Penetration of juveniles and developement of adults of *Heterodera oryzae* on different plants

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SUMMARY

The behaviour of *Heterodera oryzae* on sixteen plants belonging to several families has been studied. Millet, Guinea grass, eggplant and pepper appear exempt of parasitic relationships, no invasion of their roots by juveniles being observed. In cotton, sugar cane and sorghum penetration was low and development practically nil. In *Centrosema pubescens* and *Vigna sinensis* penetration was moderate, only males developed and these species are regarded as potential trap crops. In soybean, *Pueraria phaseoloides*, tomato and rice cv. Guissy, a small to very small proportion of females developed and these plants are regarded as good material for pathotype research. In maize and *Mariscus umbellatus*, many females developed although sex-ratio was greater than one. In only one plant, rice cv. Moroberekan, all juveniles which had penetrated, developed into adults and sex-ratio equalled one.

In maize and *Centrosema pubescens*, more than 50 % of juveniles which penetrated developed into males, indicating that male juveniles had penetrated more readily than females, because sex is genetically controlled in *H. oryzae*.

The meaning of the term "host" is discussed.

RÉSUMÉ

*Pénétration des juvéniles et développement des adultes d'*Heterodera oryzae *dans différentes plantes*

Le comportement d'*Heterodera oryzae* a été étudié en présence de seize plantes appartenant à des familles très diverses. En utilisant une méthode qui consiste à observer la pénétration par coloration des racines et le développement par mise en culture hydroponique des plantes, on a observé, pour chaque espèce, la pénétration, le développement des mâles et celui des femelles. Pour chacun de ces critères, les taux les plus divers ont été observés et tous les degrés existent entre la plante sans aucune relation avec le parasite et le meilleur hôte. Non sans une part d'arbitraire inévitable, on a tenté de classer les plantes en six groupes :

A — Tous les juvéniles ayant pénétré se développent et le rapport mâles/femelles est égal à 1 : riz cv. Moroberekan.

B — Tous les juvéniles ayant pénétré ne se développent pas mais les femelles sont nombreuses : maïs et *Mariscus umbellatus.*

C — Le développement des mâles est assez important, celui des femelles est très faible : soja, *Pueraria phaseoloides,* tomate, riz cv. Guissy.

D — Le développement des mâles est important bien que celui des femelles soit nul : *Centrosema pubescens* et *Vigna sinensis.*

E — La pénétration est faible et le développement pratiquement nul : coton, canne à sucre, Sorgho.

F — Aucune pénétration n'a été constatée : mil, *Panicum maximum,* aubergine, poivron.

Chez deux plantes, maïs et *Centrosema pubescens,* plus de 50 % des juvéniles ayant pénétré se développent en mâles, ce qui indiquerait une pénétration préférentielle des juvéniles à destinée mâle puisque, chez *H. oryzae,* le sexe est déterminé avant la pénétration.

La signification du terme « hôte » est discutée. Pour être qualifiée d'hôte, la plante doit héberger le parasite et le nourrir, de ce fait, on ne peut appeler hôte une plante chez laquelle la seule pénétration a été observée. On ne peut donc considérer comme plante hôte que celle chez laquelle un développement est constaté. Dans ce cas, le développement des seuls mâles est-il suffisant ? Si des femelles sont formées, faut-il exiger, pour qu'une plante soit retenue comme hôte, qu'elles soient assez nombreuses et prolifiques pour que la population du parasite soit au moins maintenue ? Il paraît aux auteurs que, du point de vue de l'agronome et du sélectionneur, cette dernière condition soit indispensable.

Heterodera oryzae Luc & Berdon, 1961, the first species of the genus described from the tropics, is amphimictic (Netscher, 1969). It has large egg masses and large numbers of second-stage juveniles are easily obtained from rice roots. For these reasons, it was selected as the test organism to study resistance-breaking under tropical conditions.

There are some indications that *H. oryzae* can reproduce on plants other than rice : one of the authors (G.M.) observed cysts on the roots of *Pennisetum purpureum* Schumach. and *Mariscus umbellatus* Wahl. in the field and made successful inoculations on maize. The other author (P.C.), attempting to induce artificial mutations, tested various plants as hosts of *Heterodera oryzae* and observed that a small number of females could develop on tomato and soybean. Behaviour of *H. oryzae* was studied on various plants concurrently with rice.

Materials and methods

A method had been developed to observe in the same time both penetration and development of males and females in plants that received the same inoculum. It has been used previously to study sex determination in *H. oryzae* (Cadet, Merny & Reversat, 1975). This method has been improved and adapted to plants other than rice which require larger containers, aeration of the liquid medium and additionnal light (Cadet & Merny, 1977).

Age of plants at time of inoculation depended on the plant species (Table 1). It is based on the root volume at the end of the experiment and the capacity of containers and was determined by preliminary trials. Number of plants per pot depends, also, on the shape and size of root systems. Generally, two plants per pot were inoculated, except for rice, *Mariscus umbellatus* and millet (four plants) and sugar cane (one 4 cm - long cutting).

Forty pots were inoculated each with 300 juveniles of *H. oryzae* hatched during the last three days and, eight days later, the plants were uprooted. Twenty root systems were stained with cotton blue-lactophenol and the number of juveniles which had penetrated was recorded. This was considered as the "true" inoculum, i.e. the number of juveniles actually available for subsequent development of both sexes. The remaining twenty plants were grown in hydroponic conditions (Cadet & Merny, 1977). Males were collected weekly from the bottoms of the containers and counted. After development of males had ceased, root systems were examined and the number of females recorded.

Results

Results obtained on sixteen plant species or cultivars are given in Table 1. Coefficients of variation are very high and inversely correlated to the observed means, due to variability of many factors such as temperature, light and planting material. Classical tests, such as the "t" test or analysis of variance could not be used. A non-parametric test (Mann & Whitney) has been made for each pair of means concerning the three variables : penetration, number of males and number of females ; very low numbers were not included in the test.

Penetration

On the basis of the statistical test, plants can be classified in five categories according to penetration by *H. oryzae:*

— Very high : soybean *(Glycine hispida).*

— High : maize *(Zea mays)*, *Pueraria phaseoloides* and *Mariscus umbellatus.*

— Moderate : rice cv. Moroberekan *(Oryza sativa)*, *Centrosema pubescens*, tomato cv. Roma *(Lycopersicon esculentum)*, cowpea *(Vigna sinensis).*

— Low : cotton *(Gossypium hirsutum)*, rice cv. Guissy *(Oryza sativa)*, sugar cane *(Saccharum officinarum)*, Sorghum cv. 51-69 *(Sorghum vulgare).*

— Nil : millet cv. Souna IV *(Pennisetum typhoides)*, Guinea grass *(Panicum maximum)*, eggplant *(Solanum melongena)*, sweet pepper *(Capsicum frutescens).*

Table 1

Penetration and development into adults of second-stage juveniles of *Heterodera oryzae* on different plants. Penetration and development of males and females are expressed in numbers of juveniles observed in the roots, numbers of males collected in hydroponic cultures and numbers of females collected on the roots. Figures bearing the same letters are not significantly different at the probability level of 0.01 (Mann & Whitney test). Figures between brackets represent the coefficients of variation (as percentages). No statistical test could be made with small figures.

Plant	*Age of plant at inoculation (in days)*	*Penetration (number) (inoculum : 300)*	*Males (number)*	*Females (number)*	*Sex ratio*	*Class (see text p 254)*
Soybean *(Glycine hispida)*	10	$134^{a}{}_{(19)}$	$40^{bc}{}_{(84)}$	0.4	100	C
Maize *(Zea mays)*	5	$98^{b}{}_{(34)}$	$67^{a}{}_{(35)}$	$22^{ab}{}_{(62)}$	3.1	B
Pueraria phaseoloides	28	$97^{b}{}_{(29)}$	$35^{b}{}_{(53)}$	$6_{(72)}$	5.8	C
Mariscus umbellatus	10	$94^{b}{}_{(38)}$	$25^{bc}{}_{(63)}$	$17^{a}{}_{(96)}$	1.5	B
Rice cv. Moroberekan *(Oryza sativa)*	10	$49^{c}{}_{(28)}$	$30^{bc}{}_{(39)}$	$30^{b}{}_{(44)}$	1	A
Centrosema pubescens	28	$33^{cd}{}_{(58)}$	$22^{c}{}_{(62)}$	0		D
Tomato cv. Roma *(Lycopersicon esculentum)*	20	$32^{d}{}_{(58)}$	$4_{(106)}$	0.25	16	C
Cowpea *(Vigna sinensis)*	14	$24^{d}{}_{(88)}$	$9_{(59)}$	0		D
Cotton *(Gossypium hirsutum)*	14	$8^{e}{}_{(153)}$	0.2	0		E
Rice cv. Guissy *(Oryza sativa)*	10	$6.5^{e}{}_{(81)}$	2.7	0.6	4.5	C
Sugar cane *(Saccharum officinarum)*	30	$5^{e}{}_{(161)}$	0.5	0		E
Sorghum vulgare	14	$2.7^{e}{}_{(104)}$	0.05	0		E
Pearl millet cv. Souna IV *(Pennisetum typhoides)*	14	0.25	0	0		F
Guinea grass *(Panicum maximum)*	35	0	0	0		F
Eggplant *(Solanum melongena)*	35	0	0	0		F
Sweet pepper *(Capsicum frutescens)*	35	0	0	0		F

Development

The proportion of juveniles that had penetrated which reached the adult stage was extremely variable, reaching 100% only in rice cv. Moroberekan.

Development of males

With the exception of millet, in which penetration was very low and of plants which were not penetrated, some males always developed and the extent of male development was roughly correlated with that of penetration.

Male development was the highest in maize in which the number of males was about two-thirds of the individuals having penetrated. The same phenomenon was observed in *Centrosema pubescens* although penetration was moderate. In rice cv. Moroberekan it was also greater than half of the number that penetrated but penetration was underestimated since total number of adults was greater than the number of juveniles that penetrated. In both rice cultivars, the proportion of juveniles that penetrated which develop into males was approximately 50%. In all other plants, male development, when observed, was always less than half of penetration. In sugar cane, cotton and sorghum, it can be considered as nil.

Development of females

Development of females was observed in seven plants, but only in rice cv. Moroberekan was it equal to half of penetration. In the six other plants, it was lower than male development, resulting in a sex ratio greater than 1.

In maize and *Mariscus umbellatus* the proportion of juveniles that developed into females, although less than 50%, was relatively high.

In soybean, tomato, *Pueraria phaseoloides* and rice cv. Guissy, the proportion of females was low to extremely low.

Discussion

According to their parasitic relations with *H. oryzae*, the plants studied can be classified into six classes, considering both penetration of juveniles and development of adults (Table 1 "class").

Class A contains only the best host (rice cv. Moroberekan) in which all juveniles wich had penetrated develop into adults with a sex-ratio of 1.

Class B includes plants in which, although not all juveniles wich penetrated developed and sex-ratio was greater than 1, females were numerous and the *H. oryzae* population can increase or, at least, be maintained.

Class C contains plants in which female development was low, male development being more or less greater but always less than 50% of true inoculum. In these plants, the *H. oryzae* population cannot be maintained.

Class D : a moderate penetration occured but was not followed by female development although male development was large, even exceeding 50%, as in *Centrosema pubescens.*

Class E : plants are resistant, with practically no development taking place after initially low penetration.

Class F : no or extremely low penetration was observed in plants belonging to this class which can be regarded as exempt of parasitic relationships under these experimental conditions.

We agree with Shepherd (1959) who, after having inoculated several plant species, including some non-hosts, with *Heterodera schachtii*, *H. goettingiana* and *Globodera rostochiensis*, observed great differences in penetration and development and concluded that "... between the extremes of complete immunity and complete susceptibility, the whole range of host efficiencies exists".

If population dynamics of the parasite are emphasized, plants belonging to classes F, E and D are resistant because, in the absence of females, no subsequent generations can develop. Moreover, plants of class D can be regarded as "trap plants" because they are invaded by soil-inhabiting juveniles which do not develop after penetration. Plants of class C are not entirely resistant since a small number of females reach the adult stage. Since the population of the second generation will be much smaller than the first, it is logical to assume that the population will decrease to a very low level and, perhaps, disappear. However if, as Triantaphyllou (1975) postulated for *H. glycines*, wild populations of *H. oryzae* are mixtures of genotypes, the selection of resistance-breaking strains is possible. These could reach a high population level after several generations and plants of this class present good material for pathotype research. Plants of class A and B are regarded as susceptible but differences are observed between them : the proportion of juveniles that penetrated which develop and the proportion developing into males are variable.

On the subject of resistance to *Globodera rotochiensis*, Jones (1954) proposed classifying plants into what he calls "three arbitrary categories" :

(1) Absolutely resistant. Not invaded.
(2) Partially resistant, invaded but juveniles fail to develop or develop into very few females.
(3) Susceptible, large number of females.

Shepherd (1959), modified this classification introducing a distinction, within partially resistant plants with a slight development, between those in which only males develop and those in which both sexes develop but in low numbers.

Although we partially agree with this classification, it appears imperfect regarding "not invaded" (=absolutely resistant) and "invaded" (=partially resistant or susceptible). From Table 1, it is seen that a penetration of 0.25 juveniles was observed in millet representing penetration of one juvenile of a total of 1,200.

Yet, if additional specimens of a "not invaded" plant (pepper, for example) are examined, perhaps an occasional juvenile will be found. Thus, using this system, millet is "partially" resistant whereas pepper is "absolutely" resistant. We consider such a distinction as meaningless since it may depend upon two different phenomena : 1) accuracy of the observation ; and 2) erratic behaviour of a single juvenile.

In "susceptible" plants, sex-ratio is seldom considered, although this is obviously of great importance in the relationship between a parasite and a plant and to the population dynamics of this relationship.

As such a classification is difficult to establish that will include all particular cases, the question is raised as to what is a host plant and what is not ?

Nusbaum and Barker (1971) state that "If the nematode feeds or attempts to feed upon a plant and in the process cells or tissues of the plant are altered, the plant may be regarded as a host regardless of whether the parasite is able to establish a successful relationship". In this case, a plant in which some juveniles penetrate should be regarded as a host provided "cells or tissues are altered". But the nematode is not a parasite of the plant if it does not feed upon it and in this case, it is difficult to consider the plant as a host. We agree with Caveness (1964) who defines a host as an "organism which is invaded or parasitized by a disease-producing agent and from which the parasite obtains its sustenance". Hence plants of class E in which practically no development occurs following a low penetration cannot be regarded as hosts. According to Caveness's definition a plant should be considered a host if the parasite can utilize it as a food source and subsequent development occurs. But if only males develop (class D), the plant cannot be regarded as a host because biological relations between plant and parasite are not balanced. *Thus a plant is a host only if females develop and some reproduction occurs* (class C). Finally from the view point of agronomists and plant breeders a plant is a host only if its presence can maintain, or increase, the population of parasites (classes A and B).

Between those plants in which a very limited penetration occured and those in which, after a high degree of penetration, development was total including the highest possible proportion of prolific females, differences are a question of degree and no sharp demarcations exist.

Sex is genetically controlled in *H. oryzae* (Cadet, Merny & Reversat, 1975). In the current study, it was observed that in maize and *Centrosema pubescens*, approximately two-thirds of the juveniles that had penetrated developed into males. It is suggested that this is the result of a previously undescribed phenomenon : preferential penetrations of male juveniles.

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References

Cadet, P. & Merny, G. (1977). Méthode d'étude de la pénétration et du développement des deux sexes chez les *Heterodera*. *Cah. ORSTOM, Sér. Biol.*, 11 (1976) : 151-155.

Cadet, P., Merny, G. & Reversat, G. (1975). Facteurs affectant le déterminisme du sexe chez *Heterodera oryzae* (Nematoda : Tylenchoidea). *Cah. ORSTOM, Sér. Biol.*, 10 : 207-214.

Caveness, F. E. (1964). *A glossary of nematological terms*. Ibadan, Nigeria, IITA, 68 p.

Jones, F. G. W. (1954). First steps in breeding for resistance to potato-root eelworm. *Ann. appl. Biol.*, 41 : 348-353.

Netscher, C. (1969). L'ovogenèse et la reproduction chez *Heterodera oryzae* et *H. sacchari* (Nematoda : Heteroderidae). *Nematologica*, 15 : 10-14.

Nusbaum, C. J. & Barker, K. R. (1971). Population dynamics In : Zukerman, B. M. & Rohde, R. A. (Eds) *Plant parasitic nematodes*, New York and London, Academic Press, Vol. 1 : 303-323.

Shepherd, A. M. (1959). The invasion and development of some species of *Heterodera* in plants of different host status. *Nematologica*, 4 : 253-267.

Triantaphyllou, A. C. (1975). Genetic structure of races of *Heterodera glycines* and inheritance of ability to reproduce on resistant soybeans. *J. Nematol.*, 7 : 356-364.

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