Genistein and methyl jasmonate inhibit nodulation of *Pisum sativum*

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Low root zone temperatures (RZT) early in the season can lower the nodulation rate of rhizobium bacteria on peas *(Pisum sativum* L.), lowering their ability to fix nitrogen. Poustini et al. (1) found that treating *Rhizobium leguminosarum* bv. *phaseoli* with either genistein or methyl jasmonate can mitigate the effects of low RZT within beans *(Phaseolus vulgaris).* We wanted to know if these chemicals would also mitigate low RZT effects on peas, specifically testing two varieties, 'Mozart' and 'Majoret'. Our results suggest that these two chemicals actually inhibit nodulation rather than stimulating it in these varieties.

The experiment consisted of exposing *R leguminosarum* bv. *pisi* cultures to genistein, methyl jasmonate, or both and inoculating imbibed pea seeds or pea seedlings with the cultures. We used a randomized complete block design consisting of 200 samples divided into four repetitions, two of which used vermiculite as a growing medium and two using Montana State University (MSU) potting soil. In addition, non-nodulating checks were included with each soil/cultivar combination that was inoculated with untreated rhizobium. The checks consisted of a non-nodulating mutant *sym14* in a 'Sparkle' background.

The rhizobium inoculant was prepared three days in advance of the inoculation date. Single colonies of *R. leguminosarum* bv. *pisi* were isolated from a commercial granular rhizobium source (NitraStik C, EMD Crop Bioscience), transferred to liquid media (2.2 mM K_2HPO_4, 1.8 mM NaCl, 0.8 mM $MgSO_4$, 1 gm/L yeast extract, pH 7.0) and shaken for 48 hours. Individual flasks of rhizobia were induced with specific treatments (genistein, 50 uM, methyl jasmonate, 20 uM) and shaken for 24 hours more. The peas were planted in conetainers and grown in a growth chamber set at 22°C during the 12 hr light phase and 16°C during the 12 hrs of dark. The seeds were initially inoculated 6 days after planting. Watering stopped two days before inoculation and began again two days later. A second inoculation was performed 4 weeks after planting, when the seeds had sprouted and the root system had formed. Harvest took place 2 weeks after the second inoculation, and the individual nodules on the roots were scored.

Nodulation rates in the various treatments are shown in Table 1. Nodulation on the control plants was relatively sparse, but it was four- to eight-fold greater than that observed in the samples treated with genistein. Samples treated with methyl jasmonate or with both chemicals displayed a complete lack of nodulation, as did the non-nodulating checks. Roots appeared healthy and vigorous under all treatments. The clear, negative impact of the treatments on nodulation rate discouraged us from pursuing similar experiments at other temperatures or with other varieties. We conclude that *R. leguminosarum* bv. *pisi* does not respond to genistein or methyl jasmonate in the same way as *R. leguminosarum* bv. *phaseoli* and that treatment with these chemicals will not aid in the establishment of a robust rhizobium/pea symbiosis under cold field conditions.

References

1. Poustini, K., Mabood, F. and Smith, DL. 2005. Acta Agriculturae Scandinavica Sec.B, 55: 293-298.

Table 1. Nodulation rate observed on roots under different treatments.

Cultivar	Treatment	Nodules per plant
Mozart		
	Distilled water	0.0
	Rhizobium only	2.1
	Rhizobium plus genistein	0.5
	Rhizobium plus methyl jasmonate	0.0
	Rhizobium plus both	0.0
Majoret		
	Distilled water	0.0
	Rhizobium only	1.2
	Rhizobium plus genistein	0.2
	Rhizobium plus methyl jasmonate	0.0
	Rhizobium plus both	0.0