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Mini Review

Rosemary Decline, an Apparent Disease of Unknown Etiology Affecting the Southwestern US.

Srijana Dura[1] and Stephen F Hanson[1,2]*

[1]*New Mexico State University, Department of Entomology, Plant Pathology, and Weed Science, Las Cruces, NM, 88011.*

[2]*Entomology, Plant Pathology, and Weed Science, and the NMSU Molecular Biology Program, New Mexico State University, USA*

***Corresponding author:** Stephen F Hanson, Entomology, Plant Pathology, and Weed Science, and the NMSU Molecular Biology Program, New Mexico State University, USA.

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Abstract

Rosemary is a low water use perennial crop that is grown for a variety of purposes ranging from food seasoning to extraction of aromatic and medicinal compounds. A slow decline resulting in the death of rosemary plants was observed in 2022 and 2023 in fields recently established in Southeastern Arizona. Rosemary had not been grown in that area previously, although several crops affected by a variety of soilborne diseases were grown in the fields previously. Affected plants showed yellowing and chlorosis for a brief period before experiencing dieback that killed individual branches before eventually killing the entire plant. Affected plants also displayed signs including root rotting that substantially reduced root volume, darkening of the root epidermis, and browning of the vasculature above the crown. The disease occurs in patches that expand slowly in all directions. Individual affected plants were observed in the first year of field establishment, with patches slowly spreading between years. By year three, approximately 30% of plants had died in the most heavily affected fields. A variety of fungi commonly associated with soilborne vascular diseases were isolated from affected plants. However, Koch's postulates have not been fulfilled as all attempts to reproduce the disease by inoculating healthy plants with isolated fungi, alone or in combination, have failed to date.

Introduction

Rosemary (*Rosmarinus officinalis* L.) has been cultivated and used as an herb and medicinal crop for over 5,000 years [1]. Today, it is grown around the world as a cold hardy low water use crop with a variety of uses. It can be grown as an ornamental, a seasoning, or as a medicinal plant [2,3]. Some rosemary is cultivated for ornamental purposes or harvested for seasoning or for use as a food preservative [4]. Several high value phytochemicals can also be extracted from rosemary including essential oils used in fragrances and carnosic acid which can be used as a fat-soluble antioxidant food preservative [5]. Antimicrobial extracts of rosemary can also be incorporated into anti-fungal biopolymers useful for food packaging [6].

Several different pathogens commonly associated with soilborne vascular disease have been reported to cause dieback and death of rosemary. *Phytophthora nicotianae* was identified as the cause of collar and root rot that emerged on both lavender and rosemary in Spain in 2004 [7]. A different study identified Fusarium oxysporum as the major cause of losses in cultivated rosemary [8]. This disease was reported to cause wilting, loss of vigor, and dieback on up to 70% of nursery plants. The identification of *P. nicotianae* was based on both morphology and ITS region sequences, and Koch's postulates were fulfilled using an infection assay where rosemary transplants were grown in potting mix infested with *P. nicatianae* isolates recovered from symptomatic rosemary. A syndrome described as rosemary wilting disease was reported to cause losses from 30-60% in Iran and attributed to infection by any of three pathogens, including *Phytophthora citrophthora*, *Rhizoctonia solani*, and *Fusarium oxysporum* [9]. *Phytopthora cytrophthora* was noted as being the most common cause in greenhouse grown rosemary, while *F. oxysporum* and *R. solani* were more commonly associated with wilting in field grown plants. Colletotrichum gloesporioides was also reported to cause dieback of rosemary in Argentina [10]. *Sclerotinia sclerotium* has also been reported to cause a dieback of greenhouse and field grown rosemary plants in Oregon [11]. Wounding of stems or roots and high humidity were reported as important factors for Sclerotinia caused dieback. Based on the wide variety of pathogens reported to cause dieback of rosemary noted above, it appears that rosemary is susceptible to dieback potentially caused by a wide variety of pathogens.

Rosemary was recently planted for the first time ever in Southeastern Arizona in 2020 and 2021 and began displaying dieback similar to previously described diseases. An investigation to determine the nature of the apparent disease was conducted in 2023. While many fungi noted as pathogens capable of dieback were isolated from diseased specimens, we have been unable to fulfill Koch's postulates by reproducing the disease with any of the isolated fungi alone or in combination. Further work is required to define the pathogen(s) and factors causing this rosemary dieback.

Results and Discussion

A significant decline and dieback of some rosemary plants was observed in 2022 and 2023 in newly established rosemary fields in Southeastern Arizona. Fields were established with transplants in 2020 and 2021. Some plants died shortly after transplant and may have been transplant failures. Other plants died after establishing and growing for a year or two (Figure 1). Other plants exhibited the same dieback symptoms after growing normally for two or more years and getting large enough to harvest (Figure 1). The syndrome appears to spread slowly in patches but does not appear to run down rows. This suggests that this dieback syndrome is not specific to transplants or associated with cultural practices like tilling and irrigation. Affected plants usually occur alone initially with the disease spreading slowly between and across rows. Rapid spread along rows, as is common for Phytophthora, was not observed. Normal looking apparently healthy plants often persist across years immediately adjacent to affected plants. While affected patches appear to spread slowly between years, some healthy plants immediately adjacent to affected plants have remained healthy for at least two years indicating that this syndrome does not spread rapidly or may require some augmenting factor that weakens a plant for it to establish. The small-scale patchy distribution that suggests abiotic causes like nutrient deficiency, hydration status, salinity or soil toxicants, or environmental factors like temperature are unlikely to be the cause since all of these would be expected to have a larger scale distribution that affected more plants. The syndrome is not correlated with a particular irrigation practice as some affected fields are drip irrigated while others are irrigated from pivot sprinklers.

Symptoms of dieback are similar in young and old plants and include individual branches showing mild to moderate chlorosis for a short time before dying (not shown). Leaves remain attached to dead branches (Figure 1). This pattern progresses to other branches until the entire plant is dead leaving a rosemary skeleton plant, with leaves attached, in the field. Stems and roots of affected plants displayed root rot and vascular wilt symptoms including a loss of many roots, a severe loss of fine lateral roots, darkening and blackening of the root epidermis and browning of the stem vasculature (Figure 2). Severely affected roots eventually blackened inside (not shown). While the root surfaces blackened, the epidermis remained attached and did not easily slough off as is typical for Phytophthora caused root roots (not shown).

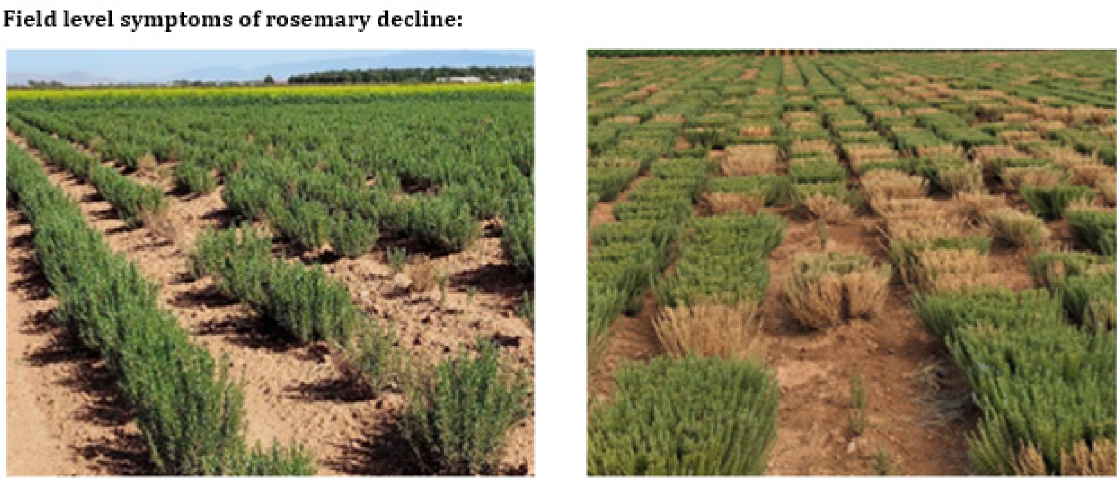

Figure 1: Rosemary declined in second year (left) and recently mowed third year (right) fields in Southeastern Arizona. Note that most of the dead plants pictured grew substantially from transplants that were ~4” tall when transplanted, and many of the dead plants in third year also grew in year three before dying.

Figure 2: Root structure and internal stem coloration shown for health (left) and affected (right) rosemary plants. Healthy plants have extensive root systems while root systems of affected plants are vastly reduced and almost devoid of fine lateral roots that are abundant in the healthy root system. Stem coloration of healthy root system is light colored vascular tissue with some light green cambium while affected stems show pronounced browning inside, especially in the cambium area.

Fungal isolations were performed from about a dozen specimens using Potato Dextrose Agar (PDA) and acidified PDA for general fungal isolation, water agar amended with pimaricin, ampicillin, and rifampicin (PARP) to enrich for isolation of Phytophthora, and Czapek-Dox medium to enrich for Verticillium. Several candidate pathogens were isolated and tentatively identified by morphology, including *Fusarium oxysporum*, *Rhizoctonia solani*, and *Alternaria* spp. Interestingly, Phytophthora and Verticillium were not isolated from any of the samples even though these pathogens are known to have affected chile peppers grown in the same fields recently.

Inoculations were performed to try and complete Koch's postulates to determine which pathogen, or combination of pathogens, is causal for the observed decline. Healthy young plants grown from rooted rosemary cuttings (~3-4" tall) were transplanted into 12" pots with three plants per pot. Plants were allowed to establish and start growing for approximately 4 weeks prior to inoculation and plants were 6-8" tall at time of inoculation. Inoculations were performed by placing 2-3 one cm diameter plugs from fungal plates in the soil between the three rosemary plants. Plants were inoculated with Fusarium, Rhizoctonia, and Alternaria cultures isolated from rosemary dieback samples. The fungi were inoculated alone and in all possible combinations with 2-3 replicates per treatment. A second inoculation was performed twenty days after the first since no symptoms were developing. Roots were also wounded in one pot from each treatment approximately 1 week after the second inoculation. Wounding was accomplished by using a dull kitchen table / butter knife to cut through the soil to the bottom of the pot between all the plants to simulate damage that could occur during tilling. Pictures of representative inoculated and non-inoculated pots at 77 days post inoculation are shown in Figure 3. All treatments remained disease free at time of writing, more than 100 after the first inoculation (not shown) with all plants (inoculated and non-inoculated) growing substantially over the course of the experiment.

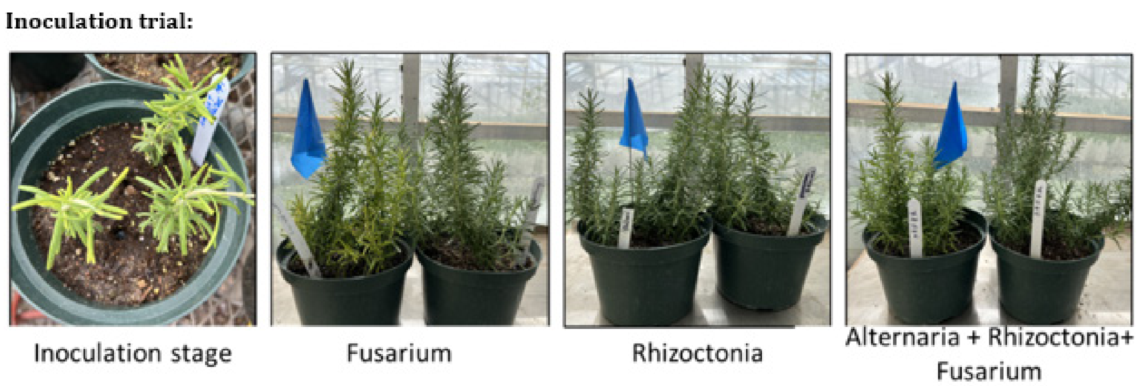

Figure 3: Representative photographs from inoculation trial. First panel shows rosemary plants at time of inoculation. Panels 2 through 4 show representative results from 77 days after inoculation for pots inoculated with Fusarium, Rhizoctonia, or Alternaria + Rhizoctonia + Fusarium, all of which were isolated from affected rosemary plants. Blue flags indicate pots where root systems were wounded by cutting through soil with a table / butter knife to the bottom of the pot between all of the plants to simulate damage from tilling. Note that all plants grew substantially from the inoculation stage, and none are showing signs of decline.

Crops planted immediately prior to rosemary varied and include chile pepper, corn, and standard rotation cover crops such as mixed brassica species so there is no apparent to tie to a previous crop. It is notable that all fields showing rosemary dieback syndrome had chile pepper in their recent history, and that soilborne diseases of chile were common in these fields. These include *Phytophthora capsici* and *Verticillium dahliae* caused vascular wilts, Fusarium and Rhizoctonia caused damping off to a lesser degree, and root knot nematodes in sandier sections of the fields. Past work in these fields showed that the root knot nematodes could enhance disease caused by some of these pathogens, particularly P. capsici, presumably due to creating wounds that facilitated infection by *P. capsici* [12].

The cause of the rosemary decline observed in Southeastern Arizona remains a mystery. The symptoms and pattern of decline are similar to those expected for a soilborne disease. Further, several types of fungi commonly associated with soilborne disease were isolated from affected rosemary plants including Fusarium, Rhizoctonia, and Alternaria. However, we have not been able to complete Koch's postulates with any of the fungi isolated from affected rosemary plants even when plants were inoculated with all three fungi simultaneously along with root wounding. There are several possibilities for the negative results obtained to date. It may be that the fungi isolated are pathogenic but require an additional factor, such as nematodes, or an environmental stress (heat, water, etc.) that we did not apply to cause disease. It is also possible that there are several too many isolates of each fungus present in the fields and to date we have isolated saprophytic but not pathogenic strains of fungi. It is also possible that there is a yet undiscovered pathogen, perhaps one that is slow growing and difficult to isolate or an unculturable obligate pathogen. Finally, it is possible that the observed rosemary decline is not a disease but due to some environmental factor that has a patchy and expanding distribution that mimics incidence patterns expected for disease. Although the cause has not been determined, the decline is severe and widespread enough to harm yields. The decline may also wipe some of the worst affected fields out if it spreads more. Therefore, additional work aimed at determining the cause of this rosemary decline will continue.

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Conflict of Interest

No conflict of interest.

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