Effects of activated charcoal on hatching and infectivity of *Globodera rostochiensis* in pot tests

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SUMMARY

The effects of various amounts of activated charcoal in charcoal/loam mixtures on the hatch, invasion and cyst production of *Globodera rostochiensis* were investigated in greenhouse pot experiments. The main feature of the results was a delay in hatching of juveniles from cysts in pots containing charcoal. The adverse effect of charcoal on hatching was confirmed in outdoor pot experiments and there was also a significant yield increase from plants in pots where charcoal had either been incorporated in the soil or used as a substrate on which the seed potato was planted. The results are discussed in the context of possible control measures using charcoal to absorb potato root diffusate.

RÉSUMÉ

Effets du charbon activé sur l'éclosion et le pouvoir infestant de Globodera rostochiensis *lors d'essais en pots*

Des expériences en pots, placés dans des serres, ont concerné les effets de quantités variables de charbon activé, dans un mélange de charbon et d'argile, sur l'éclosion, la pénétration et la production de kystes de *Globodera rostochiensis.* Le fait le plus important des résultats a été le retard de la sortie des juvéniles hors des kystes dans les pots contenant du charbon. L'effet négatif du charbon sur l'éclosion a été confirmé lors d'expériences où les pots étaient disposés à l'air libre. Il a également été observé une augmentation significative de la récolte des plants poussant, soit dans des pots où du charbon avait été incorporé au sol, soit sur du charbon utilisé comme substrat. Ces résultats sont discutés dans l'optique d'une possibilité de lutte utilisant le charbon pour absorber les diffusats radiculaires de la pomme de terre.

Measures which influence nematode behaviour may eventually become feasible as part of an integrated control programme and interference with the hatching response of cyst nematodes to host root diffusates is one possible approach (Perry, 1986). The active factors in potato root diffusate (PRD), which stimulate the hatch of *Globodera rostochiensis,* can be adsorbed on activated charcoal (Calam, Raistrick & Todd, 1949). Jones and Gander (1962) added various amounts of charcoal to PRD and found decreasing hatch with increasing amounts of charcoal in *in vitro* hatching tests. We have carried out pot tests to determine whether the addition of activated charcoal to soil influences the hatching and infectivity of *G. rostochiensis.*

Materials and methods

HATCHING TESTS

Two tests were made to determine the potency of PRD leached from pots containing potatoes grown in a mixture of steam sterilised loam and activated charcoal (Sigma Chemical Co. Ltd.). In the first test, chitted potato tuber pieces were grown in 9 cm diameter pots containing activated charcoal at 0, 0.1, 0.2 or 0.4 g kg^{-1} with four pots per treatment; a duplicate set of pots without potatoes acted as controls. In the second test, potatoes were grown in pots containing charcoal at 0, 0.05, 0.4 or 0.8 g kg^{-1}; unplanted pots containing only sterile loam without charcoal were again used as controls.

PRD and soil leachate were obtained (Fenwick, 1949) at intervals after planting the chitted potatoes (Tab. 1). For each treatment or control, PRD or leachate from the four pots was pooled and used after dilution with distilled water 1 in 4 by volume. Cysts of *G. rostochiensis* Ro 1, grown on potato cv. Arran Banner in pots, were taken from a single generation harvested in 1982 (test 1) or 1983 (test 2) and stored dry at room temperature (20°) for six months after extraction from the soil. Cysts were soaked for 1 wk in distilled water and then placed in the test solutions at 20°. Four batches of 25 cysts for each solution were tested for 5 wk; counts of hatched juveniles were made at weekly intervals when fresh solution was added, and total percentage hatch determined at the end of each trial.

Table 1

The percentage hatch of cyst contents of two populations of *G. rostochiensis* after 5 wk in leachates from potato plants of various ages grown in pots containing various amounts of activated charcoal mixed with sterile loam.
Sterile loam leachate (SLL) is from pots without potatoes or charcoal (experiment 1).

	Plant age (wk)	*Activated charcoal (g kg^{-1} of sterile loam)*				
TEST 1 (1982 population)			0	0.1	0.2	0.4
	2		3.3	3.5	5.5	4.5
	4		31.9	25.5	25.0	24.3
	8		52.9	36.2	44.2	49.4
	16		19.7	10.3	10.8	9.8
TEST 2 (1983 population)		SLL	0	0.05	0.4	0.8
	4	4.7	64.7	68.6	60.9	60.8
	8	5.3	63.0	63.7	65.2	64.3
	12	3.9	49.5	51.8	52.4	57.9
	16	4.3	35.1	28.8	35.3	36.2

Greenhouse pot tests

For greenhouse pot tests, potato tuber pieces (approximately 3 cm diameter), cv. Arran Banner, with single sprouts were potted into 9 cm diameter plastic pots containing loam plus charcoal at rates of 0, 0.1, 0.2 or 0.4 g kg^{-1}. A batch of 30 cysts raised in 1982 containing known numbers of juveniles, held in a secure polyester voile bag, was added to each pot. Ten pots were set up for each charcoal /loam mixture arranged in a fully randomised design of five rows of eight pots in a cool greenhouse (minimum temperature 15°). For each treatment, five pots were taken at random after 4 wk, tops and roots of the plants were weighed and a 2 g root sub-sample was stained in 0.1 % methyl blue and macerated to determine the number of juveniles which had invaded. The numbers of unhatched juveniles remaining in the cysts were counted and expressed as a percentage of the inoculum. The remaining five pots were kept for 16 wk and the numbers of new cysts produced were determined by standard methods (Southey, 1986); eggs remaining in the cyst inocula were also determined.

A similar pot test was done with cysts raised in 1983, with rates of charcoal of 0, 0.05, 0.4 or 0.8 g kg^{-1} of sterilised loam. The rest of the procedures and the experimental design were identical to those of the previous experiment.

Outdoor pot tests

Whole tubers, cv. Désirée, were potted into 20 cm diameter plastic pots containing loam plus charcoal at rates of 0 and 3.3 g kg^{-1}. Charcoal was either mixed with the loam, as for greenhouse tests, or it was used as a substrate on which the seed potato was planted. Two batches of 30 cysts from 1983 stock in polyester voile bags were added below tuber level to each pot. Ten pots for each treatment were plunged in sand in a randomised block lay-out in May. For each of the treatments, five pots were removed after 4 wk and plant growth and nematode hatching and invasion were determined as for the greenhouse tests. The remaining pots were kept for 20 wk, before hatch from the inocula, numbers of new cysts and tuber weight were determined. Results were subjected to two-way analysis of variance.

Results

Hatching tests

The percentage hatch from cysts after 5 wk in test leachates is given in Table 1; results were analysed statistically after arcsin transformation of percentages. Leachate from potatoes grown in sterile soil acted as controls for treatment comparisons in both hatching tests. Where leachates were also taken from pots containing loam/charcoal mixtures without potatoes (test 1), the total percentage hatch did not exceed 5 % for any rate of charcoal. Similarly, the hatch in soil leachate from pots without potatoes or charcoal (test 2) did not exceed 6 %.

For all treatments in the hatching test on the 1982 population, diffusate activity increased with increasing age of the plants up to a maximum at 8 wk; activity declines at 16 wk as the plant senesced. Hatches induced by leachates from pots containing 2 wk old potato plants did not exceed 6 %, irrespective of the soil mixture. The hatch induced by leachates from pots containing 4, 8 and 16 wk old plants grown in loam/charcoal mixtures was less than in leachates from controls without charcoal. At each plant age, the reductions in hatch were significant ($p < 0.05$) with the exception of diffusate from 8 wk old plants in 0.4 g kg^{-1} charcoal/loam (Tab. 1; test 1).

The 1983 population behaved differently. In this hatching test, leachates were taken from 4, 8, 12 and 16 wk old plants and there were no significant differences between hatches induced by leachates from potatoes grown in any of the charcoal/loam mixtures compared to leachates from potatoes in sterile loam without charcoal. Maximum hatching activity occurred with leachates from either 4 or 8 wk old plants (Tab. 1).

Greenhouse pot tests

The effects of activated charcoal on invasion and cyst formation are shown in Table 2. In all charcoal/loam

Table 2

The effect of various amounts of activated charcoal mixed with sterile loam on potato plant top and root weights and the harch, invasion and cyst formation of two populations of *G. rostochiensis* (LSD : least significant difference, P < 0.05; df = 16)

	Test 1 (1982 population) Activated charcoal (g kg^{-1} sterile loam)					*Test 2 (1983 population) Activated charcoal (g kg^{-1} sterile loam)*				
	0	*0.1*	*0.2*	*0.4*	*LSD*	*0*	*0.05*	*0.4*	*0.8*	*LSD*
Top wt (g) at 4 wk	23.1	19.7	17.9	22.7	5.1	17.9	13.1	16.3	16.6	8.1
Root wt (g) at 4 wk	4.5	3.5	3.4	4.2	1.1	7.9	4.1	6.5	6.2	3.4
Invasion (JJ2/g) at 4 wk	220.5	122.0	67.0	144.0	64.7	146.5	117.6	158.2	131.3	44.3
Eggs remaining in 30 cysts at 4 wk	3 480	5 265	4 975	4 315	1 166.4	2 015	3 065	3 385	3 388	625.0
(% of inoculum)	(38 %)	(58 %)	(55 %)	(48 %)		(37 %)	(56 %)	(62 %)	(62 %)	
No. cysts per root system at 16 wk	940	676	712	740	342.4	730	804	699	617	271.0
Eggs remaining in 30 cysts at 16 wk	1 625	1 815	1 585	1 920	613.0	635	715	990	715	502.0
(% of inoculum)	(18 %)	(20 %)	(17 %)	(21 %)		(12 %)	(13 %)	(18 %)	(13 %)	

treatments, plant top and root weights were less than in the controls but the differences were significant ($p < 0.05$) in two treatments only (top and root weights in 0.2 g kg^{-1} charcoal and root weight in 0.05 g mg^{-1} charcoal). Evidently, activated charcoal does not enhance plant growth directly or indirectly by inhibiting hatch and/or invasion. The adverse effects could be due to normal variability in plant growth from tuber pieces or because charcoal adversely affects plant growth, perhaps by adsorption of essential plant nutrients from the soil.

The proportions of eggs from the initial cyst inocula remaining unhatched after 4 wk were 38 % (test 1) and 37 % (test 2) in pots without charcoal. More eggs, 48 % (0.4 g kg^{-1}, test 1) to 62 % (0.8 g kg^{-1}, test 2), remained in all charcoal/loam mixtures. In the first test, 0.1 and 0.2 g kg^{-1} charcoal increased the number of unhatched eggs significantly ($p < 0.05$) but the increase in 0.4 g kg^{-1} charcoal was not significant. Results from cysts raised in 1983 showed that treatments with 0.4 and 0.8 g kg^{-1} charcoal both resulted in 62 % unhatched eggs, a highly significant increase ($p < 0.001$).

The results of both tests indicate that the incorporation of charcoal into sterilised loam decreased the number of juveniles emerging from cysts during the first 4 wk, but the effect did not persist, for after 16 wk there were no significant differences from any treatment in the numbers of unhatched juveniles in either population.

The effects of charcoal on hatch after 4 wk were only partially reflected in the numbers of juveniles that invaded the roots. In the first test, all three charcoal/loam mixtures significantly decreased the numbers of juveniles/g root compared with controls (Tab. 2). In the second test, charcoal had no significant effect possibly because fewer juveniles invaded control plants than in the first test.

OUTDOOR POT TESTS

Charcoal, either incorporated into the soil or used as a substrate under the seed potatoes, had no significant effect ($p > 0.05$) on plant growth (Tab. 3). In pots without charcoal 24 % of the initial cyst contents remained unhatched after 4 weeks, whereas significantly ($p < 0.001$) more eggs remained unhatched in either the incorporated (44 %) or substrate (35 %) treatments with charcoal. By contrast with the greenhouse experiment, the effect on hatch persisted, for even after 20 wk the percentage of eggs remaining was significantly greater in the charcoal treatments.

Table 3

The effect of activated charcoal (3.3 g kg^{-1}), either incorporated into sterile loam (I) or used as a substrate under seed potatoes (S), on potato plant top weight, root weight and yield and the hatch, invasion and cyst formation of *G. rostochiensis* in outdoor pot tests. Control pots (C) had no added charcoal. (LSD : least significant difference, $p < 0.05$; df = 12).

	C	*S*	*I*	*LSD*
Top wt (g) at 4 wk	32.0	38.2	41.2	9.6
Root wt (g) at 4 wk	20.3	15.6	22.3	5.0
Invasion (JJ2/g) at 4 wk	96.0	103.5	72.5	64.5
Eggs remaining per 30 cysts at 4 wk	2 473	3 588	4 533	882.7
(% of inoculum)	(24 %)	(35 %)	(44 %)	
Number of new cysts per root system at 20 wk	5 290	4 424	5 009	1 550.0
Eggs remaining per 30 cysts at 20 wk	643	1 065	2 105	265.4
(% of inoculum)	(6 %)	(10 %)	(21 %)	
Yield, as tuber weight (g)	212.1	255.2	272.2	35.1

The addition of charcoal had no significant effect ($p > 0.05$) on the number of juveniles that had invaded the roots by 4 wk or on the numbers of cysts formed after 20 wk. However, there was a significant ($p < 0.01$) increase in tuber weight from plants grown in both charcoal treatments : the increase over controls for substrate and incorporated treatments were 20 % and 28 % respectively.

Discussion

PRD has a bimodal action on the hatching of *G. rostochiensis :* besides altering eggshell permeability (Clarke & Perry, 1985), it stimulates the metabolism of unhatched juveniles (Perry, 1987). In addition, PRD enhances movement and migration of hatched juveniles (Clarke & Hennessy, 1984) and this may be important if a concentration gradient is set up (Wallace, 1958) attracting juveniles to the root area. Thus, the removal of diffusates, by adsorption on activated charcoal, may reduce hatch and disrupt stimuli required for root location. Root dips of various concentrations of actived charcoal inhibited the entry of *Pratylenchus penetrans* and *Heterodera tabacum* into roots of tomato and tobacco seedlings (Miller & McIntyre, 1976). Activated charcoal interferes with normal host seeking by larvae of the wheat bulb fly, *Delia coarctata,* by adsorbing arrestant compounds exuded from the plants (Scott & Greenway, 1984).

In the *in vitro* hatching tests with *G. rostochiensis,* the activity of diffusates from pots containing charcoal/loam mixtures was adversely affected in the first test only. It is probable that, as root diffusate is continually being produced by the root system, the amounts of charcoal used were sufficient to adsorb only the initial production of diffusate. The adsorption capacity of the charcoal may have been reached more rapidly in the second series of pot experiments where mean root weights, and therefore PRD production, were greater for all treatments than in the first test. This would also explain why juvenile invasion after 4 wk was only significantly affected by charcoal/loam mixtures in the first test but not the second. However, the results do not fully support explanations based on the adsorption capacity of charcoal because there are inconsistencies in the effects of increasing the amounts of charcoal used.

The maximum hatch reduction obtained in the first *in vitro* hatching test (17 %, 0.1 g kg^{-1} charcoal) is unlikely to be of major importance in the field because of the pyramidal age structure of cyst nematode populations; the prevention of hatch of a proportion of second-stage juveniles tends merely to remove individuals surplus to the carrying capacity of their hosts and does not lead to a reduction of cysts at the end of a growing season (Jones & Kempton, 1978). This is reflected in the present work, where results from the greenhouse and outdoor pot trials showed that there was no significant difference in the number of cysts formed on plants in any charcoal/loam mixture or controls.

The important feature of the results for both populations in greenhouse pot tests is that activated charcoal reduced the hatch during the first 4 wk but did not have any significant effect by 16 wk; thus, charcoal delays hatch but does not affect the final number of juveniles emerging. In the outdoor pot tests, charcoal caused à marked delay in hatch. Where charcoal was mixed into the loam, over 40 % of the eggs remained unhatched after 4 wk and the effect persisted up to 20 wk when 20 % of the eggs remained unhatched, significantly more than in controls; charcoal as a substrate treatment similarly delayed hatch (Tab. 3). The more marked effect in outdoor pot tests compared to greenhouse tests may be a reflection of differences in plant growth rates and times before senescence. Also, tubers were used for outdoor tests, rather than the small potato pieces used in greenhouse tests, so considerably more charcoal was used. The difference is unlikely to be due to diffusate potency : root diffusates from Désirée, the cv. used in outdoor tests, is known to stimulate less hatch than diffusates from Arran Banner, the cv. used in greenhouse trials (Evans & Franco, 1979), but more eggs remained unhatched after 4 wk in Arran Banner controls in the greenhouse than in Désirée controls outdoors.

Delayed hatching has been correlated with a reduction in infectivity of the juveniles (Robinson, Atkinson & Perry, 1986) but this happens only when the delay occurs after hatch stimulation by PRD. In the present work, delay is likely to be attributable to absence of stimulation. By contrast to hatch reduction, a delay in hatch of *G. rostochiensis* may be useful under field conditions. Hatching and invasion occur early in the season when the plants are very young (Evans, 1969); older plants, with a more extensive root system, are likely to be less affected by nematode invasion. Older roots also seem to be invaded less successfully (Rao & Peachey, 1965).

Therefore, the use of activated charcoal is unlikely to reduce the total number of juveniles hatching and invading, or the number of cysts produced; but it may delay hatch for a crucial period, sufficient to protect the first formed roots and to ensure that plants grow large enough to tolerate invasion. The benefit is apparent in increased yield. In the mining areas of West Germany, the use of coal dust by some potato growers at planting results in increased yield (Dr. R. A. Sikora, pers. comm.). The use of charcoal as a substrate under seed potatoes appears to be almost as effective as incorporating charcoal into the soil; it is certainly likely to be more practical and more cost effective in the field. The possible adverse effects on plant growth caused by soil nutrients being adsorbed onto the charcoal will be of less moment as tubers will provide the main nutrient source for early plant growth.

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