the control of various fungal infections that included diseases on various crop species caused by *Pythium* spp., *Rhizoctonia solani*, *Phytophthora parasitica*, *Fusarium oxysporum,* and *Colletotrichum gloeosprioides.*

Whereas for *B. subtilis,* the liquid fermentation yielded approximately 5 x 10^{9} cfu/ml and the bacterial propagules remained vible for more than one year when stored at 6℃. The application trials conducted indicated that the cultural broth was effective in stimulating plant growth in addition to the discouragement of infection of various fungal and bacterial pathogens. Notable protective effect covered diseases caused by *Xanthomonas* spp., *Sclerotium rolfsii*, and *Cereospora nicotiana*.

The two sets of liquid fermentation protocols were found to be easily adaptable for the production of members of antagonistic *B. subtilis* and S*treptomyces* spp. each with morphological characteristics close resemble that of the respective model isolate. One of the major breakthroughs of the technology development was the manipulation leading to the maximization of yield of bacterial spores with long durable shelf life. In regarding to the commercialized use of *Streptomyces* spp. for plant disease control,

this is the first success in mass production of long durable spore formulation.

The technology platforms was further improved and yielded over 10^{11} cfu/ml spore biomass for *Streptomyces griseobrunenneus* S3, over 10^{11} cfu/ml endospore biomass for *B. subtilis* WG6-14 and over 10^{8} cfu/ml of chlamydospore biomass for *Gliocladium virens* WJGV2. The broth cultures obtained can be directly used for field application or further processed into prowder or granule formulations; they can be applied by foliar spray, soil drenching, and seed soaking/coating like that for chemical pesticide application. The application of broth formulations of all three target microbes have been shown having growth promoting effect and were effective in controlling the diseases selectively targeted. The effectiveness of disease control was comparative to that by chemical fungicides especially for the control of soil borne diseases.

CONCLUSION AND FUTURE PERSPECTIVE

It is likely that insect management by the use of synthetic organic chemicals will still dominate the world pesticide market . However, major thrusts are under way in industrial, academic, and government research laboratories to develop more selective methods of pest control which are compatible with biological, toxicological, environmental, and societal requirements（Altstein *et al.* 1993）. In Taiwan, environmental concerns and insecticide resistance has recently led many farmers to consider the use of microbial insecticides for the control of agricultural pests. The following topics are particularly worthy of more attention.

Isolation, Screening and Strain Improvement of Indigenous Entomopathogens

Taiwan's subtropic and tropic climate, diversified topography and mosaic cropping systems provide rich microbial resources. There is good potential for finding novel agents with high pathogenicity and broad spectra. The selection of effective strains of entomopathogens, and microbial breeding and genetic manipulation to improve strains, are essential to the development of microbial insecticides（Aizawa, 1987）.

Development of Microbial Control Strategies in IPM

Microbials are ideal for use in integrated pest management (IPM) because of their selectivity and environmental safety(Starnes *et al*. 1993). To maximize the efficiency and ecological compatibility of microbial insecticides, specific methods of application should be designed for their use.

Improved Production and Formulation

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.

Opportunities exist for enhancing field effectiveness and prolonging the shelf-life through improved formulation. Suggested areas for advancement include micro-encapsulation 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

More research effort should be directed towards biotechnology. Studies such as plasmid curing and conjugation, development of transgenic microorganisms and transgenic crops all provide new insights into microbial control.

Better Risk Assessment of Genetically Modified Organisms (GMOs)

The commercialization of transgenic crops and transgenic microorganisms may pose a potential hazard to the ecosystem. The impact of GMOs on the surrounding environment needs to be carefully examined.

Emphasis on Sex Pheromone Research

In the future, research and development work for insect sex pheromones in Taiwan should include: Isolation and identification of sex pheromonesfor key insect pests; Development of methods of synthesis; Improved bioassay techniques and formulations; Designing effective trapping systems; Evaluation of the effectiveness and economic returns of pheromone use; and Extension education for farmers （Hwang 1997）.

Policies Needed to Enhance the Biopesticide Develogment and Usage

Emphases should be focused on the following aspects: Strengthening the basic research; Rinforcement of international and cross-strait collaboration to facititate the commericialization; Policy support and requlation improvement from the government to encourage the investment for new innovations; Facilitation the use of biopesticides through collaboration projects among industry, academics, government officials and farmers; and Promotion of the concept of organic farming to farmers.

Promotion of the Development of Novel Bipesticides

Application of modern biotechnology to develop novel biopesticides including polyvalent *Bacillus thuringiensis* for plant pests control, new type of microbial protein pesticides, multifunctional symbiotic bacterium, *Photorhabdus luminenscens*

and entomopathogenic *Beanveria bassiana,* as an endophyte with dual biocontrol activity.

LITERATURE CITED

Aizawa, K. 1987. Strain improvement of insect pathogens. *In*: Biotehnology in Invertebrate Pathology and Cell Culture. K. Maramorosch [ed.], Academic Press, Lnc. p. 3-11.

Altstein, M., N. Aharonson, and J. J. Menn. 1993. Overview: New target for insect management in crop protection. Arch. Insect Biochem. Physiol. 22: 5-12.

Chak, K. F., and J. C. Jen. 1993. Completc Nucieotide sequence and identification of a putative promoter region for the expression in *Escherichia colli* of the *cry1Ab* gene HD133. Proceedings of the National Science Council, ROC. Part B: Life Science 17: 7-14.

Chak, K. F., and Y. M. Young. 1990. Characterization of *Bacillus thuringiensis* strains isolated from Taiwan. Proceedings of the National Science Council, ROC. Part B: Life Science 14: 175-182.

Chak, K. F., D. C. Chao, M. Y. Tseng, S. S. Kao, S. J. Tuan, and T. Y. Feng.1994. Determination and distribution of *cry*-type genes of *Bacillus thuringiensis* isolates from Taiwan. J. Appl. Environ. Microbiol. 60: 2415-1420.

Chak, K. F., S. S. Kao, and T. Y. Feng. 1995. Characterization and *cry* gene typing of *Bacillus thuringiensis* isolates from Taiwan. *In*: *Bacillus thuringiensis* Biotechnilogy and Environmental Benefits. T. Y. Feng, K. F. Chak, R. Smith, T. Yamamoto, J. Margalit, C. Chilcott, and R. Rose [eds.], Hua Shiang Tuan Publishing Co. Taipei, ROC. Vol. 1: 105-123.

Chao, D. C. 1992. Characterization of the *Bacillus thuringiensis* strains isolated from Taiwan. M.S. Thesis, National Yang-Ming Medical College, 52pp. (In Chinese with English summary)

Chen, P. W. 1992. Isolation and application of insecticidal crystal protein genes from *Bacillus thuringiensis.* M.S. Thesis, National Chung-Hsin University, 80pp. (In Chinese).

Chen, Y. R. 1992. Molecular characteration of *cry* genes from *Bacillus thuringiensis* isolate YMB82. M. S. Thesis, National Yang-Medical College, 91 pp. (In Chinese with English summary)

Chien, N. Y. 2004. Studies of formulations for *Nomuraea rileyi* and *Trichoderma virens*. M.S. Thesis, Da-Yeh University, 125pp. (In Chinese with English summary)

Chiu, T. M. 1989. Production of the white muscardine fungus*, Beauveria bassiana,* and its infection on the Asian corn borer, *Ostrinia furnacalis.* MS Thesis, National

Chung-Hsin University, 68pp. (In Chinese with English summary)

Chiuo, W. C. 1990. Microbial control of the Asian corn borer, *Ostrinia furnacalis* with *Bacillus thuringiensis* subsp. *kurstaki*, *Beauveria bassiana* and *Metarhizium anisopliae.* M.S. Thesis, National Chung Hsing University, 60pp. (In Chinese with English summary)

Chiuo, W. C., and R. F. Hou. 1993. Infection of the Asian corn borer, *Ostrinia furnacalis* Guenee (Lep. Pyralidae), with entomopathogens under screen house conditions. Appl. Entomol. 115: 246-253.

Chou, C. M., C. J., Huang, C. F.,Lo, G. H., Kou, and C. H. Wang, 1996. Characterization of *Perina nuda* nucleopolyhedrovirus (PenuNRV). J. Invertbr. Pathol. 67: 259-266.

Chung, F. I. 1991. Studies on pathogenicity of the bacterium *Bacillus thuringiensis* and the entomgenous nematode, *Steinernema feltiae* to the diamondback moth, *Plutella xylostella* (L.). M.S. Thesis, National Chung Hsing University, 52pp. (In Chinese with English summary)

Hsiao, W. F., and C. R. Yang. 1998. Virulence of three local entomopathogenic fungal isolates to Turnip aphid, *Lipaphis erysimi* (Homoptera: Aphididae) J. Chiayi Ins. Tec. 59:61-68.

Hsiao, W. F., and Y. C. Lin. 1995. Effects of fungicides on the entomopathogenic fungus, *Beauveria bassiana Bals.* Chinese J. Entomol. 15: 295-304. (In Chinese with English summary)

Hsieh, C. M., S. J. Tuan, S. C. Wang, S. S. Kao, and Y. M. Tseng .1998b. The virulence of destruxins from *Metarhizium anisopliae* var. *anisopliae* to *Spodoptera exigua.* J. Agricul. Chem. Soc. 36: 622-629. (In Chinese with English summary)

Hsieh, C. M., W. K. Hung, S. S. Kao, S. C. Wang, and Y. M. Tzeng .1998a. Production destruxins from a local entomopthogenic fungus, *Metarhizium anispliae* var. *anisopliae*, by solid-state submerged fermentation. J. Agricul. Chem. Soc. 36: 371-379.

Hsieh, F. C., C. Y. Tzeng, and S. S. Kao. 2007. *Bioactivites of Photorhadus luminescens* subsp. *akhurstii*, a symbiont of entomopathogenic nematode, *Heterhabditis brevicudis* p.101 *In* Annual Meeting of the Invertebrate Pathology and the Ist International Forum on Entomopathogenic Nematodes and Symbiotic Bacteria August 12-16. Universite Laval in Quebec City, Canda.

Hsieh, F. C., T. C. Lin, J. T. Tseng, and S. S. Kao. 2004. An entomopathogenic-entomophilic bacterium, *Photorhabdus luminescens*, with insecticidal and antimicrobial activies. Plant Prot. Bull. 46: 163-172. (In Chinese with English summary)

Hsieh, F. C., T. C. Lin, J. T. Tseng, and S. S. Kao. 2004. An entomopathogenic nematophilic bacterium, *Photorhabdus luminescens*, with insecticidal and antimicrobial activities. Plant Prot. Bull. 46: 163-172. (In Chinese with English summary)

Hu, W. J., R. F. Hou, and N. S. Talekar. 1996. Pathogenicity of *Beauveria bassiana* to *Riprortus linearis* (Hemiptera: Coreidae), a pest of soybean. Appl. Entomol. Zool. 31: 187-194.

Hu, Y. C., C. F. Lo, and C. J. Shih. 1994. Construction of recombinant baculovirus containing the *Bacillus thuringiensis* δ-endotoxin gene. Chinese J. Entomol. 14: 445-461. (In Chinese with English summary)

Huang, L. H., and S. S. Kao. 1994. Production of *Spodoptera exigua* nuclear polyhedrosis virus in larvae. Chinese J. Entomol. 14: 343-352. (In Chinese with English summary)

Hwang, J. S. and C. C. Hung. 1994. Formulations of sex ateactant and trap designs for trapping carambola fruit borer *Eucosma notanthes* Meyrick. Plant Protection Bulletin 36: 31-40. （In Chinese with English summary）.

Hwang, J. S. and C. C. Hung. 1997a. Mating disruption for the control of carambola fruit borer, *Eucosma notanthes* Meyrick, with synthetic sex pheromone. Plant Protection Bulletin 39: 159-164. （In Chinese with English summary）.

Hwang, J. S. and C. C. Hung. 1997b. Controlled release formulations of sex pheromone for mating disruption of the carambola fruit borer *Eucosma notanthes* Meyrick. Plant Protection Bulletin 39: 275-280. （In Chinese with English summary）.

Hwang, J. S., C. C. Hung, and R.F. Hou. 1996. A bioassay metnod of the sex pheromone of the caramobla fruit borer, *Eucosma notanthes* Meyrick. Plant Protection Bulletin 38: 119-127. （In Chinese with English summary）.

Hwang, J. S., C. C. Hung, and Y. P. Yen. 1989. Evaluation of formulatios of synthetic sex pheromone and trap designs for trapping sweet potato weevil, *Cylas formicariuss elegantulus* （Summers）. Chinese Journal of Entomology 8: 37-43. （In Chinese with English summary）

Hwang, J. S., C. C. Hung, and Y. P. Yen. 1991. Formulations of synthetic sex pheromone, trap designs and their use for mass trapping sweet potato weevil, *Cylas formicarius elegantulus*. Proceedings of the Conference of Insect Chemical Ecology, Tabor 1990. pp. 365-368. Academia Prague and SPB Acad. Publ. The Hague, 1991.

Jinn, T. R., S. S. Kao, C. I. Liu, H. Y. Tzeng, and T. Y. Wu .2004. Boric acid as a synherngist of *Spodoptera exigua* and *Autograph california* Nuclear Polyhedrosis, viruses. Formosan Entomol. 24: 173-184. (In Chinese with English summary)

Jinn, T. R., S. S. Kao, J. T. C. Tzeng, and T. Y. Wu, 2005. Coral red fluorescence protein as GMBV tracer. J.Biotectnol 119: 255-259.

Kao, C. H., C. S. Chiu, and E. Y. Cheng, 1990. Field evaluation of microbial and chemical insecticides for diamondback moth and other lepidopterous pests control on cabbage. J. Agricul. Res. China 39: 221-227.

Kao, C. W., and Y. S. Tsai. 1989. Control of beet armyworm with entopathogenic fungi. Chinese J. Entomol. Special Publication No. 4. pp. 214-225. (In Chinese with English summary)

Kao, S. S., and S. H. Huang. 1992. Effectiveness of UV protectants for *Spodoptera exigua*

(Hübner) nuclear polyhedrosis virus. Chinese J. Entomol. 12: 31-40. (In Chinese with English summary)

Kao, S. S., and S. J. Tuan. 1992. *Bacillus thuringiensis* potency bioassay against *Plutella xylostella* lavae. *In*: Proceedings XIX International Congress of Entomology. Beijing, China, p. 276.

Kao, S. S., and S. J. Tuan. 1995. Registration of *Bt* prepatations in Taiwan. *In*: *Bacillus thuringiensis* Biotechnology and Environmental Benefits. T. Y. Feng, K. F. Chak, R. Smith, T. Yamamoto, J. Margalit, C. Chilcott, and R. Rose [eds.], Hua Shiang Yuan Publishing Co. Taipei, ROC. Vol.: 337-348.

Kao, S. S., C. C. Tzeng, S. J. Tuan, and Y. S. Tsai. 1996. Isolation, characterization and *cry* gene typing from stored product material samples collected around Taiwan. *In*: The 2nd Pacific Rim Converence on Biotechnology of *Bt* and its Impact to the Environment. Nov. 4-6, 1996. Chiang Mai, Thailand, pp. 132-151.

Kao, S. S., C. Y. Hsieh, and Y. S. Tsai. 2000. An oil-based recovery system for separation and concentration of lipophilic fungal spores and their metabolites (in Japanese) (Japanese patent No.344222).

Kao, S. S., C. Y. Hsieh, and Y. S. Tsai. 2002. An oil-based recovery system for separation and concentration of lipophilic fungal spores and its metabolites (In Chinese) (ROC patent No.166820).

Kao, S. S., C. Y. Hsieh, Y. S. Tsai, and D. C. Hsu, 2004. Solid-state fermenter (In Japanese) (Japanese patent No.3100300).

Kao, S. S., F. C. Hsieh, C. C. Tzeng, and Y. S. Tsai. 2003. Cloning and expression of the insecticidal crystal protein gene *Cry*1Ca9 of *Bacillus thuringiensis* G10-01A from Taiwan granaries. Current Microbiol. 47: 295-299.

Kao, S. S., L. H. Huang, Y. S. Tsai, and S. J. Tuan. 1997. Implementation of control program against *Spodoptera exigua* with NPV in green onion field. The 3rd Asian-Pacific Conference of Entomology. Nov. 16-22, 1997. Taichung, Taiwan, ROC. p. 167.

Kao, S. S., S. J. Tuan, and S. L. Hsuan. 1994. A plasmid profile technique for characterizing *Bacillus thuringiensis* subspecies and strains. Plant Prot. Bull. 36: 155-160. (In Chinese with English summary)

Kao, S. S., T. R. Jinn, and C. H. Wang. 2000. Detection and identification of five nucleopolyhedrosisvirus islates from Taiwan by PCR-RFLP. Chinese J. Entomol. 20: 23-35. (In Chinese with English summary)

Kao, S. S., W. T. Hsia, and L. H. Huang. 1991. Effectiveness of adjuvants for nuclear polyhedrosis virus against the beet armyworm, *Spodoptera exigua*. Chinese J. Entomol. 11: 330-334. (In Chinese with English summary)

Kao, S. S., W. T. Hsia, and L. H. Huang.1991. Effectiveness of adjuvants for nuclear polyhedrosis virus against the beet armyworm, *Spodoptera exigua*. Chinese J. Entomol. 11: 330-334. (In Chinese with English summary)

Kao, S. S., Y. S. Tsai, and C. W. Kao. 1989. Green muscardine fungus, *Metarhium anisopliae* var. *anisopliae,* a natural enemy of *Spodoptera exigua* on green onion. Special Report, Taiwan Agricultural Chemicals and Toxic Substances Research Institute No. 11. 5pp. (In Chinese)

Kao, S. S., Y. S. Tsai, and S. Y. Lin. 2006. A liquid inoculation system and method for automatic mass production of microorganisms. (ROC Patent NO. I265973)

Kao, S. S., Y. S. Tsai, P. S. Yang, B. C. Wang, and J. Hua. 1998. User's guide of commonly-occurred entomopathogenic fungi in Taiwan. Mycological Monograph No. 12. Food Industry Research and Development Institute, Hsinchu, Taiwan, ROC. 120pp. (In Chinese with English summary)

Kao, S. S., Y. S. Tsai, P. S. Yang, T. H. Hung, and J. L. Ko. 2002. Use of random amplified polymorphic DNA to characterize entomopathogenic fungi, *Nomuraea* spp., *Beauveria* spp., and *Metarhizium anisopliae* var. *anisopliae*, from Taiwan and China. Formosan Entomol. 22: 125-134. (In Chinese with English summary)

Kuo, W. S., and K. F. Chak. 1996. Identification of novel *cry*-type genes from *Bacillus thuringiensis* strains on the basis of restriction fragment length polymorphism of the PCR-amplified DNA. Appl. Environ. Microbiol. 62: 1369-1377.

Lee, C. S. 1993. In vitro assay of *Bacillus thuringiensis* subsp. *kursfaki* HD-1 δ- endofoxin using a *Plufella xylostella* cell line. M.S. Thesis, National Chung Hsing University, 49pp. (In Chinese with English summary)

Lee, P. C., and R. F. Hou. 1989a. Pathogenesis of *Metarhizium anisopliae* var. *anisopliae* in the smaller brown planthopper, *Laodelphax striatellus.* Chinese J. Entomol. 9: 13-19. (In Chinese with English summary)

Lee, P. C., and R. F. Hou. 1989b. Factors affecting pathogenicity of *Metarhizium anisopliae* var. *anisopliae* to the smaller brown planthopper, *Laodelphax striatellus.* Plant Prot. Bull. 31: 1-9. (In Chinese with English summary)

Lee, P. C., and R. F. Hou. 1989c. Studies on culturing and mass production of the green muscardine fungus, *Metarhizium anisopliae* var. *anisopliae*. Plant Prot. Bull. 31: 10-20. (In Chinese with English summary)

Lee, S. T. 1996. Enchancing factors of nuclear polyhedrosis virus of *Spodoptera litura* and Quantitation of *Plutella xylostella* granulosis virus. M.S. Thesis, National Taiwan Univeristy, 67pp. (In Chinese with English summary)

Li, L. 1991. Characterization and cloning of an insecticidal crystal protein gene from *Bacillus thuringiensis* YMB 96 strain isolated from Taiwan. M.S. Thesis, National Yang-Ming Medical College, 92pp. (In Chinese with English summary)

Lin, C. H., C. C. Tzeng, S. S. Kao, and L. J. Chen. 2003. Insecticidal efficacy and phytopathogenic antagonism of the epiphytic *Erwinia herbicola* strains transformed with the *Bacillus thuringiensis cry*1Aal gene. J. Agri. Assoc. China 4: 17-29. (In Chinese with English summary)

Lin, C. H., W. F. Hsiao, and R. F. Hou. 1998. Effects of fungicides and insecticides *in*

vitro on the vegetative growth and sporulation of the entomopathogenic fungus, *Verticillium lecanii.* VII the International Colloquium on Invertebrate Pathology and Microbial Control. Sapporo, Aug. 23-28. 1998. p. 29.

Lin, M. S., Y. H. Hsieh, S. S. Kao, and C. Y. Hsieh. 2003. Medium optimization for mycelium production of *Nomuraea rileyi* using response surface methodology. Taiwanese J. Agricul. Chem. Food Science 41(6): 444-450.

Lin, R. W. 1995. Construction of recombinant beculovirus containing melanin gene and the preliminary molecular studies on *Spodoptera litura* nuclear polyhedrosis virus. M.S. Thesis, National Taiwan University. 61pp.

Liu, B. L., and Y. H. Tzeng . 2000. Characterizaion study on the sporulation process of *Bacillus thuringiensis* Biotechnol. Bioeng. 68: 11-17.

Liu, B. L., and Y. M. Tzeng . 1998. Optimization of growth medium for the production of spores from *Bacillus thuringiensis* using response surface methodology. Bioproc. Eng. 18: 413-418.

Liu, S. D. 1996. The application of fungicide resistant entomopathogenic green muscardine fungus in Taiwan: Biological control of coconut leaf beetle (*Brontispa longissima*) and diamondback moth (*Plutella xylostella*). *In*: Biological Pest Control in Systems of Integrated Pest Management. FFTC. Book Series No. 47. Food & Fertilizer Technology Center. Taipei, ROC. pp. 154-163.

Liu, S. D., S. C. Lin, and J. F. Shiau. 1989. Microbial control of coconut leaf beetle (*Brontispa longissima*) with green muscardine fungus, *Metarhizium anisopliae* var. *anisopliae*. J. Invertbr. Pathol. 53: 307-314.

Lo, C. C., J. S. Hwang, S. C. Lee, and F. C. yen. 1989. Evaluation of sex hperomones formulation and development of pheromone-based trapping system for control of some important insects in Taiwan. In: Phytochemical Ecology; Alleochemicals, Mycotoxins and Insect Pheromones and Allomones. C. H. Chou and G. R. Waller （Eds.）.Institute of Botany Academia Sinica Monograph Series No. 9., Taipei, ROC, pp. 443-453.

Lo, C. C., M. D. Hung, and C. J. Lin. 1992. Replacement of carcinogenic alkylating agent ethylene oxide in the synthesis of （Z）3 dodecen-1-yl（E）-2-butenoate, sex pheromone of sweet potato weevil, C*ylas formicarius elegantulus*（Summers）and *Cylas formicarius*（F.）.*Journal of Chemical Ecology* 18: 95-103.

Lo, C. C., M. D. Hung, J. S. Hwang, and C. C. Hung. 1998. An improved Method synthesize the major component of sex pheromone of *Spodoptera litura* F. Journal of the Chinese Agricultural Chenical Society 26: 536-544.

Matten, E. R., E. A. Milewski, W. R. Schneider, and B. I. Slutsky. 1993. Biological pesticides and U. S. Environmental Protection Agency. *In*: Advanced Engineered Pesticides L. Kim (Ed.). Marcel Dekker. New York. pp. 321-350.

Peng, B. C. 1985. Pathological study of entomogenous fungus, *Verticillium lecani*i (Zimm.) Viegas in aphid. M.S. Thesis, National Chung Hsing University. 45pp. (In

Chinese with English summary)

Shih, C. H., Y. S. Tsai, and S. S. Kao. 1998. Characterization of isolates of entomopathogenic fungi *Beauveria* spp. by PCR-RFLP. Parper Presented at Annual Meeting of the Entomological Society of the ROC. (In Chinese).

Shih, C. I., and S. P. Shiue. 1994. Morphology and life history of *Neozygites* cf. *adjarica*, an entomopathogenic fungus parasitized on the two spotted spider mites, *Tetranychus urticae* in Taiwan. Chinese J. Entomol. 14: 319-330. (In Chinese with English summary)

Starnes, R. L., C. L. Lin, and P. G. Marrone. 1993. History, use and future of microbial insecticides. American Entomologist 39: 83-91.

Su, C. Y. 1988. The effect of certain pesticides on *Beauveria bassiana.* Chinese J. Entomol. 8: 157-160. (In Chinese with English summary)

Su, C. Y. 1989. The evaluation of granuiosis and nucler polyhedrosis viruses for control of three lepidopterous insect pests on cruciferous vegetables. Chinese Journal of Entomology 9: 189-196. (In Chinese with English summary)

Su, C. Y. 1991a. Field application of *Beauveria bassiana* for control of weet potato weevil, *Cylas formicarius.* Chinese J. Entomol. 11: 162-168. (In Chinese with English summary)

Su, C. Y. 1991b. Field trials of granulosis virus and *Bacillus thuringiensis* for control of *Plutella xylostella* and *Artogeia rapae.* Chinese J. Entomol. 11: 174-178. (In Chinese with English summary)

Su, C. Y. 1991c. Screening of soil pernicious to sweet potato weevil, *Cylas formicarius*, and use of *Beauveria bassiana.* Chinese J. Entomol. 11: 198-203. (In Chinese with English summary)

Su, C. Y. 1992. Use of nuclear polyhedrosis virus for control of *Spodoptera litura*. Plant Protect. Bull. 34: 227-234 (In Chinese with English summary)

Su, C. Y., S. S. Tzean, and W. H. Ko. 1988. *Beauveria bassiana* the lethal factor in a Taiwanese soil pernicious to sweet potato weevil, *Cylas formicarius.* J. Invertbr. Pathol. 52: 195-197.

Sun, C. N. 1992. Insecticide resistance in diamondback moth. *In*: Diamondback Moth and Other Crucifer Pests. Proceedings of the Second International Workshop. N. S. Talekar [ed.], Asian Vegetable Research and Development Center, Tainan, Taiwan, ROC. pp. 419- 426.

Tang, H. C. 1998. Studies on infection of *Spodopera exigua* with the entomopathogenic fungus, *Nomuraea rileyi.* M.S. Thesis, National Chung Hsing University. 55pp. (In Chinese with English summary)

Tang, L. C. 1996. Infection of the corn earworm, *Heliocoverpa annigera,* with entomopathogenic muscardine fungus, *Nomuraea rileyi,* isolated from Taiwan. Dissertation, National Chung Hsing University. 147pp. (In Chinese with English summary)

Tang, L. C., and R. F. Hou. 1998. Potential application of the entomopathenic fungus, *Nomuraea rileyi*, for control of the corn earworm *Heliocoverpa armigera.* Entomologia Experimentalis et Applicata 88:25-30.

Tang, L. C., and R. F. Hou. 2001. Effects of environmental factors on virulence of the entomopathogenic fungus, *Nomuraea rileyi*, against the corn earworm, *Helicoverpa armigera* (Lep., Noctuidae). J. Appl. Entomol. 125: 243-248.

Tang, L. C., D. J. Cheng, and R. F. Hou. 1999. Virulence of the entomopathogenic fungus, *Nomuraea rileyi*, to various larval stages of the corn earworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). Appl. Entomol. Zool. 34: 243-248.

Tsai, Y. S. 2004. Factors affecting application of *Verticillim lecanii* for insect control. Dissertation, National Taiwan University. 145pp (In Chinese with English summary)

Tsai, Y. S., C. W. Kao, R. F. Hou, and S. S. Kao. 1993a. Screening of fungicide-resistant isolates of *Metarhizium anisopliae* var. *anisopliae.* Chinese J. Entomol. 13: 45-47. (In Chinese with English summary)

Tsai, Y. S., R. F. Hou, S. S. Kao, and C. W. Kao. 1994. Effects of fungicides, commonly used in green onion field, on infection of the green muscardine fungs, *Metarhizium anisopliae* var. *anisopliae* against *Spodoptera exigua*. Plant Prot. Bull. 36: 239-246. (In Chinese with English summary)

Tsai, Y. S., S. S. Kao, C. W. Kao, and R. F. Hou. 1993b. Effect of insecticide on the virulence of the green muscardine fungus, *Metarhizium anisopliae* var. *anisopliae*, against the beet armyworm, *Spodoptera exigua.* Plant Prot. Bull. 34: 216-226. (In Chinese with English summary)

Tuan, S. J., and R. F. Hou. 1988. Enhancement of nuclear polyhedrosis virus infection by lecithin corn earworm, *Heliothis armigera.* J. Invertbr. Pathol. 52: 180-182.

Tuan, S. J., L. C., Tang, and R. F. Hou, 1989b. Factors of affecting pathogenicity of NPV preparations to the corn earworm, *Heliothis armigera.* Entomophaga 34: 541-549.

Tuan, S. J., L.C. Tang, and R. F. Hou. 1989a. Control of *Heliothis armigera* in maize with a nuclear polyhedrosis virus. Appl. Entomol. Zool. 24: 186-192.

Tuan, S. J., S. L. Hsuan, and S. S. Kao. 1993. Enzyme-linked immunosorbent assay for measuring the potency of *Bacillus thuringiensis* products. Plant Prot. Bull. 35: 1-9. (In Chinese with English summary)

Tuan, S. J., S. S. Kao, and D. .J. Cheng . 1994. Histopathology and pathogenicity of *Spodoprera exigua* nuclear polyhedrosis virus isolated in Taiwan. Chinese J. Entomol. 14: 33-45. (In Chinese with English summary)

Tuan, S. J., S. S. Kao, and U. L. Leu . 1995a. Factors affecting pathogenicity and stability of *Spodoprera litura* nuclear polyhedrosis virus isolated in Taiwan. Chinese J. Entomol. 15: 47-58. (In Chinese with English summary)

Tuan, S. J., S. S. Kao, U. L. Leu, and D. J. Cheng. 1995b. Pathogenicity and propagation of *Spodoptera litura* nuclear polydedrosis virus in Taiwan. Chinese J. Entomol. 15: 19-33. (In Chinese with summary)

Tuan, S. J., S. S. Kao, Y. C. Chao, and R. F. Hou. 1997. Investigation of pathogenicity of AcMNPV to nine lepidopteran pests in Taiwan. Chinese J. Entomol. 17: 209-225. (In Chinese with English summary)

Tuan, S. J., W. L. Chen, and S. S. Kao. 1998. *In vivo* mass production and control efficacy of *Spodoptera litura* (Lepidoptera: Noctuidae) nucleopolyhedrovirus. Chinese J. Entomol. 18: 101-116. (In Chinese with English summary)

Tzean, S. S., L. S. Hsieh, , and W. J. Wu. 1997. Atlas of entomopathogenic fungi from Taiwan. Council of Agriculture, Executive Yuan, Taiwan, ROC. 214 pp.

Tzeng, C. C., S. S. Kao, L. J. Chen, K. F. Chak, and R. F. Hou. 2002. Clonong of *cry*1Ac gene in a *Bacillus thuringiensis* isolate from Taiwan. Plant Prot. Bull. 44: 185-208. (In Chinese with English summary)

Tzeng, D. D. S., and Y. Yeh. 2003. Research and development of microbial biofungicides for plant disease control. Plant Prot. Bull. Spec. New 5 (Proceedings of the workshop on Plant Protection Management for Sustainable Development: Technology and New Dimension): 221-228. （In Chinese with English summary）

Tzeng, D. D. S., W. D. Huang, W. R. Lai, S. S. Chu, and S. W. Wang. 2004. The development of probiotic biofungicides for plant disease control. International Symposium on Future Development of Agricultural Biotechnology Park. pp. 362-369. Nov. 18-20, 2004. National Pingtung University of Science and Technolgy.

Yang, C. C., I. H. Pan, M. H. Chen, S. S. Kao, and Y. S. Tsai. 1994. Method for the preparation of a biocidal formulation containing starch and *Bacillus thuringiensis*, US patent number 5,352,579.

Yang, C. C., I. H. Pan, M. H. Chen, S. S. Kao, and Y. S. Tsai. 1995. Anti-ultraviolet biocidal composition. US patent number: 5,427,787.

Yen, D. F. 1977. Microbial control of insect pests in Taiwan. Phytopatho. Entomol. (National Taiwan University). 5: 1-14.

Yen, Y. P. and J. S. Hwang. 1990. Improved synthesis method and bioactivity sex pheromone of sweet popato weevil, *Cylas formicarius elegantulus*. Plant Protection Bulletin 32: 239-241. （In Chinese with English summary）

Yen, Y. P., J. S. Hwang , and C. C. hung . 2004. Development and application of some novel sex pheromones and attractants in Taiwan. Plant Prot. Bull. Spec. Publ. New 6 （Proceedings of the Symposium of Innovative Strategy on Plant Protection Agricultural Productivity, Product Safety and Environmental Protection）: 87-106. （In Chinese with English summary）