ENVIROSOL TECHNOLOGY - A NEW APPROACH TO FUNGICIDE APPLICATION IN GREENHOUSES

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ABSTRACT

Envirosol technology, which uses carbon dioxide to deliver pesticides as aerosol droplets into enclosed spaces, is being developed to deliver fungicides into greenhouses. Three experimental aerosol formulations for the control of powdery mildew in cucumbers, and grey mould in tomatoes, were evaluated in two separate trials. The high pressure aerosol formulations were sprayed into the greenhouse through an automated application system with spray nozzles strategically placed in the greenhouse. Severity of powdery mildew in cucumbers was reduced by 65%, and incidence of grey mould on tomatoes was reduced by 68% when treated with these formulations.
Keywords: application technology, envirosol fungicides, cucumber powdery mildew, tomato grey mould.

INTRODUCTION

The use of liquefied gases under high pressure to dissolve and propel active ingredients of pesticides as aerosols in enclosed spaces (Grant 1981; Slatter *et al.* 1981) is termed "Envirosol Technology" by BOC Gases. Premixed products, in pressurised cylinders are generally applied through automatic space sprayers. Envirosol products such as Permigas, Pestigas and Insectigas are commercially available for the control of insect pests in domestic, commercial and industrial buildings. For the export cut flower and asparagus industries, Floragas and Hortigas were developed as postharvest fumigants (Carpenter 1987; Carpenter and Stocker 1992). Phosphine, a fumigant for stored grain, is being developed by BOC Gases Australia (Karunaratne *et al.* 1997; R. Ryan pers. comm.). Approval for its use in New Zealand is currently being sought.

Greenhouse pesticide application technologies used by growers include high-volume spraying using motorised pumps and reduced-volume spraying with thermal pulse-jet foggers, air-assisted rotary mist applicators, mechanical aerosol generators (cold foggers) and electrostatic sprayers (Lindquist *et al.* 1993). The limitations of these methods include inadequate coverage of foliage with pesticide, the need to mix and dispose of pesticides, and run-off of excessive pesticide. In addition, applicator exposure to potentially harmful pesticides, particularly in closed environments, can be hazardous. High volume spraying of mature greenhouse crops in an average sized commercial enterprise is time consuming and uncomfortable for operators, especially when heavy protective garments are worn.

Powdery mildew (*Spherotheca fuliginea*) in cucumbers, and grey mould (*Botrytis cinerea*) in tomatoes are important fungal diseases of greenhouse crops (Jarvis 1992). Effective control of these diseases is often difficult, requiring frequent applications of fungicides. The chemicals used to manage these diseases are generally protectants and complete coverage of foliage is essential.

Three experimental formulations, EVF304 containing chlorothalonil only, EVF307 containing a mixture of chlorothalonil and triadimefon, and EVF900 containing iprodione only, were received from BOC Gases New Zealand Ltd for evaluation

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against powdery mildew in cucumbers and grey mould in tomatoes. There is no documented information on the efficacy of liquefied gas formulations for disease control. This paper outlines trials to evaluate their efficacy.

MATERIALS AND METHODS

Establishment of crops

The evaluation trials were conducted in heated greenhouses at the Levin Research Centre, New Zealand Institute for Crop & Food Research Ltd. Seeds of the long English cucumber (*Cucumis sativus* L.) cv. 'Fabiola' were sown directly into bags of potting mix on three sowing dates: 26/8/96, 28/11/96 and 28/2/97. Bags were held in three greenhouses with 105 m^2 floor area (volume 315 m^3) and irrigated daily through an automated irrigation system. Liquid nutrients were delivered to the bags in the irrigation water in a drain-to-waste system. Crop training was carried out according to commercial practice. When the crop reached a height of 2.2 m, it was topped to stop any further growth. Twelve rows of eight cucumber plants were established in each of the three greenhouses and border plants were established along the perimeters of the trial. Day/night temperatures in each greenhouse were set at 20/18°C, and relative humidity fluctuated between 50 and 80%, depending on watering and external weather conditions.

After the cucumber trials had been completed, the same greenhouses were sown with tomatoes (*Lycopersicum esculentum* L.) cv.'Taupo' on 15 July 1997. Seeds were sown directly into bags of potting mix and liquid nutrients were supplied to the bags as for the cucumber crop. Crop training followed commercial practice. Plants were topped at 2.2 m, leaving three leaves above the 7th truss. Fourteen rows with ten plants in each row were established in each of the greenhouses for the trial. The day/night temperatures in each greenhouse were set at 20/15°C and relative humidity fluctuated between 50 and 70%.

Experimental design and treatments

EVF307-treated plants in the cucumber trial were compared with untreated controls. Five grams of test product/m^2 (equivalent to 500 g ai/ha) were sprayed three times at weekly intervals, commencing at the first appearance of powdery mildew on the foliage. Greenhouses 1, 2 and 3 were sprayed on 4/11/96, 11/11/96, 18/11/96; 29/1/97, 5/2/97, 12/2/97; and 14/4/97, 21/4/97, 28/4/97 respectively, giving three replicates in time. Spraying was carried out using an automated spray application system with two nozzles, situated at the gable ends facing each other.

In the tomato trial (Table 1), each greenhouse received three sprays of the same treatment fortnightly which were treated as replicates in time. The spray treatments were applied through the same delivery system as in the cucumber trial.

TABLE 1: Formulations, dose rates and dates of applications of envirosol treatments to tomatoes.

Green-house	Treatments	Active ingredient	Dose rates g ai/m^2	1st application (replicate 1)	2nd application (replicate 2)	3rd application (replicate 3)
1	EVF304	chlorothalonil	0.1	19/11/97	2/12/97	15/12/97
2	EVF900	iprodione	0.1	19/11/97	2/12/97	15/12/97
3	EVF304 + EVF900	chlorothalonil + iprodione	0.05 + 0.05	19/11/97	2/12/97	15/12/97

Untreated control plants

In each greenhouse, one plant in each row was randomly selected to provide an untreated control. The control plants were surrounded by upright iron rod frames (diameter 1 m) covered with plastic sleeves (thickness 80 µm) which were sealed at the top. When not in use, the plastic covers were rolled up to the top of the frames and fastened. Before spraying, the covers were pulled down and fastened securely to prevent spray entering the enclosures. Disease intensity in the control plants was used as a check. The morning after spraying, the greenhouses were vented for 30 minutes before the plastic sleeves were rolled up again.

Tomato inoculation

Grey mould in the tomato crop was introduced artificially. Standard inoculum of an isolate of *B. cinerea* was prepared on 10 mm discs of unripe tomato fruit wall tissue and applied to petioles using the method of Morgan (1979).

Three petioles above the 7th truss on each treated plant were inoculated 48 h after spraying. An untreated control plant in each row was also inoculated at the same three positions. Twenty- four plants including 12 control plants were inoculated in each greenhouse after the first spraying. At the second spraying the same control plants were used, but treated plants next to the previously inoculated ones were used. At the third spraying the same procedure was carried out. This maintained uniformity of leaf position for the assessment of treated plants. For the untreated controls, however, progressively lower leaves were taken for inoculation.

Disease assessments

The efficacy of EVF307 against powdery mildew in the cucumber trial and its effect on the phytotoxicity were assessed between 10 and 14 days after the third spray, depending on the progression of the powdery mildew. Disease severity was assessed visually on ten control, and ten treated plants chosen randomly. The first five leaves (excluding the oldest leaf) on each treated and untreated plant were assessed for powdery mildew infection. A visual severity rating was used: 1=no colonies visible to the unaided eye; 2=few scattered, small discrete colonies; 3=large but still discrete colonies; 4=colonies merging to form large mildew colonies; 5=mildew covering half the total leaf surface; 6=most leaf surface covered by the fungus (Coyier 1986).

Disease assessment in the tomato trial was carried out between 5 and 7 days after inoculation. Disease incidence was recorded as 0 or 1 indicating absence or presence of lesions caused by *B. cinerea* on each of the three inoculated petioles.

Pesticide residues

Fruit samples were picked for fungicide residue analysis at and after the third spray for cucumber, and second and third sprays for tomato. Fruit were picked from untreated and treated plants on Days 1, 2, 3, 4, 5 and 7 after spraying. At each of these times, ten fruit were randomly picked and each fruit was weighed, placed in a plastic bag, then stored at -20°C. When the sampling was complete, the frozen fruit samples were freighted (at -20°C) to Analytical Research Laboratories (ARL) in Hastings. Fruits were analysed for quantities (mg) of chlorothalonil and iprodione per kilogram of fruit.

Statistical analysis

Analysis of variance was carried out on severity rating data from the cucumber trial using the statistical package, Genstat 5 Release 4.1 for Windows. Variability between leaves on each plant was negligible. Therefore, mean scores over five leaves for each plant were used for the analysis. The influence of time on treatment effects was removed in the analysis to ensure realistic comparisons could be made between treatments.

Data from the tomato trial are presented as a binomial response, the number of leaves infected out of three, and analysed using the Generalized Linear Mixed Model (Genstat procedure GLMM) with logit link function. GLMM provides residual maximum likelihood analysis with predicted means and SEDs in logit terms for the tests, and natural means between 0 and 3. For ease of discussion and presentation, the results are tabulated as natural means.

RESULTS

Powdery mildew on cucumber

EVF307 applied to cucumber plants as an aerosol fog reduced ($P<0.001$) powdery mildew on foliage compared to the untreated control. Untreated control plants had a disease severity rating of 4.8±0.12, while plants treated with EVF307 had a rating of 1.7±0.12. Powdery mildew colonies covered nearly 50% of the leaf area in untreated plants. Only small discrete colonies were visible on treated foliage. Phytotoxicity on leaves was negligible.

Grey mould in tomato

The proportion of lesions found on treated petioles of tomato plants was less (P<0.001) than for the untreated controls (Table 2) for all three fungicide formulations.

TABLE 2: Mean numbers of tomato petioles (out of three) that showed *B. cinerea* lesions for plants receiving different envirosol treatments.

Treatment (ai)	Disease incidence	Phytotoxicity
EVF304 (chlorothalonil)	1.52 b[1]	Negligible
EVF900 (iprodione)	0.89 a	No symptoms
EVF304 + EVF900	1.57 b	Negligible
Untreated control	2.79 c	Not applicable

[1]Pairs of means followed by the same letter are not significantly different (P>0.05).

Averaged over the three fungicide treatments, lesion incidence was suppressed by 52% compared with the untreated control. There were fewer (P=0.07) lesions on plants treated with EVF900 than on plants treated with EVF304. EVF900 suppressed disease incidence by 68% when compared with the untreated control. EVF304 and EVF304 sprayed in combination with EVF900, gave similar levels of control. Phytotoxicity seemed negligible in all of the treatments applied, although EVF304 caused some scattered leaf scorching.

Pesticide residues in fruit

Chlorothalonil and triadimefon residues found in cucumber fruit sprayed with EVF307 were generally very low, ranging between not detectable (<0.01 mg/kg) and 0.15 mg/kg of fruit. The levels of chlorothalonil and iprodione in tomatoes at each sampling time were also low and varied between not detectable (<0.01 mg/kg) and 0.08 mg/kg fruit for chlorothalonil, and not detectable (<0.02 mg/kg) and 0.44 mg/kg for iprodione. Fungicide residues were not detected in cucumber and tomato fruit from control plants, confirming that the method used to exclude spray drift from control plants was effective.

DISCUSSION

The aerosol fungicide formulations developed by BOC Gases New Zealand Ltd contain active ingredients commonly used in many conventional formulations for water-based application with well-established efficacy against important foliar diseases. Conventional formulation for water-based application was not included in the trial. The three aerosol fungicide formulations evaluated in these trials showed that when fungicide active ingredients are formulated in liquefied gases, they remain effective.

Three applications of EVF307 at approximately weekly intervals gave economic control of powdery mildew on cucumbers. The dose rate evaluated here was half the quantity used per unit area for conventional water-based formulations. The formulation did not appear to cause any leaf scorching at the rate used. The variation of powdery mildew scores between leaves on plants, and between plants, was not statistically significant, which suggests that envirosol technology satisfactorily distributed and deposited fungicide on foliage.

The automated application of EVF304 and EVF900 on petioles before inoculation with *B. cinerea* also gave excellent disease control. The tomato canopy is generally more dense than the cucumber canopy. The levels of disease control achieved indicate that the aerosol effectively penetrated the crop canopy. Excellent foliage coverage and, consequently, good disease control was probably achieved by generating aerosol droplets ranging in size between 3 and 9 μm mean diameter (Slatter *et al.* 1981).

Most natural infection in commercial greenhouse crops comes from the deposition of airborne spores on plant surfaces. Aerosol fog settles on foliage in much the same way, ensuring placement of fungicide where spores are likely to alight. Morgan (1981), working on the distribution and persistence of iprodione applied by thermal fogging in a greenhouse tomato crop, found that there was a good correlation between the residues found on plants at six positions within the greenhouse, and the incidence of infection from artificial inoculations with *B. cinerea* on petioles.

Pesticide residues found in cucumbers and tomatoes picked after spraying in the two trials were consistently lower than the maximum residue levels of 5 mg/kg allowed for fruiting vegetables (Eichelbaum 1994).

Soon after spraying, the greenhouse appeared fogged and the fog remained visible for up to an hour. Giles *et al.* (1995) have shown that airborne concentration of the pesticide permethrin applied as a thermal fog decreased by 60% in the first hour after application, and by 95% 12 hours after application.

The ease with which each spray application was made suggests that the method will be useful for growers and/or pesticide applicators. The method reduces hazards for operators, and eliminates the problems of leftover chemicals and run-off. Complete automation and the ability to integrate this system with existing greenhouse environmental control systems increase the potential of this technology for use in the greenhouse industry.

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