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RELATIONSHIP BETWEEN POPULATION STRUCTURE AND RESPIRATION ACTIVITY IN *APHELENCHUS AVENAE*

by
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Summary. Four populations of *Aphelenchus avenae* were estabilished on *Rhizoctonia solani* cultures for six months. The compatibility with *R. solani, Verticillium dalhiae, Fusarium solani, F. oxysporum* f. sp. *lycopersici, Phytophtora parasitica* and *P. capsici* was determined to detect variability among populations. Marked variations of respiration rates, and inhibition by potassium cyanide and salycilhydroxamic acid were found among the four populations tested due to their different population structures. pH and antimicyn A did not significantly affect the respiration activity of the *A. avenae* populations.

Aphelenchus avenae Bastian has been considered as an ideal biological control agent because of its ability to colonize most edaphic environments (Klink and Barker, 1968; Barnes *et al.*, 1981). The efficacy of *A. avenae* as a control agent depends on nematode and fungi densities as well as the type of crop involved (Cayrol and Combettes, 1977; Caubel *et al.*, 1981). However, ecophysiological differences among *A. avenae* isolates have been reported to occur (Evans and Fisher, 1970b; Evans and Womersley, 1980). Therefore, nematode populations adapted to the same ecological condition as the specific soil inhabiting pathogenic fungi have to be selected for biological control assays, this being a basic principle of biological control (Price, 1981). In addition, it has been estabilished that the host plays an important role in the density and the structure of *A. avenae* populations (Evans and Fisher, 1970a).

Preliminary data on respiration of *A. avenae* populations indicate the presence of thermotypes (Mendis and Evans, 1983). In the work presented here the variation in respiration has been analyzed in relation to the structure of different *A. avenae* populations.

Materials and methods

Four populations of *A. avenae* from Poland (P) and Spain (A, B, C) were grown for six months on *Rhizoctonia solani* Kühn, cultured on Potato Dextrose Agar (PDA). Four mature females of each population were transferred to plates containing, *R. solani*, *Verticillium dalhiae* Kleb, *Phytophtora parasitica* Dost, *P. capsici* Leon, *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder *et* Hansen, *F. oxysporum* f. sp. *pisi* (Van Hall) Snyder *et* Hansen, and *F. solani* (Mart.). Snyder *et* Hansen, respectively, and incubated at 19 °C. There were four replicates of each culture. After 45 days the nematodes were recovered and the ratio between females and juveniles was calculated to determine the potential of reproduction.

For respiration assay, nematodes were extracted from plates containing *R. solani* by cutting the agar into small pieces which were soaked in distilled water on a 90 µm sieve covered with paper tissue. Active specimens were recovered after 24 h and incubated for other 24 h in 0.1 M potassium phosphate buffer at pH 5, 6, 7 or 8. Then, 0.5 ml of the suspension of each nematode population was transferred to a Clark Type oxygen electrode chamber (Rank Bros., Cambridge, UK), and oxygen uptake monitored at 24 °C. When a constant rate of respiration was estabilished, respiratory inhibitors such as KCN (1.2 mM), SHAM (2 mM) and antimicyn (0.5 mM) were added, respectively. Each measurements was repeated twice. The Multiple Factorial Correspondence Analysis and Discriminant Analysis of STAT-ITCF program, 1988, were applied to relate the respiratory parameters and population structures.

Results

The numbers of juveniles and mature females of the four populations present in cultures were affected by the type of fungi as the source of food (Fig. 1). There was a high variability in the potential of reproduction (juveniles/females) when the four different nematode populations were allowed to grow on the same fungus or when the same population was grown on different fungi (Table I).

The respiratory rates at different pH values (5, 6, 7 and 8) of the four *A. avenae* populations are reported in Table II. Apparently, pH affected the rate of respiration of each

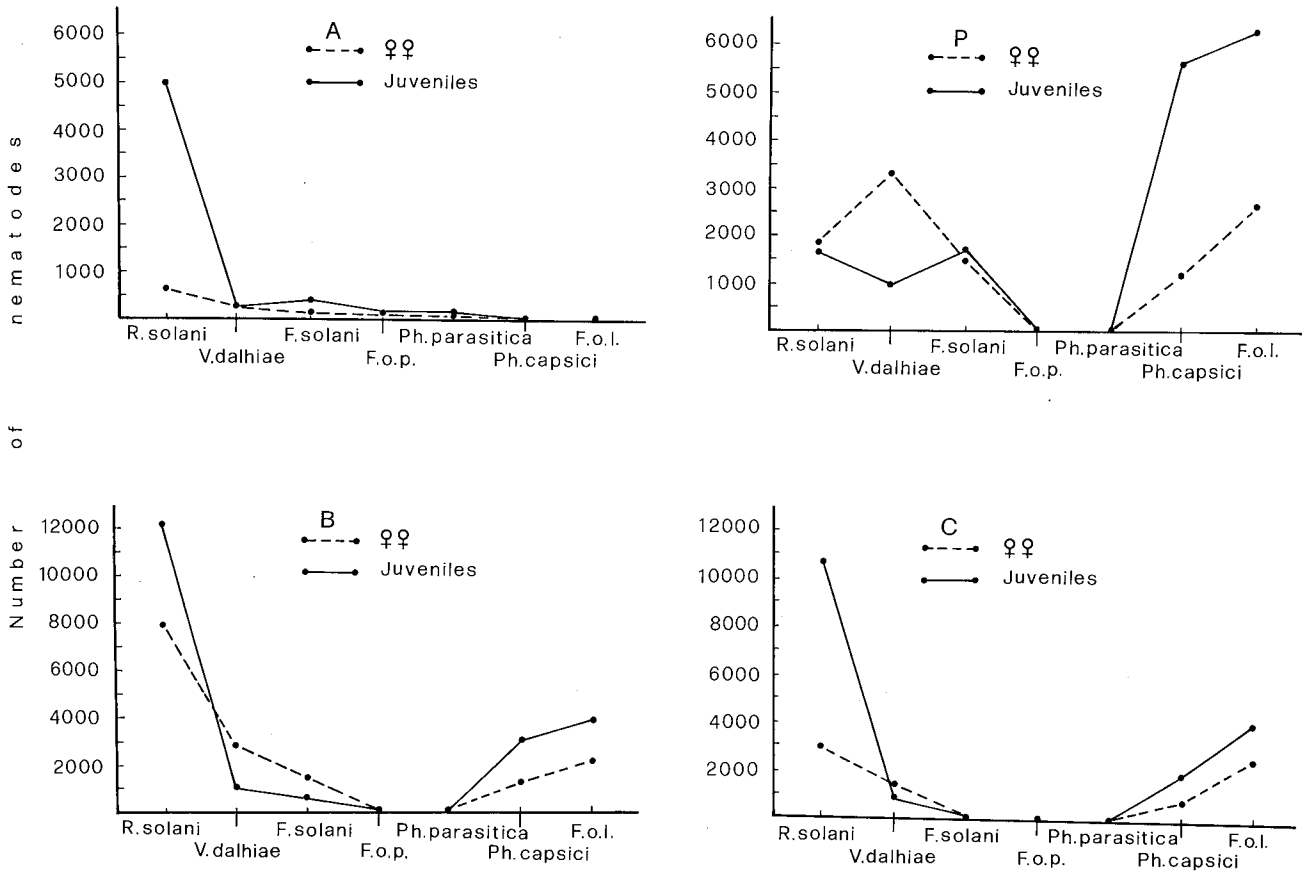

Fig. 1 - Profiles of population structure of four *Aphelenchus avenae* populations (P, A, B and C) growing on the same fungi.

population and the inhibitory effect of the chemicals tested. However, when the population structure was assayed after each polarographic measurement (Table III), it was evident that the difference in the respiratory and inhibitory values was not due to the pH *per se* but to the marked diversity of the population structure in the different polarographic samples.

Data from the relationships between respiratory activity, population structure and pH values were analysed by a multidimensional approach. Table IV shows the range of variation of all the variables considered. Data matrix (Table III) has been arranged in classes according to statistical normalization criteria. Table IV also shows that 65% of total variability could be expressed by 5 axes and the 44% by the first three axes; pH and antimicyn A inhibition are basically expressed by axes 4 and 5. This indicates that pH and antimicyn A inhibition are not associated with the remaining variables in affecting the overall variability in respiration activity. Moreover, these two variables are not correlated according to their X^2 value (9.23 with 6 D. F.) and therefore can be removed from the interpretation of the analysis.

Distribution of samples in factorial space (Fig. 2a-b) identified four populations whose geometric centres are in Fig. 2c. The distribution of samples is mainly determined by population structure and oxygen consumption, and expressed by axes 1 and 2 (young females and total respiration) while variation of juveniles and mature females is expressed by axes 1 and 3. Population structure has a direct influence on the per cent inhibition induced by KCN and SHAM since the inhibition is explained mainly by axes 2 and 3. From the association of the variables four well-defined groups were apparent (Fig. 2d). The most distant group associates a lower value of respiration with a smaller number of individuals (LLI, HJI, HHI and R1), and the other three groups follow a direction from the lowest to the

TABLE I - *Potential of reproduction of A, P, B and C populations of* Aphelenchus avenae *(Juveniles / Females) growing on different fungal hosts*

Fungus species	Nematode populations				
	A	P	B	C	$\bar{X}$
R. solani	8.38	0.88	1.53	3.57	3.58
V. dalbiae	0.77	0.29	0.37	0.57	0.50
Ph. parasitica	1.38				0.34
Ph. capsici		4.79	2.28	2.29	2.59
F. oxysp. (lyc.)	2.38	1.77	1.54		1.42
F. oxysp. (pisi)	1.23				0.30
F. solani	2.30	1.22	0.41	0.64	1.14
$\bar{X}$	2.01	1.36	0.90	1.23	

highest inhibition along the plane 1-2. These groups and the direction would correspond with characteristics of populations C, B and A, i.e., when there is a medium level of respiration due to the ranging of females between 3000-6000, the lowest respiratory inhibition is associated with the median class of young females and juveniles and an abundance of mature females between 6000-12000 (pop. B). Maximum inhibition occurs when respiration and numbers of total nematodes are at the maximum level (pop. A).

Finally, population P shows intermediate characteristics between populations A and B which is reflected in its broader distribution in the factorial space (Fig. 2a).

The classification of the populations by discriminant analysis confirms the results obtained by ordination (Table V). The populations are discriminated when inhibition by KCN and SHAM is considered, but no discrimination occurs with antimicyn A inhibition which is an indipendent variable (F and correlation values are not significant). However, inhibition by KCN and SHAM shows a very high correlation and significant F values, thus characterizing the nematode populations. On the other hand, the two first axes practically represent the entire variation within KCN and SHAM inhibition values. According to the correlation values, these axes represent basically one of these variables. That is, axis 1 represents the highest variation of SHAM inhibition and axis 2 the highest of KCN inhibition. Cosine values in Table V are indicative of the distribution of four populations along the axes variation. Accordingly, populations P and C are differentiated by class of medium values (80-96) of SHAM inhibition on total respiration rate, while populations A and B can be distinguished by class of medium value (60-80) of KCN inhibition on total respiration rate; the total percentage of classification is very high (65%) and also suggests that inhibition (especially by SHAM) could be a specific characteristic of each population.

Discussion

Ecophysiological variability frequently occurs among cosmopolitan species of nematodes. This is an adaptation mechanism to colonize different ecological conditions. *A. avenae* is an ideal model because of its widespread distribution and capability as a functional fungal feeder. Therefore, it is reasonable to suppose that *A. avenae* populations might be selected which are adapted to the same ecological conditions of the soil inhabitating plant pathogenic fungi by which they feed. Evans and Fisher (1970b) demonstrated that temperature has an important influence on the biology and ecology of *A. avenae* while Mendis and Evans (1983) suggested measurement of respiratory activity as a selective tool to define different thermotypes of the nematode. However, the definition of a thermotype cannot be always related to oxygen consumption as it has previously been shown that considerable variability in oxygen uptake occurs among nematode populations (Cooper,

TABLE II - *Respiration rate (nmol x min $^{-1}$ x ml $^{-1}$) and percentages of inhibition by antimicyn A, SHAM and KCN of four* A. avenae *populations at different pH values*

		pH 5	pH 6	pH 7	pH 8
Rate of oxygen consumption					
Popul.	P	3.0	3.9	4.45	5.3
	A	6.05	6.0	5.8	7.0
	B	2.6	3.85	3.2	4.65
	C	3.55	4.55	4.45	3.85
% inhib. antimicyn A					
Popul.	P	8	12.5	6	3
	A	21.5	8	7	18
	B	17	11	4.5	+4
	C	12	14	13.5	10
% inhib. SHAM					
Popul.	P.	94.5	98.5	92	99
	A	93.5	92.5	89	78
	B	82	84	88	93.5
	C	78.5	80.5	85.5	81.5
% inhib. KCN					
Popul.	P	83	77	68	76
	A	80.5	77.5	84	65
	B	66	62.5	66	80
	C	60	63.5	69	62

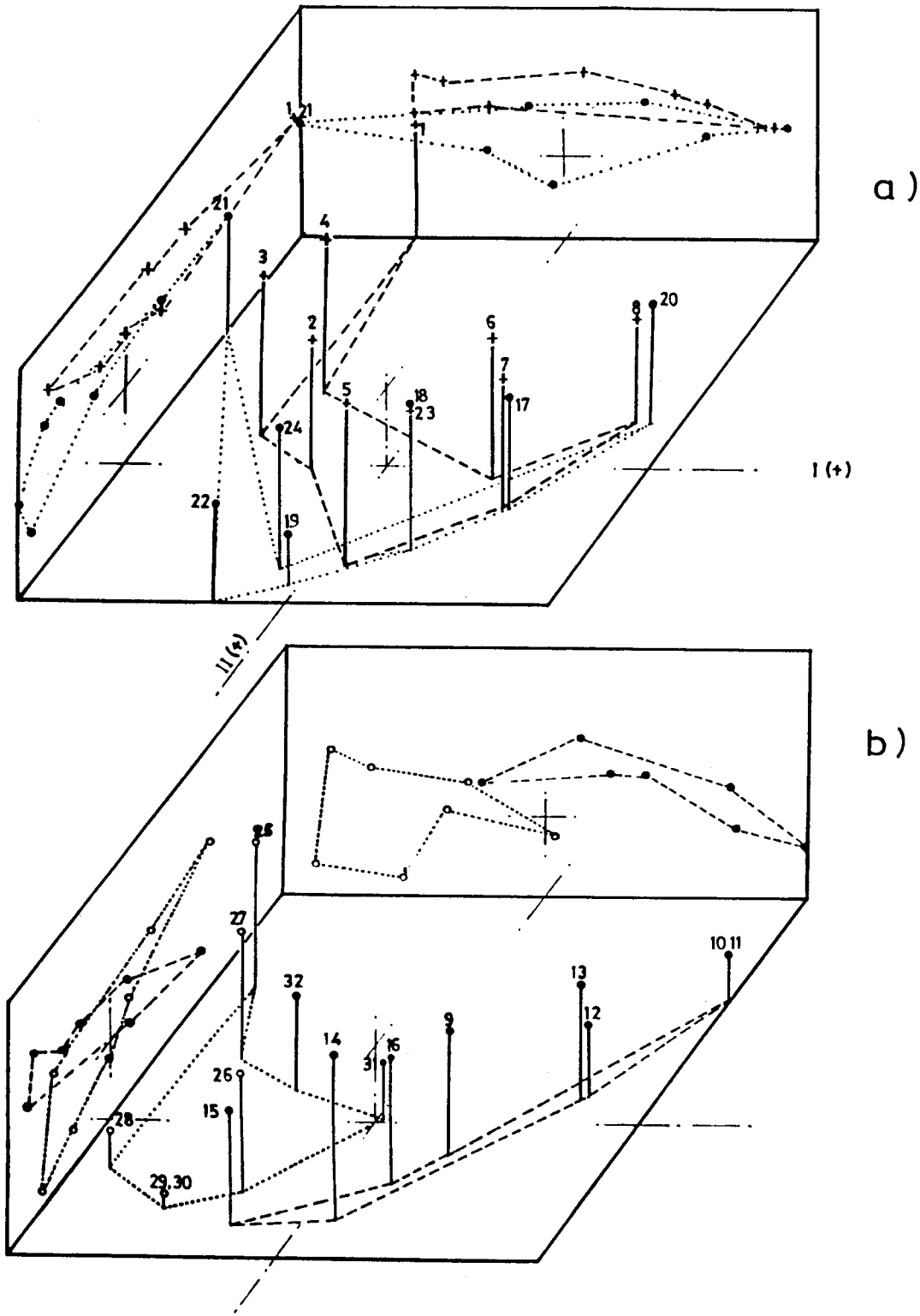

Fig. 2a-b - Representation of correspondence factorial analysis of populations P (samples 1-8) and B (samples 17-24) in (a) and of populations A (samples 9-16) and C (samples 25-32) in (b).

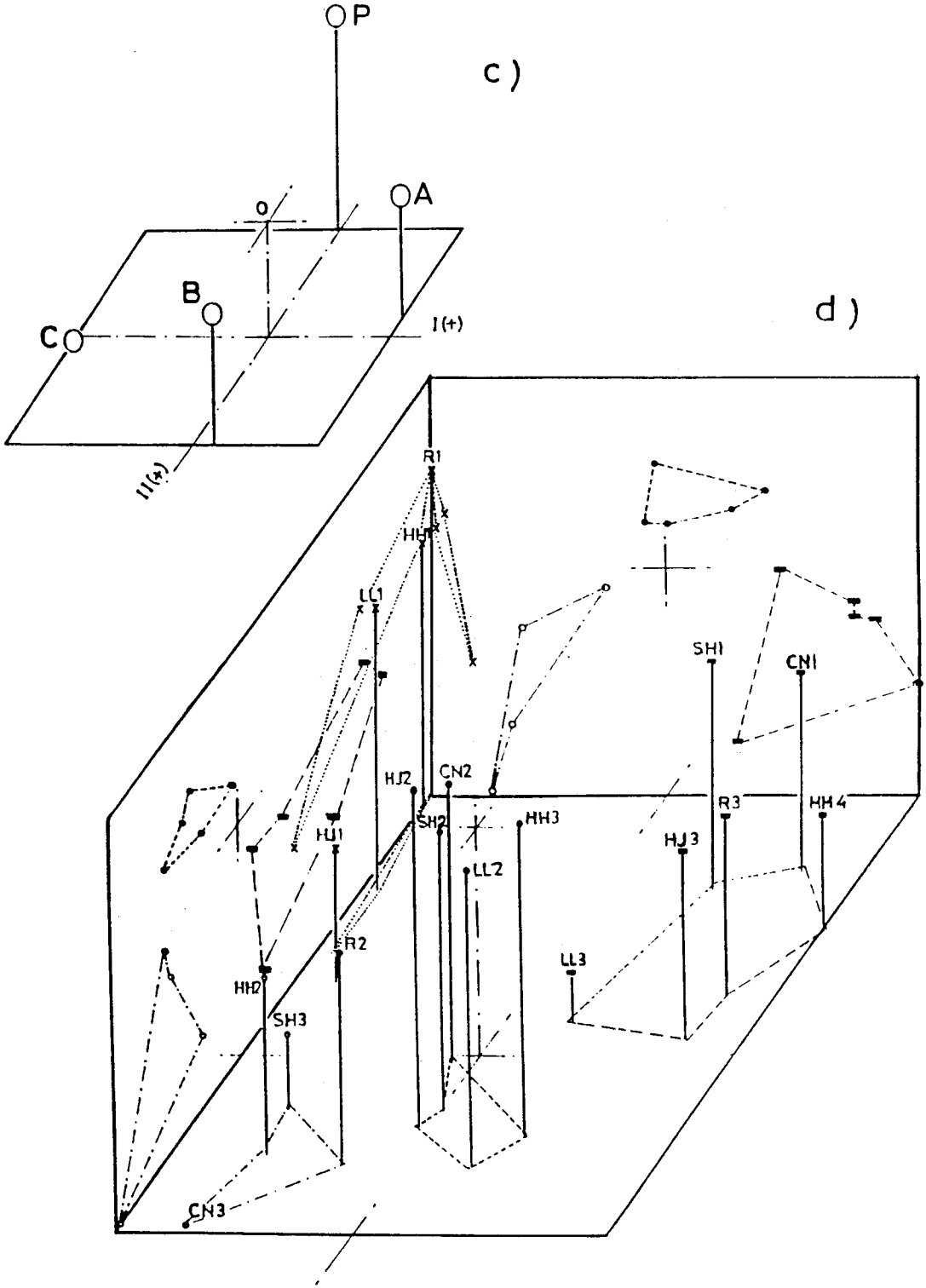

Fig. 2c-d - Gravity centers of *Aphelenchus avenae* populations (c) and disposition of variables (d) in the factorial space.

TABLE III - *Data matrix*

pH5	pH6	pH7	pH8	population P	A	B	C	specimens Juven.	Females	J. females	inhibition (%) Antim.	KCN	SHAM	Total Resp.
1				1				200	2100	900	0	-100	-100	2,2
	1			1				1400	5300	4600	- 6	- 72	- 98	3,1
		1		1				1100	3900	4100	0	- 69	- 90	2,9
			1	1				1400	6000	3400	0	- 73	-100	3,0
1				1				4300	6400	3600	-16	- 66	- 89	3,8
	1			1				6400	9400	4700	-19	- 82	- 99	4,7
		1		1				5100	9000	6700	-12	- 67	- 94	6,0
			1	1				6800	18000	9200	- 6	- 79	- 98	7,6
1					1			17000	8400	4300	-20	- 80	- 92	5,1
	1				1			24900	15000	5500	- 1	- 90	-100	7,5
		1			1			36900	14200	6100	- 7	- 91	-100	7,2
			1		1			31300	15600	5800	-10	- 64	- 82	6,1
1					1			13800	13400	4900	-23	- 81	- 95	7,0
	1				1			6900	8900	3100	-15	- 65	- 85	4,5
		1			1			12000	5100	3100	- 7	- 77	- 78	4,4
			1		1			9500	10200	4800	-26	- 66	- 74	7,9
1						1		17900	26900	11900	-17	- 71	- 92	3,4
	1					1		15000	8700	9900	-17	- 65	- 87	3,5
		1				1		5500	3100	5100	-12	- 60	- 91	3,1
			1			1		10800	9600	8500	+ 8	- 86	- 98	5,8
1						1		2000	2400	1900	-17	- 61	- 72	1,8
	1					1		3600	5700	4500	- 5	- 60	- 81	4,2
		1				1		5000	6100	5200	+21	- 72	- 85	3,3
			1			1		3400	3500	4100	0	- 74	- 89	3,5
1							1	2500	2200	1200	-14	- 65	- 83	2,9
	1						1	4800	3500	1600	- 5	- 70	- 89	4,4
		1					1	3000	1200	700	-16	- 73	- 92	3,7
			1				1	2500	3100	1700	-11	- 60	- 80	3,5
1							1	17800	4400	2600	-12	- 55	- 74	4,2
	1						1	17400	4500	1500	-23	- 57	- 72	4,7
		1					1	16500	6700	2400	-11	- 65	- 79	5,2
			1				1	17000	2500	2000	- 9	- 64	- 83	4,2

1969; Cooper and Van Gundy, 1970; Marks, 1971; Awan, 1975; Mendis, 1981).

On the other hand, the classic electron transport pathway presents considerable differences among mitochondria from different species of animal parasitic nematodes (Fry and Jenkins, 1984a; Fry and Beesley, 1985). This phenomenon could be related to the body biomass (Fry and Jenkins, 1984b) and may explain differences in the respiration among free living nematodes (Klekowsky *et al.*, 1979).

Our results indicate that the relative numbers of juveniles and females in cultures is determined by the food source. It should be noted that population A increased on *P. parasitica* and *F. oxysporium* f. sp. *pisi*, but failed to reproduce on *P. capsici* and *F. oxysporium* f. sp. *lycopersici*. Population A differed in its behaviour from populations P, D and C. On the other hand, population P reproduced very well on *P. capsici*, *F. oxysporium* f. sp. *lycopersici* and *F. solani*, while population B and C reproduced well on *R. solani*, *P. capsici* and *F. oxysporium* f. sp. *lycopersici* with a

TABLE IV - *Description of Correspondence Factorial Analysis of Table III*

Variable	Range	Classes	Fraquency	Relative contribution of axes to explained inertia				
				I (18%)	II (15%)	III (12%)	IV (10%)	V (8%)
JUVENILES	< 3000	LL1	8					
	3000 - 1500	LL2	15	16,9	8,3	26,5	2,3	0,00
	> 15000	LL3	9					
FEMALES	< 3000	HH1	5					
	3000 - 6000	HH2	11	26,0	18,7	13,9	22,1	0,8
	6000 - 12000	HH3	10					
	> 12000	HH4	6					
YOUNG FEMALES	< 2800	HJ1	10					
	2800 - 5000	HJ2	12	20,7	1,4	21,0	0,9	8,9
	> 5000	HJ3	10					
ANTIMYCIN A (% inhib.)	< 0	AN1	6					
	0 - 15	AN2	16	0,7	6,7	16,9	31,1	10,4
	> 15	AN3	10					
KCN (% inhib.)	< - 81	CN1	6					
	- 80 a - 60	CN2	21	6,1	19,7	7,9	8,2	22,7
	> - 60	CN3	5					
SHAM (% inhib.)	< - 96	SH1	8					
	- 96 a - 80	SH1	17	5,2	11,7	8,4	16,1	12,6
	> - 80	SH3	7					
TOTAL RESPIRATION	< 3,0	R1	5					
	3 - 5	R2	17	21,6	24,3	3,8	0,6	1,7
	> 5	R3	10					
pH		PH5	8					
		PH6	8	2,8	9,2	1,5	18,6	42,9
		PH7	8					
		PH8	8					

TABLE V - *Descriminant Analysis Description*

	POPULATION					CORRELATION	
	P	A	B	C	F	AXIS I	AXIS II
ANTIMICYN	7.37 ± 7.05	13.6 ± 8.24	4.87 ± 12.9	12.6 ± 4.97	NS	–	–
KCN	-76 ± 10.6	-76.7 ± 10.14	-68.6 ± 8.42	-63.6 ± 5.7	3.48**	0.291	0.666
SHAM	-96 ± 4.15	-88.2 ± 9.3	-86.8 ± 7.3	-81.5 ± 6.38	5.05***	0.853	0.1455

Quality of representation (coseno value)

	P	A	B	C
AXIS I	0.961	0.149	0.008	0.613
AXIS II	0.014	0.84	0.78	0.286
% Classification	75%	62.5%	50%	75%

Correlation between variables

	ANT	KCN	SHAM
ANT	–	0.328 (NS)	0.314 (NS)
KCN		–	0.759***
SHAM			–

** P < 0.01; *** P < 0.001; NS Not significant.

similar growing profile (Fig. 1). The four populations did not develop well on *V. dalhiae*. Population A showed a high reproductive potential in spite of its low density confirming its lower selective ability when compared with the other three populations.

An actual difference in the oxidative metabolic pathways occurring among the tested populations is suggested by the different levels of inhibition on respiration observed when KCN and SHAM were used as inhibitors. However, such a difference could be related to population structure as reported by Mendis and Evans (1983) when they recorded the reaction of *A. avenae* juveniles and adults to KCN added to the *R. solani* culture media. Mendis (1981) claimed that EDB (ethylene dibromide) and KCN had a similar action on oxygen uptake by *A. avenae*; he also supported the view that the difference in *A. avenae* response to EDB found by Marks (1971) and Awan (1975) was probably due to different metabolic states of the nematode during the experimental time and/or to intraspecific different isolates.

Our data show that the *A. avenae* populations tested can be distinguished by their differing capability of growing on different fungal hosts. Consequently, their reproductive potential has been taken into account for the statistical analysis of their respiratory activity. This analysis has revealed that, generally, the four populations present significant differences of total respiration rates as well as in the inhibition levels by either KCN or SHAM. Furthermore, these different properties have been found to be dependent on the relative contribution of the diverse nematode life stages present in the assayed samples. It was not possible to differentiate among *A. avenae* populations when antimicyn A was used as the inhibitor.

Finally, our approach does not provide the biochemical basis to explain the differences in respiratory activity of *A. avenae* populations. However, it would be useful to validate and to differentiate sets of populations coming from previously selected ecosystems. Further research in this field would provide a basis for the better understanding, for example, of the different mode of action of the same nematicide in different ecosystems and to determine the role of nematodes in the soil metabolic activity.

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