EFFECTS OF PUMPING ON ENTOMOPATHOGENIC NEMATODES AND TEMPERATURE INCREASE WITHIN A SPRAY SYSTEM

J. P. Fife, H. E. Ozkan, R. C. Derksen, P. S. Grewal

ABSTRACT. *Exposure to hydrodynamic stresses and increased temperature during hydraulic agitation within a spray system could cause permanent damage to biological pesticides during spray application. Damage to a benchmark biopesticide, entomopathogenic nematodes (EPNs), was measured after a single passage through three different pump types (centrifugal, diaphragm, and roller) at operating pressures up to 828 kPa. No mechanical damage to the EPNs due to passage through the pumps was observed. Separate tests evaluated the effect of pump recirculation on temperature increase of water within a laboratory spray system (56.8-L spray tank) and a conventional-scale spray system (1136-L spray tank). A constant volume of water (45.4 L) was recirculated through each pump at 15.1 L/min within the laboratory spray system. After 2 h, the temperature increase for the centrifugal pump was 33.6°C, and for the diaphragm and roller pumps was 8.5°C and 11.2°C, respectively. The centrifugal pump was also evaluated within the conventional spray system, under both a constant (757 L) and reducing volume scenario, resulting in an average temperature increase of 3.2°C and 6.5°C, respectively, during the 3-h test period. When comparing the number of recirculations for each test, the rate of temperature increase was the same for the conventional spray system (for both the constant and reducing volume scenarios), while for the laboratory spray system the temperature increased at a greater rate, suggesting that the volume capacity of the spray tank is the primary factor influencing the temperature increase. Results from this study indicate that thermal influences during pump recirculation could be more detrimental to EPNs than mechanical stress. Results show that extensive recirculation of the tank mix can cause considerable increases in the liquid temperature. Diaphragm and roller pumps (low-capacity pumps) are better suited for use with biopesticides compared to the centrifugal pump, which was found to contribute significant heat to the spray system.*

Keywords. *Biopesticide, Pumping, Spray system, Temperature.*

Biological pesticides (i.e., biopesticides) are receiving increased attention as safer, more environmentally friendly alternatives to chemical pesticides and as key components of integrated pest management (IPM) programs (Copping and Menn, 2000). Yet, few biopesticides are currently being used commercially as alternatives to chemical pesticides (Gan-Mor and Matthews, 2003). Grower acceptance of biopesticides has been limited primarily by the relative cost compared to chemical pesticides, limited shelf life, difficulties in handling and delivery, and variable field performance (Copping and Menn, 2000).

In contrast to chemical pesticides, biopesticides include living organisms (i.e., bacteria, fungi, viruses, predators, and parasites), which introduce additional challenges with respect to formulation and delivery because, in order to be effective, the biological organisms must remain viable during the application process. At present, there are few research-based guidelines on how biopesticides should be applied to optimize their performance in the field. Such guidelines are necessary for increased acceptance and use of biopesticides by growers.

Debate continues as to whether existing, conventional spray equipment is acceptable, or if new equipment and delivery systems need to be developed for application of biopesticides (Bateman, 1999; Steinke and Giles, 1995). In practice, different kinds of sprayers, mostly hydraulic, are being used to apply some biopesticides, as there are currently no special delivery systems commercially available. However, before new delivery systems are considered for development, it is advantageous to investigate the limitations of existing equipment.

Agricultural spray application systems vary considerably, from small plot and greenhouse application systems to large–scale boom sprayers used in field crops. Nonetheless, hydraulic spray application systems will, in general, consist of the same types of equipment components (i.e., tank, pump, valves, spray lines, and nozzles). During flow through a spray system, the biological organisms will experience a variety of physical stresses, including changes in pressure, hydrodynamic stress, increases in temperature, or deficient levels of oxygen.

Previous work has investigated the effect of pressure differentials (Fife et al., 2003) and hydrodynamic stress (Fife et al., 2004, 2005) on the viability of a benchmark biopesti-

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cide, entomopathogenic nematodes (EPNs). In general, results from these studies were consistent with the commonly held recommendation for EPN application of operating pressures less than 2000 kPa and nozzle openings greater than 50 µm (Georgis, 1990). However, there are limitations to the equipment and operating conditions. The recommendations by Georgis (1990) are largely based on information from one species, *Steinernema carpocapsae*, the most widely studied and available EPN species. More recently, Fife et al. (2003, 2004, 2005) observed greater reductions in relative viability for *Heterorhabditis* spp., in particular *Heterorhabditis megidis*, indicating that EPN species is an important factor to consider when defining spray operating conditions. Thus, it was recommended that operating pressures within a spray system should not exceed 1400 kPa. Other EPN species may require lower operating pressures. Common hydraulic nozzles (i.e., flat fan and cone) are suitable for spray application of EPNs, when following the nozzle manufacturer's rated pressure and flow rate guidelines. Larger capacity hydraulic nozzles are recommended, as the orifice should be appropriately larger than the organism to avoid hydrodynamic damage. In the case of EPNs, the organisms are rod-like in shape, and typically range in size from approximately 20 to 50 µm in width and 500 to 1000 µm in length, depending on the species (Poinar, 1990).

Less conclusive information is available on the effect of pumping and sprayer agitation on biopesticides. Even more so than chemical pesticides, biopesticide tank mixes need to be thoroughly agitated during application to maintain a constant spray concentration, to avoid sedimentation of the organisms to the bottom of the spray tank. Agitation is generally conducted by either mechanical or hydraulic means. For mechanical agitation, impellers in the tank rotate creating a turbulent flow field. For hydraulic agitiation, a pump withdraws part of the fluid from the tank and then injects it back into the tank through high-velocity jets from nozzles creating a turbulent flow field. Using hydraulic agitation, extensive recirculation within a spray system has been suggested to damage biopesticides (Steinke and Giles, 1995; Grewal, 2002). Pumps with high mechanical shearing may damage the organisms by tearing them apart. Also, some types of pumps may produce enough heat to raise the temperature of the spray liquid, which could be detrimental to the organisms.

Previous work has mainly investigated the effect of mechanical pumping on organism damage (Klein and Georgis, 1994; Nilsson and Gripwall, 1999; Vandanjon et al., 1999). Klein and Georgis found that the viability of several nematode species (*Steinernema* spp. and *H. bacteriophora*) were not affected by different pumps (centrifugal, diaphragm, piston, and roller) at standard pressures. In contrast, Nilsson and Gripwall (1999) found the survival of *S. feltiae* decreased approximately 10% during a 20-min pumping period, using a piston pump. Nilsson and Gripwall (1999) indicated that the reason for the decreased viability was probably mechanical stress from the pump and nozzles, but may also be due to the rise of temperature in the liquid.

The aim of this study was to determine which factor (i.e., mechanical stress or thermal stress) within a pumping system could be most detrimental to the EPNs. The specific objectives of this study were: 1) to investigate the effect of a single passage through several common types of pumps on mechanical damage to the nematodes (without the confounding effect of temperature increase during multiple passages through the spray system), and 2) to evaluate the temperature within a spray tank during recirculation of water through several common types of pumps.

MATERIALS AND METHODS

SINGLE PASSAGE OF NEMATODES THROUGH VALVES

Prior to testing the pumps, several valve types were investigated to determine whether passage through a control valve within a pumping system could contribute to nematode damage. The five different valves evaluated were: full ball valve (brass, 0.64 cm or 0.25 in., Milwaukee Valve Company, Inc., Milwaukee, Wis.), reduced ball valve (steel, 0.64 cm, Sharpe, Taiwan), diaphragm (A-Flo, 1.27 cm or 0.5 in., ITT Industries, Lancaster, Pa.), globe (0.64 cm, Stockham, Canada), and needle (0.64 cm, Deltrol Fluid Products, Bellwood, Ill.).

The test system consisted of a 1-L plastic bottle fitted with an inverted spray header (Model 202V, R and D Sprayers, Opelousas, La.), with a 0.32-cm air inlet, 0.64-cm spray outlet, and a pressure gauge. The spray outlet was connected with a clamp to a 0.95-cm hose, 122 cm in length, at the end of which each valve was connected.

Entomopathogenic nematodes of *H. bacteriophora* GPS 11 strain were used for the testing as they are sensitive to hydrodynamic stress (Fife et al., 2004). The nematodes were cultured *in vivo* in the laboratory using last-instar *Galleria mellonella* (L.) (Vanderhorst Canning Co., St. Mary's, Ohio) as the host and standard culture procedures (Kaya and Stock, 1997). Approximately 50-mL aliquots of the harvested suspensions were stored in 150- × 20-mm plastic Petri dishes at 10°C until tests were conducted. Prior to use, the EPNs were acclimated to room temperature, and suspensions were prepared to concentrations between 1000 and 2000 EPNs/mL for testing. All tests were conducted within two weeks following harvest.

Immediately before conducting individual tests, the EPN suspensions were thoroughly mixed and a 10-mL sample was removed to serve as an untreated control. Approximately 0.5 L of the well-mixed nematode suspension was poured into the plastic container, the container was pressurized to either 276 or 483 kPa, and then the valve was opened and a 100-mL sample from the valve exit was collected. The tests involved the valves fully open; however, some preliminary tests were conducted with the valves partially open (i.e., quarter and half), and no differences in results were observed. Each valve was tested at 276 kPa, and only the ball and diaphragm valves were tested at 483 kPa. The tests were repeated one time.

SINGLE PASSAGE OF NEMATODES THROUGH PUMPS

A laboratory spray system, developed by Reichard et al. (1996) for pump recirculation studies on spray additives, was used to evaluate the effect of a single passage through different pumps on nematode damage to *H. bacteriophora*. A schematic of the laboratory system is shown in figure 1. Three pumps commonly used in agriculture were evaluated: centrifugal (Model 9202C, Hypro Corp., New Brighton, Minn.), diaphragm (Model 9910-D19, Hypro Corp.), and roller (Model 4101C, Hypro Corp.).

A model TEFC, 3-phase, 230-V motor (The Lincoln Electric Co., Cleveland, Ohio) was used for the tests. Motor speed

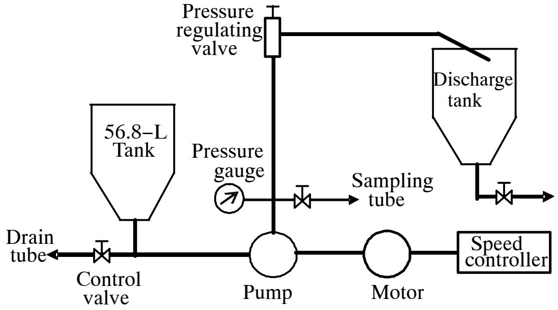

Figure 1. Schematic diagram of the laboratory spray system used to evaluate the damage to nematodes after a single passage through a centrifugal, diaphragm, and roller pump.

was controlled by a speed controller (Dayton model 1XC97, 3-phase, W. W. Grainer, Inc., Lincolnshire, Ill.). In each case, pump speed was measured with an optical tachometer (Model Tach-4A, Monarch Instrument, Amherst, N.H.).

The liquid suspension was contained within a 56.8-L, tapered bottom, Raven tank (Raven Industries, Inc., Sioux Falls, S.D.) with an antivortex fitting. The discharge line from the pump was routed to a separate Raven tank (i.e., no recirculation). The discharge line included a sampling outlet, consisting of a ball valve and a flexible tube from which the samples were collected, a pressure gauge, and a pressure regulating valve. Pressure in the discharge line was monitored with the pressure gauge, and to achieve the desired test pressure, changes were made by adjusting either the pump speed or the pressure regulating valve. The flow rate for each treatment was determined by measuring the discharge volume within approximately a 1-min period of time.

Third stage infective juveniles (IJs) of *H. bacteriophora* were supplied by Integrated BioControl Systems (Indianapolis, Ind.). Nematodes were suspended in water and stored in 150- × 20-mm plastic Petri dishes, with approximately 4 million EPNs in 50 mL of water per dish, at 10°C until tests were conducted. All tests were conducted within two weeks following the arrival of the EPN shipment from the supplier.

Based on the recommended application rate (250 million EPNs/ha) from the supplier, and spray volume of 1500 L/ha (Grewal and Georgis, 1999), the desired suspension concentration for the tests was 0.16 million EPNs/L. Prior to use, the EPNs were acclimatized to room temperature, and approximately 12 million EPNs (i.e. three Petri dishes) were added to water to get 2 L of total volume, giving a concentration of 6 million EPNs/L. This was the prepared suspension that will be referred to later.

Each trial was conducted by first adding 37.9 L of water to the tank and running the pump to set the desired operating pressure. Any adjustments to the motor speed or pressure regulating valve were made at this time. After the system was set-up, the pump was turned off. The nematodes were then added to the tank, and the suspension was stirred to evenly distribute the nematodes within the tank. To allow for enough time to collect samples during the trial, a greater volume was necessary to test the centrifugal pump. Thus, 1 L of the prepared nematode suspension was added to the tank along with water to bring the total volume to 37.9 L. This gave a tank concentration of 0.16 million EPNs/L, the desired suspension concentration based on spray recommendations. For the diaphragm and roller pumps, 0.5 L of the prepared nematode suspension was added along with water to bring the total volume to 18.9 L, which gave a tank concentration of 0.16 million EPNs/L. A 10-mL sample of the EPN suspension was withheld to serve as an untreated control. The pump was immediately turned on, and approximately 5 s after the system was up to the desired pressure, the ball valve on the sampling port was opened, and a 100-mL sample of treated suspension was collected. A second sample was collected approximately 10 s later. Both samples were then analyzed for EPN damage within an hour following the test.

The experimental conditions are reported in table 1. Three pressures were tested: 276, 552, and 828 kPa. The centrifugal pump was not tested at 828 kPa, as this pressure is beyond its operable range. Additionally, the diaphragm pump was tested at 1379 kPa. The tests were repeated two times.

QUANTIFICATION OF NEMATODE DAMAGE

Nematode damage was quantified following the procedure of Fife et al. (2003). Three 100-µL subsamples per trial of both the treated suspension and the untreated control were evaluated for all treatments. Nematode damage was determined by separately recording the number of live (L), dead whole (D), dead half pieces (HP), and dead quarter pieces (QP) of nematodes within a subsample. Nematodes were considered dead if they were broken or did not respond to prodding. The relative damage of EPNs (RD, %) after treatment was computed by the following equation.

$$RD = 100 - \left(\left(\frac{L}{L + D + \dfrac{HP}{2} + \dfrac{QP}{4}} \right) \times 100 \right) \qquad (1)$$

The treatment mean difference was evaluated using Student's *t* test at a significance level of 0.05.

EVALUATION OF TEMPERATURE DURING PUMP RECIRCULATION

Laboratory Test System

The previously described laboratory test system, with several variations, was used to evaluate water temperature during tank recirculation through the centrifugal, diaphragm, and roller pumps. For the diaphragm pump, a single phase, variable speed motor (Graham 230 M, Marathon Electric Manufacturing Corp., Wausau, Wis.) was used instead of the 3-phase motor. The centrifugal pump was operated at 3640 rpm, the diaphragm pump at 519 rpm, and the roller

Table 1. Experimental conditions for evaluating the effects of a single passage through different pump types on nematode damage to Heterorhabditis bacteriophora.

Pump Type	Operating Pressure (kPa)	Flow Rate (L/min)	Pump Speed (rpm)
Centrifugal	276	104.8	3133
	552	138.7	4470
Diaphragm	276	6.4	205
	552	5.8	205
	828	11.7	348
	1379	10.3	346
Roller	276	2.2	664
	552	4.9	995
	828	6.3	1243

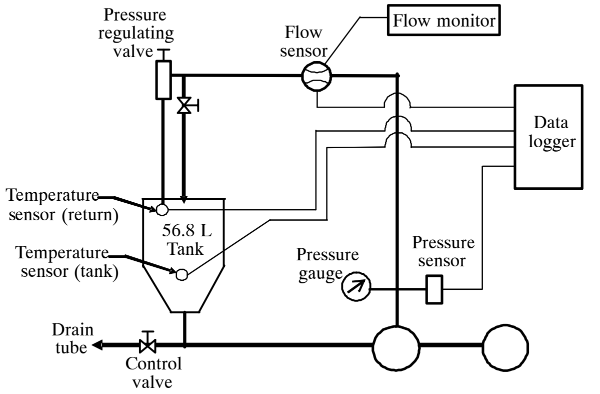

Figure 2. Schematic diagram of the laboratory spray system used to evaluate temperature during pump recirculation of a centrifugal, diaphragm, and roller pump. When the diaphragm and roller pumps were evaluated, the pressure sensor and pressure gauge were located downstream from the flow sensor.

pump at 1923 rpm. A schematic of the modified test system is shown in figure 2.

The discharge line from the pump was routed directly back into the holding tank. The discharge line from the pump to the tank included a pressure gauge, a pressure sensor (Model PX303-300G5V, Omega, Stamford, Conn.), a flow sensor (Series 4000, No. 410200, Data Industrial, Mattapoisett, Mass.), and a pressure regulating valve (Delevan-Delta, Inc., Lexington, Tenn.). To minimize turbulence when the water passed through the flow sensor, an upstream pipe length of 10 diameters (i.e., 12.7 cm), and downstream length of 5 diameters (i.e., 6.35 cm) were used. Also, the flow sensor was connected to a flow monitor (Data Industrial Series 1500, Data Industrial Corp., Mattapoisett, Mass.) to allow for easy adjustment of the flow rate during the experimental set-up. The water temperature was measured with temperature probes (Model 107, Campbell Scientific, Logan, Utah) at two locations: the center of the tank and within the return line to the tank. Additionally, a plug was removed from the centrifugal pump casing and a thermocouple was inserted and sealed, providing a temperature reading from inside the centrifugal pump. The internal pump temperature was inaccessible for the other two pumps.

For all tests, 45.4 L of water was recirculated through each pump system at a volumetric flow rate of 15.1 L/min. The length of each test was 2 h. Temperature, pressure, and flow rate were measured and recorded at a sampling rate of 1 Hz to a CR23X data logger (Campbell Scientific, Logan, Utah). Average values of the temperature and pressure were computed for each minute. The volumetric flow rate was determined by dividing 3.79 L, the volume needed to transmit a pulse by the flow sensor, by the interval of time (seconds) between each pulse. The average volumetric flow rate per minute was then computed from these values. Mean differences in the experimental parameters were evaluated using Student's *t* test.

The number of passes through the pump (i.e. number of recirculations) was computed using the following equation, modified from Reichard et al. (1996),

$$N_i = \frac{t_i Q_i}{V_i} + N_{i-1}\,, \quad \mathrm{i} = 1,2,3,...,\mathrm{k} \qquad (2)$$

where N_i is the number of recirculations through the pump within the time interval t_i (min); k is the number of time intervals within the experiment (k = 120); Q_i (L/min) is the discharge rate of liquid back into the tank; and V_i (L) is the volume of liquid remaining in the tank. Each time interval (t_i) was 1 min, and the average flow rate measured during the time interval was input in equation 2 for Q_i. The volume (V_i) remained constant.

Conventional Spray System

Because of its high volumetric flow capacity, the centrifugal pump was also evaluated within a conventional spray system. The holding container was an elliptical, polyethylene, horizontal tank with 1136-L capacity (Cagle Manufacturing Co., Inc., Coconut Creek, Fla.). A step-down (13.3 cm: 7 cm) V-belt drive was driven by the 3-phase, 230-V motor (Model TEFC, Lincoln Electric). The return line to the tank was fed at the top of the tank. The experimental set-up, including the temperature, pressure, and flow sensors, and data acquisition were the same as before. The temperature probe in the tank was located at the center, approximately 15.2 cm from the bottom of the tank.

The first test evaluated a constant volume of water, 757 L, which was recirculated through the pump system at an operating pressure of 276 kPa. The test was conducted over a 3-h time period under ambient conditions within the laboratory. The test was repeated two times.

The second test evaluated a spray scenario, where the tank volume decreased following an on/off spraying procedure. Similarly, the test was conducted over a 3-h time period under ambient conditions within the laboratory. The spray scenario was designed to represent typical application conditions encountered in the commercial lawn care industry, where the scenario allows 15 min for driving to the property, and then 30 min to spray approximately 0.1 ha (0.25 acre). The initial volume of water in the tank was 757 L. The pump system was operated at 276 kPa. For the first 15 min, the liquid was bypassed to the tank, and then for the next 30 min the liquid was sprayed with three FL-5VS (Spraying Systems Co., Wheaton, Ill.) full cone nozzles. The combined flow rate of the three nozzles was 5.9 L/min at 276 kPa. The nozzle flow rate was based on the devised spray scenario (i.e., 30 min to cover a 0.1 ha yard). Thus, at a nozzle flow rate of 5.9 L/min, the application rate of the suspension to the yard would be 1752 L/ha, which is within the upper range of the recommended 749 to 1890 L/ha (Grewal and Georgis, 1999). During the spray, the remainder of the pump output was recycled to the tank. The pump bypass (i.e., sprayer off) for 15 min, and then the sprayer turned on for 30 min, was repeated until the tank was nearly empty, approximately 3 h. The test was repeated two times.

The number of recirculations through the centrifugal pump with time was determined according to equation 2 for both the constant and reducing volume cases. For the reducing volume case, when the spray was turned on, the discharge rate back into the tank (Q_i) was the remainder of the measured flow rate and the flow rate of the nozzles (5.9 L/min). Additionally, the volume in the tank (V_i) was reduced by the amount of liquid sprayed by the nozzles for each 1-min time interval (5.9 L).

In addition, the temperature of water within the conventional spray system was measured at the center of the spray tank every hour for the duration of a work day (i.e., from 9:00 AM to 5:00 PM) under outdoor ambient conditions. The spray tank contained 757 L of water with a starting temperature of 15.5°C. The test was conducted on 9 October 2003.

Results

Effect of Valves on Nematode Damage

There was no significant difference in the mean relative viability of *H. bacteriophora* nematodes after treatment through the valves (99.8%, SE = 0.07%, n = 24) compared to the untreated control (99.8%, SE = 0.08%, n = 24), for data pooled over the experimental trials (P = 0.6). Thus, any nematode damage observed during flow through the pumping system will be due to mechanical stress in the pump.

Effect of Pumps on Nematode Damage

The mean relative viability of *H. bacteriophora* nematodes after treatment with the pumps was 97.3% (SE = 0.15%, n = 108), which was significantly higher (P = 0.001) than the untreated control (96.5%, SE = 0.22%, n = 54), for data pooled over the experimental trials. Thus, it is reasonable to conclude that the nematodes did not experience any physical damage during passage through the pumps for the different test conditions.

Comparison of Temperature Increase by Pumps during Recirculation

Table 2 summarizes the experimental conditions for each trial during the evaluation of pump recirculation within the laboratory spray system (i.e., 56.8-L tank).

Figure 3 compares the temperature measured within the 56.8-L tank for the centrifugal, diaphragm, and roller pump. For the centrifugal pump, at the end of the 2-h test period, the temperature within the tank had risen to 55.8°C (SE = 0.5°C, n = 2), which was an increase of 33.6°C. While for the diaphragm and roller pumps, the increase in temperature was considerably less, 8.5°C and 11.2°C, respectively.

The differences in temperature between the bypass line and the tank were, on average, 1.0°C (SE = 0.02°C), 0.2°C (SE = 0.002°C), and 0.3°C (SE = 0.003°C) for the centrifugal, diaphragm, and roller pumps, respectively, and in each case were not significant (P > 0.37). For the centrifugal pump, the average difference in temperature between the pump and tank was 1.5°C (SE = 0.02°C) and was not significant (P = 0.25).

Table 2. Experimental conditions for evaluating temperature within the 56.8-L tank (laboratory spray system) during pump recirculation.

		Temperature in Tank (°C)		Pressure[a]	Flow Rate[a]
Pump Type	Rep	Initial	Final[b]	(kPa)	(L/min)
Centrifugal	1	22.0	56.3	429.0 (0.7)	15.1 (0.04)
	2	22.5	55.4	427.6 (0.7)	15.4 (0.04)
Diaphragm	1	22.4	29.9	931.7 (1.4)	14.9 (0.02)
	2	20.8	30.3	933.1 (1.4)	15.1 (0.01)
Roller	1	20.1	31.3	731.0 (4.1)	15.0 (0.03)
	2	21.2	32.5	738.6 (3.4)	15.2 (0.07)

[a] Average values computed over the 120-min test period; values in parentheses are the standard error of the mean, n = 121.

[b] Temperature at the end of the experiment, after 120 min.

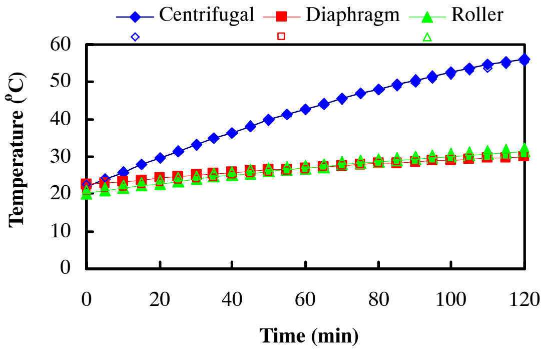

Figure 3. Temperature in the 56.8 L tank (laboratory spray system) during recirculation of 45.4 L of water at a volumetric flow rate of 15.1 L/min using centrifugal (◆), diaphragm (■), and roller pumps (▲). Every fifth data point shown.

Evaluation of Centrifugal Pump within a Conventional Spray System

Table 3 summarizes the experimental conditions during recirculation of the centrifugal pump within a conventional spray system.

Figure 4 presents the temperature within the tank during recirculation of a constant volume and a reducing volume due to repeated spraying. During recirculation of the constant volume, the temperature in the tank increased an average 3.2°C during the 3-h test period. For the reducing volume, the temperature within the spray system increased an average 6.5°C. There was a decrease in temperature after 155 min in the first replication, which occurred because the water level within the tank went below the temperature probe, and the probe was measuring the air temperature in the tank. Thus, the bypass temperature, with the average difference between the bypass line and tank removed (0.16°C), was used to estimate the liquid temperature in the tank from 155 min to the end of the test. The differences in temperature between the bypass line and the tank were, on average, 0.2°C (SE = 0.001°C), and were not significant (P > 0.05). Similarly, the differences in temperature between the pump and the tank were, on average, 0.2°C (SE = 0.001°C), and were

Table 3. Experimental conditions for evaluating temperature within the 1136-L tank (conventional spray system) during pump recirculation for conditions when the tank volume either remained constant or decreased according to repeated spraying.

		Temperature in Tank (°C)		Pressure[a]	Flow Rate[a]
Test Volume	Rep	Initial	Final[b]	(kPa)	(L/min)
Constant	1	22.5	25.4	276.6 (0.03)	59.36 (0.08)
	2	23.1	26.6	275.9 (0.06)	60.76 (0.04)
Reducing	1	25.9	31.4[c]	257.9 (0.14)	59.47 (0.11)
	2	19.1	26.7	257.9 (0.03)	59.01 (0.11)

[a] Average values computed over the 180-min test period; values in parentheses are the standard error of the mean, n = 181.

[b] Temperature at the end of the experiment, after 180 min.

[c] The water level in the tank went below the temperature probe near the end of the test (at 155 min), so the end temperature in the tank was estimated using the bypass line temperature.

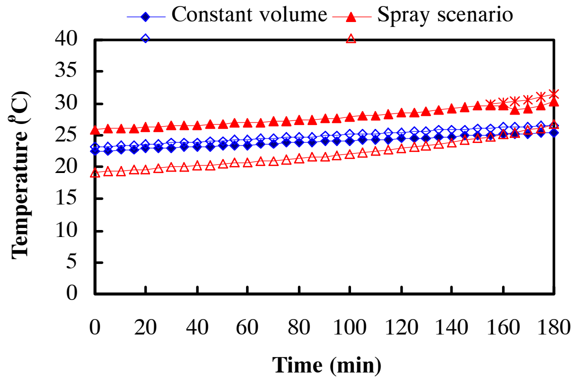

Figure 4. Temperature in the 1136-L tank (conventional spray system) during recirculation of 757 L of water at a system operating pressure of 276 kPa through a centrifugal pump. A constant volume (◆) and a spray scenario (▲) were evaluated. Solid data points are rep 1 (* indicates the adjustment to rep 1 of the spray scenario using values from the bypass line) and unfilled data points are rep 2. Every fifth data point shown.

insignificant (P > 0.09; with one exception, P = 0.02 for rep 1 of the constant volume test).

The number of recirculations through the centrifugal pump was compared to the corresponding increases in temperature within the tank, averaged over the two replications, and are presented in figure 5 for the three test cases (45.4-L constant volume, 757-L constant and reducing volume). In the conventional spray system, the temperature increase was the same with respect to the number of recirculations, regardless of whether the volume remained constant or decreased with time. In the laboratory spray system, the temperature increased at a greater rate with the number of recirculations, owing to the small volume of liquid that was recirculated through the pump compared to the conventional system.

Finally, the contribution of outdoor ambient conditions to the temperature within the conventional spray system is presented in figure 6. During the course of the work day, the temperature of water within the spray tank increased 5.0°C, starting at 15.5°C and increasing to 20.5°C. The average ambient temperature was 23.8°C.

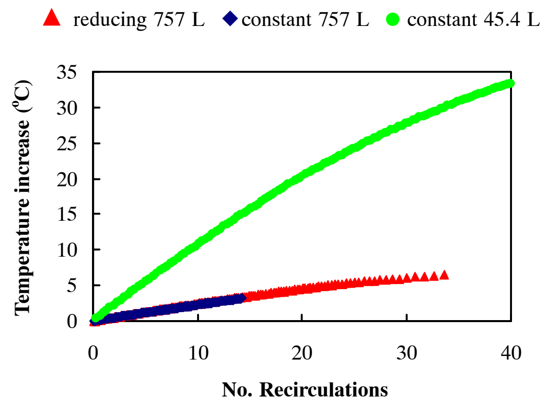

Figure 5. Temperature increase during pump recirculation compared to the number of passages through the centrifugal pump for the three experimental conditions: 757-L reducing volume (▲) and constant volume (◆) (conventional spray system), and 45.4-L constant volume (●) (laboratory spray system).

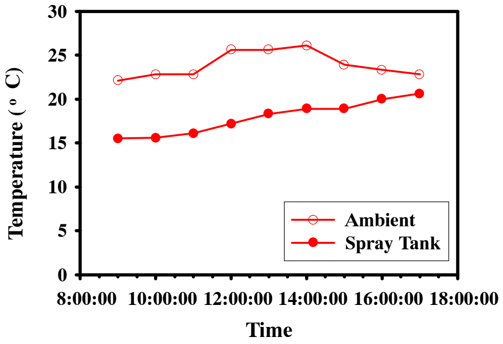

Figure 6. Temperature increase of water within the conventional spray system during the course of a work day under outdoor ambient conditions.

DISCUSSION

No mechanical damage to the *H. bacteriophora* nematodes occurred during a single passage through the pumps, which was consistent with other work (Klein and Georgis, 1994). Previous studies that evaluated the effects of pressure differentials (Fife et al., 2003) and hydrodynamic conditions (Fife et al., 2004, 2005) on nematode damage have demonstrated that the EPNs are relatively robust organisms with respect to mechanical stress. Therefore, this suggests that reductions in nematode viability during pump recirculation are likely the result of temperature influences and not mechanical stress. However, this study considered only a single passage through the pumps as a means to remove the confounding affect of temperature increase during recirculation, and repeated passes could have a cumulative effect on mechanical damage. Vandanjon et al. (1999) studied the effects of recirculation of marine microalgae in pumps and valves, and found that cell degradation increased with the number and frequency of passes, suggesting that repeated passes into the pump body cause shocks which lead to breaking of the cells. Further work is necessary to evaluate whether multiple passes in pumps cause damage to nematodes. In order to test the effect of multiple passages on EPN damage, without increasing liquid temperature, the liquid would need to be appropriately cooled to remove the possibility of viability loss due to temperature increase.

Extensive recirculation of the tank mix will cause increases in the liquid temperature because of heat contributed during the pump's operation. Results from this study indicate that the centrifugal pump, a high-output pump, contributes substantial heat to the spray system (fig. 3). A centrifugal pump consists of an impeller with curved vanes, which rotate at high speeds inside a disk-shaped casing. During rotation, the viscous energy between the impeller vanes and the liquid is dissipated in the form of heat. Due to the high-speed rotation of the impeller vanes, the heat energy contributed by the centrifugal pump is more pronounced than with the other pumps. Thus, a lower capacity pump, like the diaphragm or roller pump, is better suited for use with biopesticides.

Temperature increase due to pump recirculation is primarily a function of the volume of liquid in the tank. Each time a unit volume of liquid passes through the pump, a certain amount of heat energy is added to the liquid. With a smaller volume of liquid in the tank, a unit volume of liquid passes through the pump more times during a pumping period. Thus, more heat per unit of volume is added to the spray system. Additionally, a larger volume of liquid in the tank provides a heat sink with respect to the ambient. This is why, for the same number of passes through the centrifugal pump, the temperature increase in the conventional spray system was much less than in the laboratory system (fig. 5). During the spray scenario, the volume of liquid decreased in the conventional system, and the number of recirculations increased. Consequently, the temperature in the tank increased at a greater rate compared to when the volume was held constant (fig. 4). However, in terms of the number of recirculations, the temperature increase was the same in both of these cases (fig. 5).

Temperature is one of the most important factors affecting EPNs. It affects their motility, infectivity, development, reproduction and longevity (Kaya, 1990). The common recommendation is to avoid exceeding temperatures of 30°C either in the tank, delivery hose, or nozzles (Grewal, 2002). Results from this study indicate that temperatures of 30°C can be reached and exceeded during pump recirculation within a relatively short period of time. The recirculation studies were conducted indoors, under controlled conditions. In practice, the effects of outside temperature and solar radiation will contribute additional heat loads to the system, as evidenced in Figure 6, causing the liquid temperature in the tank to be even higher. Also, the initial temperature of the tank mix, flow rate and volume will influence the temperatures reached.

Survival of EPNs during extended periods of exposure to high temperature varies considerably with species and strain (Grewal et al., 2002; Somasekhar et al., 2002). After 2 h at 40°C, the survival rate of *H. bacteriophora* GPS 11 strain was nearly 90% (Grewal et al., 2002), while *S. carpocapsae* All strain was reduced to only 60% (Somasekhar et al., 2002). Less information is available about how exposures to increased levels of temperature affect EPN infectivity in the long term. Finnegan et al. (1999) exposed *Heterorhabditis* spp. to various temperatures ranging between 20°C and 39°C for 1 h, and evaluated the infectivity of those that survived the temperature treatment against *G. mellonella*. They found that the mortality of *G. mellonella* larvae was reduced considerably by prior exposure to high temperatures (35°C and above). After the temperature treatments of 35°C, 37°C, and 39°C, the EPN infectivity was reduced by approximately 35%, 60%, and 85%, respectively. Information on the interaction between temperature and time of exposure is needed to fully understand how temperature increases during pump recirculation affect the viability and infectivity of EPNs being delivered.

CONCLUSIONS

Results from this study indicate that a single passage through the spray system does not cause physical damage to the nematodes. Multiple passages through the spray system, however, introduce thermal influences during pump recirculation that may be more detrimental to EPNs than mechanical stress. Extensive recirculation of the tank mix can cause considerable increases in the liquid temperature. Diaphragm and roller pumps (low-capacity pumps) are better suited for use with biopesticides compared to the centrifugal pump, which was found to contribute significant heat to the spray system. The volume of liquid in the spray tank was determined to be a primary factor influencing the temperature increase. The smaller the volume of liquid in the tank, the more times the liquid will pass through the pump during a pumping period, causing the temperature of the liquid in the tank to increase at a greater rate. A larger volume of liquid provides a heat sink in the tank which moderates the temperature rise.

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