Influence of some factors on sex-ratio in *Heterodera oryzae* and *H. sacchari* (Nematoda : Heteroderidae)

Patrice CADET and Georges MERNY

Laboratoire de Nématologie, ORSTOM, B.P. V51, Abidjan, Côte d'Ivoire
and Laboratoire de Biologie des Sols, ORSTOM, 70-74 route d'Aulnay, 93140 Bondy, France

SUMMARY

Influence of several factors on penetration and development of *Heterodera oryzae* and *H. sacchari* has been studied.

Increasing the inoculum of *H. sacchari* from 100 to 12,800 juveniles resulted in an increase in the proportion of juveniles developing into males whereas the proportion of females decreased and the proportion of juveniles reaching the adult stage remained 100%. This indicated that in *H. sacchari* sex determination is epigenetic.

The reaction of *H. sacchari* to age of rice plants was different than that of *H. oryzae*, penetration and development being very low in one-week-old plants.

Time of contact between rice plant and juveniles of *H. oryzae* had a direct influence on the proportion of males and females developed : male juveniles enter the roots more rapidly than female ones, the normal balance of both sexes being reached only after eight days.

The reaction of both species to periods of darkness applied to the plant before inoculation, during contact with the inoculum and during the development of parasites was nearly the same : penetration was reduced by darkness before and during contact between parasite and plant while the development of both sexes was strongly affected by darkness after penetration.

These results suggest that :

(*1*) An important part can be played by attractive or repulsive substances present in root exudates ; and

(*2*) Reduced development is not always caused by shortage of food but can be due to the presence, in the roots, of inhibiting substances or the absence of stimulating substances.

RÉSUMÉ

Influence de certains facteurs sur l'indice andrique chez Heterodera oryzae *et* H. sacchari *(Nematoda : Heteroderidae)*

Il avait été constaté (Cadet, Merny & Reversat, 1975) que chez l'espèce amphimictique *Heterodera oryzae*, l'indice andrique, égal à 1 dans les conditions normales pouvait être modifié par les variations de certains facteurs tels que le niveau de l'inoculum ou l'âge de la plante hôte. L'influence de ces deux facteurs a été étudiée chez *H. sacchari*, autre espèce africaine parasite du riz, mais parthénogénétique.

Des inoculums variant de 100 à 12.800 juvéniles d'*H. sacchari* ont été appliqués à des plants de riz âgés de dix jours. A tous les niveaux d'inoculum, pratiquement tous les juvéniles ayant pénétré ont atteint le stade adulte. La proportion de juvéniles se développant en mâles varie dans le même sens que l'inoculum alors que celle des juvéniles se développant en femelles varie en sens inverse. Ceci indique que, contrairement à ce qui a été constaté chez *H. oryzae*, la détermination du sexe est tardive et épigénétique, un environnement défavorable amenant à se développer en mâles des juvéniles qui, dans des conditions normales, se seraient développés en femelles.

Concernant la pénétration, *H. sacchari* montre une sensibilité à l'âge de la plante supérieure à celle d'*H. oryzae*. Chez cette dernière espèce, la pénétration présente un minimum très accentué chez les plantes âgées de deux semaines. Chez *H. sacchari* le minimum apparaît dès la première semaine. En outre, alors que chez *H. oryzae* le développement est pratiquement maximum quel que soit l'âge de la plante, chez *H. sacchari* il est très fortement réduit dans les plantes âgées d'une semaine. Le fait que cette réduction affecte à la fois les deux sexes montre qu'il ne s'agit pas seulement d'un manque de nourriture.

De plus, l'influence du temps de contact entre la plante et l'inoculum a été étudiée chez *H. oryzae* et celle de l'influence de l'obscurité chez les deux espèces.

Si les juvéniles d'*H. oryzae* sont laissés en contact avec les racines du riz deux, quatre ou huit jours on constate que, tous les juvéniles qui ont pénétré atteignant le stade adulte, la proportion de mâles est d'autant plus importante que le temps de contact a été court, l'indice andrique normal de 1 n'étant atteint qu'après un contact de huit jours. Comme chez cette espèce le sexe est prédéterminé, il ne peut s'agir que de la pénétration plus rapide des juvéniles à destinée mâle.

Les plants de riz ont été soumis à diverses périodes d'obscurité totale : avant l'inoculation, pendant le temps de contact entre le parasite et la plante et pendant le développement du nématode. Chez les deux espèces, la pénétration a été plus ou moins réduite par l'obscurité avant l'inoculation et pendant le contact alors que c'est la proportion de juvéniles se développant en adultes qui était très sensiblement réduite par l'obscurité pendant le développement du nématode. Le fait que le développement des individus des deux sexes est affecté montre qu'il ne s'agit pas seulement d'une réduction de la nourriture disponible.

Ces résultats indiquent que, si certaines différences observées entre les deux espèces sont dues au fait que l'une est amphimictique et l'autre parthénogénétique, comme par exemple l'influence du niveau d'inoculum, il n'en est pas toujours ainsi, notamment en ce qui concerne l'influence de l'âge de la plante et celle de l'obscurité.

Ils indiquent également que :

1) Un rôle important peut être joué par des substances répulsives ou attractives éventuellement présentes dans les exsudats radiculaires (pénétration plus rapide des juvéniles mâles ou défaut de pénétration chez les plantes d'un certain âge).

2) La réduction du développement n'est pas toujours causée par le manque de nourriture mais peut être due à la présence dans les racines de substances inhibitrices ou à l'absence de substances stimulatrices (influence de l'obscurité).

Cadet, Merny and Reversat (1975) have observed variations in sex ratio of *Heterodera oryzae* Luc & Berdon, 1961, associated with external factors such as overcrowding and age of the plants. The influence of the same factors has been studied on another rice parasite, *H. sacchari* Luc & Merny, 1963, as well as the influence of two additional factors : time of contact between plant and parasite and effect of artificial periods of darkness to which the plant was subjected at different stages of its relations with the parasite.

Materials and methods

Second-stage juveniles of *H. oryzae* were obtained from egg masses, collected from rice roots, kept three weeks in Dropkin's solution (NaCl, 17,5 g/l) and put in tap water for not more than two days. In *H. sacchari* they were collected from cysts placed in rice root exudates obtained as described by Merny (1972*a*). They were inoculated to rice *(Oryza sativa* L.*)* cv. Moroberekan in 750 cm³-pots, each containing four seedlings in sterilised soil. Except in the experiment concerning plant age, rice plants were inoculated ten days after the seeds had been put in wet conditions for germination. Except in the experiment concerning time of contact between plant and parasite, juveniles remained in contact ten days before plants were uprooted.

Rates of penetration and development into adult males and females were observed using a device described by Cadet and Merny (1977). Each treatment was applied to 40 pots, each containing four rice plants. After uprooting, they were randomly divided into two lots of twenty. In one lot, the roots were stained to observe penetration and the plants of the other were put in hydroponic conditions to observe male and female development. As only juveniles which have penetrated will actually play a part in development, numbers of animals observed in the roots will be referred to as "true inoculum". Due to normal variations in populations of parasites and plants, penetrations in both lots could differ. Moreover, observations of development were generally more accurate than observations of penetration. For these reasons, rate of development, in some cases, was higher than the observed rate of penetration.

Inoculum level

Six different inoculum levels consisting of 100, 400, 1,600, 3,200, 6,400 or 12,800 *H.*

sacchari second-stage juveniles, were applied to pots containing ten-day-old rice plants which were processed as described above.

Age of plants

Rice plants, one-, two-, three- or four- week-old were uniformly inoculated with 300 *H. sacchari* second-stage juveniles and the inoculum was left eight days in contact with root systems. "True inoculum" as well as numbers of males and females reaching the adult stage were recorded as previously described.

Time of contact

In order to balance the rates of penetration, inoculum levels were inversely correlated with times of contact. Inoculum consisting of 2,100, 1,500 or 300 *H. oryzae* second-stage juveniles were left in contact with the plants two, four and eight days, respectively. Penetration and adult development were evaluated as described above.

Variable periods of darkness

Rice plants were inoculated with 400 second-stage juveniles of *H. oryzae* (first series) or 500 second-stage juveniles of *H. sacchari* (second series). Each series was divided into four lots of 40 pots. One lot (A) was put in darkness from four days before inoculation until one day after ; the second lot (B) was put in darkness from the day of inoculation until two days after being put into hydroponics ; the third lot (C) was kept in the dark from one day before being put into hydroponics until the end of the experiment. The fourth lot, the control, was kept in normal alternating light and darkness, about twelve hours each in equatorial conditions. At the end of the ten-days contact period each of these four lots was subdivided and half of the plants were stained as previously described, to determine penetration and the other half were placed in hydroponics to study development.

Results and discussion

Inoculum density and sex determination in H. sacchari

The observed penetration figures in relation to inoculum density fit the formula based on Nicholson's competition curve (Merny, 1972*b*) :

$$y = 608 \left[1 - \left(1 - \frac{0.23}{608} \right)^n \right]$$

where y is the number of juveniles that penetrated (true inoculum), n=the number of inoculated juveniles, 608=the maximum number of juveniles which can penetrate and 0.23=the probability of a single juvenile entering a root (Fig. 1).

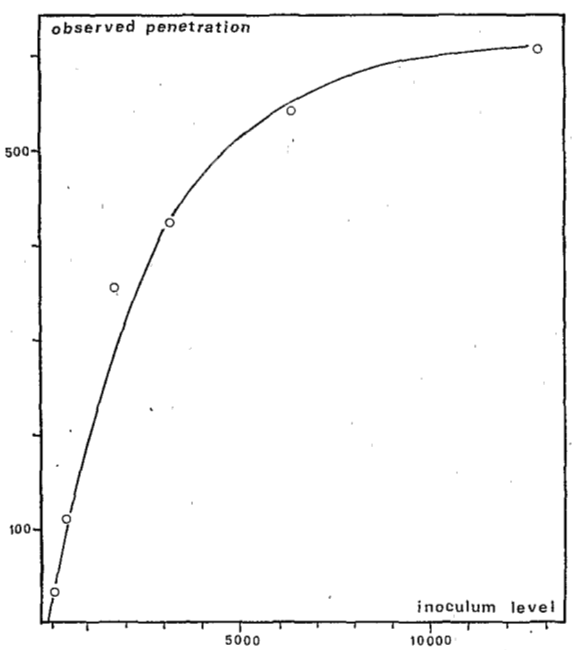

Fig. 1. Number of penetrated juveniles of *H. sacchari* in rice roots at different inoculum levels. The curve fits the equation $y = 608 \left[1 - \left(1 - \frac{0.23}{608} \right)^n \right]$. Circles = experimental observations.

Taking into accrount that rate of development was determined more accurately than rate of penetration and that both were observed on different plants, practically all juveniles that penetrated reached the adult stage (Fig. 2A).

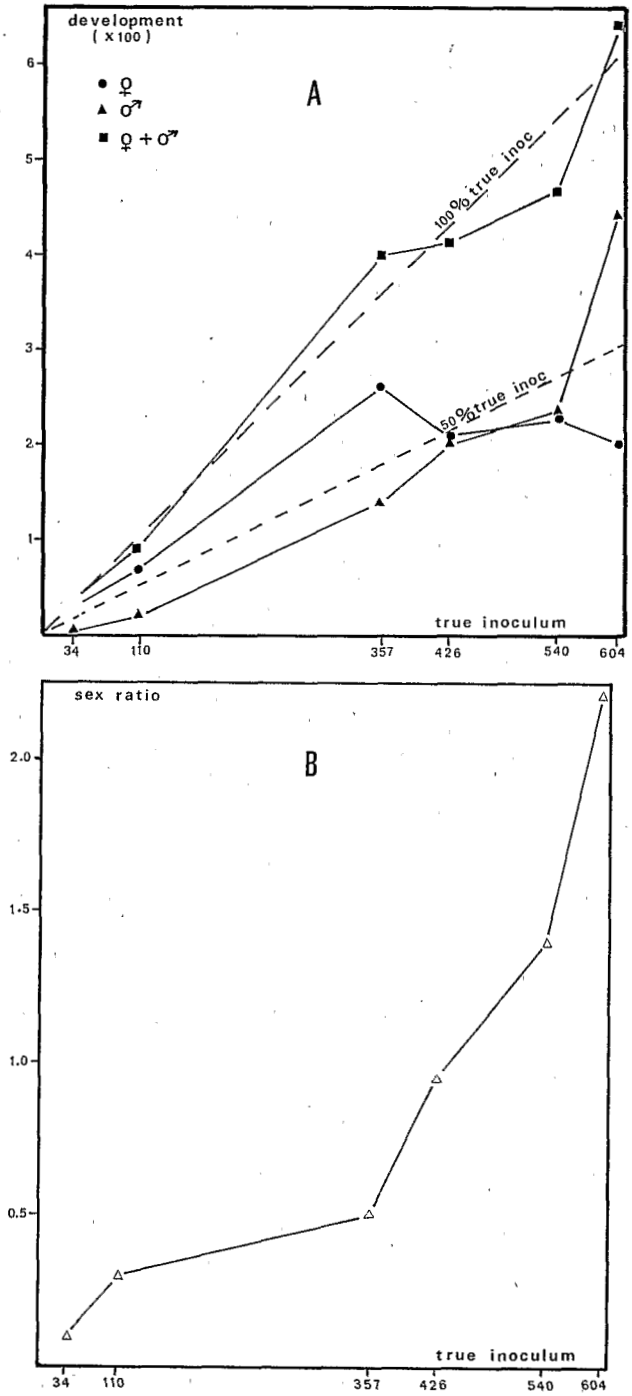

Fig. 2. Development (number of adults) of *H. sacchari* in rice roots in terms of true inoculum. A : number of males, females and total number of adults observed. B : sex ratio.

The number of males, very low for a true inoculum less than 100, increased slowly to a true inoculum of about 350 and then more quickly, whereas the number of females decreased. Above a true inoculum of about 550, many more males than females were observed. At the same time, sex ratio increased gradually from 0.1 for the lowest inoculum to 2.22 for the highest (Fig. 2B).

When sex is determined at an early stage, as in *Heterodera schachtii* (Kerstan, 1969), *H. glycines* (Koliopanos & Triantaphyllou, 1972) or *H. oryzae* (Cadet, Merny & Reversat, 1975) increase of sex ratio with the inoculum level is due to preferential death rate of female juveniles under conditions created by overcrowding. As food stress increases, more female juveniles die before reaching maturity. At the same time, all male juveniles that have penetrated reach maturity up to a high infestation level, where their development begins to be prevented by lack of food.

In the case of epigenetic sex determination as in *Globodera rostochiensis* (Ross & Trudgill, 1969), when the inoculum increases more and more juveniles which would have normally developed into females develop into males, due to food stress, and the proportion of males increases as the proportion of females decreases.

H. sacchari obviously belongs to the second category. Curves expressing development of juveniles in both sexes plotted against true inoculum (Fig. 2A) ressemble those obtained by Trudgill (1967) for *G. rostochiensis*. As practically all juveniles present in the roots reached the adult stage, it can be stated that, in *H. sacchari*, each second-stage juvenile is able to develop into a male or a female adult according to some external factors, such as overcrowding.

Age of plants and rates of penetration and development of H. sacchari

Whereas in *H. oryzae* a minimum of penetration was observed in two-week-old plants (Cadet, Merny & Reversat, 1975) rate of penetration of *H. sacchari* was low in one- and two-week-old plants and then increased with the age of the plants (Fig. 3A).

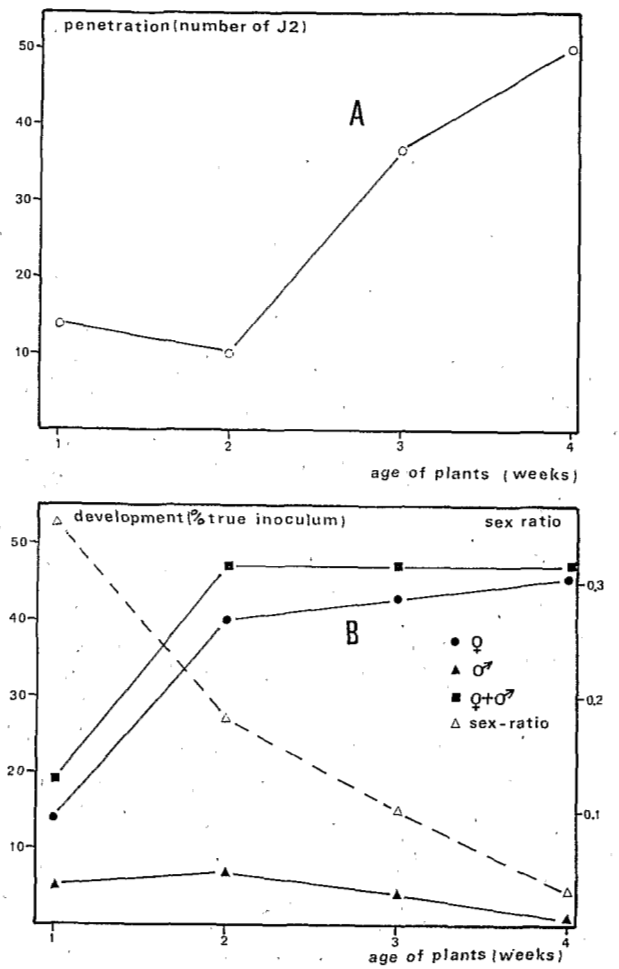

Fig. 3. Penetration and development of *H. sacchari* on 1—, 2—, 3—, and 4—week—old rice plants. A : penetration. B : development.

That penetration was lower in younger plants could be attributed to the size of root systems but, in the case of *H. oryzae*, maximum penetration was observed in one-week-old plants and the low penetration observed in two-week-old plants could not be attributed to the size of root systems. Penetration ability of *H. sacchari* may differ from that of *H. oryzae*, its importance increasing with plant age, but it is more likely that the reduced penetration observed, for *H. sacchari*, in one- and two-week-old plants was due to another factor as for *H. oryzae* in two-week-old plants.

Development into adults, expressed in percentage of true inoculum, was very low in one-week-old plants and reached a little less than 50% in older plants (Fig. 3B). This low percentage of development is in disagreement with results previously obtained in the experiment involving inoculum size, and thus, these results must be interpreted with caution. It appears that plant age exerts a different influence on *H. sacchari* and *H. oryzae*. In *H. oryzae*, practically all juveniles that had penetrated developed into adults regardless of plant age. In *H. sacchari* development of juveniles in one-week-old plants was very low and sex ratio relatively high. This cannot be due to higher susceptibility of *H. sacchari* to shortage of food. In this case, as observed in a previous experiment, more males than females would develop but the total development would not be reduced.

Effects of time of contact between H. oryzae and roots

Differences in the importance of inoculum size were thought to balance the differences in contact times. In fact, penetration was higher with high inocula. The observed average numbers of juveniles having penetrated was 259 at the inoculum level 2,100, 185 at 1,500 and 49 at 300. This disparity in true inoculum levels could have influenced the sex ratio, less females developing at high inoculum levels (Cadet, Merny & Reversat, 1975). However, the proportion of true inoculum reaching the adult stage has never been less than 98% and it can be assumed that inoculum size has played no part in the determination of the sex ratio.

As shown in Figure 4, sex ratio was very high when time of contact was short and lessened regularly when contact time increased to reach 1 after eight days. It can be stated that penetration of male juveniles takes place more rapidly than that of female ones. Hence, every study concerning sex ratio must be done with a contact time not less than eight days.

This experiment was not made with *H. sacchari*. In this species sex is determined after penetration has taken place and juveniles are all of the same kind and must be equally attracted and penetrate at the same rate.

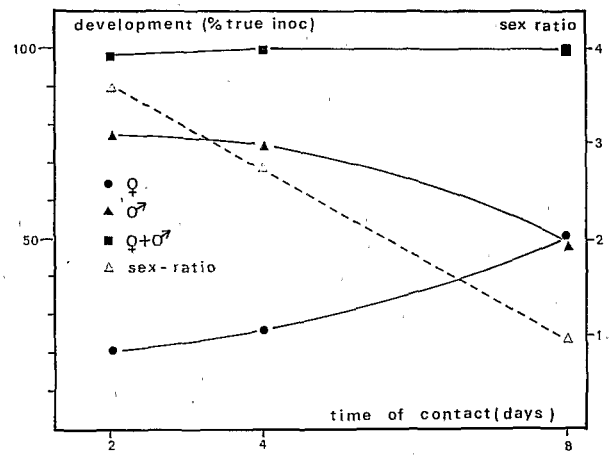

Fig. 4. Kinetics of penetration of male and female juveniles of *H. oryzae* in rice roots. Circles=number of females; black triangles=number of males; squares=total number of adults, expressed as percentage of true inoculum; white triangles=sex ratio.

Effects of variable periods of darkness on penetration and development of H. sacchari and H. oryzae

Penetration of *H. oryzae* was significantly reduced by darkness before and during the period of contact between inoculum and plants (Table 1). In *H. sacchari*, penetration is significantly reduced by darkness before inoculation but darkness during time of contact had a smaller effect and the observed penetration appears intermediate.

Table 1

Numbers of juveniles of *H. oryzae* and *H. sacchari* that penetrated under different darkness conditions, data with the same letter are not significantly different (Mann & Whitney test)

	Inoculum	
	400	*500*
Series	*H. oryzae*	*H. sacchari*
D (control)	83 a	41 a
A (1)	31 b	21 b
B (2)	35 b	30 ab
C (3)	71 a	42 a

(1) In darkness from four days before inoculation until one day after; (2) In darkness from day of inoculation until two days after being placed in hydroponics; (3) In darkness from one day before being placed in hydroponics until end of experiment.

In both species the development of males was not affected by darkness before and during the period of contact, whereas female development was slightly affected (Fig. 5). On the other hand, development of both sexes was strongly affected by darkness during hydroponic conditions. Males and females were equally affected in *H. oryzae*, resulting in the same sex ratio as in the control, whereas females were more affected in *H. sacchari*. This observation supports the former conclusion that, in *H. sacchari*, sex determination occurs at an advanced stage of development and the observed changes in sex ratio must be due to the fact that, in adverse conditions, more juveniles develop into males.

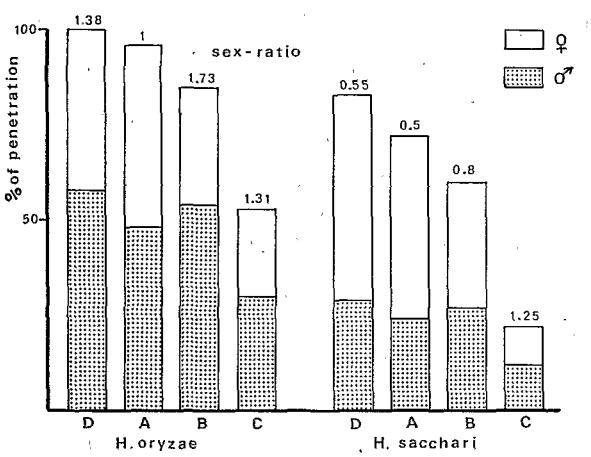

Fig. 5. Development in percentage of penetration of *H. oryzae* and *H. sacchari* in plants subjected to different periods of darkness. A, B, C : different treatments (see text and Table 1). D : control.

Conclusions

Influences of factors such as overcrowding, age of host plant, time of contact between inoculum and host plant and variable periods of darkness are different on an amphimictic species *(H. oryzae)* and a parthenogenetic species *(H. sacchari)* partly due to the fact that sex is determined before penetration in the first and not in the second. Selection of female juveniles at penetration stage observed in some cases with *H. oryzae* did not occur

with *H. sacchari* where juveniles are all of the same kind, sex being determined later.

Differences were observed affecting penetration as well as development of juveniles which have penetrated.

With regard to penetration an important part can be played by attractive or repulsive substances present in root exudates :

(1) The reduced penetration by *H. oryzae* in two-week-old plants and in one- and two-week-old plants by *H. sacchari* can be based upon the activity of such substances.

(2) One could assume that, in plants kept in the dark before and just after inoculation, the root system is reduced by lack of food but this has not been observed and it is more likely that the decrease in the rate of penetration be due to another factor, for examples lack of attractive or presence of repulsive substances in root exudates. In both cases, *H. oryzae* would be more susceptible than *H. sacchari*.

(3) The differences in penetration rates of male and female juveniles of *H. oryzae* can be due to differences in sensitivity to such substances.

One does not know whether juveniles of both species are attracted by the roots of rice or other plants. The hypothesis of the influence of substances emitted by roots on the penetration of their juveniles has to be investigated.

With regard to development, differences observed cannot always be attributed to availability of food :

(1) In the case of *H. sacchari* on one-week-old rice plants, the low percentage of development of juveniles must be due to the presence or absence of an unknown factor in the roots.

(2) In both species, in plants kept in the dark during development of the parasite, the reduction in development percentage of males and females is probably caused partly by lack of food in the root, but this assumption is not sufficient to explain the results obtained : in *H. oryzae*, shortage of food affects the development of females much more than development of males (Cadet, Merny & Reversat, 1975). If the reduction in development was caused by food shortage, the sex ratio in treatment C (Fig. 5) would have been much higher. It seems to be caused by the presence or absence of some substances in the roots which prevent development of juveniles regardless of their sex.

Accepté pour publication le 17 février 1978.

ACKNOWLEDGMENTS

The authors thank Dr. D. P. Taylor for reviewing the manuscript and translating it into "true American".

REFERENCES

CADET, P. & MERNY, G. (1977). Méthode d'étude de la pénétration et du développement des deux sexes chez les *Heterodera* (Nematoda : Tylenchida). *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.

KERSTAN, U. (1969). Die Beeinflussung des Geschlechterverhältnisses in der Gattung *Heterodera*. II. Minimallebensraum - Selektive Absterberate der Geschlechter-Geschlechterverhältnis *(Heterodera schachtii)*. *Nematologica*, 15 : 210-228.

KOLIOPANOS, C. N. & TRIANTAPHYLLOU, A. C. (1972). Effect of infection density on sex ratio of *Heterodera glycines*. *Nematologica*, 18 : 131-137.

MERNY, G. (1972 *a*). Les nématodes parasites des rizières inondées de Côte d'Ivoire. III. Études sur la dynamique des populations de deux endoparasites : *Hirschmanniella spinicaudata* et *Heterodera oryzae*. *Cah. ORSTOM, Sér. Biol.*, n° 16 : 31-87.

MERNY, G. (1972 *b*). Les nématodes parasites des rizières inondées de Côte d'Ivoire. IV. Essai d'interprétation mathématique de l'intensité de l'infestation des racines par les nématodes endoparasites en fonction de l'inoculum. *Cah. ORSTOM, Sér. Biol.*, n° 16 : 129-140.

ROSS, G. J. S. & TRUDGILL, D. L. (1969). The effect of population density on the sex ratio of *Heterodera rostochiensis* ; a two dimensional model. *Nematologica*, 15 : 601-607.

TRUDGILL, D. L. (1967). The effect of environment on sex determination in *Heterodera rostochiensis*. *Nematologica*, 13 : 263-272.