The influence of certain herbicides in pelletted form on the hatch and invasion of *Globodera rostochiensis, G. pallida* and *Heterodera schachtii*

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SUMMARY

The herbicides, chloridazon, metribuzin, tri-allate and vernolate, can be effectively incorporated into and released from sodium alginate pellets. In hatching tests, chloridazon and tri-allate significantly reduced the hatch from cysts of *Globodera rostochiensis* but had no effect on the hatch of this species and *G. pallida* in pot tests. In pot tests, tri-allate increased host invasion by *Heterodera schachtii* but decreased host invasion by *G. rostochiensis.* The possible use of pelletted formulations to overcome problems of soil incorporation of herbicides to affect nematode hatch and invasion is discussed.

RÉSUMÉ

Action de certains herbicides en formulation granulée sur l'éclosion et la pénétration de Globodera rostochiensis, G. pallida *et* Heterodera schachtii

Les herbicides chloridazone, metribuzin, tri-allate et vernolate peuvent être efficacement incorporés à et libérés par des granulés d'alginate de sodium. Les tests d'éclosion démontrent que le chloridazon et le tri-allate diminuent significativement l'éclosion hors des kystes chez *Globodera rostochiensis,* mais celle-ci, tant chez cette espèce que chez *G. pallida,* n'est pas influencée lors d'expérimentations en pots. Lors d'expériences en pots, le tri-allate augmente l'infestation de l'hôte par *Heterodera schachtii;* elle est par contre diminuée dans le cas de *G. rostochiensis.* Il est discuté des possibilités d'utilisation de formulations granulées pour surmonter les problèmes de l'incorporation d'herbicides dans le sol en vue d'influencer l'éclosion et le pouvoir pénétrant des nématodes.

Several reports have been published on the effects of herbicides on nematodes. For example, Dmowska and Kozcowska (1986) found that paraquat and glyphosate had a negligible effect on free living nematodes, whereas Saly and Ragala (1984) found that glyphosate at 12 l ha^{-1} resulted in increased numbers of free living nematodes but at concentrations of 30 l ha^{-1} numbers were reduced substantially. Weischer and Müller (1985) found that of 29 herbicides tested, some promoted nematode multiplication and thus resulted in increased plant damage or affected plant resistance while others, such as dalapon and monolinuron, were found to be toxic to nematodes and reduced damage.

Certain herbicides have been reported to stimulate hatching of *Heterodera schachtii* (Staly & Stanova, 1976). Kraus and Sikora (1981, 1983) found that di-allate significantly increased the hatch of *H. schachtii.* By contrast, *in vitro* treatment of cysts of *Globodera rostochiensis* and *H. schachtii* with four other thiocarbamate herbicides (cycloate, pebulate, vernolate and tri-allate) at medium field application rates prevented hatch in root diffusate, whereas chloridazon and metribuzin had slight or no adverse effects on hatch (Perry & Beane, 1989). The effect of these thiocarbamate herbicides on hatch was reduced markedly on dilution (Perry & Beane, 1989), so any control strategy based on their use would have to ensure that cysts were exposed to the correct concentrations. Herbicides are usually applied to the soil surface and do not penetrate very deeply into the soil profile. By using herbicides in a pelletted form to facilitate incorporation into the soil it was hoped to overcome this problem and enable the herbicides to be released in close proximity to the cysts. This paper describes the effects of pelletted forms of certain herbicides on the *in vitro* hatch of *G. rostochiensis, G. pallida* and *H. schachtii,* and the effects of such pellets on the numbers of nematodes invading host roots in pot experiments.

Materials and methods

Cysts of both *G. rostochiensis* and *G. pallida* were

from single generations cultured in 1985 and 1986 respectively on potatoes, cv. Désirée. *H. schachtii* cysts were from a single generation cultured in 1985 on cabbage, cv. Hispi. Collection and storage of cysts and collection of host root diffusates were described previously (Perry & Beane, 1989).

Before hatching tests, cysts of *G. rostochiensis* and *G. pallida* were soaked in glass distilled water (GDW) for 7 days at 20° C and cysts of *H. schachtii* were pretreated in GDW for 1 day. For each treatment, hatching tests were done on four batches of 25 cysts, each batch held at 20° C in a covered excavated glass block containing approximately 2 cm^3 of solution. Counts of hatched juveniles were made at weekly intervals when fresh GDW or diffusate was added. At the end of each test, cysts were broken open and the number of viable unhatched juveniles were counted in order to determine the percentage hatch. Batches of cysts in GDW, potato root diffusate (PRD) and sugar beet root diffusate (SBRD) were used routinely as controls.

Four herbicides were used, each made up to the manufacturer's recommended medium rate for field application : " Vernam " (Stauffer Chemical Co., now ICI Plant Protection) a.i. vernolate (2.8 kg a.i. ha^{-1}), " Sencorex " (Bayer UK Ltd) a.i. metribuzin (0.6 kg a.i. ha^{-1}), " Pyramin " (BASF UK Ltd) a.i. chloridazon (2.5 l a.i. ha^{-1}) and " Avadex BW " (Monsanto plc) a.i. tri-allate (1.7 kg a.i. ha^{-1}). A 100 cm^3 quantity of each herbicide was mixed with a kaolin-sodium alginate solution and pelletted by dropping into 0.25 M gluconic acid using a 101-1 000 µl size pipette tip (Walker & Connick, 1983; Flavel *et al.*, 1985). For hatching experiments, pellets (pH 6.0-7.0) were used either moist or after drying on glass Petri dishes at 50 % RH for 1 or 7 days or stored for 10 weeks at 20° C. In all hatching tests, moist pellets began to disintegrate after 3 weeks. Determination of the weight of a subsample of counted pellets enabled the weight and the total number of pellets from each 100 ml of herbicide solution to be estimated; this gave an equivalent recommended field rate of 9-10 pellets per 10 cm diameter pot.

All pot tests were done in a cool greenhouse (minimum temperature 15° C) with pots arranged in a fully randomised design. Pots contained steam sterilised loam.

Results for each experiment were subjected to two way analysis of variance after arcsin transformation of percentages where necessary. The level of significance selected for comparisons using the l.s.d. test is the 5 % level; results are reported as significant or non-significant with reference to this level only.

Hatching tests

Experiment 1

Cysts of *G. rostochiensis* and *H. schachtii* were set to hatch in PRD and SBRD respectively containing pellets of two of the herbicides, tri-allate and chloridazon (moist or dried for 7 days or 10 weeks), at the rates of 0, 5 and 10 per glass block. After 5 weeks, the pellets were removed and hatch continued in fresh diffusate alone for a further 5 weeks when the percentage hatch was determined.

Experiment 2

The ingredients used to formulate the pellets may affect hatch, so hatching tests with *G. rostochiensis* and *H. schachtii* were set up using pellets containing GDW in place of the herbicides. Pellets, either moist or dried for 7 days, were used for hatching tests in either PRD or SBRD at rates of 0, 1, 5 and 10 per glass block for 4 weeks and for a further week after removal of the pellets.

Greenhouse pot tests

Experiment 1

To determine whether herbicides affected plant growth and diffusate production, chitted potato tuber pieces (approximately 3 cm diameter), cv. Désirée, were planted into 10 cm diameter pots containing pellets of chloridazon, metribuzin, vernolate and tri-allate at recommended field rate (10 pellets per pot); controls were set up with GDW pellets and no pellets. Five pots per treatment were used. Sugar beet, cv. Monoire, was grown in 14 cm diameter pots, eight seeds per pot, with similar treatments to the potato test except that pellets were used at the rate of fifteen per pot with two pots per treatment.

SBRD was collected 6 weeks after planting and PRD after 8 weeks using 2 × 250 ml and 2 × 125 ml GDW per pot respectively (Fenwick, 1949). Four batches of 25 cysts of *G. rostochiensis, G. pallida* and *H. schachtii* were set to hatch for 4 weeks in the appropriate diffusate from each treatment. Counts of hatched juveniles and total percentage hatch were determined as before.

After diffusate collection, plants were washed out, top and root weights were recorded and root length was measured with a Commair Root Scanner (Commonwealth Aircraft Corp, Ltd) using a 1 g subsample run though twice.

Experiment 2

To determine the effects of chloridazon and tri-allate on hatch and invasion, pellets were incorporated into 10 cm diameter pots of sterilised loam at the rates of 0, 5, 10 and 20 per pot with four pots per treatment. Each pot was inoculated with 30 cysts of *G. rostochiensis* or *H. schachtii* contained in a polyester voile bag. The pots were planted with either chitted potato tuber pieces, cv. Désirée, or two seeds of sugar beet, cv. Monoire. After 4 weeks, plant growth and percentage hatch from the cyst inoculum were determined and a 2 g root subsample was stained in 0.1 % methyl blue and macerated

to determine the numbers of juveniles which had invaded.

Experiment 3

To determine if herbicides could affect hatch from cysts prior to planting, pellets of tri-allate, chloridazon, metribuzin and vernolate were incorporated into loam in 10 cm diameter pots (ten pots per treatment) containing an inoculum of 30 cysts of *G. rostochiensis* and *G. pallida* in polyester voile bags. After 5 weeks chitted potato tuber pieces, cv. Désirée, were planted into pots and grown for 4 weeks when five pots per treatment were used to assess root invasion, plant growth and percentage hatch of the original inoculum. The remaining pots were grown for a further 12 weeks when percentage hatch from the inoculum and numbers of new cysts produced were determined by standard methods (Southey, 1986).

Results

HATCHING TESTS

Experiment 1

Neither chloridazon nor tri-allate had any marked effect on the hatch of *H. schachtii* at any pellet rate or drying period (Tab. 1). The hatch in SBRD control was 71 %, while the hatch from chloridazon treated cysts ranged from 64-84 %. Although the hatch from cysts exposed to tri-allate pellets (range : 76-89 %) was greater than the control, the differences were not significant.

By contrast, the hatch of *G. rostochiensis* was significantly affected by exposure to pellets of both herbicides, except with tri-allate pellets dried for 7 days and used at the one pellet rate (Tab. 1). With all other treatments, tri-allate had a marked effect; tests with New Blue R (Shepherd, 1962) showed that tri-allate killed up to 70 % (with moist pellets), 60 % (dried for 7 days) and 40-50 % (dried for 10 weeks) of cyst contents. With dried pellets, the percentage hatch was significantly reduced with increase in pellet rate. Chloridazon was less effective, giving 40 % kill of cyst contents with moist and 7 days pellets and up to 50 % kill with pellets dried for 10 weeks and used at the ten pellet rate; however, the lower rates of one and five pellets resulted in egg mortality of only 10 and 20 % respectively. There was no increase in hatch of either species after removal of the pellets.

Experiment 2

Substituting GDW for herbicide in pellets did not affect the total hatch of either species, irrespective of treatment rate or drying period for pellets. There is a suggestion from the results (Fig. 1) that the hatch of *G. rostochiensis* is delayed at the highest rate of moist pellet treatment, the bulk of the juveniles hatching in the second week rather than the first week as in controls. With *H. schachtii*, the dried pellets at all rates seem marginally to increase the rate of hatching with more juveniles hatching during the first week than from control cysts.

GREENHOUSE POT TESTS

Experiment 1

Chloridazon was phytotoxic to potatoes causing senescence by 8 weeks; the other herbicides had no effect on growth of potato plants (Tab. 2). There were no significant differences in the hatch from cysts of *G. rostochiensis* or *G. pallida* exposed to diffusates collected from pots with herbicide pellets, GDW pellets or no pellets.

Metribuzin was phytotoxic to sugar beet and no plants survived. Vernolate significantly reduced root weight and length and top weight and diffusate from these plants gave significantly reduced hatch of *H. schachtii*. Diffusates from sugar beet with chloridazon, tri-allate or GDW pellets did not differ from controls in their hatching activity.

Experiment 2

Chloridazon again proved to be phytotoxic to potatoes and, with the exception of root weight at the five pellet

Table 1
Total percentage hatch of *Globodera rostochiensis* and *Heterodera schachtii* after exposure for 5 wk to different rates of chloridazon and tri-allate pellets (means of 4 × 25 cysts).

Treatment rate	*Moist pellets*			*Dried 7 d*			*Dried 10 wk*			
	1	*5*	*10*	*1*	*5*	*10*	*1*	*5*	*10*	
G. rostochiensis										PRD control
Chloridazon	6.7	6.3	5.7	26.8	28.6	5.9	39.2	38.1	10.1	51.2
Tri-allate	22.6	11.0	3.8	51.2	10.9	9.0	38.4	31.0	7.0	
H. schachtii										SBRD control
Chloridazon	69.5	84.1	64.8	76.6	77.9	63.5	82.5	77.0	81.3	71.2
Tri-allate	80.8	83.5	88.9	76.0	81.5	80.6	85.5	83.0	82.2	

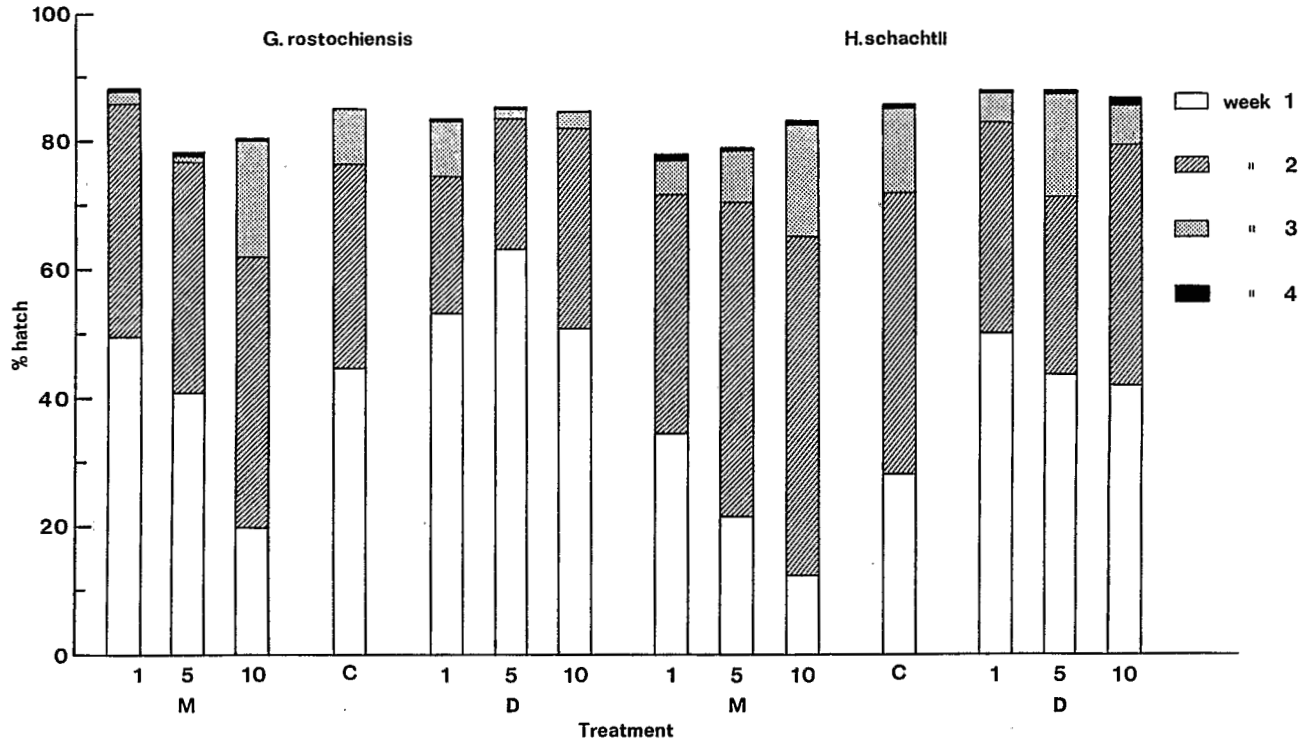

Fig. 1. The cumulative percentage hatch of *Globodera rostochiensis* and *Heterodera schachtii* over 4 weeks exposed to pellets containing GDW at the rate of 1,5 and 10 pellets used as moist (M) or dried for 7 days (D), Potato root diffusate (PRD) and sugar beet root diffusate (SBRD) used as controls (C).

treatment rate, all top and root weights were significantly reduced compared to controls. Tri-allate had no effect on top growth but root weight was significantly increased at all treatment rates (Table 3). The hatch from cysts of *G. rostochiensis* was unaffected by either herbicide, with a mean hatch in treated pots of 88 % and in control pots of 85 %. Root invasion was significantly reduced in pots containing chloridazon pellets. The effect of tri-allate on invasion was unexpected. Used at the 20 pellet rate, tri-allate did not affect root invasion

Table 2

The effects of herbicides on plant growth and on the total percentage hatch from cysts of *Globodera rostochiensis, G. pallida* and *Heterodera schachtii* exposed to root diffusates collected from treated plants (potatoes-mean of 5 pots per treatment grown for 8 wk; sugar beet mean of 2 pots per treatment grown for 6 wk; hatching test means of 4 × 25 cysts).

Treatment	*Potatoes*					*Sugar Beet*			
	Top wt. (g)	*Root wt (g)*	*Root length (m)*	G. rostochiensis *% hatch*	G. pallida *% hatch*	*Top wt. (g)*	*Root wt. (g)*	*Root length (m)*	H. schachtii *% hatch*
Vernolate	28.3	4.0	50.8	78.0	34.3	17.3	2.9	38.5	39.5
Metribuzin	34.4	4.0	54.0	71.6	40.9		no data		
Chloridazon	21.2	2.0	21.6	79.3	31.9	34.4	3.8	54.5	79.3
Tri-allate	29.3	3.5	45.2	71.6	35.4	35.0	3.8	61.7	75.1
GDW pellets	32.5	2.8	37.4	80.3	34.2	34.2	4.4	71.8	63.4
Untreated	31.9	3.0	40.2	80.9	35.9	39.9	5.3	74.4	85.2
L.S.D. ($P < 0.05$)	5.6	0.3	7.4			9.0	1.9	28.9	

Table 3

Effects of chloridazon and tri-allate on the growth of potatoes (cv. Désirée) and sugar beet, (cv. Monoire) at 4 wk and on the invasion by juveniles of *Globodera rostochiensis* and *Heterodera schachtii* (mean 4 reps per treatment).

Treatment rate	*Chloridazon*			*Tri-allate*			*Control*	*LSD* $P < 0.05$
	5	*10*	*20*	*5*	*10*	*20*		
Potatoes								
Top wt. (g)	11.4	9.0	5.6	18.4	18.6	17.6	18.1	3.5
Root wt. (g)	1.8	1.5	1.1	3.1	3.4	3.3	2.3	0.73
G. rostochiensis (g root^{-1})	204.5	233.3	318.6	260.0	457.5	637.5	643.6	167.0
Sugar beet								
Top wt. (g)	3.1	2.7	2.1	3.1	3.5	4.3	3.4	1.0
Root wt. (g)	0.3	0.1	0.1	0.2	0.2	0.4	0.3	0.21
H. schachtii (g root^{-1})	412.5	1 275.0	725.0	1 905.6	1 556.3	959.8	686.3	1 065.0

but significantly fewer juveniles invaded at the ten pellet rate and a further significant reduction in invasion occurred at the five pellet rate.

Table 3 shows that sugar beet seedlings were mainly unaffected by either herbicide, although chloridazon pellets at the highest rate reduced top growth significantly. Chloridazon did not affect root invasion by juveniles of *H. schachtii* and, although tri-allate appears to increase invasion, this was only significantly greater than controls at the five pellet rate. Probably because of the small root mass, the within treatment variation in numbers of juveniles invading was large; thus, results on this aspect serve only to indicate that the two herbicides had no marked effect on the ability of *H. schachtii* juveniles to invade. There was also no significant difference in the hatch from cysts in treated and control pots.

Experiment 3

Using pelletted herbicides as pre-plant treatments had no effect on plant growth or invasion and hatch of *G. rostochiensis* and *G. pallida* after 4 weeks (Table 4). Plants treated with chloridazon showed the phytotoxic effects found previously but with less damage in the pots inoculated with *G. rostochiensis* than in pots inoculated with *G. pallida*, where plant growth was significantly reduced. The marginally reduced hatch and enhanced invasion of *G. rostochiensis* in the metribuzin treatment were not significant. Hatch of *G. pallida* was unaffected by treatment and was far greater (90.3-91.7 %) than that of *G. rostochiensis*.

After 16 weeks, plants exposed to chloridazon treatment had died. The other treatments did not affect the number and weight of tubers or the hatch from the cyst

Table 4

The effects of tri-allate (A), vernolate (B), chloridazon (C) and metribuzin (D) as pre-plant treatments on the hatch, invasion and new cyst production of *Globodera rostochiensis* and *G. pallida* on potatoes (cv. Désirée) 4 and 16 wk after planting (mean 5 pots per treatment). (L SO : $P < 0.05$)

Treatment	G. rostochiensis						G. pallida					
	Control	*A*	*B*	*C*	*D*	*LSD*	*Control*	*A*	*B*	*C*	*D*	*LSD*
4 wk Root wt. (g)	3.1	2.6	3.3	2.4	2.4	0.86	2.5	3.5	2.8	1.7	3.2	0.63
top wt. (g)	8.9	7.6	10.7	9.7	8.6	3.5	9.3	11.2	9.3	6.5	13.2	3.8
JJ[2] (g root^{-1})	336.8	310.0	383.5	373.8	425.7	173.6	675.2	833.8	743.1	902.1	709.5	175.2
% hatch	52.2	52.2	50.9	52.0	41.6	—	90.6	91.7	90.3	91.4	91.4	—
16 wk tuber wt. (g)	14.1	16.9	17.4	—	17.5	8.6	16.0	16.8	14.3	—	12.4	6.6
% hatch	81.4	88.0	83.5	—	83.6	—	92.3	91.9	92.3	—	92.0	—
new cysts	1 007	1 025	1 084	—	1 079	374	1 160	1 012	1 096	—	1 129	323

inoculum or the numbers of new cysts for either species. Hatch of *G. rostochiensis* increased to between 81.4-88.0 %.

Discussion

Results from this work indicate that herbicides can be effectively incorporated into sodium alginate pellets and released on use from moist or dry pellets. None of the herbicides used enhanced hatch. The ingredients used to formulate the pellets had no effect on root diffusate activity and did not affect total hatch and only slightly altered the initial rate of hatch. With *G. rostochiensis*, a delay in hatch may be beneficial as late emerging juveniles have reduced lipid content and impaired infectivity (Robinson, Atkinson & Perry, 1985).

The population of *H. schachtii* used in this work was cultured on cabbage, resulting in cysts having similar hatching characteristics to *G. rostochiensis* with negligible hatch in GDW and a need for root diffusate to stimulate substantial hatch. The role of the eggshell membrane and the absence of fungal contamination as factors in the hatching response of this population have been discussed (Perry & Trett, 1986). The present work indicates that it is unlikely that tri-allate, chloridazon, metribuzin or vernolate will be of use to perturb hatch of *H. schachtii* under field conditions. Although solutions of each herbicide at field application rate with SBRD effectively prevented hatch of *H. schachtii* in *in vitro* hatch tests (Perry & Beane, 1989), the effect could not be reproduced with chloridazon or tri-allate in pelletted form. The effect of solutions of these herbicides was reduced markedly on dilution (Perry & Beane, 1989), so it is likely that the herbicides were not released from pellets sufficiently rapidly to give the required concentration for hatch inhibition. Greater amounts of herbicides would be economically unrealistic for field control.

Hatch from cysts of *H. schachtii* in pot experiments was also unaffected by chloridazon and tri-allate treatments. There were indications that tri-allate increased invasion of *H. schachtii* and invasion at the five pellet rate was significantly greater than controls. Although the caveat concerning the large within treatment variation means the results have to be interpreted with caution, it is interesting to note that Kraus and Sikora (1983) found increased invasion of sugar beet roots by *H. schachtii* following treatment with " Avadex " (a.i. di-allate). In the present work, tri-allate did not affect the growth of sugar beet so any enhanced invasion may be the result of a direct effect on the hatched juveniles or may have affected root physiology. Metribuzin and vernolate pellets were phytotoxic to sugar beet so, although they inhibit hatch when used in solution (Perry & Beane, 1989), their possible use as control agents for *H. schachtii* is obviated.

Pellets containing chloridazon and tri-allate effectively reduced the hatch of *G. rostochiensis* in *in vitro* tests. Tri-allate had the most marked effect on hatch, killing up to 70 % of cyst contents. Moist pellets appeared to be more effective, presumably because the herbicide dissolved into the hatching medium more rapidly than from dry pellets which had to hydrate during the course of the test. These results confirm previous data from experiments with *G. rostochiensis* (Perry & Beane, 1989) where solutions of PRD mixed with tri-allate or chloridazon at field application rates killed up to 90 % and 60 % respectively of cyst contents. However, chloridazon was phytotoxic to potatoes and the inhibitory effects of tri-allate on hatch were not evident in pot tests.

Prior exposure of *G. rostochiensis* cysts to solutions of vernolate and tri-allate irreversibility inhibited subsequent hatch in PRD (Perry & Beane, 1989) but a similar effect was not observed with *G. rostochiensis* or *G. pallida* when pelletted herbicides were used as preplant treatments. This was probably again because the cysts in the soil were exposed to insufficient concentrations of the herbicides. Retaining cysts in bags to enable percentage hatch to be determined may have contributed to this dilution effect, as only a few of the pellets may have been in close proximity to the cysts.

Problems associated with effective soil incorporation of herbicides to ensure that the correct concentration reaches the cysts to inhibit hatch appear not to have been surmounted by using pelletted formulations. Pot tests did demonstrate an effect by tri-allate on invasion of *G. rostochiensis* which was significantly reduced at the 5 and 10 pellet rate but not at the 20 pellet rate. As yet, this effect cannot be explained but it illustrates that even low concentrations of tri-allate in the soil, while not affecting hatch may be important in reducing or delaying invasion of *G. rostochiensis*. It also illustrates the different responses of nematodes to the same herbicide, for tri-allate significantly increased the invasion of *H. schachtii*.

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