Tumambing J, Mallari R, Widayanti S, Hararap IS, Salazar G (2012) Phosphine fumigation protocols using ECO-$_2$FUME® for complete control of strongly resistant cigarette beetle, *Lasioderma serricorne*, in Indonesia. In: Navarro S, Banks HJ, Jayas DS, Bell CH, Noyes RT, Ferizli AG, Emekci M, Isikber AA, Alagusundaram K, [Eds.] Proc 9th. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products, Antalya, Turkey. 15 – 19 October 2012, ARBER Professional Congress Services, Turkey pp: 624-632

PHOSPHINE FUMIGATION PROTOCOLS USING ECO$_2$FUME® FOR COMPLETE CONTROL OF STRONGLY RESISTANT CIGARETTE BEETLE, *LASIODERMA SERRICORNE,* IN INDONESIA

Justin Tumambing[1], Remil Mallari[1], S Widayanti[2], Idham S. Hararap[2] and Gabriel Salazar[3]

[1]CYTEC Australia Holdings Pty Ltd, P O Box 7125, Baulkham Hills, NSW 2153 Australia
[2]SEAMEO BIOTROP, Jl. Raya Tajur Km 6, Bogor, Indonesia
[3]PT Sterix Indonesia, Epicentrum Walk, Lantai 6, Suite A-623, Kuningan, Jakarta Selatan, Indonesia
*Corresponding author's e-mail: *Justin.Tumambing@cytec.com*

ABSTRACT

Efficacy trials were conducted at BIOTROP to establish phosphine fumigation protocols using ECO$_2$FUME® achieving complete mortality of all stages of strongly resistant cigarette beetle, *Lasioderma serricorne*, infesting dry tobacco leaves in Indonesia. Tobacco leaves in Indonesia have been fumigated with metal phosphides such as aluminum phosphide tablets and magnesium phosphide plates for a long time. Over time, metal phosphides have proved unable to achieve 100% control of all stages of cigarette beetle and with repeated failed fumigations, phosphine resistance developed with strains of insects exhibiting strong and weak levels of resistance to the current protocols. Experimental treatments with combinations of three phosphine concentrations (1000 ppm, 700 ppm and 350 ppm) and three exposure times (5 days, 8 days and 12 days), at average temperatures of 28°C or higher were used to determine the conditions achieving 100% mortality of all stages of both the strongly and weakly resistant cigarette beetle strains.

ECO$_2$FUME® (2% phosphine, 98% CO_2 by weight) is a cylinderized gas formulation of phosphine which offers the advantages of absence of fire risk, enhanced worker safety, effective and rapid distribution of gas, easy application and control, no waste generation or disposal thus being environmentally friendly, and cost effectiveness.

Results of the study suggest potential fumigation protocols achieving 100% efficacy for all stages of strongly resistant cigarette beetle in Indonesia are 1000 ppm for 5 days, 700 ppm for 8 days and 350 ppm for 12 days at average temperatures of 28°C or higher. The minimum recommended phosphine concentration should be maintained through regular phosphine concentration monitoring and top up throughout the required exposure period. The larva of the strongly resistant strain appeared to be the most tolerant stage.

Key words: phosphine gas, ECO$_2$FUME®, *Lasioderma serricorne*, tobacco fumigation, resistance.

INTRODUCTION

Tobacco is one of the most important crops in Indonesia as its leaves are used in the production of cigarettes and cigars for domestic and international markets. Tobacco leaves just like any other plant commodity are subject to insect pest infestation during storage which leads to both significant qualitative and quantitative losses. The major insect pests of tobacco leaves are the cigarette beetle, Lasioderma serricorne (F.) and tobacco moth, Ephestia elutella (Hubner). Of the two insect species, cigarette beetle is the most damaging and more tolerant to treatment.

The general treatment for insects infesting tobacco leaves is with the use of phosphine from metal phosphides, solid formulations of magnesium phosphide or aluminum phosphide. Metal phosphide had been used for decades in fumigating tobacco leaves against cigarette beetle and over time resistance has developed. Phosphine resistance of cigarette beetle has progressed substantially in Indonesia so that it is now very difficult to achieve 100% mortality of all developmental stages (adults, pupae, larvae and eggs) using the current protocols developed and recommended by CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco). CORESTA is an international association of companies, research institutes and independent laboratories with R & D interest in the production, manufacture and use of tobacco. CORESTA has separate recommended phosphine fumigation protocols for susceptible and resistant strains of cigarette beetle. The recommended protocols for susceptible cigarette beetle are 300 ppm for 6 days at 16 – 20°C and 200 ppm for 4 days at 20°C or higher. For resistant cigarette beetle, the recommended protocols are 700 ppm for 10 days at 20 – 25°C and 600 ppm for 6 days at >25°C.

Many factors enhance the development of phosphine resistance in insects such as: 1) use of sub-lethal doses, 2) not maintaining the minimum recommended phosphine concentration over the required exposure time, 3) lack of regular monitoring of phosphine concentration 4) repeated fumigation with phosphine and 5) no monitoring or action taken regarding insect survival that later can give rise to resistant strains.

This paper describes the efficacy trials conducted to establish fumigation protocols using ECO2FUME® phosphine fumigant that will achieve 100% efficacy against strongly resistant cigarette beetle that infests dried tobacco leaves in Indonesia. ECO2FUME® is a non-flammable and ready-to-use cylinderized formulation of 2% phosphine (PH3) and 98% carbon dioxide (CO2) by weight. It is packaged in high pressure steel cylinders with net content of 31 kg of PH3/CO2 mixture and equivalent phosphine amount of 620 g. ECO2FUME® (formerly known as Phosfume) was first commercially applied in Australia in 1988 by BOC Gases Australia who produced and patented the phosphine/CO2 blend and developed special dispensing equipment for fumigating grains and oilseeds in unsealed and good sealed silos and horizontal sheds (Cavasin et al., 2000). From then on, ECO2FUME® has been used globally for treatment of other commodities such as cut flowers, nuts, tobacco, and wheat flour and non-food applications such as structural fumigation.

The use of ECO2FUME® has the advantages over solid metal phosphide phosphine formulations of 1) safer operation by application outside the structure, 2) quick dispensing and attainment of target concentration throughout the structure and possibility of maintaining the concentration by measured top-ups, 3) independence of humidity and temperature for reaction, 4) lower phosphine dosages due to maintenance of phosphine concentration close to the target concentration, 5) eliminating dust or solid waste generated from using solid

formulations, 6) no waste deactivation and disposal, and 7) no ammonia emissions that require additional scrubbing.

The objectives of the study were 1) to test the effectiveness of ECO2FUME® against strains of cigarette beetle showing weak and strong resistance to phosphine in dried tobacco leaves in Indonesia and 2) to define parameters and propose phosphine fumigation protocols for complete control of all stages of these cigarette beetle strains.

MATERIALS AND METHODS

The fumigation trials were conducted during the period 12 - 24 February 2012 with a further two weeks for the bioassay efficacy assessment. The trials took place at the postharvest warehouse of BIOTROP (Tropical Biology Institute) in Bogor, Indonesia. Preparation of test insects and bioassay was performed at the BIOTROP entomology laboratory. The climatic conditions during the trials were 23 – 33°C ambient temperature and 44 - 89% relative humidity.

The materials used in the trials were as below:

1. Tobacco bales, 36 boxes (approx.1 m^3 each)
2. Wooden pallets, one under each bale
3. Polypropylene tarpaulin sheet (150 micron)
4. Sand snakes for sealing tarp covered stock
5. $ECO_2FUME^®$ cylinder and dispensing hose with special gun injector for small doses
6. SILOCHEK® phosphine monitor (0 – 2000 ppm)
7. Digital weighing scale (100 kg with 1 g precision)
8. ¼ inch plastic tubing for gas sampling
9. Industrial fan to control exposure levels
10. Full face gas masks with phosphine canisters

The efficacy trials were designed in Completely Randomized Design (CRD), with times and dosages as factors in the experiment. The four level of dosages tested were; 0, 25, 50, and 70 g/m3 of ECO2FUME® and 3 levels of exposure time tested; 5, 8 and 12 days. Each treatment combination consisted of 3 replications. The tobacco bale boxes were arranged in a fully randomized fashion.

The test insects used in these trials were all life stages of the cigarette beetle, L. serricorne (eggs, larvae, pupae and adults). There were two sets of test insects collected. The strongly resistant test insects were collected from a leading cigarette company and the weaker resistant test insects were from a leading cigar company. Each stage was assigned a plastic tube with dry tobacco leaves as a culture media. The plastic tubes covered with gauze which allowed the fumigant to penetrate into the tube through it. There were about 40 individuals of each stage of both strains of cigarette beetles. The plastic tubes were then placed into the center of tobacco bales and buried with dry tobacco leaves. Gas sampling hoses were installed into the space between pallets and tobacco bales and into the center of tobacco bales, for monitoring the phosphine concentration during fumigation.

Each tobacco bale with test insects was then covered with PVC fumigation sheets (thickness 0.15 mm = 150 µm) to make fumigation enclosures. The edges of the top covering sheet and the under liner sheet were folded together into interlocking folds and pressed down

with sand snakes for sealing. The folded plastic sheets were also secured with Teflon tape on to the concrete floor for extra sealing.

The equivalent amount of phosphine from $ECO_2FUME^{®}$ was injected inside the tarp using a braided stainless steel hose and gun type gas injector with gas flow rate of as low as 1 g/sec. The exact amount of $ECO_2FUME^{®}$ dispensed was determined by the weight change of the cylinder on top of a digital weighing scale accurate to 0.001 kg or 1 g. Fumigation was terminated at 5, 8 and 12 days of exposure time and followed by aeration of the slightly opened enclosure until the phosphine concentration reached the threshold limit value (TLV) of 0.3 ppm or lower. The plastic cover sheets were completely removed afterwards.

Phosphine gas concentrations were monitored using two lengths of ¼ inch diameter plastic tubing as gas sampling lines, one located at the core of the bale and one below the bale within the frame of the wooden pallet. Monitoring was conducted at each of the following times; 1) 6, 12, 24, 48, 72, 96, 120 h for 5 days exposure time, 2) same as item 1 plus 144, 168, 192 h for 8 days exposure time and 3) same as item 2 plus 216, 240, 264, 288 h for 12 days exposure time. Phosphine concentration readings were made with a calibrated SILOCHEK® phosphine monitor (0–2000 ppm).

When the phosphine concentration fell below the target concentration, top up of $ECO_2FUME^{®}$ dosing was conducted to bring the concentration back to or above the target concentration. The top up procedure was conducted in the same way as the initial gas dispensing, the exact amount calculated based on the difference between the target concentration and the actual reading.

Mortality of adult stage test insects was evaluated shortly after the fumigation period was completed (after aeration). To ensure accurate assessment of mortality of larvae, pupae and eggs stages, tubes were examined 5 – 7 days after fumigation. The eggs mortality was further assessed within two weeks of the first mortality assessment if any larvae emerged from eggs that survived.

RESULTS AND DISCUSSION

1. Five Days Exposure Time

Mortality of the test insects exposed to 5 days fumigation showed that the test insects from the cigarette company appeared to be of stronger resistance than the test insects from the cigar company. As shown in Table 1, dosages of 350 ppm and 700 ppm did not achieve 100% mortality for all stages of insects. The larval stage also appeared to be more tolerant than the inactive egg and pupal stages at dosages of 350 ppm and 700 ppm. A dose of 1000 ppm had two replicates (R2 and R3) achieving 100% mortality for all stages of insects. Replicate 1 (R1) achieved 100% mortality for the adults, pupae and eggs but only 82.4% for the larvae.

Based on the phosphine concentration profile of the three replicates as shown in Fig. 1, it can be seen that R1 which showed <100% mortality for the larvae had two days (day 4 and day 5) with phosphine concentration way below the target concentration of 1000 ppm. The other two replicates (R2 and R3) had phosphine concentration below 1000 ppm at day 3 but a top up of phosphine was conducted to bring the phosphine concentration back to the target of 1000 ppm. The operator missed the top up of R1 which resulted in a further decline in

phosphine concentration. This is a good learning experience on the importance of regular monitoring of gas concentration and the topping up of phosphine to ensure that the target lethal concentration is maintained. From the results, it can be deduced that maintaining the target concentration for the whole fumigation period will ensure the achievement of minimum concentration-time (ct) product for 100% mortality of all stages of insects. If the target phosphine concentration of 1000 ppm had been maintained in R1 then 100% mortality of larvae could have been achieved. In this trial, top up of phosphine with ECO_2FUME was done safely and quickly using a stainless steel quick dispensing hose with gun type gas injector.

Table 1. Mortality of the different stages of cigarette beetle at the cigarette company at four levels of phosphine concentration (0, 350, 700 and 1000 ppm) for the three replicates (R1, R2, R3) at 5 days of exposure time

Treatment	Adult % Mortality	Pupae % Mortality	Larvae % Mortality	Eggs % Mortality
Control R1	0.0	0.0	0.0	0.0
Control R2	0.0	0.0	0.0	0.0
Control R3	0.0	0.0	0.0	0.0
350 ppm R1	97.5	100.0	94.1	80.0
350 ppm R2	100.0	60.0	88.2	85.0
350 ppm R3	92.5	60.0	88.2	90.0
700 ppm R1	100.0	60.0	88.2	100.0
700 ppm R2	95.0	100.0	94.1	100.0
700 ppm R3	100.0	100.0	94.1	100.0
1000 ppm R1	100.0	100.0	82.4	100.0
1000 ppm R2	100.0	100.0	100.0	100.0
1000 ppm R3	100.0	100.0	100.0	100.0

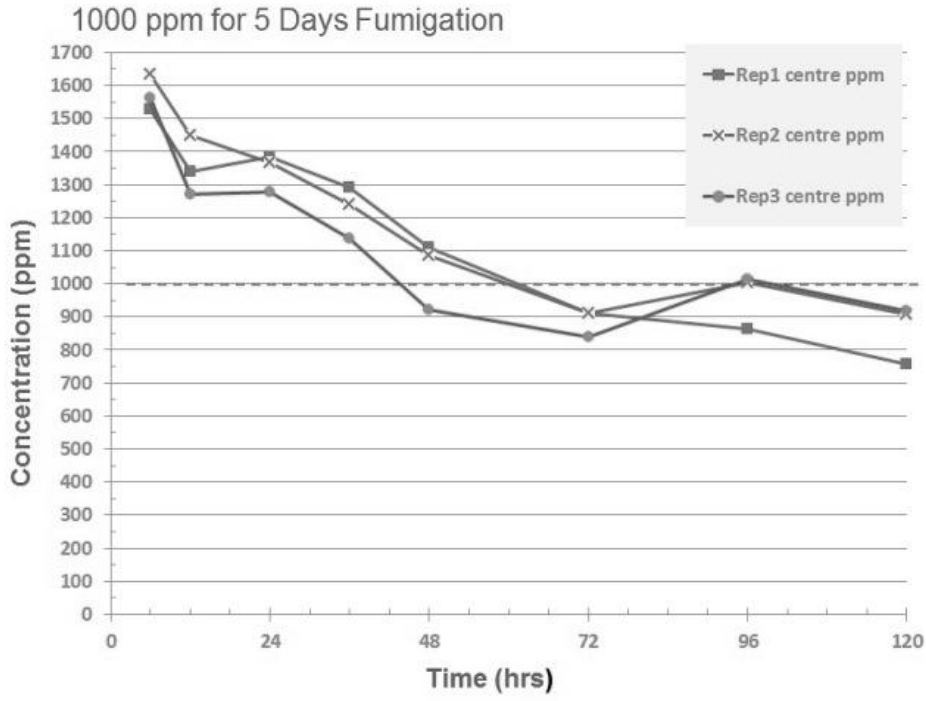

Fig. 1- Phosphine concentration at five days fumigation for the three replicates.

In Fig. 1, the initial phosphine concentration for the three replicates was much higher than the target concentration of 1000 ppm since the phosphine gas was occupying only the empty air space around the tobacco bale and in between tobacco leaves and not the whole volume of stock. The phosphine dosage was based on the total volume of the stock such that a higher concentration of 1550 – 1650 ppm was reached initially. As fumigation progressed, there were gas losses due to gas leakage and phosphine sorption into the tobacco leaves.

The test insects from the cigar company were less resistant with almost all of the replicates of the 700 ppm and 1000 ppm treatments achieving 100% mortality at 5 days exposure time (Table 2). The 350 ppm treatment achieved <100% mortality for the four stages of insects.

2. Eight Days Exposure Time

Table 3 shows that mortality of the four stages of test insects of the strongly resistant (SR) strain at four levels of phosphine concentration (0, 350, 700 and 1000 ppm) for the three replicates (R1, R2, R3) at 8 days of exposure time. The 350 ppm dose was not sufficient to control stages other than eggs. In the treatment of 700 ppm, replicate 2 achieved only 76.5% mortality of larvae as compared to 100% mortality for replicates 1 and 3. This can be explained from the phosphine concentration profile of replicate 2 which showed that the concentration fell below the target concentration for 5 out of 8 days and thus had a much lower ct-product than replicates 1 and 3. In contrast the three treatments of 350 ppm, 700 ppm and 1000 ppm for 8 days achieved 100% mortality for all stages of the weaker resistant (WR) test insects from the cigar company.

Table 2. Insect mortality of the four stages of test insects at the cigar company at four levels of phosphine concentration (0, 350, 700 and 1000 ppm) for the three replicates (R1, R2, R3) at 5 days of exposure time

Treatment	Adult % Mortality	Pupae % Mortality	Larvae % Mortality	Eggs % Mortality
Control R1	0.0	0.0	0.0	0.0
Control R2	0.0	0.0	0.0	0.0
Control R3	0.0	0.0	0.0	0.0
350 ppm R1	100.0	100.0	100.0	100.0
350 ppm R2	100.0	80.0	100.0	100.0
350 ppm R3	95.0	80.0	80.0	90.0
700 ppm R1	100.0	100.0	100.0	100.0
700 ppm R2	100.0	100.0	100.0	100.0
700 ppm R3	100.0	100.0	100.0	100.0
1000 ppm R1	100.0	100.0	100.0	100.0
1000 ppm R2	100.0	100.0	100.0	100.0
1000 ppm R3	100.0	100.0	100.0	100.0

Table 3. Insect mortality of the four stages of test insects at the cigarette company at four levels of phosphine concentration (0, 350, 700 and 1000 ppm) for the three replicates (R1, R2, R3) at 8 days of exposure time

Treatment	Adult % Mortality	Pupae % Mortality	Larvae % Mortality	Eggs % Mortality
Control R1	0.0	0.0	0.0	0.0
Control R2	0.0	0.0	0.0	0.0
Control R3	0.0	0.0	0.0	0.0
350 ppm R1	97.5	60.0	100.0	100.0
350 ppm R2	100.0	100.0	88.2	100.0
350 ppm R3	92.5	80.0	76.5	100.0
700 ppm R1	100.0	100.0	100.0	100.0
700 ppm R2	100.0	100.0	76.5	100.0
700 ppm R3	100.0	100.0	100.0	100.0
1000 ppm R1	100.0	100.0	100.0	100.0
1000 ppm R2	100.0	100.0	94.1	100.0
1000 ppm R3	100.0	100.0	100.0	100.0

3. Twelve Days Exposure Time

The three treatments of 350 ppm, 700 ppm and 1000 ppm achieved 100% mortality for both resistant strains except for one replicate (R2) which showed only 94.1% mortality of larvae of the SR strain from the cigarette company at 350 ppm. The lower than 100% mortality of test insects in R2 was again due to the phosphine concentration falling way below the target phosphine concentration of 350 ppm in 6 out of 12 days.

CONCLUSIONS AND RECOMMENDATIONS

1. Effective control of all stages of both SR and WR strain insects could be achieved in 5 to 12 days.
2. The larvae stage of SR cigarette beetle is the most tolerant stage.
3. Based on the phosphine concentration profile analysis the proposed fumigation protocols are:
   a. 1000 ppm for 5 days at 28°C average temperature or higher for strong resistant strain
   b. 700 ppm for 5 days at 28°C average temperature or higher for weak resistant strain
   c. 700 ppm for 8 days 28°C average temperature or higher for strong resistant strain
   d. 350 ppm or 500 ppm for 8 days 28°C average temperature or higher for weak resistant strain
   e. 350 ppm for 12 days 28°C average temperature or higher for strong resistant strain
   f. 350 ppm or 200 ppm at 28°C average temperature or higher for weak resistant strain
4. These protocols are suitable for tropical climates in Indonesia and other SE Asian countries with similar phosphine resistance issue.
5. The proposed protocols can be effectively obtained using cylinderized phosphine gas such as $ECO_2FUME^{®}$.
6. Validate the proposed fumigation protocols in larger scale trials before adoption in Indonesia and other tropical countries.
7. Optimize the dose for the weakly resistant strains of cigarette beetle.
8. Replicate these trials to cover lower temperature ranges common in temperate climates where a similar issue of strong phosphine resistance is encountered.

REFERENCES

Cavasin, R., McSwigan, B., Ryan, R. and Gock, D. ECO2FUME® global application updates. Paper presented at the 6th International Controlled Atmosphere and Fumigation Conference 2000, Fresno, CA USA, 29 October – 3 November 2000.

DISCLAIMER

Cytec Industries Inc. in its own name and on behalf of its affiliated companies (collectively, "Cytec") decline any liability with respect to the use made by anyone of the information contained herein. The information contained herein represents Cytec's best knowledge thereon without constituting any express or implied guarantee or warranty of any kind (including, but not limited to, regarding the accuracy, the completeness or relevance of the data set out herein). Nothing contained herein shall be construed as conferring any license or right under any patent or other intellectual property rights of Cytec or of any third party. The information relating to the products is given for information purposes only. No guarantee or warranty is provided that the product and/or information is adapted for any specific use, performance or result and that product and/or information do not infringe any Cytec and/or third party intellectual property rights. The user should perform its own tests to determine the suitability for a particular purpose. The final choice of use of a product and/or information as well as the investigation of any possible violation of intellectual property rights of Cytec and/or third parties remains the sole responsibility of the user.

Trademark Notice: The ® indicates a Registered Trademark in the United States and the ™ or * indicates a Trademark in the United States. The mark may also be registered, the subject of an application for registration or a trademark in other countries.