In vitro hatching of *Globodera pallida* in response to *Solanum vernei* and *S. tuberosum* × *S. vernei* hybrids

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Summary

The hatching activity of diffusates collected from *Solanum tuberosum*, *S. vernei* and *S. tuberosum* × *S. vernei* hybrids was studied. Diffusates were collected at weekly intervals and assessed by adding them in sequence to batches of cysts of *Globodera pallida* (Pa2) and individually in dilution series. *S. vernei* produced the least active diffusate though it stimulated a substantial hatch. The *S. vernei* hybrids hatched fewer eggs than the *S. tuberosum* cv. Pentland Crown. The use of *in vitro* techniques for assessing the hatching activity of clones is discussed.

Résumé

Influence de Solanum vernei *et d'hybrides* S. tuberosum × S. vernei *sur l'éclosion* in vitro *de* Globodera pallida

Des techniques *in vitro* ont été utilisées pour étudier la stimulation de l'éclosion chez *Globodera pallida* par les exsudats de racines *i*) du cultivar sensible de *Solanum tuberosum* Pentland Crown, *ii*) de deux hybrides résistants *S. vernei* × *S. tuberosum* (8917 b(3) et 12380 abc(2)), et *iii*) de *S. vernei* CPC 4078. Les diffusats de racines ont été collectés chaque semaine pendant une période de dix semaines. Leur activité a été évaluée tout d'abord en les appliquant à la suite les uns des autres, par ordre d'obtention, aux mêmes lots de kystes de *Globodera pallida*. Ensuite, les exsudats collectés chaque semaine ont été évalués séparément après diverses dilutions (1:1, 1:2, 1:4, 1:8, 1:16). Les exsudats les moins stimulants sont ceux de *S. vernei* bien qu'en fin d'expérience on obtienne avec eux une éclosion de 82,7 %. Avec les hybrides de *S. vernei*, l'éclosion a été de 89 et 91 % alors qu'avec le cultivar le plus actif (Pentland Crown) elle était de 95,1 %. L'exsudat le plus actif est produit, par tous les clones, pendant la troisième semaine. La dilution diminue généralement l'activité des exsudats quoique de manière inégale suivant les semaines. L'utilisation des techniques *in vitro* pour étudier l'influence des clones sur l'éclosion est discutée.

Williams (1956, 1958) showed that *Solanum vernei* (Bitt. & Wittm.) differed from *S. tuberosum* in its ability to induce juveniles of the potato cyst nematode *(Globodera pallida)* to hatch, the former producing a less active root diffusate. Forrest and Phillips (in press) examined cysts exposed to growing potato roots and found that partially resistant *S. vernei* × *S. tuberosum* hybrids hatched fewer juveniles than susceptible clones derived from *S. tuberosum*. We have further investigated the *in vitro* hatching activity of hybrids between *S. vernei* and *S. tuberosum* and of *S. vernei* itself.

Materials and methods

Four clones were used in the experiments described below : the susceptible cv. Pentland Crown, two resistant hybrids derived from *S. vernei* (8917 b (3) and 12380 abc (2)) and *S. vernei* CPC 4078 (derived from CPC 2487 and CPC 2488 the original sources of resistance of the hybrid clones). Clones 8917 b (3) and 12380 abc (2) have susceptibility indices of 10 % and < 1 % respectively (Phillips, Forrest & Farrer, 1982).

Diffusate collection

Potato root diffusate was collected from plants grown in 13 cm plastic pots containing sandy loam (1:3) in a glasshouse (Widdowson, 1958*b*). Sprouted tubers of each clone were planted singly in April 1981 and the diffusate collected weekly for ten weeks,

starting one week after planting, by saturating the soil with tap water and then adding a further 50 ml water. This was recycled through the pot three times, filtered to remove soil particles and stored in the dark at 4° (Widdowson, 1958*a*).

Twelve tubers per clone, subdivided into three groups of four pots were laid out in a randomised complete block design. Each block also included four pots containing only soil. The diffusate from each group of pots of any one clone was bulked to give three replicate batches of diffusate per clone.

"In vitro" hatching tests

Diffusates were tested on batches of 100 cysts of *G. pallida* (Pa2) in 20 ml glass vials, with four batches per treatment. Before each test the cysts were soaked in tap water for one week and following the addition of 3 ml of diffusate were incubated at 20° in the dark. The hatched juveniles were counted weekly and at the end of each experiment the numbers of viable eggs remaining were counted (Forrest & Farrer, in press) and the percentage hatch determined. Batches of cysts exposed to water only were included in every test. Percentage hatch data were transformed to Arc sin $\sqrt{x}$ before analysis of variance.

In the *first experiment* in May 1981 batches of cysts were treated in sequence with individual undiluted diffusates collected for the first seven weeks. Each diffusate was applied to the cysts for one week, removed and replaced with the next weeks diffusate. This initial experiment indicated that there were no significant differences between clonal replicates of diffusate and they were bulked for the remaining experiments.

The *second series of experiments* compared the weekly diffusates in a dilution series (1:1, 1:2, 1:4, 1:8, 1:16). These were carried out at monthly intervals between August and December 1981. Batches of cysts were exposed to the same stored diffusates for four weeks, the hatched juveniles being counted and fresh diffusate added weekly.

In the *third experiment* four treatments were used to investigate the effect of a low stimulus to hatch followed by desiccation and a treatment with an active diffusate. Batches of cysts were exposed to either Pentland Crown diffusate (week 2) or *S. vernei* (week 2) or to tap water. The diffusate was replaced weekly for four weeks after which the cysts were dried and stored at 20° for two weeks before being soaked in water for one week. Following this, those cysts which had been exposed to diffusates, together with one of the water controls were all exposed to Pentland Crown diffusate (week 3) replaced weekly for a further four weeks.

Results

The results of the sequential treatment of cysts are shown in Fig. 1. Throughout the experiment a consistently lower hatch was recorded from cysts exposed to diffusate collected from *S. vernei* than from the other treatments, these differences being significant ($p < 0.05$) except in week 3. Clone 8917 b (3) stimulated the most rapid hatch initially but after four weeks the rate of hatch decreased resulting in a final hatch comparable to that produced by Pentland Crown. Diffusates from 12380 abc (2) stimulated a slower hatch than Pentland Crown and 8917 b (3). The total hatch produced by 12380 abc (2) was significantly lower than that produced from Pentland Crown ($p < 0.05$).

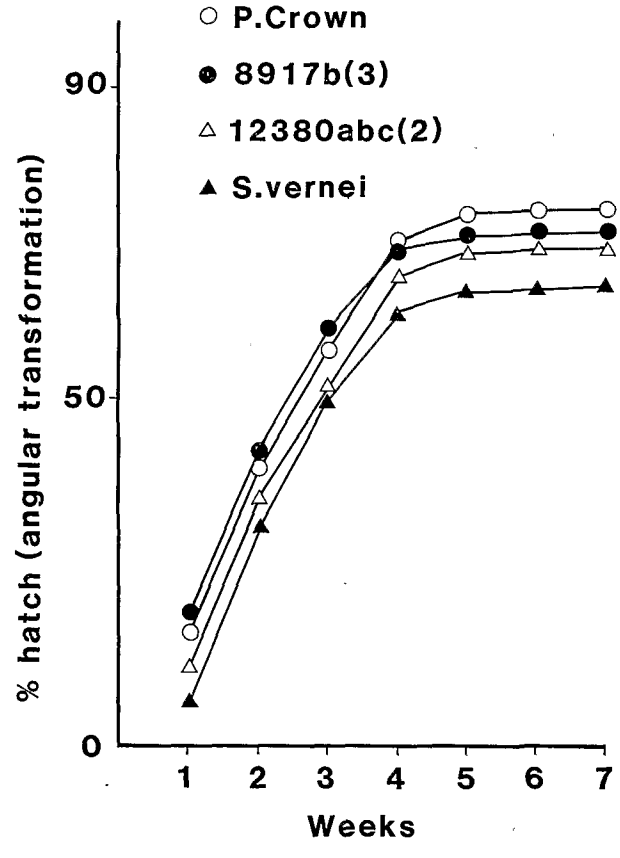

Fig. 1. Cumulative percentage hatch (angular transformation) of juveniles from cysts exposed to a sequence of diffusates collected weekly.

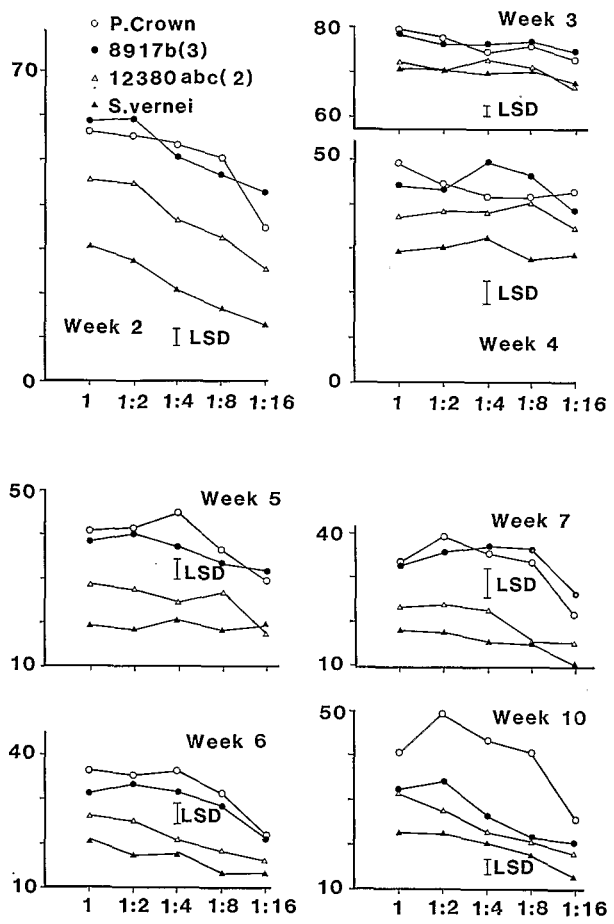

Fig. 2. Percentage hatch (angular transformation) of juveniles from cysts exposed to diffusates collected in individual weeks.

Analyses of variance of the data from examination of diffusates from individual weeks in the second experiment showed significant clone × dilution interactions for weeks 2 and 3 ($p < 0.01$), and for weeks 4 and 10 ($p < 0.001$). Therefore it was not possible to examine the effects of clones independently of dilutions for these diffusates and all the data are presented in Fig. 2. An error occurred in the addition of some diffusates to cysts during the investigation of week 1 diffusate and these data are omitted. It is clear from the data that the activity of diffusates is at a maximum in the first weeks with a peak at three weeks. Thereafter activity decreased and remained steady.

The effect of dilution was generally to reduce the activity of diffusates but this effect was variable, being at its most marked in the second week and lowest when the diffusates were most active (week 3). Diffusates from Pentland Crown appeared most susceptible to dilution, these also showed a trend towards slight stimulation with low dilutions in diffusates collected from week 5 onwards. The diffusates from 8917 b (3) also exhibited this in weeks 4, 6 and 7 but there was no consistent pattern.

Pentland Crown and 8917 b (3) produced the most active diffusates there being little difference between them except in week 10 when the hatch recorded from 8917 b (3) was significantly lower ($p < 0.001$). The least active diffusates were produced by 12380 abc (2) and *S. vernei*. The latter was the least effective and frequently produced a significantly lower hatch than 12380 abc (2) diffusate.

The results of the third experiment are given in Fig. 3. After the initial four week treatment Pentland Crown diffusate and *S. vernei* diffusate had stimulated a 52.9 % and 12.2 % hatch respectively, the hatch from the water controls being less than 1 %. Following the period of drying, rehydration and exposure to Pentland Crown diffusate, the hatch from those cysts initially treated with Pentland Crown diffusate showed a very small hatch not significantly different from that of the water control. The cysts previously exposed to *S. vernei* diffusate gave a final total hatch of 28.1 % while those cysts initially kept in water gave 42.7 % ($p < 0.001$).

Discussion

The results of the first experiment confirm the findings of Forrest and Phillips (in press) in that the hatch induced by 8917 b (3) diffusate is lower than that of Pentland Crown diffusate whilst the more resistant 12380 abc (2) stimulated the least number of juveniles to hatch. The importance of these findings with respect to their contribution to resistance is discussed by Forrest and Phillips (in press). The findings with *S. vernei* confirmed that the wild species produced the least active diffusate (Williams, 1958), though the hatch was nevertheless substantial as shown by Stelter (1959) and Deshmukh and Weischer (1970).

Examination of the activities of the individual diffusates indicated that the most active was produced by all clones in week 3, *S. vernei* hatching 89.1 % as many juveniles as Pentland Crown. In all other weeks examined *S. vernei* diffusate stimulated a 59.9 % hatch or less (Tab. 1) when compared to Pentland Crown. One explanation of the difference

Table 1

Hatch induced by *S. vernei* and *S. vernei* × *S. tuberosum* hybrids as a percentage of Pentland Crown after exposing cysts of *G. pallida* to undiluted diffusate applied singly or in sequence.

Week collected	*2*	*3*	*4*	*5*	*6*	*7*	*10*	*In sequence*
Source of diffusate	*Percentage hatched*							
8917 b (3)	105.0	98.5	90.8	94.1	86.7	97.7	80.2	95.7
12380 abc (2)	80.0	90.9	75.0	70.0	71.8	67.7	78.9	92.9
S. vernei	54.0	89.1	59.9	48.3	56.4	53.0	56.6	85.4

between the results of Williams (1958) and Deshmukh and Weischer (1970) is that plants of different ages were used for collecting diffusate. There is also no indication from Williams (1958) at what time of year his experiments were carried out. Deshmukh and Weischer (1970) reported seasonal differences between May, when they obtained a high hatch from *S. vernei*, and September when hatches were low.

Widdowson (1958*a*) showed that the most active diffusate was produced by young plants within the first four weeks of plant growth and that differences between cultivars were greatest after six weeks. The results presented here are similar (Tab. 1) differences between clones being least at three weeks when the diffusates were most active, and greater thereafter. Widdowson (1958*a*) investigated the periodicity of diffusate production whereas our purpose was to study differences between clones. It is important, in this case, to know at what stage diffusates should be collected to give the best estimate of total hatching activity among clones. Apart from those collected in week 2 the relative activity of all the diffusates produced in individual weeks reflected the performance of clones for hatching activity (Forrest & Phillips, in press) but not their total relative effectiveness. The total hatches induced by the three resistant clones after sequential treatment correspond most closely with the individual diffusates collected in week 3. Had diffusates from any other week been used to predict the total hatching activity, the reduction in hatching would have been over estimated.

Hague (1958) showed that concentrated diffusate could inhibit hatching while the data presented by Fenwick (1957) showed that at high concentrations total hatches were not reduced. In the present experiments the effect of the most active diffusates was not enhanced by dilution. Those occasions when dilution did increase the degree of hatch were relatively few and were mainly confined to Pentland Crown diffusates collected in weeks 5 to 10. Had the experiments been conducted with undiluted diffusates the conclusions drawn would not have been substantially different.

The sequential hatching test showed initial differences in hatch which may reflect differences in the rate of root growth after only one week. After this time the rate of hatch induced by all clones was similar despite differences in their individual activities. From the fourth week the numbers of juveniles hatching reflected more the cumulative effects of the individual diffusates.

Resistance to the potato cyst nematode is usually defined in terms of the reduction in the number of eggs remaining in the soil after a potato crop. It has been suggested by Deshmukh and Weischer (1970) that resistance coupled with active diffusate production may be advantageous for effective control of the nematode. Stimulation of cysts by diffusate followed by desiccation has been shown to produce a decrease in viability of remaining eggs by Forrest and Farrer (in press) and a decrease in hatch compared to untreated cysts when treated again with diffusate was shown by Perry and Hill (1962). The respective authors suggested that these findings are due to an increased susceptibility to desiccation as a result of an alteration to eggshell permeability.

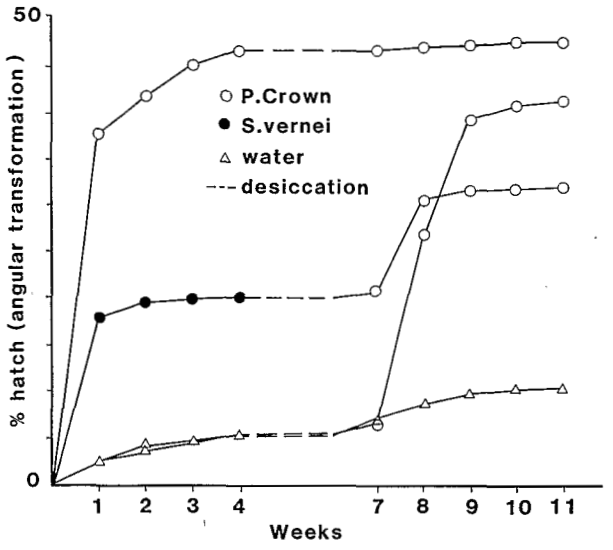

Fig. 3. Percentage cumulative hatch (angular transformation) of juveniles from cysts exposed to various regimes of diffusates from Pentland Crown and *S. vernei*.

The period of drying in this study was imposed in order to simulate a period of environmental stress such as would be encountered between one season and the next. The results of the third experiment indicated that there was a significant reduction in hatch from previously stimulated cysts and these findings complement the studies mentioned above suggesting that stimulated eggs are more susceptible to desiccation than unstimulated. Thus it would appear that a clone that induces a low hatch could effectively lower the initial population to a greater extent than would be expected from examination of its hatching activity alone. The experiment did not take account of the possibility that following a treatment with diffusate eggs were rendered insensitive (e.g. through diapause). While this possibility cannot be ruled out Forrest and Farrer (in press) showed a reduction in viability by staining with new blue R (Shepherd, 1972). In the relatively short time scale of this experiment it is possible that many eggs observed as viable were not so and that they had not had time to degenerate by the time counts were made.

In vitro techniques used here have given results in good agreement with those obtained by observations on cysts exposed to growing roots (Forrest & Phillips, in press), a method that might be thought to be most likely to represent field conditions. The use of *in vitro* methods would allow greater numbers of clones to be assessed for hatching activity as pots could be used repeatedly. If, however, information is required on relative total hatch, diffusates need to be collected on a number of occasions and applied in sequence to cysts.

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