

Submission ID #: 69678

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21251838>

Title: Grower-Based In Vivo Propagation of Entomopathogenic Nematodes

Authors and Affiliations:

Shawn A. Steffan^{1,2}, Sehrish Gulzar³, Camila Oliveira-Hofman³, David I. Shapiro-Ilan³

¹US Department of Agriculture, Agricultural Research Service, Vegetable Crops Research Unit

²Department of Entomology, University of Wisconsin-Madison

³US Department of Agriculture, Agricultural Research Service, Southeast Fruit and Tree Nut Research Unit

Corresponding Authors:

Shawn A. Steffan

steffan@entomology.wisc.edu

David I. Shapiro-Ilan

david.shapiro@usda.gov

Email Addresses for All Authors:

Sehrish Gulzar

sehrishgulzar41@gmail.com

Camila Oliveira-Hofman

coliveirahofman@gmail.com

Shawn A. Steffan

steffan@entomology.wisc.edu

David I. Shapiro-Ilan

david.shapiro@usda.gov

Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar?

No

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 17

Number of Shots: 32

Introduction

Videographer: Please film the interviews and protocol section 2.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Answers are edited in accordance with journal guidelines for length

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **David Shapiro-Ilan:** Our research develops a sustainable, grower-friendly method for producing entomopathogenic nematodes, addressing cost and technical barriers to enable on-farm pest control solutions.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~What are the most recent developments in your field of research?~~

- 1.2. **Sehrish Gulzar:** This low cost and low input approach for entomopathogenic nematode production leads to sustainable production and is easily adaptable to various species.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~How will your findings advance research in your field?~~

- 1.3. **Stacy Byrd:** Grower-oriented EPN production methods represent lower costs, adoptability, sustainability, longevity, and higher yields for pest control programs. EPNs are suitable for a wide diversity of cropping systems.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

(Affiliation of Stacy: US Department of Agriculture, Agricultural Research Service, Southeast Fruit and Tree Nut Research Unit)

What advantage does your protocol offer compared to other techniques?

- 1.4. **Sehrish Gulzar:** Unlike prior methods, our grower-based approaches, once initiated, are fully self-sustaining. Medium gel can be stored for months under refrigeration for later use, making it cost effective.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~What new scientific questions have your results paved the way for?~~

- 1.5. **David Shapiro-Ilan:** This research provides a clear path for the farmers and stakeholders to propagate EPNs in small areas without much technical difficulty. In the future, further optimization and increases in efficiency can be explored.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

- 1.6. **David Shapiro-Ilan (Research Leader, Southeast Fruit and Tree Nut Research Unit, US Department of Agriculture)**: (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

How does the video format of your article help you to share your work with the broader scientific community?

- 1.7. **Shawn Steffan (Research Entomologist, Vegetable Crops Research Unit, US Department of Agriculture, Agricultural Research Service, Madison, WI, USA)**: (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

NOTE: 1.7. was APF

Protocol

2. Entomopathogenic Nematode Culturing in *Galleria mellonella*

Demonstrator: Stacy Byrd

- 2.1. To begin the initial inoculum, obtain the desired entomopathogenic nematode species [1]. Use the infective juvenile stage of *Steinernema carpocapsae* All (A-L-L) strain and *Heterorhabditis bacteriophora* VS (We-Yes) strain [2].
 - 2.1.1. Talent placing culture containers containing the desired entomopathogenic nematode species on the work surface.
 - 2.1.2. Talent picking up or showing the infective juvenile stage cultures of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*.
- 2.2. For individual infections, inoculate 50 live *Galleria mellonella* larvae with infective juveniles at a density of 100 infective juveniles per insect [1]. Keep the inoculated insects at 25 degrees Celsius until infective juveniles are near emergence [2].
 - 2.2.1. Talent adding infective juveniles to live *Galleria mellonella* larvae.
 - 2.2.2. Talent placing the Petri plate into a temperature-controlled area set to 25 degree Celsius.
- 2.3. For mass inoculation, place 1 infected cadaver with 50 live *Galleria mellonella* larvae into a plastic container with a volume between 5.7 to 14.2 liters lined with a moist paper towel [1] and keep the container at 25 degree Celsius [2].
 - 2.3.1. Talent placing an infected cadaver and live larvae into a plastic container lined with a moist paper towel.
 - 2.3.2. Talent closing the container and placing it into an incubator or controlled environment set to 25 degrees Celsius.
- 2.4. After 72 hours, remove cadavers that do not show appropriate color changes to avoid

contamination [1]. Identify tan or brown cadavers for *Steinernema* infections and red or orange cadavers for *Heterorhabditis* infections [2].

2.4.1. Talent removing and discarding cadavers that lack the appropriate color changes.

2.4.2. Talent showing brown and red cadavers.

2.5. Next, moisten the polyacrylamide gel with water at a ratio of 20 milliliters per dry gram of gel [1]. Place the cadavers on top of a nylon screen mesh with a 2 by 2 millimeter pore size [2] and transfer the appropriately colored cadavers into the clear plastic container containing polyacrylamide gel [3].

2.5.2, Talent adding the appropriate volume of water to moisten the gel.

2.5.3, Talent placing a nylon screen mesh over the gel and positioning cadavers on top of the mesh.

2.5.1, Talent transferring the appropriately colored cadavers into clear plastic containers containing polyacrylamide gel.

Author's NOTE: Place 2.5.1 after 2.5.3, VO adjusted

2.6. Then, remove and discard depleted cadavers once infective juvenile production diminishes substantially [1]. Harvest infective juveniles from the infected hosts that remain on the screen above the gel [2]. Continue harvesting into the gel for 5 to 7 days after infective juveniles begin to emerge [3].

2.6.3,. Talent removing and discarding depleted cadavers from the screen.

2.6.1. Talent collecting infective juveniles that migrate into the gel.

2.6.2. Shot of infective juvenile emerging.

Author's NOTE: Move 2.6.3 before 2.6.1

Order is 2.6.3, 2.6.1, 2.6.2, VO adjusted

2.7. To develop inoculum for subsequent production rounds, expose healthy *Galleria mellonella* larvae to infective juveniles within the gel in 90-millimeter Petri dishes. Use these newly infected hosts to inoculate the next group of *Galleria mellonella* larvae [1].

2.7.1. Talent placing healthy larvae into Petri dishes containing gel with infective juveniles.

~~Talent pointing to the infected hosts.~~ **Author's NOTE:** Not filmed, VO merged

- 2.8. For entomopathogenic nematode applications, rinse infective juveniles that have entered the gel using tap water and screens with a pore size smaller than 0.3 centimeter [1]. Alternatively, apply the nematodes directly in the gel to small arenas without rinsing [2].
 - 2.8.1. Talent rinsing infective juveniles from the gel using water and fine screens.
 - 2.8.2. Talent applying gel containing infective juveniles directly to small experimental arenas.

3. *Oscheius onirici* and *Heterorhabditis georgiana* Propagation

Demonstrator: Shawn Steffan

NOTE: This part was APF, use section 3 revised videos in the lab media folder, labeled as per shot numbers. The authors have given an audio explanation throughout

- 3.1. Obtain infective juveniles of *Oscheius onirici* and *Heterorhabditis georgiana* from in vivo nematode cultures using *Tenebrio molitor* as the host and gently irrigate the round cotton pad with water [1]. Collect aliquots of nematode slurry to observe and count the live infective juveniles under a microscope [2].
 - 3.1.1. Talent has placed a Petri dish with a nematode culture on the work-surface, then gently irrigates the cotton rounds with water to flush out the nematodes from the cotton. **Author's NOTE:** This step and VO was modified

Added shot 3.1.2: Talent collecting small aliquots of the nematode slurry to observe and count the live infective juveniles under a microscope.
- 3.2. Now, position 4 to 6 *Tenebrio molitor* larvae, either live or freeze-killed, in a non-overlapping pattern between the two cotton pads [1] and add 10 to 15 milliliters of water [2].
 - 3.2.1. Talent positioning *Tenebrio molitor* larvae between the cotton rounds without overlap. **Author's NOTE:** This step was combined in 3.2.1
 - 3.2.2. Talent add 10-15 ml of water onto the top cotton round.
- 3.3. Add 10 milliliters of a nematode slurry with a concentration of 400 to 500 infective juveniles per milliliter onto the top cotton pad and allow the infective juveniles to infect the mealworms [1].
 - 3.3.1. Talent pouring the nematode slurry onto the top cotton round.

3.4. Harvest infective juveniles from the Petri dishes by pouring 20 to 25 milliliters of water over the top cotton pad [1], then collect the slurry that has percolated through the cotton [2].

3.4.1. Talent pouring water over the top cotton round.

3.4.2. Talent collecting the percolated nematode slurry into a container. **Author's**

NOTE: This step was combined in 3.2.1

3.5. To infect insect hosts in large, shallow trays, cut and place the wool bilayer within the tray [1]. Then, distribute the *Tenebrio molitor* hosts between the wool sheets [2] before thoroughly drenching the wool sheets with approximately 400 milliliters of a nematode suspension at a concentration of 80,000 to 90,000 nematodes per liter. Incubate the tray at 21 to 22 degrees Celsius for 4 weeks [3].

3.5.1. Talent cutting wool sheets and placing within a shallow tray, which will serve as the nematode propagation vessel.

3.5.2. Talent evenly distributing *Tenebrio molitor* larvae between the two wool sheets.

3.5.3. Talent drenching wool sheets in shallow trays with the nematode suspension.

3.5.4. ~~Talent placing the trays into an incubation area set to 21 to 22 degrees Celsius.~~

NOTE: Could be merged or not filmed

3.6. After 4 weeks, harvest the nematodes by irrigating each tray with 500 milliliters of tap water and then collecting the solution. Repeat the irrigation a second time and collect a total of 1 liter of nematode slurry [1].

3.6.1. Talent pouring tap water over a tray to irrigate the wool sheets and collecting the combined nematode slurry.

3.7. Finally, determine the nematode density by counting the number of nematodes per milliliter under a dissecting microscope and tally the counts for each replicate tray [1].

~~Talent placing the sample under a dissecting microscope.~~

3.7.1. SCOPE: Live nematodes viewed under a microscope.

Results

4. Results

- 4.1. In the *Steinernema carpocapsae* experiment, setting 30 cadavers in polyacrylamide gel in 14.2 liter-clear-plastic containers produced significantly more infective juvenile nematodes per cadaver [1] compared to that in larger White traps [2].

4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the bar labelled T4*

4.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the bar T2*

- 4.2. In the *Heterorhabditis bacteriophora* experiment, significantly more infective juvenile nematodes per cadaver were produced in the grower-oriented gel treatment [1] compared to the White trap treatment [2].

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the bar labelled Gel*

4.2.2. LAB MEDIA: Figure 3. *Video editor: Highlight the bar labelled White Trap*

1. Entomopathogenic
Pronunciation link: <https://www.howtopronounce.com/entomopathogenic>
IPA: / ˌɛn.tou.moʊ.pæθəˈdʒɛnɪk/
Phonetic Spelling: en·toh·moh·path·uh·jen·ik
2. Nematode
Pronunciation link: <https://www.merriam-webster.com/dictionary/nematode>
IPA: / ˈni:məˈtoʊd/
Phonetic Spelling: nee·muh·tohd
3. Steinernema
Pronunciation link: <https://www.howtopronounce.com/steinernema>
IPA: / ˌstɪnərˈni:mə/
Phonetic Spelling: sty·ner·nee·muh
4. Carpocapsae
Pronunciation link: <https://www.howtopronounce.com/carpocapsae>
IPA: / ˌkɑːrpoʊˈkæpsiː/
Phonetic Spelling: kar·poh·kap·see
5. Heterorhabditis
Pronunciation link: <https://www.howtopronounce.com/heterorhabditis>
IPA: / ˌhɛtərərɒʊˈhæbdɪtɪs/
Phonetic Spelling: het·uh·roh·hab·duh·tiss

6. Bacteriophora
 Pronunciation link: <https://www.howtopronounce.com/bacteriophora>
 IPA: /bækˌtɪriˈɑːfərə/
 Phonetic Spelling: bak·teer·ee·of·uh·ruh
7. Infective
 Pronunciation link: <https://www.merriam-webster.com/dictionary/infective>
 IPA: /ɪnˈfektɪv/
 Phonetic Spelling: in·fek·tiv
8. Juvenile
 Pronunciation link: <https://www.merriam-webster.com/dictionary/juvenile>
 IPA: /ˈdʒuːvəˌnaɪl/
 Phonetic Spelling: joo·vuh·nyl
9. Galleria mellonella
 Pronunciation link: <https://www.howtopronounce.com/galleria-mellonella>
 IPA: /ˌɡæləˈriːəˌmɛləˈnɛlə/
 Phonetic Spelling: gal·uh·ree·uh mel·uh·nel·uh
10. Cadaver
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cadaver>
 IPA: /kəˈdævər/
 Phonetic Spelling: kuh·dav·er
11. Polyacrylamide
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polyacrylamide>
 IPA: /ˌpɑːliəˈkrɪləˌmɑːd/
 Phonetic Spelling: pol·ee·uh·kril·uh·myd
12. Nylon
 Pronunciation link: <https://www.merriam-webster.com/dictionary/nylon>
 IPA: /ˈnaɪˌlɑːn/
 Phonetic Spelling: ny·lon
13. Oscheius
 Pronunciation link: <https://www.howtopronounce.com/oscheius>
 IPA: /ˈɒskiəs/
 Phonetic Spelling: os·kee·uhs
14. Onirici
 Pronunciation link: <https://www.howtopronounce.com/onirici>
 IPA: /ooˈnɪrɪtʃi/
 Phonetic Spelling: oh·neer·ee·chee
15. Georgiana
 Pronunciation link: <https://www.howtopronounce.com/georgiana>
 IPA: /ˌdʒɔːr.dʒiˈæn.ə/
 Phonetic Spelling: jor·jee·an·uh
16. Tenebrio molitor
 Pronunciation link: <https://www.howtopronounce.com/tenebrio-molitor>
 IPA: /təˈniːbrɪoʊ ˈmɒlɪtɔːr/
 Phonetic Spelling: tuh·nee·bree·oh mol·ih·tor

17. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /'æləˌkwɑ:t/
Phonetic Spelling: al·uh·kwaht
18. Slurry
Pronunciation link: <https://www.merriam-webster.com/dictionary/slurry>
IPA: /'slɜːi/
Phonetic Spelling: slur·ee
19. Percolated
Pronunciation link: <https://www.merriam-webster.com/dictionary/percolate>
IPA: /'pɜːkəˌleɪtəd/
Phonetic Spelling: per·kuh·lay·tuhd
20. Irrigating
Pronunciation link: <https://www.merriam-webster.com/dictionary/irrigate>
IPA: /'ɪrɪˌɡeɪtɪŋ/
Phonetic Spelling: ir·uh·gay·ting
21. Dissecting
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissecting>
IPA: /dɪˈsektɪŋ/
Phonetic Spelling: dye·sek·ting
22. Replicate
Pronunciation link: <https://www.merriam-webster.com/dictionary/replicate>
IPA: /'rɛpləˌkeɪt/
Phonetic Spelling: rep·luh·kayt
23. Contamination
Pronunciation link: <https://www.merriam-webster.com/dictionary/contamination>
IPA: /kənˌtæməˈneɪʃən/
Phonetic Spelling: kuhn·tam·uh·nay·shuhn
24. Inoculum
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculum>
IPA: /ɪˈnɔːkjələm/
Phonetic Spelling: ih·nok·yuh·luhm
25. Emergence
Pronunciation link: <https://www.merriam-webster.com/dictionary/emergence>
IPA: /ɪˈmɜːdʒəns/
Phonetic Spelling: ih·mur·juhns