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Aspects of resistance in deepwater rice to the stem nematode *Ditylenchus angustus*

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Summary – A seedling based technique for screening for resistance to *D. angustus* in deepwater rice was developed and used to examine components of variability in host suitability. The techniques mimicked natural infection from water and achieved 100 % infection of susceptible check cv. NC492. Approximately 10 % of inoculum invaded seedlings, the infective stages being predominantly J3, J4 and adult. Reproduction of *D. angustus* was rapid on susceptible plants (period of life cycle was 10-20 days at 30 °C). Symptom expression and nematode multiplication were studied on a range of resistant and susceptible deepwater rice cvs and lines. Resistance is in part conferred by a rapid necrotic response to feeding in the host. The response was exhibited by some plants of lines, namely, CNL 319, Bazail 65, Rayada 16-02, Rayada 16-03 and Rayada 16-06 to Rayada 16-09, which, in the field, have been consistently resistant across locations and years. The response is qualitatively different from the well known susceptible response and provides a basis for genotypic selection. The relationship between water depth and seedling stature appears to be important in determining infection and early symptom development. *D. angustus* invades primarily at the water surface so submergence of the leaf sheath delays infection. Symptoms develop more quickly if the water level during infection is adjacent to, or just below, the collar at the top of the leaf sheath. The expression of symptoms and hence early damage is delayed in shallower water.

Résumé – ***Aspects de la résistance du riz flottant au nématode des tiges,* Ditylenchus angustus** – Une technique utilisant les plants de riz a été mise au point pour cribler la résistance du riz flottant à *Ditylenchus angustus*. Cette technique copie l'infestation naturelle à partir de l'eau et permet une infestation de 100 % sur le cv. témoin sensible NC492. Environ 10 % de l'inoculum pénètre dans les racines, les stades infestants étant surtout représentés par les J3, J4 et adultes. La reproduction de *D. angustus* est rapide sur les cvs sensibles (la durée du cycle est de 10 à 20 jours, à 30 °C). L'expression des symptômes et la multiplication du nématode ont été étudiées sur une série de cultivars et lignées de riz flottant, tant sensibles que résistants. La résistance est en partie conférée par une réaction nécrotique rapide de l'hôte lors de la prise de nourriture du nématode. Cette réaction est présente chez quelques lignées (CNL 319, Bazail 65, Rayada 16-02, Rayada, 16-03, Rayada 16-06 à Rayada 16-09) qui se sont montrées en champ résistantes en divers endroits et au cours du temps. Une telle réaction est qualitativement différente de celle, bien connue, montrée par les plantes sensibles, et peut ainsi fournir une base en vue de la sélection génotypique. La relation entre profondeur de l'eau et taille des plants de riz est d'une importance cruciale pour le déclenchement de l'infestation et le développement des symptômes. *D. angustus* pénètre dans les plants d'abord au niveau de la surface de l'eau, aussi une submersion des gaînes des feuilles retardera-t-elle l'infestation. Les symptômes se développent plus rapidement si, durant l'infestation, le niveau de l'eau est voisin de ou très peu inférieur à celui du collet situé au sommet de la gaîne foliaire. L'expression des symptômes, et par conséquent les dégâts précoces, sont ralentis en abaissant le niveau de l'eau.

Key-words : Host-parasite relationships, resistance, screening, *Ditylenchus angustus,* deepwater rice, population dynamics, life cycle, Ufra.

The stem nematode of rice, *Ditylenchus angustus,* which causes the disease known as Ufra, occurs in several Asian countries where it has been predominantly associated with deepwater rice (Bridge *et al.,* 1990). Its pest status arises from its potential, in common with other stem nematodes, to overrun its host which has sporadically resulted in complete crop failure, sometimes over large areas. As rice cropping practices shift away from deepwater rice towards more productive lowland systems, the importance of the deepwater crop has been diminished. In Bangladesh, however, where the same shift in cropping practices is taking place (Islam, 1993), *D. angustus* now also infects the lowland transplanted *Aman* and *Boro* crops (Miah & Mondal, 1988). Although the distribution of the nematode in these different crops requires assessment, it is clear that the characteristic pattern of sporadic complete crop failure in endemic areas is occuring in lowland rice in Bangladesh (M. L. Rahman pers. comm.).

Rice cultivars with resistance to *D. angustus* could become a central component of Ufra management. Being preventative rather than curative they would be effective against outbreaks of the disease where perhaps, for socioeconomic reasons, other control measures are

not implemented. Measures such as stubble burning, destroy valuable fodder and chemical protection requires treatment before symptoms are apparent to the farmer. Sources of resistance to *D. angustus* have been identified e.g; Bazail 65, CNL 319 and the Rayada group of lines (Rahman, 1987) which, despite some variation across years and locations, have been effective against populations of the nematode from Vietnam, Bangladesh, India and Burma (Anon., 1986, 1987, 1988). These selections, made in pot, deepwater tank (Rahman & McGeachie, 1982) and field experiments, were based on assessments of the proportion of stems or tillers infected by the nematode, supported in some cases by counts of nematode populations. The exploitation of resistance requires additional screening techniques to enable the testing of large numbers of plants. Techniques are required to characterise genotypes and facilitate selection in the early generation screening of breeding programmes.

The broad objective of this work was to provide information on the host-parasite interaction to support the development of a higher volume screen for resistance to *D. angustus*. The work is presented under two headings. The first deals with practical aspects of inoculating *D. angustus* with associated biological information and the second deals with population dynamics and variability in the host-parasite interaction. The discussion brings together information from both areas to enable an assessment of the technique and the components of resistance to *D. angustus*.

Examination of the precision of inoculation techniques

Several methods have been used to infect rice with *D. angustus,* (Hashioka, 1963; Sein, 1977; Rahman & McGeachie, 1982; Ou, 1985; Rahman & Evans, 1987). Techniques have usually mimicked the field condition described by Cox *et al.* (1980) where secondary inoculum is water borne spreading from primary infection loci within the field. This emphasis has been based on the assumption that barriers to, or escape from, infection may be important components of resistance. Injecting young seedling or leaf sheaths of older tillers would circumvent these mechanisms.

The success of inoculation methods has been assessed in terms of the proportion of susceptible tillers which become infested. However, to discriminate single plant genotypes within segregating generations or heterogeneous rice lines it is necessary to have inoculation techniques which enable homogeneous infection and reduce environmental and error components of variation within and between lines. Precise control of the initial population density/plant (P_i) of *D. angustus* is vital if rates of reproduction are to be used to separate levels of resistance. Variability in P_i may also influence the assessment of variation in host responses of a qualitative nature.

Two of the factors which are likely to affect initial invasion by *D. angustus,* i.e. inoculum infectivity and water depth, were the subject of this study.

Materials and methods

D. angustus was maintained in monoxenic culture on seedlings of lowland rice cv. IR 36 (Plowright & Akehurst, 1992). All the work reported here used a population of *D. angustus* from Hau Giang Province, Vietnam. Unless stated otherwise' experiments were done in a heated glasshouse (25-35 °C) with a 10 h minimum photoperiod. When preparing suspensions of inoculum, J4 and adult stages of *D. angustus* tended to aggregate, particularly if population densities exceeded 500 nematodes/ml. Aggregates were dispersed by prolonged agitation. Counting of extremely active nematodes was achieved by cooling, anaesthetising with CO_2 or by trapping nematodes in a shallow layer of water in a Bridge counting dish (Hooper, 1990). For the latter treatment a trace of detergent was used to lower surface tension. Rice seed was provided by the International Rice Research Institute.

Infectivity of stages of D. angustus

Developmental stage distribution analysis of inoculum from several experiments has indicated that populations harvested from cultures 40-60 days after inoculation have a stable demography and comprise eggs (21 %), J2 (7 %), J3 (14 %) J4 (33 %) and adults (25 %). In order to determine whether equal weight should be given to all stages in terms of infection potential, the infectivity of each was compared.

Developmental stages of *D. angustus* were isolated manually using a low power binocular microscope. Stages were defined from measurements of body length of 246 nematodes (Fig. 1). Adult male and female body lengths overlapped.

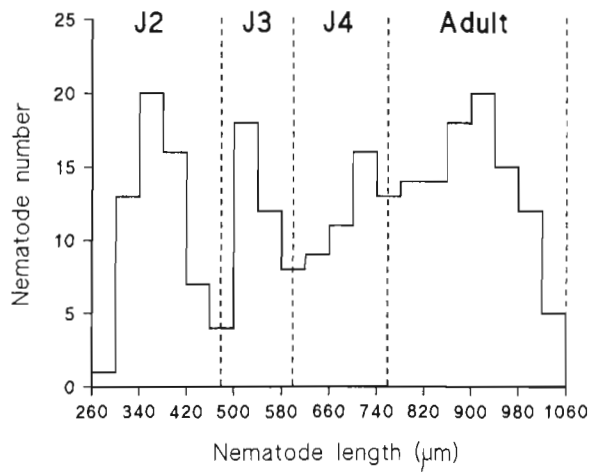

Fig. 1. *The frequency distribution of 40 µm size classes of 246 vermiform stages of* Ditylenchus angustus.

Seedlings or rice cv. NC492 were inoculated using a modification of the coleoptile method (Hashioka, 1963). Seedlings were inoculated in pairs, when they measured 3-5 cm, by introducing 75 nematodes or eggs into 2 ml of water in 7 ml sterile bijou bottles (Sterilin). Replication was four fold.

Seven days after inoculation (DAI) the bottles were filled with boiling acid fuchsin (0.1 % w/v) in glycerol, lactic acid and distilled water (1:1:1) (Bridge *et al.,* 1982). The plants were teased in distilled water and counts of all nematode stages in the seedlings and water were made.

Comparison of inoculation methods

The objective of these experiments was to compare direct injection methods with methods varying the proximity of inoculum confinement at two depths of water at inoculation. Plastic drinking straws provided 6 mm diameter tubes for confining inoculum close to plants. Each straw was cut 4 cm longer than the water depth to allow room for the inoculum volume. Ten, 100 ml pots (vacapots, H. Smith Plastics) of steam sterilised clay loam soil were placed in each of eight deep boxes (30 × 24 × 17 cm) and sown with rice cv. NC492 at the rate of 1 seed/pot. Each box was assigned one of a number of inoculation treatments (Table 1) viz : **1 :** 5 µl of inoculum injected into stems, severed 5 cm above the soil; **2 :** 5 µl of inoculum injected between the leaf sheath and the emerging new leaf and maintained thereafter in a saturated environment which was achieved using an unvented, clear Perspex propagator; **3 :** same as treatment 2 but with diurnal fluctuations in glasshouse relative humidity which was monitored for the duration of the experiment and varied between 60 % and 80 %; **4 :** 500 µl of inoculum released into water of depth 5 cm, adjacent to each seedling but not confined, **5 :** same as treatment 4 but with water depth 10 cm; **6 :** 500 µl of inoculum confined within a 6 mm diameter tube in water of depth 5 cm; **7** : same as treatment 6 but with water of depth 10 cm; **8 :** 500 µl of inoculum confined using a 2 cm diameter tube in water of depth 5 cm. One hundred nematodes and eggs/plant were inoculated 12 days after sowing and periodic checks of inoculum homogeneity were made in duplicate. Water levels were adjusted and left for 24 h before inoculation to enable water temperature to equilibrate at 30 °C.

Seven days after inoculation all above ground tissue was harvested and stored in plastic bags at – 20 °C. Defrosted plants were stained with hot acid fuchsin (0.1 % w/v/) in equal parts glycerol, lactic and distilled water (Bridge *et al.,* 1982). Stained tissues were cut into 3-5 mm lengths and macerated in 50 ml of distilled water for 5-8 s. Debris was washed strongly on a 313 µm sieve with a further 50 ml of distilled water from a hand pumped spray. Counts of nematodes were made following settling and large numbers were estimated from duplicate 5 % (v/v) subsamples.

Influence of water depth at inoculation

Five rice cvs were selected to provide susceptible and resistant plants of different seedling stature, viz. NC492, tall susceptible, Ratna, short susceptible, Hashiamon, short susceptible, Bazail 65 tall resistant, Rayada 16-06, short resistant. Seed were sown in 100 ml vacapots in deep trays assigned a water depth of 0, 1, 5, 7.5 or 10 cm. A randomised block design with four replicates was used. Twelve days after sowing, seedling height measured to the collar at the top of the leaf sheath was recorded. An inoculum of 250 nematodes was introduced into the volume of water confined by a 6 mm diameter tube placed around each seedling. Water levels were again established 24 h before inoculation. Each day after inoculation observations of symptom occurrence and type (resistant or susceptible) were made on the most recently emerged or emerging leaf. The severity of susceptible symptoms were also assessed by scoring symptoms according to a scale shown in Fig. 2 (Plowright *et al.,* 1992). The number of nematodes/plant was determined, as described above, 7 days after inoculation.

Table 1. *The precision of different techniques for inoculating* Ditylenchus angustus

Inoculation technique	Water depth (cm)	Infected plants (%)	P_i	
			mean	CV
1 Injection severed shoots	5	100	32	22
2 Leaf sheath 100 % r.h.	5	100	22	24
3 Leaf sheath 60-80 % r.h.	5	80	2	39
4 Free in water	5	40	1	41
5 Free in water	10	80	4	39
6 Confined in 6 mm diam. tube	5	100	8	16
7 Confined in 6 mm diam. tube	10	100	6	46
8 Confined in 2 cm diam. tube	5	70	3	42

1-3 : inoculum volume = 5 µl
4-8 : inoculum volume = 500 µl
CV = Coefficient of variation

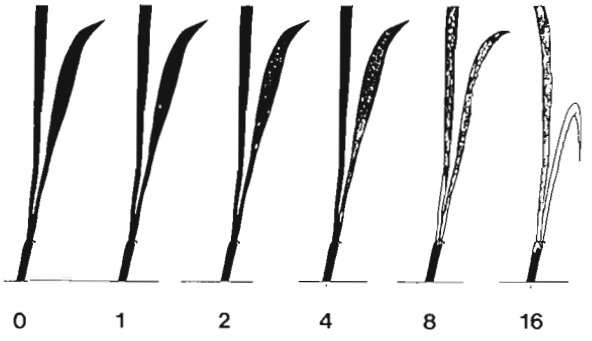

Fig. 2. *A scheme for scoring the severity of susceptible symptoms of infection by* Ditylenchus angustus *on rice.*

Results

Infectivity

All vermiform stages invaded the plant (Fig. 3). The proportion of infective J2's that were either hatched from eggs or inoculated was very low and these did not moult. Only J3, J4 and adult inoculum established reproducing populations composed of eggs and all vermiform stages. An estimate of the infectivity of each stage (the sum of the inoculated and older stages [up to adult] in plants, expressed as a proportion of the number inoculated) indicated that infectivity was ranked J4 > adult > J3 > J2 > egg and that approximately 23 % of an inoculated population of vermiform stages in equilibrium is infective. The J4 also had the highest reproductive potential (Fig. 3). Low numbers of J3's were found in reproducing populations suggesting a rapid moult to J4 and adult which is further supported by the predominance of these stages in the demographic equilibrium of cultures (see above). The presence of J4 in plants only 7 days after inoculation with adults indicates a very short life cycle of approximately 10 days at 30 °C.

Inoculation methods

A 100 % infection of inoculated plants was achieved by direct injection of severed shoots, leaf sheath inoculation and by confining inoculum within a 3 mm radius of plants (Table 1). Successful infection by leaf sheath inoculation required a high, stable relative humidity. Fluctuating humidity, wider or no confinement of inoculum, produced a reduction in P_i (number of nematodes/plant after 7 days) and the proportion of infected plants. Direct injection and leaf sheath inoculation in a saturated environment established a higher P_i than other treatments which mimicked natural invasion from water. Leaf sheath inoculation required small (5-10 µl) inoculum volumes which created the additional problems of inoculum heterogeneity through nematode aggregation. Coefficients of variation were generally high but indicated that confinement of inoculum in a water depth of 5 cm was the least variable (C of V 16 % Table 1).

Effect of water depth on infection

At inoculation, rice cv. NC492 was taller than the other cultivars (P = 0.05) and the cultivar differences in seedling stature were consistent with the basis of selection although the differences were small (Table 2). As in the previous experiment the population in plants 7 DAI was equivalent to ⩾ 10 % of inoculated number. Combining water depth treatments indicated that more nematodes invaded rice cvs Ratna and NC492 than Rayada 16-06 or Hashiamon; Bazail 65 was intermediate between both pairs (P > 0.01). This ranking of cultivars, however, was not the same at each water depth (cultivar × water depth interaction P > 0.05). In general infection of cvs NC492, Bazail 65, Rayada 16-06 and Hashiamon was independent of water depth whilst more nematodes invaded cv. Ratna in water depths of 0, 1 and 5 cm than at 7.5 and 10 cm (Table 2).

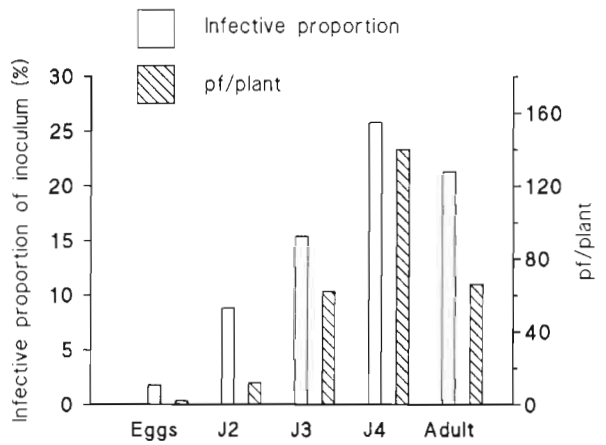

Fig. 3. *The relative infectivity of stages of* Ditylenchus angustus *in rice.*

Table 2. *Influence of water depth on the initial infection of rice by* Ditylenchus angustus

Rice cv/line	Host suitability	Leaf sheath height (cm)	*D. angustus*/plant 7 DAI at water depth (cm) 0	1	5	7.5	10	mean
Rayada 16-06	R	7.6 *bc*	13 *ab*	7 *b*	6 *b*	4 *a*	3 *a*	6 *b*
Bazail 65	R	8.0 *b*	10 *b*	5 *b*	21 *a*	6 *a*	7 *a*	10 *ab*
Hashiamon	S	6.8 *c*	7 *b*	15 *ab*	6 *b*	2 *a*	2 *a*	6 *b*
NC492	S	11.0 *a*	17 *ab*	10 *b*	18 *a*	4 *a*	12 *a*	12 *a*
Ratna	S	7.0 *bc*	22 *a*	24 *a*	15 *ab*	5 *a*	1 *a*	13 *a*

Data mean of four replicates. In a column treatment means having a common letter are not significantly different. P = 0.05. Host suitability : R = resistant, S = susceptible.

The appearance of symptoms in the young leaf varied with rice cultivar and water depth. With some exceptions, more plants had symptoms within 7 days of inoculation, when the water level at inoculation was adjacent to, or just below the collar at the top of the leaf sheath (Table 3). Combining cultivars, more plants showed symtoms in water of depth 7.5 and 10 cm, than at 0 and 1 cm P = 0.05 (Table 3). Although all the cultivars were infected at water depth 0 cm, only Bazail 65 exhibited symptoms within 7 days of inoculation. In Ratna and NC492 and to some extent Rayada 16-06 symptom expression appeared to be delayed in shallower water, for example, NC492 showed symptoms 3 D.A.I. only in 10 cm of water and cv. Ratna showed symptoms within 24 h in 7.5 cm water but after 5 days in 5 cm. We have now found that at optimum water depth for symptom expression both resistant and susceptible responses can be expressed within 24 h of infection.

Nematode population dynamics, symptomatology and variations in host responses

Materials and methods

Population dynamics

Seeds of resistant cvs Rayada 16-06 and Bazail 65 and susceptible cv. Habiganj Aman I were sown in vacapots in deep boxes. Fifty seeds were sown in boxes, each containing a single cultivar, and five seeds of each cultivar were sown in a further eight boxes. Ten days after sowing each plant was inoculated with 100 *D. angustus* using 6 mm diameter tubes; water depth being adjusted to 7-8 cm. This depth approached the collar at the top of the leaf sheath in the majority of plants. The tubes were removed 7 DAI. Five randomly identified plants from single cultivar boxes were harvested at 2 day intervals following inoculation. All the plants in one of the remaining boxes were harvested at 7 day intervals after day 20, giving a total of 18 harvests. At each harvest, counts of eggs and all vermiform stages were made as above. One plant of each cultivar was cut into 2 cm sections, above the peduncle, to examine the distribution of nematodes within the leaf sheath and basal leaf regions.

Variations in host response

Forty predominantly deepwater rice cultivars and lines (Table 4) were selected for inclusion in this experiment. Selection was based on citations of their susceptibility or resistance to *D. angustus* in the field, although ten untested lines from the 1989 International Ufra screening set were also included. The entries were sown in four completely randomised blocks in 100 ml vacapots in deep boxes (20 planting stations/box). Twenty plants of a susceptible check entry NC492 were sown at random throughout each block. Seedlings were inoculated with 300 *D. angustus,* as described above, 11 days after sowing and tubes were removed 7 days after inoculation. The plants were examined daily and symptoms of *D. angustus* infection were scored 7, 14, 21 and 28 days after inoculation using the scale shown in Fig. 2 (Plowright *et al.,* 1992). After the final assessment, all plants were cut at soil level and stored in plastic bags at – 20 °C. Counts of eggs and nematodes were made in the usual manner.

In a second experiment examining intravarietal variation in host response, 100 seeds of deepwater rice Rayada 16-06 were screened following precisely the same procedure.

Results

Population dynamics

Approximately 10 % of the inoculated nematodes invaded seedlings of both resistant and susceptible varie-

Table 3. *The influence of water depth on occurrence of symptoms in rice plants within 7 days of infection by* Ditylenchus angustus

Rice cv/line	Host suitability	Leaf sheath height (cm)	Proportion of plants with symptoms (%) water depth (cm)				
			0	1	5	7.5	10
Rayada 16-06	R	7.6 *bc*	0 *a*	0 *a*	0 *a*	25 *a*	25 *a*
Bazail 65	R	8.0 *b*	50 *ab*	25 *b*	75 *ab*	100 *a*	100 *a*
Hashiamon	S	6.8 *c*	0*a*	50 *a*	0 *a*	50 *a*	25 *a*
NC492	S	11.0 *a*	0 *b*	25 *ab*	0 *b*	0 *b*	75 *a*
Ratna	S	7.0 *bc*	0 *b*	0 *b*	75 *a*	75 *a*	50 *a*
Mean			10	20 *b*	30 *ab*	50 *a*	55 *a*

Host suitability : R = resistant; S = susceptible. Leaf sheath heights with a common letter are not significantly different (P = 0.05). In a row, means of symptom occurrence with a common letter are not significantly different P = 0.05.

Table 4. *Reproduction of* Ditylenchus angustus *on 40 rice cultivars and lines, 28 days after inoculation.*

Rice cv/line	IRTP No.	ST	*D. angustus*/ plant	Rice cv/line	IRTP No.	ST	*D. angustus*/ plant
Rayada 16-09	13876	R	13	BKNFR 76046-10/2/5/1/6/0/4	13082	S	588
Rayada 16-11	13867	R	24	BR 425-189-1-6-2-1-2	14902	S	851
CNL 319	06200	R	75	CR 156-5021-207	03199	S	911
Rayada 16-07	13874	R	76	CN 506-147-14-2	11472	S	950
Rayada 16-08	13875	R	80	BKNFR 76046-10-2-5-1-6-0-2	13081	S	998
Bazail 65	13861	R	129	Ratna	01007	S	1066
Hashiamon	13864	S	158	IR 36	00266	S	1074
Karkati 161	13866	R	191	NC 492	12789	S	1118
Gowai 50-9	13865	S	217	BKN 6986-66-2	04528	S	1164
Rayada 16-02	13870	R	222	BR 539-83-4-2-2	14918	S	1164
Rayada 16-06	13873	R	236	BKNFR 76046-10-2-5-1-1-0-1	13079	S	1190
BR 425-189-1-6-1-2	14578	S	284	BR 716-7-2-1-1	14574	S	1195
Habiganj Aman I	06059	S	353	BR 1185-2R-6	14580	S	1196
IR 2307-247-2-2-3	04477	S	374	IR 40905-11-3-3-4-2-21	16122	S	1292
CNL 231B/B	06201	S	408	Rangabao	15057	S	1357
IR 50	07847	S	435	CNM 539	11470	S	1472
Padmapani	15025	S	458	BR 425-189-1-6-2-1-2	14902	S	1578
Rayada 16-03	13871	R	542	IR 40905-11-3-1-6-3-21	16119	S	1625
PJNB 96-10-1	15053	S	565	BKNFR 76045-85-1-1-1-0-1	13078	S	1665
Cula	05689	S	585	BR 539-83-4-2-2	14918	S	2002

Data are means of four repetitions. Standard error of difference between two means = 441 (P = 0.01). ST = Symptom type.

ties. J3, J4 and adult stages had invaded by day 2, J2's were not present until day 8 and were associated with a peak in egg numbers. The dynamics of the population were characterised by a series of rising peaks and troughs (Fig. 4) and, within a variety, the timing of fluctuations in number of eggs and all vermiform stages coincided. The period between peaks of egg production, 10-20 days, was considered to approximate to the nematode generation time and indicated little difference between resistant and susceptible varieties (Fig. 5). Similar proportions of the harvested plants of resistant and susceptible rice were infected by 8 DAI, but observations of all plants in the experiment revealed symptoms in a greater proportion of resistant plants than in susceptible. By 80 DAI more plants of Habiganj Aman I remained infected with *D. angustus* than either of the resistant cultivars (Table 5). Nematode numbers increased more rapidly and to higher levels in the susceptible cultivar (P = 0.05) (Fig. 6). The population of *D. angustus* declined to zero in Rayada 16-06 after flowering; a small population of eggs remained in one plant of Bazail 65 whilst in Habiganj Aman I a population of J4's and adults remained. Nematodes were distributed throughout the leaf sheath above the peduncle, numbers declining toward the base and top of the culm and distributed about a peak density. There is some evidence that whilst the distribution of nematodes remained the same, the position of peak population density migrated upwards as the plant matured (Table 6).

Variations in host response

Symptoms were apparent on the most recent leaf of some cultivars within 2-3 days of infection. Symptoms on some plants of resistant entries, namely, Bazail 65, CNL 319, Karkati 161, and the Rayada group of lines differed from normal Ufra symptoms. In these plants a browning response rapidly followed chlorosis of affected tissue and was visible 1-2 days after initial symptoms. The response was varied but took one or more of three

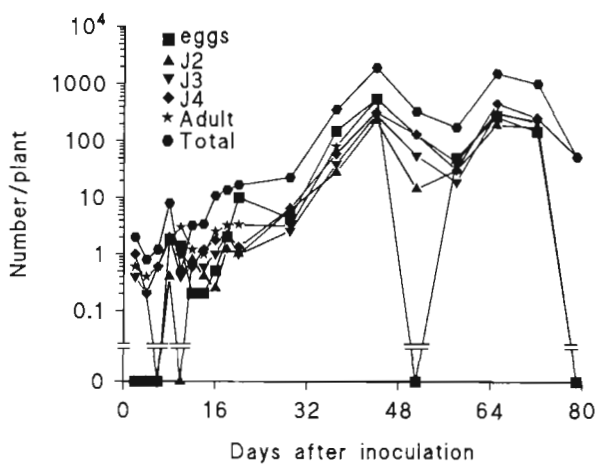

Fig. 4. *The population dynamics of* Ditylenchus angustus *on deepwater rice cv. Habiganj Aman I.*

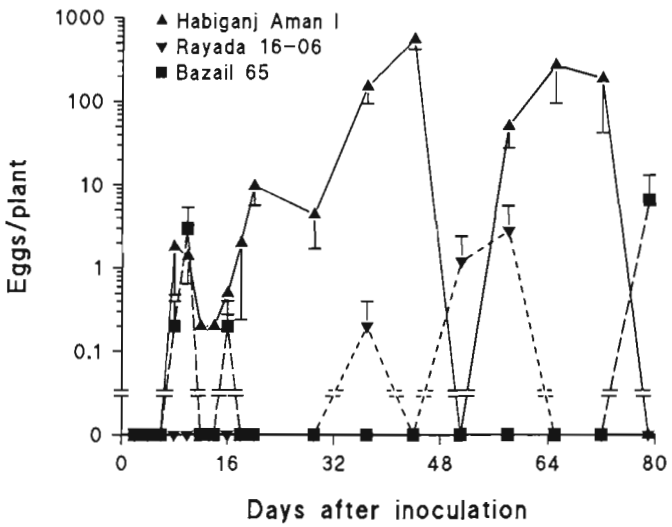

Fig. 5. *The dynamics of egg populations of* Ditylenchus angustus *on susceptible deepwater rice cv. Habiganj Aman I and resistant cvs Rayada 16-06 and Bazail 65.* (Error bars indicate standard error of means).

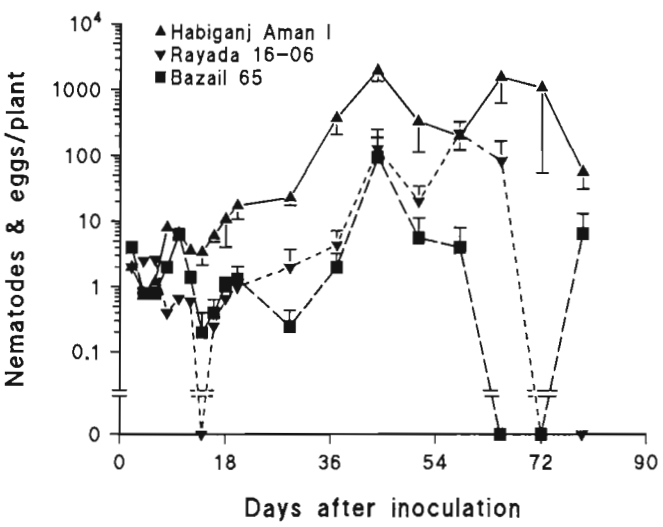

Fig. 6. *The population dynamics of* Ditylenchus angustus *on susceptible deepwater rice cv. Habiganj Aman I and resistant cvs Rayada 16-06 and Bazail 65.* (Error bars indicate standard error of mean).

Table 5. *Differences in infection by* Dytilenchus angustus *of resistant and susceptible deepwater rice.*

Rice cv/line	Host suitability	Infected plants (%)		Plants with symptoms (%)
		8 DAI*	80 DAI**	8 DAI**
Bazail 65	R	56	35	91
Rayada 16-06	R	47	35	53
Habiganj Aman I	S	65	80	28

Host suitability : R : resistant, S : susceptible, from field assessments
*n = 20 observations
**n = 90 observations

Table 6. *The distribution of* Ditylenchus angustus *within the leaf sheath of deep water rice prior to the onset of flowering.*

Days after infection	Population range and peak density height above penducle (cm)	
	Range	Peak
8	2-6	4
14	0-12	2
20	8-12	8
27	0-12	6
34	0-18	8
41	0-16	6
48	0-20	10
55	0-22	12

forms : *i)* Browning of the penultimate leaf base; *ii)* Browning of the midrib, sometimes along its entire length; *iii)* Browning within a more or less discrete, yellowish halo on the leaf lamina (Fig. 7). Subsequent leaves were often symptomless. Occasionally the response was noted in later leaves, both in plants which had previously exhibited the symptom or those that had been symptomless. Some plants of resistant entries remained symptomless throughout the 30 day experiment.

All plants of the susceptible entries exhibited symptoms, but the speed of development and severity of symptoms varied between entries. For example 7 DAI, symptoms on cv. Ratna had reached a severity of 8, whilst symptoms on cv. Habiganj Aman I required 30 days to reach the same severity and were symptomless 7 DAI. There was slight variation between plants of the susceptible check cv. NC492. Seven DAI, 25 % were symptomless and 44 % were scored 4, although at 30 DAI 97 % of plants were scored 8 or 16 and none escaped infection. In some cultivars, for example Hashiamon and Gowai 50-9, which exhibited a susceptible response, the mean reproduction of *D. angustus* was equivalent to that of some resistant lines (Table 4).

Some plants of all the resistant entries supported reproduction of *D. angustus* e.g. one replicate plant of Bazail 65, CNL 319 and Karkati 161 supported populations of 450, 300 and 735 *D. angustus* respectively. A similar variation was evident amongst the Rayada group and 11 of 28 plants supported a multiplication factor of > 2. Within a population of 100 plants of Rayada 16-06 individuals displayed either resistant (49 %) or susceptible (38 %) responses or were symptomless (13 %) the

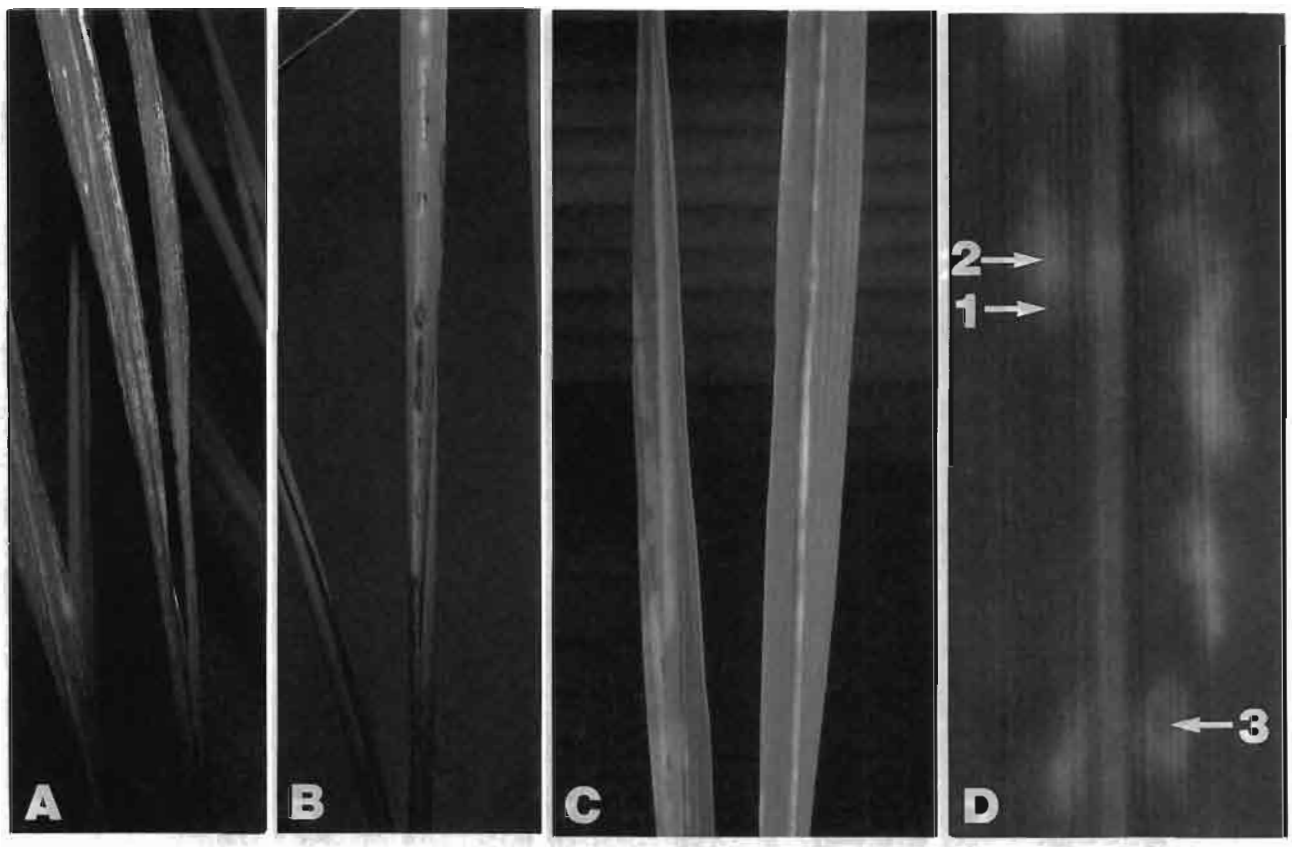

Fig. 7. *Responses of deepwater rice to infection by* Ditylenchus angustus. *A : Susceptible : B-D : Resistant : note necrosis of mid-rib and pale green-white patches on the leaf blade sometimes with a rectangular appearance. In high magnification of leaf blade (D) note pale green halo (1) around discrete yellow-white area (2) which precedes necrosis (3).*

response being consistent with the presence and number of nematodes (Table 7). Within the susceptible group, symptom severity rating 28 DAI was linearly correlated with number of *D. angustus* ($r = 0.697$, $P < 0.001$). No such correlation was found in the resistant group.

Scores of symptom severity for all entries made 28 DAI, were linearly correlated with number of *D. angustus*/entry ($r = 0.735$, $P < 0.001$) (Fig. 8). The relationship did not hold for symptoms assessed at earlier times. Analysis of the mean number of *D. angustus*/plant of all entries indicated significant differences ($P = 0.001$) (Table 4). Variations among resistant entries were not significant, but among susceptible entries (resistant entries removed from the analysis) there were differences in the level of susceptibility ($P = 0.01$).

Table 7. *Variations of host response of deep water rice, Rayada 16-06 to infection by* Ditylenchus angustus.

Host response	Proportion of plants	*D. angustus* pf/plant
Symptomless	13	0
Resistant	49	2 (0-13)
Susceptible	38	46 (6-572)

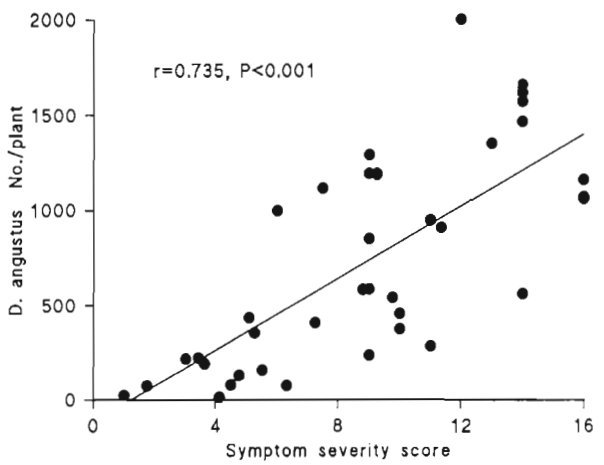

Fig. 8. *The relationship between symptom severity and reproduction of* Ditylenchus angustus *in deepwater rice 28 days after inoculation.*

The coefficient of variation of susceptibility for the susceptible check was high (40-50 %) which enabled differentiation of levels of susceptibility differing by a multiplication factor (P_f : actual P_i) of approx. 29-30, i.e. approx. 900 nematodes. Estimates of error variance suggest that 10 fold replication would be required to reduce the Least Significant Difference to 300 nematodes.

Discussion

The screening techniques used in this work revealed significant differences in resistance to *D. angustus* (defined solely in terms of the influence of the host on nematode reproduction, see Cook and Evans, 1987) between cultivars and lines of deepwater rice. In common with other stem nematode host-parasite relationships (Bingefors, 1970; Williams, 1972; Cook & Evans, 1988; Stanton *et al.,* 1984; Whitehead *et al.,* 1987), there was a good correlation between symptom type and severity and the number of nematodes infecting the plant (Fig. 8). Resistant plants were symptomless or exhibited a response involving rapid necrosis (Fig. 7). The prevalence and severity of symptoms which precede browning is initially greater in resistant plants (Table 5) which suggest that the response is of a hypersensitive nature. The current whole plant studies precluded observations of new leave until they emerged from the leaf sheath so the time scale of events is imprecise. Subsequent studies (Plowright & Gill, unpubl.) have indicated that the response has a growth cost which is particularly obvious in very young seedlings (< 10 Days). The consistently resistant lines from field trials e.g. CNL 319 and the Rayada lines exhibit this necrotic response to feeding by *D. angustus* in glasshouse tests and it is reasonable to assume that resistance, at least in part, is derived from this host response. Symptomless plants are escapes, which have avoided infection either through a failure of technique or as a consequence of a disease avoidance strategy. The occurrence of symptomless plants harbouring *D. angustus* has been reported (Rahman & McGeachie, 1982; Rahman & Evans, 1987), but we have never found *D. angustus* in plants which remain symptomless throughout the 28 day period of assessment.

In general, low populations of *D. angustus* developed on entries exhibiting the resistant response. However between the extremes of resistance and susceptibility some entries exhibiting resistant and susceptible responses supported similar levels of reproduction (Table 4) and showed no differences in the time required for the nematode to complete a life cycle (Fig. 5). Comparable levels of susceptibility disguise differences in the rate of symptom development and, for example, although mean numbers of *D. angustus* were the same on Rayada 16-06 and Habiganj Aman I at 58 DAI, the rate of population increase was initially greater on the susceptible cv. Habiganj Aman I (Fig. 6). To some extent nematode reproduction on resistant entries can be explained by genotypic variability within a population of plants, for example, the Rayada lines comprise resistant and susceptible genotypes (Table 7). The isolation of nematodes in necrotic host lesions is involved in resistance to *Ditylenchus dipsaci.* But, it is also possible to envisage how such a necrotic reaction might fail to completely restrict an active ectoparasite such as *D. angustus* and thus allow some reproduction.

The principal concern for resistance screening is the extent to which genotypic, environmental and error sources contribute to the observed variability. Answers to these questions indicate important components of resistance. Escapes due to inoculum failure are a possible source of error, particularly when mimicking natural infection of rice by *D. angustus.* The proportion of inoculum invading the plant was consistently low, usually around 10 % of ≤ 300 inoculated individuals and, although increasing inoculum sizes would reduce the risk of errors from escapes, it would be wasteful of nematodes. Achieving P_i's higher than 30/plant is probably unnecessary in view of the very rapid life cycle of *D. angustus.* Variations caused by differences in the demography of populations of *D. angustus* between experiments are likely to be slight, but populations composed of unusually high proportions of the less infective stages, viz. eggs, J2 and J3 (Fig. 3), should not be used.

The relationship between plant stature and water depth appears to be important in determining infection and early symptom development. Table 2 suggests that infection of rice by *D. angustus* is reduced if the leaf sheath is completely submerged. It follows that the nematodes invade primarily at the water surface. Symptom development is more rapid if the water level coincides with the top of the leaf sheath and is delayed in shallower water. The appearance of symptoms at the base of the leaf blade in rice at all growth stages points to a preference for tissue with meristematic activity. Intercalary meristematic activity occurs in this region (Esau, 1965), and probably explains the rapidity of symptom development. Subsequent studies (Plowright & Gill, unpubl.) have confirmed these observations and demonstrated that if infection takes place after tillering has commenced then symptom development is more rapid in the younger, shorter, tiller providing it is not submerged. Severe damage can lead to death of these younger tillers. Disease escape then, can play a role in resistance to *D. angustus.* In the field, however, assuming that nematodes become active for widespread infection at flooding, escape mechanisms would be sensitive to the interrelationships of sowing date, plant growth rate and the onset and rate of flooding. At a given stage of maturity, water depth determines which tillers become infected and the severity of the initial damage to those tillers. Premature flooding could circumvent escape mechanisms and result in early damage of crucial

importance in establishing infection and determining subsequent crop loss.

Barriers to infection may play a role in resistance. Older plants are known to be less easily infected (Rahman & Evans, 1987). Among the few rice cultivars and lines examined in detail in this work (Table 2), there were no consistent differences in the initial infection of resistant and susceptible seedlings. Likewise, similar proportions of resistant and susceptible plants initially become infected, the reduction in the proportion of infected resistant plants (Table 5) being expressed post-infectionally.

From the point of view of screening, seedling and young plant responses agree closely with field assessments made of the host suitability of mature plants and provide a strong basis for genotypic selection. Poor correlations between assessments of levels of susceptibility made in successive screening trials and the sensitiviy of these assessments to inoculation technique, appear to be a feature of screens for resistance to stem nematodes e.g. (Williams, 1972). Such variability has been a feature of our studies and is also evident from field screening results. Rice cv. NC492, which is a susceptible cultivar, was among the best resistant entries in field trials in 1987 (Anon., 1988). This variability is also expressed in the sporadic nature of crop losses (Bridge *et al.*, 1990). Differences in seedling stature and rate of growth make it difficult to optimise water depth for nematode infection and symptom development for mixed entries in a screen. It follows that it is equally difficult to conduct successive screening trials in precisely the same manner because of temporal fluctuations in plant growth rates. Variability due to differences in seedling stature can be minimised by inoculating younger seedlings where the height of the leaf sheath and water depth is $\geqslant 5$ cm. Repeated inoculations by direct inoculation would be required to effectively eliminate this source of variability.

In conclusion, resistance in deepwater rice is determined, in part, by a postinfectional response (probably of a hypersensitive nature) and seedling growth rate. Selection of the former is possible through seedling based techniques which provide a reliable and field relevant means of increasing the volume of screening programmes. The techniques should be adapted, probably by using repeated, direct inoculations, to eliminate escapes. In this work, symtomless plants have been treated with caution because of their occurrence in known susceptible cultivars. Their occurrence in resistant lines is the subject of further investigation of the mechanisms involved.

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