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THE APPLICATION OF FUNGICIDE RESISTANT ENTOMOPATHOGENIC GREEN MUSCARDINE FUNGUS IN TAIWAN: Biological Control of Coconut Leaf Beetle (*Bronstispa longissima*) and Diamondback Moth (*Plutella xylostella*)

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FOREWORD

There is a growing interest in the use of biological methods rather than toxic chemicals to control insect pests. This important Bulletin looks at the use of a green muscardine fungus which is entomopathogenic (pathogenic to insects) to control two important pests in Taiwan, coconut leaf beetle and diamondback moth. Since the fungicide strain originally isolated was sensitive to benomyl, a widely used fungicide, a mutated strain was produced. This was as virulent against the target insects as the original strain, but was resistant to benomyl.

The mutated strain, called MA-126, has been mass produced and applied to coconut trees for some years in Taiwan. It has caused a significant drop in populations of coconut beetle. It is also giving good control of diamondback moth in greenhouses, when applied together with low doses of chemical insecticides. The paper was first presented by the author at an international seminar on the "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.

THE APPLICATION OF FUNGICIDE RESISTANT ENTOMOPATHOGENIC GREEN MUSCARDINE FUNGUS IN TAIWAN: Biological Control of Coconut Leaf Beetle (*Bronstispa longissima*) and Diamondback Moth (*Plutella xylostella*)

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(Chinese Abstract)

摘　　要

許多本土分離之黑殭菌（*Metarhizium anisopliae* var. *anisopliae*）也可從自然被感染的可可椰子紅胸葉蟲體內分離出來。其中MA-1分離體對鞘翅目及鱗翅目昆蟲具有很強的致病性，但對氨基甲酸鹽類殺菌劑很敏感。利用紫外光及化學藥劑的誘變處理，可以得到抗免賴得之MA-126分離體，其對標的昆蟲的毒性程度與親本相同。穀類、大豆和一些農業副產品如米糠及稻桿浸出液可用爲添加物，製成固態醱酵培養基以大量生產孢子；酵母菌浸出液、葡萄糖液和Sabouraud液則用爲生產菌絲之深層醱酵培養基。許多用來製備以孢子和菌絲爲主的真菌型殺蟲劑中之分散劑，保護劑和惰性載體，則分析其對孢子發芽及成活的影響。1986至1988年在臺灣南部進行兩個以MA-1和MA-126孢子製劑之小規模田間噴施試驗，會顯著降低被感染之椰子樹上的可可椰子紅胸葉蟲的族群。目前利用這類生物防治推廣計劃，已防治了80,000株以上的椰子樹免於蟲害。小菜蛾（*Plutella xylostella*）爲臺灣田間及溫室栽培十字花科蔬菜的主要害蟲，但接種每毫升10^6孢子濃度的MA-126懸浮液後三日，有90%以上的小菜蛾幼蟲死亡。當真菌菌絲經由體表氣孔侵入蟲體後，孢子發芽管會直接貫穿蟲體。目前在臺灣小菜蛾綜合防治推廣計劃下，有120種蔬菜的網室栽培是利用MA-126可濕性粉劑和液劑劑型，配合低劑量的化學藥劑來防治小菜蛾。

(Japanese Abstract)

摘　　要

緑きょう病菌、*Metarhizium anisoplia var. anisopliae*、に自然感染をしているココナツハムシから分離した系統のなかのMA-1は鞘翅目、鱗翅目に強い毒性を示すが、バノミル感受性が高い。紫外線および化学処理によって得られたMA-126は、バノミル抵抗性で毒性はMA-1と同等である。胞子を大量増殖するため米、大豆、こめぬか、とき汁などを使って発酵培地を作成した。菌糸体の生産培地にはイースト抽出液、ブドウ糖、サブロー液状培地をつかった。また製剤に使うキャリヤーなどの補助剤による分生胞子への影響も調べた。MA-1およびMA-126の分生胞子の製剤を使った小規模の野外試験をココナツハムシならびにコナガを対象に行った。その結果にもとずき、現在8万本のココナツの防除を実施している。コナガ幼虫にMA-126の懸濁液（分生胞子濃度$10^{\ }$/ml）を散布した場合3日後で90%の死亡率が得られた。分生胞子の発芽管は昆虫体に直接侵入するが、菌糸体は気門から侵入する。台湾ではコナガの総合管理の一環として120のハウスでMA-126が低濃度の殺虫剤と共に水和剤もしくは液剤の形でつかわれている。

(Korean Abstract)

초 록

녹강균(*Metarhizium anisopliae* var. *anisopliae*)의 국내종 분리주 몇가지가 자연감염된 야자수잎 해충(잎벌레 종류)으로부터 분리되었다. 그중에서 분리주 MA－1은 딱정벌레목과 나비목 곤충에 매우 강한 병원성을 지니나, benzimidazole carmarbate 살균제에는 감수성이다. 자외선이나 화학적 돌연변이 처리를 하면 어버이 분리주와 같은 수준에서 대상 곤충에 악성으로 작용하는 benomyl 저항성 분리주 MA－126이 얻어진다. 쌀, 콩 그리고 쌀기울, 쌀뜨물과 같은 농산부산물 등이 포자의 대량생산을 위한 고형배지의 첨가제로 사용되었다. Yeast extract, dextrose, sabouraud broth 등이 균사생산을 위한 영양제로 이용되었다. Fungal insecticide preparation의 포자와 균사 base를 제제화하기 위한 포자 발아와 생존력에 관한 영향을 알기 위하여 dispersents, protectants, inert carriers 등이 시험되었다. 대만 남부에서 야자수잎 해충(*Brontispa longissima*)에 감염된 coconut palm trees에 MA－1과 MA－126 conidial base preparation을 2개의 소규모 시설에 접종한 결과 1986～1988의 기간에 leaf beetle의 밀도를 상당히 감소시켰다. 80,000그루 이상의 야자수가 이러한 생물적 방제 지도사업에 의해 보호되었다. 배추좀나방(*Plutella xylostella*)은 대만의 야외, 온실이나 방실에서 십자화과 식물에 대한 매우 주요한 해충이다. MA－126 suspension을 10^7 conidia/ml의 농도로 접종하였을 때 배추좀나방의 유충사망률은 3일 후 90% 이상을 보였다. 진균의 균사가 곤충 기문의 표면을 통하여 침입하는 반면에, 포자의 germ tube는 곤충체내로 직접 관통한다. 배추좀나방 종합관리 지도프로그램에 따라 대만 전역의 120개 채소생산 방실에서 배추좀나방을 방제하기 위하여 MA－126수화제와 liquid formulated preparation을 낮은 양의 화학살충제와 같이 살포하였다.

THE APPLICATION OF FUNGICIDE RESISTANT ENTOMOPATHOGENIC GREEN MUSCARDINE FUNGUS IN TAIWAN: BIOLOGICAL CONTROL OF COCONUT LEAF BEETLE (*BRONSTISPA LONGISSIMA*) AND DIAMONDBACK MOTH (*PLUTELLA XYLOSTELLA*)

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ABSTRACT

Several domestic isolates of green muscardine fungus, Metarhizium anisopliae var. anisopliae, were isolated from naturally infected coconut leaf beetles. Among them, isolate MA-1 had strong pathogenicity against Coleoptera and Lepidoptera insects, but was sensitive to benzimidazole carmarbate fungicide. Using ultraviolet and chemical mutation treatment, a benomyl resistant isolate MA-126 was obtained, with a target insect virulence at the same level as its parent isolate. 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. Several dispersents, protectants, and inert carriers were assayed for their influences on conidial germination and viability in order to formulate the conidial and mycelial base of the fungal insecticide preparation. Two small-scale field applications of MA-1 and MA-126 conidial base preparation to coconut palm trees infested with coconut leaf beetle (Brontispa longissima) in southern Taiwan significantly reduced leaf beetle populations in the period 1986 to 1988. Over 80,000 coconut trees were protected under this biocontrol extension program. Diamondback moth (Plutella xylostella) is a major pest of crucifer vegetables in both the open field and greenhouses and nethouses in Taiwan. The larval mortality of the diamondback moth is 90% three days after inoculating MA-126 suspension at a concentration of 10^7 conidia per ml.. The conidia germ tubes penetrated directly into the insect body, while the fungal mycelia invaded the insect through the surface spiracle. The MA-126 wettable powder and liquid formulated preparations were applied with low dosages of chemical insecticide to control diamondback moth in 120 vegetable production net houses over Taiwan under an extension program for the integrated management of diamondback moth.

INTRODUCTION

This paper summarizes the first attempts and acnievements in the biological control of coconut leaf beetle and diamondback moth by the application of entomopathogenic fungus in Taiwan. Coconut leaf beetle (CLB), *Brontispa longissima* (Chrysomelidae), was first found to be a coconut pest in Taiwan in July 1975 and since then has spread widely and rapidly, causing severe damage and loss (Chen 1976). A pupal parasitoid, *Tetrastichus brontispae*, was successfully introduced and liberated for biological control of CLB in Taiwan (Chiu *et al.* 1985), but parasitoid populations were difficult to establish in coastal areas and in poorly sterilized plantations. The use of microbial agents such as baculovirus and green muscardine fungus for controlling coconut palm rhinoceros beetle and other coconut palm

Keywords: biological control, *Brontispa longissima*, coconut leaf beetle, diamondback moth, green muscardine fungus, isolate MA-1, isolate MA-126, *Metarhizium anisopliae*, *Plutella xylostella*, Taiwan

pests has been observed (Ferron *et al.* 1975, Young 1974), but no microbial control of CLB has yet been reported.

Diamondback moth (DBM), *Plutella xylostella*, one of the most damaging pests of crucifer vegetables, easily produces strains resistant to organic phosphorus, carbamate, synthetic pyrethrins, insect growth regulators and many other insecticides, making control very difficult (Hwang 1990, Talekar 1992). Although spraying *Bacillus thuringiensis* can obtain effective control, its high cost and low level of effectiveness due to high temperature and UV irradiation are its major handicaps. DBM resistance to chemical sprays, and the growing concern about risks to farmers, consumers and the environment, have prompted studies using entomopathogenic fungis as biocontrol agents as an alternative method for DBM management. Several microorganisms including viruses (granulosis virus and multiple-embedded nucleopolyhedrosis virus), bacteria (*Bacillus thuringiensis* (bt)), and fungi (*Zoophthora radicans*, *Erynia blunckii*, *Beauveria bassiana*, *B. brongnartii*, *Metarhizium anisopliae*, and *Paecilomyces fumoso-roseus*) were potentially effective as biocontrol agents against DBM (Wilding 1986). Most of the fungi were tested in the laboratory for their DBM infectivity, but were not tested in large-scale field application.

Screening for Benomyl Resistant Mutant of *M. anisopliae* var. *anisopliae* (MAA)

Two strains, MA-1 and MA-2, of MAA and one strain of *Beauveria bassiana* were isolated from naturally infected CLB. MA-1 was found to have the highest virulence, with 100% mortality on larvae, pupae and adults of CLB at an inoculation density of 10^7 conidia per mL (Table 1) (Liu *et al.* 1989). In testing for sensitivity to pesticides, MA-1 showed spore germination and mycelial growth was vulnerable in the presence of a low benomyl concentration (Fig. 1). Induction of fungicidal resistance was essential if the biocontrol agent was to survive with insect control effects under the stress of a fungicide applying environment. Using ultraviolet (UV) irradiation, chemical mutagens (Diethyl sulfate, ethyl methanesulfonate, and sodium nitrite) and treatments on conidial suspension prepared from MA-1 culture, several benomyl resistant mutants were obtained from ethyl methanesulfonate treatment (Fig. 2). Among these

Table 1. Virulence of two strains of *Metarhizium anisopliae* var. *anisopliae* and *Beauveria bassiana* against coconut leaf beetle, *Brontispa longissima*

	Mortality (%)											
	Larvae				Pupae				Adults			
Pathogen	10^7	10^5	10^3	0	10^7	10^5	10^3	0	10^7	10^5	10^3	0
M. anisopliae var. *anisopliae*												
MA-1	100^a	97^a	47^a	0	100^a	40^a	60^a	0	100^a	17^b	6^a	0
MA-2	97^a	76^b	23^b	0	60^b	29^b	15^b	0	52^b	13^b	13^a	0
B. bassiana	91^a	47^c	37^b	0	35^c	20^b	20^b	0	46^b	27^a	10^a	0

Note: The percentages of mortality in each vertial column with different letters are significantly different according to Duncan's multiple range test ($p<0.05$). The conidia of entomopathogenic fungi were harvested from Sabouraud dextrose agar plates, and incubated for 14 days at 28°C in adjusted conidia suspensions to three concentrations (2.17×10^7, $\times 10^5$, $\times 10^3$ conidia/mL) with 0.1% (v/v) Tween 20. Each treatment had three replicates. Each replicate had 50 insects in five Petrie dishes fed with young coconut leaves. After 8 days, the infected and dead insects were counted and the mortality of the insects was determined.

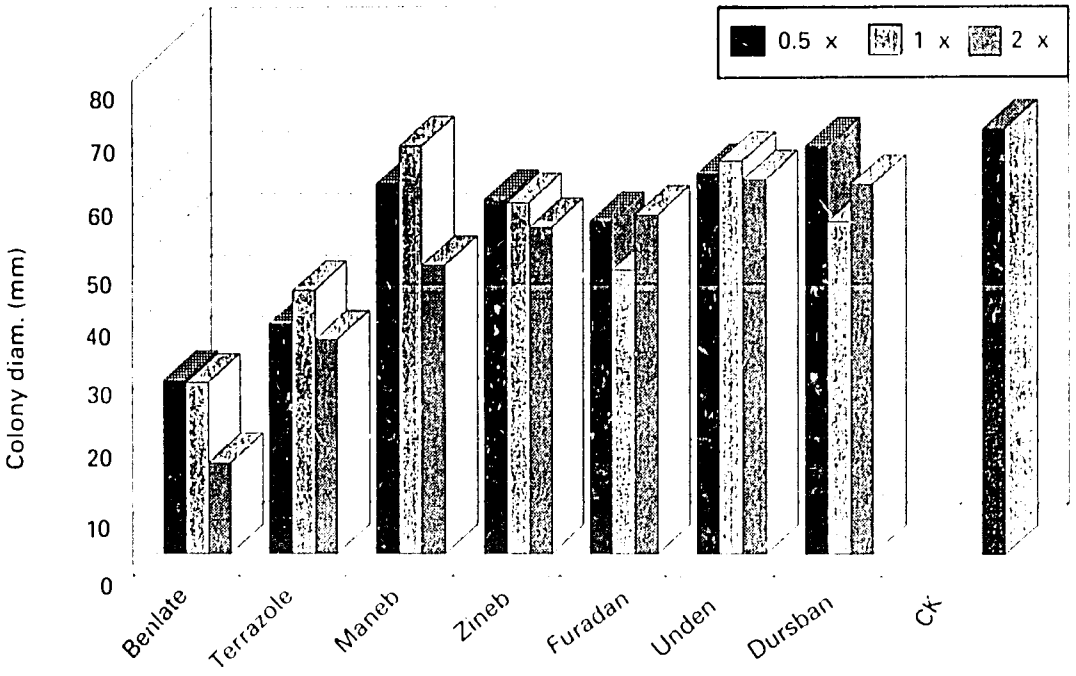

Fig. 1. The fungicidal effect of various pesticides on the mycelial growth of *Metarhizium anisopliae* var. *anisopliae*, MA-1. The mycelia disc (diam. 5mm) was inoculated at the center of a Saboraud dextrose agar plate containing various concentrations of pesticides (0.5, 1 and 2 x concentrations are recommended concentrations for field application). The colony diameter was measured after incubation at 28°C for 14 days.

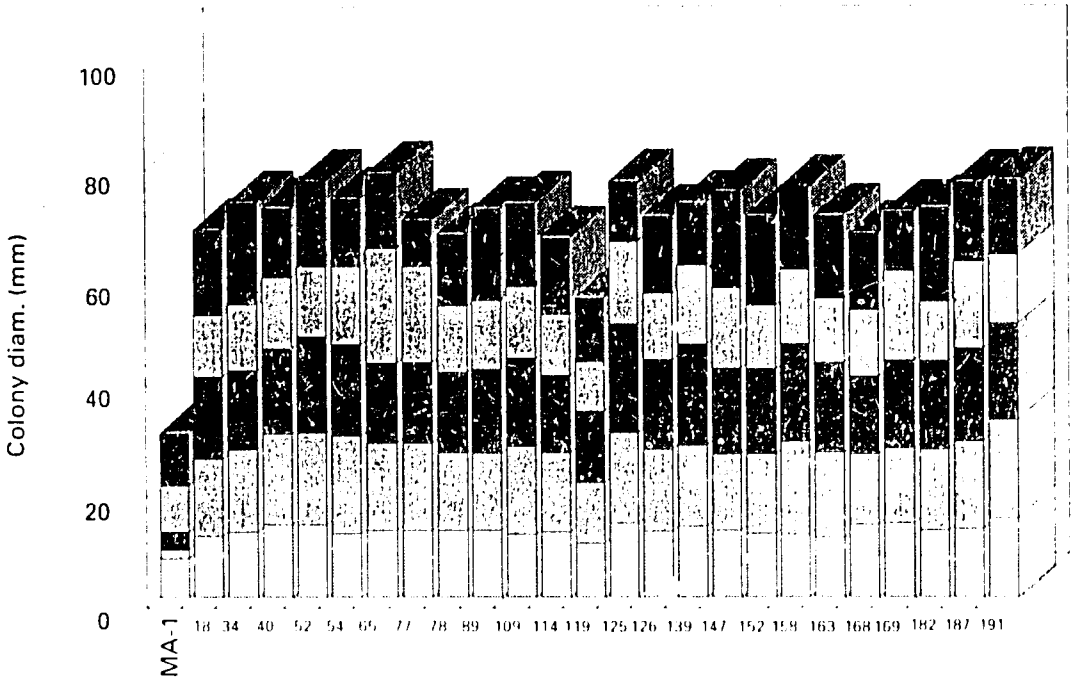

Fig. 2. The mycelial growth of benomyl resistant mutants of *M. anisopliae* var. *anisopliae*, MA-1, on Sabouraud dextrose agar plates containing 2000 ppm (a.i.) of benomyl. The colony diameters were measured after inoculating the mycelia disc (diam. 5mm) at the center of the plate and incubating it at 28°C for 14 days.

mutants or new biotypes, MA-126 was shown to be highly resistant to UV irradiation (at 150 uW per cm^2 254 nm wavelength) compared with the others (Table 2). While its fungicidal and UV irradiative resistance improved, the insecticidal pathogenicity of MA-126 was not reduced (Liu 1990).

Mass Production of MA-126 by Solid and Submerge Fermentation and Recovery System

Several substrates containing carbohydrates and proteins, such as rice, rice bran, soybean, wheat bran, rice steep, peptone and yeast extract, were used as the main ingredients or additives to formulate the culture media for solid or submerged fermentation. Polished rice grain with a moisture content of 43% to 45% (W/W) was inoculated with conidial suspension and incubated at 28 C for 12 days, producing 10^{10} conidia per g fresh biomass (Fig. 3). Complete sterilization by autoclaving, partial semi-sterilization by $KMnO_4$, benomyl and nonsterilization of culture media were compared for sporulation ability. Even the non-sterilized culture media produced 1.6 x 10^9 conidia per g fresh biomass at harvesting if the inoculum density was high enough to establish predominance (Table 3). Washing and then centrifuging, shaking through a fine screen, and floating drying methods were all tested for a satisfactory conidia harvesting recovery system. Floating and drying proved to be the most effective. Sabouraud broth was the best liquid media for MA-126 mycelia production. Paper filtration was applied for mycelia harvesting.

Formulation of Conidia and Mycelia Type Microbial Insecticide

Anionic, non-ionic and zwitter dispersants were tested to evaluate their effect on conidial germination. Chaps and Vannox 565 were found to be the proper additives for conidia dispersion (Table 4). Yeast extract

Table 2. Effect of UV irradiation on conidial germination of various mutant biotypes of *Metarhizium anisopliae* var. *anisopliae*

Biotypes	Conidial germination (%)								
	254 nm					366 (150 uw/cm^2)			
	0	10	20	30	60	10	20	30	60 (min)
MA-1	93.2^a	0^d	0^d	0.2^d	0^d	48.5^{bc}	59.5^b	55.9^{bc}	16.2^{cd}
MA-1-52	92.5^a	0.2^c	0^c	0^c	0^c	96.7^a	99.0^a	51.8^b	0^c
MA-1-54	76.1^a	0^c	0^c	0^c	0^c	46.3^b	38.9^b	0.7^c	0^c
MA-1-65	72.5^a	0^c	0^c	0^c	0^c	92.6^a	96.8^a	82.5^a	62.1^b
MA-1-109	69.2^a	0.5^c	0^c	0^c	0^c	68.3^a	32.4^b	51.1^{ab}	0.5^c
MA-1-126	90.2^{ab}	56.0^{bc}	30.1^c	0^d	0^d	94.1^a	94.5^a	84.0^b	33.1^c
MA-1-139	94.2^a	0^c	0^c	0.1^c	0^c	91.1^a	97.1^a	96.8^a	41.2^b
MA-1-158	95.4^a	0^c	0.1^c	0^c	0^c	65.9^b	72.0^b	57.6^b	36.7^c
MA-1-169	89.4^a	0^c	0^c	0^c	0^c	89.6^a	76.7^a	83.0^a	61.4^b
MA-1-182	58.9^a	0^c	0^c	0^c	0^c	32.1^{ab}	21.0^{bc}	10.4^c	0^c
MA-11-187	90.8^a	0^c	0^c	0^c	0^c	94.7^{ab}	89.8^{bc}	90.1^c	9.9^c

Note: Conidial suspensions (5×10^4 conidia/mL) were irradiated under 150 uw/cm^2 250 nm intensity wavelengths. Means in the same row with different letters were significantly different according to Duncan's multiple range test ($p = 0.05$).

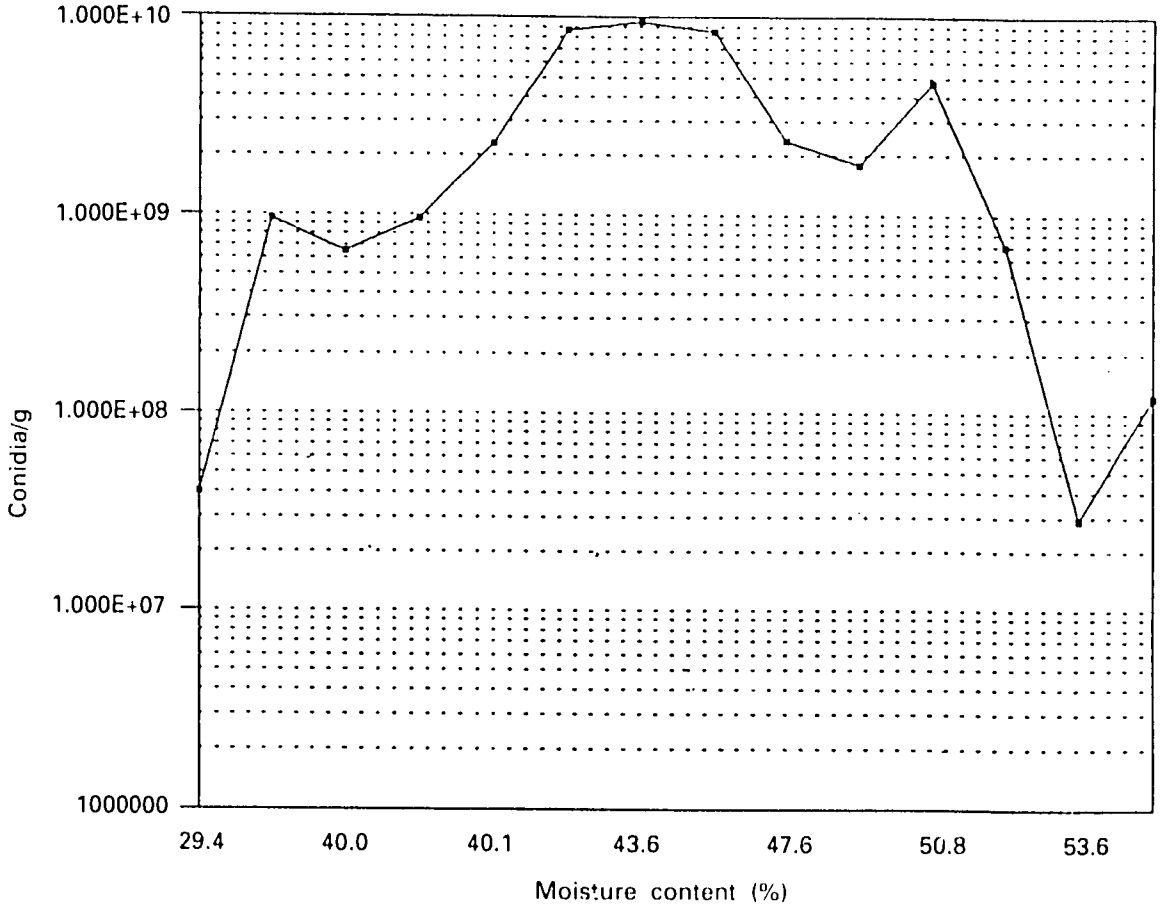

Fig. 3. The effect of moisture content of rice grain medium on the conidia production of MA-126. The rice grains were steamed for various periods to adjust the moisture content, then packed in autoclavable polypropane bags for sterilizing. After being inoculated with conidal suspension and incubated at 28°C for 12 days, the culture biomass was counted for sporulation.

Table 3. Effect of sterilization and inoculation methods on sporulation of *Metarhizium anisopliae* var. *anisopliae* in mass production

Sterilization	Inoculation	Conidial density (conidia/g)
Autoclaving	granule	3.3×10^9b
	suspension	9.8×10^9a
Semi-sterilizing w/KMn04, Benlate	granule	1.3×10^9d
	suspension	2.9×10^9bc
Non-sterilizing	granule	1.6×10^9d
	suspension	2.4×10^9c

Note: Means with different letters were significantly different according to Duncan's multiple range test (p=0.05)

Table 4. Effect of dispersant on conidial germination of *Metarhizium anisopliae* var. *anisoppliae*

Dispersant	Ion type	Conidial germination (%)				
		4%	2%	1%	0.5%	0.1%
Agral-90	non				67.8	86.8
PEG20000	non		45.9			88.7
Temol W	non & an	81.8			67.8	77.2
AG-WP	non & an					69.1
Zupol S95	an	0			0	0
Tensiofix BCE	an		0		0	0
Tensiofix LX	an		0		0	0
Vannox 565	an	38.1				86.7
Vannox 560	an					78.2
Chaps	Zwitter			47.6		92.4

Note: Conidial suspensions were mixed with dispersant at concentrations of 4% to 0.1% (w/v) and conidial germination was observed by the slide glass method after 24 hr incubation

Table 5. Effect of UV protectant on conidial germination of *Metarhizium anisopliae* var. *anisopliae*

UV protectant	Conidial germination (%)			
	0	5	10	15 (min)
3% peptone	100	100	95.0	83.3
3% yeast extract	100	100	99.0	90.0
3% beef extract	100	100	95.0	90.0
3% skim milk	95.0	88.5	37.9	25.5
1% tannic acid	78.3	0	0	0
1% nicotinic acid	65.4	66.1	62.4	48.6
1% para-amino-benzonic acid	99.0	0	0	0
1% d-cellobiose	99.0	74.6	1.7	0
Check	85.0	36.8	0	0

Note: Conidial suspension (5×10^3 conidia/mL) were irradiated under an intensity of 100 uv/cm^2 254nm wavelength.

and beef extract provided a better UV protection effect compared with peptone, skim milk, tannic acid, nicotinic acid, para-amino-benzonic acid and d-cellobiose (Table 5). Based on these data, several wettable powder and liquid formulations of the conidia (Daoust 1983) and mycelia type (Pereiva *et al* 1990, 1991) were made and their suspensibilities and conidial germinations were compared (Fig. 4). In addition, their packing and storage conditions were studied to compare the shelf life of the products. The conidial type wettable powder formulation product stored at 4°C appear had a better shelf life compared with storage at 28°C or mycelial type products.

The Biocontrol Effect of MA-126 Formulations on CLB and DBM

In 1988 a field trial was carried out in which microbial agents were applied to 120 coconut trees through liquid spraying of conidia suspension and solid granule treatment. The CLB population density decreased significantly after this treatment compared with

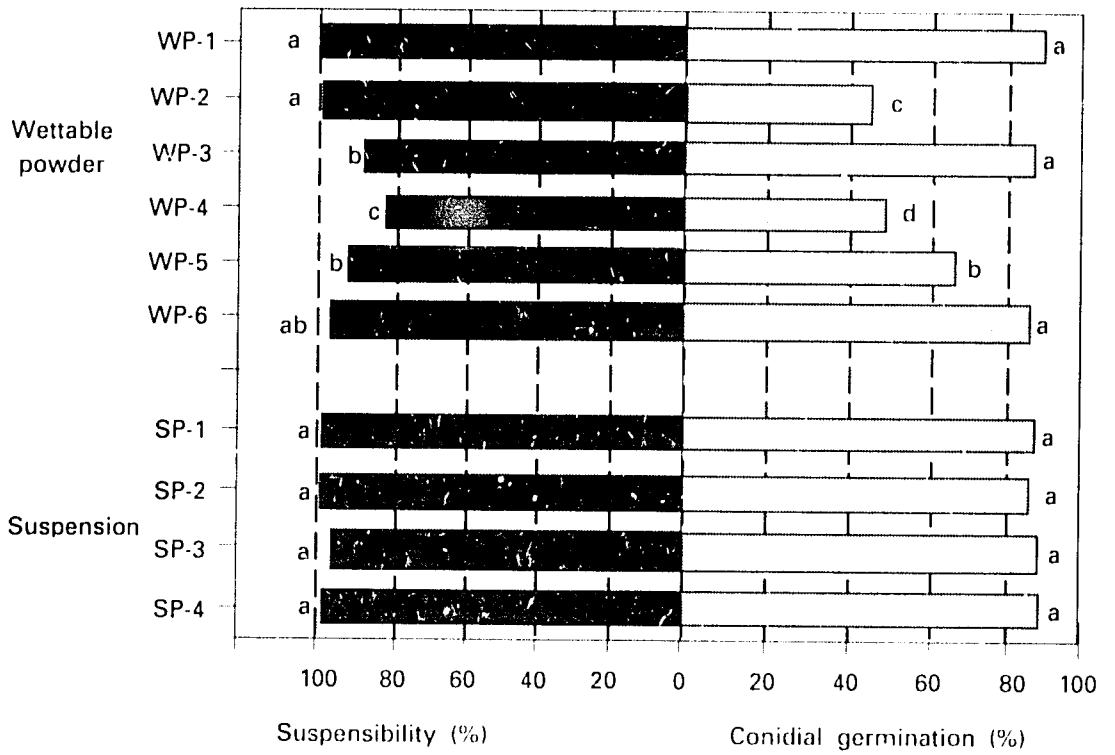

Fig. 4. The suspensibility and conidial germination of wettable powder and liquid formulated products of *M. anisopliae* var. *anisopliae* MA-126. WP-1 to WP-6 were wettable powders, SP-1 to SP-4 were liquid suspensions containing various kinds and concentrations of dispersents, UV protectants and inert carriers.

the control group. In the granule treatment group, CLB density was significantly lower than in the group that received spraying treatment (Table 6). After treatment, the new leaves grew without CLB damage, in contrast to the necrotic leaves of untreated trees. Since 1989, satisfactory biocontrol effects have been achieved through the application of liquid and wettable powder formulations of MA-126 to more than 80,000 coconut trees under the CLB integrated management extension program.

The mortality of DBM 4th instar larva treated by wettable MA-126 powder was significantly higher than those treated by MA-2, *Beauveria bassiana* (BB-1), or *Paecilomyces fumosus* (PF) (Liu *et al.* 1990), and the same as those treated by MA-1 (Fig. 5). Scanning electronic microscopic study showed that the mycelia of MA-126 penetrated through the spiracle of the DBM body surface; the new mycelia grew out from the spiracle after invasion and proliferation of the fungus (Fig. 6). MA-126 wettable powder and liquid formulations were applied to Chinese kale and Chinese mustard growing in net houses in the northern, central and southern regions of Taiwan to observe the biocontrol effect on DBM compared with traditional chemicals and bt spraying. Trials conducted in two successive years showed a rational control effect after fungal insecticide application. The DBM larval population remained at the same level after MA-126 treatment compared with bt treatment, and in some instances the levels were lower than after chemical treatment (Table 7). The MA-126 formulated products were distributed and applied by 120 vegetable growers growing pesticide-free vegetables in net houses as part of an extension program in 1992 and 1993.

Table 6. Biological control of coconut leaf beetle (*Brontispa longissima*) with *Metarhizium anisopliae* var. *anisopliae*

	Population per coconut palm tree								
	Spraying treatment			Granule treatment			CK		
	L	P	A	L	P	A	L	P	A
Before treatment	170.0	12.1	36.3	138.0	11.5	40.3	62.7	2.7	23.8
3 months after	32.4	0.9	15.1	10.6	0.0	10.1	69.0	9.1	30.9
7 months after	3.9	0.0	1.3	10.9	0.9	7.2	65.5	7.4	34.7
Change (%)	-97.7	-100	-96.4	-92.1	-92.2	-82.1	+4.6	+174	+45.8

Note: Conidia suspension with Tween 20 (10^{7} conidia/mL) were sprayed using spraying treatment. Granule application was 100-120g per tree of MA-1 formulated from rice grain medium. Each treatment was applied three times at 1-month intervals. Each treatment had four replicates, each replicate used 30 coconut trees.

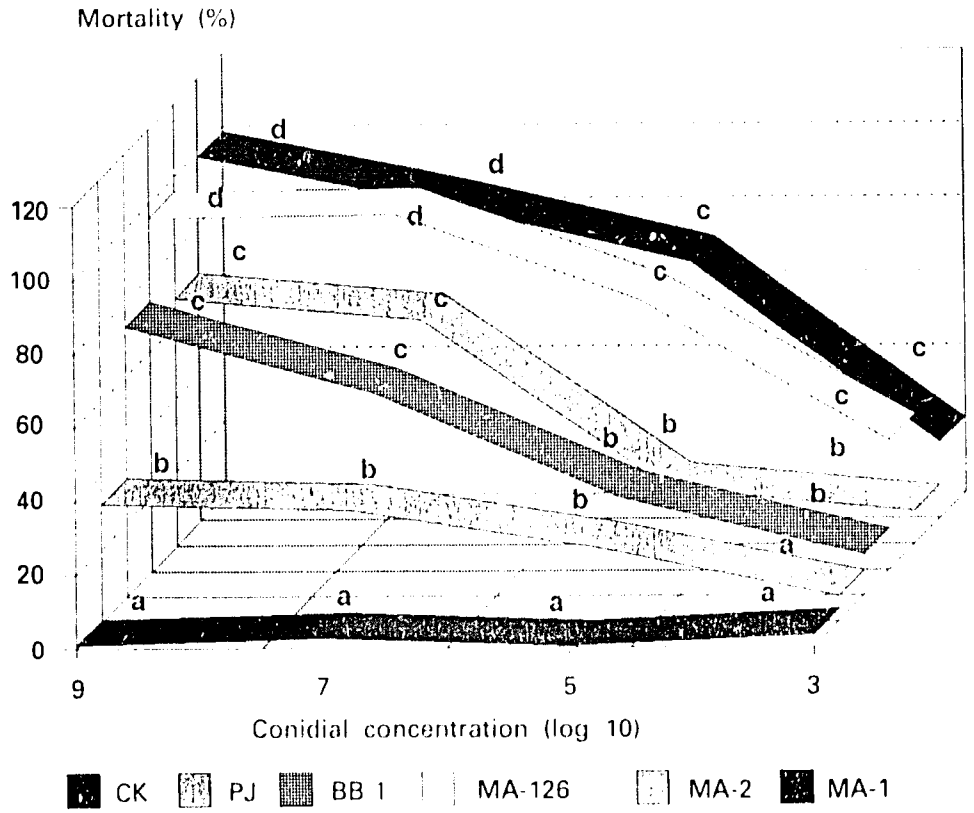

Fig. 5. The mortalities of 4th instar larva of diamondback moth (*Plutella xylostella*) treated by conidial suspensions of entomopathogenic fungi including *M. anisopliae* var. *anisopliae*, MA-1, MA-2, MA-126; *Beauveria bassiana*, BB-1; and *Paecilomyces javanicus*, PJ. The instar larva were sprayed with various concentrations of conidia suspension and fed on seedlings of Chinese mustard, then the mortalities counted after a three-day infection period.

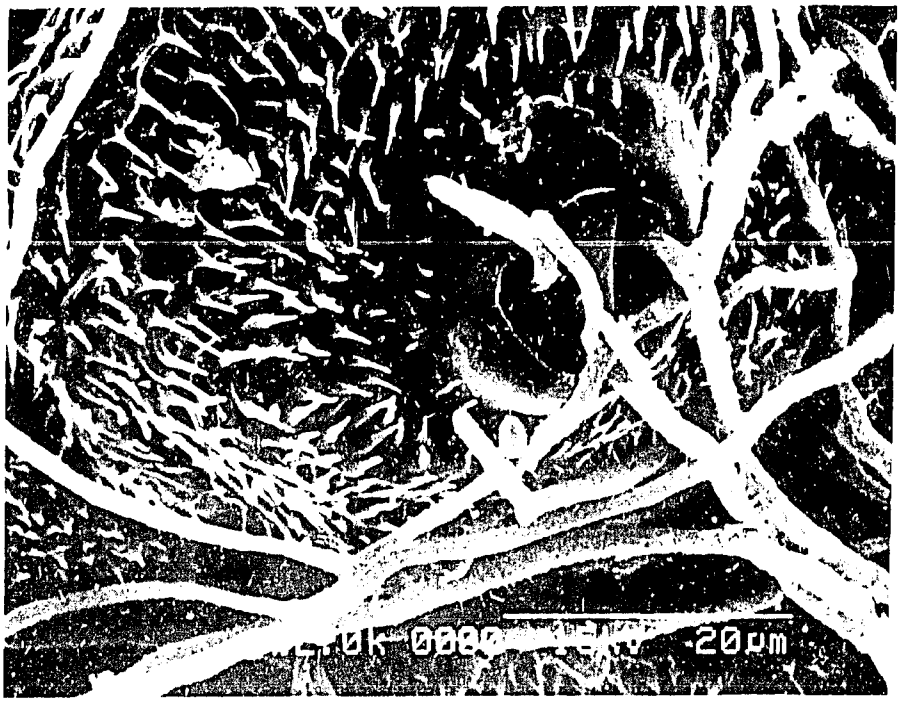

Fig. 6. The penetration and invasion of fungal mycelia of *Metarhizium anisopliae* var. *anisopliae*, MA-126, through the spiracle of the diamondback moth (*Plutella xylostella*).

Table 7. The biological control effect of wettable powder and liquid formulations of MA-126 (*Metarhizium anisopliae* var. *anisopliae*) on diamondback moth (*Plutella xylostella*) in crucifer vegetable production in net houses

Treatment	Larval population (no./30 plants)			
	plot 1	plot 2	plot 3	average
On Chinese kale (1991)				
Spraying with				
MA-126 liquid	2.0	5.2	1.0	2.7 b
MA 126 W.P.	1.6	4.5	1.7	2.6 b
BT	1.7	5.0	1.9	2.9 b
chemicals	3.2	10.1	6.6	6.6 b
Untreated	6.0	19.9	8.7	11.5 a
On Chinese mustard (1992)				
Spraying with				
MA 126 liquid	4.2	6.5	5.4	5.4 bc
MA 126 W.P.	3.5	3.0	2.8	3.1 c
BT	5.7	3.0	2.7	3.8 c
chemicals	6.2	7.9	7.8	7.3 b
Untreated	15.4	10.5	11.0	12.3 a

Note: Each treatment was sprayed at one week intervals. The conidial densities of spraying suspension were approximately $10^{[illegible]}$ conidia per mL when MA 126 W.P. was diluted 400X. Thuricide 1500X for BT treatment, cypermethrin 1500X and acephate 800X alternatively applied for chemical treatment. Each plot had 2 net houses (ca. 200 m²). Mean with different letters were significantly different according to Duncan's multiple range test.

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DISCUSSION

Dr. Liu was asked how often the green muscardine fungus was applied against diamondback moth. He replied that applications are made at seven-day intervals, the number of applications depending on the tuype of crop. For example, three to four applications are needed for Chinese cabbage. Dr. Kaya asked whether repeated applications of the fungus would produce the same kind of resistance in the diamondback moth that had occurred in the case of *Bacillus thuringiensis*. Dr. Liu felt that killing of insects by the fungus is a more complex process than infection with b.t.. The fungus has to penetrate the insect's body, but van also produce toxic enzymes against the insect. Perhaps for this reason, there is no record of any resistant strains.

One participant pointed out that when IPM programs are being planned, a fungicide's effect on natural enemies should be taken into account. He asked about the effect of green muscardine fungus on natural enemies. Dr. Liu replied that surveys taken in vegetable fields after application seemed to show that most natural enemies were not affected by the fungus.