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Greenhouse and Field Evaluation of a New Male Annihilation Technique (MAT) Product, SPLAT-MAT Spinosad ME™, for the Control of Oriental Fruit Flies (Diptera: Tephritidae) in Taiwan【Research report】

溫室及田間網籠評估新的滅雄劑型 SPLAT-MAT Spinosad ME™ 對東方果實蠅 (Diptera: Tephritidae) 的誘殺效果【研究報告】

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Abstract

The attraction and toxicity of a novel male annihilation technique (MAT) product, SPLAT-MAT Spinosad ME™ (SPLAT-Spin-ME), to the male of the oriental fruit fly (Bactrocera dorsalis) was evaluated under both greenhouse and field conditions in Taiwan. SPLAT-Spin-ME is a viscous, MAT formulation containing low doses of spinosad (2%) mixed with the powerful male-specific parapheromone attractant methyl eugenol (ME). The proprietary carrier formulation, SPLAT®, provides a metered release of the toxicant and the attractant into the environment. In greenhouse cage trials the attraction rates of B. dorsalis males for the two MAT treatments (SPLAT-Spin-ME, naled+ME) were equally high at 0, 1, and 2 weeks weathering time-points, and uniformly low for the two bait spray treatments (GF-120 bait spray, malathion protein bait spray). The mortality of B. dorsalis males in greenhouse cage studies for the two MAT treatments was highest with SPLAT-Spin-ME outperforming naled+ME at the 1 and 2 weeks weathering periods both at the 24 h and 48 h grading intervals, with neither bait spray treatments providing a substantial mortality. The results from the field cage studies were the reverse of the results from the greenhouse evaluations. In the field cage studies naled+ME (8 mL) maintained a consistent ca 70-76% attraction rate of up to 7 weeks, whereas SPLAT-Spin-ME (1 g) demonstrated a relatively rapid decline in attraction from an initial 51% to 32% and below starting at week 4. Based on a 48 h grading period, naled+ME generated 92-100% mortality in the field mortality bioassay at all weathering time-points from 0-8 weeks, whereas SPLAT-Spin- ME provided 88-100% mortality, but only through week 3. The markedly better performance of naled+ME in the field vs. the greenhouse bioassays is attributed to a rate change that took place and which dramatically increased the amount of ME attractant, relative to SPLAT-Spin-ME, used in the field studies, as well as the fact that naled+ME was infused into caneite blocks for the field bioassays which extended the residual of the ME attractant. Under these test conditions the overall efficacy of SPLAT-Spin-ME for controlling B. dorsalis males outdoors was around 3-4 weeks whereas naled+ME overall efficacy was around 8 weeks. Although apparently with a shorter residual than the naled+ME MAT standard, SPLAT-Spin-ME was shown to be a highly effective MAT treatment for controlling B. dorsalis and a viable alternative or replacement for current, more toxic MAT products.

摘要

在台灣利用溫室及田間網籠評估一種新的滅雄技術 SPLAT-MAT Spinosad ME™ (SPLAT-Spin-ME) 對東方果實蠅 (Bactrocera dorsalis) 雄蟲的誘殺效果。SPLAT-MAT Spinosad ME™ 是道禮農業科學公司提供的一種具黏性的滅雄製劑，其內含低劑量的賜諾殺 (有效成份 2%) 混合對東方果實蠅雄蟲具強吸引力的甲基丁香油 (50%)。以風化資材在溫室網籠比較 1 及 2 週誘殺率，其中二種滅雄試驗組 (SPLAT-Spin-ME, naled+ME) 的雄蟲誘殺率較高，不過二種誘餌試驗組 (GF-120 bait spray, malathion protein bait spray) 雄蟲誘殺率則很低。在溫室網籠 1 到 2 週的試驗中，24 及 48 小時的死亡率 SPLAT-Spin-ME比 naled+ME高，但差異不顯著。田間網籠的試驗結果卻相反。以 1 小時誘引率而言，naled+ME (8 mL) 在 7 週內的試驗期皆維持 70 ~ 76% 的誘引率，而 SPLAT-Spin-ME (1 g) 則從 51 到 32%，其中在第 4 週後開始下降。另外，48 小時誘殺率試驗中，naled+ME 在 8 週的試驗期間皆維持於92 ~ 100%的誘殺率，而 SPLAT-Spin-ME 僅於 3 週內維持 88 ~ 100% 的誘殺率。二種滅雄處理在田間及溫室網籠誘殺率相反的結果，主要是因 naled+ME 田間使用的量比 SPLAT-Spin-ME 來得大 (甲基丁香油的量差 15 倍)，且其吸附於蔗板中，能達到緩釋的效果。以本試驗的量，SPLAT-Spin-ME (1 g) 在戶外可維持大約 3 到 4 週，而 naled+ME (8 mL) 可以有 8 週的效果。雖然 SPLAT-Spin-ME 1 克的量比現行使用的含毒甲基丁香油 (蔗板吸附 8 ml) 的殘留時間較短，SPLAT-Spin-ME 仍是一個有效防治東方果實蠅且可替代目前較毒滅雄產品的製劑。

Key words: spinosad, naled, methyl eugenol, male annihilation, Bactrocera dorsalis

關鍵詞: 賜諾殺、乃力松、滅雄法、甲基丁香油、東方果實蠅。

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Greenhouse and Field Evaluation of a New Male Annihilation Technique (MAT) Product, SPLAT-MAT Spinosad ME™, for the Control of Oriental Fruit Flies (Diptera: Tephritidae) in Taiwan

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ABSTRACT

The attraction and toxicity of a novel male annihilation technique (MAT) product, SPLAT-MAT Spinosad ME™ (SPLAT-Spin-ME), to the male of the oriental fruit fly (*Bactrocera dorsalis*) was evaluated under both greenhouse and field conditions in Taiwan. SPLAT-Spin-ME is a viscous, MAT formulation containing low doses of spinosad (2%) mixed with the powerful male-specific parapheromone attractant methyl eugenol (ME). The proprietary carrier formulation, SPLAT®, provides a metered release of the toxicant and the attractant into the environment. In greenhouse cage trials the attraction rates of *B. dorsalis* males for the two MAT treatments (SPLAT-Spin-ME, naled+ME) were equally high at 0, 1, and 2 weeks weathering time-points, and uniformly low for the two bait spray treatments (GF-120 bait spray, malathion protein bait spray). The mortality of *B. dorsalis* males in greenhouse cage studies for the two MAT treatments was highest with SPLAT-Spin-ME outperforming naled+ME at the 1 and 2 weeks weathering periods both at the 24 h and 48 h grading intervals, with neither bait spray treatments providing a substantial mortality. The results from the field cage studies were the reverse of the results from the greenhouse evaluations. In the field cage studies naled+ME (8 mL) maintained a consistent ca 70-76% attraction rate of up to 7 weeks, whereas SPLAT-Spin-ME (1 g) demonstrated a relatively rapid decline in attraction from an initial 51% to 32% and below starting at week 4. Based on a 48 h grading period, naled+ME generated 92-100% mortality in the field mortality bioassay at all weathering time-points from 0-8 weeks, whereas SPLAT-Spin-ME provided 88-100% mortality, but only through week 3. The markedly better performance of naled+ME in the field *vs*. the greenhouse bioassays is

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attributed to a rate change that took place and which dramatically increased the amount of ME attractant, relative to SPLAT-Spin-ME, used in the field studies, as well as the fact that naled+ME was infused into caneite blocks for the field bioassays which extended the residual of the ME attractant. Under these test conditions the overall efficacy of SPLAT-Spin-ME for controlling *B. dorsalis* males outdoors was around 3-4 weeks whereas naled+ME overall efficacy was around 8 weeks. Although apparently with a shorter residual than the naled+ME MAT standard, SPLAT-Spin-ME was shown to be a highly effective MAT treatment for controlling *B. dorsalis* and a viable alternative or replacement for current, more toxic MAT products.

Key words: spinosad, naled, methyl eugenol, male annihilation, *Bactrocera dorsalis*

Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is widely distributed throughout Southeast Asia and the Pacific region (Christenson and Foote, 1960) and is considered one of the most economically damaging pests in Taiwan. Protein baits or bait sprays (for females) and male annihilation treatments (MAT's) consisting of a toxicant mixed with the powerful, male-specific parapheromone attractant methyl eugenol (ME) form the basis of most control programs for *B. dorsalis*. Control by MAT products is achieved though the selective removal of males from a fruit fly population, thus reducing mating and eventually leading to a population crash. Eradication of *B. dorsalis* has been achieved in many locations through the area-wide application of MAT products containing ME (Vargas *et al*., 2003). Current protein sprays/baits and MAT options depend heavily on organophosphate (OP) insecticides such as malathion, naled, or dichlorvos as the toxic component of these attract-and-kill mixtures. Recently there have been numerous recent reports of OP resistance in fruit flies, including *B. dorsalis* in Taiwan, *B. oleae* in Europe and *Ceratitis capitata* in Spain (Hsu and Feng, 2002; Hawkes *et al*., 2005; Magaña, 2007; Skouras *et al*., 2007). Additionally, organophosphate insecticides have received widespread scrutiny for their negative impacts on non-target animals and human health and thus the identification of reduced-risk alternatives for use in these area-wide control programs is of high priority (Mau *et al.*, 2007; Vargas *et al*., 2008).

Spinosad, an insecticide derived from metabolites of the soil bacterium *Saccharopolyspora spinosa,* is an effective insecticide with highly favorable mammalian toxicology and environmental profiles (Cleveland *et al*., 2001). Spinosad was initially registered in 1997 and is considered a naturally derived product. As a result, certain formulated products have been approved for use in the organic agriculture by the Organic Materials Review Institute (OMRI) and numerous other national and international certification bodies (Cleveland, 2007; Racke, 2007). Spinosad affects the insect's nervous system at unique sites on the nicotinic acetylcholine and GABA receptors and is non-cross-resistant to any other known insecticides (Salgado, 1998; Watson, 2001; Salgado and Sparks, 2005). Spinosad is very active against the oriental fruit fly, is safer to handle and more environmentally friendly than many current products presently on the market, and has less impact than malathion on populations of beneficial parasitoids (Vargas *et al*., 2001, 2002). Initial identification of spinosad as a promising active insecticide for MAT purposes was reported by Vargas *et al*. (2003). More recent studies by Vargas *et al*. (2008, 2009) have demonstrated the utility of spinosad-based MAT products for the control of the oriental fruit fly and the melon fly in Hawaii.

Dow AgroSciences and ISCA Technologies co-developed and recently introduced a novel MAT formulation for the control of *Bactrocera* species designed to provide enhanced performance and safety relative to the existing organophosphate-based MAT sprays and bait station options. This new MAT product, termed "SPLAT-MAT Spinosad ME™" (hereafter referred to as SPLAT-Spin-ME), consists of a low rate of spinosad infused into a wax emulsion matrix, SPLAT® (Specialized Pheromone and Lure Application Technology), containing the long-distance attractant ME. The single most important benefit of the SPLAT carrier formulation is that it protects the attractant and toxicant components from environmental decay (via rain or UV light) while at the same time providing a metered time release of the essential components. The unique nature of SPLAT-Spin-ME greatly enhances its efficacy as it can create up to 100's of point sources per hectare instead of the ca 20-50 traps/hectare recommended for current bait station MAT products. The pattern possible with SPLAT-Spin-ME also obviates the need for labor-intensive and costly preparations, servicing and retrieval of current MAT bait stations. SPLAT-MAT Spinosad ME™ recently received EPA registration approval in the USA for the control of fruit flies in non-crop areas and for fruit fly eradication programs in public areas.

The objective of the present study was to evaluate the efficacy of SPLAT-Spin-ME for area-wide control of *B. dorsalis* males under outdoor conditions in Taiwan and to compare its performance to the current MAT standard product used in Taiwan (naled+ME dispensed on caneite blocks) and two non-MAT, conventional protein bait sprays (malathion protein bait spray and GF-120 bait spray). Greenhouse and field cage studies were used to measure the attraction and mortality of male *B. dorsalis* to SPLAT-Spin-ME relative to the standard commercial treatments when all treatments were conducted outdoors under ambient Taiwan weather conditions.

Materials and Methods

Insects

The laboratory strain of the oriental fruit fly, *B. dorsalis*, has been collected from Taichung in Taiwan and reared in an incubator at 24 ± 2°C with a 12:12 (L:D) photoperiod since 1994. The newly emerged adults, 500 to 2000 flies per cage (L/W/H: 39/19/6 cm), were provided with water and a standard laboratory diet consisting of a mixture of 4 parts granulated sugar to 1 part peptone (Kyokuto Seiyaku). Three- to five-day-old male adults were starved for 24 h prior to use in these experiments.

Treatments

Five treatments with four replications were compared:

1) SPLAT-Spin-ME (containing 2% spinosad a.i., 50% methyl eugenol, and 48% SPLAT; Dow AgroSciences, Indianapolis, IN), a novel MAT product recently introduced by Dow AgroSciences in partnership with ISCA Technologies. For greenhouse cage evaluations, a single 5 mm diameter droplet (ca 50 mg/lid) of SPLAT-Spin-ME was applied to the center of a Teflon-lined, 70 mm diameter round plastic lid (Fig. 1C). For field cage experiments, a single 30 mm diameter droplet of SPLAT-Spin-ME (ca 1.0 g/lid) was applied to the center of a Teflon-lined plastic lid, as above (Fig. 1D). Thus, the ME concentrations were 25 mg a.i. (active ingredient) per lid for the greenhouse

evaluations and 0.5 g a.i. per lid for the field experiments.

2) naled+ME (containing 5% naled and 90% methyl-eugenol, Sinon Corporation, Taiwan), the most widely-used MAT product in Taiwan. For greenhouse cage evaluations, a 10 µL droplet of naled+ME was applied to each of four, 6 mm-diameter filter papers, and these 4 filter papers were then secured to the inside Teflon surface of a 7 mm-diameter plastic lid (Fig. 1A). Later, this greenhouse evaluation treatment was modified to apply a single 40 µL droplet of naled+ME to a small caneite block (8 × 4 × 2 mm) in order to promote absorption thereby reducing the evaporation rate of the highly-volatile ME attractant. This caneite block was then secured to the inside Teflon surface of a 7 mm-diameter plastic lid (Fig. 1 B). For field cage experiments, 8 mL of naled+ME (ca 7.5 g/lid) was applied to a larger caneite block (40 × 40 × 8 mm) (Fig. 1E). This rate is equivalent to the recommended field use rate of naled+ME in Taiwan. Thus, the ME concentrations were 37 mg a.i. per lid for the greenhouse evaluations and 7.0 g a.i. per larger caneite block for the field experiments (based on a 1.036 specific density of ME).

3) GF-120 (0.24% a.i. spinosad, Dow AgroSciences, Indianapolis, IN), a commercial bait spray product based on food attractants and containing a low amount of spinosad as the toxicant. It attracts both male and female fruit flies. Although not a MAT product, GF-120 is included for comparison because it represents a direct competitor in the marketplace for MAT products. The stock solution of GF-120 was first diluted 4-fold with water, which is the recommended field application rate. For greenhouse cage evaluations, a single 5 mm-diameter droplet of the pre-diluted GF-120 (ca 60 µL/lid) was dispensed onto the inside Teflon surface of a 7 mm-diameter plastic lid. GF-120 was not included in the comparative field experiments. GF-120 does not contain ME.

4) malathion 50 EC protein bait spray (malathion Probelte®, Dow AgroSciences, Spain + Buminal®, hydrolyzed proteins 30% p/v, SL, Bayer, Spain), another commercial bait spray product commonly used in Taiwan based on hydrolyzed protein attractants. It contains malathion as the toxicant, and it attracts both male and female fruit flies. Although not a MAT product, malathion bait spray is included for comparison because it represents a direct competitor in the marketplace for MAT products. First, a stock solution of malathion bait spray was prepared according to field label use rate directions as practiced in Spain-6 mL of malathion Probelte® 50% EC was mixed with 1 L of water, and then 6 mL of Buminal® hydrolyzed protein 30% SL p/v was added to the solution. For greenhouse cage evaluations, a total of ten, 20 µL droplets of the malathion bait spray mixture were dispensed onto the inside Teflon surface of a 7 mm-diameter plastic lid. Malathion bait spray was not included in the comparative field experiments. Malathion bait spray does not contain ME.

5) Untreated control (water): a total of ten, 20 µL droplets of distilled water were applied onto the inside Teflon surface of a 7 mm-diameter plastic lid for both greenhouse and field cage experiments.

Outdoor Weathering of Treatments for Greenhouse Evaluations and Field Experiments

Round plastic lids of 7 cm diameter with a Teflon lining were treated with SPLAT-Spin-ME, with competitor fruit fly MAT and with bait spray products at scaled field use rates at time 0. The treated lids were then aged outdoors under ambient Taiwan weather conditions. Treated lids were weathered in the field for 0, 1, 2, and 3 weeks for the greenhouse evaluations. For the field experiments, treated lids were weathered outdoors for 0, 1, 2, 3, 4, 5, 6, 7, 8 weeks. The treated lids

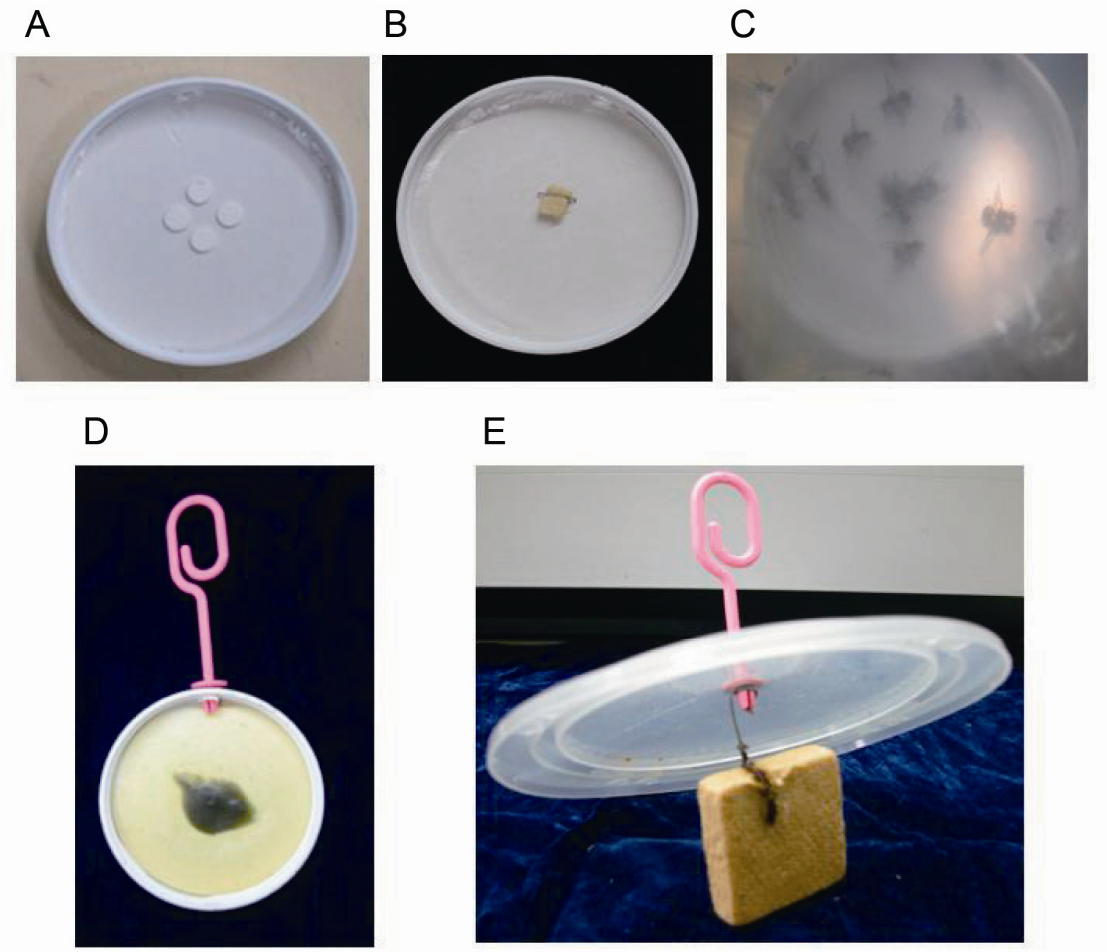

Fig. 1. The naled-ME (prior sticking) and SPLAT-spinosad-ME treatments of the Teflon surface of the lids that were then put into the trap for these experiments. A. 40 μL of naled+ME placed onto filter papers, at 10 μL each; B. 40 μL of naled+ME added into a caneite block; C. 50 mg of SPLAT-spinosad-ME; D. 1 gram of SPLAT-spinosad-ME; E. A caneite block with 8 mL of naled-ME.

were hung 30 cm apart from a clothes line positioned in the shade (Fig. 2A). A randomized complete-block experimental design was used with 4 replicates. The mean (± SD) daily temperatures during the weathering period for the replications were 30.7 ± 0.93 (range: 26.4-35.1), 28.78 ± 1.38 (23.5-35.0), 28.39 ± 1.00 (24.61-34.14), and 28.04 ± 1.73 (24.71-33.51) for the greenhouse trials, and 20.05 ± 2.40 (15.2-29.7), and 19.56 ± 2.55 (14.40-29.60) for the field trials, respectively. Rainfall was sporadic, but significant during the weathering period due to an approaching typhoon during the greenhouse trials. The cumulative rainfall values for the replications were 10 mm, 458 mm, 282 mm and 268.5 mm for the greenhouse trials, and 32.5 mm, and 30.5 mm for the field trials, respectively. Each week 5 (greenhouse trial) or 3 (field trial) weathered lids (including an untreated check) from each treatment were removed from the clothes line and transported to

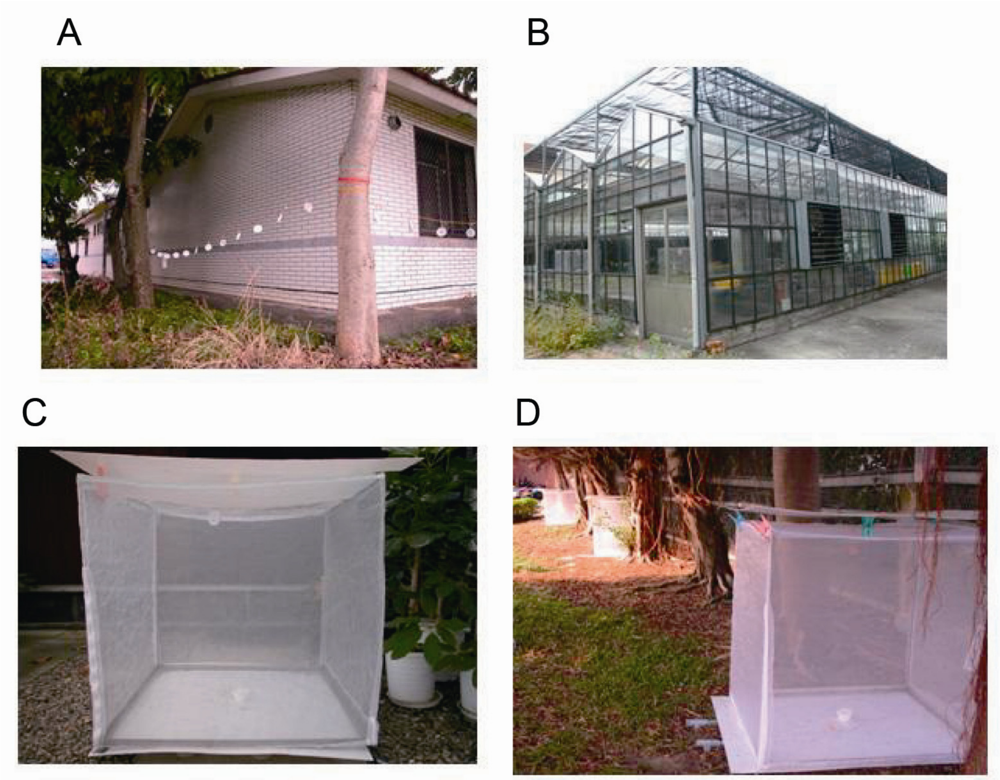

Fig. 2. Trials evaluating the efficacy of various fruit fly control products weathered outdoors under ambient conditions in Taiwan. A. Discs weathered outdoors; B. Greenhouse; C. Cage with roof in greenhouse; D. Field cage experiment.

the greenhouse cages or to the field cages where bioassays of male fruit flies were conducted to assess the residual efficacy of these treatments over time.

Greenhouse Valuations:

Greenhouse evaluations were carried out between 10 July-21 Oct, 2007 at the Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Wufeng, Taichung County, Taiwan. The greenhouse cage trials evaluated the relative attraction and mortality of *B. dorsalis* males based on SPLAT-Spin-ME and 4 reference treatments (naled+ME MAT product, GF-120 bait spray, malathion protein bait spray, Untreated check) using a field-lab bioassay system.

Greenhouse Attraction Bioassays. Attraction bioassays were conducted inside a ventilated greenhouse (14.5 × 10 × 3.6 m, L × W × H, temperature 21-33°C, relative humidity 70-100%) (Fig. 2B). A treatment of freshly-prepared SPLAT-Spin-ME (50 mg/lid) was included at weathering time-points 0, 1, 2 and 3 weeks for comparison. Each of 5 treated lids from each treatment/weathering time-point were placed inside of a perforated plastic fruit fly trap. These traps were then suspended by means of a wire from the roof of a large, organdy 1 m^3 cage (Fig. 2C). A total of 50 male flies were then introduced into the cage and supplied with cotton wicks soaked in a protein/sugar/water solution as a food source. Relative attraction was assessed as the percentage of the 50 original male fruit flies found within the perforated plastic trap hanging within the cage after 1 hour of exposure.

Greenhouse Mortality Bioassays. Mortality bioassays took place directly following the attraction bioassays in the same ventilated greenhouse, using the same organdy 1 m^3 cage, and using the same weathered, treated plastic lids. The treated lids were removed from the perforated plastic traps used in the previous attraction bioassay and were hung directly from the roof of the organdy cage, thus allowing unimpeded access to the weathered lids from all sides (the plastic traps were removed from the organdy cages and discarded). A total of 50 new male fruit flies were introduced into the organdy cages and observed for an additional 48 h. During that time they were supplied with water and a dry protein/sugar mixture (ratio: 1/4) as a food source. Dead flies within the cage were recorded after 24 h and 48 h exposure periods. Efficacy data is expressed as percent-mortality of male fruit flies at the 0, 1, 2, and 3 weeks weathering time-points.

Field Attraction and Mortality Bioassays. Field experiments were carried out from 2 November, 2007-5 January, 2008 at the Taiwan Agricultural Chemicals and Toxic Substances Research Institute. Field (outdoor) cage trials evaluated the relative attraction and mortality of *B. dorsalis* males based on SPLAT-Spin-ME and 2 reference treatments (naled+ME MAT product, untreated check) over a period of 8 weeks of field weathering using a field-lab bioassay system. These field cage (Fig. 2D) trials were similar in most respects to the greenhouse bioassays described above, except that the weathering period extended to 8 weeks, the field bioassays were run in cages held outdoors, and higher rates of SPLAT-MAT Spinoad ME (1.0 g/lid) and naled+ME (7.5 g/lid) were used in these field cage trials relative to the prior greenhouse evaluations. Two SPLAT-Spin-ME treated lids, two naled+ME treated lids, and a single untreated water check lid from each treatment/ weathering time-point were evaluated. For each replicate, a treated, weathered lid was initially presented to the male fruit flies within a perforated plastic trap suspended from the roof of a 1 m^3 organdy cage. Test cages were maintained outdoors in the shade under ambient Taiwan weather conditions (Temp. 6-32°C). A total of 50 male flies were introduced into the cage and supplied with water and a dry protein/sugar mixture (ratio: 1/4) as a food source. Attraction was measured as the percentage of the 50 original male fruit flies found within the perforated plastic trap hanging within the cage after the first hour. After the one hour attraction exposure, the plastic traps and flies were removed from the cages, and the weathered lids (SPLAT-Spin-ME) or caneite blocks (naled+ME) were suspended directly from the roof of the cages, thus allowing unimpeded access to the treated lids from all sides. A total of 50 new male fruit flies were introduced into the organdy cages and observed for an additional 48 h. During this time they were supplied with water and a dry protein/sugar mixture (ratio: 1/4) as a food source. Dead flies inside the cage were recorded after 24- and 48-h exposure periods. Efficacy was measured as the percent- mortality of male fruit flies at the 0, 1, 2, 3, 4, 5, 6, 7, and 8 weeks weathering time-points.

Statistical Analyses. Statistically significant differences (at $p < 0.05$) between the attraction and mortality rates of treatments were analyzed using a multivariate analysis of variance (MANOVA) test, followed by LSD tests for the comparison of treatment means (StatSoft, Inc. 2003).

Results

Greenhouse Attraction Bioassays. Attraction rates (%) were significantly

Table 1. Attraction of *Bactrocera dorsalis* males (based on the capture rate within a baited trap during a 1 h exposure period) to four MAT and bait spray treatments versus a water control in a greenhouse cage experiment (mean ± SD of 4 replications)

Treatments	Attraction (%) at weathering time-points of weeks			
	0	1	2	3
SPLAT-MAT	42 ± 5.0Aa[1]	23 ± 23Ab	29 ± 25Aab	15 ± 16Ab
Naled+ME MAT	42 ± 5.5Aa	17 ± 25Ab	18 ± 17Ab	6.0 ± 7.1Ab
GF-120 bait	9.0 ± 3.5Ba	14 ± 8.6ABa	8.5 ± 7.5Bb	3.5 ± 3.0Ab
Malathion protein	4.5 ± 5.0Ba	6.5 ± 5.3Ba	11 ± 6.2Ba	2.5 ± 2.5Aa
Water	4.0 ± 2.3Ba	4.0 ± 3.7Ba	7.0 ± 8.9Ba	4.5 ± 4.4Aa

[1] Means within a column followed by different capital letters or the different lower case letters in a row were significantly different by Fisher's LSD test ($p < 0.05$).

different among treatments ($F_{4, 72} = 9.96$, $p < 0.05$) and weathering time-points ($F_{4, 72} = 4.29$, $p < 0.05$). SPLAT-Spin-ME and naled+ME were equally attractive at time = 0 (42% capture rates), but SPLAT-Spin-ME appeared to maintain its attractiveness longer than naled+ME, especially when the weathering periods were extended to 1, 2 and 3 weeks (Table 1). GF-120 bait spray exhibited significantly more attraction than malathion protein bait spray, but only at the 1 week time-point. The malathion protein bait spray proved to be not significantly different in its level of attraction when compared with the water control throughout the 0-3 weeks weathering periods.

Greenhouse Mortality Bioassays. Mortality rates (%) were significantly different among treatments ($F_{4, 72} = 21.94$, $p < 0.05$) and weathering time-points ($F_{3, 72} = 14.15$, $p < 0.05$). At the 48 h grading period, SPLAT-Spin-ME generated 96, 78, 71 and 16% male fruit fly mortality at the 0, 1, 2, and 3 weeks weathering time-points, respectively (Table 2). The comparable data for naled+ME were 95, 56, 18 and 12% mortality, respectively. The comparable data for malathion protein bait spray were 23, 21, 8 and 3% mortality, respectively. The comparable data for GF-120 bait spray were 28, 26, 17 and 11% mortality, respectively. Untreated control mortality at 0, 1, 2, and 3 weeks weathering time-points were 6, 10, 6 and 3%, respectively. Similar relative differences among treatments were evident at the 24-h grading period, although mortality rates were lower overall and the rank order of treatments in terms of %-mortality were essentially the same.

Under the conditions of this greenhouse test method, SPLAT-Spin-ME at the relatively low dose of 50 mg/lid demonstrated an initial and residual efficacy that was superior to the commercially-used MAT product, naled+ME, throughout the 0-21 d weathering period. It should be noted that in these greenhouse experiments the naled+ME treatment was presented on filter papers instead of being impregnated into caneite blocks, as is recommended. SPLAT-Spin-ME also significantly outperformed the commercial protein bait sprays, malathion protein bait and GF-120 bait.

Field Attraction Bioassay. The attraction rates (%) measured after male fruit flies were exposed for 1 h to the two MAT treatments were significantly different at various weathering time-points in the field cage (Table 3). The untreated water check exhibited a very low attraction and residual efficacy at all weathering time-points, so it was excluded from the MANOVA analysis. In this field attraction bioassay, naled+ME was significantly more attractive to male *B.*

Table 2. Mortality of *Bactrocera dorsalis* males within baited cages to 4 MAT and bait spray treatments and a water control in a field cage experiment at 24 h and 48 h grading periods (mean ± SD of 4 replications)

Treatments	Mortality (%) at weathering time-points of weeks			
	0	7	14	21
24 h				
SPLAT-MAT	82 ± 9.1Aa[1]	65 ± 20Aab	58 ± 24Ab	10 ± 9.8Ac
Naled+ME MAT	88 ± 12Aa	49 ± 49Ab	15 ± 15BC	6.5 ± 7.9Ac
GF-120	27 ± 8.7Ba	21 ± 17Ba	14 ± 6.3Ba	7.5 ± 8.4Aa
Malathion protein	19 ± 5.7BCa	16 ± 9.4Ba	6.0 ± 8.2Ba	0.5 ± 1.0Ba
Water	4.5 ± 3.0Ca	6.5 ± 4.1Ba	2.5 ± 3.8Ca	1.5 ± 1.9Ba
48 h				
SPLAT-MAT	96 ± 8.0Aa	78 ± 20Aab	71 ± 28Ab	16 ± 14Ac
Naled+ME MAT	95 ± 6.0Aa	56 ± 50Ab	18 ± 15Bc	12 ± 18Ac
GF-120	28 ± 9.2Ba	26 ± 16Ba	17 ± 10Ba	11 ± 14Aa
Malathion protein	23 ± 5.0Ba	21 ± 4.7Ba	7.5 ± 11Ba	2.5 ± 2.5Aa
Water	5.5 ± 4.1Ba	10 ± 5.2Ba	5.5 ± 6.4Ba	2.5 ± 2.5Aa

[1]Means within a column followed by different capital letters or the different lower case letters in a row were significantly different by Fisher's LSD test ($p < 0.05$).

dorsalis than SPLAT-Spin-ME at nearly all of the 0-8 weeks weathering time-points. Naled+ME maintained a 70-76% attraction rate for 7 weeks whereas SPLAT-Spin-ME demonstrated a rapid decline in attraction from an initial 51% at week 0 to 34% or below starting at week 2 and continuing these relatively low attraction rates to week 8.

Field Mortality Bioassay. The mortality rates (%) of *B. dorsalis* males for the two treatments were significantly different at various weathering time-points and grading periods (Table 4). At the 24 h grading period, SPLAT-Spin-ME showed a 99, 81, 91 and 67% male fruit fly mortality at the 0, 1, 2 and 3 weeks weathering time-points, respectively, after which time the mortality fell off considerably. On the other hand, naled+ME provided 85-98% control throughout all 0-8 weeks weathering periods. Untreated control mortalities at these same weathering time-points were all at or below 4%. At the 48 h grading period, SPLAT-Spin-ME resulted in 100, 95, 97, 88, 61, 59, 65, 53 and 37% male fruit fly mortality at the 0, 1, 2, 3, 4, 5, 6, 7, and 8 week weathering time-points, respectively. The comparable data for naled-ME at these same time-points were 100, 96, 98, 92, 99, 96, 93, 98, and 95%, respectively. Untreated control mortalities at the same weathering time-points were 7% or lower. Under the conditions of this field bioassay test method, the mortality rates for SPLAT-Spin-ME were similar to those of naled+ME during the 0-3 week weathering time-points, but after that the mortality rates of SPLAT-Spin-ME were much lower and statistically different from those of naled+ME. It should be noted that in these field cage experiments the naled+ME treatment was presented impregnated into caneite blocks, as is commonly recommended.

Discussion

This series of greenhouse and field experiments compared the attraction and toxicity over time of SPLAT-Spin-ME and other commercially-used MAT products (naled+ME) and bait spray products (GF-120 bait and malathion protein bait) in controlling the *B. dorsalis* populations

Table 3. Attraction of *Bactrocera dorsalis* males (based on the capture rate within a baited trap during a 1 h exposure period) to two MAT treatments and water control in a field cage experiment (mean ± SD of 4 replications)

Treatments[1]	Attraction (%) at weathering time-points of weeks								
	0	1	2	3	4	5	6	7	8
SPLAT-MAT	51 ± 13Aa[2]	33 ± 15Aab	34 ± 28Aab	11 ± 6.2Ac	32 ± 5.7Aab	18 ± 3.8Abc	19 ± 18Abc	29 ± 20Abc	19 ± 6.0Ab
Naled+ME MAT	74 ± 15Ba	71 ± 12Ba	72 ± 12Ba	60 ± 21Ba	75 ± 5.0Ba	76 ± 12Ba	32 ± 18Bb	70 ± 15Ba	48 ± 19Bab
Water	3 ± 1.4	1 ± 1.4	0 ± 0	1 ± 1.4	0 ± 0	1 ± 1.4	2 ± 2.8	2 ± 2.8	2 ± 2.8

[1] The replications of water treatment were used only two times.

[2] Means within a column followed by different capital letters or the different lower case letters in a row were significantly different by Fisher's LSD test ($p < 0.05$).

Table 4. Mortality of *Bactrocera dorsalis* males within baited cages to two MAT treatments and a water control in a field cage experiment at 24 h and 48 h grading periods (mean ± SD of 4 replications)

Treatments[1]	Mortality (%) at weathering time-points of weeks								
	0	1	2	3	4	5	6	7	8
24 h									
SPLAT-MAT	99 ± 1.0Aa[2]	81 ± 15Aa	91 ± 8.9Aa	67 ± 16Ab	35 ± 13Ac	38 ± 31Ac	58 ± 33Abc	31 ± 27Ac	22 ± 15Ac
Naled+ME MAT	98 ± 0Aa	93 ± 6.6 Aa	96 ± 3.4Aa	86 ± 14Aa	92 ± 8.1Ba	95 ± 3.0Ba	90 ± 7.1Ba	85 ± 4.1Ba	86 ± 8.5Ba
Water	1 ± 1.4	3 ± 1.4	3 ± 1.4	4 ± 0	1 ± 1.4	0 ± 0	4 ± 2.8	1 ± 1.4	3 ± 4.2
48 h									
SPLAT-MAT	100 ± 0Aa	95 ± 6.8Aa	97 ± 3.4Aa	88 ± 16Aa	61 ± 8.9Ab	59 ± 30Abc	65 ± 35Aab	53 ± 35Acd	37 ± 29Ad
Naled+ME MAT	100 ± 0Aa	96 ± 5.3Aa	98 ± 3.8Aa	92 ± 9.3Aa	99 ± 1.0Ba	96 ± 1.0Ba	93 ± 6.8Ba	98 ± 1.6Ba	95 ± 4.2Ba
Water	4 ± 2.8	4 ± 2.83	6 ± 2.8	3 ± 1.4	5 ± 1.4	0 ± 0	7 ± 1.4	5 ± 1.4	4 ± 5.7

[1] The replications of water treatment were used only two times.

[2] Means within a column followed by different capital letters or the different lower case letters in a row were significantly different by Fisher's LSD test ($p < 0.05$).

in Taiwan under ambient outdoor conditions.

In the greenhouse attraction bioassays SPLAT-Spin-ME and naled+ME were the most attractive treatments for male *B. dorsalis* based on the trap captures after a 1 h exposure period. SPLAT-Spin-ME was numerically but not significantly superior to naled+ME over all 0-3 weeks weathering time-points. Weathered deposits of GF-120 bait spray were attractive to male *B. dorsalis* for the first week of outdoor exposure, but then the attraction fell to levels not statistically different than the water control. The attraction of malathion protein bait spray was never significantly different than that of the water control. These results were not unexpected given that both MAT products employ the powerful male-specific kairomone, methyl eugenol. Both bait spray products depend on food additives for their attraction properties to *B. dorsalis,* and although of proven utility, the attraction of these baits when in the presence of supplied protein and sugar (as they were in this experiment) is relatively low in comparison to methyl eugenol.

In the field attraction bioassays the relative attraction of SPLAT-Spin-ME *vs* naled+ME was reversed compared to the greenhouse results, with naled+ME the more attractive and longer-lasting treatment in these field cage studies. A likely reason for this reversal in measured attraction is the fact that in the field evaluations the overall applied concentration of ME in the naled+ME treatment was raised from 37 mg/lid in the greenhouse evaluations to 7.5 g/lid in the field experiments. The overall applied concentrations of ME in the SPLAT-Spin-ME treatments were 25 mg/lid in the greenhouse evaluations and 0.5 g/lid in the field experiments. This resulted in a disproportionate increase in relative concentration of the ME attractant in the field trials, with naled+ME having ca 1.5-fold more ME than SPLAT-Spin-ME in the greenhouse evaluations, but ca 15-fold more overall ME in the field experiments. In addition , naled+ME was presented on filter paper in the greenhouse evaluation , but it was infused into caneite blocks for the field experiments-the latter presentation method being more akin to the recommended commercial use pattern and designed to retard the evaporation of the highly volatile ME attractant.

In the greenhouse mortality bioassays SPLAT-Spin-ME consistently resulted in higher levels of *B. dorsalis* mortality than naled+ME at nearly all of the 0-3 weeks weathering time-points. This was true for both the 24 h and 48 h grading periods.

In the field mortality bioassays there was a reversal in the relative efficacy of SPLAT-Spin-ME vs. naled+ME relative to the greenhouse results, with naled+ME now enjoying a relative advantage. Mortality rates for SPLAT-Spin-ME and naled+ME were uniformly high and generally equivalent through weathering time-points 0-3 weeks, but by the 4th week SPLAT-Spin-ME mortality fell off markedly and continued at these lower levels through weathering time-points 4-8 weeks, whereas naled+ME maintained its effectiveness for the entire 0-8 weeks weathering period. The longer effective residual effect of the naled+ME in the field *vs.* the greenhouse mortality bioassays mirrors a trend previously observed in the field. This is similar to the results of the attraction bioassay, where naled+ME seemed to maintain its attraction much longer in the field *vs.* the greenhouse bioassays. The root cause in both cases is likely the same-the fact that the overall rate of the naled+ME treatment was raised from 40 mg/lid in the greenhouse bioassays to 7.5 g/lid in the field bioassays (which in turn raised the ME concentration from 37 mg to 7.0 g/lid) while the SPLAT-spinosad-ME concentration only increased from 50 mg/lid in greenhouse bioassays to 1.0 g/lid in field evaluations (raising the ME concentration from 25 mg to 0.5 g/lid). As a result, the naled+ME field treatments contained 15 times more ME than SPLAT-spinosad-ME field treatments whereas in the greenhouse bioassays the rates and ME concentrations of naled+ME and SPLAT-spinosad-ME were much closer (only a 1.5-fold differential in ME concentration between the two greenhouse treatments). It should be noted that the 7.5 g/lid rate of naled+ ME is the scaled-down recommended field use rate and thus probably best represents its field performance.

Taken together, these results indicate that the overall efficacy of SPLAT-Spin-ME for controlling *B. dorsalis* males outdoors under the conditions of these tests is ca 3-4 weeks whereas the overall efficacy of naled+ME is maintained for ca 8 weeks. The major determinant for this difference in overall efficacy appears to be the prolonged attraction of naled+ME vs SPLAT-Spin-ME under field conditions, and this in turn is likely linked to the much higher concentrations of ME in the naled+ME field bioassays.

Vargas *et al*. (2008) conducted similar outdoor weathering studies with an earlier, experimental SPLAT-Spin-ME formulation, comparing it to a standard Min-U-Gel+ naled+ME treatment for control of *B. dorsalis* males in Hawaii. Min-U-Gel is an attapulgite clay carrier formulation and Min-U-Gel+naled+ME formulations have been used for a number of years as spot applications in California MAT programs for the eradication of *B. dorsalis* (Chambers *et al*., 1974; Cunningham and Suda, 1985).

First, treatments were applied to wooden discs and presented to wild populations of *B. dorsalis* males within bucket traps situated inside a papaya, guava or grapefruit orchard. Their results demonstrated that the SPLAT-Spin-ME formulation they used captured *B. dorsalis* males on par with the Min-U-Gel+naled+ ME standard treatment for up to 6 weeks, and was superior in terms of trap captures from weeks 7-12. In addition, freshly-prepared treatments were compared with weathered treatments to estimate the length of exposure time before the trap catches for fresh and weathered treatments began to diverge significantly. It was found that SPLAT-Spin-ME showed an effective residual of 3 weeks in these trials *vs.* 3 weeks for the Min-U-Gel+ naled+ME standard. The amount of ME in the SPLAT-Spin-ME experimental formulation on each wooden disc was ca 2.0 g a.i.. They then concluded that SPLAT-Spin-ME represented a promising substitute for organophosphate-based MAT products.

Vargas *et al*. (2009) conducted a second set of experiments comparing an experimental SPLAT-Spin-ME formulation with a Min-U-Gel+naled+ME standard treatment, but this time the treated wooden tongue depressors were weathered for up to 8 weeks in outdoor southern California conditions and then shipped to Hawaii for behavioral bioassays employing populations of *B. dorsalis* males. Their results showed a general equivalency in trap captures between the two treatments over 1, 2, 4, and 8 weeks weathering periods when the treated tongue depressors were presented in bucket traps in a guava orchard (in 2007) and in non-host plants adjacent to a papaya orchard (in 2008). In outdoor cage studies conducted at the Hawaii Waiakea Experiment Station the cumulative mortality of *B. dorsalis* males exposed to treated tongue depressors for 24 h within cages was not statistically different for either MAT treatment, and this was similar for all weathering time-points, as well as for both the 2007 and 2008 repetition of this trial. In laboratory cage feeding studies, *B. dorsalis* males were allowed to feed for up to 5 minutes on weathered deposits before being transferred to clean containers where they were provided with untreated sugar-water solutions for food. No differences in cumulative 24 h mortality were detected in these feeding studies between SPLAT-Spin-ME and Min-U-Gel+ naled+ME treatments at the 1, 2, or 4 weeks weathering time-points in either the 2007 or 2008 repetition of this trial, although by the 8 weeks weathering time-point there was a significant drop-off in SPLAT-Spin-ME toxicity vs Min-U-Gel+ naled+ME in both years. The amount of ME in the SPLAT-Spin-ME experimental formulation on each tongue depressor was ca 0.9 g a.i.. Vargas *et al*. (2009) note that SPLAT-Spin-ME can be considered an excellent replacement for organophosphate insecticides in MAT sprays.

Results of the present study, combined with those of other authors, suggest that SPLAT-Spin-ME can be considered a viable and effective MAT treatment for the control of *B. dorsalis*, and thus represents a valuable new tool for the integrated control of this damaging fruit fly pest. Under the stringent conditions of these tests, the outdoor residual efficacy of SPLAT-Spin-ME appeared to be ca 3-4 weeks which was shorter than the ca 8 weeks apparent residual of at least one alternative MAT treatment, naled+ME on caneite blocks. However, it remains to be seen how these field-lab results translate to commercial usage. There is evidence from other studies (Vargas *et al*., 2008, 2009) that SPLAT-Spin-ME provides superior control with a longer residual than some other, alternative MAT treatments (e.g. Min-U-Gel+naled+ME). Given the convenience of a MAT product, the re-application of SPLAT-Spin-ME on a monthly schedule could prove to be perfectly acceptable to growers.

In addition, SPLAT-Spin-ME possesses a number of unique attributes that serve to make it particularly attractive for use in area-wide control programs: 1) it utilizes very low rates of both the spinosad toxicant and the ME attractant, much lower than in alternative MAT products, 2) spinosad has highly favorable mammalian toxicological and environmental profiles and is regarded as a reduced-risk alternative to more toxic MAT or bait spray toxicants, 3) the use pattern of SPLAT-Spin-ME, along with the flexibility of its application, should prove to be highly convenient to growers, 4) the use pattern possible with SPLAT-Spin-ME obviates the need for the labor-intensive and costly preparation, and the servicing as well as the retrieval of current MAT bait stations, and 5) the waxy SPLAT® formulation matrix is designed to protect against environmental degradation and wash-off.

Further evaluation of SPLAT-Spin-ME for area-wide control of *B. dorsalis* in Taiwan appears to be warranted in light of these promising preliminary results.

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溫室及田間網籠評估新的滅雄劑型 SPLAT-MAT Spinosad ME™ 對東方果實蠅 (Diptera: Tephritidae) 的誘殺效果

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摘　要

在台灣利用溫室及田間網籠評估一種新的滅雄技術 SPLAT-MAT Spinosad ME™ (SPLAT-Spin-ME) 對東方果實蠅 (*Bactrocera dorsalis*) 雄蟲的誘殺效果。SPLAT-MAT Spinosad ME™ 是道禮農業科學公司提供的一種具黏性的滅雄製劑，其內含低劑量的賜諾殺 (有效成份 2%) 混合對東方果實蠅雄蟲具強吸引力的甲基丁香油 (50%)。以風化資材在溫室網籠比較 1 及 2 週誘殺率，其中二種滅雄試驗組 (SPLAT-Spin-ME, naled+ME) 的雄蟲誘殺率較高，不過二種誘餌試驗組 (GF-120 bait spray, malathion protein bait spray) 雄蟲誘殺率則很低。在溫室網籠 1 到 2 週的試驗中，24 及 48 小時的死亡率 SPLAT-Spin-ME 比 naled+ME 高，但差異不顯著。田間網籠的試驗結果卻相反。以 1 小時誘引率而言，naled+ME (8 mL) 在 7 週內的試驗期皆維持 70～76% 的誘引率，而 SPLAT-Spin-ME (1 g) 則從 51 到 32%，其中在第 4 週後開始下降。另外，48 小時誘殺率試驗中，naled+ME 在 8 週的試驗期間皆維持於 92～100%的誘殺率，而 SPLAT-Spin-ME 僅於 3 週內維持 88～100% 的誘殺率。二種滅雄處理在田間及溫室網籠誘殺率相反的結果，主要是因 naled+ME 田間使用的量比 SPLAT-Spin-ME 來得大 (甲基丁香油的量差 15 倍)，且其吸附於蔗板中，能達到緩釋的效果。以本試驗的量，SPLAT-Spin-ME (1 g) 在戶外可維持大約 3 到 4 週，而 naled+ME (8 mL) 可以有 8 週的效果。雖然 SPLAT-Spin-ME 1 克的量比現行使用的含毒甲基丁香油 (蔗板吸附 8 ml) 的殘留時間較短，SPLAT-Spin-ME 仍是一個有效防治東方果實蠅且可替代目前較毒滅雄產品的製劑。

關鍵詞：賜諾殺、乃力松、滅雄法、甲基丁香油、東方果實蠅。

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