Parasitism of banana by *Heterodera oryzae*

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SUMMARY

A large population of an unidentified species of *Heterodera* was found parasitizing banana in Senegal, the first report of parasitism of banana by a member of this genus. Under greenhouse conditions this isolate reproduced well on both banana and rice. *H. oryzae* from the type locality in the Ivory Coast also reproduced on banana. The isolate from banana responded positively to rice root exudates. No morphological or cytological differences were found between the banana isolate and *H. oryzae*. Reciprocal matings between the banana isolate and *H. oryzae* were successful producing progeny which reproduced on rice. It is concluded that the isolate from banana is conspecific with *H. oryzae*.

RÉSUMÉ

Parasitisme d' Heterodera oryzae *envers le bananier*

Une population importante d'un *Heterodera* a été trouvée sur bananier au Sénégal, première observation d'une espèce du genre sur ce végétal. Une expérimentation en serre a permis de préciser que cette population se reproduit sur bananier et sur riz et que *H. oryzae* (population topotype) se reproduit sur bananier ; d'autre part la population isolée de bananier répond positivement aux exsudats radiculaires de riz. Aucunes différences morphobiométriques ou cytologiques n'ont pu être notées entre ces deux populations. Les fécondations croisées entre individus des deux populations ont été positives et ont donné des descendants se reproduisant sur riz. De ces observations, l'auteur conclut que la population isolée du bananier appartient à l'espèce *Heterodera oryzae*.

During a routine survey of nematodes associated with banana in Senegal, a soil sample collected from a small planting at Keur Moussa, approximately 40 km east of Dakar, was found to contain large numbers of juveniles and males of a species of *Heterodera* Schmidt, 1871. An additional soil and root sample was collected from which was extracted a large number of cysts (Fig. 1A) and root fragments on which were found white *Heterodera* females. This is believed to be the first report of a species of *Heterodera* attacking banana. Subsequently, specimens of *Heterodera* sp. were collected from one field in a large banana cooperative near Goudomp in the Casamance region of southern Senegal.

Greenhouse culture and host range trial

Cysts containing eggs as well as egg masses were selected from the Keur Moussa sample and used to infest pasteurized greenhouse soil. Suckers of banana, *Musa acuminata* Colla, were heat-treated in water (55° C for twenty minutes), planted in the infested soil and maintained in the greenhouse. After four months one plant was sacrificed ; large numbers of juveniles and males were recovered from the soil and many females with egg masses were observed on the roots (Fig. 1B). Thus, parasitism of this isolate on banana was confirmed.

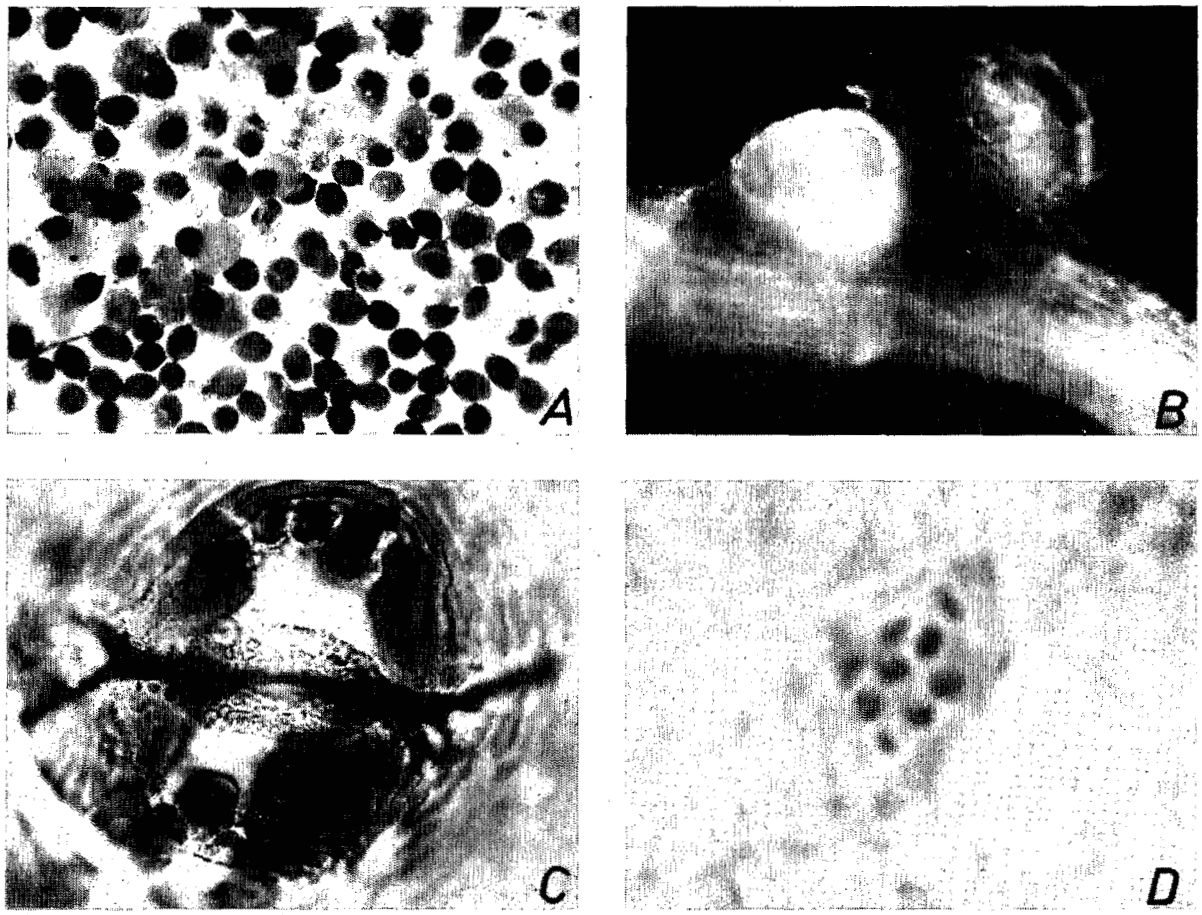

Fig. 1. *Heterodera oryzae*, banana isolate from Senegal. A : Cysts and egg masses from original collection ; B : Female and egg mass on banana root (greenhouse inoculation) ; C : Underbridge and bullae ; D : Metaphase in egg (n=9).

Additional cysts from the original site were added to pasteurized soil in pots and the following species planted : rice, *Oryza saliva* L. ; sorghum, *Sorghum vulgare* Pers. ; millet, *Penniselum typhoides* Stapf & Hubbard ; Guinea grass, *Panicum maximum* Jacq. ; maize, *Zea mays* L. ; sugarcane, *Saccharum officinarum* L. ; wheat, *Triticum aestivum* L. ; greenbean, *Phaseolus vulgaris* L. ; cowpea, *Vigna unguiculata* Walp. ; and turnip, *Brassica rapa* L. After three months, pots were examined for the presence of *Heterodera* cysts. Cysts and egg masses were abundant in pots containing rice ; a slight increase occurred on maize ; and no increase was detected on the other species.

Juveniles of *H. oryzae* Luc & Berdon, 1961, derived from the type locality were added to pasteurized soil and a heat-treated banana sucker was planted. Four months after inoculation a portion of the soil was processed for cyst recovery. Cysts and egg masses of *H. oryzae* were abundant establishing that banana was a host of *H. oryzae*.

Effect of rice root exudates on the banana isolate of *Heterodera* sp.

Merny (1972) had established that exudates from rice roots had a positive effect of emergence of *H. oryzae* from cysts. A small experiment was initiated to determine if the same effect would be obtained using cysts of the banana isolate of *Heterodera* sp.

Groups of five cysts of the banana isolate were placed in each of twelve small watch glasses. Six of these were filled with the water in which four-day-old rice seedlings had been placed for 24 hours ; the remaining six were filled with sterile distilled water. Every 24 hours the number of juveniles per dish was recorded and the liquids replaced. Results are given in Figure 2. A mean number of 362 juveniles emerged per dish during the seven days duration of the experiment in the dishes containing crude rice root exudates ; a mean of 222 emerged in water. Thus, rice root exudates provoked a positive response of emergence in the banana isolate similar to that reported for *H. oryzae*.

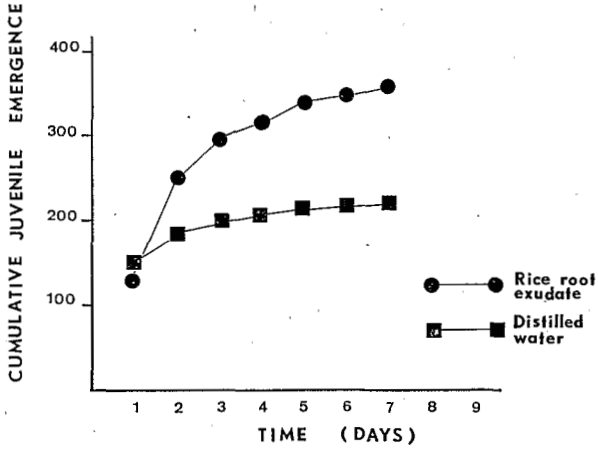

Figure 2. Emergence of juveniles from cysts of the banana isolate of *Heterodera oryzae* in distilled water and in crude rice root exudate. Each figure is the mean of six replicates, each containing five cysts.

Morphology

Morphological observations and measurements of the banana isolate of *Heterodera* sp. and *H. oryzae* are given in Table 1. It is evident from Table 1 that there are no consistant differences in morphometrics between the two isolates, with the possible exception of the mean length of juveniles and the lengths of the hyaline portion of the tail. Netscher and Pernès (1971) have already shown great differences in lengths of juveniles of *H. oryzae* when isolates were inbred. The differences measured in this study fall between the maximum and minimum lengths reported by these authors.

Cytology

No differences in chromosome number or morphology were observed between the banana isolate (Fig. 1D) and *H. oryzae* as described by Netscher (1969). In both cases, n=9.

Mating experiments

Second-stage juveniles were collected from cysts from greenhouse cultures of *H. oryzae* and the banana isolate of *Heterodera* sp. Five-day-old rice seedlings were placed in Petri dishes and a suspension of 50 juveniles of an isolate were poured over the root systems. Sufficient sterilized fine sand was added to each dish to cover the root system and absorb excess water. Seedlings were left in contact with the nematodes for five days after which the roots were thoroughly washed to remove sand particles and juveniles that had not penetrated. These infected seedlings were placed individually in test tubes 25×250 mm containing water. Seedlings were supported by a cotton plug in such a way that the roots were suspended in the water with the leaves in the air similar to the techniques of Trudgill (1967) and Cadet, Merny and Reversat (1975). The plants were maintained for 34 days after which the roots were examined for protruding females. Males, which had developed, had fallen to the bottom of the tubes and thus were unable to fertilize the females. In many cases females were found with a small gelatinous matrix attached to the posterior end, but in no case were eggs observed in the matrix. Small root segments, approximately 1 cm long and each containing a single female, were excised and placed in small watch glasses. To each dish were added two males of the opposite isolate that had been freshly extracted from greenhouse cultures. The following combinations were made : eighteen dishes with males of *H. oryzae* and females of the banana isolate and two dishes with the males of the banana isolate and females of *H. oryzae*. The root segments and the water

containing the males were covered with sterile fine sand and incubated at room temperature. Eight days later one dish was sacrificed and the female examined. Two eggs were found in the uteri and sperm in the region of the uteri. The remaining dishes were incubated for 21 days after which the females were removed from the root segments, cut open, and eggs present were removed and allowed to develop. The first juvenile hatched 22 days after males and females were placed together. Viable juveniles were obtained in seven of the eighteen *H. oryzae* males x females of the banana isolate and in both *H. oryzae* females x males of the banana isolate. Thus, viable offspring were produced in 45% of the attempted crosses and in both reciprocal crosses of the two isolates. One concludes that there is no barrier to interbreeding between the banana isolate and *H. oryzae*.

The experiment was repeated, the only difference being that all virgin females from each isolate attached to 1 cm root segments were placed in a single watch glass, covered with fine sand, and 50 males of the other isolate were added. These watch glasses were incubated at room temperature in the dark for two weeks after which all females and/or cysts were examined for the presence of eggs or juveniles. All eggs and juveniles recovered from each cross were placed together in a small quantity of water and placed in a depression in the soil in a pot containing four ten-day-old rice seedlings. Forty days later the rice plants in the two pots were examined for cysts or females. Several white females with attached egg masses were found on the roots in both pots, establishing that the progeny from the reciprocal crosses were infective and capable of reproduction on rice.

Table 1

Morphological characters of banana isolate and *Heterodera oryzae*

	Banana Isolate	Heterodera oryzae (1)
Males	(n=25)	
L (mm)	.85(.73-.98)	.90(.79-.97)
a	36.5(26.6-40.3)	38.6(31.1-46.3)
Stylet length (µm)	22.8(20.7-24.9)	24(23-25)
Spicule length (µm)	25.1(20-30)	32-36
Number of lines in lateral field	4	4
Juveniles (2nd stage) :	(n=25)	
L (mm)	.39(.36-.40)	.44(.37-.51)
a	23.4(20.3-26.1)	24.7(22.6-28)
Stylet (µm)	20.4(19.6-21.2)	21(19.5-22)
Hyaline tail portion (µm)	31.2(26.5-35.5)	38(31-45)
Number of lines in lateral field	3	3
Cysts :	(n=50)	
Length (mm)	.56(.40-.66)	.57(.31-.81)
Width (mm)	.43(.32-.55)	.46(.22-.69)
Type (after Mulvey)	Group 4	Group 4
Fenestration	ambifenestate	ambifenestrate
Bullae	present	present
Underbridge	present, no finger-like projections (2)	present, no finger-like projections
Expanded hyaline portion of underbridge	present, 48×25 µm	present, 50×25 µm
Egg masses	present, large	present, large

(1) Data after Luc and Taylor (1977).
(2) See Figure 1C.

Ten juveniles each from two of the successful crosses in the first experiment were fixed (Netscher & Seinhorst, 1969) and measurements were made of their lengths and the length of the hyaline portion of the tails. Means obtained were, respectively, .42 mm (.41-.45) and 34 µm (30-40) for cross no. 6 (*H. oryzae* males x female of the banana isolate) ; and .43 (.41-.46) and 33 µm (29-37) for cross no. 8 (also *H. oryzae* males x female of the banana isolate). These figures are within the range reported for *H. oryzae* (see Table 1) : the length of juveniles being closer to that of *H. oryzae* than that of the banana isolate. The length of the hyaline tail tip of the juveniles from these crosses was closer to the figure for the banana isolate.

Conclusions

On the basis of all the evidence presented, it is concluded that the isolate of *Heterodera* found parasitizing banana in Senegal is conspecific with *H. oryzae*. Banana is considered a host of *H. oryzae*. Reactions of other test plants, including maize, are in agreement with the results of Merny and Cadet (1978).

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