EFFECT OF LEAF AND ROOT EXTRACTS OF *PHYTOLACCA AMERICANA* L. ON HATCHING OF *GLOBODERA ROSTOCHIENSIS* AND *MELOIDOGYNE ARTIELLIA*

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Summary. An investigation was conducted to assess the hatching response of Italian populations of the potato cyst nematode, *Globodera rostochiensis*, and the British root-knot nematode, *Meloidogyne artiellia*, to pokeweed (*Phytolacca americana*) leaf and root extracts under laboratory conditions at 20 ± 1 °C. Cysts of *G. rostochiensis* were exposed to a series of increasing pokeweed leaf or root extract aliquots (0.25, 0.5, 0.75 or 1 ml), which were added to 3 ml of a 0.6 mM sodium metavanadate ($NaVO_3$) solution. Egg masses of *M. artiellia* were exposed to the same rates of leaf and root extracts but the extracts were, instead, added to 3 ml of distilled water. Controls were $NaVO_3$ 0.6 mM and both extracts alone for *G. rostochiensis* and distilled water for *M. artiellia* experiments. No egg hatched from cysts of *G. rostochiensis* incubated in pokeweed leaf or root extract alone, but 47% hatched from cysts in $NaVO_3$ alone in 7 weeks. Hatch of *G. rostochiensis* was greatly stimulated by incubation in $NaVO_3$ with the addition of 0.25 ml of leaf or root extract, reaching 79.5% and 68.2%, respectively. Egg hatch increased further in $NaVO_3$ plus 0.5, 0.75 and 1 ml of pokeweed leaf or root extract, and reached cumulative hatches of 86-90.5% of eggs from cysts. Egg hatch of *M. artiellia* was 76.5% in distilled water alone; it was not affected by extracts of pokeweed leaf at 0.25 and 0.5 ml or by extracts of pokeweed root at 0.25 ml, but was significantly suppressed at larger concentrations of both extracts.

Key words: Juvenile emergence, pokeweed, potato cyst nematode, root-knot nematode.

Pokeweed (*Phytolacca americana* L.) is a common weed plant species of the family Phytolaccaceae, which contains several genera and species of herbs, shrubs, vines and trees (Krochmal and LeQuesne, 1970) and whose members now occur world-wide. Pokeweed is common in Italy, especially in the south of the country (Di Vito *et al*., 2007). The plant has many medicinal uses.

The roots of pokeweed are mentioned in old medicinal texts as the source of a powerful drug, which in small doses was thought useful for the treatment of ulcers and skin disorders (Jenkins, 1929). However, toxic effects were observed when it was used in large doses. The extracts contain phytolaccagenin, a highly oxygenated structure characterised by several physiologically active triterpenoids (Stout *et al*., 1964). It is closely related to the well known oleanic acid, a powerful molluscicidal saponin isolated from *Phytolacca dodecandra* L'Her. Phytolaccagenin is the most poisonous component of both *Phytolacca* species and, since it exhibits some chemical similarities to extracts from a related species that has shown molluscicidal properties, it is suggested that it may have potential for controlling fresh-water snails.

We further hypothesized that pokeweed could also affect the biology of plant parasitic nematodes. Because of interesting results obtained earlier by Di Vito *et al*. (2007) with fruit extract of this plant, on the hatching of eggs of *Globodera rostochiensis* (Woll.) Behrens and *Meloidogyne artiellia* Franklin, further investigations were undertaken to assess the effect of leaf and root extracts of pokeweed on hatching of eggs of the above mentioned nematodes.

MATERIALS AND METHODS

Preparation of the extract. To obtain leaf and root extracts, pokeweed was reared in 2-dm^3 clay pots filled with steam-sterilized sandy soil (sand 88%, silt 5%, clay 7% and organic matter 2.5%) in a glasshouse at 26 ± 2 °C. When four months old, leaves and roots of pokeweed were separately homogenized in a blender and the liquid extracts were filtered, centrifuged at 1500 rpm for 30 minutes and stored at -20 °C until use. These were considered as standard leaf and root extracts.

Test with Globodera rostochiensis. The Italian population of *G. rostochiensis* was from a field located at Polignano a Mare (Apulia region) in which a potato crop had been grown the previous season. Soil from the field was kept in plastic trays outdoors in the shade to dry before use. Cysts were extracted from 200 cm^3 aliquots of soil with the Fenwick can. The water-cyst suspension was poured onto a coarse 15-cm-diameter filter paper and cysts were separated from soil debris. Batches of 50 cysts each (averaging 13,000 eggs) were put in 1.5-cm-diameter sieves of 215 µm aperture and each of these was placed in a 3-cm-diameter plastic Petri dish (Greco *et al*., 1982). Each Petri dish received 3 ml of 0.6 mM sodium metavanadate ($NaVO_3$) solu-

tion, to which was added 0.25, 0.5, 0.75 or 1 ml of standard pokeweed leaf or root extract. Controls were cysts in 3 ml of 0.6 mM $NaVO_3$ solution only and in distilled water with 0.5 ml of either extract.

The experiment was set out in a completely randomised design comprising four replicates per treatment. All dishes were incubated at 20 ± 1 °C for a period of seven weeks. Emerging second stage juveniles were counted weekly and the hatching media renewed at same time. At the end of the test the cysts in each dish were crushed according to the method of Seinhorst and den Ouden (1966) and the remaining unhatched eggs were counted. The sum of these and the total number of juveniles emerged was considered to be the total number of eggs per dish at the start of the test. The emerging juveniles were then expressed as cumulative weekly percentages of the cyst content at the beginning of the test.

Test with Meloidogyne artiellia. An Italian population of *M. artiellia* was collected from roots of chickpea (*Cicer arietinum* L.) at Monopoli (Apulia region) and reared on the same host in a glasshouse at 21 ± 2 °C. Fifty egg masses of uniform size (averaging 14,000 eggs and juveniles each) were collected and put singly into sieves of 1.5-cm-diameter and 75 µm aperture. Each sieve was placed in a plastic Petri dish of 3-cm-diameter (Ekanayake and Di Vito, 1985) and 3 ml of a distilled water solution containing 0.25, 0.5, 0.75 or 1 ml of the standard pokeweed leaf or root extracts were added to each dish. Egg masses in distilled water served as controls. Each treatment was replicated four times and arranged in a completely randomised design. All dishes were incubated in a growth chamber at 20 ± 1 °C.

Weekly counts of emerging juveniles and changes of the hatching media were made for four consecutive weeks. Then the gelatinous matrix of the egg mass in each dish was dissolved in 20 ml of a 1% sodium hypochlorite (NaOCl) solution in a 50 ml glass bottle, according to Hussey and Barker (1973), and the bottles were shaken for 3 minutes. The unhatched eggs were counted and the sum of these and of the emerged juveniles per dish was considered to be the number of eggs per dish at the start of the test. The emerged juveniles were expressed as cumulative percentages of the initial population.

Statistical analysis. All data were statistically analysed by ANOVA and means compared by least significant difference (LSD).

RESULTS

No juvenile emerged from cysts of *G. rostochiensis* incubated in leaf or root extracts of pokeweed alone (data not reported), while 47% of the eggs hatched from cysts incubated in $NaVO_3$ by the end of the test

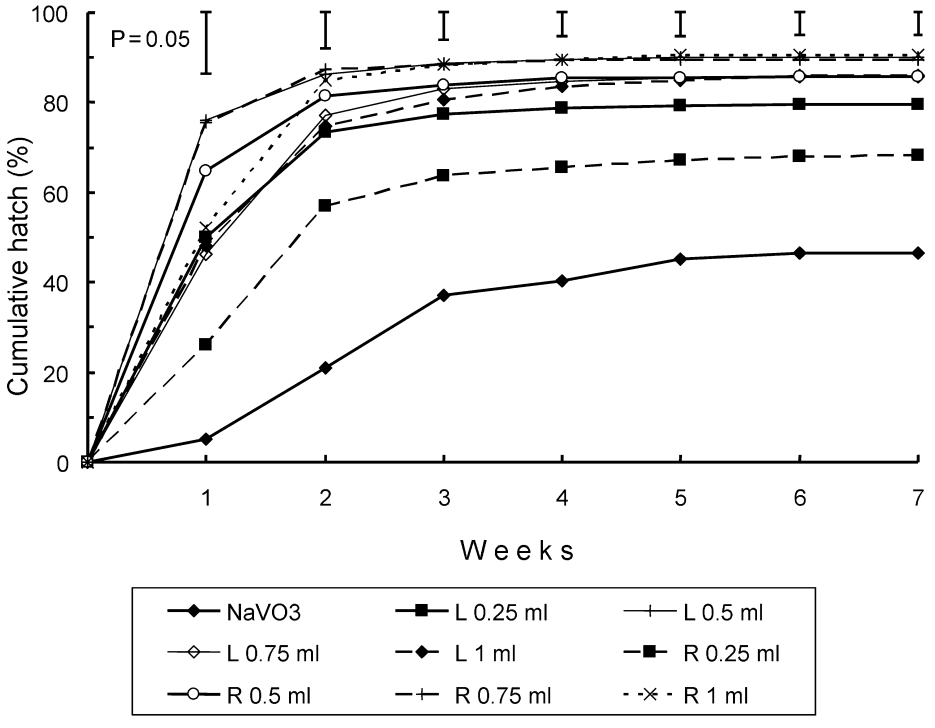

Fig. 1. Per cent cumulative hatch of eggs from cysts of *Globodera rostochiensis*, in 0.6 mM of sodium metavanadate ($NaVO_3$) and different concentrations of leaf (L) or root (R) extracts of pokeweed, and incubated at 20 ± 1 °C for seven weeks.

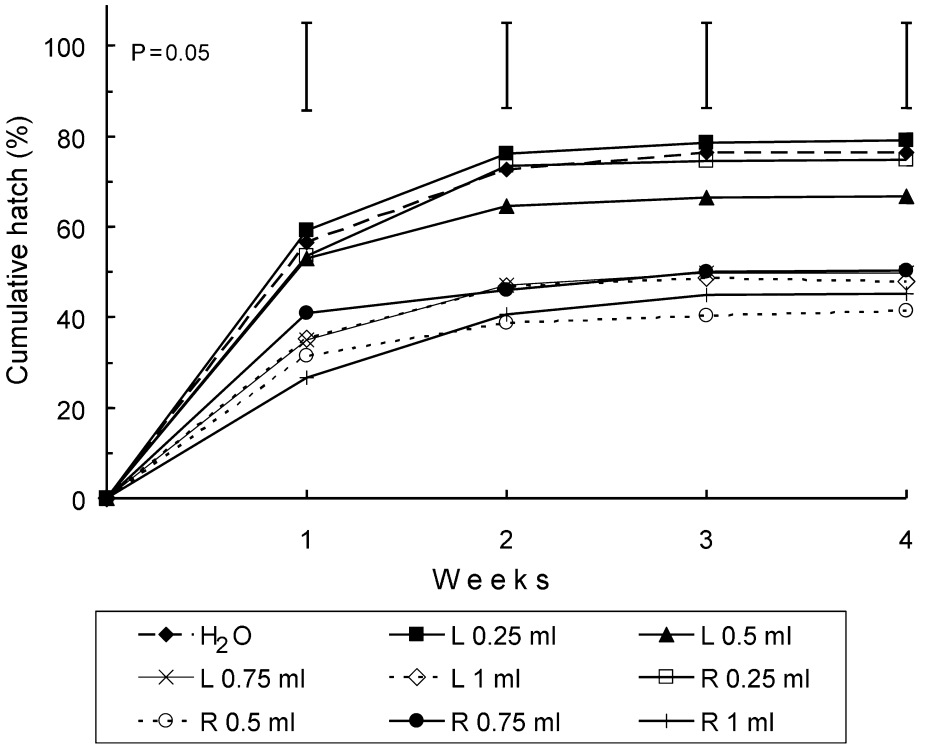

Fig. 2. Per cent cumulative hatch of eggs within egg masses of *Meloidogyne artiellia*, in different concentrations of leaf (L) or root (R) extracts of pokeweed, and incubated at 20 ± 1 °C for four weeks.

(seventh week) (Fig. 1). Compared with hatch in $NaVO_3$ alone, significant increases of egg hatch were observed when the $NaVO_3$ solution was supplemented with 0.25 ml of pokeweed leaf or root extract, with percentages of cumulative egg hatch of 79.5 and 68.2%, respectively. Egg hatches were even greater (85.7-90.5%) from cysts incubated in $NaVO_3$ supplemented with 0.5, 0.75 and 1 ml of pokeweed leaf or root extract (Fig. 1), with hatches in $NaVO_3$ plus 0.75 and 1 ml of pokeweed root extract and 0.5 ml of leaf extract significantly larger than those in all other treatments. Overall, after two weeks of incubation, emergence of juveniles from cysts incubated in $NaVO_3$ plus all concentrations of leaf and root extract was significantly larger (55-87%) than that in $NaVO_3$ alone.

By the end of the test (after four weeks), the emergence of juveniles from egg masses of *M. artiellia* in distilled water was 76.5% (Fig. 2). Similar percentages of hatch occurred from egg masses incubated in distilled water plus 0.25 and 0.5 ml of pokeweed leaf (66.7, 79%) or 0.25 ml of pokeweed root (74.7%) extracts, thus indicating that the extracts had no effect on egg hatch. At larger concentrations of both leaf and root extracts, emergence of juveniles was significantly reduced (41.5-50.2%), with no significant differences among these concentrations. In general, no hatch stimulation of *M. artiellia* was observed with pokeweed leaf and root extracts.

DISCUSSION

The hatch of eggs of *G. rostochiensis* was stimulated by all concentrations of leaf or root extracts of pokeweed added to a 0.6 mM solution of $NaVO_3$. The possible explanations for this effect have already been discussed (Di Vito *et al.*, 2007) and these results have confirmed the stimulating effect of extracts from *P. americana* on *G. rostochiensis*. However, some remarkable differences were observed among extracts from different plant parts. Whether the observed differences depend on the chemical nature of the ingredients present in the extracts or on their concentrations cannot be inferred from our tests. In an experiment with fruit extracts (Di Vito *et al.*, 2007), a stimulating effect occurred when 3 ml of 0.6 mM of $NaVO_3$ were enriched with 0.1-0.8 ml of the fruit extracts and a suppressing effect when they were enriched with 1 ml of extract. In the current experiment, both root and leaf extracts significantly enhanced hatching at all tested concentrations, with the greatest hatching occurring with 0.5-1 ml of extracts. Moreover, in fruit extract hatch was negligible during the first week and most of the hatchable eggs hatched by the fourth week. In leaf and root extracts, 48-76% of the eggs hatched by the end of the first week in 0.5-1 ml of extract (Fig. 1) and only a negligible hatch occurred during the second week. However, in the test with fruit extracts, probably because of the physiological state of the eggs within cyst, the cumulative hatch of the nematode by the end of the test was only 20.7% (Di

Vito *et al*., 2007), while it was 47% in our test (Fig. 1). The observed stimulating effect would be a useful reaction whenever second stage juveniles of *G. rostochiensis* are required for any purpose, or to estimate the proportion of hatchable eggs of the nematode. It would be interesting to test whether the stimulating effect observed with *G. rostochiensis* also occurs with other cyst nematodes and whether the extracts overcome the reluctance to hatch found during diapause or dormancy of eggs within cysts.

In contrast, no hatching stimulation by pokeweed leaf and root extracts was observed on eggs in the egg masses of *M. artiellia* (Fig. 2), as egg hatches in these extracts were similar to that in distilled water (66.7-76.5%) at low concentrations (0.25-0.5 ml of leaf extract and 0.25 ml of root extract). With this nematode, larger concentrations of both extracts significantly suppressed egg hatch (41.5-50.2%), thus confirming the response observed earlier with fruit extract (Di Vito *et al*., 2007).

However, more insights are required to understand which of the ingredients have stimulating or suppressive effects, or both, on egg hatching.

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