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## Evaluation of a Plant-Based Antimicrobial Formulation on Control of Damping-Off and Other Seedling Pathogens in Vegetable Seedlings

### Abstract

Damping-off disease is a major challenge in greenhouse and nursery vegetable transplant production and can result in significant economic losses due to seedling mortality. The disease is caused by soilborne fungi and oomycetes, including *Pythium*, *Fusarium*, *Rhizoctonia*, and *Phytophthora* species, and is often exacerbated by environmental stress conditions common in greenhouse production systems. Conventional fungicides are commonly used to manage damping-off; however, concerns about environmental impacts and the development of fungicide-resistant pathogen populations have increased interest in alternative management strategies. This study evaluated the efficacy of a plant-based antimicrobial formulation containing clove oil for controlling damping-off in tomato (*Solanum lycopersicum*) seedlings under greenhouse conditions. A dilution curve of clove oil concentrations (0.05–0.15%) was evaluated using pathogen-inoculated seedlings with four replicates per treatment. Greenhouse trials were repeated three times to confirm treatment efficacy. Results demonstrated that a 0.05% clove oil concentration provided the most effective disease suppression without causing phytotoxic injury. Higher concentrations resulted in plant injury, while lower concentrations did not adequately suppress pathogen development. Seedlings treated with the 0.05% formulation showed improved survival and reduced

disease symptoms compared to untreated controls. These results indicate that clove oil formulations may provide growers with a plant-based alternative to conventional fungicides for managing damping-off in vegetable transplant production systems.

**Keywords:** *Solanum lycopersicum*, damping-off, antimicrobial formulation, clove oil, tomato seedlings, eco-friendly

## Introduction

According to the Louisiana Summary of Agriculture and Natural Resources (2020), 40 vegetable crops were produced commercially in Louisiana. Tomatoes ranked as the top vegetable crop in the state in 2020 with a gross farm value (GFV) of \$15.6 million, while the total GFV of all commercial vegetable production reached \$45.5 million.

Greenhouse vegetable production also contributes to the state's vegetable industry. In 2020, 58 growers reported commercially producing greenhouse vegetables, with production primarily consisting of tomatoes. Greenhouse tomatoes accounted for a GFV of \$1,886,545 and contributed to an estimated total greenhouse vegetable GFV of approximately \$1.96 million in Louisiana. Because tomatoes represent the dominant greenhouse-grown vegetable crop in Louisiana, seedling losses during transplant production can significantly impact commercial greenhouse profitability.

Damping-off disease poses a major challenge for greenhouse and nursery vegetable seedling production and can result in substantial economic losses due to seedling mortality. Damping-off is one of the most common and difficult diseases to manage in greenhouse and nursery crop production systems (Pscheidt and Ocamb, 2019). The disease typically occurs shortly after germination and is caused by several soil-borne pathogens, including *Fusarium spp.*, *Rhizoctonia spp.*, and oomycetes such as *Phytophthora spp.* and *Pythium spp.* (Agrios, 2005). These pathogens infect seeds or young seedlings, often leading to pre-emergence damping-off, where seeds rot before emerging, or post-emergence damping-off, where seedlings collapse shortly after emergence.

Environmental conditions strongly influence disease development. Excessive soil moisture, overhead irrigation, overcrowding, unfavorable soil temperatures, and improper soil pH can reduce germination and promote pathogen infection. These pathogens, combined with favorable environmental conditions, can cause severe seedling mortality depending on host susceptibility and pathogen pressure (Lamichhane, 2017). Diseases caused by *Fusarium* and *Rhizoctonia* species are associated with warm, dry conditions, whereas *Phytophthora* and *Pythium* species tend to develop under cool, wet soil conditions.

Symptoms of damping-off typically appear within the first four to six weeks after sowing. Infected seedlings often develop dark, water-soaked lesions near the soil line that progress to stem rot and seedling collapse. Root rot may also occur, disrupting water and nutrient uptake and resulting in plant death. The disease may also be exacerbated by poor seed quality, improper planting depth, or early spring planting when soil temperatures are suboptimal.

Economic losses associated with damping-off arise from both direct and indirect costs. Direct losses include seed and seedling mortality, while indirect costs result from replanting expenses, delayed planting schedules, and reduced yields (Lamichhane, 2017). Historically, chemical fumigation practices such as methyl bromide were widely used to control soilborne pathogens in greenhouse and nursery systems. However, methyl bromide has been phased out in many countries, including the United States and the European Union, due to its negative environmental impacts (Moultet et al., 2014). Although conventional fungicides continue to play an important role in managing damping-off pathogens, frequent fungicide use has contributed to the development of resistant pathogen populations and increasing management challenges for growers.

Because of the economic impact of damping-off and the environmental concerns associated with conventional fungicide-based disease control strategies, there is increasing interest in developing alternative and sustainable management approaches. Integrated pest management (IPM) strategies emphasize preventive measures,

improved seed health, and cultural practices that reduce disease pressure. Practices such as improving soil drainage, optimizing organic matter and nutrient management, adjusting soil pH, and creating growing conditions that promote rapid seed germination and seedling growth can help reduce disease incidence. However, cultural practices alone may not provide sufficient disease suppression in intensive greenhouse production systems.

Plant-derived antimicrobial compounds have emerged as promising alternatives for sustainable disease management. Essential oils (EOs) are volatile compounds extracted from various parts of edible and medicinal plants, including buds, bark, flowers, leaves, fruits, and seeds (Edris, 2007). Many essential oils, such as cinnamon, clove, thyme, rosemary, peppermint, and spearmint, are classified as generally recognized as safe (GRAS) food additives and are also registered as EPA minimum-risk pesticide products. Essential oils possess antimicrobial properties that may inhibit fungal and bacterial pathogens by disrupting cell membranes and interfering with cellular metabolism (Dagli et al., 2015). Several essential oil-based products have been developed for disease management in horticultural systems. For example, Sporatec EO fungicide contains rosemary, clove, and thyme oils and is used as a broad-spectrum contact fungicide for greenhouse, nursery, and landscape crops. However, the efficacy of some essential oil formulations may be limited due to instability when diluted in water.

Therefore, the development of stable plant-based antimicrobial formulations that effectively control damping-off pathogens while minimizing environmental impact is needed. The objective of this study was to evaluate the efficacy of a plant-based antimicrobial formulation containing clove oil for controlling damping-off and related diseases in vegetable seedlings, with a focus on tomato production under greenhouse conditions. The formulation was evaluated at multiple concentrations to determine its ability to prevent and treat damping-off in tomato seedlings. One advantage of this formulation is its improved water solubility and stability, which may facilitate application and product storage, as demonstrated in previous work evaluating tea tree essential oil for the control of pathogenic bacteria (Liu et al., 2017).

## **Materials and Methods**

### **Location and Plant Material**

'Abe Lincoln' tomato (*Solanum lycopersicum* L.) seeds were sown in commercial propagation medium in 4-inch pots and grown in a greenhouse located at the USDA Plant Materials Center at the LSU AgCenter Central Research Station in Baton Rouge, Louisiana (30.3606° N, -91.1772° W).

### **Preparation of Clove Oil Concentrate**

A clove oil concentrate (COC) was prepared using a proprietary formulation developed in the Medicinal Plant Laboratory at the LSU AgCenter. The formulation contained clove essential oil at 5% (v/v) combined with a solubilizing agent to produce a water-soluble concentrate at a pH of 7.2

The concentrate was stored under refrigeration and protected from light. Prior to application, the concentrate was diluted with deionized water containing 0.001% citric acid. Final clove oil concentrations evaluated were:

- 0.05%
- 0.075%
- 0.10%
- 0.125%
- 0.15%

### **Experimental Design**

The study consisted of two phases:

1. In vitro antimicrobial assays
2. Greenhouse efficacy trials

## ***In Vitro* Antimicrobial Assays**

Laboratory assays were conducted to determine the antimicrobial activity and effective dose range of a clove oil formulation prior to greenhouse evaluation. Results from these assays were used to guide treatment concentrations evaluated in subsequent greenhouse trials. *In vitro* antimicrobial activity of the clove oil formulation was evaluated in the LSU AgCenter Plant Diagnostic Laboratory using agar plate assays. Pathogens associated with damping-off disease were cultured on potato dextrose agar (PDA) and included *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., and *Phytophthora* spp.

Each pathogen group was evaluated separately to reduce confounding effects among pathogens and to allow for clearer assessment of the clove oil formulation against individual causal agents of damping-off disease. Evaluating pathogens independently during initial screening is important because mixed infections can alter disease severity, pathogen interactions, and treatment responses, making it difficult to determine the specific effectiveness of the product against a particular pathogen. The clove oil formulation contained 5% clove oil (v/v) combined with a proprietary solubilizer to produce a water-soluble product. Two concentrations of the formulation were incorporated into PDA to evaluate pathogen growth inhibition.

Treatment 1 consisted of 10 mL product added to 90 mL PDA, resulting in a final clove oil concentration of 0.5% (v/v). Treatment 2 consisted of 25 mL of product added to 75 mL PDA, resulting in a final clove oil concentration of 1.25% (v/v). An untreated PDA medium served as the control treatment. Each pathogen–treatment combination was prepared in duplicate to confirm treatment consistency.

Following inoculation, plates were incubated under laboratory conditions and monitored for pathogen growth. Radial colony growth was measured to evaluate inhibition of fungal and oomycete growth relative to untreated controls. Plates were photographed 10 days after inoculation to document treatment effects on pathogen development (Figure 1). Dose–response analyses were conducted to estimate median lethal

concentration ( $LC_{50}$ ) values for each pathogen. These results were used to guide the selection of treatment concentrations evaluated in subsequent greenhouse experiments.

The clove oil concentrations used in agar assays were intentionally higher than those later evaluated in greenhouse trials. Agar plate assays are commonly conducted at elevated concentrations to rapidly screen antimicrobial activity and establish inhibitory thresholds under controlled conditions. Lower concentrations were subsequently evaluated in greenhouse trials to determine the minimum effective rate capable of suppressing disease while minimizing potential phytotoxic effects on tomato seedlings.

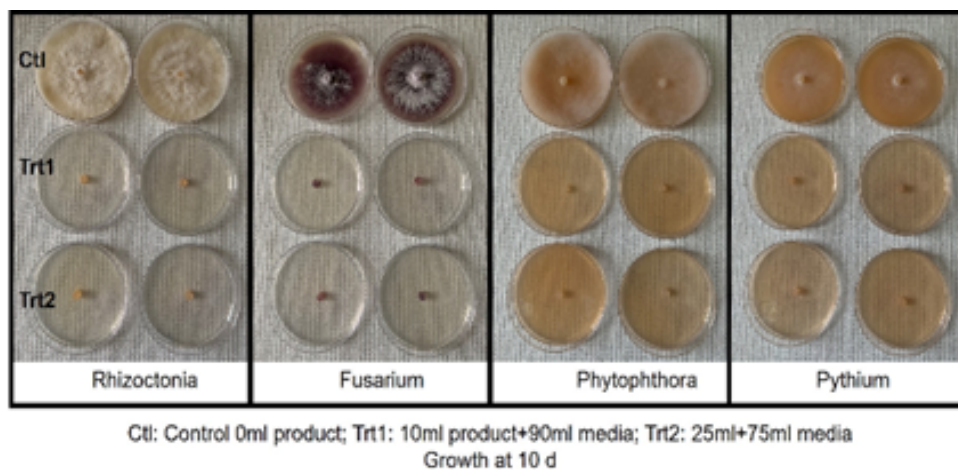


Figure 1. In vitro inhibition of damping-off pathogens by clove oil treatments on potato dextrose agar (PDA). Treatments included a control (Row 1), Treatment 1 with a final concentration of 0.5% clove oil (Row 2), and Treatment 2 with a final concentration of 1.25% clove oil (Row 3).

### Greenhouse Efficacy Trial

Greenhouse trials were conducted to evaluate the effectiveness of the clove oil formulation in suppressing damping-off disease in tomato seedlings.

Tomato plants were inoculated with individual pathogens previously evaluated in laboratory assays. Each plant was infected with only one pathogen to prevent cross-

infection. The greenhouse experiment included four replicates per treatment and was repeated three times to confirm treatment efficacy.

### **Pathogen Inoculation**

To initiate infection, six fungal inoculum disks (colonized agar) were placed in the soil near the root zone of each tomato plant. Plants were incubated for 3–4 days to allow pathogen establishment prior to treatment application.

### **Treatments Evaluated**

Dose-response treatments included:

- 0.05% clove oil
- 0.075% clove oil
- 0.10% clove oil
- 0.125% clove oil
- 0.15% clove oil
- Captan™ fungicide control (positive control, a.i. (*N*-Trichloromethylthio-4-cyclohexene-1, 2-dicarboximide, Oriskany, NY) Deionized water control (negative control)

Following dose-response experiments, validation trials were conducted using:

- 0.05% clove oil
- Captan™ control
- Water control

### **Treatment Application**

Treatments were applied when visible fungal growth was observed. Diluted clove oil formulations or control treatments were applied directly to the soil surrounding the root zone of each plant.

## **Data Collection**

Plant health was monitored for 14 days following treatment. Observations included:

- seedling survival
- disease symptom development
- percent injury
- damping-off symptoms
- phytotoxicity symptoms

Symptoms of damping-off included water-soaked stem lesions, stem rot at the soil line, leaf curl, and seedling collapse associated with pathogen infection. In contrast, phytotoxicity symptoms from clove oil treatments were characterized by uniform chlorosis, necrosis, tissue burn, and stem collapse occurring without visible pathogen-associated stem rot or lesions. Percent injury was visually estimated and compared across treatments and replicates based on symptom type and severity.

## **Results**

### **Pathogen Severity**

Clove oil formulations demonstrated antimicrobial activity against multiple damping-off pathogens, including *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., and *Phytophthora* spp. Disease severity varied among pathogens. *Pythium* spp. produced the most severe symptoms, causing rapid stem rot, seedling collapse, and high mortality in untreated controls. *Fusarium* spp., *Rhizoctonia* spp., and *Phytophthora* spp. caused comparatively less severe disease symptoms but still resulted in substantial plant decline and mortality in water-treated control plants.

Symptoms of damping-off included water-soaked stem lesions, stem rot at the soil line, leaf curl, root rot, and seedling collapse. Untreated plants exhibited progressive disease development throughout the 14-day evaluation period.

## Phytotoxicity Effects

Plant response to clove oil treatments varied according to concentration. Higher concentrations of clove oil resulted in increasing phytotoxic effects on tomato seedlings. Treatments containing 0.125% and 0.15% clove oil caused severe chlorosis, necrosis, stem collapse, and rapid plant decline. In several trials, plants treated with 0.15% clove oil died within nine days following application, particularly under severe *Pythium* spp. infection pressure.

In contrast, lower concentrations minimized phytotoxic injury but were less effective at suppressing pathogen development. Concentrations below 0.05% did not consistently suppress disease symptoms across trials.

## Optimal Treatment Concentration

Dose-response trials identified 0.05% clove oil as the optimal treatment concentration for disease suppression while minimizing phytotoxic effects. Plants treated with 0.05% clove oil exhibited the highest survival rates and maintained healthy stem and foliar tissue throughout the experiment. Repeated greenhouse trials confirmed the effectiveness of the 0.05% concentration across all evaluated pathogens.

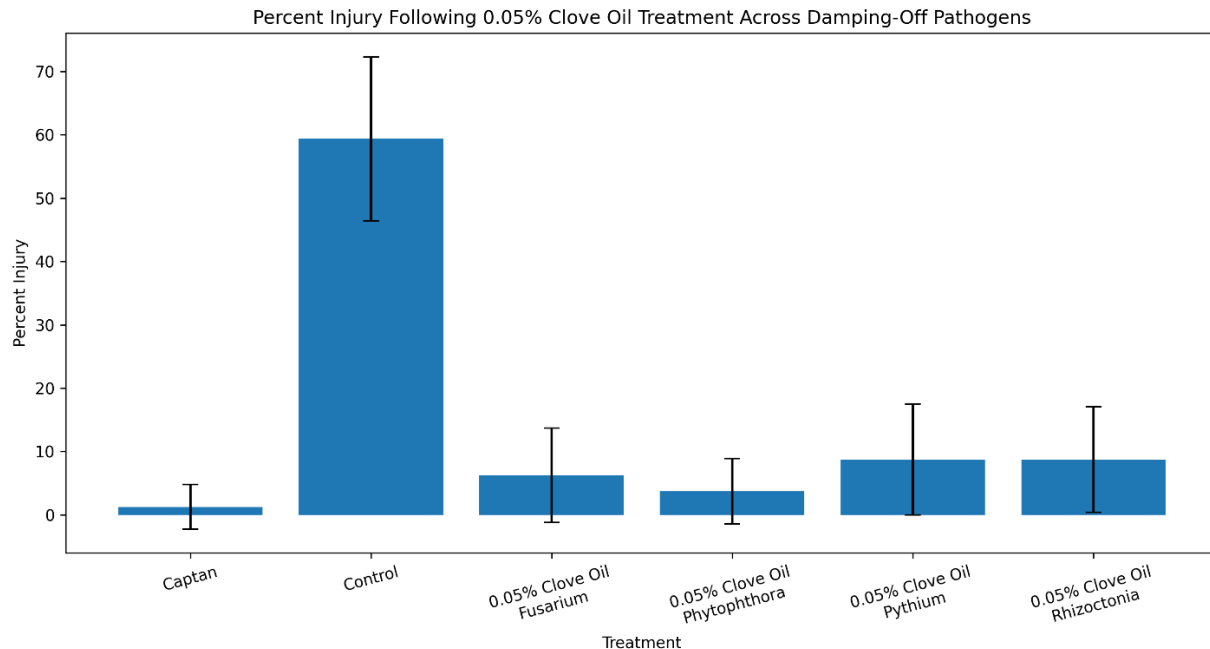
**Table 1.** Effect of Clove Oil Concentration on Damping-Off in Tomato Seedlings

Treatment	Disease Control	Phytotoxicity
Water Control	Severe disease, high mortality	None
Captan™	Moderate disease suppression	None
0.05% Clove Oil	High disease suppression	None
0.075% Clove Oil	Moderate suppression	Slight injury

<b>Treatment</b>	<b>Disease Control</b>	<b>Phytotoxicity</b>
0.10% Clove Oil	Moderate suppression	Mild phytotoxicity
0.125% Clove Oil	Disease suppression	Severe phytotoxicity
0.15% Clove Oil	Plant mortality	Severe phytotoxicity

Additional validation experiments conducted using *Fusarium* spp., *Rhizoctonia* spp., *Phytophthora* spp., and newly propagated *Pythium* spp. isolates further confirmed that the 0.05% clove oil treatment effectively suppressed disease symptoms without causing phytotoxic damage. Plants maintained for an additional four weeks following treatment showed no further disease progression or symptom development, indicating sustained suppression of damping-off pathogens.

Figure 2. Percent injury of tomato seedlings 14 days after inoculation with damping-off pathogens and treatment with 0.05% clove oil formulations. Pathogens evaluated separately included *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., and *Rhizoctonia* spp. Captan and untreated controls were included for comparison. Bars represent mean percent injury  $\pm$  standard deviation (SD) based on eight total plant replicates across two greenhouse trials (n = 8 per treatment).



## Statistical Analysis

Treatment significantly affected percent injury of tomato seedlings 14 days after inoculation ( $F_{5,42} = 57.32, p < 0.0001$ ). Untreated control plants exhibited significantly greater injury than all clove oil and Captan™ treatments. Mean injury in water-treated controls reached  $59.38 \pm 12.94\%$ , whereas plants treated with 0.05% clove oil formulations exhibited substantially lower injury levels across pathogen groups. No significant differences were detected among the 0.05% clove oil pathogen treatments and the Captan™ treatment according to Tukey's HSD multiple comparison test ( $p > 0.05$ ). These findings demonstrate that the 0.05% clove oil treatment provided disease suppression comparable to the conventional fungicide standard.

## Discussion

This study demonstrated that a plant-based antimicrobial formulation containing clove oil can effectively suppress major damping-off pathogens affecting tomato seedlings under greenhouse conditions. The identification of 0.05% clove oil as an effective and non-phytotoxic concentration provides a clear and actionable treatment recommendation for greenhouse transplant production systems.

The antimicrobial activity observed in this study is consistent with previous reports demonstrating the antifungal properties of essential oils. Essential oils, including clove oil, contain biologically active compounds capable of disrupting microbial cell membranes and interfering with cellular metabolism (Dagli et al., 2015). Clove oil is rich in eugenol, a compound widely recognized for its antimicrobial activity against fungal and bacterial pathogens. The results of this study support previous findings that plant-derived compounds may serve as reduced-risk alternatives to conventional synthetic fungicides in horticultural production systems.

The ability of the 0.05% clove oil treatment to suppress disease progression without phytotoxic injury is particularly important for greenhouse transplant production, where young seedlings are highly susceptible to both pathogen infection and chemical damage. While higher clove oil concentrations increased antimicrobial activity, they also increased phytotoxicity, demonstrating the importance of identifying biologically effective but plant-safe application rates. These findings establish a practical concentration threshold that balances disease suppression with seedling safety.

The consistency of disease suppression observed across repeated greenhouse trials suggests that clove oil formulations may provide growers with a sustainable management tool that could supplement or partially replace conventional fungicides such as Captan™. The observed reduction in stem necrosis, absence of visible mycelial growth, and improved plant survival further supports the potential practical utility of this treatment in vegetable transplant production systems.

A limitation of this study is that a single isolate representing each pathogen group was evaluated, and greenhouse trials were conducted using a limited number of tomato cultivars. Therefore, isolate variability and cultivar-specific responses were not fully assessed. In addition, pathogens were evaluated separately to reduce confounding effects and allow clearer assessment of treatment efficacy against individual causal agents of damping-off disease. Mixed infections commonly occur in greenhouse production systems and may alter disease severity and treatment response.

Future research should evaluate multiple pathogen isolates, additional tomato cultivars, and mixed-pathogen inoculations under greenhouse and field conditions. Preventative treatment applications and application timing should also be investigated to determine whether clove oil formulations can reduce infection prior to symptom development. Additional research evaluating the formulation in other specialty crops, including ornamental nursery crops, forestry seedlings, and fruit crops, may further expand the applicability of this plant-based disease management strategy.

### **Conclusions**

This study demonstrated that a 0.05% clove oil formulation effectively suppressed major damping-off pathogens in tomato seedlings while avoiding phytotoxic injury. Among the concentrations evaluated, 0.05% consistently provided the greatest disease suppression and highest seedling survival across repeated greenhouse trials. In contrast, higher concentrations caused significant phytotoxicity, while lower concentrations did not provide adequate pathogen control.

The 0.05% clove oil treatment performed similarly to the conventional fungicide Captan™, and reduced disease progression caused by *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., and *Phytophthora* spp. Treated plants also showed sustained suppression of disease symptoms for up to four weeks following treatment.

These findings support the potential use of clove oil formulations as a plant-based, reduced-risk alternative for damping-off management in greenhouse vegetable transplant production systems. Adoption of such products may help reduce reliance on synthetic fungicides while improving transplant survival and production sustainability.

Future research should evaluate preventative applications, mixed-pathogen systems, additional tomato cultivars, and field-scale validation in other specialty crop production systems, including ornamental, forestry, and fruit crops.

## Implications for Extension

This research provides growers with a practical, research-supported disease management strategy that may improve transplant survival while supporting sustainable production practices. Results from this project were disseminated through grower workshops, field days, and presentations reaching more than 200 vegetable producers, increasing awareness of botanical disease management tools for specialty crop systems.

## Acknowledgments

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## Literature Cited

Agrios, G. N. (2005). *Plant pathology*. 5th ed. Elsevier Academic Press, Burlington, MA.

Dagli, N., R. Dagli, R. S. Mahmoud, and K. Baroudi. (2015). Essential oils, their therapeutic properties, and implications in dentistry: A review. *Journal of International Society of Preventive and Community Dentistry* 5(5):335–340.  
<https://doi.org/10.4103/2231-0762.165933>

Edris, A. E. (2007). Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. *Phytotherapy Research* 21(4):308–323.  
<https://doi.org/10.1002/ptr.2072>

Lamichhane, J. R., C. Dürr, A. A. Schwanck, M. Robin, J. P. Sarthou, V. Cellier, Y. Messéan, and J. N. Aubertot. (2017). Integrated management of damping-off diseases: A review. *Agronomy for Sustainable Development* 37:10.  
<https://doi.org/10.1007/s13593-017-0417-y>

Liu, Z., W. E. Owens, Y. Dong, and J. Zhang. (2017). Antibacterial botanical formulations. *Louisiana Agriculture* 60(1):11–13.

Mouttet, R., Escobar-Gutiérrez, A., and Esquibet, M. (2014). Banning of methyl bromide for seed treatment: Could *Ditylenchus dipsaci* again become a major threat to alfalfa production in Europe? *Pest Management Science* 70:1017–1022.  
<https://doi.org/10.1002/ps.3745>

Pscheidt, J. W., and Ocamp. C.M. (2019). Pacific Northwest plant disease management handbook. Oregon State University Extension Service, Corvallis, OR.