uterus. The part of the uterus anterior to the egg is pushed forward, flexed and folded in the vulvar region. Whether this condition is a result of inability to oviposit can only be speculated : similar disorders are sometimes observed in normal, especially egg-producing females. As a consequence, the posterior ovejector and vagina are severely deformed, whereas the anterior one is only slightly deformed. The position of the specimen on the slide is somewhat ventrolateral. Both circumstances prevent accurate comparison of vaginae and ovejectors and attribution of abnormality status to anatomical details. The following only can be firmly stated : both vaginae are fully developed. The ovejectors are fused and the anterior one seems regular in the non-deformed part, whereas the posterior one seems less developed.

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A NEW AFRICAN ISOLATE OF *MONACROSPORIUM BEMBICODES* ACTING SIMULTANEOUSLY AS PREDATOR AND HATCHING INHIBITOR OF NEMATODES

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A new isolate of a nematophagous fungus similar to *Monacrosporium bembicodes* (Drechsler) Subram was isolated in a moist soil cultivated with tomatoes during a study of the nematofauna, in soils of vegetable crops of the Bobo-Dioulasso region of Burkina Faso (West Africa). Despite the presence of numerous root-knot galls, only few eggs were isolated from roots, indicating a possible predacious activity against *Meloidogyne.* Our objectives were to : *i)* determine the distinctiveness of the new African isolate in comparison to the original isolate of Drechsler : *ii)* to study the predatory activity of the fungus against different species of nematodes; and *iii)* to observe its ability to inhibit the hatching of *Meloidogyne.*

Materials and methods

Petri plates filled with selective medium (8.5 g corn meal agar; 8.5 g agar in a liter of distilled water) were simply sprinkled with soil using the technique of Drechsler (1941). After 7 days at 23 °C the fungus was well developed and produced erect conidiophores. Pure cultures were obtained by picking off individual conidia under the dissecting microscope, and transfering them on corn meal agar medium in Petri dishes. Morphologically, the African isolate closely resembles *M. bembicodes* (Drechsler) Subram, as conidia and conidiophores are the same in shape and sizes (Fig. 1A), but it differs significantly by the organs of capture. In the original description, nematodes were captured by three-celled constricting rings whereas in the new isolate they are captured by adhesive nets (Fig. 1B, C). Also, chlamydospores were not formed in the original Drechsler strain, whereas these resting spores were commonly observed in the new African isolate. When agar cultures are two months old, the fungus produces abundant intercalary or terminal chlamydospores. These are thick-walled, globose in shape, measuring 8 to 20 µm in diam., with dense globuliferous contents and are arrang-

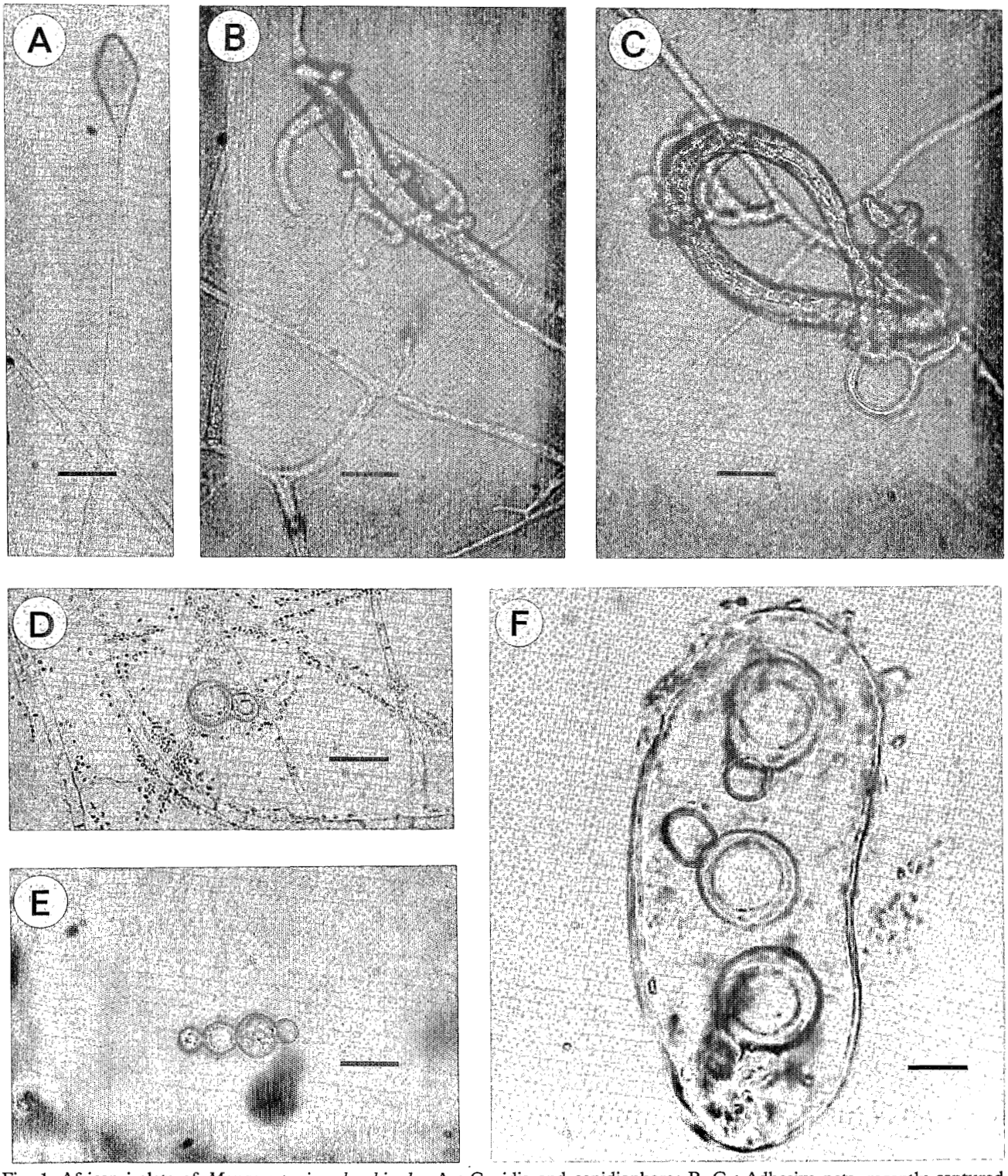

Fig. 1. African isolate of *Monacrosporium bembicodes.* A : Conidia and conidiophore; B, C : Adhesive nets, recently captured nematodes; D : Chlamydospores arranged in pair; E : Chlamydospores arranged in short chain; F : Chlamydospores inside a *Meloidogyne* egg. *(Bar equivalent : A — E = 20 µm; F = 10 µm).*

ed in pairs or in short chains (Fig. 1D, E). These morphological differences are not sufficient to establish the African isolate as a new species, as confirmed by G. S. de Hoog *(in litt.)*. The degree of predatory activity of the African isolate was tested with the following nematode species : *Caenorhabditis elegans, Ditylenchus dipsaci, Pratylenchus penetrans* (adults and juveniles) *Meloidogyne arenaria* (second stage juveniles).

Two hundred specimens of each species were introduced in a water droplet into one week old fungal cultures growing in Petri dishes (50 mm diam.) on the medium described above. The same number of nematodes was also introduced in Petri dishes plated with uninoculated agar medium as a control. After one and two days the viable nematodes from the different Petri dishes were extracted in Baermann funnels. Each treatment was replicated four times. By comparing the numbers of nematodes recovered in inoculated and uninoculated Petri dishes, it was possible to estimate the predatory activity of the fungus. The inhibitory effect of the fungus on the hatching was estimated by introducing *Meloidogyne arenaria* egg masses onto one week old fungal cultures in Petri dishes. After 3 and 6 days the egg masses were recovered and placed on 0.1 mm aperture nylon sieves in Petri dishes, with just enough water to cover the mesh. Every three days, the water was changed and the number of hatched juveniles counted. Egg masses from uninoculated Petri dishes were treated similarly. The observations were continued for one month. In a second experiment, egg masses taken from inoculated or uninoculated Petri dishes were used to infest two-week old tomato seedlings transplanted in a 50 ml plastic tube containing 40 ml of autoclaved sandy soil. Twenty days after inoculation, tomato roots were washed free of soil and stained with cold cotton blue lactophenol (de Guiran, 1967). Juveniles in the roots were extracted and counted using a dissecting microscope.

In all experiments, each treatment included four replications with ten egg masses picked at random from a homogenous sample.

Results

Predatory activity

No nematodes were trapped during the two-day experiment. Predacious organs appeared four to six days after nematodes were added to the fungus. Consequently, the predatory activity was delayed but all nematode species, except the saprophagous *C. elegans*, were trapped in one week. The fact that *C. elegans*, normally highly susceptible to trapping fungi, was not captured in this experiment, could be attributed to heavy contamination of the agar medium with nematode associated bacteria that may have suppressed trapping activity.

Hatching inhibitory effect

Results of the two experiments (counting of hatched juveniles and infestation of tomato seedlings) are summarized in Table 1. The results show that the new African isolate acts simultaneously as a trapping predacious fungus, and as a hatching inhibitor. Live nematodes did not stimulate rapid trap formation and consequently the predatory activity was delayed, although still effective. Hatching of nematodes in fungus infected egg masses was very reduced in comparison with the check (about 80 %). An important reduction in the tomato root penetration was also observed when treated egg masses were used for infestation. We have not proved that the observed hatching reduction is due to fungal egg parasitism. There may only be a suppressive effect of the fungus, or of its metabolites on nematode emergence. However, the presence of numerous chlamydospores inside eggs taken from treated egg masses (Fig. 1F) supports our assumption.

A considerable range of fungi have been reported to colonize eggs of cyst and root-knot nematodes (Brown & Kerry, 1986), but only one species of *Monacrosporium (M. lysipagum)* has been described parasitizing juveniles and eggs of *Meloidogyne* (Esser, 1983). The new African isolate is the second occurrence of predacious fungus acting also as hatching inhibitor. Research is needed before this very promising fungus can be properly evaluated as biological control agent in African soils.

Table 1

Effect of the new African isolate of *Monacrosporium bembicodes* on the hatching of *Meloidogyne arenaria* and the tomato roots penetration by juveniles.

	Nematode hatching in fungus infected egg masses (% of the check)		Nematode penetration into tomato roots (% of the the check)	
	Contact period of the egg masses with the fungus			
Repetition	3 days	6 days	3 days	6 days
1	23.87	21.49	6.32	10.20
2	22.32	46.15	19.48	15.00
3	16.71	20.76	51.98	8.92
4	20.49	18.16	14.32	10.41
$\bar{X}$	20.85	26.64	23.03	11.13
s	3.08	13.08	20.05	2.66

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DISCRIMINATION ENTRE *HETERODERA CAROTAE* ET *H. CRUCIFERAE* PAR L'ÉLECTROPHORÈSE (SDS PAGE) DE PROTÉINES SOLUBLES EXTRAITES À PARTIR DE KYSTES INDIVIDUALISÉS

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En culture légumière de plein champ, les infestations simultanées par plusieurs espèces de nématodes phytoparasites, sont fréquentes. Ainsi, dans les régions productrices de carottes où diverses crucifères entrent dans la rotation culturale, les sols peuvent être infestés par des kystes de *Heterodera carotae* Jones, 1950 et de *H. cruciferae* Franklin, 1945 en mélange. La reconnaissance spécifique est alors très difficile. Outre l'utilisation de tests biologiques qui permettent de déceler les parasites sur les racines de leur plante hôte respective (technique longue à mettre en œuvre), l'identification peut se faire par la morphométrie des juvéniles (Wouts & Weischer, 1977), mais les critères pris en compte, notamment la mesure de la partie hyaline de la queue, présentent des variations intraspécifiques importantes et les recouvrements interspécifiques sont possibles. De plus cela représente un travail particulièrement long. Pour pallier ces difficultés, une technique plus discriminante a été recherchée. Parmi les techniques biochimiques utilisables en taxonomie (Hussey, 1979), l'électrophorèse s'est avérée être un outil performant (Bergé & Dalmasso, 1985). L'électrophorèse de protéines dénaturées provenant de kystes individualisés a été utilisée.

Matériel et méthode

Différentes populations françaises (cinq pour *H. carotae* et quatre pour *H. cruciferae*) sont élevées en chambre climatisée à 20-22 °C, sur leur plante hôte respective : *Daucus carota* cv. Nandor pour *H. carotae* et *Brassica oleracea* cv. Brutor pour *H. cruciferae.*

Après lavage à l'eau distillée, le kyste est placé dans un tube de 1,1 mm de diamètre contenant une solution de Tris HCl à 0,01Ml^{-1} (PH 7.4) et de Chaps à 20 gl^{-1}. Après broyage et centrifugation à 5 000 *g* pendant 3 min, on ajoute 5 µl d'une solution de dithioérytritol à 0,15Ml^{-1} et de SDS à 100 gl^{-1} et on centrifuge à 10 000 *g* pendant 10 min. Les protéines sont alors dénaturées par trempage des tubes au bain-marie à 100 °C pendant 5 min. Avant utilisation, le surnageant est mélangé à 10 µl d'une solution de saccharose à 200 gl^{-1}.

La technique électrophorétique employée est celle de Laemmli (1970), avec gel de concentration à 4 % et de séparation à 12,5 %, d'une épaisseur de 0,5 mm. La migration est contrôlée par le déplacement du bleu de bromophénol.

La coloration au nitrate d'argent est réalisée selon la technique décrite par Heukeshoven et Dernick (1985) et modifiée par Damerval *et al.* (1987). Le Rf des bandes discriminantes est calculé par rapport à la migration du bleu de bromophénol.

Résultats

Au niveau intraspécifique, on ne décèle pas de différences discriminantes au sein des populations.

Au niveau interspécifique, quatre différences principales permettent l'identification aisée de chaque espèce (Fig. 1). On note une bande de Rf : 0,40, assez ténue, présente chez *H. carotae* et absente chez *H. cruciferae.* Une deuxième différence facile à repérer existe pour la bande de Rf : 0,46 qui est présente pour chacune des espèces, mais de forte intensité pour *H. carotae* alors qu'elle est faible pour *H. cruciferae.* Inversement, la bande Rf : 0,49 est plus intense chez *H. cruciferae* que