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The role of betaines in alkaline extracts of *Ascophyllum nodosum* in the reduction of *Meloidogyne javanica* and *M. incognita* infestations of tomato plants

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Summary – Soil application to the roots of tomato plants (*Lycopersicon esculentum* cv. Ailsa Craig) of a commercially available alkaline extract of the brown alga, *Ascophyllum nodosum,* resulted in a significant reduction in the number of second stage juveniles of both *Meloidogyne javanica* and *M. incognita* invading the roots, compared to those of plants treated with water alone. Egg recovery from the seaweed extract treated plants was also significantly lower. The three major betaines found in the seaweed extract (γ-aminobutyric acid betaine, δ-aminovaleric acid betaine and glycinebetaine), when applied at concentrations equivalent to those in the extract, also led to significant reductions in both the nematode invasion profile and egg recovery. This led to the conclusion that the betaines present in the seaweed extract play a major role in bringing about the observed effects. Application as a soil drench of the inorganic constituents of the extract also resulted in significant reductions in egg recovery, but these reductions were not so pronounced as those produced by application of the betaines.

Résumé – ***Rôle des bétaïnes provenant de l'extraction alcaline d'*Ascophyllum nodosum *dans la réduction des infestations de pieds de tomates par* Meloidogyne javanica *et* M. incognita.** – L'application sur les racines de plants de tomates d'un extrait alcalin de l'algue brune *Ascophyllum nodosum,* disponible dans le commerce, produit une réduction significative du nombre de juvéniles de deuxième stade de *Meloidogyne javanica* et *M. incognita* pénétrant dans les racines, en comparaison avec des plants de tomates traités uniquement avec de l'eau. Le nombre d'œufs récupérés sur les plants traités par l'extrait d'algue est également significativement plus faible. Lorsque les trois bétaïnes essentielles (bétaïne d'acide-γ-aminobutyrique, bétaïne d'acide δ-aminovalérique, glycine bétaïne) présentes dans l'extrait d'algue sont appliqués – à des concentrations équivalant à celles de l'extrait – on observe également une réduction significative de l'infestation par les nématodes et du nombre d'œufs récupérés. Il peut donc être conclu que les bétaïnes présentes dans l'extrait d'algue jouent un rôle important dans le processus occasionnant les effets observés. Lorsque le sol est humidifié par une application des constituants inorganique de l'extrait, on observe également une réduction significative du nombre d'œufs récupérés, cette réduction étant plus faible que celle produite par application de bétaïnes.

Key-words : *Ascophyllum nodosum,* betaines, biological control, *Meloidogyne,* nematodes, seaweed extract.

There are several reports which show that the application of seaweed extracts to plants resulted in decreased incidence of nematode attack. Tarjan (1977) demonstrated that when an extract of *Ascophyllum nodosum* was applied to citrus seedlings infected with *Radopholus similis,* the seedlings weighed more and had fewer nematodes than the water control seedlings. Morgan and Tarjan (1980) showed that commercial extracts of *A. nodosum,* when applied to the soil, were effective in controlling *Belonolaimus longicaudatus* on centipede grass 1 month after application. Whapham *et al.* (1994) reported that the use of a commercially-available, alkaline extract of *A. nodosum* as a root drench resulted in significantly reduced numbers of *Meloidogyne javanica* eggs recovered after one generation from the roots of tomato plants.

A commercially-available seaweed concentrate prepared from *Ecklonia maxima,* when applied as a soil drench, was found to reduce root-knot nematode (*Meloidogyne* spp.) infestation of tomato plants (Featonby-Smith & van Staden, 1983). The use of the same commercial product also suppressed the reproduction of *Pratylenchus zeae* on excised maize roots (De Waele *et al.,* 1988). The *E. maxima* concentrate, when used as a soil drench, was also demonstrated to produce a significant increase in plant growth and reduced infestation by *Meloidogyne incognita* (Crouch & van Staden, 1993).

Despite the various reports on the effects of seaweed extracts on reducing the levels of nematode attack on plants, the active compounds in the extracts have not been determined, although Featonby-Smith and van Staden (1983) suggested that the effects could be due to cytokinins present in the seaweed extracts. We have considered the possibility of the involvement of betaines. These compounds, when applied at low concentrations, have been shown to increase the resistance of plants to attack by fungi (Tyihák *et al.,* 1988; Kraska & Schönbeck, 1992; Manninger *et al.,* 1992). Betaines are known constituents of seaweed extracts; for example, the commercial products derived from *A. nodosum* contain γ-aminobutyric acid betaine, δ-aminovaleric acid betaine and glycinebetaine (Blunden *et al.,* 1986). In this communication we report the effects on *M. javanica* and *M. incognita* infestations of tomato plants following soil application of an alkaline extract of *A. nodosum* and of the betaines present in the extract.

Materials and methods

Seaweed extract

The seaweed extract used was Maxicrop Original (Maxicrop International Ltd), an aqueous alkaline extract of the brown marine alga, *Ascophyllum nodosum,* that contains approximately 8 % dissolved solids, of which approximately 58 % is organic and 42 % inorganic. The extract has a pH value of 9.0 ± 0.6 and a specific gravity of 1.045 ± 0.015. A typical analysis of the inorganic constituents is given in Table 1, although these values will vary from batch to batch of the product. When assayed for betaines using the method of Blunden *et al.* (1986), the extract was shown to contain γ-aminobutyric acid betaine (51.7 mg/l), δ-aminovaleric acid betaine (14.7 mg/l) and glycinebetaine (3.9 mg/l).

Betaines

Glycinebetaine hydrochloride was purchased from Sigma Chemical Co., γ-aminobutyric acid betaine and δ-aminovaleric acid betaine were synthesized by N-methylation of the corresponding amino acids using methyl iodide under mildly basic conditions as described by Benoiton and Chen (1976).

Nematodes

Meloidogyne javanica and *M. incognita* were obtained from stock cultures maintained on tomato plants (*Lycopersicon esculentum* cv. Ailsa Craig). The method used for the collection of eggs from the entire root ball was that of Whapham *et al.* (1994).

The eggs obtained were hatched to release infective second stage juveniles (J2s) over a period of 7 days at 25 °C in beakers containing aerated water.

Inoculation and treatment

In each experiment, a sample of 80 tomato plants, all at the four-leaf stage and planted in 450 cm^3 (approximately) of John Innes No. 1 compost, was divided into two groups of eight experimental sets containing five plants per set, all arranged randomly. One set of eight was used to determine invasion profile, and the second set of eight for egg recovery. The experimental sets were treated with 30 ml per plant of one of the following: i) water as a control; *ii)* 3.6 % Maxicrop Original; *iii)* γ-aminobutyric acid betaine solution; *iv)* δ-aminovaleric acid betaine solution; *v)* glycinebetaine solution (each betaine being applied in the same quantity as that present in the 3.6 % Maxicrop Original); *vi)* a mixture of γ-aminobutyric acid betaine, δ-aminovaleric acid betaine and glycinebetaine in the concentrations applied in *iii, iv* and *v; vii)* the residue of 3.6 % Maxicrop Original after evaporation to dryness, incineration at 800 °C for 4 h (Maxicrop ash) and resuspension in water; and *viii)* Maxicrop ash mixed with the three test betaines in the concentrations used in *vi.* The application rate of 30 ml per plant, although based on earlier work by Whapham *et al.* (1994), was lower than the 50 ml per plant used by them.

Two plastic pipette tips with additional perforations were pushed into the soil, on either side of the tomato stem, to a depth of 2 cm. The inoculum (750 J2s of

Table 1. *Typical composition (mg/kg) of the inorganic components of the solids obtained after evaporating Maxicrop Original to dryness.*

Nitrogen	(N)	7500
Phosphorus	(P_2O_5)	500
Potassium	(K_2O)	192 800
Aluminium	(Al)	10
Boron	(B)	30
Bromine	(Br)	800
Calcium	(Ca)	3500
Chlorine	(Cl)	25 500
Copper	(Cu)	12
Iodine	(I)	1100
Iron	(Fe)	290
Magnesium	(Mg)	2000
Manganese	(Mn)	6
Molybdenum	(Mo)	2
Rubidium	(Rb)	20
Selenium	(Se)	8
Sodium	(Na)	5500
Strontium	(Sr)	126
Sulphur	(S)	29 000
Tellurium	(Te)	66
Titanium	(Ti)	100
Zinc	(Zn)	56

either *M. javanica* or *M. incognita*) was applied through the pipettes. Plants were inoculated 2 days after treatment and were watered every day. The plants were maintained under glasshouse conditions at 20 °C ± 4 °C.

COUNTING JUVENILES AND EGGS

Fourteen days post inoculation, the roots of each plant were washed free from soil, bleached, stained with acid fuchsin and the numbers of juveniles in the root tissues counted using the method described by Southey (1986).

After one generation, at 49 *(M. javanica)* and 63 *(M. incognita)* days post inoculation, the egg sacs from each plant in each experimental set were removed using 1 % sodium hypochlorite solution and the total numbers of eggs recovered from the sacs were counted under a microscope (Southey, 1986).

Means and standard error of the means are shown in the results section (Fig. 1), using T test comparisons for significance.

Results and discussion

Application of a 3.6 % solution of an alkaline extract of *Ascophyllum nodosum* (Maxicrop Original) as a soil drench to tomato plants resulted in a significant reduction ($P \leqslant 0.05$), compared to water-treated controls, in the number of second stage juveniles of *Meloidogyne incognita* invading the roots (Fig. 1A). Significant reductions ($P \leqslant 0.05$) also resulted when γ-aminobutyric acid betaine, δ-aminovaleric acid betaine and glycinebetaine were used in the same quantities as those applied in Maxicrop Original. A mixture of the three betaines also produced a significant reduction. However, the inorganic constituents of Maxicrop Original, obtained by incineration of the organic components of the extract, did not produce a significant reduction in the number of invading juveniles, although when mixed with the solution of betaines, a significant reduction was obtained (Fig. 1 A). Similar results were recorded when *M. javanica* was the invading nematode (Fig. 1 B), but the effects were not so pronounced as those obtained with *M. incognita*. It is to be expected that the response of different nematode species may vary, but we can offer no explanation for the extent of the variation observed.

The soil drench treatment with Maxicrop Original also led to a significant ($P \leqslant 0.05$) reduction in egg recovery from plants infected with *M. incognita* and *M. javanica* after one generation (49 and 63 days, respectively, post inoculation; Fig. 1 C, D). Similar significant reductions were achieved with the use of γ-aminobutyric acid betaine, δ-aminovaleric acid betaine, glycinebetaine and a mixture of all three betaines, when used in the same quantities as those when Maxicrop Original was used. A significant reduction, although not so pronounced, was achieved by the application of the inorganic constituents of Maxicrop Original (Maxicrop ash).

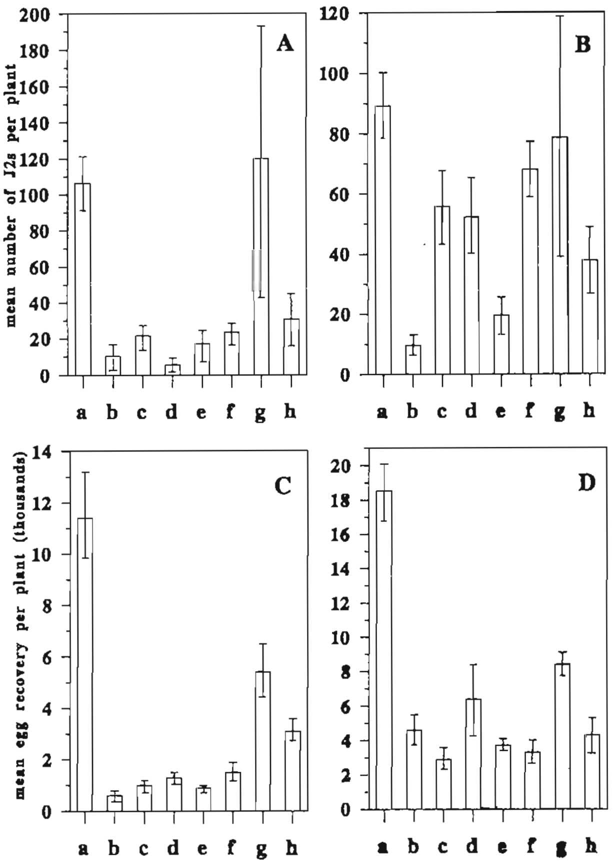

Fig. 1. *A : Number of second-stage juveniles of* Meloidogyne incognita *per tomato plant 14 days post inoculation; B : Number of second-stage juveniles of* Meloidogyne javanica *per tomato plant 14 days post inoculation; C : Number of eggs of* Meloidogyne incognita *per tomato plant 63 days post inoculation; D : Number of eggs of* Meloidogyne javanica *49 days post inoculation. (Mean ± standard error of five replicates). Treatment : a = water control; b = Maxicrop Original; c = γ-aminobutyric acid betaine; d = δ-aminovaleric acid betaine; e = glycinebetaine; f = mixture of the three betaines; g = Maxicrop ash; h = Maxicrop ash plus a mixture of the three betaines.*

The reduction in the numbers of eggs of *M. javanica* recovered from tomato plants treated with Maxicrop Original after one generation has been reported by us previously (Whapham *et al.*, 1994). The studies reported in the present communication show that similar results are produced when both *M. javanica* and *M. incognita* are treated with the seaweed extract. It is also clear that the effects of the seaweed extract on the nematodes is due, at least in part, to the betaines contained in the extract. However, the inorganic components of Maxicrop Original also produced significant reduction in the egg recoveries from both *M. javanica* and *M. incognita,* but the mechanism for this activity is not known.

The data presented indicate clearly the major role played by betaines present in the seaweed extract in reducing both the number of J2s invading the roots of tomato plants and the number of eggs recovered. Further studies to define the role of betaines are in progress.

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