

## Entomopathogenic Nematodes Were Effective against Western Flower Thrips on Chrysanthemum Plants, But Not in Gerbera Flowers, under Greenhouse Conditions

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Raymond A. Cloyd and Nathan J. Herrick

Department of Entomology, Kansas State University

Western flower thrips, *Frankliniella occidentalis*, is a major insect pest of cut flower crops that can cause direct economic damage. Producers are looking for alternative strategies to manage western flower thrips because populations have developed resistance to many insecticides. Consequently, some producers are using entomopathogenic nematodes, although most information on how well they work is anecdotal. Therefore, we conducted a research project, funded by the Association of Specialty Cut Flower Growers Research Foundation, to determine the efficacy of the entomopathogenic nematode *Steinernema feltiae* against western flower thrips in gerbera, *Gerbera jamesonii*, flowers and on chrysanthemum, *Chrysanthemum × grandiflorum*, plants under greenhouse conditions.

### Materials and Methods

The following study involved two greenhouse experiments conducted at Kansas State University (Manhattan, KS).

#### Experiment One

In the first experiment, sixty gerbera daisy cut flower stems were placed into glass vials containing tap water. There was one cut flower per glass vial. All vials ( $n=60$ ) were placed on a wire-mesh bench in a greenhouse with an open frame composed of PVC piping, which held a 50% black knit shade cloth placed on top. The experiment was set up as a completely randomized design. Each cut flower was artificially infested with 10 to 15 western flower thrips adults, obtained from our laboratory colony, and allowed to acclimate in the cut flowers for 24 hours. The glass vials were refilled as needed with tap water. The treatments were prepared in 32 fluid ounces (946 milliliters) of tap water and applied to the flowers using 32-ounce spray bottles. Each cut flower received 0.85 fluid ounces (25 milliliters) of spray solution, which thoroughly saturated the flower surface and

allowed the solution to penetrate the disc floret portion of the flowers. There were six treatments, which are presented in Table 1, with five replications per treatment. Thirty gerbera cut flowers were harvested four days after the first application, and the remaining 30 cut flowers were harvested four days after the second application. For both evaluations, cut flowers were placed into plastic Petri dishes with lids and the number of live, dead, and total number of western flower thrips adults per cut flower were counted. Percent mortality associated with each treatment was calculated by dividing the number of dead western flower thrips adults by the total number recovered per cut flower or replicate. Percent mortality estimates for all the data were subject to analysis with treatment as the main effect.

Percent western flower thrips adult mortality was not significantly different ( $P>0.05$ ) across the treatments associated with one application of the entomopathogenic nematodes. There were no statistical comparisons made affiliated with two applications of the treatments because of the low recovery of western flower thrips adults, which may have been due to the cut flowers being excessively moist.

#### Experiment Two

The second greenhouse experiment involved using 35 clear plastic observation cages [18 x 18 x 24 inches (length x width x height)] arranged in rows on top of two wire-mesh greenhouse benches. Each cage had a lid with a hole and two holes on opposing sides covered with thrips screening to allow for ventilation and prevent western flower thrips adults from escaping.

Thirty-five chrysanthemum ‘Manhattan’ young plants were transplanted into 6-inch diameter containers with a growing medium composed of 75 to 85% coarse sphagnum peat moss, perlite, vermiculite, and a wetting agent. Plants were grown for 44 days before they were infested with western flower thrips adults.

Table 1. Treatments and rates used for experiments one and two associated with the effects of the entomopathogenic nematode, *Steinernema feltiae* on western flower thrips, *Frankliniella occidentalis*, adults in gerbera flowers (experiment one) and on chrysanthemum plants (experiment two).

<b>Experiment One</b>	
<b>Treatments</b>	<b>Rates</b>
1. <i>Steinernema feltiae</i> (Nemasys): one application	50 million nematodes/50 gallons (125 thousand nematodes/50 milliliters)
2. <i>Steinernema feltiae</i> (Nemasys): two applications	50 million nematodes/50 gallons (125 thousand nematodes/500 milliliters)
3. Water: one application	---
4. Water: two applications	---
5. <i>Steinernema feltiae</i> (Nemasys) + <i>Beauveria bassiana</i> PPRI 5339 (Velifer): one application	50 million nematodes/50 gallons (125 thousand nematodes/500 milliliters) + 13 fluid ounces/100 gallons (0.96 milliliters/32 ounces)
6. <i>Steinernema feltiae</i> (Nemasys) + <i>Beauveria bassiana</i> PPRI 5339 (Velifer): two applications	50 million nematodes/50 gallons (125 thousand nematodes/500 milliliters)
<b>Experiment Two</b>	
1. <i>Steinernema feltiae</i> (Nemasys): one application	50 million nematodes/50 gallons (125 thousand nematodes/500 milliliters)
2. <i>Steinernema feltiae</i> (Nemasys): two applications	50 million nematodes/50 gallons (125 thousand nematodes/500 milliliters)
3. <i>Steinernema feltiae</i> (Nemasys): three applications	50 million nematodes/50 gallons (125 thousand nematodes/500 milliliters)
4. Water: one application	---
5. Water: two applications	---
6. Water: three applications	---
7. Untreated check	---

Chrysanthemum plants were randomly placed among the 35 clear plastic observation cages. Plants were watered as needed with 6.7 to 10.1 fluid ounces (200 to 300 milliliters) of tap water. Western flower thrips adults were released onto each of the 35 plants with 10 to 15 per plant. These were allowed to acclimate on the plants for three days. The experiment was set up as a completely randomized design. There were seven treatments, which are presented in Table 1, with five replications per treatment.

The chrysanthemum plants received 2.1 fluid ounces (60 milliliters) of spray solution using 32-ounce plastic spray bottles. Seven days after each application, they were inspected to assess the level of thrips feeding damage based on the following foliar damage ranking scale:

- 0=no damage
- 1=very slight damage (minimal leaf scarring; less than 10% of leaf surface area affected)
- 2=slight damage (minimal leaf scarring; 11 to 25% of leaf surface area affected)
- 3=moderate damage (leaf scarring; 26 to 50% of leaf surface area affected)
- 4=severe damage (51 to 75% of leaf surface area affected; leaf scarring and distortion)
- 5=very severe damage ( $\geq 76\%$  of leaf surface area affected; leaf scarring and leaf distortion/minimal leaf expansion)

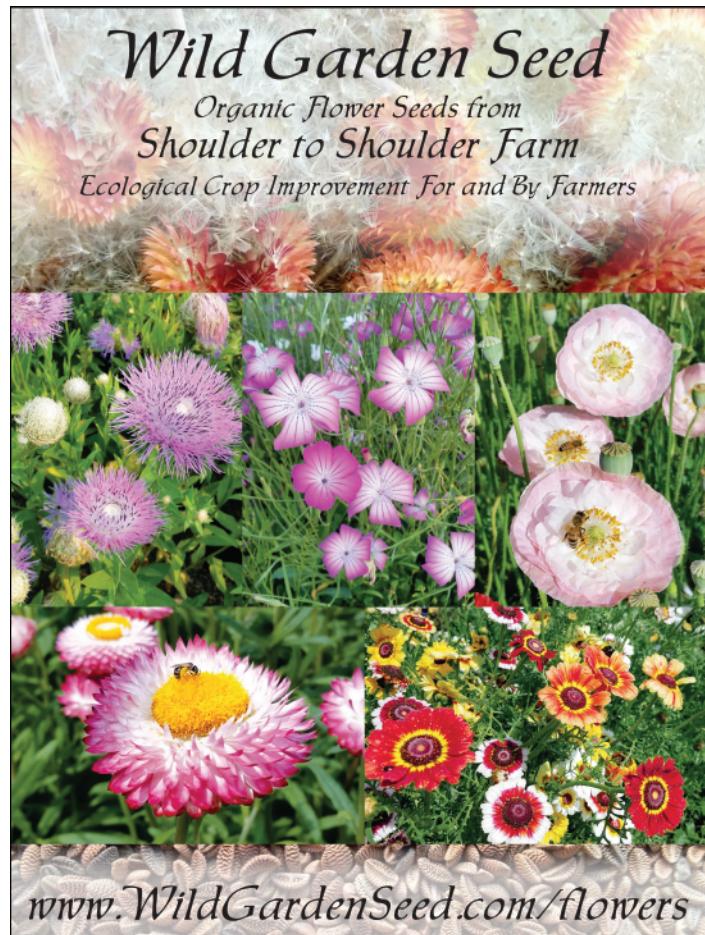
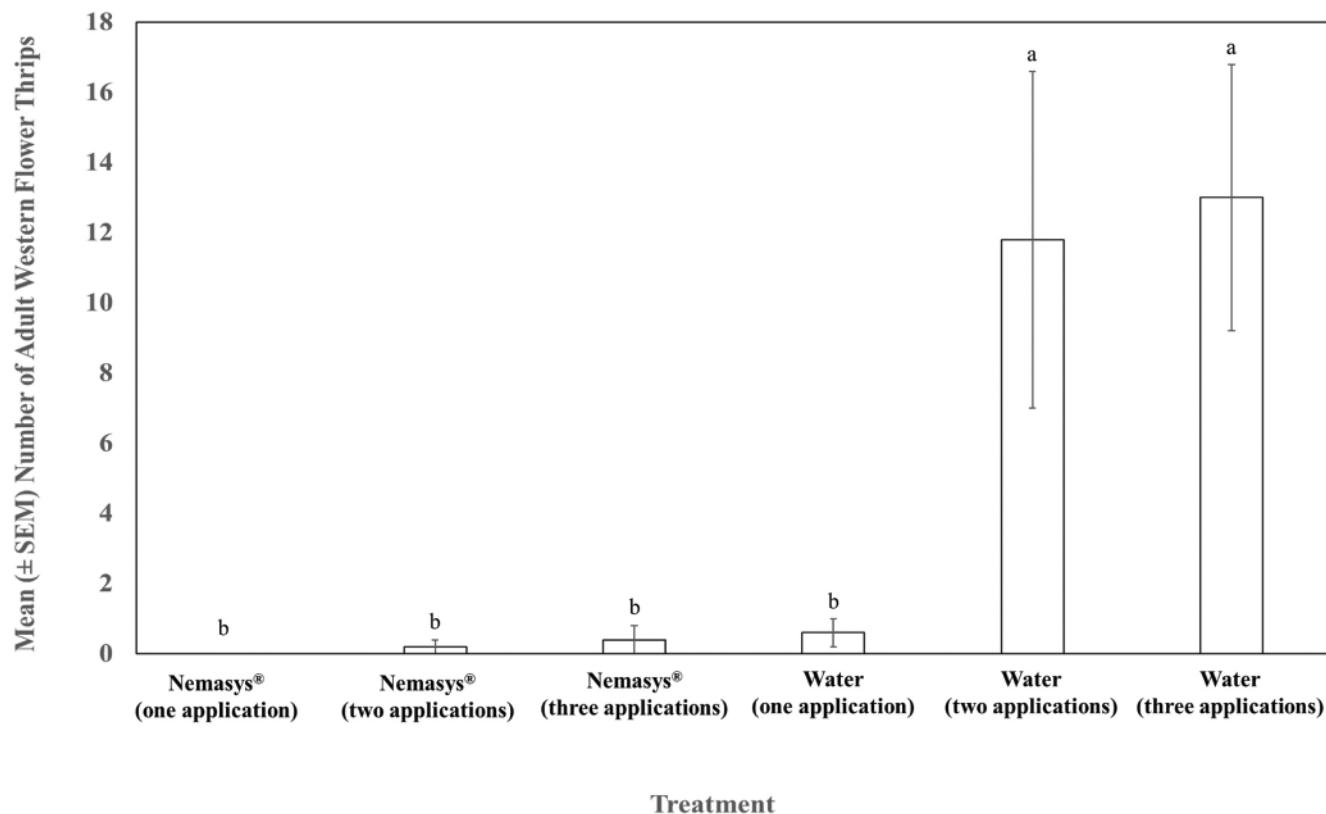


Figure 1. Mean ( $\pm$  SEM) number of western flower thrips, *Frankliniella occidentalis*, adults recovered from growing medium associated with each of the six treatments. Means followed by the same letter are not significantly different ( $P>0.05$ ) as determined by Tukey's honestly significance difference test. The vertical bars represent the standard error of the mean (SEM).



After recording the foliar damage rankings, chrysanthemum plants were cut at the base and all plant material was discarded. A yellow sticky card was placed on top of each 6-inch diameter container and returned to the observation cage. The yellow sticky card (3.0 x 5.0 inches) was inspected after seven days and the number of western flower thrips adults captured was recorded. The use of the yellow sticky card was an indirect assessment of western flower thrips pupal survival among the treatments.

Data were analyzed with treatment and number of applications as the main effects. The foliar damage rankings never exceeded 1 (i.e. very slight damage: minimal leaf scarring; less than 10% of leaf surface area affected) across all treatments and number of applications (one, two, and three). There was a significant treatment effect associated with the mean number of western flower thrips adults recovered from the growing medium ( $F=7.07$ ;  $df=5, 20$ ;  $P=0.0006$ ) (Figure 1). The mean number of western flower thrips adults recovered from the growing medium in the untreated check was  $23.8 \pm 9.5$  adults.

**In summary, the mean number of western flower thrips adults recovered from the growing medium was significantly lower for the entomopathogenic nematode treatments after one, two, and three applications compared to the water control after two and three applications** (Figure 1). The water control after one application had very low number of thrips and was not similar to the other water controls. The reason for this result is the short amount of time between the single application and measuring the number of adult thrips that emerged from the growing medium and were captured on the yellow sticky cards. There was no significant effect ( $P>0.05$ ) affiliated with the number of spray applications on the mean number of western flower thrips adults recovered from the growing medium.

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For more information regarding the study,  
contact Dr. Raymond A. Cloyd at (785) 532-4750  
or [rcloyd@ksu.edu](mailto:rcloyd@ksu.edu)

## Identification of *Fusarium commune* causing meltdown issue in zinnia cut flowers

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Ravi Bika and Fulya Baysal-Gurel

Tennessee State University, Otis L. Floyd Nursery Research Center, McMinnville, Tennessee

Commercial cut flower growers in the eastern and southeastern United States have for many years experienced a postharvest issue with zinnias, commonly called “zinnia meltdown”. The most frequent symptom is bent stems, usually just below the flower head. Meltdown reduces yields of cut flower stems, and therefore, income for the grower. The project started in 2017 with an online survey that reported 29 responses from 17 states: Connecticut (1), Georgia (3), Illinois (2), Iowa (1), Louisiana (1), Maine (1), Maryland (1), Massachusetts (1), Mississippi (2), New Jersey (4), New York (1), North Carolina (3), Ohio (2), Tennessee (2), Texas (1), Utah (1), and Virginia (1), as well as Ontario (1) in Canada. According to the survey results, ‘Benary’s Giant’ was the series most susceptible to the zinnia meltdown issue.

The next step was to identify possible causes of zinnia meltdown. Fifteen zinnia cut stems with flowers exhibiting meltdown symptom were collected from a flower farm in Lincoln County, Tennessee. Small sections of zinnia stems and leaves were isolated from the symptomatic zinnia samples and plated on potato dextrose agar (PDA) and *Fusarium*-selective media. The plates were incubated for five to seven days in laboratory ambiance (21 °C, 60% RH and 12-h fluorescent light and dark cycle).

The morphological characterization (pigmentation, growth pattern, shape and size of micro and macro conidia) and molecular analysis confirmed that *Fusarium commune* was the causal agent for zinnia meltdown issue.

To confirm the pathogen ‘*F. commune*’, a pathogenicity study was performed on three zinnia cultivars: ‘Benary’s Giant Golden Yellow’, ‘Benary’s Giant Pink’, and ‘Benary’s Giant Lime’ at vegetative stage (two weeks after transplantation) or flower bud stage (one month after transplantation).

The fungal conidial suspension (inoculum) was prepared by flooding a 10–14 day old culture of *F. commune*. Three different methods of inoculation were tested on the vegetative and flower bud stages: drench (25 mL of conidial suspension was applied to the substrate near zinnia plant root area); stem injection (30 µL of conidial suspension was injected into

zinnia stem using a 1-mL syringe with a disposable needle); and foliar spray (conidial suspension was sprayed on zinnia plant [including leaves, stem and flower bud] until runoff using a handheld sprayer). Control plants received sterile distilled water.

Zinnia stems were harvested when the outer petals of the flowers were fully expanded, and displayed under laboratory conditions. Similar symptoms, such as stem bending just below the flower, were observed on inoculated zinnia cut flowers of all three cultivars two days after harvesting.

*Fusarium commune* was re-isolated from the infected flower stems of all three cultivars of zinnia, but not from the non-inoculated zinnia flower stems. We observed that the zinnia stem colonization by *F. commune* was statistically similar in all three tested cultivars regardless of plant growth stage and method of inoculation. The typical meltdown issue observed in zinnia cut flowers in postharvest condition might have been due to vascular occlusion; the microconidia of *F. commune* may form a cluster in vascular tissue, hindering the water uptake that can lead to bending of the stem below the flower during postharvest vase life.

In conclusion, the morphological and molecular analysis, as well as pathogenicity tests, confirmed that *Fusarium commune* is the causal organism for zinnia meltdown in Tennessee. The next steps in this project would be to screen cultivars for sensitivity to *F. commune*, and identify possible sources, such as irrigation water, transplants, soils etc. of *F. commune* in the zinnia production area. Additionally, we are still accepting infected zinnia samples from other states, and conducting pathogenicity studies on the zinnia meltdown issue.

So, what can be done to prevent zinnia meltdown? Control methods are still being developed, but growers report that it helps to be sure buckets and cutters are clean, and fresh solutions are used. Growers report success with using commercial hydration or slow-release chlorine right after harvest.

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For more information, please contact  
Dr. Fulya Baysal-Gurel ([fbaysalg@tnstate.edu](mailto:fbaysalg@tnstate.edu)).