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NOVEL INDUCIBLE PHYTOCHEMICAL DEFENCES AGAINST PLANT PARASITIC NEMATODES

IMELDA RIZALINA SORIANO

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School of Agriculture and Wine
The University of Adelaide
South Australia

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SUMMARY

The insect moulting hormone, 20-hydroxyecdysone (20E), is inducible in spinach and has been demonstrated to provide defence against insect herbivory. It is not known if such phytoecdysteroids are inducible by and defensive against plant parasitic nematodes. However, given that insects and nematodes belong to the same clade, the ecdysozoa, this is possible. Therefore, plants were tested for the presence of inducible phytoecdysteroids and effects on the nematodes tested.

Induction of possible defence compounds in common cultivars of spinach, two *Briza* spp., *B. maxima* and *B. minor*, oats and lucerne cultivars with varying degree of resistance to stem nematode, *Ditylenchus dipsaci*, was undertaken by treatment with methyl jasmonate and by challenging plants with nematodes. The influence of nematode inoculum density on the induction of phytoecdysteroid was also assessed in spinach. In addition, the relationship between the levels of inducible compounds and resistance response of lucerne cultivars to the stem nematode were evaluated.

Treatment with methyl jasmonate induced methanol extractable compounds in all plants tested. *Pratylenchus neglectus* induced the same compounds at levels equivalent to methyl jasmonate induction in all plants except lucerne, which was not tested. An inoculum rate of 500 to 10,000 *P. neglectus* induced similar levels of phytoecdysteroids in spinach. *Heterodera schachtii* induced phytoecdysteroids in both roots and shoots of spinach. *H. avenae* induced methanol extractable compounds in the roots of *B. minor* and shoot and roots of oats. *Meloidogyne javanica* was only found to increase levels of phytoecdysteroids in the shoots of spinach. Among the plants inoculated with the stem nematode, induced compounds were detected only in some lucerne cultivars resistant to the nematode.

The methanol extracts from the induced plants were further tested for biological activity using *Drosophila melanogaster* B_{II} cell microplate-based bioassay to screen and

detect biologically active ecdysteroids. The extracts were subjected to mass spectrometry to confirm the presence of ecdysteroids. The biological and chemical characterisation of the inducible compounds in the plants tested provided evidence that spinach, *Briza* spp. and lucerne contained the ecdysteroids 20E and polypodine B, which were biologically active based on the B_{II} cell bioassay, except for lucerne. Lucerne shoots appeared to contain compounds or conjugate groups that inhibit ecdysteroids. In addition to the ecdysteroids above, *B. maxima* also contained ecdysone. On the other hand, inducible flavonoids were observed in the shoots and roots of oats.

Two plant parasitic nematodes, *P. neglectus* and *Anguina tritici*, were examined for the presence of similar ecdysteroids induced in the plants tested. This information will corroborate the involvement of these compounds in plant defence against nematodes. Based on HPLC and mass spectrometry data, both nematodes did not contain the ecdysteroids induced in the plants. However, compounds with masses similar to 20,26-dihydroxyecdysone, 20,26-dihydroxyecdysone 22-acetate, makisterone A, and possibly an unreported ecdysteroid were observed in *P. neglectus*. No ecdysteroid was observed in *A. tritici,* which consisted only of second stage juveniles in the anhydrobiotic survival state as opposed to the presence of all stages of an actively developing population of *P. neglectus*.

In order to establish that ecdysteroids are potential defence compounds against parasitic nematodes, the effects of direct application of 20E on nematodes was assessed by treating cereal cyst nematode, *H. avenae*, juveniles with concentrations of 20E from 8.2×10^{-8} to 5.2×10^{-5} M before applying to wheat. *H. avenae*, *H. schachtii*, *M. javanica* and *P. neglectus* were treated with 5.2×10^{-5} 20E and incubated in moist sand. To test the protective effects of 20E in plants, *H. schachtii* and *H. avenae* were applied to spinach and quaking grass, respectively, and the latter two nematodes in both plants, in which elevated concentrations of 20E had been induced by methyl jasmonate. Abnormal moulting, immobility, reduced invasion, impaired development and death occurred in nematodes

exposed to 20E either directly at concentration above $4.2x10^{-7}$ M or in plants. Phytoecdysteroid induction apparently protected spinach and *B. maxima* from plant parasitic nematodes and may confer a mechanism for nematode resistance.

Green manure is an alternative option to deliver the defence compound, as high constitutive production in a crop plant might impose unacceptable metabolic cost. Induced spinach when applied as green manure suppressed invasion of *H. avenae* in wheat but the direct involvement of 20E was not established because of the highly toxic effects of the treatment on the nematode.

Three inducible compounds, isolated in methanolic root and shoot extracts of oats were identified as flavone-*C*-glycosides by mass spectrometry. The effect of the flavone-*C*-glycosides on the invasion by and development of cereal cyst nematode, *H. avenae,* was assessed using methanolic extracts of shoots and roots from methyl jasmonate treated plants. Both extracts impaired nematode invasion and development. When the extracts were fractionated by high voltage paper electrophoresis, only one flavone-*C*-glycoside, *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside, inhibited nematode invasion. The protective effect of the induction of flavone-*C*-glycosides in oats by methyl jasmonate was evaluated against *H. avenae* and *P. neglectus*. Treatment with methyl jasmonate reduced invasion of both nematodes and increased plant mass, compensating for damage caused by the nematodes, and is attributed to the active flavone-*C*-glycoside. The active compound, *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside, has not been implicated previously in plant defense against any pest or pathogen, and appears to provide protection against the major cereal nematodes *Heterodera* and *Pratylenchus*.