# Effects of micronutrients on *Fusarium oxysporum* f. sp. *spinaciae* and limestone-mediated suppression of spinach Fusarium wilt

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## INTRODUCTION

The maritime Pacific Northwest is the sole region of the US suitable for producing spinach seed, a crop that requires long days for flowering and a cool, dry summer climate. However, the acid soils of this region are highly conducive to Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. *spinaciae*, and rotations of 10 years or more are necessary to reduce risk of losses to the disease (1).

Application of agricultural limestone ($CaCO_3$) to soil can suppress spinach FW (2-5). This practice has been adopted widely, yet the disease remains a constraint to spinach seed production. Furthermore, the mechanism(s) of suppression remains unknown. Research on the use of limestone to manage FW of other crops suggests that pH-mediated reduction in availability of iron (Fe), manganese (Mn), and zinc (Zn) may limit growth and aggressiveness of the pathogen (6,8). However, at elevated soil pH spinach plants may also be vulnerable to deficiencies of these micronutrients, which are involved in plant defense (7).

**The objectives of this study were to evaluate the effects of Fe, Mn, and Zn concentration on biomass and sporulation of *F. oxysporum* f. sp. *spinaciae in vitro*, and the influence of these micronutrients on FW suppression induced by limestone.**

## MATERIALS AND METHODS

***In vitro* micronutrient assays:**
*F. oxysporum* f. sp. *spinaciae* was grown in a liquid broth medium with a range of Fe, Mn, and Zn concentrations. A stock solution of each micronutrient was added to a basal medium at concentrations ranging from 0 to 2 mg/L. The pathogen was grown in each solution for 7 days on a rotary shaker, the culture filtrate vacuum-removed, and the mycelial mat dried and weighed to estimate fungal biomass. Spore production and germination were measured using similar experiments.

***In planta* interaction of limestone and micronutrient amendments:**
A field soil naturally-infested with *F. oxysporum* f. sp. *spinaciae* was used in greenhouse experiments to evaluate the effects of limestone and Mn, Zn, and Fe concentrations on FW development. Separate experiments were completed for each micronutrient, each with a 3-way factorial design: 1) +/- steam-pasteurization of the soil; 2) amendment with $CaCO_3$ at 0 or 2 tons/acre; and 3) application at planting of 0, 19, or 190 mg/L soil (w/v) of the appropriate micronutrient (chelated), with a 1.9 mg/L treatment added in repeat experiments. FW symptoms were rated weekly (0 to 5 scale, with 0 = healthy plant and 5 = dead plant) and converted to a disease index of 0 to 1, with 1 = maximum FW severity. Aboveground plant biomass was measured. Pre- and post-trial soil samples were tested for pH and the *F. oxysporum* population quantified on Komada's agar medium.

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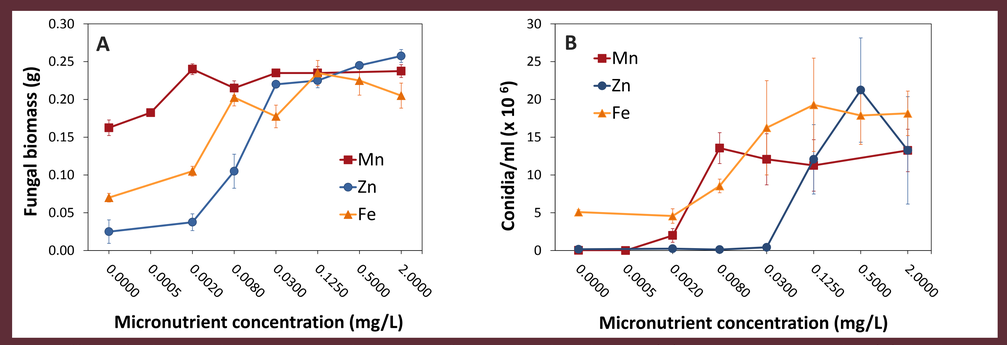


Fig. 1. Effect of micronutrient concentration on *F. o.* f. sp. *spinaciae* biomass (A) and spore production (B) *in vitro*.

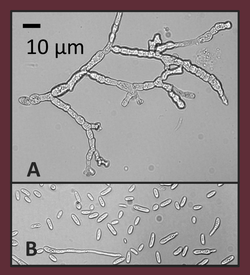


Fig. 2. A = 0 mg Mn/L → shortened, swollen hyphae, few spores. B = 2 mg Mn/L → normal spore production and hyphal morphology.

Fig. 3. Pigmentation of *in vitro* cultures of *Fusarium oxysporum* f. sp. *spinaciae* at increasing concentrations of Mn (left to right). Similar effects were observed with Zn and Fe (not shown).

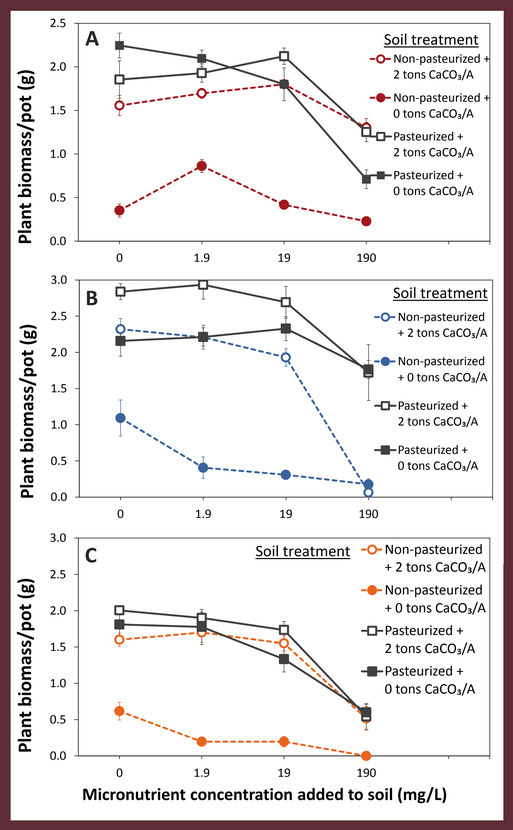


Fig. 4. Effect of micronutrient concentration on plant biomass in pasteurized and non-pasteurized soils. A = Mn, B = Zn, and C = Zn.

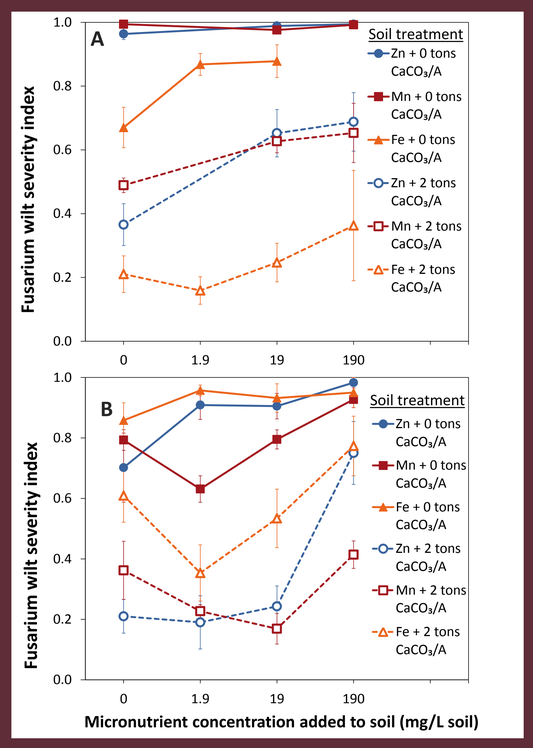


Fig. 5. Effect of micronutrient concentration on Fusarium wilt severity at 28 days after planting (non-pasteurized soil only). A and B = repeat experiments, with each micronutrient tested independently.

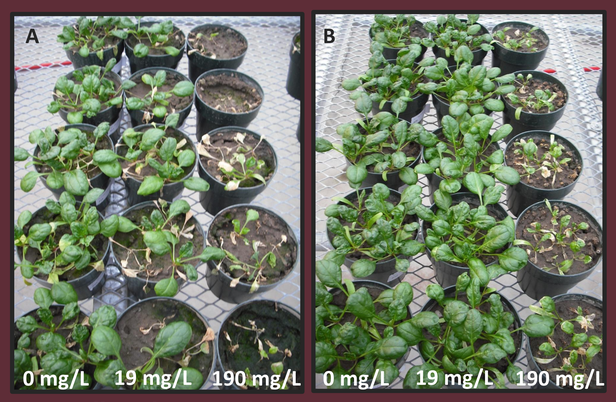


Fig. 6. Effect of Zn amendment on spinach plants in pasteurized (A) and non-pasteurized soils (B) amended with $CaCO_3$.

## RESULTS

***In vitro* micronutrient assays:**
- Increasing Fe, Mn, and Zn from 0 to 2 mg/L increased fungal biomass, with the greatest effect observed for Zn (Fig. 1).
- At the two lowest Mn concentrations, reduction in fungal biomass compared to higher concentrations was minimal, but sporulation was inhibited >1,000-fold (Fig. 1).
- Cultures in Mn-deficient broth produced abnormal mycelium with short, highly branched hyphae (Fig. 2).
- Pigmentation of the nutrient broth by the pathogen was affected by micronutrient concentration (Fig. 3).

***In planta* interaction of $CaCO_3$ and micronutrients**
- $CaCO_3$ suppressed FW and increased plant biomass across all micronutrient treatments in non-pasteurized soil (Figs. 4 and 5).
- In pasteurized soil, the effect of $CaCO_3$ on plant biomass was inconsistent (Fig. 4).
- The highest level of each micronutrient (190 mg/L soil) appeared phytotoxic to spinach in pasteurized and non-pasteurized soil based on reduced plant biomass and stunted, chlorotic plants (Figs. 4 and 6).

| 25 Mn 54.938 | 30 Zn 65.409 | 26 Fe 55.845 |
|---|---|---|
| **Manganese** | **Zinc** | **Iron** |
| 1st experiment (Fig. 5A): FW severity increased with added Mn in $CaCO_3$-amended soils. Without $CaCO_3$, FW severity developed rapidly at all micronutrient levels.<br><br>2nd experiment (Fig. 5B): No added Mn = more FW compared to soil with 1.9 and 19 mg/L, but FW severity increased in plants with 19 vs. 1.9, and 190 vs. 19 mg/L (non-$CaCO_3$ amended soil only). | 1st experiment: Area under disease progress curve increased with added Zn in $CaCO_3$-amended soils.<br><br>2nd experiment: No consistent change in FW associated with Zn treatment.<br><br>3rd experiment (Fig. 5B): FW severity increased with 1.9 and 19 mg Zn/L vs. no added Zn (non-$CaCO_3$ amended soil). | Increasing amounts of Fe applied to soil did not affect FW severity except at the highest level, at which severe phytotoxicity confounded FW ratings (Fig. 5). |

## CONCLUSIONS

The results indicate that minimum levels of Fe, Mn, and Zn are required by the spinach FW pathogen for optimal growth and sporulation. While the relationship between soil micronutrient levels and pathogen aggressiveness on spinach is less distinct, this study demonstrates that increasing concentrations of Zn and Mn in soil may enhance FW development. In some iterations of the experiments, however, there was evidence that spinach may be more susceptible to FW at low levels of these micronutrients. Given the complexity of soil biological and chemical interactions that can affect this disease, caution should be exercised in attempts to manipulate soil micronutrient status for management of FW.

**REFERENCES**

1. Correll, J.C., Morelock, T.E., Black, M.C., Koike, S.T., Brandenberger, L.P., and Dainello, F.J. 1994. Economically important diseases of spinach. Plant Dis. 78:653-660.
2. du Toit, L.J., Derie, M.L., and Brissey, L.M. 2008. Effect of agricultural limestone amendments on Fusarium wilt and Verticillium wilt in a spinach seed crop, 2007. Plant Disease Management Reports 2:V042.
3. du Toit, L.J., Derie, M.L., Brissey, L.M., and Cummings, J.A. 2007. Evaluation of limestone amendments for control of Fusarium wilt in a spinach seed crop, 2006. Plant Disease Management Reports 1:V091.
4. du Toit, L.J., Derie, M.L., Gatch, E.W., Brissey, L.M., and Holmes, B. 2011. Effect of agricultural limestone amendments on Fusarium and Verticillium wilts in a spinach seed crop, 2008. Plant Disease Management Reports 5:V117.
5. Gatch, E.W., Derie, M.L., Brissey, L.M., Holmes, B.J., and du Toit, L.J. 2011. Effect of agricultural limestone amendments and nitrogen source on Fusarium wilt in a spinach seed crop, 2009. Plant Disease Management Reports 5:V118.
6. Jones, J.P., and Wolz, S.S. 1970. Fusarium wilt of tomato: Interaction of soil liming and micronutrient amendments on disease development. Phytopathology 60:812-813.
7. Marschner, H. 1995. Mineral Nutrition of Higher Plants, 2nd Ed. Academic Press, London, UK.
8. Woltz, S.S. and Jones, J.P. 1968. Micronutrient effects on the in vitro growth and pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici*. Phytopathology 58:336–338.