Journal of Visualized Experiments

Plate competition assay as quick preliminary assessment of disease suppression

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58767R1
Full Title:	Plate competition assay as quick preliminary assessment of disease suppression
Keywords:	bioassay; Biological control; Rhizoctonia solani; root diseases; soilborne fungal pathogens; soil microbial communities
Corresponding Author:	Deborah A Neher University of Vermont Burlington, VT UNITED STATES
Corresponding Author's Institution:	University of Vermont
Corresponding Author E-Mail:	Deborah.Neher@uvm.edu
Order of Authors:	Deborah A Neher
	Thomas R Weicht
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	University of Vermont, 63 Carrigan Drive, Jeffords Hall Room 342, Burlington, Vermont 05405

The University of Vermont

July 11, 2018

Dear Lyndsay Toyer,

Thank you for the invitation to submit the attached manuscript for publication in JOVE. I have followed the instructions provided to my best ability. The style is straightforward and hopefully sufficient for publication. Please let me know if there are any revisions necessary before the review process or acceptance for publication.

Sincerely,

Deborah A. Neher, Ph.D.

Professor

Department of Plant and Soil Science, College of Agriculture and Life Sciences
Jeffords Hall, 63 Carrigan Drive, Burlington, VT 05405
Telephone: (802) 656-2630 • Fax: (802) 656-4656 • www.uvm.edu/~pss/
Equal Opportunity/Affirmative Action Employer

TITLE:
A Plate Competition Assay as a Quick Preliminary Assessment of Disease Suppression

AUTHORS & AFFILIATIONS:
Deborah A Neher[1], Thomas R Weicht[1]

[1] *Department of Plant and Soil Science, University of Vermont, Burlington, VT USA*

Corresponding Author:
Deborah A Neher
Email address: dneher@uvm.edu

Email Addresses of Co-authors:
Thomas Weicht (tweicht@uvm.edu)

KEYWORDS:
Biological control, soil microbial communities, *Rhizoctonia solani*, root diseases, soilborne fungal pathogens, bioassay

SUMMARY:
Presented is a protocol for a plate competition assay to identify whether a specific compost is likely to contain bacteria and fungi that suppress growth of *Rhizoctonia solani*.

ABSTRACT:
The goal was to develop and optimize a simple, affordable, and effective bioassay to detect disease suppressive ability of a specific compost against soilborne fungus *Rhizoctonia solani*. *R. solani* is a pathogen of a wide range of plant hosts worldwide. The fungus survives in soils as a saprophyte and grows rapidly on simple water agar media. The plate assay is a rapid method to compare composts for their ability to slow the growth of *R. solani*. The assay also correlates well with suppression of other soilborne fungal pathogens that survive as saprophytes in soils such as Alternaria early blights, Fusarium wilt, Phytophthora root rot, and Pythium root rot.

INTRODUCTION:
Rhizoctonia represents a broad complex of fungi, of which *Thanatephorus cucumeris* (Frank) Donk (anamorph = *Rhizoctonia solani* Kühn) is the pathogen causing root rot and damping-off[1]. *Rhizoctonia solani* is an aggressive pathogen and a saprophyte that can survive as sclerotia under adverse environmental conditions[1]. As a result, it has a global distribution and can cause disease on wide range of plant hosts including Solanaceae, Fabaceae, Asteraceae, and Brassicaceae resulting in serious economic losses.

Compost has the capacity to harbor biocontrol agents for certain plant pathogens[2]. However, not all composts are alike nor do they affect all pathogens similarly[3]. Wood-based carbon has higher lignin to cellulose ratios than hay or straw-carbon based composts. *R. solani* prefers readily available carbon found in straw. In contrast, biological control fungi, such as

Trichoderma spp., are more effective when carbon is less readily available. Beneficial fungi and bacteria into compost can suppress plant disease through competition, antagonism or regulating plant growth[3]. The proposed assay primarily detects antagonism created by production of antibiotics, ecoenzymes or chelators that are detrimental to the pathogen.

Plant bioassays are a gold standard to determine whether composts favor or deter plant growth[4]. However, plant bioassays are time-consuming (weeks to months) to complete which may be longer than desired and requires more labor to extract plants with roots to quantify severity of root systems. Comparably robust, but quicker (days) assays would be ideal for quality control programs. The goal of this paper is to demonstrate a relatively quick and accurate test to predict suppressive potential of compost. The method was patterned after Alfano *et al.*[5] with two exceptions, compost extracts were diluted and water agar was used in place of potato dextrose agar (PDA). *R. solani* grows rapidly on simple water agar media whereas PDA promoted growth of bacteria and other fungi that contaminated the culture[6].

This plate assay serves as an indicator of suppression that applies to a range of plant pathogens that survive in soil as saprophytes including *R. solani*[7], Alternaria early blights, Fusarium wilt, Phytophthora root rot, and Pythium root rot. The plate competition assay is useful to screen a range of compost products for containing communities of microbes that serve as biological control agents of soil pathogens. The assay was one of the most consistent indicators of disease suppression in commercial compost products[6,8]. Products were chosen for their variation in recipe, maturity, and production process.

PROTOCOL:

1. Prepare in Advance

1.1 Master fungal culture (test organism)

1.1.1 Order from the American Type Culture Collection, Microbiology Collection[9], by its teleomorph *Thanatephorus cucumeris* (Frank) (ATCC 10154) or MYA 4031.

1.1.2 Alternatively: Collect local isolates as done by the authors. Seedlings of red radish (*Raphanus sativus*) work well as a bait plant; collect soil from a location known to have a history of damping-off or root rot caused by *R. solani*. Sow seeds in the soil and isolate from root lesions when seedlings are 3 to 4 weeks old.

1.1.2.1 Remove seedlings from soil and rinse with tap water. Look for brown tissue in the hypocotyl region between the leaves and the root (**Figure 1**).

[Place **Figure 1** here]

1.1.2.2 Use a single edged razor blade to cut 1 cm segments of the hypocotyl or root that have a brown color. Use flame-sterilized forceps to dip the segments in 10% solution of household bleach for 1 min, followed by a 10 s rinse in sterile water.

Note: The top and bottom of a sterile Petri dish are useful containers for this step.

1.1.2.3 Use flame-sterilized forceps to transfer the plant pieces to the inside of a paper towel to pat dry and then place on the surface of a Petri dish with water agar (15 g in 1 L).

1.1.2.4 Place the Petri dish inside a plastic container with a lid and incubate at room temperature (approximately 20 °C). Wipe the interior of the container with 10% bleach or 75% ethanol and let it air dry before inserting the dish.

1.1.2.5 Keep the isolates in long-term storage on a minimal media of corn meal agar (17 g in 1 L) slants (at 5 °C).

1.2 Use potato dextrose agar plates (39 g in 1 L) for inoculum for this plate assay.

1.2.1 Establish a daughter culture of *Rhizoctonia* culture on potato dextrose agar one or two days before the assay, to insure it is well established on the culture plate before the assay. Start the daughter culture by transferring a small piece (10 mm diameter) of *R. solani* from the master culture to the center of a fresh plate of potato dextrose agar (**Figure 2**).

Note: Work in a laminar flow hood or wipe down a laboratory bench with 10% bleach or 75% ethanol to minimize risk of contamination.

1.3 Autoclave 125 mL conical flasks of with 10 mL aliquots of distilled water (2 x number of samples.

2. Preparation of Samples to Test

2.1 Add two independent 0.5 g samples of each compost test sample to 10 mL of autoclaved water in 125 mL Shake the conical flasks overnight.

2.1.1 Label the conical flasks with unique sample numbers, and each member of the pair as A (reference) or B (sample).

2.2 After 24 h, add 1.5 g of plain agar to 90 mL of distilled water for two conical flasks.

2.3 Autoclave both conical flasks with agar, and the A sample of each pair for 20 min with a slow exhaust setting.

2.4 After the autoclave, place the conical flasks with agar into a 45 °C water bath until they reach equilibrium (about 30 min). Do not put any sample slurries in the water bath until it has cooled to 45 °C.

[Place **Figure 2** here]

2.5 Pour the A sample (autoclaved reference) compost slurry into the molten agar of the A agar flask. Swirl to disperse compost into the agar.

2.6 Pour the B sample (living sample) compost slurry into the molten agar of the B agar flask. Swirl to disperse compost into the agar.

2.7 Pour the mixture of each flask into five plastic Petri dishes that are 100 mm in diameter.

2.8 Let the plates cool overnight so the agar hardens.

3. Add the *Rhizoctonia* Challenge

3.1 Using aseptic technique, transfer equal size pieces of *R. solani* to each pair of sample agar plates (3 to 5 mm corkborer works well to establish equal sized pieces). Take pieces of the colony from the outside margin of the colony to insure mycelium is actively growing.

Note: Work in a laminar flow hood or wipe down a laboratory bench with 10% bleach or 75% ethanol.

3.2 Incubate the plates at room temperature for 1-2 days until the colony growth reaches about half way to the edge of the A plates.

4. Measure *R. solani* Growth

4.1 Measure the radius of the mycelium in each plate to the nearest 1 mm with a clear flat ruler using a stereo microscope with transmitted illumination.

Note: Oblique or dark field illumination will make it easier to see and measure the transparent hyphae. At this point, zones of inhibition will be visible around compost fragments in the B plate if the compost is suppressive.

4.2 Record the radius at three places per plate and compute a mean of the three to serve as a representative measure.

4.3 Subtract the mean of the B plates from the A plates. If B < A then there are microbes in the B plates that are suppressive to the *R. solani* pathogen.

4.4 Divide the mean radius by the number of days of challenge to express units of relative suppression as a rate, mm mycelium per day.

REPRESENTATIVE RESULTS:

Finished compost should be stable and mature, two terms that are often used interchangeably, so it can be safely packaged and transported, and not cause adverse effects during its end use[4]. *Stability* is a resistance to decomposition and is usually determined using indices of microbial activity. General measures of microbial respiration may measure compost stability but not necessarily disease suppression of an aggressive pathogen and saprophyte such as *R. solani*[7]. This study focused on *maturity* which infers the material is ready for a particular use, and, for horticultural purposes, is determined by plant germination and growth assays.

Extracts of the living composts suppressed *R. solani* growth significantly more than autoclaved samples ($P \leq 0.0001$). Autoclaving kills off microbial activity, pointing to a microbially mediated suppression. This confirms that Plate A in the method serves as a negative control for microbially mediated biological control (**Figure 2**).

[Place **Figure 3** here]

Growth of *R. solani* mycelium on compost extract was affected by compost method and raw material in the recipe of compost but not duration of maturation or curing[6,8]. Growth was reduced by vermicompost and windrow processes and recipes containing hardwood bark (**Figure 3**). Suppressiveness represents a reduction in growth of *R. solani.*

[Place **Figure 4** here]

FIGURE AND TABLE LEGENDS:

Figure 1. Disease symptoms. Soil containing R. solani results in patchy germination and establishment (left). Symptoms on radish seedlings occur as brown lesions at the hypocotyl (right).

Figure 2. Illustration of protocol. Plugs of *R. solani* are placed on Petri dishes containing compost water extract. The diameter of mycelial growth is measured after 1-2 days with the aid of a stereo microscope to improve resolution and contrast.

Figure 3. Effects of autoclaving on *R. solani* growth *in vitro*, as measured by percentage change in mycelial growth from control. A test material is 'suppressive' to the fungus if the radius of mycelial growth is less than an autoclaved control. Illustrated are means ± 1 standard error. The error bar for the autoclaved control is too small to see.

Figure 4. Use of plate competition assay to compare the suppressiveness of compost products. Means ± 1 standard error of the change in *R. solani* growth on an autoclaved reference plate. Both controls and treatment comparisons were inoculated with virulent *R.*

solani. Values below zero represent suppressiveness. Suppressiveness of *R. solani* was affected by compost A) method, B) duration of curing or maturation, and C) recipe. Processes compared were aerated static pile (ASP), windrow (W), vermicompost (V) and anaerobic digestion (AD). Recipe ingredients compared were food waste (F), poultry manure (P), food waste and poultry manure (FP), dairy manure (M), and hardwood bark (H). Contrasting letters above bars denote treatments that are significantly different. This figure has been modified from Neher *et al.*[8].

DISCUSSION:

We know from previous research, that certain composts are effective at suppressing *R. solani* and that the suppressive effects are due to the microbes living in the compost, not the abiotic properties of compost[6,8]. The use of autoclaving as a means to 'kill' microbiota has been criticized because it affects the carbon chemistry of the media[10]. We compared the use of autoclaving to vacuum filtration through Whatman No. 1 paper. Treatments that were neither filtered nor autoclaved showed the greatest suppression of *R. solani* growth[6]. Filtration removed the larger, solid particles that were apparently important to disease suppression by likely harboring antibiotic producing microbes.

Advantages of the method are the simplicity and affordability. Similar to the conclusions and recommendations of Alfano *et al.*[2], the plate competition assay is a quick preliminary assessment of disease suppression but not reliable as a standalone assay because only the pathogen, and not the plant host, is present. Besides, disease would not establish if the environment was unfavorable; *R. solani* diseases are favored by warm and moist conditions[1]. Overall, there is more complexity in the soil and compost ecosystem than could be mimicked entirely by a single laboratory assay[4,8]. The plate assay is comparable to other indicators including a nematode index of compost maturity and three microbial ecoenzymes, phosphatase, β-glucosidase and β-1,4-N-acetylglucosaminidase[8]. The ecoenzymes reflects the state of decomposition and the carbon and nutrient needs of the microbial community. Confirmation of suppressiveness can be achieved with plant assays but they take longer to complete. Isolating and maintaining a pure culture of a fungus takes care to avoid contamination. ATCC[9] offers tips and techniques for culturing yeasts and filamentous fungi.

The method is a potential candidate for commercial certification of disease suppressive properties. The result reflects suppression as a soil function. It does not specify mechanisms or specific microbial species responsible for disease suppression. A future application could be to cut out a portion of the inhibition zone for extraction of DNA for sequencing and identification of community members.

ACKNOWLEDGMENTS:

The Vermont Agricultural Experiment Station Competitive Hatch Program VT-HO1609 funded the research. Lynn Fang used the method as part of her M.S. thesis at University of Vermont[10].

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Gonzalez Garcia, G., Onco, M.A.P., Susan, V. R. Review. Biology and systematics of the form genus *Rhizoctonia*. *Spanish Journal of Agricultural Research* **4** (1), 55-79 (2006).

2. Bonanomi, G., Antignani, V., Pane, C., Scala, E. Suppression of soilborne fungal diseases with organic amendments. *Journal of Plant Pathology* **89**, 311-324 (2007).

3. Noble, R. Risks and benefits of soil amendment with composts in relation to plant pathogens. *Australasian Plant Pathology* **40,** 157-167 (2011). (1)

4. Wichuk, K.M., McCartney, D. Compost stability and maturity evaluation - a literature review. *Canadian Journal of Civil Engineering* **37**, 1505-1523 (2010).

5. Alfano, G., Lustrato, G., Lima, G., Vitullo, D., Ranalli, G. Characterization of composted olive mill wastes to predict potential plant disease suppressiveness. *Biological Control* **3**, 199-207 (2011). (2)

6. Fang L (2015) Biological indicators of compost-mediated disease suppression against the soilborne plant pathogen *Rhizoctonia solani*. M.S. Thesis, University of Vermont.

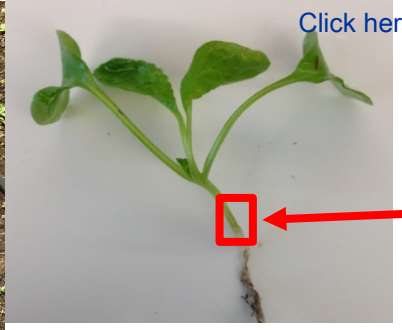
7. Bonanomi, G., Antignani, V., Capodilupo, M., Scala, F. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology and Biochemistry* **42**, 136-144 (2010).

8. Neher, D.A., Fang, L., Weicht, T.R. Ecoenzymes as indicators of compost to suppress *Rhizoctonia solani*. *Compost Science and Utilization* **25** (4), 251-261 (2017).

9. American Type Culture Collection, https://www.atcc.org/ Microbiology Collection. https://www.atcc.org/~/media/PDFs/Culture%20Guides/Mycology_Guide.ashx.

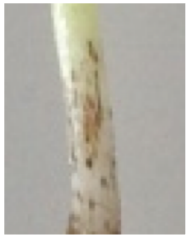
10. Berns, A.E., Phillip, H., Narres, H. –D., Burauel, P., Vereecken, H., Tappe, W. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. *European Journal of Soil Science* **59** (2008).

Figure 1



Disease severity


None


Moderate


Severe

Pre-emergence damping off by *Rhizoctonia solani*

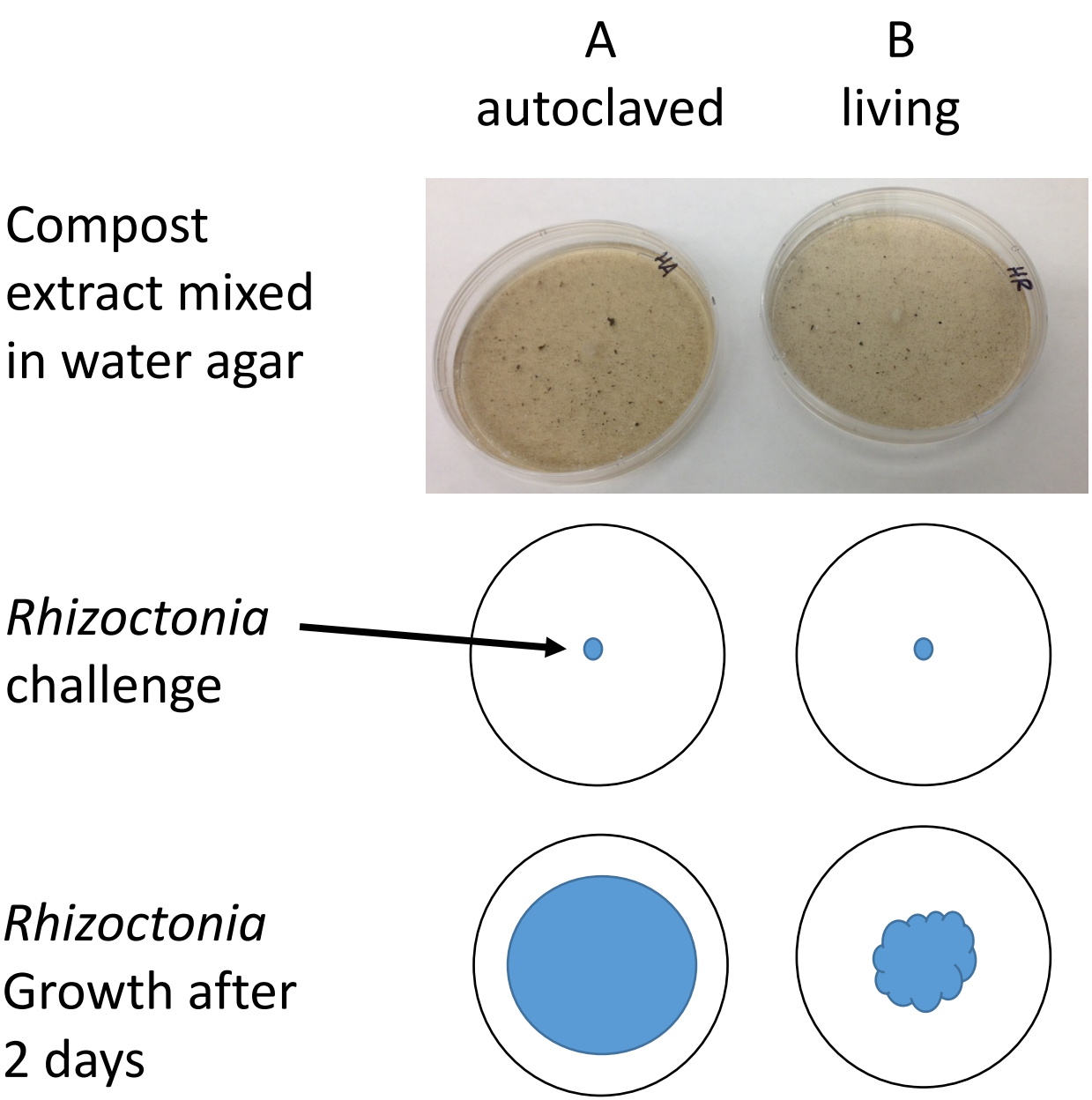


Figure 3

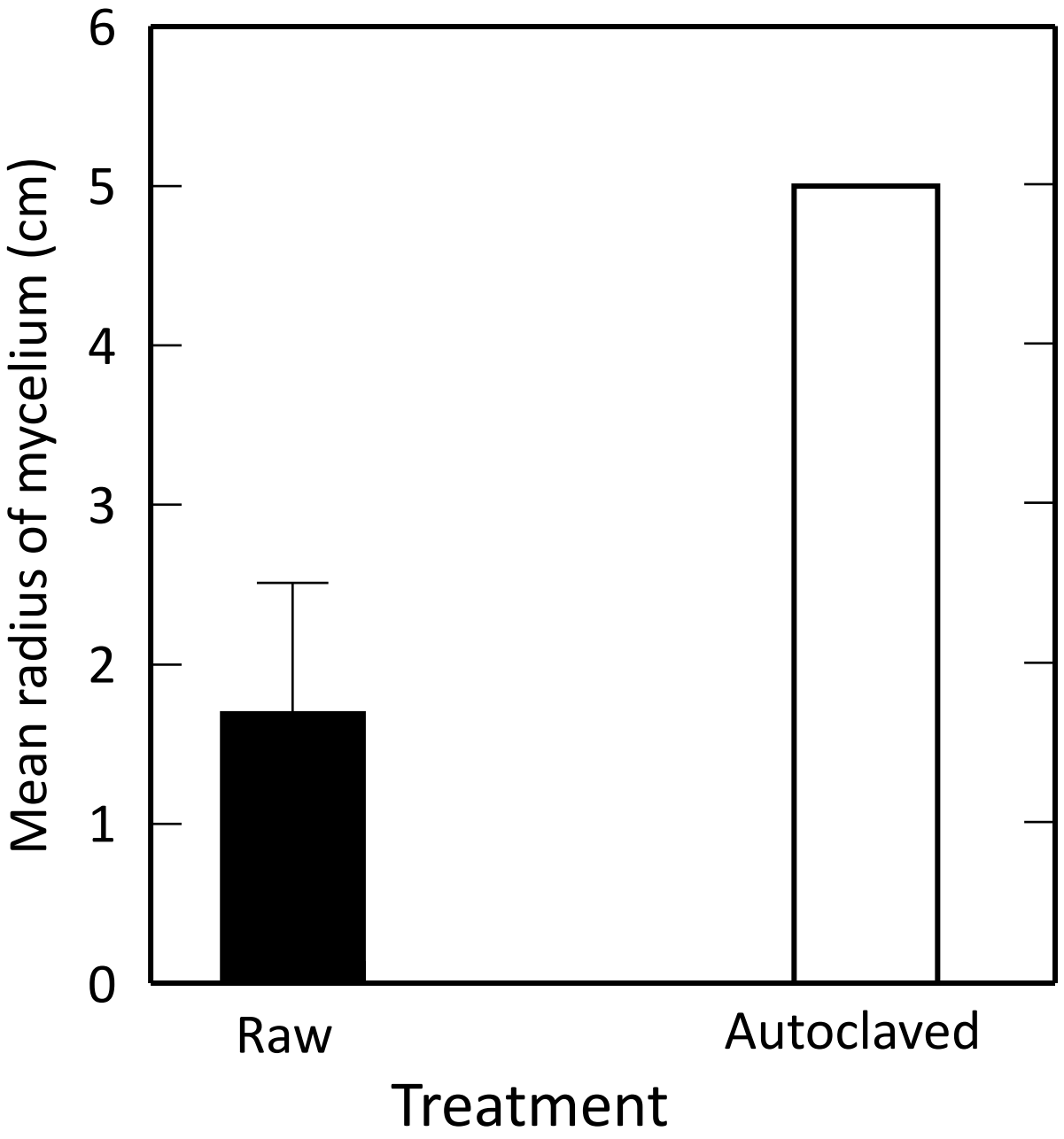


Figure 4

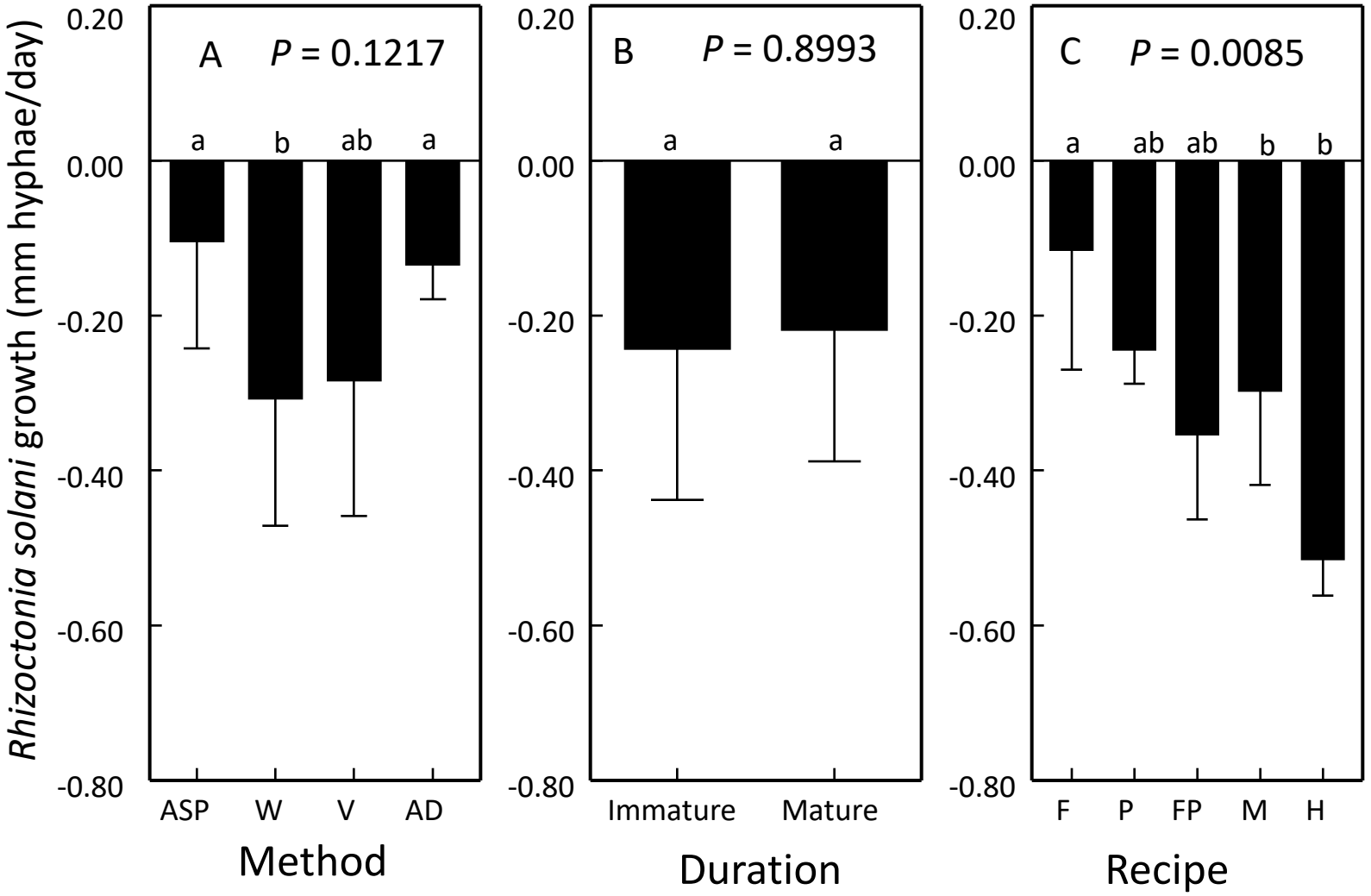


Table of Materials

Click here to access/download;Table of Materials;JoVE_Materials.xls

Name of Material/ Equipment	Company	Catalog Number	Comments/Description			
autoclavable narrow-neck glass conical flask	Fisher Scientific	10-040D	125-mL			
dehydrated granulated agar	Fisher Scientific	DF0145-17-0	500 g quantity			
heat resistant gloves	Fisher Scientific	MEMGG1314WL	several brands available			
Parafilm (strips of 2-3 cm wide)	Fisher Scientific	PM992 13-374-16	5 or 10 cm widths work			
disposable polystyrene petri dishes	Fisher Scientific	R80116	comes in sleeves of 20/ea or cases of 500			
dehydrated potato dextrose agar	Fisher Scientific	DF0013-15-8	comes in quantities of 100, 500 and 2000 grams			
benchtop reciprocal shaker	Thomas Scientific	1227Y31	other models will work			
water bath	ThermoScientific	S37363	5L general purpose			
clear ruler, flat, at least 10 cm	Any		use metric rule			
ATCC culture	American Type Culture Collection	ATCC 10154	teleomorph *Thanatephorus cucumeris* (Frank) (ATCC 10154) or MYA 4031;			
lab tape	Fisher Scientific	15935	autoclavable and removable, 1" wide preferred			
water resistant marker	office or scientific supply	Sharpie fine tip	write sample number on tape			

jove JOURNAL OF VISUALIZED EXPERIMENTS

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Plante competition assay as quick preliminary assessment of disease suppression

Author(s): Deborah A. Neher and Thomas R. Weicht

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/author) via: [x] Standard Access [ ] Open Access

Item 2 (check one box):

[x] The Author is NOT a United States government employee.

[ ] The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

[ ] The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name:	Deborah A. Neher
Department:	Dept of Plant and Soil Science
Institution:	University of Vermont
Article Title:	Plate competition assay as quick preliminary assessment of disease suppression
Signature:	Deborah A Neher
Date:	July 11, 2018

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

From: em.jove.145d9.5d0ee5.a0fac39d@editorialmanager.com
<em.jove.145d9.5d0ee5.a0fac39d@editorialmanager.com> **On Behalf Of** Vineeta Bajaj
Sent: Monday, August 06, 2018 1:51 PM
To: Deborah Neher <Deborah.Neher@uvm.edu>
Subject: Revisions required for your JoVE submission JoVE58767 - [EMID:9f2e9c61d2374625]

CC: tweicht@uvm.edu

Dear Dr. Neher,

Your manuscript, JoVE58767 Plate competition assay as quick preliminary assessment of disease suppression, has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.

After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually. Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300 dpi.

Your revision is due by **Aug 20, 2018**.

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision".

Best,

Vineeta Bajaj, Ph.D.
Review Editor
JoVE
617.674.1888
Follow us: Facebook | Twitter | LinkedIn
About JoVE

Editorial comments:
Changes to be made by the Author(s) regarding the written manuscript:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

Requested on 18 August 2018.
3. Keywords: Please provide at least 6 keywords or phrases.
Added 3 words: root diseases, soilborne fungal pathogens, bioassay
4. Please expand the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …"
Done
5. Long Abstract: Please do not include references here. Removed the reference
6. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.
Updated; not sure if days is d, so left it as days or weeks
7. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.
Updated
8. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: American Type Culture Collection, etc. The only one that I noticed was Erlenmeyer which is now changed to conical flask.
9. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.). Done

10. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Removed 'should be' from 4.1.1
Added laminar hood or bleached counter top to the Notes

11. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Some examples:
1.1.2: Please describe how to isolate from root lesions.
1.1.2.1: How to excise lesions? Added 1.1.2.1 through 1.1.2.3
1.1.2.2: What container is used? Added 1.1.2.4
1.2.1: Please describe how to establish a daughter culture. Added a note
12. Please include single-line spaces between all paragraphs, headings, steps, etc. Done
13. Please remove the weblink and use a superscripted numbered reference instead. I removed the web links in the references
14. For in-text references, the corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text (before punctuation but after closed parenthesis). The references should be numbered in order of appearance. Done.
15. References: Please do not abbreviate journal titles. Wrote them out

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
The manuscript details a methodology of a plate-based assay to identify plant disease suppression capacity of compost.

Major Concerns:
The authors have done a decent attempt of highlighting the steps but the manuscript needs refinement. The intro paragraph is very weak and does not really provide a hook to keep the readers reading. The discussion section also reads weak. The authors should really highlight the strength of the protocol rather than ending on a negative note. The methods section also needs work.
being introduced like multiple indicators, etc.
Line 2: Disease suppression in what/where? Plant roots
Line 34-38: Please choose a better word than tool. Perhaps "medium" or "bioproduct". I also don't necessarily agree that it allows manipulation of soil microbial communities? I would suggesting rephrasing this entire paragraph. Reworked the paragraph
Feedstocks used to prepare compost? Also what process are these? Do you want to convey biological processes? Please provide clarity. I can only draw vague understanding of the message here. Changed feedstocks to raw materials (ingredients)
LIne 35: Feedstocks used to prepare compost? Also what process are these? Do you want to convey biological processes? Please provide clarity. I can only draw vague understanding of the message here. Changed 'process' to 'method'
Line 36: Fungi and bacteria in compost? is this plant disease? No, beneficial microbes
Line 37: What do you mean by antagonism? Please elaborate. Leaves the readers wondering what kind of antagonism it detects. Even straightaway highlighting which process is being detected here in this manuscript will also help. I would take some time to craft this paragraph. It is the opening paragraph but desires much to be understood. The message is not clear, and the paragraph reads choppy at its best. Reworked the paragraph.
Line 40: Provide examples of such time consuming assays. There are no more examples
Line 42-43: What does finer degrees of suppression mean? Finer than what? Bioassays or excavation of roots
Line 45: Potential of what? suppression
Line 47-48: Why should the readers care about R. solani with respect to the bigger picture of the manuscript? This is the first instance that R. solani has been introduced in this manuscript, but I haven't understood why. Added some text for explanation
Line 62: What kind of infested soil is this? Is this easily available/safe to use? Those naturally containing R. solani
Line 63: Provide composition of water agar.(water plus agar)
LIne 64: Provide composition of corn meal agar. What percent? Are the isolates kept in dark? added
Line66: Provide composition and what percent added
Line 73: What is meant by each compost? How many are there? clarified to a single compost

Line 77: After 24 hours? changed
Line 94: How many wells per plate? And are the wells+R solani placed centrally in the plate or dispersed throughout the plate? A petri dish only has one well
Line 95: Perhaps elaborate this for readers who are not familiar with this measurement technique?
Line 101: Sentence seems to have error. Recheck for grammar. Thanks, changed.
Line 108: days of challenge? Or incubation? Is this a common term in plant pathogenesis? Challenge relates to the definition in the prior section
Line 112: Avoid using such words in scientific literature. Significantly is appropriate. Also, background information of the representative results should be provided. Changed to significantly.
Line 113: Again, avoid such phrases….how much is almost all?? Changed
Line 114: How can your method serve as a negative control? Please edit/rephrase. Explained that it was the autoclaved plate A of the 2 plate pair
Line 118-119: How did you test this? Your methods don't highlight this. You tested one type of compost, unless you tested more. If you did, this should be highlighted in the methods. Added text to explain that a. variety of composts were tested
Line 118: Avoid words like most/more/almost all….this is an unscientific way of discussing results. Ok, removed them
Line 120: check grammar. Done
Line 135: Why isn't there error bars in control? Were replicates not performed for controls? Too small to see
Line 139: ??? Check for grammar. Reads incorrect. Good catch, thanks.
Line 150: This is a new concept being introduced in the discussion. Is filtering an established protocol? If so, it should be highlighted in the introduction. Added some text so it is less abrubt.
Line 154-156: References? Is this something you did or are you citing others' results?? Yes, we did this, but I also added references.
Line 158: Figure 2 is place above in line 116…..Why should it be placed here again? Good catch, the second mention is removed.
Line 170: Table 1 seems to be placed at line 127, although it would make more sense to place it here. Removed the Table per Reviewer 2
LIne 173-174: "takes care to avoid contamination". Rephrase sentence, incorrect grammar; Done
Line 177-178: Was expecting the discussion to end on a positive note. Since you just highlighted the limitation, that is what sticks in mind. Is there a way you can highlight the benefits of your method and discuss some potential applications of it, rather than ending it in a negative note and one random sentence about the future application. The future application sentence reads odd as well, since the future application is not particularly relevant to your method's future application. But I can see how using the plate assay would allow you to access the inhibition zone and attempt community identification. I would recommend highlighting applications like this to end on a robust note. It should make readers want to adopt this protocol for their work. Added a positive ending.
Line 182: Why is Lynn Fang not a co-author on a method that she/he tested and published???? Tom developed the method for Lynn to use and Deb ran statistics, created figures, and wrote manuscripts.

Minor Concerns:

Grammatical errors.

Reviewer #2:
The paper titled "Plate competition assay as quick preliminary assessment of disease suppression" by Deborah A Neher and Thomas R Weicht submitted to JoVE, reports a methodological study addressing the development of a rapid and affordable test to assess the suppressive capability of compost towards the pathogen Rhizoctonia solani.

The idea of developing a rapid and affordable assay is very interesting. Moreover, the authors report a correct materials & methods section to reproduce the experiment. The results are interesting and support the basic idea of the authors. My only concerns is about the way of data and method presentation. The Introduction is too short and only some of the topic are properly introduced. So, the reader when arrive to the results become confused for the appearance of concepts and details that were not previously introduced. Moreover, the quality of several figure must be improved.

Below I summarize a list and my comments that may improve future version of this manuscript:
1. Line 34. Compost allow manipulation of soil microbial communities to modify soil functioning, including disease suppression. Thanks for the specific suggestion
2. Line 36. "Beneficial fungi and bacteria into compost…" Completed
3. Line 41. "from week to months" instead of "2-4 weeks" Completed
4. Lines 46-48. I think that a short sentence that explain the reasons for the selection of R. solani is required. A short paragraph was added
5. Lines 49-50. A couple of sentence describing the experiment could be very useful here. The experiment, in the present version, is described only in the caption of Figure 3. So, is quite difficult to follow the rationale of the experiment. I think that the authors can make a synthetic description of the factors included in the experiment: i.e. decomposition time and feedstock types. A couple of sentences were added
6. Lines 120-121. Please, check English. Good catch, thanks.
7. Lines 124-127, Table 1. I do not understand the logic of this table. What is the nematode index? Why reporting some enzymatic activities only here? A short background is necessary to explain their significance. In fact, presented in this way the table create only confusion to the reader. This section must be properly introduced explaining the importance of identify the suppressiveness of compost not only with bioassay but also with microbiological, chemical and/or biochemical parameters. Removed the table.
8. Figure 1. This figure must be improved. A structure with some arrow showing autoclaved and autoclaved sample can be useful. The quality of the cartoon is low and can be misleading. For instance, the R. solani culture seems liquid instead is solid in water agar or PDA. I think that the use of pictures would be better than the proposed cartoon. For instance, a panel with pictures of water agar, water agar + compost and these two materials inoculated with R. solani can be very useful. In case of the plate with R. solani, please report a plate with not complete growth but where the mycelium cover only 50-75% of the plate. This picture will be more explicative. Remade the figure
9. Figure 2. Why standard deviation is not present for autoclaved bar? Too small to see

10. Figure 3. Y axe label is wrong because growth cannot be negative. The correct info are reported in the caption of figure 3. Please, change the label and make this consistent with the caption. Revised the legend to explain the negative values

Reviewer #3:

Manuscript Summary:

Based on a master thesis and a published journal article by the authors, the protocol described a plate assay with comparison of mean radius of mycellium to detect disease suppressive ability of compost against R solani, a soilborne fungus pathogen. The protocol is well written. The procedures are clear, and results are well described and supportive to conclusion.

Major Concerns:

Line 45. please provide more details about the method by Alfano, then explicitly explain what the current method differs from the one by Alfano. Display the advantages if any. More explicitly stated the two differences and why those changes were made

Lines 140-142. To improve the readability and clarity, the batch of compost terminology need to be explained and elaborated. Attempted to use simpler terms

Minor Concerns:

Line 73. 2.1 rephrase the sentence; Done

Line 78. 2.3. what is a liquid cycle? please explain or be explicit. Rephrased as a slow exhaust setting

Line 120, delete is. Done

Lines 140-142. this is confusing. separate the stats results from those abbreviations. Moved the statistics to the graphs and rearranged the legend.

Figure 2. what is the error bar for Autoclaved? Too small to see

Reviewer #4:

Manuscript Summary:

This is a useful assay for studying disease suppression potential of mixed microbial communities in compost.

Major Concerns:

The introduction is clear and the methods are fairly good as well, but then in the results you talk a lot about different assays (ie. ecoenzymes) that you never explained and never end up showing how the results in your plate assay relate to disease suppression potential in plants (the gold standard for estimating compost disease inhibition potential). I would ask that you delete table 1 (you can still mention those other assays in the discussion if you want, but don't dwell on them), and instead replace it with a direct comparison of plate assay results juxtaposed against plant disease assay results. Some photos of what disease results in the plant assay look like, as well as what plate assay growth inhibition looks like would also be useful.

Deleted Table 1 but let the explanation in the text.

Minor Concerns:

You missed a few details that might be important. For example, what genotypes of radish should

one use as bait? What composts did you test and where did you obtain them? What could one use as positive controls to know the technique is properly being conducted? Did you get your R. solani from ATCC or did you isolate it yourself? Why is maintaining moist, warm conditions a limitation? Please do a better job of explaining the drawbacks of plant bioassays (maybe just stick to your original explanation that they take too long).
Added the Latin name for radish, and clarified that the authors isolated their own Rhizoctonia solani.

You need to prove or better reference why it would not be reliable as a standalone assay. Please reference: Shehata, Hanan R., et al. "Relevance of in vitro agar based screens to characterize the anti-fungal activities of bacterial endophyte communities." BMC microbiology 16.1 (2016): 8. That paper shows that plate competition assays are reliable as standalone assays, so why shouldn't your assay be as well? Please elaborate. Added some explanation in the text to provide more clarification.