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Assessment of the North Carolina differential host test for identification of Australian populations of root-knot nematodes (*Meloidogyne* spp.)

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Abstract

Forty-one Australian populations of *Meloidogyne* were assessed for their host-race status by the North Carolina differential host test which relies on the combination of resistant and susceptible reactions of six differential hosts to the nematode population. Although in most cases the distinction between reactions of the NC differential hosts to nematode populations was clear, there was sufficient discrepancy to make the test unreliable for species and race identification in Australia when used alone. Five atypical combinations of reactions were found.

Additional keywords: host range

Introduction

Meloidogyne spp. (root-knot nematodes) are one of the most important groups of nematodes attacking the world's agricultural crops. More than 60 species of *Meloidogyne* have been described (Eisenback and Triantaphyllou 1991) and together they attack almost every crop grown. Even with current management practices, which are often based on chemical nematicides, they cause an estimated 12% crop loss annually worldwide (Sasser and Freckman 1987). To manage these pests with species- or race-specific strategies such as the use of crop rotation, resistant cultivars or biological control, it is necessary to identify accurately the nematode to be controlled.

Meloidogyne spp. have been differentiated by various morphological (Jepson 1987), cytological (Triantaphyllou 1985) and biochemical characters (Eisenback and Triantaphyllou 1991; Hugall *et al.* 1994; Stanton *et al.* 1997). The North Carolina differential host test (Table 1) (Hartman and Sasser 1985) was devised to identify the four most common species, *M. javanica* (Treub) Chitwood, *M. hapla* Chitwood, *M. arenaria* (Neal) Chitwood and *M. incognita* (Kofoid & White) Chitwood and host races of the last two species. The NC differential host test relies on combinations of resistant and susceptible reactions to nematodes of *Capsicum frutescens* L. (capsicum) cv. California Wonder, *Gossypium hirsutum* L. (cotton) cv. Deltapine 16, *Arachis hypogaea* L. (peanut) cv. Florunner, *Lycopersicon esculentum* Mill. (tomato) cv. Tiny Tim, *Nicotiana tabacum* L. (tobacco) cv. NC95 and *Citrullus vulgaris* Schrad. (watermelon) cv. Charleston Gray (Taylor and Sasser 1978).

The response to nematodes of NC differential hosts has been described as 'fairly reliable' for identification of the four common species (Eisenback *et al.* 1981). However, this test does not distinguish between *M. javanica* and *M. arenaria* race 2. Furthermore, atypical host reactions have been found (Taylor *et al.* 1982). For example, although peanut and capsicum are considered non-hosts of *M. javanica*, they are susceptible to some populations. Also, some populations of *M. arenaria* race 2 reproduce on capsicum which is usually a non-host.

Hugall *et al.* (1994) showed that identification of some Australian populations of *Meloidogyne* by the NC differential host test was not consistent with esterase phenotype or mitochondrial DNA

(mtDNA) type, whereas esterase phenotype and mtDNA type were perfectly correlated. We considered that reduction of eggmass counts to assignment as a 'resistant' or 'susceptible' reaction ignored a lot of information and may have masked the true relationship between host range and biochemical characters.

The purpose of this study is firstly to report some atypical reactions by NC differential hosts to *Meloidogyne*. Secondly, we used numbers of eggmasses and eggmass ratings rather than resistance/susceptibility to assess the test's usefulness in identifying Australian *Meloidogyne* populations by determining the degree of similarity between host reactions to different nematode populations.

Methods

Nematode populations were collected from within Australia (Table 2) and maintained in a glasshouse as single eggmass cultures on tomato cv. Tiny Tim. Eggs were removed from roots in 0.5% sodium hypochlorite (Southey 1986), and plants were inoculated with 6000 eggs per pot, replicated four times. The NC differential host plants used were capsicum, cotton, peanut, tomato, tobacco and watermelon. Sixty days after inoculation, roots were washed and eggmasses stained with 0.15% phloxine B and counted.

The standard differential host test uses eggmass ratings according to the following scale: 0 = 0 eggmasses per plant, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100 and 5 = more than 100. In the NC differential host test, plants with average eggmass ratings of 2 or less are classified as resistant and those with ratings greater than 2 are classified as susceptible. The reaction to each nematode population of the six differential hosts is used to assign a species and race (Table 1).

Agglomerative hierarchical cluster analyses were carried out with similarity matrices based on Euclidean distances (Genstat 5 Committee 1993). The average link method was used to determine how similarities between clusters were redefined at each merge. Dendrograms were constructed to indicate the level of similarity at which clusters were used to compare nematode populations. In these analyses, counts of more than 100 eggmasses were considered to be 100. Therefore, in any host-nematode reaction, where at least three of the four replicates had more than 100 eggmasses, the mean eggmass rating was considered to be 5, even if the resulting mean number of eggmasses was less than 100.

Table 1 Usual responses of the four common *Meloidogyne* species and their host races to the North Carolina differential host test (Hartman and Sasser 1985) and atypical reactions of some Australian populations

Meloidogyne species and race		NC differential hosts[A]					
		CP	CT	PN	TB	TM	WM
M. incognita	race 1	+[B]	–[B]	–	–	+	+
	race 2	+	–	–	+	+	+
	race 3	+	+	–	–	+	+
	race 4	+	+	–	+	+	+
M. arenaria	race 1	+	–	+	+	+	+
	race 2	–	–	–	+	+	+
M. javanica		–	–	–	+	+	+
M. hapla		+	–	+	+	+	–
Atypical (1)		+	–	–	+	+	–
Atypical (3)		–	–	–	+	+	–
Atypical (4)		–	–	+	+	+	–
Atypical (5)		+	–	+	–	+	–
Atypical (6)		+	–	+	–	+	+

[A]CP, *Capsicum frutescens* (capsicum) cv. California Wonder; CT, *Gossypium hirsutum* (cotton) cv. Deltapine 16; PN, *Arachis hypogaea* (peanut) cv. Florunner; TB, *Nicotiana tabacum* (tobacco) cv. NC95; TM, *Lycopersicon esculentum* (tomato) cv. Tiny Tim; WM, *Citrullus vulgaris* (watermelon) cv. Charleston Gray.

[B]+,– = resistant, susceptible as defined by Hartman and Sasser (1985).

Table 2 Origins of *Meloidogyne* populations used in this study and identification by the North Carolina differential host test and by mitochondrial DNA type

Pop	Original host	Collection site[A]	No. eggmasses on NC differential hosts[B]						NC differential host test[C]			mtDNA type[E]
			CP	CT	PN	TB	TM	WM	Numerical	Rating	Identity[D]	
11	Grape	Ballandean, Q	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	D
47	Banana	Wamuran, Q	0	0	0	85	>100	81	000555	RRRSSS	j/a2	D
63	Tobacco	Glasshouse, Q	0	0	0	87	>100	90	000555	RRRSSS	j/a2	–
65	Leucaena	Crow's Nest, Q	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	A
72	Lucerne	Monto, Q	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	D
82	Lucerne	Langhorne Creek, SA	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	D
101	Grape	Eden Valley, SA	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	–
106	Tomato	Bunbury, WA	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	–
NQ1	Tobacco	Mareeba, Q	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	A
NQ2	Tobacco	Mareeba, Q	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	D
NQ5	Tobacco	Mareeba, Q	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	C
NQ7	Tobacco	Mareeba, Q	0	0	0	>100	>100	>100	000555	RRRSSS	j/a2	C
51	Tomato	Bundaberg, Q	0	0	0	>100	14	48	000534	RRRSSS	j/a2	D
92	Grape	Loxton, SA	0	0	0	67	>100	85	000455	RRRSSS	j/a2	–
Y	Taro	Malanda, Q	0	0	0	40	78	20	000453	RRRSSS	j/a2	C
60	Tomato	Bundaberg, Q	0	0	0	22	62	72	000344	RRRSSS	j/a2	–
94	Ginger	Eumundi, Q	5	0	0	>100	>100	>100	100555	RRRSSS	j/a2	D
16	Kiwifruit	Maleny, Q	6	1	0	81	>100	65	100554	RRRSSS	j/a2	D
87	Fig	Loxton, SA	4	0	0	61	>100	55	100454	RRRSSS	j/a2	–
B	Tomato	Toogoolawah, Q	1	0	0	47	>100	56	100454	RRRSSS	j/a2	D
1	Banana	Redlands, Q	6	0	0	>100	>100	>100	200555	RRRSSS	j/a2	D
69	Leucaena	Mt Cotton, Q	3	0	0	>100	>100	>100	200555	RRRSSS	j/a2	D
102	Carrot	Wellard, WA	4	0	13	>100	>100	2	203551	RRSSSR	Atypical (4)[F]	E
A	Tomato	Bundaberg, Q	1	0	0	49	>100	1	100451	RRRSSR	Atypical (3)	D
G	Thunbergia	Atherton, Q	>100	0	0	1	>100	>100	500155	SRRRSS	i1	B
19	Ginger	Yandina, Q	>100	0	0	0	>100	>100	500055	SRRRSS	i1	–
35	Banana	Mission Beach, Q	55	0	0	0	33	64	400044	SRRRSS	i1	B
15	Pineapple	Elimbah, Q	13	0	0	1	>100	>100	300155	SRRRSS	i1	A

Table 2 continued

12	Grape	Applethorpe, Q	20	0	0	12	>100	0	300250	SRRSSR	atypical (1)	G
H	Home garden	Burdekin, Q	>100	0	0	>100	>100	>100	500555	SRRSSS	i2	B
44	Peanut/maize	Wooroolin, Q	39	0	0	>100	>100	>100	400555	SRRSSS	i2	D
39	Grape	Herberton, Q	66	0	0	34	>100	>100	400455	SRRSSS	i2	A
Z	Banana	Batavia Downs, Q	66	0	0	46	>100	13	400452	SRRSSS	i2	B
42	Sugarcane	Mulgrave, Q	28	0	0	>100	>100	>100	300555	SRRSSS	i2	D
86	Pecan	Loxton, SA	15	0	0	>100	>100	>100	300555	SRRSSS	i2	D
D	Olive	Mitchell, Q	9	0	0	>100	>100	78	300554	SRRSSS	i2	D
33	Pasture	Pavgola Park, Q	21	0	0	84	>100	18	300553	SRRSSS	i2	C
113	Peanut	Redvale, Q	39	0	93	8	>100	0	405250	SRSRSR	Atypical (5)	E
115	Peanut	Tongola, Q	97	0	18	0	>100	>100	503055	SRSRSS	Atypical (6)	–
48	Kiwifruit	Maleny, Q	53	0	>100	58	>100	1	405451	SRSSSR	h	F
114	Peanut	Mareeba, Q	>100	0	>100	86	>100	96	505455	SRSSSS	a1	E

[A]SA, South Australia; Q, Queensland; WA, Western Australia.

[B]CP, *Capsicum frutescens* (capsicum) cv. California Wonder; CT, *Gossypium hirsutum* (cotton) cv. Deltapine 16; PN, *Arachis hypogaea* (peanut) cv. Florunner; TB, *Nicotiana tabacum* (tobacco) cv. NC95; TM, *Lycopersicon esculentum* (tomato) cv. Tiny Tim; WM, *Citrullus vulgaris* (watermelon) cv. Charleston Gray.

[C]Reactions of host plants are listed in the same order as for number of eggmasses. An average eggmass rating of <2 indicates resistance (R); an average rating of >2 indicates susceptibility (S); plants with a rounded-off, numerical rating of 2 were assigned an R or S rating depending on whether the original value was 2 or >2. For example, ratings of 1.5–2.0 are considered indicative of resistance (R), ratings of 2.1–2.4 indicative of susceptibility (S).

[D]j, *M. javanica*; a, *M. arenaria*; i, *M. incognita*; h, *M. hapla*. Numbers denote standard differential host races.

[E]A, C, *M arenaria*; B, *M. incognita*; D, *M. javanica*; E, F, *M. hapla*; G, *M. hispanica* (Hugall *et al.* 1994).

[F]Reactions (see Table 1) which differ from those described by the North Carolina differential host test.

Results

Details on populations studied, along with eggmass numbers and ratings, are shown in Table 2. In populations 16, 33, 47, 63, 92, 114 and Y, more than 100 eggmasses were counted but were recorded as 100 when averaging over four replicates. This resulted in apparent anomalies between eggmass numbers and ratings.

There was poor correlation between mitochondrial DNA type and identification by the NC differential host test (Table 2). For example, only 70% of populations of type A, C (*M. arenaria* race 2) or D (*M. javanica*) elicited the appropriate reactions by the NC differential hosts. Also, populations 12, 102, 113, 115 and A produced atypical reactions in the NC differential hosts.

Number of eggmasses When numbers of eggmasses were compared by cluster analysis (Figure 1), groups containing populations which produced reactions in NC differential hosts indicative of *M. javanica*/*M. arenaria* race 2 and *M. incognita* race 2 were not clearly differentiated. For example, populations D, 42, 44 and 86 (identified as *M. incognita* race 2 using the NC differential host test) were more closely related to many populations identified as *M. javanica*/*M. arenaria* race 2 than to Z, 33, 39 or H (identified as *M. incognita* race 2). Also some populations identified as *M. javanica*/*M. arenaria* race 2, e.g. Y, 51 and 60, were more closely correlated to populations identified as *M. incognita* race 2 than to other populations of *M. javanica*/*M. arenaria* race 2.

Similar biochemical types were not clustered reliably. Therefore, the relationship between biochemical characterisation and identification by the NC differential host test was not improved by comparing total numbers of eggmasses produced on differential hosts.

Eggmass rating When eggmass ratings were compared by cluster analysis (Figure 2), there was little discrepancy between per cent similarity and assignment of species/race based on eggmass rating. All populations identified as *M. javanica*/*M. arenaria* race 2 with the NC differential host test were clustered, as were populations identified as *M. incognita* race 1 or *M. incognita* race 2.

Similar biochemical types were not clustered reliably. Therefore, the relationship between biochemical characterisation and identification by the NC differential host test was not improved by comparing eggmass ratings of differential hosts.

Discussion

In a worldwide collection of 662 populations from 76 countries, about 47% were *M. incognita*, 40% *M. javanica*, 7% *M. arenaria* and 6% *M. hapla* (Taylor *et al.* 1982). In the current study, we found a similar spread of Australian populations when the NC differential host test was used. However, we also found that 14% of populations induced host reactions which were atypical, i.e. an identity could not be based on the NC differential host test, but none of these reactions was reported by Taylor *et al.* (1982). Taylor *et al.* (1982) reported peanut as a host of Egyptian variants of *M. javanica* and also found populations of *M. javanica* and *M. arenaria* race 2 that infected capsicum which is usually a non-host of these races. We did not observe these reactions, either in the present study or in other unpublished work in this laboratory.

Our study suggests that, although differentiation of populations by the NC differential host test often corresponded with mtDNA type, there are sufficient discrepancies to make it unreliable. Reliability was not improved by characterising populations by number of eggmasses produced on differential hosts or by eggmass ratings. The NC differential host test was developed for use in corn-cotton-peanut-tobacco rotations and was intended for use in combination with identification by perineal patterns of adult females (Hartmann and Sasser 1995). However, perineal patterns are also very variable and unreliable as an indicator of species (Hugall *et al.* 1994).

When NC differential host tests were repeated with the same nematode populations, there was usually some variability between tests (data not shown). Numbers of eggmasses were always similar in these tests but sometimes eggmass ratings were affected if close to a cut-off value between consecutive rating classes, further contributing to lack of confidence in the test.

In this study, cotton was resistant and tomato susceptible to all nematode populations tested. Therefore, in effect, only four plant genotypes were being used to differentiate several species and races of *Meloidogyne*. It is unlikely that the reactions of four plant genotypes to even the four most common species and their races would represent the total

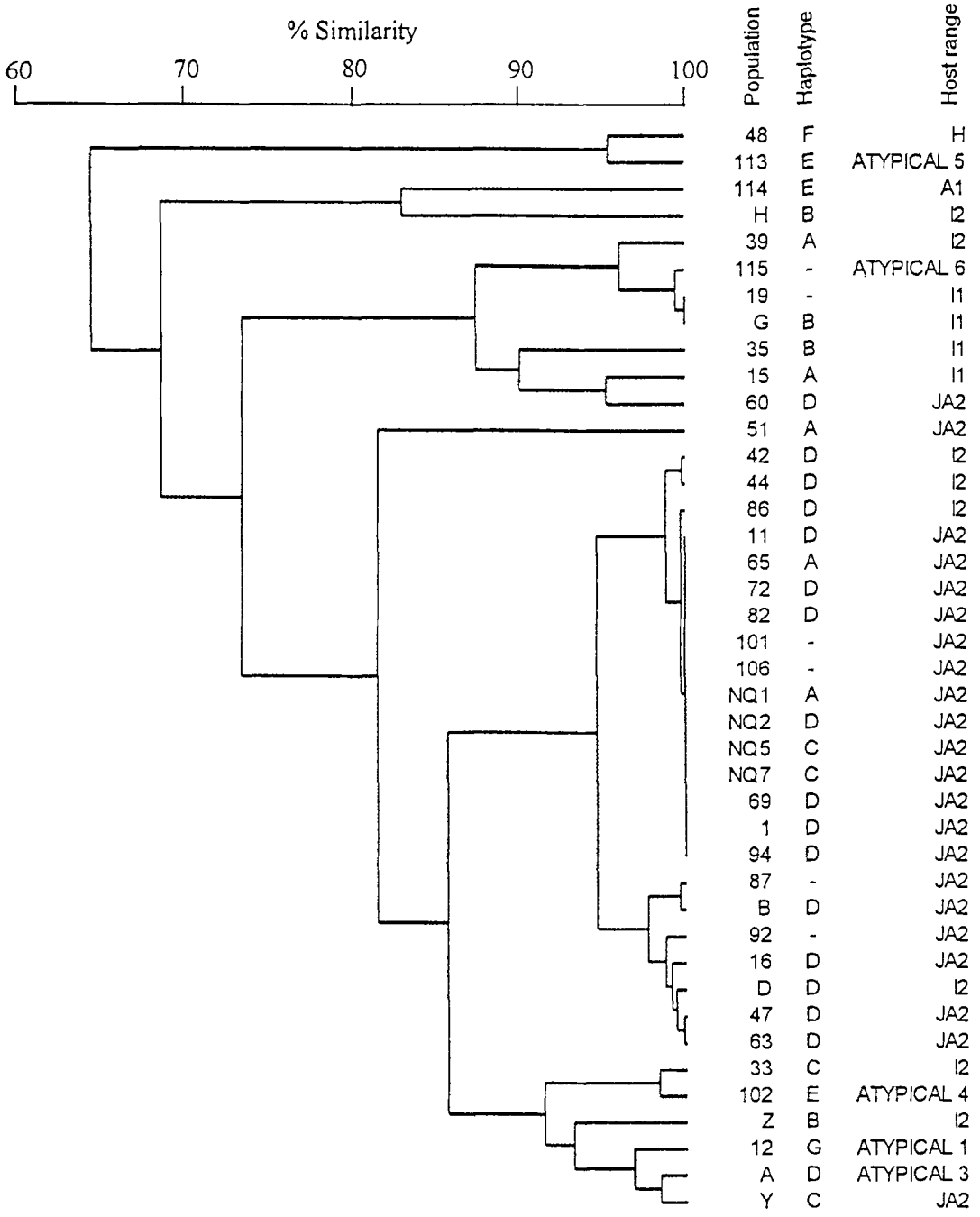

Figure 1 Dendrogram displaying groupings generated by the agglomerative hierarchical cluster analysis of numbers of eggmasses produced by various populations of *Meloidogyne* spp. on six North Carolina differential host test plant cultivars.

pathotype variability. If the set of differential hosts were expanded, more differences between populations may be demonstrated.

A disadvantage of this type of host range test is that it forces populations into groups which may not reflect pathotype variability, e.g. the reactions of capsicum to populations 15 (13 eggmasses) and D (9 eggmasses) are very similar but the difference between them indicates a host race difference. A suitable host range test for identification of species

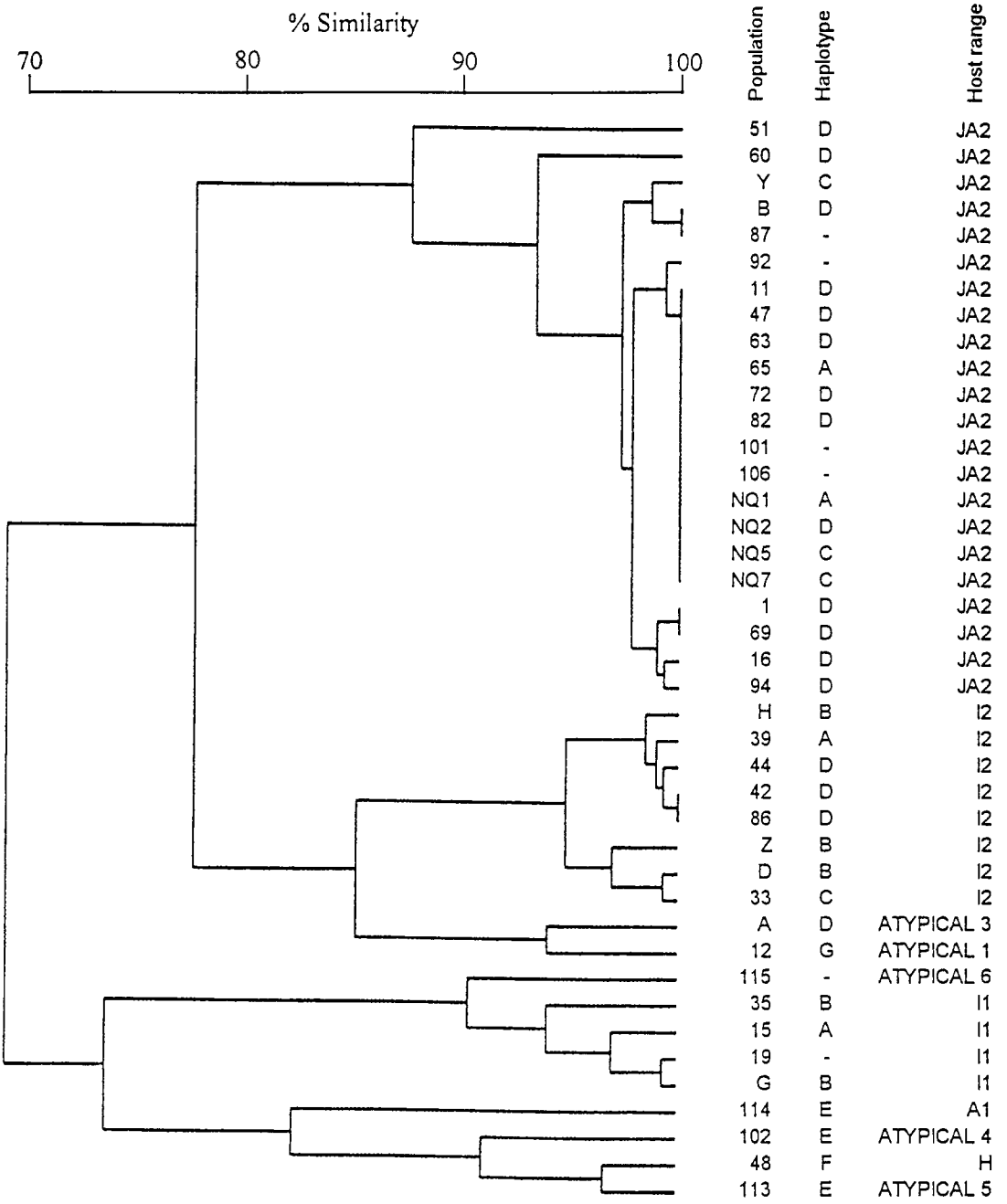

Figure 2 Dendrogram displaying groupings generated by the agglomerative hierarchical cluster analysis of numbers of eggmass ratings of various populations of *Meloidogyne* spp. on six North Carolina differential host test plant cultivars.

and races should be based on major gene differences which produce clear host range groupings.

Roberts (1995) has developed an improved scheme for characterising the large variability in host range of *Meloidogyne*. It relies on the reaction of a number of differentials, each with a single resistance gene, to the nematode population. Nevertheless, given the wide variability within the genus, it may not be possible to develop a single scheme to characterise the reactions of all nematode populations to a wide range of crops throughout the world. However, knowledge of the host range is essential when developing management systems based on resistant cultivars and non-host rotation crops. An immediate practical solution is to use a molecular test (Stanton *et al.* 1997) to identify nematode populations in the farming system and region of interest and then screen potentially useful rotation crops for resistance to those nematode populations only. In practice, it is preferable to recommend rotation crops which are resistant to all or most of the nematode populations present rather than to make recommendations for individual crops. This approach has been successful in developing suitable rotations for Queensland's vegetable (Stirling *et al.* 1996) and tobacco (Stanton 1994) industries.

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