Abstract. Mechanized application of biological pesticides is a challenge because the organisms must remain viable during the application process to be effective. The wide variety of spray equipment components commercially available makes it impossible to test each component for compatibility with each biological agent under varying operating conditions. A unique approach to this problem is to use computational fluid dynamics (CFD) as a tool to determine equipment suitability with respect to the viability of the biological agent. FLUENT, a commercial CFD program, was used to perform numerical simulations of the internal flow within a standard flat fan nozzle (Spraying Systems XR8001VS). Aqueous suspensions of a biological pest control agent, entomopathogenic nematodes (EPNs), were passed through the nozzle within an experimental, opposed-pistons flow device at flow rates of 21.5, 28.1, 34.7, and 41.3 cm³/s. Four EPN species were evaluated: Heterorhabditis bacteriophora, H. megidis, Steinernema carpocapsae, and S. glaseri. Nematode damage was quantified by counting the number of living and dead EPNs. An empirical model relating EPN damage as a function of energy dissipation rate was developed using data from a previous study. The model parameters were calibrated for each of the EPN species. Average energy dissipation rates within the flat fan nozzle exit orifice (1.1E+8, 1.9E+8, 2.8E+8, and 4.0E+8 W/m³) were computed in FLUENT for the range of experimental conditions and were input to the mathematical model. Overall, the model was able to predict EPN damage well, in many cases within 5%. The ability to predict the damage response of the EPNs within a different system and under varying operating conditions shows the robustness of the mathematical model and the versatility of energy dissipation rate to characterize the hydrodynamic conditions responsible for the EPN damage. The results from this study show that CFD is a feasible method to evaluate the flow field conditions within an equipment component to assess its compatibility with a biological agent.

Keywords. biopesticides, entomopathogenic nematodes, flat fan nozzle, CFD, mathematical model, energy dissipation rate, damage
Introduction

Overuse of some chemical pesticides, resulting in pest resistance, a reduction in the number of chemicals registered for use, and public concern for safety and environmental quality has accelerated interest in alternative pest management practices. Biological pesticides (i.e., biopesticides) are receiving increased attention as benevolent alternatives to conventional 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), representing little more than 1% of the total world pesticide market (Menn and Hall, 1999). In contrast to chemical pesticides, biopesticides are living systems (e.g., bacteria, fungi, viruses, predators, parasites), which introduces additional challenges with respect to formulation and delivery because the biological agents must remain viable during the application process to be effective. At present, there are few research-based guidelines on how biopesticides should be applied to optimize their performance in the field. Such guidelines are critical for increased acceptance and use of biopesticides by growers.

Commercially, the implementation of biopesticides is most likely to come about with the development of products that can be applied using existing, conventional spray application equipment, as growers are unlikely to invest in new equipment or radically alter their practices (Bateman, 1999). In a conventional hydraulic spray system, the suspension is pumped from a tank reservoir, through pressure regulators and flow valves, to a nozzle where the suspension is forced under high pressure through an orifice to the atmosphere. A variety of hydrodynamic stresses are developed during flow through the spray system. In some cases, the stresses may be large enough to disrupt the structural membrane of an organism, causing permanent damage or death. Understanding the hydrodynamic stresses within a spray system is important to begin identifying the equipment characteristics and operating conditions that are least detrimental to the biological agents.

The wide variety of spray equipment components commercially available makes it impossible to individually test each component for compatibility with each biological agent under varying operating conditions. A unique approach to this problem is to use computational fluid dynamics (CFD) as a tool to numerically simulate the complex flow conditions within different equipment components. Important flow field parameters from the numerical simulations can then be evaluated to determine whether the conditions within a particular equipment component are suitable to avoid hydrodynamic damage to the organisms. Because of its practical and theoretical appeal, the scalar quantity of energy dissipation rate has been used in several studies to characterize local hydrodynamic conditions resulting in cell (Gregoriades et al., 2000; Ma et al., 2002) and organism damage (Fife et al., 2003b).

A recent study (Fife et al., 2003b) evaluated the effect of flow through an abrupt contraction on damage to entomopathogenic nematodes (EPNs), a biological pest control agent. An opposed-pistons flow device generated flow through an orifice plate (orifice diameter 0.0635 cm, orifice length 0.1778 cm) ranging between 8.3 cm$^3$/s and 41.3 cm$^3$/s. The experimental flow field was completely described using FLUENT, a commercial CFD program. Average energy dissipation rates within the contraction were computed in FLUENT for each of the experimental conditions and were compared to the corresponding observed nematode relative damage. This relationship provides benchmark information for development of an empirical model to describe EPN damage as a function of energy dissipation rate.

Therefore, the overall goal of this study was to determine the feasibility of using CFD to predict the suitability of an equipment component for safe delivery of a biological pest control agent.
entomopathogenic nematodes. The specific objectives of this study were: 1) to develop an empirical model describing the relationship between EPN damage and energy dissipation rate using data from a previous study (Fife et al., 2003b), 2) to simulate the internal flow through a standard flat fan nozzle using numerical methods, and 3) to predict EPN damage after flow through the standard flat fan nozzle using computed energy dissipation rates from the numerical simulations as input for the mathematical model.

Safety Emphasis

Modern pest control is shifting away from reliance on persistent, broad-spectrum chemical pesticides in favor of a more integrated approach to pest management, which can include use of biological pest control agents. Entomopathogenic nematodes belonging to the Steinernematidae and Heterorhabditidae families have been recognized as excellent biological control agents of soil-dwelling insect pests (Klein, 1990). Entomopathogenic nematodes have been observed to infect over 200 species of insects in the laboratory, and numerous tests have shown the safety of these EPNs to plants and vertebrates (Woodring and Kaya, 1988). The United States Environmental Protection Agency (USEPA) has ruled that EPNs, with their associated bacteria, are exempted from registration under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Ultimately, the increased use of EPNs and other biological pest control agents will result in a reduction in the amount of chemical pesticides currently being used.

Materials and Methods

Entomopathogenic Nematodes

Four species of entomopathogenic nematodes (EPNs) were studied: *Heterorhabditis bacteriophora* (Poinar) GPS 11 strain, *H. megidis* (Poinar, Jackson and Klein) UK strain, *Steinernema carpocapsae* (Weiser) All strain, and *S. glaseri* (Steiner) NJ strain. The EPNs were cultured *in vivo* in the laboratory using last-instar *Galleria mellonella* (L.) (Vanderhorst Canning Co., St. Mary's, OH) as the host and standard culture procedures (Kaya and Stock, 1997). The harvested EPNs, in the infective juvenile (IJ) stage, were placed in a 500-mL beaker, and then after 15 min the liquid from the top, which contained dead EPNs and debris, was decanted. The concentrated suspension was then slowly poured over a tissue paper, wetted and draped over a mesh screen on top of a 150x20-mm petri dish to separate the living nematodes from the dead nematodes. The filtered suspensions were diluted with tap water to concentrations between 1000 and 2000 EPNs/mL, and 60 mL aliquots were individually stored in 150x20-mm petri dishes at 10 ºC until tests were conducted. All tests were conducted within two weeks following harvest.

Experimental Flow Device

The experiments were conducted using an opposed-pistons flow device developed by Clay and Koelling (1997). The device consists of two high-pressure pipes (stainless steel, diameter 1.45034 cm, length 35.56 cm) (High Pressure Equipment, Erie, PA) that are coupled with an orifice plate (stainless steel, orifice diameter 1.45034 cm, plate thickness 0.1778 cm). A stainless steel gasket was specially made to hold the plastic casing of the nozzle within the orifice plate opening. Two pistons (stainless steel, diameter 1.44145, stroke length 30.48 cm) (High Pressure Equipment) are cycled in phase by a hydraulic system, so that as one piston moves forward, forcing the EPN suspension through the pipe and nozzle, the other piston retracts on the collection side. Prior to each test, the piston speed was measured using a digital tachometer (DT-107 hand digital tachometer, Shimpo, Japan), and any adjustments to the
Piston speed were made at this time. All tests were within 0.04 cm/s (SE=0.002 cm/s) of the desired piston speed.

A standard flat fan nozzle (Spraying Systems XR8001VS, Spraying Systems Co., Wheaton, IL) was tested. Immediately before conducting the individual tests, the EPN suspensions were thoroughly mixed and approximately 50 mL of the suspension was loaded into the pipe, between the piston and the nozzle. The total time for running each test was less than 10 min. Tests were conducted at piston speeds of 13 cm/s, 17 cm/s, 21 cm/s, and 25 cm/s, corresponding to volumetric flow rates of 21.5 cm$^3$/s, 28.1 cm$^3$/s, 34.7 cm$^3$/s, and 41.3 cm$^3$/s, respectively. Tests at each flow rate were repeated two times.

**Quantification of Nematode Damage**

Nematode damage was quantified following the procedure of Fife et al. (2003a). Three 100-µL subsamples per replication were counted for all treatments. Nematode damage was determined by separately recording the number of live (L), dead (D), half pieces (HP), and 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.

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

## Simulation of the Flat Fan Nozzle

The internal flow field of the XR8001VS flat fan nozzle was simulated using FLUENT (Version 6.0, FLUENT, Inc., Lebanon, NH). The internal dimensions of the XR8001VS flat fan nozzle were measured using a digital caliper (Model 500-155, Mitutoyo, Japan) and a scanning electron microscope (Hitachi Model S-4700, Nissei Sangyo Instruments, Inc., Mountain View, CA) micrograph of the nozzle cross section. A diagram of the internal structure of an XR8001VS nozzle tip is presented in Figure 1. The three-dimensional geometry was created in GAMBIT (Version 2.0.4, FLUENT Inc., Lebanon, NH), the preprocessor for FLUENT. The geometry consists of three superimposed cylinders with the following radii (R, mm) and lengths (L, mm): $R_1=2.365$ mm, $L_1=3.5$ mm, $R_2=1.78$ mm, $L_2=1.5$ mm, $R_3=0.52$ mm, and $L_3=2.0$ mm. A sphere of radius 0.5279 mm was split by a plane located 0.091 mm from the center of the sphere. The smaller portion of the sphere was superimposed onto the end of the third cylinder. A wedge (height 0.417 mm, base 0.286 mm) was removed from the sphere to create the elliptical exit orifice. A Cartesian coordinate system was used, with $z$ in the axial direction.

The grid mesh used in the FLUENT simulations is presented in Figure 2. The mesh was a hexahedral and tetrahedral hybrid. The grid nodes were non-uniform with a concentration of nodes around the nozzle axis and outlet. There is a tradeoff between the resolution of the simulation results (i.e., total number of computational nodes) and the time to run the simulation, so several different mesh schemes were analyzed to determine an appropriate grid independent solution. A total of 159,080 nodes were used in the model, with 70% of those nodes located in the exit orifice region. The grid dimension within the exit orifice region is approximately 10 µm, which is over two times smaller than the average width of an EPN.

The assumptions for the problem were standard (i.e., incompressible fluid, no slip along the walls, fluid is a continuum). Also, it was assumed that all the experiments were conducted in
the laminar regime. The boundary conditions assigned for the problem were a uniform velocity profile at the domain inlet and outflow at the domain exit. The fluid medium was water at standard atmospheric conditions. The solver settings (i.e., pressure, pressure-velocity coupling, and under-relaxation) were set to the default.

Simulations of the nozzle flow field were conducted for each of the experimental flow rates. A user-defined function was created in FLUENT to compute the energy dissipation rate from the flow field information. The rate of viscous energy dissipated \( \frac{dQ_f}{dt} \) per unit of volume \( \Delta V \) for three-dimensional flow is computed by the following equation (Schlichting, 1955),

\[
\frac{dQ_f}{dt} = \mu \left[ 2 \left( \frac{\partial u_x}{\partial x}^2 + \frac{\partial u_y}{\partial y}^2 + \frac{\partial u_z}{\partial z}^2 \right) + \left( \frac{\partial u_y}{\partial x} + \frac{\partial u_x}{\partial y} \right)^2 + \left( \frac{\partial u_z}{\partial y} + \frac{\partial u_y}{\partial z} \right)^2 + \left( \frac{\partial u_z}{\partial x} + \frac{\partial u_x}{\partial z} \right)^2 \right]
\]

where, \( \mu \) (kg m\(^{-1}\) s\(^{-1}\)) is the fluid viscosity, and \( u_x \) (m/s), \( u_y \) (m/s), and \( u_z \) (m/s) are the velocity components in the \( x \) (m), \( y \) (m), and \( z \) (m) directions, respectively. It was assumed that all the viscous energy being dissipated by the fluid element would be fully realized by the organism.

The most representative value of the energy dissipation rate from within the exit orifice region, for each experimental condition, was determined by averaging the values from computational nodes located on cross-sections at 0.0417 mm intervals within the exit orifice region. The energy dissipation rate values from nodes located within 31.75 \( \mu \)m from the nozzle wall were not included in the mean calculation because values near the wall were significantly higher than the bulk flow due to the assumption of no-slip at the wall, and would skew the mean. The distance of 31.75 \( \mu \)m was used in the previous study for an abrupt contraction (Fife et al., 2003b), and represents approximately one EPN width from the wall.

**Development of the Mathematical Model**

From Fife et al. (2003b), the average energy dissipation rates within the abrupt contraction that were computed in FLUENT and the corresponding observed nematode relative damage after flow through the contraction provided the data for development of an empirical model. On observation, the experimental nematode damage increases in a sigmoidal fashion from 0% to near 100% with increases in energy dissipation rate for each of the EPN species. Thus, two models whose curves are both sigmoid were evaluated: the logistic model and the Gompertz model (Batschelet, 1976). The logistic model is defined by the following equation,

\[
y_{RD} = \frac{a_L}{1 + \exp(-b_L + c_L x_{EDR})}
\]

where, \( y_{RD} \) (%) is the model predicted nematode relative damage, \( x_{EDR} \) (W/m\(^3\)) is the model input average energy dissipation rate from FLUENT simulations, and \( a_L \), \( b_L \), and \( c_L \) are model parameters. The Gompertz model is defined by the following equation,

\[
y_{RD} = a_G \exp(-b_G e^{-c_G x_{EDR}})
\]

where, \( a_G \), \( b_G \), and \( c_G \) are model parameters.

In the cases of both models, parameter \( a \) scales the maximum value to which the function is asymptotic, \( b \) is a positioning parameter, and \( c \) is a rate parameter. Parameter estimates \( b_L \) and \( c_L \) for the logistic model (Equation 3) and \( b_G \) and \( c_G \) for the Gompertz model (Equation 4) were determined in MATLAB (Version 6.0, The Mathworks, Inc., Natick, MA) using non-linear least-squares data fitting by the Gauss-Newton method. Parameters \( a_L \) and \( a_G \) were set to 100.
because the maximum EPN damage that is possible is 100%. The parameter estimates were individually calibrated for each of the EPN species. The total sum of squares of the residuals between the model and the observed relative damage values was computed for each calibrated model and was used as a measure to compare the two models.

**Nematode Damage Predictions**

The nematode relative damage after flow through the XR8001VS flat fan nozzle was predicted for each of the EPN species using the calibrated model that was determined to be most appropriate in the model development. The average energy dissipation rate from within the exit orifice of the flat fan nozzle computed in FLUENT was input to the mathematical model for each of the experimental conditions. The adequacy of the mathematical models to predict the independent nematode relative damage data was assessed by regression of the experimental observations onto the model predictions. An F-statistic (Dent and Blackie, 1979) that tests the regression intercept and slope simultaneously was used to determine whether the models were statistically indistinguishable from the data. This statistic follows the F distribution with 2 and n-2 degrees of freedom, and tests the null hypothesis that the regression intercept and slope are equivalent to 0.0 and 1.0, respectively. The critical F value for 2 and 2 degrees of freedom (n=4) is 19.00. Consequently, small values of F indicate that the model provides a good fit to the data.

**Results**

**Mathematical Model Calibration**

Tables 1 and 2 list the model parameter estimates $a_l$, $b_L$, and $c_c$ for the logistic model (Equation 3) and $a_G$, $b_G$, and $c_G$ for the Gompertz model (Equation 4) that were calibrated for each of the EPN species, respectively. The model computed- and the observed mean-nematode relative damage after treatment with the abrupt contraction as a function of the average energy dissipation rates computed in FLUENT (Fife et al., 2003b) are presented in Figure 3 for *H. bacteriophora* (A), *H. megidis* (B), *S. carpocapsae* (C) and *S. glaseri* (D) for both models. The total sum of squares of the residuals, summed over the EPN species, was lower for the Gompertz model (SS=23,463) compared to the logistic model (SS=25,247). Thus, the Gompertz model was selected for further analysis and is referred to as the model in the remainder of the study.

**Nematode Damage Predictions within the Flat Fan Nozzle**

The average energy dissipation rates within the XR8001VS flat fan exit orifice computed in FLUENT for each of the experimental conditions were 1.1E+8 W/m$^3$, 1.9E+8 W/m$^3$, 2.8E+8 W/m$^3$ and 4.0E+8 W/m$^3$. These energy dissipation rates were input to the Gompertz model (Equation 4, Table 2) for each of the EPN species. The resulting model predictions of nematode relative damage and the corresponding observed mean nematode relative damage after flow through the XR8001VS flat fan nozzle are presented in Figure 4 for *H. bacteriophora* (A), *H. megidis* (B), *S. carpocapsae* (C), and *S. glaseri* (D), for each of the experimental conditions.
Table 1. Logistic model parameter estimates $a_L$, $b_L$, and $c_L$ (Equation 3), and the 95% confidence intervals of parameters $b_L$ and $c_L$, that were calibrated for *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema carpocapsae*, and *S. glaseri*.

<table>
<thead>
<tr>
<th>Entomopathogenic nematode species</th>
<th>Model parameter estimates$^1$</th>
<th>95% confidence interval</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a_L$</td>
<td>$b_L$</td>
<td>$b_L$, $c_L$</td>
</tr>
<tr>
<td><em>H. bacteriophora</em></td>
<td>100</td>
<td>-2.7294</td>
<td>-0.6342</td>
</tr>
<tr>
<td><em>H. megidis</em></td>
<td>100</td>
<td>-7.7327</td>
<td>-2.2302</td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>100</td>
<td>-4.4299</td>
<td>-0.5774</td>
</tr>
<tr>
<td><em>S. glaseri</em></td>
<td>100</td>
<td>-5.9321</td>
<td>-1.2838</td>
</tr>
</tbody>
</table>

$^1$ Model calibrations were conducted using experimental data from a previous study using an abrupt contraction (Fife et al., 2003b). Average energy dissipation rates within the abrupt contraction that were computed in FLUENT for each of the experimental conditions were input for the model. Model output was compared to observed nematode relative damage to determine the best fitting model parameters according to the Gauss-Newton method. (n=60, except for *S. glaseri* n=24)

Table 2. Gompertz model parameter estimates $a_G$, $b_G$, and $c_G$ (Equation 4), and the 95% confidence interval of parameters $b_G$ and $c_G$, that were calibrated for *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema carpocapsae*, and *S. glaseri*.

<table>
<thead>
<tr>
<th>Entomopathogenic nematode species</th>
<th>Model parameter estimates$^1$</th>
<th>95% confidence interval</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a_G$</td>
<td>$b_G$</td>
<td>$b_G$, $c_G$</td>
</tr>
<tr>
<td><em>H. bacteriophora</em></td>
<td>100</td>
<td>3.9838</td>
<td>1.3504</td>
</tr>
<tr>
<td><em>H. megidis</em></td>
<td>100</td>
<td>26.1589</td>
<td>22.4402</td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>100</td>
<td>7.4334</td>
<td>2.0156</td>
</tr>
<tr>
<td><em>S. glaseri</em></td>
<td>100</td>
<td>25.6634</td>
<td>13.1104</td>
</tr>
</tbody>
</table>

$^1$ Model calibrations were conducted using experimental data from a previous study using an abrupt contraction (Fife et al., 2003b). Average energy dissipation rates within the abrupt contraction that were computed in FLUENT for each of the experimental conditions were input for the model. Model output was compared to observed nematode relative damage to determine the best fitting model parameters according to the Gauss-Newton method. (n=60, except for *S. glaseri* n=24)

Table 3 provides a summary of the sum of squares of the residuals and the regression of the observed mean nematode relative damage onto the model predictions for each of the EPN species. The Gompertz model adequately predicted the independent nematode relative damage data for *H. megidis*, *S. carpocapsae*, and *S. glaseri* (F#1.0). However, for *H. bacteriophora* the model was not statistically acceptable (F=57.4). On average, the model over-predicted the observed data by 26.1% for *H. bacteriophora*, while for the other species the mean differences were only 6.0%, 2.3%, and 4.2% for *H. megidis*, *S. carpocapsae*, and *S. glaseri*, respectively (Table 4).
Table 3. Summary of the statistics of predicting nematode relative damage using the Gompertz model for *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema carpocapsae*, and *S. glaseri* after flow through an XR8001VS flat fan nozzle.

<table>
<thead>
<tr>
<th>Entomopathogenic nematode species</th>
<th>Sum of Squares of Residuals $^1$</th>
<th>Regression $^2$</th>
<th>Intercept, 95% confidence interval</th>
<th>Slope</th>
<th>Slope, 95% confidence interval</th>
<th>F statistic $^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. bacteriophora</em></td>
<td>3016</td>
<td>Interception</td>
<td>16.29</td>
<td>12.09</td>
<td>1.56</td>
<td>0.53</td>
</tr>
<tr>
<td><em>H. megidis</em></td>
<td>187</td>
<td>Interception</td>
<td>3.30</td>
<td>18.48</td>
<td>1.05</td>
<td>0.34</td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>33</td>
<td>Interception</td>
<td>2.31</td>
<td>7.25</td>
<td>0.78</td>
<td>0.66</td>
</tr>
<tr>
<td><em>S. glaseri</em></td>
<td>97</td>
<td>Interception</td>
<td>-1.31</td>
<td>12.85</td>
<td>1.10</td>
<td>0.27</td>
</tr>
</tbody>
</table>

$^1$ The sum of squares of the residuals between the model predicted values (Equation 4, Table 2) and the observed mean nematode relative damages after flow through the XR8001VS flat fan nozzle (n=4).

$^2$ Regression of the experimental observations onto the model predictions.

$^3$ F statistic (Dent and Blackie, 1979), critical F value is 19.00, with 2 and 2 degrees of freedom.

**Discussion**

Damage to a suspended cell is defined as the disruption of the cell structure that results from cell deformation (Garcia-Briones and Chalmers, 1994). Deformation of a body immersed within a fluid results from the superposition of normal and tangential forces from the flow field acting on the surface of the body (i.e., stresses). Mathematically, stress is described by velocity gradient components. Thus, energy dissipation rate (Equation 2) is an appealing flow field parameter because it captures the local effect of the nine velocity gradient components into a scalar value. Practically, energy dissipation rate represents the rate of viscous energy being dissipated by the fluid element directly to a body that is in the control volume of that fluid element.

The concept that energy dissipation can be related to cell damage was first suggested by Bluestein and Mockros (1968), with respect to hemolysis of red blood cells. More recently, Gregoriades et al. (2000) sought to test the hypothesis that energy dissipation can characterize the hydrodynamic conditions within a flow field that result in cell detachment from microcarriers. Their study was unique because it was the first to combine an experimental study of cell damage with high-resolution, numerical simulations of the flow field (an abrupt contraction) which created the cell damage. Encouragingly, they found that the magnitude of energy dissipation rate at which cell detachment began to be detected ($1E+3$ W/m$^3$) was comparable to the estimated maximum energy dissipation in bioreactors operated under conditions where cell detachment was observed (Venkat et al., 1996). In a similar study using a more advanced microfluidic flow device, Ma et al. (2002) evaluated four mammalian cell lines in suspension and found that the suspended cells were able to withstand relatively intense energy dissipation rates ($1E+7$ to $1E+8$ W/m$^3$). These energy dissipation levels are several orders of magnitude higher than measured maximum energy dissipation rates generated by impellers in bioreactors, $0.94E+5$ W/m$^3$ (Wernersson and Tragardh, 1999) and $0.899E+5$ W/m$^3$ (Zhou and Kresta, 1996). The quantitative results of Ma et al. (2002), which indicate the robustness of these cells, reflect the successful culturing of mammalian cells observed in practice using large-scale bioreactors. Based on the above discussion, energy dissipation rate is a meaningful parameter to characterize the damaging effects of a flow field on a biological material.
Table 4. Differences between Gompertz model predicted and observed mean nematode relative damage of *Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema carpocapsae*, and *S. glaseri* after flow through an XR8001VS flat fan nozzle for each of the experimental conditions.

<table>
<thead>
<tr>
<th>Energy dissipation rate (W/m$^3$)</th>
<th>Model predicted$^1$ – Observed mean nematode relative damage$^2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>H. bacteriophora</em></td>
</tr>
<tr>
<td>1.1E+8</td>
<td>13.8</td>
</tr>
<tr>
<td>1.9E+8</td>
<td>22.5</td>
</tr>
<tr>
<td>2.8E+8</td>
<td>32.0</td>
</tr>
<tr>
<td>4.0E+8</td>
<td>36.0</td>
</tr>
<tr>
<td>Mean difference</td>
<td>26.1</td>
</tr>
</tbody>
</table>

$^1$ Nematode relative damage was predicted for each of the EPN species according to the Gompertz model (Equation 4, Table 2).

$^2$ Nematode damage was quantified by counting the number of living and dead (whole and pieces) EPNs and the relative damage was computed according to Equation 1.

Using methods similar to Gregoriades et al. (2000), Fife et al. (2003b) have extended the body of knowledge to include the effects of hydrodynamics on EPNs, a multi-cellular organism. Fife et al. (2003b) found that appreciable damage to EPNs occurred at energy dissipation rates ranging between 1.2E+8 W/m$^3$ and 3.7E+8 W/m$^3$, which is comparable to the levels reported by Ma et al. (2002) for suspended cells. More interestingly, there is a clear trend in the damage response of the EPNs with respect to increases in energy dissipation rate, which was also observed in the studies for mammalian cells (Gregoriades et al., 2000; Ma et al., 2002). The relative damage remains low until a certain level of energy dissipation rate, and then increases rapidly toward saturation at 100% at high levels of energy dissipation rate (Figure 3). The consistency in the relationship between relative damage and energy dissipation rate, termed sigmoid, provided incentive to model the relationship using an empirical function. The logistic (Equation 3) and Gompertz (Equation 4) functions were selected because they capture the sigmoidal relationship. The main difference between these two functions is that the logistic curve is symmetrical about its central point of inflection, while the Gompertz curve is asymmetrical about its point of inflection. Thus, the derivative of the logistic function is normally distributed, while the Gompertz function derivative is skewed slightly to the right. Both functions were able to describe the calibration data well, however the Gompertz function provided a better fit to the data (i.e., lower sum of squares of the residuals). This is likely due to the Gompertz’ asymmetric sigmoid growth curve, which better captures the rapid increase in nematode relative damage at energy dissipation levels where appreciable damage starts to be observed. The Gompertz growth model has been frequently used by ecologists to explain biological phenomena (Batschelet, 1976), and based on the above discussion this function provides a reasonable model for predicting cell or organism damage with respect to the flow field parameter energy dissipation rate.

Overall, the Gompertz model (Equation 4, Table 2) was able to predict nematode relative damage extremely well. For *H. megidis*, *S. carpocapsae*, and *S. glaseri*, 9 out of the 12 model predictions were within 5% of the observed nematode relative damage, and all of the model
predictions were within 10% (Table 4). A possible explanation for the poor model performance of *H. bacteriophora* may be the considerable variance of the experimental data that was used in the model calibration (Figure 3A). The quality of the calibration data effects the model parameter estimates that are determined using the non-linear least squares data fitting algorithm. Additional data for *H. bacteriophora* is necessary to redefine the parameter estimates, and possibly improve the model performance.

The robustness of the empirical model is demonstrated by the fact that not only was the model evaluated with nematode relative damage data that was independent from the model calibration, but also the two experimental systems were different. Average energy dissipation rates from the numerical simulations of an abrupt contraction were input for the model calibration, while average energy dissipation rates from the simulations of the XR8001VS flat fan nozzle were input for the model evaluation. It is important to note that selection of the most representative value of the energy dissipation rate from the flow field for input into the model is critical. Analysis of the distribution of energy dissipation values within the nozzle exit orifice showed that values remain relatively constant away from the nozzle wall. Near the nozzle wall, the energy dissipation values increase rapidly because of the assumption of no-slip at the wall (i.e., a large velocity gradient near the wall). Thus, it was determined that the average energy dissipation rate within the nozzle exit orifice, not including values near the wall, was the most representative value of the flow field.

The ability to successfully predict the damage response of the EPNs within different systems and under varying operating conditions shows the versatility of using energy dissipation rate as a flow field parameter to characterize the hydrodynamic conditions responsible for the observed nematode damage. Moreover, these results show that CFD is a feasible method to evaluate the flow field conditions within an equipment component, and provides the necessary flow field information (i.e., energy dissipation rate) to predict whether or not an equipment component is suitable for a biological agent. This study provides a standard procedure that can be used to evaluate other equipment components and biological agents in order to develop guidelines for selection of appropriate spray equipment and operating conditions for effective application of biopesticides.

**Conclusions**

An empirical model was developed using data from a previous study (Fife et al., 2003b) that related observed nematode relative damage to average energy dissipation rates within an abrupt contraction. The model was calibrated for each of the EPN species. A separate experiment using a common agricultural hydraulic nozzle (XR8001VS flat fan) was conducted to evaluate the model. The nozzle flow field was numerically simulated using FLUENT for the range of experimental conditions, and the average energy dissipation rates (1.1E+8, 1.9E+8, 2.8E+8, and 4.0E+8 W/m$^3$) within the nozzle exit orifice computed in FLUENT were input for the model. Overall, the model was able to predict nematode damage well. For *H. megidis*, *S. carpocapsae*, and *S. glaseri*, 9 out of 12 of the model predictions were within 5% of the observed nematode relative damage, and all were within 10%. However, the model was statistically unacceptable for *H. bacteriophora*, where the observed damage was over-predicted by 14 to 36% for the range of experimental conditions. The considerable amount of variance in the data used for calibrating the *H. bacteriophora* model may have contributed to the poor performance of the model in this case. The ability to successfully predict the damage response of the nematodes within a different system and under varying operating conditions shows the robustness of the empirical model and the versatility of energy dissipation rate to characterize the hydrodynamic conditions responsible for the nematode damage. The results from this study...
demonstrate the feasibility of using CFD methods to evaluate the flow field within an equipment component to assess compatibility with a biological agent.

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**References**


Figure 1. Diagram of the XR8001VS flat fan nozzle.

Figure 2. The grid mesh of the XR8001VS flat fan nozzle used in FLUENT to simulate the experimental flow field.
Figure 3. The logistic (—) and Gompertz (––) model calibration results for *Heterorhabditis bacteriophora* (A - □), *H. megidis* (B - 9), *Steinernema carpocapsae* (C - 0), and *S. glaseri* (D - ∈). Calibration data were from a previous study of an abrupt contraction (Fife et al., 2003b). Error bars represent the standard error of the mean (n=6).
Figure 4. Observed mean relative damage of *Heterorhabditis bacteriophora* (A), *H. megidis* (B), *Steinernema carpocapsae* (C), and *S. glaseri* (D) after treatment with the XR8001VS flat fan nozzle, and the corresponding Gompertz model (Equation 4, Table 2) predictions of nematode damage of each species for the range of experimental conditions. Error bars represent the standard error of the mean (n=6).