EFFECT OF POKEWEED (*PHYTOLACCA AMERICANA* L.) EXTRACT ON HATCHING OF *GLOBODERA ROSTOCHIENSIS* AND *MELOIDOGYNE* SPP.

M. Di Vito[1], V. Alba[2], E. Alba[3], and F. Catalano[1]

[1] *Istituto per la Protezione delle Piante, Sezione di Bari, C.N.R., 70126 Bari, Italy*
[2] *Dipartimento di Biologia e Chimica Agroforestale, Università di Bari, 70126 Bari, Italy*
[3] *Dipartimento di Biologia, Difesa e Biotecnologie Agroforestali, Università della Basilicata, Potenza, Italy*

Summary. The effect of pokeweed fruit extract on hatching of the potato cyst nematode, *Globodera rostochiensis*, and the British root-knot nematode, *Meloidogyne artiellia*, was studied in Italy under laboratory conditions at 20 ± 1 °C. Cysts of *G. rostochiensis* were exposed to a series of increasing pokeweed fruit extract aliquots of 0.01, 0.02, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml, which were added to 3 ml of sodium metavanadate 0.6 mM. Egg masses of *M. artiellia* were exposed to the same series of increasing fruit extract aliquots that were instead added to 3 ml of water. Controls were the hatching agent sodium metavanadate 0.6 mM, pokeweed root leachate and an aliquot of 1 ml of pokeweed fruit extract added to 3 ml of water for *G. rostochiensis,* and distilled water only for *M. artiellia*. The control treatment sodium metavanadate induced 20.7% hatching of *G. rostochiensis*, whereas the other two controls, pokeweed root leachate and 1 ml fruit extract in 3 ml of water, did not induce any hatching. The hatching of *G. rostochiensis* was not enhanced by adding to sodium metavanadate aliquots of 0.01-0.05 ml of pokeweed fruit extract, but was significantly increased by adding larger aliquots of 0.1-0.8 ml and suppressed (7.2%) by 1 ml. The greatest hatch increase of *G. rostochiensis* (81.6%) was provided by adding an aliquot of 0.4 ml of pokeweed fruit extract to 3 ml of sodium metavanadate. Hatching of *M. artiellia* was of 80.5 % in the control distilled water alone and was not enhanced by adding 0.01-0.2 ml of pokeweed fruit extract. However, water enrichment with increasing aliquots of 0.4-1 ml fruit extract significantly suppressed (17.5-37.7%) *M. artiella* hatching compared to the control. Pokeweed was a very good host for *M. incognita* and *M. javanica* and a non-host for *M. artiellia*.

Key words: Egg hatching, plant extract, potato cyst nematode, root-knot nematodes.

Pokeweed (*Phytolacca americana* L.) is a native North American perennial shrub that has been introduced to many other parts of the world including Italy (Krochmal and LeQuesne, 1970). This shrub produces berries that are juicy, shiny, dark purplish black that ripen in early summer in warm climates and in autumn in temperate regions (Chittendon, 1956).

Pokeweed root extracts were used in folk medicine for the treatment of ulcers, skin disorders and fungal infections (Jenkins, 1929). Stout *et al.* (1964) isolated a toxic compound from pokeweed roots called phytolaccatoxin, which under acid hydrolysis degrades into glucose and xylose. Root tissues are also rich in alkaloids, antiviral protein, phytolactic and formic acids, lectins, tannin, fatty oil, resin and sugar (Lampe and Fagerstrom, 1986).

Pokeweed has an unusually high potassium content and its ashes, which contain over 45% caustic potash, have been used as a salve for ulcers and cancerous growths (Castro, 1990). The antiviral proteins of this plant (Phytolacca Antiviral Proteins = PAP) and glycosidases belong to a group of Ribosome-Inactivating Proteins (RIPs), which show potent activity against many viruses causing plant and animal diseases (Wyatt and Shepherd, 1969; Owens *et al.*, 1973; Tomlison *et al.*, 1974; Barbieri *et al.*, 1982; Endo *et al.*, 1988; Chen *et al.*, 1991; Irvin, 1995; Tumer *et al.*, 1998) and also fungal plant diseases (Zoubenko *et al.*, 1997).

A sapogenin (phytolaccagenin) having potential molluscidal activity, has also been identified in pokeweed roots by paper chromatography. Laboratory tests and field observations confirmed this toxic activity against molluscs. Tap-water extracts of the dried and powdered pokeweed fruit had an LC_{100} (lethal concentration) of 10 ppm against snails (*Biomphalaria*, *Bulinus* and *Lymnaea* spp.) (Krochmal and LeQuesne, 1970).

The wide spectrum of toxic activity of pokeweed extracts against many harmful organisms has promoted our interest in determining, under laboratory conditions, whether pokeweed extracts have adverse or favourable effects on hatching of the golden nematode, *Globodera rostochiensis* (Woll.) Behrens, and the British root-knot nematode, *M. artiellia* Franklin. The host status of pokeweed to economically important root-knot nematodes such as *M. artiella*, *M. incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Citw. was also assessed.

MATERIALS AND METHODS

Hatching test of Gobodera rostochiensis. Soil infested by *G. rostochiensis* was collected from a field located at Polignano a Mare (Bari province, Apulia) after potato harvest in the late spring of 2005 and kept in plastic bags in a shed until November of the same year. The nematode was identified on the basis of the perineal patterns

and isoelectrofocusing patterns of the superoxide dismutase (SOD) isozymes of the cysts (Molinari *et al.*, 2005).

Nematode cysts were extracted in a Fenwick can from 200 cm^3 sub-samples of non-dried soil and separated from soil debris. Then, batches of 50 cysts each (averaging 10,000 eggs and juveniles) were placed in 1.5-cm-diam. sieves of 215 µm aperture and arranged in 3-cm-diam. plastic Petri dishes (Greco *et al.*, 1982). Each Petri dish received 3 ml of 0.6 mM sodium metavanadate ($NaVO_3$, an artificial hatching agent) alone or enriched with aliquots of 0.01, 0.02, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of full-strength pokeweed fruit extract. Controls were cysts in 3 ml of distilled water containing 1 ml of pokeweed fruit extract, pokeweed root leachate, and 0.6 mM of $NaVO_3$.

The Petri dishes were placed in a growth chamber at 20 ± 1 °C. There were four replicates per treatment according to a completely randomised design. Counts of emerging juveniles and changes of hatching media were done weekly for seven consecutive weeks. At the end of the experiment, the cysts were crushed as described by Seinhorst and Den Ouden (1966) and unhatched eggs were counted to ascertain the total number of eggs in the cysts at beginning of the test. Cumulative numbers of juveniles emerging weekly were expressed as percentages of the total content of the cysts.

Preparation of pokeweed root leachate and fruit extract. Pokeweed root leachate was obtained by drenching with distilled water the soil in 14-cm-diam. pots in which two-month-old pokeweed plants were growing and collecting the leachate from the pots over a 24-hour period. The leachate was then centrifuged at 1500 rpm for 30 minutes and stored at 4 ± 2 °C. To prepare the full-strength pokeweed fruit extract, mature berries of pokeweed were collected in the autumn of 2005 from a field at Galatina (Lecce province, Apulia). The berries were crushed and the liquid extract was filtered, centrifuged at 1500 rpm for 30 minutes and stored at -20 °C . Increasing aliquots of this full-strength fruit extract were used as described above in 2006.

Hatching test of Meloidogyne artiellia. The population of *M. artiellia* was collected from roots of chickpea (*Cicer arietinum* L.) at Monopoli (province of Bari, Apulia) and reared on the same host in a glasshouse at 21 ± 2 °C. When large egg masses had formed on the roots of chickpea, fifty of uniform size (averaging 13,000 eggs and juveniles) were collected and each was placed in a 1.5-cm-diam. sieve of 75 µm aperture. Each sieve was placed in a 3-cm-diam. plastic Petri dish (Ekanayake and Di Vito, 1985) and received 3 ml of distilled water enriched with increasing aliquots of 0.01, 0.02, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml full-strength pokeweed fruit extract. Egg masses in distilled water only were used as a control. There were four replicates per treatment according to a completely randomised design and all Petri dishes were placed in a growth chamber at 20 ± 1°C.

Emerging juveniles were counted and hatching media renewed weekly for four consecutive weeks. At the end the experiment, unhatched eggs and juveniles were counted by dissolving the gelatinous matrix of the egg masses of each replicate in a 50 ml glass bottle containing 20 ml of a 1% sodium hypochlorite solution (NaOCl) (Hussey and Barker, 1973) and shaking for 3 minutes. The sum of emerged juveniles and unhatched eggs was considered as the initial population of the nematode at the start of the hatching test. Emerged juveniles were expressed as cumulative percentage hatch of the initial population.

Statistical analysis. The data were statistically analysed by ANOVA and means compared by LSD.

Host status of pokeweed to root-knot nematodes. Three groups of ten 40-day-old pokeweed seedlings were transplanted into three separate plastic trays (50 × 32 × 10.5 cm) containing 10 dm^3 of steam-sterilized sandy soil. Three days later, the 10 seedlings in each tray were inoculated separately with 10,000 eggs and juveniles of *M. incognita*, *M. javanica* or *M. artiellia* per seedling. The identification of these species was based on the morphological characteristic of the second stage juveniles and perineal and esterase patterns of the females. Two trays planted with ten 20-day-old seedlings of tomato cv. Rutgers and inoculated with *M. incognita* and *M. javanica*, as described above, served as susceptible controls. For *M. artiellia*, the control was a tray planted with chickpea and inoculated with 10,000 eggs of the nematode per plant. Trays infested with *M. incognita* and *M. javanica* were then placed in a glass-house at 26 ± 2 °C and trays infested with *M. artiellia* in a glasshouse at 21 ± 2 °C. Forty days after inoculation, all plants were uprooted and the roots gently washed free of the adhering soil. Gall and egg mass indices of the roots were assessed according to a 0-5 scale, where 0 = 0 gall and/or egg mass, 1 = 1-2 galls and/egg masses, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 more than 100 galls and/or egg masses (Taylor and Sasser, 1978).

RESULTS

Hatching tests. In general, emergence of juveniles from cysts of *G. rostochiensis* (Fig. 1) was low during the first week of incubation. It increased rapidly during the second and third weeks and less rapidly from the fourth to the sixth weeks. Few or no juveniles emerged during the seventh week. Consideration of the percentage cumulative hatch at the end of the test (seventh week) showed that no juvenile emerged from cysts incubated in root leachate or fruit extract of pokeweed in water (1 ml/3 ml), while 20.7% of the eggs hatched in $NaVO_3$. When $NaVO_3$ was enriched with 1 ml fruit extract of pokeweed, the emergence of the juveniles from cysts of *G. rostochiensis* decreased to 7.2%. No signifi-

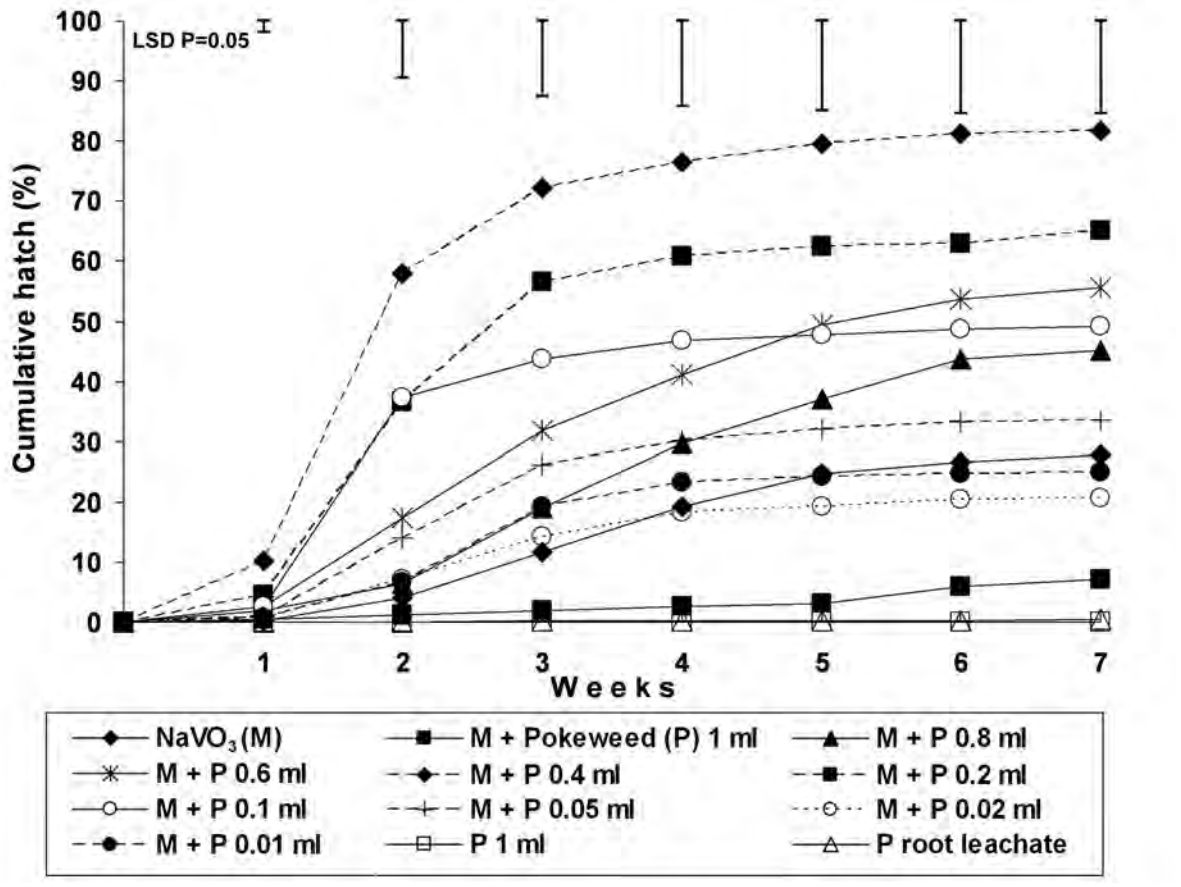

Fig. 1. Effect of increasing concentrations of pokeweed fruit extract on cumulative percentage hatch of eggs in cysts of *Globodera rostochiensis* incubated at 20 ± 1 °C for seven weeks.

cant hatching increase was observed by adding 0.01-0.05 ml aliquots of pokeweed fruit extract to 3 ml of $NaVO_3$. Hatching was, however, significantly enhanced (45.2, 49.2 and 55.7%) compared to the control $NaVO_3$ alone, by enriching $NaVO_3$ with 0.1-0.8 ml aliquots of pokeweed fruit extract. Greatest cumulative hatch (65% and 81.6%) occurred from cysts incubated in $NaVO_3$ enriched with 0.2 and 0.4 ml of pokeweed fruit extract, respectively (Fig. 1).

The cumulative hatch of *M. artiellia* was 80.5% after four weeks of incubation in distilled water (Fig. 2). Similar cumulative hatch values were obtained by adding 0.01-0.2 ml aliquots of pokeweed fruit extracts to 3 ml of water. Greater aliquots (0.4-1 ml) of pokeweed fruit extract suppressed cumulative hatch significantly (17.5-37.7%) compared to the control.

Host status. Root gall and egg mass indices (5) were high on pokeweed and tomato inoculated with *M. incognita* and *M. javanica* (Table I). *Meloidogyne artiellia* failed to infect pokeweed and no egg masses or galls were observed on pokeweed roots. A low gall index (1.3) but a high egg mass index (5) was observed on the control chickpea. This indicates that pokeweed is a good host for *M. incognita* and *M. javanica* and a non-host for *M. artiellia*.

Table I. Response of pokeweed to infection by *Meloidogyne artiellia* (Ma), *M. incognita* (Mi) and *M. javanica* (Mj), compared to that of chickpea inoculated with Ma and tomato inoculated with Mi or Mj.

Plant species	Gall index (0 - 5)			Egg mass index (0 - 5)		
	Ma	Mi	Mj	Ma	Mi	Mj
Pokeweed	0	4.8	5	0	4.8	5
Tomato "Rutgers" (check)	NT[1]	5	5	NT	5	5
Chickpea "Gab 1" (check)	1.3	NT	NT	5	NT	NT

[1]NT = Not tested

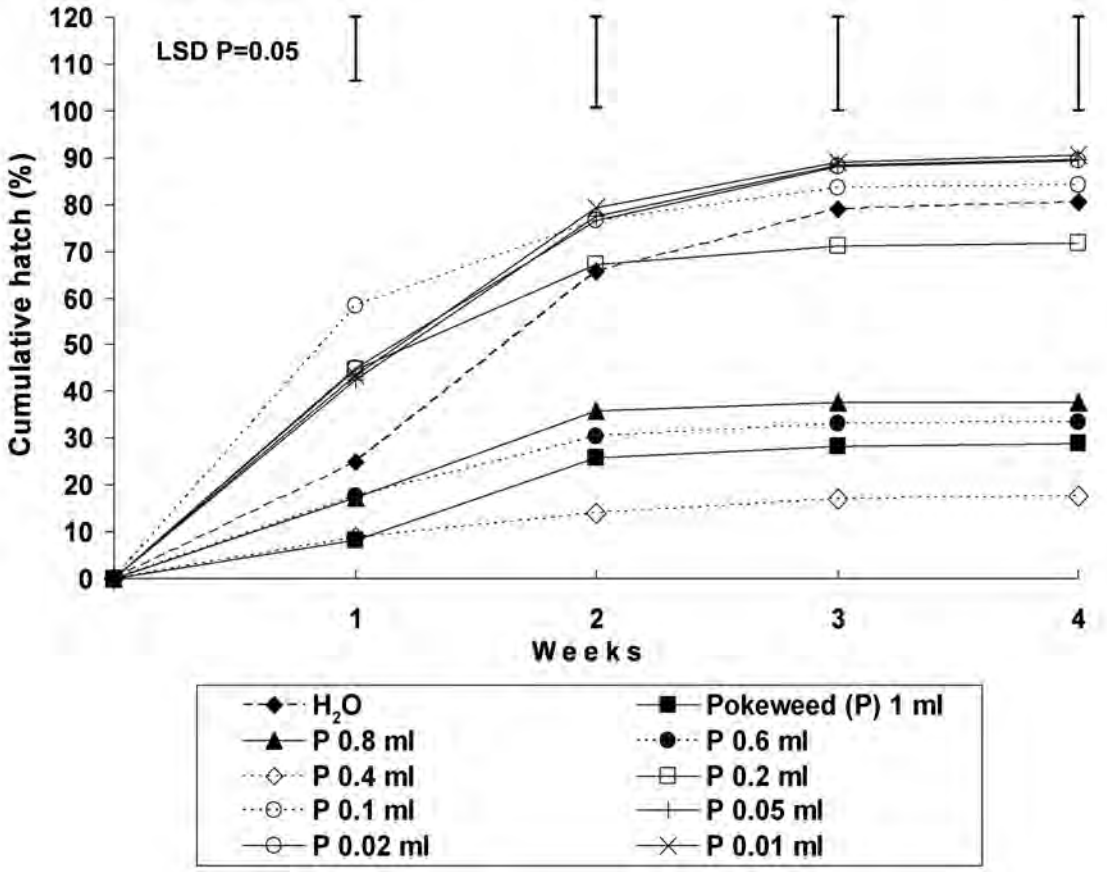

Fig. 2. Effect of increasing concentrations of pokeweed fruit extract on cumulative per cent hatch of eggs within egg masses of *Meloidogyne artiellia* incubated at 20 ± 1 °C for four weeks.

DISCUSSION

Hatch of eggs of *G. rostochiensis* was suppressed at the largest (1 ml) and stimulated at intermediate (0.2-0.8 ml) concentrations of pokeweed fruit extract. The high content of potassium and saponins in pokeweed fruits may be responsible for this effect. Saponins are known to increase cell membrane permeability to macromolecules (Francis *et al*., 2002) and probably also eggshell permeability, which is a key event for hatching to occur (Perry, 2002). However, whether pokeweed fruit extracts increased egg hatch as a consequence of a true stimulating effect or by increasing eggshell permeability, thus enhancing the stimulating effect of $NaVO_3$, cannot be inferred from this study.

No stimulating effect of pokeweed fruit extract was observed on *M. artiellia*. The tendency of *M. artiellia* eggs to hatch freely (80.4%) in water prevented the detection of any potential hatch stimulating effect by pokeweed. In contrast, the modest (20.7%) cumulative *G. rostochiensis* hatch in $NaVO_3$ favoured the detection of the stimulating effect of pokeweed extract concentrations.

The different responses of *G. rostochiensis* and *M. artiellia* eggs that we found to low and high concentrations of pokeweed extracts has been reported for other chemicals such as the oximecarbamate nematicide aldicarb, which increases hatch of *M. javanica* at 0.48 µg/ml, but suppresses it at 4.8 µg/ml (Hough and Thomason, 1975). It is noteworthy that pokeweed concentrations of 0.4-0.8 ml suppressed the emergence of juveniles from egg masses of *M. artiellia* but stimulated that from cysts of *G. rostochiensis*. Different response to the same rate of a nematicide by different nematode species has also been observed. The nematicide aldicarb completely suppressed emergence of *Heterodera schachtii* Schmidt second-stage juveniles at 4.8 µg/ml, but required higher concentrations of 48 µg/ml to suppress emergence of *M. javanica* juveniles (Hough and Thomason, 1975). Moreover, the organophosphate nematicide fenamiphos, at 0.48 µg/ml, suppressed hatch of *M. javanica* but had no effect on that of *H. schachtii* (Greco and Thomason, 1980).

This is the first evidence of the stimulating and suppressing effects of increasing concentrations of pokeweed extracts on nematode eggs. However, the potential nematicidal activity of pokeweed extracts needs further assessment. The fact that pokeweed was easily infected by *M. incognita* and *M. javanica* would indicate that these nematodes are able to detoxify the harmful compounds or that these compounds are not toxic to the tested nematodes.

Cysts of *G. rostochiensis* may undergo diapause (Shepherd and Cox, 1967; Evans, 1982; Hominick *et*

al., 1985) and hatching rates can be modest, as observed in our test (20.7%). The use of 0.4 ml of pokeweed fruit extract combined with a hatching agent would be a useful treatment whenever information on the proportion of hatchable (viable) eggs of the nematode is required.

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