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# Circumventing the diapause of potato cyst nematodes

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**Abstract**

The diapause of potato cyst nematodes was bypassed by avoiding desiccation of the cysts. Larvae were artificially hatched by cutting the cysts in halves and subsequent incubation in potato root diffusate. Approximately 40% of the cyst content hatched. These treatments had no influence on viability and fecundity as ascertained by rearing nematodes in pots and on roots of sprouts grown on water agar in Petri dishes. With the artificial hatching procedure it is possible to produce five to six generations a year in Petri dishes and three to five generations in pots.

*Additional keywords:* potato cyst nematodes, diapause, artifical hatching, controlled single matings.

## Introduction

In the international pathotype scheme of Kort et al. (1977) potato cyst nematode populations are designated virulent or avirulent for a given differential if the multiplication factor ($P_f/P_i$) is $>1$ or $\leq 1$, respectively, implicating that populations of pathotypes are defined as a response to a certain resistance source. In compatible combinations the multiplication factors may range from 1 to approximately 40 indicating that the numbers of virulent individuals vary considerably among populations classified as identical pathotypes. Generally those variations for virulence are not taken into account in studying the biochemical and histopathological aspects of the host-parasite interactions. Moreover, current knowledge on the genetics of the host-parasite interaction is inadequate in obtaining conclusive results.

Genetic research is hampered by the diapause. Preventing the potato cyst nematodes from going into diapause will accelerate genetic studies on the *Globodera rostochiensis* (Woll.) Behrens and *G. pallida* (Stone) Behrens pathotypes. Although a substantial body of literature is available on the hatching process (e.g. Clark and Perry, 1977) no detailed information is published on bypassing the diapause. There are reports which point at this problem. Cunningham (1960), Ellenby and Smith (1967), Guile (1967) and Rode (1971) showed that if there is a diapause it varies considerably in length. Shepherd and Cox (1967) found that fresh brown cysts, if crushed, gave a 39% hatch of the total cyst content. Promising results were also obtained by McKenna and Winslow (1972) who managed to produce six generations a year by subsequent inoculation of pots with soil containing cysts of the former generation.

Preliminary experiments involving temperature shocks were not satisfactory. Storing moist and air-dried cysts during various alternating time intervals at −80°, −20°, +4° and 21 °C did not result in any significant acceleration of the hatching process.

The research described in the present paper investigates the possibilities to accelerate genetic research by shortening the time between subsequent generations.

## Materials and methods

The *G. rostochiensis* populations $Ro_1$-Mierenbos, $Ro_3$-$C_{133}$, $Ro_4$-$F_{515}$ and *G. pallida* populations $Pa_2$-Dutch D and $Pa_3$-$E_{1202}$ were obtained from the Plant Protection Service (Wageningen, the Netherlands). $Ro_5$-Harmerz was from Dr H.J. Rumpenhorst (Münster, Federal Republic of Germany) and $Pa_2$-$HPL_1$ from Ir A. Mulder (Hilbrands Laboratorium, Assen, the Netherlands). The pathotype classification of *G. rostochiensis* ($Ro_1$-$Ro_5$) and *G. pallida* (*Pa*$_1$-$Pa_3$) are according to the scheme of Kort et al. (1977) and the codes refer to the registration in the original collections. The populations were maintained on *Solanum tubersosum* ssp. *tuberosum* L. 'Eigenheimer' susceptible to all pathotypes.

Females were reared on roots of sprouts grown on water agar in Petri dishes (Mugniéry and Person, 1976; Mugniéry, 1982). Two second-stage larvae were inoculated per root tip and incubated at 21 °C. Four to six weeks after inoculations each female was mated with a single male. The males from the seven populations were reared in pots on 'Eigenheimer' grown in sand in a climate chamber at 21 °C. A slow release N-P-K fertilizer (Osmocote®) was added. One month after inoculation the males were recovered from the soil with an Oostenbrink elutriator (Oostenbrink, 1960).

In pot experiments with three *G. rostochiensis* populations young cysts were reared on 'Eigenheimer' as described above for males. Six, 8 and 12 weeks after inoculation the young wet cysts were picked from the rootballs or collected from the soil with a Kort elutriator (Kort, 1960).

Root diffusate was obtained by placing a tuber of *S. tuberosum* ssp. *tuberosum* L. 'Bintje' in the dark, above a beaker filled with tap water. Approximately one month later, when the roots were developed, the solution was filtered, stored at 4 °C and used as hatching agent.

Hatching tests were performed in micro-titer plates (Linbro® ) with one cyst per hole (25 $mm^3$) at 21 °C. The hatching agent was refreshed weekly.

## Results

*Artificial hatching from newly formed fresh cysts reared in Petri dishes.* The percentage of inoculated second-stage larvae of the *G. rostochiensis* and *G. pallida* populations that developed into females ranged from 52-84%. The number of fertilized females obtained after single matings varied from 33-60% of the larvae inoculated. The average content was 193 eggs per cyst. The number of successful matings expressed as a percentage of the total number of females obtained was 59% on the average for the *G. rostochiensis* populations and 68% for the *G. pallida* populations.

From the cysts thus obtained 30 per population were used in an artificial hatching test immediately and the remainders were stored air-dry and tested for hatchability

Table 1. Numbers of nematodes and hatched second-stage larvae ($F_1$ generation) from newly formed cysts obtained 10-12 weeks after controlled inoculations and singular matings (P generation) on roots of potato sprouts grown on water-agar in Petri dishes.

| Pathotype population | P | | | | | $F_1$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | number of inoculated larvae[1] | females developed | | fertilized females | | number of eggs/ cyst | artificial hatch[3] | | | |
| | | | | | | | 2 days | 2 weeks | 1 month | |
| | | No. | (%)[2] | No. | (%)[2] | | No. | No. | No. | (%)[4] |
| $Ro_1$-Mierenbos | 104 | 73 | (70) | 47 | (45) | 156 | 18 | 52 | 75 | (48) |
| $Ro_3$-$C_{133}$ | 102 | 86 | (84) | 43 | (42) | 120 | 12 | 52 | 58 | (48) |
| $Ro_4$-$F_{515}$ | 90 | 63 | (70) | 36 | (40) | 205 | 39 | 66 | 69 | (33) |
| $Ro_5$-Harmerz | 90 | 71 | (79) | 47 | (52) | 151 | 27 | 83 | 97 | (64) |
| $Pa_2$-Dutch D | 120 | 62 | (52) | 46 | (38) | 180 | 21 | 56 | 72 | (40) |
| $Pa_2$-$HPL_1$ | 102 | 80 | (78) | 61 | (60) | 244 | 12 | 41 | 110 | (45) |
| $Pa_3$-$E_{1202}$ | 100 | 60 | (60) | 33 | (33) | 297 | 16 | 119 | 127 | (42) |
| LSD ($P<0.05$) | | | | | | | | | 6.10 | |

[1] Obtained from air-dried 1 year old cysts.
[2] Percentage of inoculated larvae.
[3] Cumulative number of hatched larvae; mean of 30 cysts.
[4] Percentage of average number of eggs per cyst.

after one year. Artificial hatching was performed by cutting cysts in halves with a scalpel and subsequent incubation in root diffusate. Hatched larvae were counted after two days, two weeks and one month (Table 1).

In one month about 46% of the total cyst content hatched. Cysts from the same batches stored air-dry for one year - a standard method - gave an average hatch of 71% when the whole cysts were incubated in root diffusate.

As shown in Table 2 the viability and fecundity of females from the artificially hatched larvae was not significantly affected. The development, the fertilization as well as the egg content of the females were for all populations in the same range as those of the starting generation.

Using the method outlined here we were able to produce four to five generations a year. In two weeks sufficient larvae were obtained by artificial hatching to start a new generation. It took 8 to 10 weeks to obtain fertile females and two additional weeks for the eggs to develop from which the second-stage larvae could be artificially hatched again.

*Artificial hatching from newly formed fresh cysts reared in pots.* Subjected to artificial hatching, on an average 68 active second-stage larvae were obtained per brown cyst of *G. rostochiensis* recovered from the roots 12 weeks after inoculation. This figure corresponds with 40% of the total egg content. For comparison, a number of the cysts was stored air-dry for a year and then placed in hatching agent. The number of active larvae thus obtained averaged 75% of the cyst content (Table 3).

Table 2. Numbers of nematodes and hatched second-stage larvae ($F_2$ generation) from newly formed cysts obtained 10-12 weeks after controlled inoculations and singular matings ($F_1$ generation) on roots of potato sprouts grown on water-agar in Petri dishes.

| Pathotype population | $F_1$ | | | $F_2$ | | |
|---|---|---|---|---|---|---|
| | number of inoculated larvae | females developed | fertilized females | number of eggs/ cyst | artificial hatch[2] | |
| | | No. (=%)[1] | No. (=%)[1] | | No. | (%)[3] |
| $Ro_1$-Mierenbos | 100 | 66 | 40 | 127 | 50 | (39) |
| $Ro_3$-$C_{133}$ | 100 | 67 | 56 | 169 | 96 | (57) |
| $Ro_4$-$F_{515}$ | 100 | 57 | –[4] | – – | – | – – |
| $Ro_5$-Harmerz | 100 | 69 | 45 | 153 | 58 | (38) |
| $Pa_2$-Dutch D | 100 | 57 | –[4] | – – | – | – – |
| $Pa_2$-$HPL_1$ | 100 | 69 | 46 | 201 | 98 | (49) |
| $Pa_3$-$E_{1202}$ | 100 | 51 | –[4] | – – | – | – – |
| LSD ($P<0.05$) | | | | | 7.02 | |

[1] Percentage of inoculated larvae.
[2] Mean of 30 cysts; hatch determined after 1 month.
[3] Percentage of average number of eggs per cyst.
[4] No males for fertilization.

Table 3. Effect of cyst age on artificial and normal hatching of active second-stage larvae from newly formed cysts and 1 year old air-dry stored cysts reared on potato roots grown in pots. Numbers of larvae are averages of 60 cysts after one month of incubation.

| Pathotype population | Age (weeks after inoculation) | Cyst[1] colour | Number of eggs/ cyst | Hatching from new cysts | | | | Normal hatching after 1 year | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | artificial[2] | | normal[3] | | | |
| | | | | No. | (%)[4] | No. | (%)[4] | No. | (%)[4] |
| $Ro_1$-Mierenbos | 6 | W | 168 | 46 | (27) | 6 | ( 4) | 110 | (65) |
| | 12 | B | 167 | 71 | (43) | 3 | ( 2) | 134 | (80) |
| $Ro_3$-$C_{133}$ | 8 | Y | 141 | 17 | (12) | 4 | ( 3) | 103 | (73) |
| | 12 | B | 173 | 76 | (44) | 7 | ( 4) | 105 | (61) |
| $Ro_5$-Harmerz | 6 | W | 145 | 30 | (20) | 7 | ( 5) | 128 | (88) |
| | 12 | B | 175 | 57 | (33) | 3 | ( 2) | 148 | (85) |
| LSD ($P<0.05$) | | | | 5.1 | | 2.5 | | 3.3 | |

[1] W = white, Y = yellow, B = brown.
[2] Halved cysts incubated in root diffusate.
[3] Whole cysts incubated in root diffusate.
[4] Percentage of average number of eggs per cysts.

Evidently, cutting of cysts in halves as well as moisture conditions favour the hatchability. When 12-week-old fresh brown cysts were placed in hatching agent without cutting them the hatchability decreased to about 3% of the cyst content (Table 3). Also when 12-week-old fresh brown cysts were stored air-dry during two weeks, cut in halves and placed in hatching agent the percentage of active larvae obtained dropped to 12% as compared to 40% for artificial hatching.

To investigate possibilities to obtain several generations a year in pot experiments, population $Ro_3$-$C_{133}$ was studied in detail. Crushed young cysts were inoculated two weeks after planting potatoes in pots. Ten to 14 weeks later new cysts were harvested and inoculated again as described above. Using this method with an initial density of 1 cyst per 10 g soil the multiplication factor of the four generations was on an average 16.4 per generation.

## Discussion

The artificial hatching procedure described here enables the propagation of three to five generations a year in pots and five to six generations a year in Petri dishes, which considerably facilitates genetic analysis of potato cyst nematodes. When females are reared in Petri dishes (Mugniéry and Person, 1976; Mugniéry, 1982) controlled matings can be made and rapid analysis of the progeny is possible. Furthermore, this system allows rearing large quantities of a population or inbred line of interest in a very short time. For instance, artificially hatched larvae from population $Ro_3$-$C_{133}$ gave an average multiplication factor of 16.4 in pots, which results, taking five generations a year, in an overall multiplication factor of $1.2 \times 10^6$. Such an approach is desirable in studying the inheritance of virulence of pathotypes of potato cyst nematodes and in producing populations which are homozygous for (a)virulence.

In Petri dishes the number of successful inoculations is high; 70% of the larvae developed into females. This high percentage is in part the result of favourable conditions in Petri dishes. Only two second-stage larvae were inoculated per root tip, which minimizes the competition for food. Our findings support the theory that sex determination is epigenic (Mugniéry and Fayet, 1984). During the course of this study males were hardly observed. However, in genetic studies using controlled matings single larva inoculation per Petri dish is recommended to avoid contamination with developed males.

That the remaining 30% of the second-stage larvae did not reach maturity may be the result of various factors. For instance, we observed that larvae left the inoculation site, started wandering through the Petri dish and starved. Also some larvae failed to develop to maturity after penetration.

From the figures in Table 1 it can be calculated that 63% of the developed females were fertilized. This percentage is high compared with 45% obtained by Green et al. (1970). This disparity may in part be explained by differences in the methods.

Artificial hatching of second-stage larvae reared in Petri dishes and pots did not affect the viability and fecundity. The number of successful inoculations and fertilizations in Petri dish experiments and the multiplication factor in pot experiments did not decline in subsequent generations.

Our results show that the water regime in the cysts and cutting the cyst wall is essen-

tial in bypassing the natural diapause. When cysts were left to desiccate the number of artificially hatchable larvae was strongly reduced. Similarly the hatchability dropped to about 3% when whole wet brown cysts, not being cut, were placed in hatching agent.

It is noted that the storage for a year favours the hatchability. The number of hatched larvae was 75% on an average of the eggs for one year air-dry stored cysts from the pot experiment, whereas this figure was 40% for new moist cysts when artificial hatching was applied. However, the number of larvae obtained by the artificial hatching procedure accomodates the scope of genetic research.


## Samenvatting

*Het omzeilen van de diapauze van aardappelcysteaaltjes*

De diapauze van aardappelcysteaaltjes kan worden omzeild door te voorkómen dat de cysten uitdrogen. Hiertoe worden de cysten opgekweekt op wortels van aardappelspruiten in Petrischalen met wateragar of in potten en zorgvuldig vochtig gehouden.

De larven worden uit de eieren gelokt door de cysten met een scalpel te halveren of zorgvuldig door te drukken zonder de eieren te beschadigen en deze vervolgens te incuberen in lokstof. Op deze wijze wordt ongeveer 40% van de cysteïnhoud gelokt. Deze behandeling heeft geen nadelige invloeden op de vitaliteit van de larven en de vruchtbaarheid van de hieruit ontwikkelde mannetjes en vrouwtjes. Dit geldt zowel voor eieren uit cysten opgekweekt in Petrischalen als die in potten.

Op deze wijze is het mogelijk drie tot vijf generaties per jaar in potten te kweken en vijf tot zes generaties in Petrischalen.


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