# Distribution of Chemical and Microbial Pesticides Delivered Through Drip Irrigation Systems

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## 1. Introduction

The current lack of scientific data for effective delivery of pest-control agents for soil pests leaves the nursery or greenhouse industries vulnerable to possible crop loss or damage, rendering the crop unmarketable either due to appearance or quarantine. Spray is the most used method to apply agrichemicals and bio-products because spray application is timely and convenient to control pests, but there are several impediments with spraying pesticides. For example, pesticides may not be efficiently applied to intended targets because of large gaps between trees where pesticides are not needed; spray drift may affect residential areas nearby nurseries; plants physically disturbed or damaged by spraying may have lower market values; and extensive skills are required for applicators to properly operate sprayers. Also, applying pesticides uniformly and sufficiently to target pests under the soil or container substrates is a challenging constraint of current spray technologies.

Drip irrigation, noted for its highly efficient water distribution capability to increase crop yields (Grabow et al., 2006; Plaut et al., 1996; Bryla et al., 2003; Lamm and Trooien, 2003; Zhu et al., 2004), offers an alternative strategy to carry the label-allowed pest control agents to the target areas in the soil for effective insect or disease control (Felsot et al., 1998). Uniform chemical distribution pattern in the soil plays an important role to achieve high pest control efficiency and a sustainable safe environment. Chemigation includes applications of insecticides, fungicides, herbicides, fertilizers, microbial biopesticides and nematicides by means of irrigation systems. In production nurseries with drip irrigation systems in place, injection of pesticides into irrigation lines (or chemigation) offers an alternative strategy for efficient and economical application of pesticides to targeted zones in soil or container substrates The drift problem caused by spraying pesticides and costs associated with sprayers can be eliminated by using chemigation. This method has been shown to increase crop yields and reduce chemical leaching (Leib et al., 2000).

Injection of pesticides into drip irrigation lines takes advantage of the fact that active ingredients can be carried by water into root zones (Lamm et al., 2007). The ingredient distribution pattern in the soil plays an important role in pest control efficacy. Drip irrigation was demonstrated as an effective technique to apply water-soluble fumigants to

the target soil (Ajwa et al., 2002). Leib and Jarrett (2003) reported limited leaching of Imidacloprid with 70% of the pesticide still located in the root zone at the end of the 40-day evaluation period when efficacy ended.

Because leaching potential is a major concern for using drip chemigation to apply agrichemicals (Jaynes et al., 1992), only few chemicals such as Imidacloprid are registered for drip applications. Delivery of alternative fumigants through drip irrigation system to treat soil to kill nematodes, fungi, and weeds greatly reduced use of fumigants and emission of fumigants from soil (Schneider et al., 2006; Ajwa et al., 2002). Considerable research has been conducted to investigate the efficacy and potential damage to the environments by applying Imidacloprid through irrigation systems (Fleischer et al., 1998; Van Iersel et al., 2000; Felsot et al., 2000; Byrne and Toscano, 2006) after it was released into the market. A multi-year field study reported that the level of Imidacloprid aphicide leaching after chemigation through a subsurface drip system could be significantly reduced if irrigation schedule matched the crop needs (Foslot et al., 2003). This is own to the mobility of Imidacloprid in the soil was very low (Leib et al., 2000).

Due to concerns about health and environmental hazards with traditional pesticides, the use of biopesticides to control pests and diseases has been dramatically increased for past decades (Fuxa and Richter, 1999; Gopal et al., 2001; Hajek et al., 2007; Dorner and Lamb, 2006; Grewal et al., 2001). Lumsden and Locke (1989) reported a very promising control of diseases caused by fungal root rot organisms in the greenhouse production of bedding plants by adding a microbial pesticide *Gliocladium virens* into the soilless substrate before planting seeds.

Since the fact that bio pest control agents are friendly to the environment with no leaching concerns, drip irrigation has become a convenient method to deliver them in the root zone. Entomopathogenic nematodes delivered through drip irrigation had very promising control of different soil pests (Wennemann et al, 2003; Ellsbury et al., 1996; Becker et al., 1989; Reed et al., 1986; Kramer and Grunder, 1998). Wennemann et al. (2003) applied entomopathogenic nematodes through drip irrigation with 2.0 L/h emitters in a vineyard and found the recovery rate of nematodes from drip emitters in 51 m long drip lines ranged from 42 to 92%. Reding et al. (2008) reported that Imidacloprid, clothianidin and entomopathogenic nematodes applied through drip irrigation effectively controlled white grubs in the root zones of various ornamental nursery trees. They also reported that nematodes applied through drip irrigation, injected into the soil, and surface drenched at a curative timing all significantly reduced numbers of grubs compared to untreated trees. These data illustrate drip irrigation is a viable delivery system for control of white grubs in nursery crop production.

Improving water distribution uniformity of drip irrigation systems has been studied extensively (Wu et al., 1979; Lamm et al., 1997; Camp et al., 2003; Clark et al., 2005; Grabow et al., 2006). However, the specific evaluation of a designated pest control agent's uniformity throughout drip lines is lacking, especially for the microbial bio-pesticides before they are used for field trials. Uniform distribution of the pest control agents throughout drip lines and in targeted areas is essential to assure drip chemigation can achieve both efficient pest/disease control and environmental safety. Physical properties of pest control agents, especially bio-pesticides, are quite variable. There are questions whether drip chemigation can uniformly distribute them throughout drip lines and within targeted areas. Efficacy of chemical and biopesticides is dependent on the amounts of water applied to facilitate movement of the chemical into the root zone. Deliverability and uniformity of many these materials have not been evaluated comprehensively under controlled conditions before they are released for field uses. Biopesticides are usually granular compounds or living organisms and are normally suspended in water. Their movement and mixture uniformity inside drip lines are influenced by fluid motion in either turbulent or laminar flow status, which varies with flow rate. Also, water use efficiency and irrigation practice are greatly affected by emitter flow capacities. Little information is available on distribution patterns of different water soluble or insoluble materials discharged from different flow-capacity (or flow-rate) emitters throughout drip lines. A quantitative relationship among emitter size, flow rate and chemical type may be helpful to develop strategies to apply suspendible granular biopesticides through drip irrigation systems.

Studies on water soluble agro-chemicals and nematodes injected into drip lines under field conditions have been reported, but there is no comprehensive study comparing distribution profiles of agro-chemicals and bio-compounds in the soil after they are injected into a system with different emitter flow capacities under controlled irrigation conditions. Little information is available on distribution patterns of different water soluble or insoluble materials in the soil under different emitter sizes and flow capacities. Another important consideration is that the chemical and microbial control agents may be less effective when surface applied due to active agent becoming bound in organic or other material at the surface. Consequently, excessive levels of surface application are required to achieve efficacy, at greater expense. Scientific information on distribution patterns of active agents in the soil with different emitter sizes and flow rate is essential to help develop strategies to apply suspendible granular biopesticides uniformly by using drip irrigation systems.

The objectives of this research were: (1) to investigate the capability of drip irrigation systems for delivering water soluble chemicals, suspendible microbial bio-insecticides and bio-fungicides, and entomopathogenic nematodes; (2) to investigate distribution patterns of these chemical and microbial pesticides in the soil. To achieve these objectives, the distribution uniformity and recovery rate of these materials throughout drip lines and in the soil were evaluated under controlled conditions as they were discharged from emitters of three flow capacities, and thus to verify whether increasing emitter flow capacity would significantly change distribution patterns of these materials along the drip lines and in the soil.

#### 2. Materials and methods

#### 2.1 Drip irrigation system design

A drip irrigation system was developed to test the application uniformity of agrochemicals and microbial bio-pesticides throughout drip lines (Wang et al., 2009). Variables including emitter flow, amount of injected materials and injection time could be individually controlled with the system. The system included three, 79 m long drip lines, a portable chemical injection unit, a shutoff valve for pressure control, a pressure sensor (Model 242PC60G, Micro Switch, Freeport, IL), a flow meter (Model DFS-2, DGH Corporation, Manchester, NH), and a backflow prevention check valve (Model T-413, Nibco Inc., Elkhart, IN). The portable chemical injection unit was installed at the beginning (upstream end) of each drip line.

The injection unit (fig. 1) included an injection valve assembled with a 1.27 cm (nominal  $\frac{1}{2}$  inch) thread PVC tee, a nominal 1.27 cm (nominal  $\frac{1}{2}$  inch) NPT electric wire connector (Kleinhuis North America, Inc., Worthington, OH), a bladder valve removed from a 40 cm diameter plastic toy ball (Item# 3314903313, Ball, Bounce, and Sport Inc. Ashland, OH), and

a modified 50 ml Pro-Pistol<sup>TM</sup> pistol grip syringe (Model 1005, Neogen Corporation, Lexington, KY). The bladder valve performed as a one-way check valve for chemicals injected into the drip line with the syringe. After the syringe was removed, the valve prevented leakage of the pressurized liquid at the injection point. The backflow prevention valve was installed in the drip line upstream of the injection valve, to prevent chemicals flowing upstream to the main water line. The pressure and flow rate near the injection point were measured with the pressure sensor and flow meter which were connected to a micro data logger (Model CR23X, Campbell Scientific, Logan, UT). The data logger was programmed to acquire these data at 1-second intervals during the experiment.

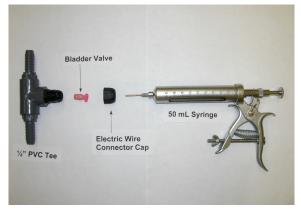


Fig. 1. Chemical injection unit assembly used to inject pesticide into drip lines.

The drip lines were three polyethylene tubes with external pressure-compensating emitters (Model WPC, Netafim USA, Fresno, CA) of three different flow capacities. The nominal flow capacity of emitters on the three drip lines was 1.9 L/h (line 1), 3.8 L/h (line 2), and 7.6 L/h (line 3), which covers the flow capacity ranges normally used for drip irrigation systems in ornamental nursery applications. The flow path of pressurized liquids within each emitter was controlled with a flexible diaphragm in the center donut-shaped chamber that reduces the flow path dimension with increasing pressure and with a series of baffles projecting from inside and outside the walls of the chamber. The diaphragm functioned as a variable-flow storage compartment as well as for a pressure compensation function. The inside and outside baffles alternating within the emitter formed a resistive flow path to discharge the pressurized liquid. The length, depth and width of the flow path in the chamber of the emitters for line 1 were 61, 1.07 and 1.17; for line 2 they were 1, 60, 1.30 and 1.40 mm; and for line 3 they were 17, 1.60 and 1.60 mm, respectively.

Each drip line contained a polyethylene tubing with 13.2 mm nominal inside diameter and 1.27 mm nominal wall thickness. The total number of emitters on each 79 m drip line was 87, spaced at 0.9 m intervals. In nurseries, it is common to grow crops in a row with less than 79 m length. The barb of emitters was inserted 4.2 mm inside the drip line tubing. Distance from the injection point to the first emitter was 0.45 m. For each replication of the treatment, only one line was used while the other two lines were disconnected.

To determine the flow rate and pressure to be used, water flow rate uniformity from emitters throughout each drip line was examined at four pressures of 69, 103, 138 and 276 kPa, respectively. The amount of water from 7 emitters at 4.1, 17.6, 31.1, 44.6, 58.1, 71.6 and

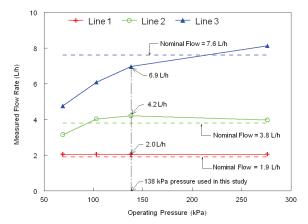


Fig. 2. Measured flow rate vs. pressure of the three different emitter capacities used in this study.

77.9 m (or 13.5 m apart) from the injection point was measured for 10 minutes and repeated three times. Figure 2 shows the relationship between the average water flow rate of the 7 emitters and the operating pressure for the three lines of different nominal flow capacities tested. The emitter flow rates on line 1 (nominal flow capacity of 1.9 L/h) and line 2 (nominal flow capacity of 3.8 L/h) were almost constant over pressure ranging from 69 kPa to 276 kPa, but on line 3 (nominal flow capacity of 7.6 L/h) the emitter flow rate increased as the pressure increased from 69 kPa to 276 kPa. The measured flow rate at 138 kPa on line 1 and line 2 was 2.0 and 4.2 L/h respectively which were very close to the nominal values, but on line 3 it was 6.9 L/h which was 15% (or 1.14 L/h) lower than the nominal value. Because of these preliminary test results, the operating pressure of 138 kPa was chosen for all treatments in this research. Also, the pressure 138 kPa was within the pressure range between 50 and 310 kPa recommended by the drip line manufacturer.

#### 2.2 Materials tested

Tests were conducted with five different materials with different types and formulations (table 1): Brilliant Sulfaflavine (BSF, MP Biomedicals, Inc., Aurora, OH), Imidacloprid (Marathon II, OHP, Inc., Mainland, PA), an entomopathogenic fungus (EPF) (Novozymes Biologicals, Inc., Salem, VA), a microbial soil fungicide (SF) (SoilGuard 12G, Certis USA, LLC., Columbia, MD), and entomopathogenic nematodes (EPN, cultured by The Ohio State University, Entomology Department, Wooster, OH).

BSF is a water soluble, non sun-light degradable fluorescent tracer normally used to track pesticide deposition (Zhu et al., 2005). Also, the BSF solution has a nearly constant intensity over the pH range of 6.9–10.4. It was selected for this test because results of BSF could provide a reference to compare performances of other materials discharged through drip emitters.

Imidacloprid is the active ingredient of Marathon II which is a flowable formulation (suspension concentrate dispersible in water) with a viscosity of 84 mPa s. It is a systemic chloronicotinyl insecticide normally applied to the soil for control of root feeding beetles such as scarab larvae (white grubs) or foliar feeding insects such as scale and leafhoppers in ornamental trees. The concentration of Imidacloprid in Marathon II is 0.24 kg/L.

Treatment -	CV (%)*			DU*			
	2.0 L/h emitter	4.2 L/h emitter	6.9 L/h emitter	-	2.0 L/h emitter	4.2 L/h emitter	6.9 L/h emitter
BSF	2.3	1.7	1.8		0.95	0.97	0.96
Imidacloprid	43	36	54		0.76	0.80	0.76
EPF	90	104	119		0.56	0.26	0.33
SF	98	72	51		0.44	0.62	0.68
Nematodes	8.8	8.0	4.9		0.80	0.83	0.91

\* Each value of CV or DU is the mean from 7 emitters in each drip line with three replications. CV and DU were calculated with equations (1) and (2), respectively.

Table 4. Mean coefficient of variation (CV) and distribution uniformity (DU) of BSF, Imidacloprid, EPF, SF and nematodes discharged from emitters with three different flow capacities (2.0, 4.2 and 6.9 L/h) at 138 kPa pressure.

EPF is a suspendible conidial powder microbial insecticide. Its active ingredient is Met52G and Tick EX EC with 2.0% by weight *Metarhizium anisopliae* Sorokin strain F52. The conidial powder contains approximately 5x10<sup>10</sup> conidia per gram. The product has very uniform size. The sizes of conidia are typically 3-4 um wide and 7-9 um long while the average length of the powder granule is about 0.039 mm. The material is insoluble in water and remains on the top of water after standing for 10 minutes. Therefore, for this study, a suspension of conidia was made in 0.05% non-ionic surfactant Tween 80 (Sigma-Aldrich, St Louis, MO) to improve dispersion. The EPF was a fungus normally used to control insects, primarily beetle larvae and ticks on non-food use greenhouse and nursery crops.

SF is a suspendible, granular formulation of a naturally occurring soil fungus (*Gliocladium virens* strain GL-21), containing 12% by weight fungal fermentor biomass with 2x10<sup>7</sup> viable propagules per gram. The compound was formulated to be applied through irrigation lines or drenched into growing media to control diseases caused by fungal root rot pathogens such as *Pythium* and *Rhizoctonia*. The suspendible granules had irregular shapes with a considerably wide range of equivalent diameter: 10% volume less than 0.136 mm, 50% volume less than 0.32 mm, and 90% volume less than 1.06 mm. The maximal granule equivalent diameter was 1.47 mm, and the average diameter was about 0.345 mm. The granules suspend in the water when injected into the drip line.

Lastly, nematodes used in this study were *Heterorhabditis bacteriophora* Poinar strain GPS11 with a concentration of  $2.0x10^6$  nematodes per 100 ml of solution, estimated by counting with a microscope (Woodring and Kaya, 1988). They are typically 500 to 1000 µm long and 18 to 50 µm wide, and are normally used to carry and introduce symbiotic bacteria (*Xenorhabdus* spp.) into the body cavities of insects that eventually kill them within 48 hours. The species and strain of nematodes has shown efficacy for controlling scarab larvae in ornamental nurseries (Reding et al. 2008). The nematodes can be suspended in water but normally settled out within a few minutes if not agitated.

#### 2.3 Experiments through drip lines

Drip lines were attached horizontally to three 2.5-mm diameter high-tensile electric fence wires suspended over 30 cm above the ground (fig. 3). Only one drip line was connected to the water source for each application. In each replication, the tested line was filled with water at a stabilized pressure (138 kPa) before injection and water sample collection.

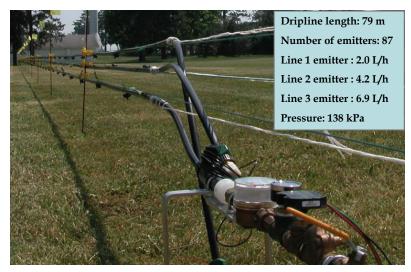


Fig. 3. Three drip lines with three different capacity emitters suspended 30 cm above the ground used in this study.

The 50 mL syringe with a 0.9 mm inside diameter needle was modified to inject a fixed amount of materials into the drip line through the chemical injection unit (fig. 1). Each of the five materials was injected over a 1-minute period for each replication. All materials were mixed with water before they were injected into the drip line. Water samples mixed with each injected material were collected with 3.8 L plastic bottles from 7 emitters at 4.1, 17.6, 31.1, 44.6, 58.1, 71.6 and 77.9 m, respectively. The 7 emitters were the same ones at the same locations used for the water uniformity distribution test as mentioned above. The water flow test verified that the drip lines were able to uniformly distribute water flow through the drip lines as discussed in Results and Discussion section. The sampling began one minute before the start of injections. The collection time for samples from line 1 was 30 minutes, and 15 minutes from line 2 and 3. An estimated time for materials to flow from the injection point to the last emitter was 16.3 minutes in line 1, 7.8 minutes in line 2, and 4.7 minutes in line 3, respectively. These estimated times were calculated with a plug-flow equation similar to the flow equation used for boom sprayers (Zhu et al., 1998). The volume of collected sample from each emitter in line 1 was 1.0 L, 1.05 L in line 2, and 1.72 L in line 3. Each sample was shaken and sub-samples were decanted into glass bottles, and then taken to the laboratory for analysis. After samples were collected, the drip line was flushed by opening the end of the line for 10 minutes and a sample of the flush water was collected from the end of the drip line at the beginning of the flushing cycle. These flushing water samples were analyzed for observation only but not for quantitative comparison because it was very hard to control the amount of water collected at the end of lines during the flushing process. The above process that included the injection of a material into the drip line, the collection of samples and the flushing of drip line was repeated for three times representing three replications for each material and each drip line.

The starting concentration of the BSF solution was 3 g BSF per liter of water which was selected based on pre-trial tests for fluorescent intensity, and fell within the detection range of the spectrometer (Perkin-Elmer Limited, Beaconsfield, Buckinghamshire, England) used

in this research. The viscosity of the solution was 0.887 mPa s, and the amount of BSF solution injected into the drip line for each replication was 50 mL, equaling 0.06 g of BSF. Water samples collected from the emitters were taken to the laboratory where the BSF concentration was measured with the spectrometer calibrated to detect an emission wavelength of 500 nm (Zhu et al., 2005).

The Imidacloprid mixture for each replication was 13 mL of Marathon II and 25 mL of water. This rate was calculated based on the label rate of 50 mL Marathon II per 305 m row. After sample collection, the concentration of Imidacloprid was measured by filtering samples and adjusting the pH to 2. Aliquots of 2 mL were subsequently analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS). The LC-MS/MS system consists of a ProStar<sup>®</sup> 210 solvent delivery module with a ProStar 430 autosampler and a 1200L triple-stage quadrupole mass spectrometer with a dual off-axis ESI interface (Varian Inc., Walnut Creek, CA). A standard concentration of 5 mg/L prepared in methanol and water was used to optimize the instrument and attain precursor and transition ions using argon as the collision gas. A molecular mass to charge ratio of 256 in positive ion mode was used with transition ions (collision energy voltages in parenthesis) 208.8 (12.5), 175.1 (12.0) and 212.0 (8.5). Optimized parameters were 350 °C for the drying gas; ion transfer capillary, nebulizer needle and shield voltages were 40, 4500 and 200, respectively. Injection volume was 20 uL and scan time was 0.1 seconds. A Nova-Pak® C18 column (4 µm, 150 mm × 3.9 mm) and packing-matched guard were used for retention of the analyte (Waters Corporation, Milford, MA). A gradient elution using 0.1% formic acid (A) and 0.1% formic acid in acetonitrile (B) was used at a flow rate of 0.4 mL/min. Solvent B was held for one minute at 5%, and ramped to 95% over four minutes, held for three minutes, then gradually returned to initial conditions over one minute and held for three additional minutes for equilibration. A standard calibration curve, using matrix-matched standards, used the most abundant transition ion for quantitative analysis. The remaining ions, along with retention time as compared to the spiked matrix samples, were used for further confirmation in the sample matrix. The system was calibrated with Imidacloprid at known concentrations ranging from 0.036 to 3600 mg/L.

In the EPF trials, 5.5 g of the EPF formulation was mixed with 50 mL of water and 25 uL of Tween 80 for each replication. The mixture was stored at 5 °C for 24 h and then shaken well before the injection. After water samples were collected at seven locations, they were shaken and sub-samples were poured into 15 mL plastic vials, then sent overnight with an ice pack to a laboratory for analysis. In the laboratory, the water samples were placed in a sonicator for 20 minutes, and 1 mL of each sample was added to 99 mL of phosphate buffer, then 100 uL of this solution was spread on selective (Veen's) media for CFU's incubation (Veen and Ferron, 1966). The media plates were incubated for 4 to 5 days at 27°C and then fungal colonies were counted.

The SF mixture injected into the drip line for each replication was 10 g SF mixed with 100 mL water, and was shaken well before injecting into the pressurized drip line system. After samples were collected from emitters for each replication, they were transferred to the laboratory. Each collected sample was diluted two or three times and then 1 mL aliquoits from the diluted sample were deposited onto the surface of a semi-selective medium containing antibiotics to suppress bacterial growth (3 plates/dilution/sample). Following a period of incubation, colonies were counted and calculations made to determine the number of units (CFU) of active ingredient that were dispensed through each emitter.

The nematode mixture for each replication was 100 mL water and 2.0x10<sup>6</sup> nematodes. It was stored at 5°C for 24 h before the application. After nematode samples were collected, each plastic bottle was held at room temperature 20°C for 24 h to allow the nematodes to settle at the bottom of the bottles, and then most of the water was poured off. The solution remaining in the bottle was then poured into a glass bottle and allowed to set for another 24 h until the nematodes again settled at the bottom. A pipette was then used to remove most of the water until 15 mL of nematode suspension remained. Three 10 µL drops containing nematodes were taken from this suspension and spread on glass microscope slides, then the all the nematodes on a slide were counted under a stereoscopic microscope (Model SZX12, Olympus, Japan) at 50× magnification. The mean number of nematodes in three drops was reported.

After all samples were analyzed, the amount of materials discharged from emitters for each test was normalized for the specific emitter flow rate tested. To determine the effect of emitter flow capacity on the amount of materials discharged throughout the drip line, each group of data for the specific material treatments was first analyzed by one-way ANOVA to test the null hypothesis that all treatments had equal means of the material quantity with Duncan's methods using ProStat version 3.8 (Poly Software International, Inc., Pearl River, NY). If the null hypothesis was rejected, the multiple comparison procedure was used to determine differences among means of the material distributed throughout the drip line. Multiple comparisons for recovery rates across the drip line were also conducted among the five materials and three flow capacities. All differences were determined at the 0.05 level of significance.

Coefficient of variation (CV) and distribution uniformity (DU) were used to quantify the uniformity of distribution of each of the five materials throughout the drip line. CV and DU were calculated by replacing the flow rate with the amount of materials discharged from each emitter defined by ASAE Standards (ASAE EP405.1, 2008), Kruse (1978), and Keller and Bliesner (2000),

$$CV = \frac{s}{q_{ave}} \times 100 \tag{1}$$

$$DU = \frac{q}{q_{ave}} \tag{2}$$

where *s* is the standard deviation of the amount of materials discharged from emitters,  $q_{ave}$  is the mean amount of materials discharged from emitters, and *q* is the mean of the lowest onefourth of the amount of materials discharged from each emitter sampled. These equations have also been used for evaluation of fertigation and chemigation performances such as the effect of injection methods and injection rates on fertigation uniformity (Bracy et al, 2003; Li et al., 2007). Since there were 7 samples of each material collected for each replication, the value of q was calculated by the following equation with expansion of the 7 data to 28 data,

$$q = \frac{4q_{L1} + 3q_{L2}}{7} \tag{3}$$

where,  $q_{L1}$  and  $q_{L2}$  are the lowest and second lowest amounts of the material among the 7 samples, respectively.

## 2.4 Experiments in the soil

To test distribution pattern of chemical and microbial pesticides in the soil after they were discharged from emitters, four materials were selected for the trials. They were BSF, Imidacloprid, EPF, and EPN. SF was not included in the soil test because previous tests demonstrated its uniformity and recovery rate through drip lines were very low.

The distribution patterns of the four materials were determined in a cultivated, 33.5 m long and 12.2 m wide field. No insecticides or other chemicals had been applied to this field for at least three years. Before the experiment, the plot was plowed and rototilled. Soil samples were collected from three depths and bulk density, water holding capacity, porosity, EC (electric conductivity), and pH were determined (Table 2). Water holding capacity was determined with the method used by Cassel and Nielsen (1986).

Soil Depth	Dry bulk density (g/ml)	Water holding capacity (%)	Total porosity (%)	EC (mS/cm)	pН
Top (7.6-10.2 cm)	1.16	29.8	49.3	601	5.6
Middle (15.2-20.3 cm)	1.20	30.2	51.8	202	5.8
Bottom (22.9-30.5 cm)	1.24	28.8	50.2	121	5.6

\* All the values are the mean of three samples.

Table 2. Properties of the soil used for chemical distribution pattern tests\*

The same drip irrigation system reported above was used to test the distribution pattern of agrichemical and microbial materials in the soil. The three drip lines were placed on the surface of the soil with the lines 5 m apart. The drip lines were longer than the soil plot so the excess tubing was coiled at the downstream border of the plot. The rates of BSF, Imidacloprid, EPF, and nematodes used for each trial were 150 mg, 2.8 mL, 5.5 g, and 2,000,000, respectively (Table 1).

A modified 50 mL syringe was used to inject a fixed amount of materials into the drip lines through a bladder valve. Each material was injected, one material at a time, into each line. Only one line at a time was connected to the water source. Irrigation was turned on and pressure was allowed to stabilize at 138 kPa before injection of each material. Injection of each material into the drip line took approximately 1 minute, and then the irrigation continued for 31 minutes for line 1, 14 minutes for lines 2, and 8 minutes for line 3. This irrigation schedule was repeated every 8 hours for 24 hours before soil samples were collected. A total 3.0 L of water was applied through each emitter with all the three flow capacities.

Soil samples of each material were collected 24 hours after last irrigation at 28 different locations near the emitter. A hollow auger with an inner diameter of 25 mm was used to take soil cores. Figure 4 shows the soil auger sampling geometry for each test. Soil samples were taken directly under emitters and 15, 30, 45 cm upstream and downstream from the emitters along the drip line laterally, and from the surface (0 cm depth) and at depths of 10, 20 and 30 cm vertically, with about 15 cm<sup>3</sup> sample from each depth. Thus, for each test, total 28 soil samples were taken from 28 locations evenly spread in the 30 x 91.2 cm section area under the drip line.

During the test period from injection of materials into drip lines to completion of soil sample collection, the ambient temperature ranged from 18° to 29 °C, relative humidity ranged from 45 to 86%, and soil moisture content at the non-irrigation point ranged from 36 to 41%. The

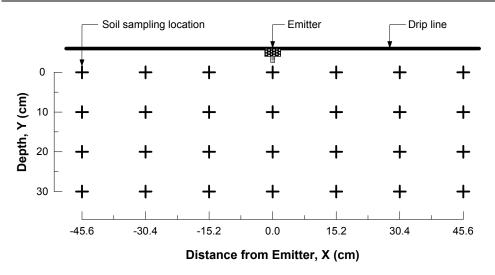


Fig. 4. Locations of soil samples collected for evaluation of chemical distribution patterns in the soil.

moisture content was measured with a Theta probe soil moisture sensor (Delta-T Devices Ltd, Cambridge, England) placed 10 cm in the soil at a point 2.5 m away from the drip lines. There was no precipitation during the experiments.

Amount of BSF in soil samples was determined by a method similar to that used by Barber and Parkin (2003) for a fluorescent tracer CBS-X. A pre-test was conducted to ensure that the concentrations of BSF soil samples obtained from spectrometer were linear with the actual BSF concentrations on the soil. An amount of 10 g soil was weighed into six 100 mL glass jars with six stock solutions (4  $\mu$ L, 8  $\mu$ L, 12  $\mu$ L, 20  $\mu$ L, 40  $\mu$ L and 60  $\mu$ L) at concentration of 3 g BSF per liter water and 50 mL distilled water was added, respectively. Samples were mixed in a rotating drum for 10 minutes under 500 rpm. To clear the samples after mixing, 0.5 g gypsum was added to each sample jar, shaken by hand for 10 s, and then left in the refrigerator until the supernatant had cleared. Once the supernatant was clear, fluorescence concentration readings were taken with the same method as the BSF-water samples mentioned above. The linear corresponding curve between readings and actual BSF concentrations had been obtained and used as a standard. After all soil samples were collected from the field, they were treated using the same methods as used for the pre-test, and the actual BSF concentration in the soil was then measured by the spectrometer.

Soil samples containing Imidacloprid were placed in glass jars, transported to the laboratory, and stored in a freezer at -40 °C until analysis. Methanol was used to extract Imidacloprid from the soil samples (Felsot et al., 1998) and ELISA kits (Envirologix, Inc., Portland, ME, USA) for Imidacloprid were used to determine the amount of Imidacloprid in the soil (Castle et al. 2005).

Soil samples containing EPF were placed in glass jars, and sent overnight with an ice pack to a laboratory for analysis. In the laboratory, the one gram of soil samples were placed in 99 mL of phosphate buffer, then 100 uL of this solution was spread on selective (Veen's) media for CFU's (Veen & Ferron, 1966). The media plates were incubated for 4 to 5 days at 27°C and the fungal colonies were counted.

Presence of nematodes in the soil samples was detected with larvae of the greater wax moth (*Galleria mellonella* L.), an available test for presence of nematodes in soil (Bedding and Akhurst, 1975). Wax moth larvae turned brick red when infected by the strain of nematodes used, and thus nematodes were considered present if larvae changed to that color. Soil samples were placed in metal containers (54 mm in diameter, 37 mm in height), and 3 wax moth larvae were carefully placed on top of the soil, the lid was placed on each container. The containers were incubated in the dark at room temperature for 5 days. During this time, color change and death of the larvae were monitored daily. Samples of untreated soil from the same field were collected, treated as above, and used for comparison.

To summarize soil experimental results, the mean concentration was calculated from three replications at each sampling point for BSF, Imidacloprid and EPF samples. For nematodes, the presence of nematodes was chosen as "Yes" if two or three replications showed the presence of nematodes at each sampling point. The presence was chosen as "No" if there was no or one replication showed the presence of nematodes.

## 3. Results and discussion

#### 3.1 Water flow rate distribution throughout drip lines

Figure 5 shows the amounts of water collected from emitters at seven different distances from the injection point on line 1 for 30 minutes and lines 2 and 3 for 15 minutes, respectively. Data in the figure illustrates that there was little variation in the amount of water discharged from emitters along the drip lines 1 and 2 while there was 6% variation in line 3. That is, water distribution throughout the 79 m drip line varied very little with the 2.0 and 4.2 L/h flow rate emitters and varied considerably with the 6.9 L/h flow rate emitters. It is understandable that the 6.9 L/h emitters could not maintain a constant flow rate at various pressures because their short flow path and limitation of the flexible diaphragm limited the pressure compensation capability. Based on Bernoulli's equation in hydraulics,

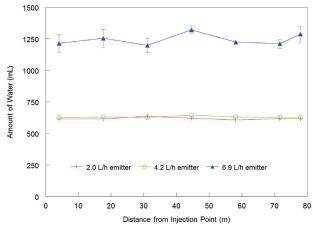


Fig. 5. Actual amounts of water samples collected from 7 locations throughout the 79 m long drip lines for each replication of the tests for the 2.0 L/h (line 1), 4.2 L/h (line 2) and 6.9 L/h (line 3) emitters. Error bars represent standard deviations around means.

flow rates from any hydraulic components are proportional to the multiplication of a flow constant, the cross section area of the flow path, and the squared root of pressure. For pressure compensated emitters with low flow capacity, the flexible diaphragm automatically maintain the flow constant and cross section area very well, and pressure changes within a small range will not produce noticeable changes in the flow rate.

## 3.2 Distribution of tested materials through drip lines

Amounts of measured BSF, Imidacloprid, EPF bio-compound insecticide, SF bio-compound fungicide, and nematodes throughout the three drip lines are shown in Figures 6 through 10. Table 3 shows the predicted and measured amounts of the five materials discharged from three different capacity emitters. The predicted amount was calculated by the amount of material injected into the drip line divided by 87 (the number of emitters in the drip line). The recovery rate shown in Table 3 is the percentage of the average measured amount of materials divided by the predicted amount of materials. Table 4 reports the mean CV and DU of the five materials throughout the entire drip lines with three different capacity emitters, respectively.

There was no significant difference in the amount of BSF discharged from different flow capacity emitters on three drip lines (fig. 6). The recovery rate of BSF was from 86% to 93% for the three drip lines (table 3). The average CV and DU of BSF throughout the 79 m drip line for all three drip lines was 1.9% and 0.96, respectively (table 4). The flow velocity near

Material	Emitter	Mean c	uantity of material per e	emitter	Recovery
flow (L/h)		Predicted	Measured*	Unit	rate (%)**
BSF	2.0	1724	1586 (99)A	μg	92a
BSF	4.2	1724	1486 (47)A	μg	86a
BSF	6.9	1724	1608 (98)A	μg	93a
Imidacloprid	2.0	35.9	18.0 (7.5)B	mg	50b
Imidacloprid	4.2	35.9	28.1 (10.7)A	mg	78a
Imidacloprid	6.9	35.9	12.3 (6.6)B	mg	34bcd
EPF	2.0	5.69x10 <sup>7</sup>	5.1 x106 (2.8 x106) A	CFU	9.0de
EPF	4.2	5.69x10 <sup>7</sup>	3.4 x106 (2.9 x106) A	CFU	6.0e
EPF	6.9	5.69x10 <sup>7</sup>	5.4 x10 <sup>6</sup> (6.6 x10 <sup>6</sup> ) A	CFU	9.5de
SF	2.0	2.3 x10 <sup>6</sup>	3.89x105 (3.52x105)A	CFU	17cde
SF	4.2	2.3x10 <sup>6</sup>	4.75x10 <sup>5</sup> (3.31 x10 <sup>5</sup> )A	CFU	21cde
SF	6.9	2.3x10 <sup>6</sup>	2.74x105 (1.45x105)A	CFU	12de
Nematode	2.0	$2.3 \times 10^{4}$	9387 (826)A	number	41bc
Nematode	4.2	$2.3 \times 10^4$	9679 (774)A	number	42bc
Nematode	6.9	$2.3 \times 10^4$	10754 (528)A	number	47b

\* Means for the measured quantity of the same material in a column followed by a different uppercase letter are significantly different (p<0.05), but not for the comparison between materials.

\*\* Recovery rate (%) = Measured quantity x 100 / Predicted quantity. Means for the recovery rate in a column followed by a different lowercase letter are significantly different among all the materials (p<0.05).

Table 3. Comparison of predicted and measured amounts of five materials discharged from individual emitters throughout drip lines with three different size emitters. Standard deviation is presented in parenthesis.

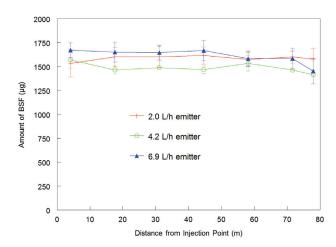


Fig. 6. Amounts of BSF discharged from individual 2.0, 4.2 and 6.9 L/h emitters throughout three different drip lines. Error bars represent standard deviations around means.

Treatment -		CV (%)*			DU*			
	2.0 L/h emitter	4.2 L/h emitter	6.9 L/h emitter	2.0 L/h emitter	4.2 L/h emitter	6.9 L/h emitter		
BSF	2.3	1.7	1.8	0.95	0.97	0.96		
Imidacloprid	43	36	54	0.76	0.80	0.76		
EPF	90	104	119	0.56	0.26	0.33		
SF	98	72	51	0.44	0.62	0.68		
Nematodes	8.8	8.0	4.9	0.80	0.83	0.91		

\* Each value of CV or DU is the mean from 7 emitters in each drip line with three replications. CV and DU were calculated with equations (1) and (2), respectively.

Table 4. Mean coefficient of variation (CV) and distribution uniformity (DU) of BSF, Imidacloprid, EPF, SF and nematodes discharged from emitters with three different flow capacities (2.0, 4.2 and 6.9 L/h) at 138 kPa pressure.

the injection point was 0.38 m/s in Line 1, 0.80 m/s in Line 2, and 1.32 m/s in Line 3. The Reynolds' number was 4807, 10120, and 16697 for the three lines, respectively. The flow near the injection point in all three lines was a turbulent flow. The flow rate in drip lines had little influence on the amount of BSF discharged from emitters. Therefore, the water soluble material could be well delivered throughout drip lines regardless of the emitter capacity.

The amount of Imidacloprid discharged from individual emitters varied with the emitter flow capacity (fig. 7). Emitter capacity also significantly influenced the distribution uniformity of Imidacloprid discharged throughout the drip line. The amount of Imidacloprid discharged from all emitters throughout drip line 3 (6.9 L/h emitters) was significantly lower than the other two drip lines. High concentrations of Imidacloprid were found in the flushing water samples collected from drip line 3. A large portion of the chemical injected into line 3 might have been carried by the high-speed water to the end of the line and then trapped. Drip line 2 (4.2 L/h emitters) had the highest amount of Imidacloprid discharged from emitters and highest recovery rate among the three lines (table 3). The average CV and DU of Imidacloprid throughout the 79 m drip line for all three drip lines was 44% and 0.78, respectively (table 4). Compared to BSF, Imidacloprid had a considerable high variation with the emitter flow capacity and the emitter location throughout the drip line.

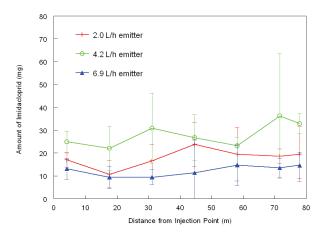


Fig. 7. Amounts of Imidacloprid discharged from individual 2.0, 4.2 and 6.9 L/h emitters throughout three different drip lines. Error bars represent standard deviations around means.

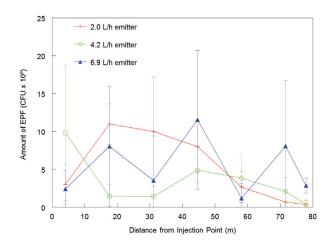


Fig. 8. Amounts of EPF discharged from individual 2.0, 4.2 and 6.9 L/h emitters throughout three different drip lines. Error bars represent standard deviations around means.

The emitter flow capacity did not significantly affect the amount of EPF discharged from individual emitters throughout the drip lines (fig. 8), which might be because the recovery rate of EPF was very low. Less than 10% EPF was recovered from all three drip lines (table 3). The low recovery rate might be caused by the adherence of EPF to the wall of drip lines. During preparation of the mixture of EPF and water, some suspensions were observed to adhere to the wall of plastic cups after standing for several minutes. The hydrophobic nature of *M. anisopliae* conidia combined with an emulsifiable concentrate formulation might require greater agitation to maintain suspension in water than that could be provided by a drip irrigation system. The CV for the amount of EPF throughout the entire drip line increased as the capacity of emitters increased, while the DU tended to decrease as the emitter capacity increased (table 4). The average CV and DU of EPF throughout the 79 m drip line for all three drip lines was 104% and 0.39, respectively (table 4).

Similar to the Imidacloprid, the amount of SF discharged from emitters was also affected by the emitter flow capacity (fig. 9). The amount of SF discharged from all emitters throughout drip line 3 was significantly lower than the other two drip lines while line 2 with 4.2 L/h emitter flow capacity had the highest amount of SF discharged (table 3). The recovery rate of SF ranged from 12 to 21% for all three emitter capacities. During three replications, there were three emitters clogged by materials in line 1, and 3 emitters clogged in line 2, but no emitters were clogged in line 3. The clogging problem was caused by particles with sizes larger than the depth of emitter flow path. Very high concentrations of SF remained in flushed water samples, which indicated that most SF remained in the drip line. Unlike EPF, the CV for the amount of SF throughout the entire drip line decreased and DU increased as the emitter capacity increased (table 4). The average CV and DU of SF throughout the 79 m drip line for all three drip lines was 74% and 0.58, respectively. The lowest and highest amounts of SF among all the emitters investigated in this study were 1.22x10<sup>6</sup> CFU and 6.67x10<sup>6</sup> CFU, respectively, both of which occurred on line 1, but the difference was not significant at the 0.05 probability level.

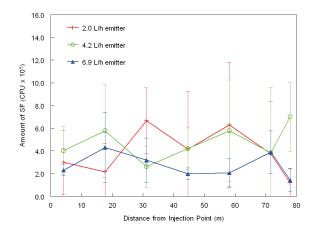


Fig. 9. Amounts of SF discharged from individual 2.0, 4.2 and 6.9 L/h emitters throughout three different drip lines. Error bars represent standard deviations around means.

The average number of nematodes discharged from individual emitters slightly increased as the emitter capacity increased, but the differences were not significant (table 3). The recovery rate of nematodes discharged from the 6.9 L/h emitters on line 3 was higher than that from the 2.0 and 4.2 L/h emitters. The emitter flow capacity influenced the distribution uniformity of nematodes throughout the drip lines (fig. 10). The value of CV decreased from 9.1% to 5.0% and DU increased from 0.80 to 0.91 when the emitter flow rate changed from 2.0 to 6.9 L/h (table 4).

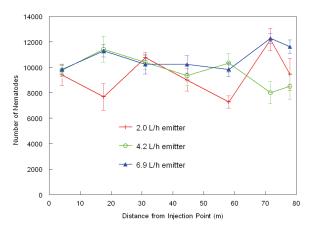


Fig. 10. Number of nematodes discharged from individual 2.0, 4.2 and 6.9 L/h emitters throughout three different drip lines. Error bars represent standard deviations around means.

Among the five materials tested, BSF had the highest recovery rate, the lowest CV and the highest DU across the three 79 m drip lines, followed by nematodes, Imidacloprid, and SF, while EPF had the highest CV and lowest DU and recovery rate for all three lines (tables 3, 4). The amount of BSF and number of nematodes discharged throughout all three drip lines had excellent distribution patterns (DU>0.80). The Imidacloprid DU was greater than 0.76 for all three drip lines. For the suspendible SF and EPF, their DU was less than 0.70, which was possibly caused by their particles not easily mixing with water. To compensate for the non-uniform delivery and low recovery rate of EPF and SF throughout the drip lines, the rate of EPF and SF required for effective insect/disease control must be determined before they are used in the field.

Both BSF and Imidacloprid solutions were soluble in water, but the viscosity of the Imidacloprid solution was much higher than the BSF solution. The Imidacloprid did not mix as well with the water in the drip line as the BSF after it was injected, which might be the reason that the Imidacloprid had higher CV and lower DU throughout all drip lines than the BSF. For nematodes and the bio-compound suspensions, the former were tiny worms suspended in water, while the latter were suspended organic particles with foam presumably from the wetting agent. Although the nematodes behaved as suspendible particles in solution, due to their small size and easy flow with water, their recovery rate and distribution uniformity throughout the entire drip lines performed much better than the two bio-compound suspensions EPF and SF.

The movement of chemical and microbial pesticides throughout the drip line is a complicated two-phase flow. Many factors influence the recovery rate and distribution uniformity of those

materials throughout drip lines. The findings in this paper demonstrate the importance of evaluating these materials under controlled conditions before they are applied in the field. Future studies should further discover the influence of specific physical properties of these materials on the chemigation performances, and develop methods to improve the recovery rate and distribution uniformity of the suspended powder formulation of the microbial insecticide EPF and the suspended granular formulation of the microbial fungicide SF.

## 3.3 Distribution of tested materials in the soil

As mentioned before, BSF was used to track water movement in the soil and determine mobility of Imidacloprid, EPF and nematodes. Data in Table 5 illustrate lateral and vertical distribution patterns of BSF concentration in the soil for three different emitter capacities. BSF was detected at all 28 locations in the soil within the range from -45.6 to 45.6 cm laterally and from 0 to 30 cm vertically near each emitter. Within the 30 cm by 91.2 cm area, BSF presented the highest concentration at soil depths between 0 and 10 cm directly under the emitter. Concentration tended to decrease with the distance from the emitter for all three emitters, but the degree of such decrease was not very strong at soil depths of 20 and 30 cm. The BSF concentration of BSF concentration was 29 % for the 2.0 L/h emitters in line 1, 33% for the 4.2 L/h emitters in line 2, and 16% for the 6.9 L/h emitters in line 3, respectively. Thus, water discharged from the emitters travelled to the entire 91.2 cm by 30 cm area under the emitters with 2.0, 4.2 and 6.9 L/h flow capacities, with more water remaining near the emitters.

Soil	Emitter		Distance from emitter (cm)						
Depth (cm)	flow (L/h)	-45.6	-30.4	-15.2	0	15.2	30.4	45.6	
0	2.0	0.215	0.258	0.337	0.492	0.323	0.268	0.230	
10	2.0	0.228	0.241	0.313	0.462	0.380	0.289	0.297	
20	2.0	0.237	0.192	0.316	0.359	0.330	0.253	0.284	
30	2.0	0.199	0.172	0.181	0.212	0.249	0.215	0.171	
0	4.2	0.339	0.318	0.309	0.431	0.310	0.289	0.319	
10	4.2	0.301	0.325	0.424	0.585	0.423	0.330	0.283	
20	4.2	0.252	0.284	0.468	0.408	0.433	0.368	0.267	
30	4.2	0.195	0.162	0.198	0.174	0.184	0.229	0.141	
0	6.9	0.376	0.349	0.370	0.430	0.404	0.419	0.393	
10	6.9	0.332	0.315	0.410	0.415	0.404	0.405	0.321	
20	6.9	0.323	0.279	0.395	0.344	0.339	0.329	0.337	
30	6.9	0.297	0.244	0.288	0.292	0.264	0.253	0.354	

Table 5. Mean BSF concentration  $(\mu g/g)$  in the soil at various depths and lateral distances from the emitter with three flow capacities.

Unlike the distribution pattern of BSF in the soil, most Imidacloprid was distributed within a very narrow zone under the emitter (Table 6). Very little Imidacloprid was found at any depth when lateral distance from the emitter was greater than 30.4 cm. This was because the distribution coefficient of Imidacloprid in the soil was very small Cox et al. 1997). For the soil area 30 cm deep, 15.2 cm to the left and 15.2 cm to the right of the emitter, the CV of Imidacloprid concentration was 173 % for the 2.0 L/h emitters in line 1, 117% for the 4.2 L/h

emitters in line 2, and 110% for the 6.9 L/h emitters in line 3, respectively. That is, higher
flow provided better Imidacloprid distribution in the section close to the emitter, but the
lateral distribution was very poor. The contour of Imidacloprid concentration in the soil also
supported this statement (fig. 11).

Soil	Emitter	Distance from emitter (cm)						
Depth (cm)	flow (L/h)	-45.6	-30.4	-15.2	0	15.2	30.4	45.6
0	2.0	0.26	0.64	12.1	2581	3.7	2.62	0.53
10	2.0	0.24	0.21	1.2	3099	3.1	0.85	0.30
20	2.0	0.28	0.16	9.5	2379	2.7	0.78	0.41
30	2.0	0.21	0.17	1.2	296	6.0	0.83	0.33
0	4.2	0.45	0.45	131.1	1170	957.4	4.05	0.49
10	4.2	0.25	0.18	4.6	1276	961.1	5.94	0.73
20	4.2	0.31	0.26	0.5	38	390.9	2.61	0.24
30	4.2	0.27	0.15	0.3	158	79.9	6.71	0.31
0	6.9	0.27	0.27	226.5	775	465.8	4.33	0.17
10	6.9	0.14	0.02	7.7	1066	805.3	8.84	0.68
20	6.9	0.21	0.09	0.3	11	610.0	2.97	0.17
30	6.9	0.15	0.02	0.2	140	54.4	10.67	0.12

Table 6. Mean Imidacloprid concentration ( $\mu g/kg$ ) in the soil at various depths and lateral distances from the emitter with three flow capacities.

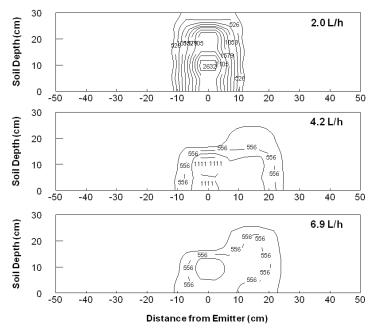


Fig. 11. Contour of Imidacloprid concentration in the soil within the 30 cm by 91.2 cm cross section area under the emitters at flow rates of 2.0, 4.2 and 6.9 L/h, respectively.

Soil	Emitter		Distance from emitter (cm)							
Depth (cm)	flow (L/h)	-45.6	-30.4	-15.2	0	15.2	30.4	45.6		
0	2.0	3700	8600	6333	40000	5533	7067	6867		
10	2.0	6467	2733	5150	6000	3733	7800	3867		
20	2.0	2533	2267	800	16067	15800	2200	1867		
30	2.0	1333	933	600	1850	5000	900	467		
0	4.2	18267	1667	6133	18000	4933	7067	5733		
10	4.2	2450	13733	5067	4867	3067	2800	4800		
20	4.2	6667	2267	3467	5200	9933	2800	2400		
30	4.2	2800	14000	1600	1400	1600	1867	933		
0	6.9	7600	5200	7133	6200	7067	7533	9333		
10	6.9	5533	6533	4200	16867	3200	5933	5200		
20	6.9	3733	2467	6267	5000	4733	4000	3067		
30	6.9	467	533	5900	3067	1467	1800	1467		

Table 7. Mean EPF concentration (CFU/g) in the soil at various depths and lateral distances from the emitter with three flow capacities. (new data)

The EPF had better distribution uniformity than Imidacloprid. The fungus spores of EPF were found at every sampling location for all three emitter flow-capacities (Table 7). Among the 28 sampling locations, the minimum number of spores was 467 CFU/g,which was located at 30 cm depth and 45.6 cm away from the emitter with both emitter flow-capacities of 2.0 and 6.9 L/h. Throughout the entire 91.2 cm wide and 30 cm deep section, the CV of EPF concentration was 130, 88, and 64% for the 2.0, 4.2 and 6.9 L/h emitters, respectively. Contours of EPF concentrations also showed that 6.9 L/h emitters had a better distribution uniformity of EPF than the 4.2 L/h emitters, while the 4.2 L/h emitters had a better distribution uniformity of EPF than the 2.0 L/h emitters (fig. 12). Therefore, higher flow could reduce variation in EPF distribution in soil.

Compared to their creamy-white color when healthy (Figure 13a), wax moth larvae turned brick red in color when infected with nematodes (Figure 13b). Detection of applied nematodes in soil samples was based on color changes in wax moth larvae. No moth larvae became infected when exposed to soil samples from untreated locations. Table 8 shows the presence of nematodes at different distances in the soil laterally and vertically away from the emitters with three different flow capacities. Among 28 locations, nematodes were found at 15 locations for the 2.0 L/h emitters, 15 locations for the 4.2 L/h emitters, and 20 locations for the 6.9 L/h emitters. Except for the absence of nematodes at all 12 locations in the 30.4 cm wide and 30 cm depth area under all three emitter capacities. Nematodes moved further laterally in deeper soil locations when discharged from 6.9 L/h emitters as compared with 2.0 and 4.2 L/h emitters. Therefore, the higher presence of nematodes laterally at greater depths may be related to water application rates.

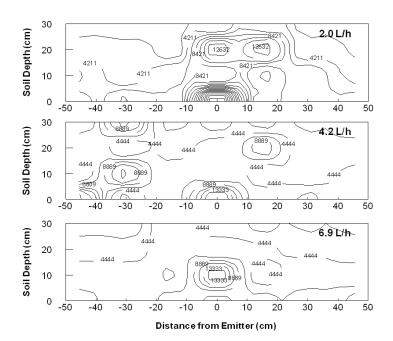


Fig. 12. Contour of EPF concentration in the soil within the 30 cm by 91.2 cm cross section area under the emitters at flow rates of 2.0, 4.2 and 6.9 L/h, respectively.



(a) No nematodes presented in soil

(b) Nematodes presented in soil

Fig. 13. Comparison of wax moth larva color between (a) no nematodes and (b) nematodes found in soil samples. The soil sample shown in (a) was taken from the location 45.6 cm laterally and 20 cm vertically from the 4.2 L/h emitter. The soil sample shown in (b) was taken from the location 15.2 cm laterally and 20 cm vertically from the 4.2 L/h emitter.

Soil	Emitter	Distance from emitter (cm)							
Depth (cm)	capacity (L/h)	-45.6	-30.4	-15.2	0	15.2	30.4	45.6	
0	2.0	No	Yes	Yes	Yes	Yes	No	No	
10	2.0	No	Yes	Yes	Yes	Yes	Yes	No	
20	2.0	No	Yes	Yes	Yes	Yes	No	No	
30	2.0	No	No	Yes	Yes	Yes	No	No	
0	4.2	No	Yes	Yes	Yes	Yes	No	No	
10	4.2	Yes	Yes	Yes	Yes	Yes	No	No	
20	4.2	No	Yes	Yes	Yes	Yes	No	No	
30	4.2	No	No	Yes	Yes	No	No	No	
0	6.9	No	Yes	Yes	Yes	Yes	No	No	
10	6.9	Yes	Yes	Yes	Yes	Yes	Yes	No	
20	6.9	Yes	Yes	Yes	Yes	Yes	Yes	No	
30	6.9	No	Yes	Yes	Yes	Yes	No	No	

Table 8. Presence of nematodes in the soil at various depths and lateral distances from the emitter with three flow capacities.

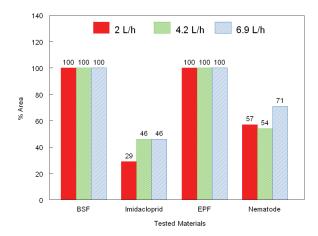


Fig. 14. Percent presence area of tested materials (BSF, Imidacloprid, ERF and nematodes) in the 30x91.2 cm<sup>2</sup> soil area under emitters with the flow capacities of 2, 4.2 and 6.9 L/h. (Change % Area to % Presence Area)

In the 30 cm by 91.2 cm sampling area under each emitter, percent area of the BSF and EPF presence was 100 for all three emitter flow capacities (fig. 14). Of the four materials tested in the soil, Imidacloprid had the lowest percent area for all three emitter flow capacities. The percent presence area of Imidacloprid was 29, 46 and 46 at 2.0, 4.2 and 6.9 L/h emitter flow rates, respectively. Presence area of Imidacloprid at 4.2 and 6.9 L/h was considerably higher than that at 2.0 L/h, while the flow capacities of 4.2 and 6.9 L/h did not significantly affect the percent presence of Imidacloprid in the sampling area. Flowable Imidacloprid did not extend laterally in the soil. More than half the tested area showed the presence of nematodes for all three flow rates. The nematode presence area was 71% at 6.9 L/h flow rate while it

was 57 and 54% at 2.0 and 4.2 L/h flow rates, respectively. In general, Imidacloprid and nematodes could be distributed in a larger area in the soil by higher flow capacity emitters while distribution of BSF and EPF in the soil was not apparently affected by the emitter flow capacity. Nematodes, as living organisms, travel substantial distances in soil when target insects are present (Grewal et al., 2005). Thus, the potential area that nematodes presented in the soil should be much larger than the 30 cm by 91.2 cm.

Previous tests for the distribution throughout drip lines reported that the recovery rate of EPF and nematodes were very low for all three flow-capacity emitters. The recovery rate of EPF discharged from 2.0, 4.2 and 6.9 L/h flow capacity emitters was 9.0, 6.0 and 9.5%, respectively. For the same flow capacity emitters, recovery rate for nematodes was 41, 42 and 47%, respectively. Presence of EPF and nematodes in the entire area of 30 cm by 91.2 cm area under the emitters verified that drip irrigation systems could be an alternative method to apply suspendible microbial pesticides. Also, application of these microbial pesticides; however, rates of EPF and nematode application that effectively control target pests must first be determined to compensate for their low recovery rates.

## 4. Conclusions and summary

Drip irrigation uniformly dispensed the water-soluble BSF, water-dispersible insecticide Imidacloprid and suspended nematodes throughout drip lines, but not the suspended powder formulation of the microbial insecticide EPF or the suspended granular formulation of the microbial fungicide SF. The uniformity of distribution of the various test agents throughout the drip line varied with their physical properties of the individual product formulation. The distribution uniformity of EPF discharged from emitters throughout the drip line was the lowest among the five materials tested, followed by SF, Imidacloprid, nematodes, and BSF.

Except for BSF and Imidacloprid, flow capacity of emitters affected the distribution uniformity of the other test agents throughout drip lines. Among the three emitters tested, EPF had the highest DU at 2.0 L/h flow rate. The uniformity of SF and nematode distribution throughout drip lines increased as the flow capacity of emitters increased. For the emitters with flow rates ranging from 2.0 to 6.9 L/h, DU averaged over 0.95 for BSF, over 0.80 for nematodes, over 0.75 for Imidacloprid, ranged from 0.44 to 0.68 for SF, and ranged from 0.33 to 0.56 for EPF.

Emitter size and flow capacity affected the recovery rates of Imidacloprid and SF discharged throughout the drip line, but not of BSF, EPF and nematodes. The recovery rates greatly varied with the physical properties of the individual product formulation. For the emitters with flow rates ranging from 2.0 to 6.9 L/h, the recovery rate was below 9.5% for EPF, 21% for SF, 50% for nematodes, and 78% for Imidacloprid.

Active agent distribution patterns in soil varied with the formulation. Water-insoluble microbial insecticides, EPF and nematodes, exhibited better distribution patterns than water-soluble systemic insecticide, Imidacloprid. EPF spores were found in the entire 91.2 cm by 30 cm cross-section under all three emitter flow-capacities. Imidacloprid showed a very narrow distribution pattern directly under the emitters.

Active-agent distribution patterns in the soil also varied with emitter flow capacity. EPF was present in the entire 30 cm by 91.2 cm area under the emitter for all three emitter flow capacities. Nematodes presented 57% of the area at 2.0 L/h flow rate, 54% at 4.2 L/h flow

rate and 71% at 6.9 L/h flow rate, while Imidacloprid presented 29, 46 and 46% area at 2.0, 4.2 and 6.9 L/h emitter flow rate, respectively.

Uniformity of EPF and nematode distributions in the soil improved as emitter flow capacity increased. Presence area of Imidacloprid at 4.2 and 6.9 L/h was significantly higher than that at 2.0 L/h while there was no significant difference in the presence of Imidacloprid in the sampling area between flow capacities of 4.2 and 6.9 L.

These results demonstrated that drip irrigation could be a viable alternative method for water-soluble pesticide applications. However, the use of drip irrigation systems for the delivery of suspended powders and granular agents, e.g. EPF and SF, for pest control may be limited because of their poor uniformity and low recovery rates throughout drip lines. Any materials with sizes larger than the width or depth of emitter flow paths would clog emitters and should not be applied through drip irrigation systems.

#### 5. References

- Ajwa, H. A.; Trout, T.; Mueller, J.; Wilhelm, S.; Nelson, S. D.; Soppe, R. & Shatley, D. (2002). Application of alternative fumigants through drip irrigation systems. *Phytopathology*, 92(12):1349-1355.
- ASAE Standards, EP405.1, APR1988 (R2008). (2008). Design and Installation of Microirrigation Systems. St. Joseph, MI:ASABE.
- Barber, J.A.S. & Parkin, C. S. (2003). Fluorescent tracer technique for measuring the quantity of pesticide deposited to soil following spray applications. *Crop Protection*, 22(1):15-21, 42.
- Becker, J. O.; Monson, M.; Van Gundy, S. D. & Schroth, M. N. (1989). Drip irrigation as a delivery system for infestation of Peld plots with nematodes. J. Nematol., 21: 524-529.
- Bedding, R.A. & Akhurst, R. J. (1975). A simple technique for the detection of insect pathogenic nematodes in soil. *Nematologica*, 21:109-110.
- Bryla, D. R.; Banuelos, G. S. & Mitchell, J. P. (2003). Water requirements of subsurface dripirrigated faba bean in California. *Irrigation Science*, 22: 31-37.
- Bracy, R.P; Parish, R.L. & Rosendale, R.M. (2003). Fertigation uniformity affected by injector type. *Hortechnology*, 13:103-105.
- Byrne, F.J. & Toscano, N.C. (2006). Uptake and persistence of imidacloprid in grapevines treated by chemigation. *Crop Protection*, 25: 831–834.
- Camp, C.R.; Sadler, E.J. & Busscher, W.J. (2003). A comparison of uniformity measures for drip irrigation systems. *Transactions of the ASAE*, 40(4):1013-1020.
- Cassel, D.K. & Nielsen, D.R. (1986). Field capacity and available water capacity. In: *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods. 2nd ed. Agron. Monogr. no.* 9. A. Klute (ed.). p. 901–926. ASA and SSSA, Madison, WI.
- Castle, S.J.; Byrne, F.J.; Bi J.L. & Toscano, N.C. (2005). Spatial and temporal distribution of Imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* populations. *Pest Management Science*, 61:75-84.
- Clark, G. A.; Lamm, F. R. & Rogers, D.H. (2005). Sensitivity of thin-walled drip tape emitter discharge to water temperature. *Applied Engineering in Agriculture*, 21(5):855-863.
- Cox, L.; Koskinen, W. & Yen, P. (1997). Sorption-desorption of imidacloprid and its metabolites in soils. J. Agric. Food Chem., 45(4): 1468–1472.
- Dorner, J.W. & Lamb, M.C. (2006). Development and commercial use of afla-guard®, an aflatoxin biocontrol agent. *Mycotoxin Research*, 21:33-38.

- Ellsbury, E. E.; Jackson, J. J.; Woodson, W. D.; Beck, D. L. & Strange, K. A. (1996). Efficacy, application distribution, and concentration by stemflow of *S. carpocapsae* (Rhabditida: Steinernematidae) suspension applied with a lateral-move irrigation system for corn rootworm (Coleoptera: Chrysomelidae) control in maize. *Journal of economic entomology*, 89:74-81.
- Felsot, A.S.; Cone, W.; Yu, J. & Ruppert, J.R. (1998). Distribution of Imidacloprid in soil following subsurface drip chemigation. *Bull. Environ. Contam. Toxicol.*, 60: 363-370.
- Felsot, A.; Evans, R. G. & Tallman, L. (2000). Soil distribution and plant uptake of imidacloprid under drip and furrow irrigation. *Proceedings of the 4th Decennial National Irrigation Symposium*, pp. 416-427. Phoenix, AZ. R. G. Evans, B. L. Benham, and T. P. Trooien, eds. American Society of Agricultural Engineers, St. Joseph, MI.
- Felsot, A. S.; Evans, R. G. & Ruppert, J. R. (2003). Field studies of Imidacloprid distribution following application to soil through a drip irrigation system. Chapter 13 (pp. 189-205) in *Terrestrial Field Dissipation Studies: Purpose, Design and Interpretation*. E. L. Arthur, A. C. Barefoot, and V. E. Clay eds. ACS Symposium Series No. 842, American Chemical Society, Washington, DC. ISBN# 0-8412-3769-7. 368 pp.
- Fleischer, S.J.; Orzolek, M.D.; De Mackiewicz, D. & Otjen, L. (1998). Imidacloprid effects of Acalymma vittata (Coleoptera: Chrysomelidae) and bacterial wilt in cantaloupe. *Journal of economic entomology*, 91(4): 940-949.
- Fuxa, J.R. & Richter, A.R. (1999). Classical biological control in an ephemeral crop habitat with Anticarsia gemmatalis nucleopolyhedrovirus. *Biol. Control*, 44: 403–419.
- Gopal, M.; Gupta, A.; Sathiamma, B. & Radhakrishnan Nair, C.P. (2001). Control of the coconut pest Oryctes rhinoceros L. using the Oryctes virus. *Insect Sci.*, 21(4): 93–101.
- Grabow, G. L.; Huffman, R. L.; Evans, R.O.; Jordan, D. L. & Nuti, R.C. (2006). Water distribution from a subsurface drip irrigation system and dripline spacing effect on cotton yield and water use efficiency in a coastal plain soil. *Transactions of the* ASABE, 49(6): 1823-1835.
- Grewal, P.S.; Power, K.T. & Shetler, D.J. (2001). Neonicotinoid insecticides alter diapause behavior and survival of overwintering white grubs (Coleoptera: Scarabaeidae). *Pest Management Science*, 57:852–857.
- Grewal, P. S.; Ehlers, R. U. & Shapiro-Ilan, D. I. (2005). *Nematodes as Biocontrol Agents*, CABI Publishing, CAB International, Wallingford, Oxfordshire, UK, 505pp.
- Hajek, A.E.; McManus, M.L. & Ju'nior, I.D. (2007). A review of introductions of pathogens and nematodes for classical biological control of insects and mites. *Biological Control*, 41: 1-13
- Jaynes, D. B.; Rice, R. C. & Hunsaker, D. J. (1992). Solute transport during chemigation of a level basin. *Transactions of the ASAE*, 35(6): 1809-1815.
- Keller, J. & Bliesner, R.D. (2000). Sprinkler and Trickle Irrigation. The Blackburn Press, Caldwell, NJ. 652 p.
- Kramer, I. & Grunder, J. (1998). Efficacy of EPNs application by irrigation systems on black vine weevil larvae control. *International Organization for Biological Control (IOBC) Bulletin*, 21: 179-182.
- Kruse, E.G. (1978). Describing irrigation efficiency and uniformity. J. Irrig. Drain. Div. ASCE, 104(IR):35-41.
- Lamm, F. R.; Ayars, J. E. & Nakayama, F. S. (2007). Microirrigation for Crop Production -Design, Operation and Management. Elsevier Publications. 608 pp.
- Lamm, F. R.; Stone, L. R., Manges, H. L. & O'Brien, D. M. (1997). Optimum lateral spacing for subsurface drip-irrigated corn. *Transactions of the ASAE*, 40(4):1021-1027.

- Lamm, F. R. & Trooien, T. P. (2003). Subsurface drip irrigation for corn production: A review of 10 years of research in Kansas. *Irrigation Science*, 22(3-4):195-200.
- Leib, B.G. & Jarrett, A.R. (2003). Comparing soil pesticide movement for a finite-element model and field measurements under drip chemigation. *Computers and Electronics in Agriculture*, 38: 55-69.
- Leib, B.G.; Jarett, A.R.; Orzolek, M.D. & Mumma, R.O. (2000). Drip chemigation of Imidacloprid under plastic mulch increased yield and decreased leaching caused by rainfall. *Transactions of the ASAE*, 43(3):615-622.
- Li, J.; Meng, Y. & Li, B. (2007). Field evaluation of fertigation uniformity as affected by injector type and manufacturing variability of emitters. *Irrigation Science*, 25:117-125.
- Lumsden, R.D. & Locke, J.C. (1989). Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless mix. *Phytopathology*, 79:361-366.
- Plaut, Z.; Carmi, A. & Grava, A. (1996). Cotton root and shoot responses to subsurface drip irrigation and partial wetting of the upper soil profile. *Irrigation Science*, 16: 107-113.
- Reding, M. E.; Zhu, H. & Derksen, R. C. (2008). Drip chemigation with Imidacloprid and nematodes for control of scarab larvae in nursery crops. J. Environ. Hort., 26: 93-100.
- Reed, D. K.; Reed, G. L. & Creighton, C. S. (1986). Introduction of entomogenous nematodes into trickle irrigation systems to control striped cucumber beetle (Coleoptera: Chrysomelidae). *Journal of economic entomology*, 79:1330-1333.
- Schneider, S.M.; Ajwa, H.A. & Trout, T.J. (2006). Chemical alternatives to methyl bromide for vineyard replant conditions. *American Journal of Enology and Viticulture*, 57(2): 183-193.
- Van Iersel, M. W.; Oetting, R. D. & Hall, D. B. (2000). Imidacloprid applications by subirrigation for control of silverleaf whiteßy (Homoptera: Aleyrodidae) on poinsettia. *Journal of economic entomology*, 93: 813-819.
- Veen, K.H. & Ferron, P. (1966). A selective medium for the isolation of Beauveria tenella and Metarrihizium anisopliae. J. Invert. Pathol., 8:268-269.
- Wang, X.; Zhu, H.; Reding, M.E.; Locke, J.C.; Leland, J.E.; Derksen, R.C.; Spongberg, A.L. & Krause, C.R. (2009). Delivery of chemical and microbial pesticides from drip irrigation emitters. *Applied Engineering in Agriculture*, 25(6): 883-893.
- Wennemann, L.; Cone, W.W.; Wright, L.C.; Perez, J. & Conant, M.M. (2003). Distribution patterns of entomopathogenic nematodes applied through drip irrigation systems. *Journal of economic entomology*, 96(2):287-291.
- Woodring, J.L. & Kaya, H.K. (1988). Steinernematid and Heterorhabditid Nematodes: A handbook of techniques, Southern cooperative Series Bulletin 331. Arkansas Agricultural Experiment Station, Fayetteville, AR.
- Wu, I.P.; Howell, T.A. & Hiler, E.A. (1979). Hydraulic design of drip irrigation systems. Hawaii Agricultural Experimental Station Technical Bulletin 105. Honolulu, Hawaii: University of Hawaii.
- Zhu, H.; Lamb, M.C.; Butts, C. L. & Blankenship, P. D. (2004). Improving peanut yield and grade with surface drip irrigation in undulating fields. *Transactions of the ASAE*, 47(1): 99-106.
- Zhu, H.; Derksen, R.C.; Krause, C.R.; Fox, R.D.; Brazee, R.D. & Ozkan, H.E. (2005). Effect of solution pH conditions on fluorescence of spray deposition tracers. *Applied Engineering in Agriculture*, 21(3):325-329.
- Zhu, H.; Fox, R.D.; Ozkan, H.E.; Brazee, R.D. & Derksen, R.C. (1998). Time delay for Injection sprayers. *Transactions of the ASAE*, 41(3): 525-530.