Beneficial effects of *Trichoderma* on nitrogen fixation and root rot disease symptoms in pea

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Background Information

Pea (*Pisum sativum* L.) is a grain legume used as a valuable source of protein for food and feed. In addition, pea provides important agro-ecological benefits for sustainable cropping systems by increasing soil fertility. This is due to its ability to fix atmospheric nitrogen in symbiosis with diazotrophic soil bacteria, known as rhizobia, that induce the formation of root nodules. However, the cultivation of pea is severely compromised by various root rot pathogens. Species of the genus *Trichoderma* are widely studied for their ability to promote plant health and antagonise a wide range of pathogens. However, it is not known how *Trichoderma* affects the rhizobia-legume symbiosis, notably the relationship between nitrogen fixation capacity and fungal resistance in legumes. It was shown that *F. solani,* one of the pathogens causing root rot in peas (Wille et al. 2020), is stimulated by the same group of plant-exuded flavonoids that are involved in the interaction with *Rhizobium leguminosarum* bv. viciae, the rhizobial symbiont of pea (Ruan et al., 1995). This points at complex plant-microbe feedbacks eventually determining plant fitness.

Objective

Our study aimed to investigate the effects of *Trichoderma atrobrunneum* (*harzianum)* on pea root rot and the rhizobia symbiosis in order to determine a possible interaction between *Trichoderma* inoculation and the rhizobia-legume symbiosis and the existence of a relationship between N-fixation and pathogenic resistance in legumes.

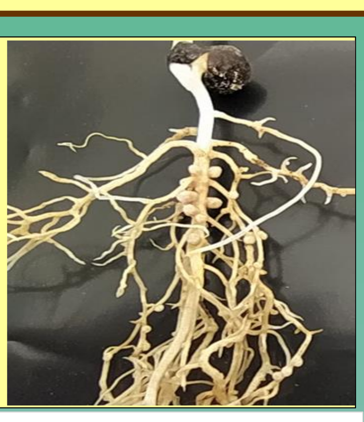
Methods

Two pea varieties ('Respect' and 'EFB 33'), differing in their tolerance to soil-borne pathogens, were either grown in naturally-infested field soil (NS) or in sterilized soil (SS) in ports of 500 ml with 4 plants per pot. Each pot was co-inoculated with 4 ml of *Rhizobium leguminosarum (10⁹ cells/ml)* and *Trichoderma atrobrunneum* (*harzianum)* at a concentration of 10^5 spores/g soil . Root rot (RRI), plant growth (RFW and SDW) and nodule formation (NN) were assessed at 10, 21 and 31 days after sowing.

1. ***Disease symptoms***

2. ***Biomass production***

3. ***Symbiosis with Rhizobia***



10d

21d

31d

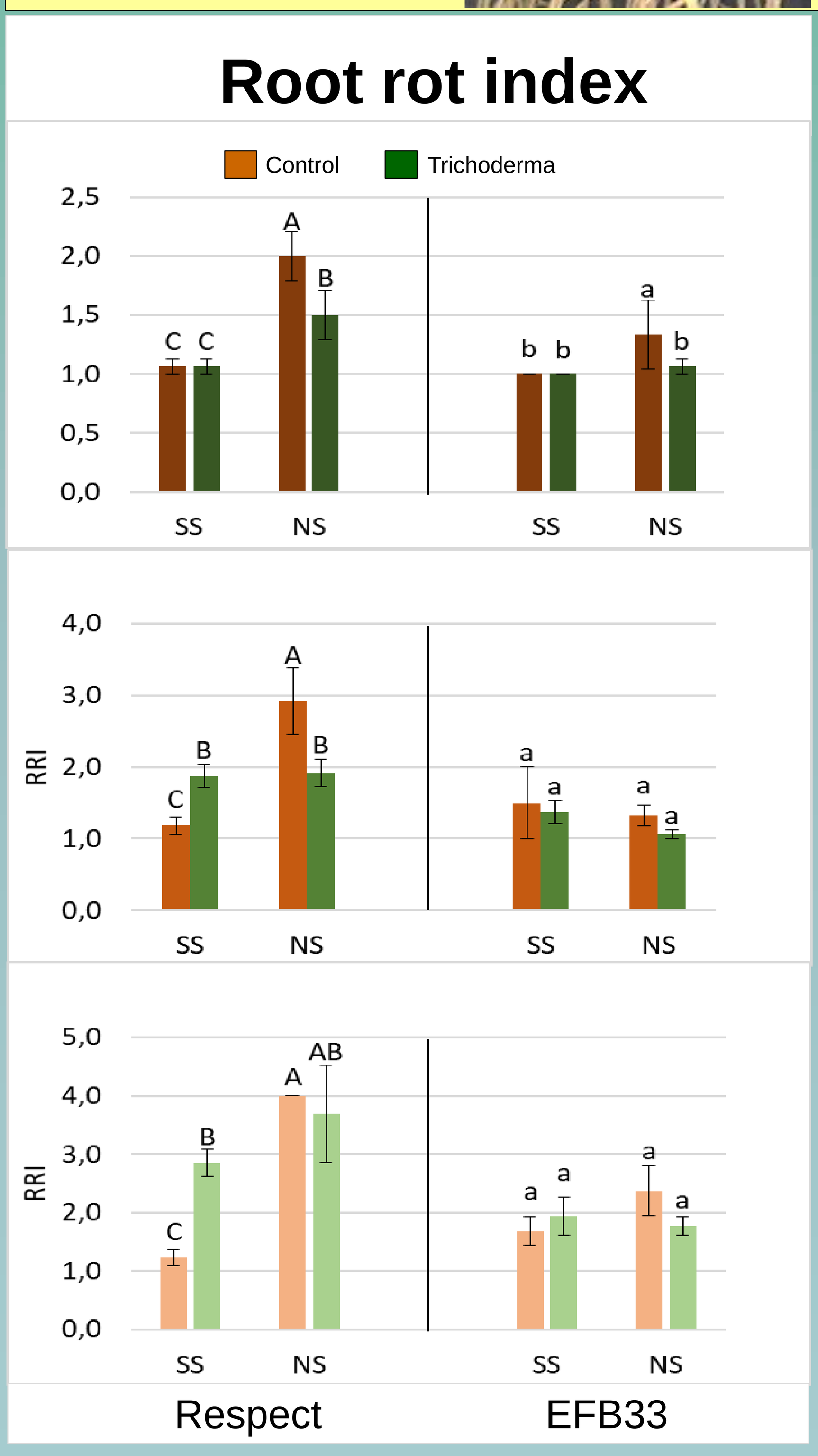


Figure 1. Root rot index (RRI) in pea varieties Respect and EFB33 harvested 10, 21 and 31 days after sowing in soil inoculated with *Rhizobium leguminosarum* and *Trichoderma atrobruneum* 48h before sowing. Data are means + SE (n=4). Mean followed by the same capital or lowercase letter do not differ (P<0.05) using the LSD test.

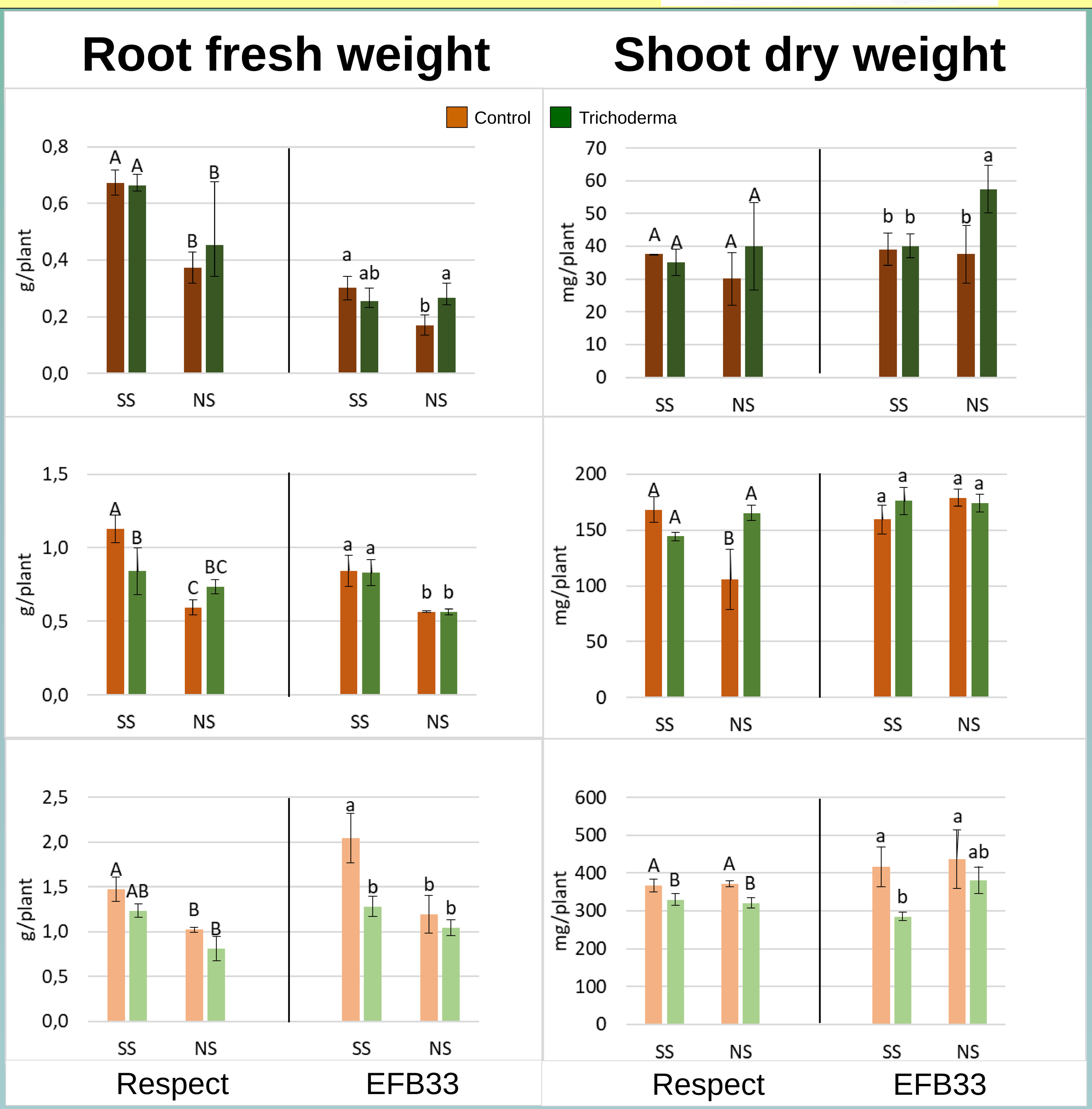


Figure 2. Root fresh weight (RFW) and shoot dry weight (SDW) in pea varieties Respect and EFB33 harvested 10, 21 and 31 days after sowing in soil inoculated with *Rhizobium leguminosarum* and *Trichoderma atrobruneum* 48h before sowing. Data are means + SE (n=4). Mean followed by the same capital or lowercase letter do not differ (P<0.05) using the LSD test.

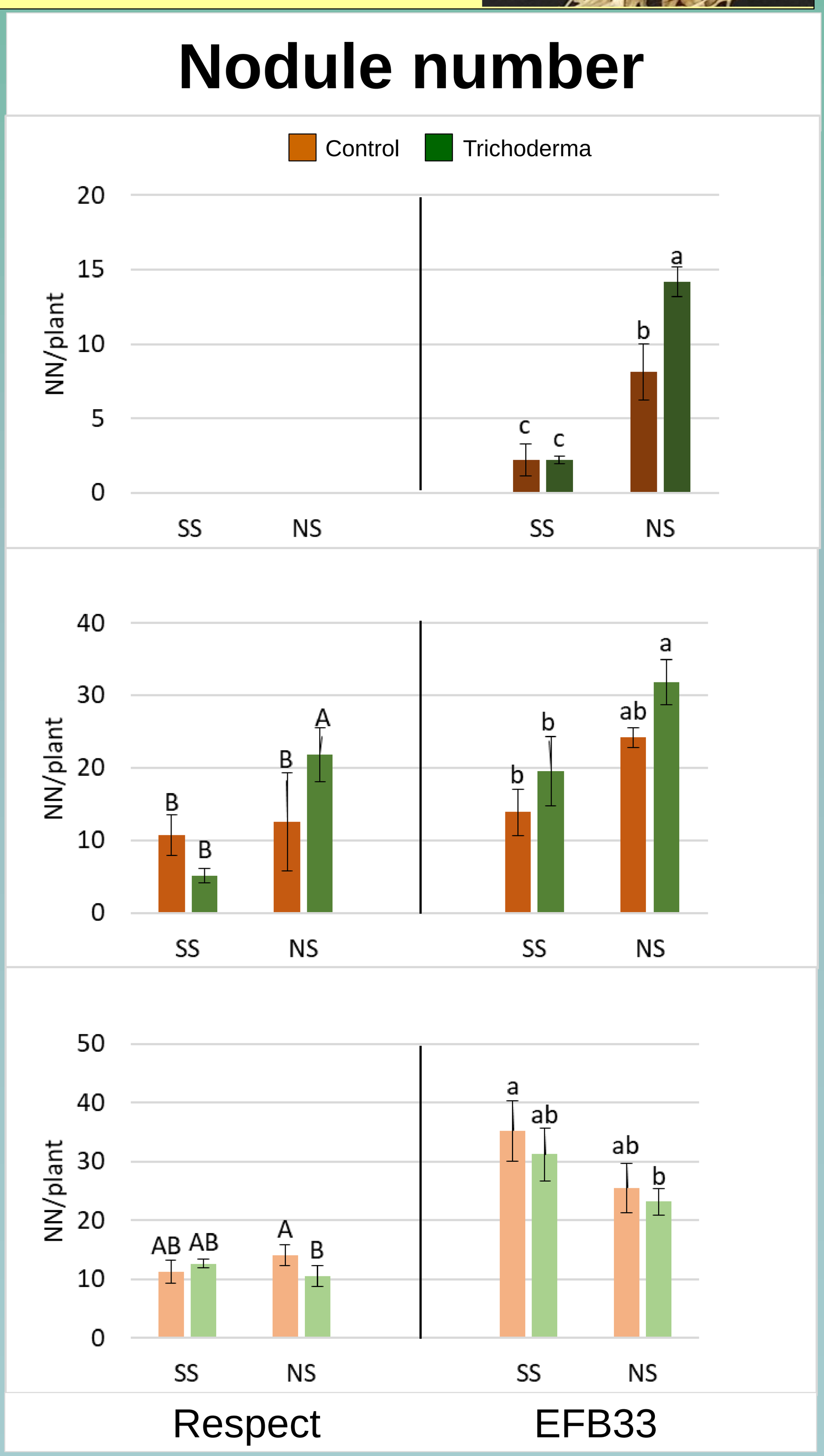


Figure 3. Nodule number (NN) in pea varieties Respect and EFB33 harvested 10, 21 and 31 days after sowing in soil inoculated with *Rhizobium leguminosarum* and *Trichoderma atrobruneum* 48h before sowing. Data are means + SE (n=4). Mean followed by the same capital or lowercase letter do not differ (P<0.05) using the LSD test.

Results

1. Disease symptoms developed more evidently in the susceptible variety (Respect) in which *Trichoderma* significantly reduced the RRI after 10 and 21 days. In the tolerant cultivar (EFB33) 10 days after sowing a slight increment in the RRI could be detected in the NS soil that was inhibited by the *Trichoderma* inoculation.
2. Root biomass in both cultivars was significantly reduced in the pathogenic soil, while the shoot biomass was only reduced in the susceptible cultivar (Respect) after 21d. *Trichoderma* inoculation provoked a reduction in the biomass of both cultivars after 31d.
3. Nodule number is higher in the tolerant cultivar (EFB), and under disease pressure it is favoured by the inoculation with *Trichoderma* 10 and 21 days after sowing.

Conclusion

Trichoderma produce a positive effect on plant biomass and nodule number in the infested soil, which indicates disease suppression and a synergistic effect of *Rhizobia* and *Trichoderma* under disease pressure conditions. However, these positive effect is restricted to early stages of the plant development.

The results arising from this study will contribute to increment the utilization of beneficial plant-associated microorganisms in the promotion of pea and also other leguminous crops of agro-ecological importance.

References

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