# Nematode parasites of deepwater and irrigated rice in the Mekong River Delta

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***Abstract.*** *The major root-parasitic nematodes present in deepwater and irrigated rice in the Mekong River Delta (MRD) are* Hirschmanniella oryzae, H. mucronata, *and* Meloidogyne graminicola. *The first two infest more than 90% of the fields and are potentially important pests of irrigated rice in the Delta; the third may be of local importance. Tiem Dot Sam disease (or ufra), caused by the nematode* Ditylenchus angustus, *was a major disease of floating, deepwater, and rainfed lowland rice in the MRD. The nematode is still present and the cultivars grown are susceptible but the incidence of the disease has been drastically reduced within the past 10 yr especially in areas where a winter–spring (W–S) crop is grown. To understand the factors that are responsible for this change, the effects of different water regimes and cultural practices were tested. Direct seeding into water, which is used by many farmers during the W–S cropping season, appears to reduce the invasion of the seedling by the nematode. The absence of deep flooding does not limit the reproduction of the nematode but reduces the severity of the disease and yield loss. The combination of direct seeding in water and water control during the W–S cropping season may be responsible for the decline in importance of Tiem Dot Sam disease in the MRD.*

Many plant-parasitic nematodes are associated with deepwater, rainfed lowland, and irrigated rice. Those of proven or potential economic importance in South and Southeast Asia are rice-root nematodes, *Hirschmanniella* spp.; rice root-knot nematode, *Meloidogyne graminicola*; stem nematode, *Ditylenchus angustus*; and white tip nematode, *Aphelenchoides besseyi* (Bridge et al 1990).

The rice-root nematodes are pathogenic to rice (Fortuner 1974, Babatola and Bridge 1979) and capable of causing yield losses under field conditions (Jairajpuri and Baqri 1991, Prot et al 1992). *Meloidogyne graminicola* causes damage in rainfed lowland and deepwater rice (Bridge and Page 1982, Jairajpuri and Baqri 1991). *Aphelenchoides besseyi* causes white tip disease, which is seed borne and subject to quarantine regulations — it can cause severe yield loss in severely infested fields (Fortuner and Orton Williams 1975).

*Ditylenchus angustus*, the causal agent of Tiem Dot Sam disease (ufra disease in Bangladesh and India) occurs mostly in floating and deepwater rice. However, it can also cause serious damage in irrigated and rainfed lowland rice (Cuc and Kinh 1981). It was a serious problem in four provinces of the Mekong River Delta (MRD) of Vietnam: Dong Thap, An Giang, Hau Giang, and Cuu Long (Cuc 1982) and yield losses ranged from 50 to 100% (Cuc and Kinh 1981). However, its occurrence in the MRD seems to have been drastically reduced in the past 10 yr.

Preliminary and limited surveys have been conducted previously (Cuc et al 1985) but information on the distribution and intensity of the major parasitic nematodes associated with rice in the MRD was limited. In addition, the distribution and importance of *D. angustus*, which has been reported to affect 60,000–100,000 ha of rice in the Delta (Catling and Puckridge 1984), must be reassessed. Moreover, it was important to identify the causes of the decline in importance of Tiem Dot

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Sam disease. This understanding may result in better control of the disease in areas of the MRD where it still occurs as well as in other countries where it is still a major problem (Bangladesh and India) and prevent its resurgence in areas where it is currently under control.

The objectives of the surveys and experiments reported here were twofold:

- To assess the prevalence and intensity of parasitic nematodes associated with deepwater and irrigated rice in the MRD; and
- To study the effects of different water regimes combined with transplanting and direct seeding on the infestation of the seedlings by *D. angustus*, the multiplication of the nematode, and the development of Tiem Dot Sam disease.

## Materials and methods

### Surveys

Two surveys were conducted to estimate the distribution and intensity (population density) of the major parasitic nematodes associated with irrigated and deepwater rice. During the first survey, 276 soil, root, and stem samples were collected in March 1989 in Hau Giang and Cuu Long provinces from the winter–spring (W–S) irrigated crop. During the second survey, conducted in November 1989 and November 1990, 1,640 (10 samples per field) samples of soil, root, and stem were collected in 164 deepwater fields selected at random over five provinces: An Giang (7 fields), Cuu Long (23), Dong Thap (8), Hau Giang (47), and Minh Hai (79). During both surveys, samples were collected between flowering and maturity stage. Information on the area of rice affected by Tiem Dot Sam disease and the area grown in deepwater rice and in irrigated rice during the W–S and the summer–autumn (S–A) cropping seasons since 1976 were collected from the plant protection services in each province.

Nematodes were extracted from 200 $cm^3$ of soil with a combination of sieving and modified Baermann funnel methods (Hooper 1986a) and from 3 g of roots by macerating them for 15 s in a blender and then placing them for 24 h in a modified Baermann funnel (Hooper 1986b). Nematodes were also extracted from 30 g of stems by cutting the stems into 1-cm pieces, teasing them apart, and incubating them for 24 h in a modified Baermann funnel (Hooper 1986b).

### Effect of cultural practices and water regime on Tiem Dot Sam disease

The specimens of *D. angustus* used in all experiments were derived from a culture maintained in a 15-$m^2$ bed at the University of Cantho on variety IR42. The soil used in all experiments was a clay soil collected from a rice field on the experimental farm of the university. This soil was sterilized by heating (100°C) for 30 min. IR42 was used in all experiments. At harvest of all experiments, nematodes were extracted from the stems by cutting the stems into 1-cm pieces, teasing them apart, and incubating them for 12 h in a modified Baermann funnel (Hooper 1986b).

*Effects on infestation.* An experiment was conducted to study the combined effects of water regime and transplanting or direct seeding on the infestation of the young plants by *D. angustus*. The experiments was conducted in 17 cm diam × 20 cm high Wagner pots filled with 1 kg of soil. Three 8-d-old seedlings or three 3-d-old pregerminated seeds of IR42 were transplanted into each pot and at the same time 300 nematodes were inoculated at the soil surface. Fifteen days after inoculation, nematodes were extracted from the stems of the three seedlings.

Eight treatments were used: direct seeding in saturated soil without standing water; direct seeding in 1, 5, 10, or 15 cm of standing water; transplanting in mud without standing water; transplanting in standing water reaching the apex of the stem, or the collar of the leaf sheath. Water levels were maintained at the same levels for the duration of the experiments by daily watering. Treatments were arranged in a randomized complete block design with 10 replications. Average numbers of nematodes observed in the stems in each treatments were compared in pairs using the Mann–Whitney U test. The experiment was replicated twice.

*Effects of flooding on the nematode and the development of the disease.* Two experiments were conducted in 75 $dm^3$, 60-cm high pots filled with 10 kg of soil.

In the first experiment, the soil was saturated and two 20-d-old seedlings were transplanted in each pot. Seven days after transplanting (DAT), 300 *D. angustus* were inoculated in half of the pots and the water level was raised to the level of the highest stem apex and four flood durations were tested in combination with presence and absence of the nematode. The treatments were maintained until 77 DAT; high water level maintained for 7 d, 3 wk, and 5 wk and then reduced to 5 cm. Numbers of stems and infected stems were recorded at 10, 20, 30, 40, and 55 DAT. At harvest, nematodes were extracted from the stems and the grain yield was measured. Treatments were arranged in a randomized complete block design with seven replications. Data were analyzed using the Mann–Whitney U test.

In the second experiment, the soil was saturated and a 15-d-old seedling transplanted into each pot. Five days after transplanting, 150 *D. angustus* were inoculated in half of the pots. Five water regimes or treatments were tested in combination with presence and absence of *D. angustus*: water level raised to 5 cm above the soil surface and maintained at this level from 5 DAT until harvest; water level raised to the level of the apex of the stem at 5 DAT, at 15 DAT, at 25 DAT, or at 35 DAT, maintained at this level until 75 DAT, and then reduced to 5 cm until harvest. Numbers of stems and infected stems were recorded at 15, 25, 35, 45, and 55 DAT. Plants were harvested at the hard-dough stage and numbers of flowers and of filled spikelets were recorded. Nematodes were extracted from all the stems. Treatments were arranged in a randomized complete block design with six replications. Data were analyzed using the Mann–Whitney U test.

## Results

### Surveys

During the survey for root-parasitic nematodes conducted in W–S irrigated rice, *Hirschmanniella oryzae* was detected in all but one sample collected (Table 1). High population densities of this parasite were observed in more than 50% of the samples. Low population densities of *H. mucronata* were recorded in 75% of the samples. *Meloidogyne graminicola* was present in 57% of the samples but at high population levels in only 10% of these.

With a prevalence (percentage of fields infested) of 99% and a mean intensity (population density) of 1,238/$dm^3$ of soil, *H. oryzae* was the most prevalent root-parasitic nematode observed in deepwater rice (Table 2). *Hirschmanniella mucronata* was observed in 96% of the fields but at a lower intensity than *H. oryzae. Meloidogyne graminicola* was observed only in Hau Giang and Cuu Long provinces and *Tylenchorhynchus annulatus* in An Giang, Dong Thap, and Hau Giang provinces.

**Table 1. Numbers of samples[a] with low, medium, and high population densities of three nematodes from the winter–spring irrigated rice crop.**

| | Population density[b] | | |
|---|---|---|---|
| | Low | Medium | High |
| *Hirschmanniella oryzae* | 16 | 108 | 151 |
| *Hirschmanniella mucronata* | 131 | 42 | 3 |
| *Meloidogyne graminicola* | 108 | 24 | 25 |

Source: Cuc and Prot 1992a.

[a] Total of 276 samples taken.

[b] Soil densities of nematodes (per $dm^3$) are low, 0–200; medium, 201–1,000; and high, >1,000. Root densities (per g) are low, 0–20; medium, 21–100; and high, >100.

Table 2. Mean intensity (MI, per dm³ of soil or per g of root) and prevalence (P, % of fields where the species was detected) of root-parasitic nematodes associated with deepwater rice in the Mekong River Delta.

| Nematode species and sample site | Mekong River Delta provinces | | | | | |
|---|---|---|---|---|---|---|
| | An Giang | | Cuu Long | | Dong Tap | |
| | MI | P | MI | P | MI | P |
| *Hirschmanniella oryzae* | | | | | | |
| Soil | 63 | 100 | 1,402 | 100 | 526 | 100 |
| Root | 32 | 100 | 76 | 100 | 12 | 100 |
| *Hirschmanniella mucronata* | | | | | | |
| Soil | 61 | 75 | 274 | 100 | 53 | 73 |
| Root | 7 | 100 | 3 | 100 | 2 | 87 |
| *Meloidogyne graminicola* | | | | | | |
| Soil | 0 | 0 | 115 | 44 | 0 | 0 |
| Root | 0 | 0 | 36 | 52 | 0 | 0 |
| *Tylenchorhynchus annulatus* | | | | | | |
| Soil | 0 | 0 | 0 | 0 | 550 | 50 |
| Root | 9 | 25 | 0 | 0 | 26 | 87 |

| | Mekong River Delta provinces | | | | | |
|---|---|---|---|---|---|---|
| | Hau Giang | | Minh Hai | | Total | |
| | MI | P | MI | P | MI | P |
| *Hirschmanniella oryzae* | | | | | | |
| Soil | 1,224 | 98 | 1,353 | 100 | 1,238 | 99 |
| Root | 82 | 98 | 26 | 100 | 49 | 99 |
| *Hirschmanniella mucronata* | | | | | | |
| Soil | 123 | 96 | 232 | 85 | 191 | 96 |
| Root | 5 | 96 | 4 | 63 | 4 | 77 |
| *Meloidogyne graminicola* | | | | | | |
| Soil | 547 | 42 | 0 | 0 | 408 | 20 |
| Root | 43 | 42 | 0 | 0 | 39 | 20 |
| *Tylenchorhynchus annulatus* | | | | | | |
| Soil | 30 | 10 | 0 | 0 | 261 | 5 |
| Root | <1 | 20 | 0 | 0 | 12 | 11 |

Source: Cuc and Prot 1992a.

*Aphelenchoides besseyi* and *D. angustus* were not observed in any of the samples collected at random. During the surveys, however, severe symptoms of Tiem Dot Sam disease were observed in Dong Thap, Cuu Long, and Hau Giang and *D. angustus* was observed in samples collected from these fields. When statistics on the areas of rice grown during the flood season and of rice affected by Tiem Dot Sam disease are compared, the decrease in Tiem Dot Sam disease seems to be related, at both the provincial (Table 3) and the district (Table 4) levels, to the reduction in area of rice grown during the flood season and an increase in the area of irrigated rice grown during the W–S season.

**Table 3. Areas damaged by *Ditylenchus angustus* and of two forms of land use in five provinces of the Mekong River Delta.**

| Province and year | Damaged area (ha) | Type of rice planted (ha) | |
|---|---|---|---|
| | | Deepwater | Winter–spring irrigated |
| Cuu Long | | | |
| 1985 | 2,798 | 141,970 | 40,824 |
| 1987 | 106 | 108,000 | 58,186 |
| 1988 | 1,133 | 105,845 | 75,366 |
| 1990 | 906 | 95,245 | 80,870 |
| Dong Thap | | | |
| 1976 | 60,000 | 110,839 | 30,165 |
| 1980 | 32 | 110,000 | 72,059 |
| 1981 | 10,000 | 80,000 | 60,815 |
| 1983 | 30,000 | na | 61,785 |
| 1985 | 10 | na | 71,116 |
| 1988 | 8 | 59,000 | 99,273 |
| 1990 | 20 | 15,000 | 141,803 |
| 1992 | 0 | 7,000 | na |
| Hau Giang | | | |
| 1982 | 17,900 | 320,517 | 27,378 |
| 1985 | 7,200 | 296,242 | 56,100 |
| 1988 | 1,284 | 252,068 | 71,287 |
| 1990 | 804 | 210,830 | 104,323 |
| An Giang | | | |
| 1976 | 2,500 | 152,692 | 31,509 |
| 1980 | 210 | 145,875 | 79,066 |
| 1985 | 5 | 83,964 | 97,632 |
| 1990 | 0 | 37,347 | 141,210 |
| 1992 | 0 | 27,000 | na |

Note: na, not available.

**Effect of cultural practices and water regime on Tiem Dot Sam disease**

In both experiments, the number of *D. angustus* observed in the stems of the seedlings 15 d after inoculation was significantly lower when pregerminated seeds were sown in 5, 10, and 15 cm of standing water than when seeds were sown in the absence of water or in 1 cm of standing water (Table 5). When 8-d-old seedlings were used, the numbers of nematodes observed in the stems 15 d after transplanting did not vary with the water level and were not significantly different from the number observed after seeding in the absence of standing water.

**Effects of flooding on *Ditylenchus angustus* and Tiem Dot Sam disease**

In the first experiment, the number of stems observed at 40 and 55 DAT was significantly reduced in the presence of *D. angustus* in all treatments except when plants were maintained under deep flooding for 5 wk (Fig. 1). In the presence of the nematode and under all water regimes, the numbers of stems showing the symptoms of Tiem Dot Sam disease were smaller ($P < 0.05$) than the total number of stems. The number of *D. angustus* present in the stems at harvest appeared to decrease when the period of deep flooding increased (Table 6) — the number of nematodes in plants flooded for 10 wk was

**Table 4. Areas (ha) infested by *Ditylenchus angustus* and of two forms of land use in two districts of Hau Giang Province.**

| District and year | Infested area (ha) | Type of rice planted (ha) | |
|---|---|---|---|
| | | Deepwater | Winter–spring irrigated |
| Phung Hiep | | | |
| 1982 | 2,560 | 27,006 | 1,034 |
| 1985 | 1,199 | 23,468 | 2,900 |
| 1988 | 620 | 22,550 | 4,355 |
| 1990 | 300 | 19,640 | 7,260 |
| Ke Sack | | | |
| 1983 | 1,700 | 10,600 | 2,580 |
| 1985 | 1,300 | 9,070 | 8,000 |
| 1987 | 250 | 7,793 | 10,600 |
| 1990 | 185 | 7,913 | 9,587 |

Source: Cuc and Prot 1992b.

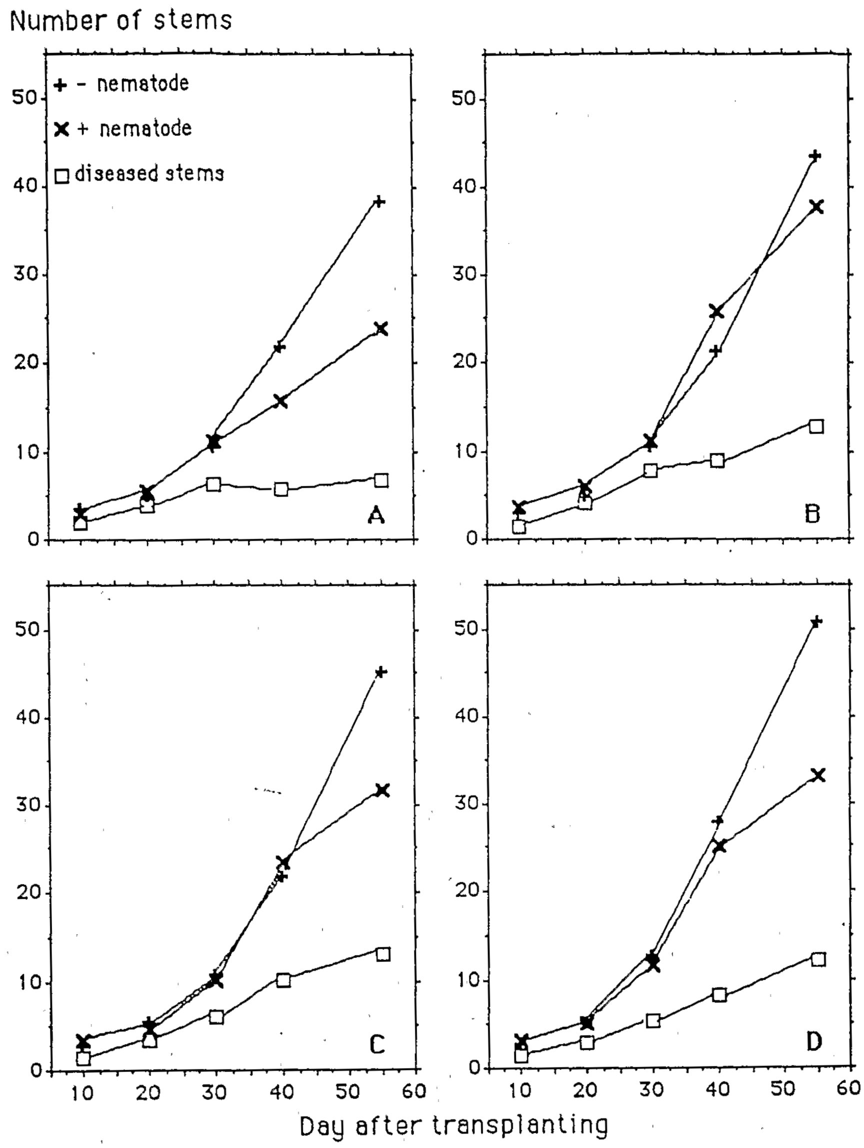
**Table 5. Effects of establishment method and water regime on infestation of young seedlings of IR42 by *Ditylenchus angustus*.**

| Establishment method and water level above soil surface | Number of nematodes in the stems[a,b] | |
|---|---|---|
| | 1st experiment | 2nd experiment |
| Direct seeding | | |
| 0 cm | 107a | 20a |
| 1 cm | 73ab | 15a |
| 5 cm | 9b | 2b |
| 10 cm | 8b | 0b |
| 15 cm | 13b | 0b |
| Transplanting | | |
| 0 cm | 45ab | 15a |
| Stem apex | 53ab | 23a |
| Top of sheath collar | 34b | 19a |

[a] Average of 10 replications.
[b] Within a column, values followed by the same letter do not differ significantly according to a Mann–Whitney U test ($P$ = 0.05).

significantly smaller than in plants flooded for 1, 3, or 5 weeks. Under all water regimes, the grain yield was significantly reduced by the nematode. In the absence of nematode, the yield was significantly reduced by 10 wk of deep flooding when compared to 1, 3, and 5 wk of flooding. In the presence of *D. angustus*, the yield was independent of the duration of the deep flooding.

In the second experiment, *D. angustus* did not significantly increase or decrease the number of stems observed until 55 DAT (Fig. 2). In nematode-infested plants, the number of stems showing the symptoms of Tiem Dot Sam disease was almost equal to the total number of stems in all treatments. The number of panicles was reduced in the presence of nematodes when deep flooding was started at 5, 15, and 25 DAT (Table 7). It was not affected when deep flooding was not applied or started at 35 DAT. In the absence of the nematode, there was no significant difference in number of




1. Effect of duration of deep flooding starting after transplanting on numbers of stems per two hills in presence or absence of *Ditylenchus angustus* and on the number of diseased stems in the presence of the nematode: A, 10 wk of deep flooding; B, 5 wk; C, 3 wk; and D, 1 wk.

filled spikelets between plants subjected to different water regimes. When plants were not subjected to deep flooding, the number of filled spikelets was not reduced by the nematode. When plants were flooded, the number of filled spikelets was significantly reduced by the nematode. Deep flooding at 5, 15, and 25 d seemed to reduce the number of nematodes present in the stems at the hard-dough stage. However, this reduction was significant only when flooding was started at 15 DAT.

## Discussion

*Hirschmanniella oryzae* and *H. mucronata* are omnipresent in irrigated as well as deepwater rice. Their effect on deepwater rice has never been assessed but they can cause yield loss in irrigated rice (Jairajpuri and Baqri 1991, Prot et al 1992) — they are potentially important pests especially if irrigated rice replaces deepwater rice. *Meloidogyne graminicola*, present in 20% of the irrigated fields

**Table 6. Effects of duration of deep flooding (first experiment) on numbers of *Ditylenchus angustus* and grain yield obtained at harvest of IR42 in presence and absence of nematodes.**[a,b]

| Period of deep flooding after transplanting (wk) | Number of nematodes in the stems | Grain yield (g) |
|---|---|---|
| *Ditylenchus angustus* present | | |
| 10 | 551b | 45c |
| 5 | 1,684a | 60bc |
| 3 | 1,805a | 56c |
| 1 | 2,481a | 51c |
| *Ditylenchus angustus* absent | | |
| 10 | 0 | 70b |
| 5 | 0 | 94a |
| 3 | 0 | 106a |
| 1 | 0 | 100a |

[a] Average of 7 replications.
[b] Within a column, values followed by the same letter do not differ significantly according to a Mann–Whitney U test (*P* = 0.05).

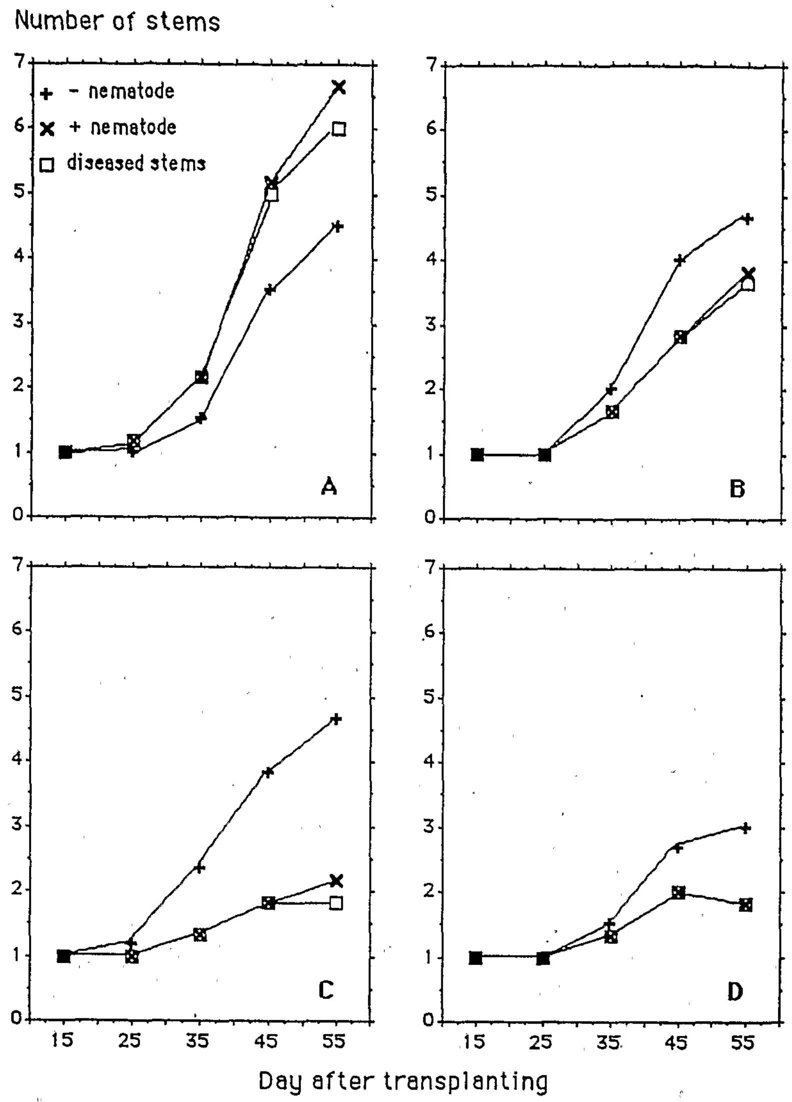
**Table 7. Effects of time and duration of a deep flooding (second experiment) on numbers of panicles, filled spikelets, and *Ditylenchus angustus* observed at hard-dough stage with IR42 in presence and absence of nematodes.**[a,b]

| Period of flooding | Number of panicles | Number of filled spikelets | Number of nematodes/ plant |
|---|---|---|---|
| *Ditylenchus angustus* present | | | |
| No flooding | 9.5a | 691bc | 2,480a |
| 5–75 DAT[c] | 3.3bc | 266c | 1,355ab |
| 15–75 DAT | 4.0bc | 357c | 1,010b |
| 25–75 DAT | 2.7c | 243c | 1,417ab |
| 35–75 DAT | 7.2ab | 560c | 2,699a |
| *Ditylenchus angustus* absent | | | |
| No flooding | 8.3a | 870ab | 0 |
| 5–75 DAT | 8.2a | 1,057ab | 0 |
| 15–75 DAT | 9.2a | 1,206a | 0 |
| 25–75 DAT | 7.8ab | 1,009ab | 0 |
| 35–75 DAT | 10.0a | 1,435a | 0 |

[a] Average of 10 replications.
[b] Within a column, values followed by the same letter do not differ significantly according to a Mann–Whitney U test (*P* = 0.05).
[c] Days after transplanting.

and 57% of the deepwater rice fields, may be of local importance. Because it can damage other crops, the economic importance of the rice root-knot may increase with any increase in crop diversification.

Surveys conducted over the last 5 yr have indicated that the occurrence of Tiem Dot Sam has been drastically reduced but it still causes limited damage in Cuu Long Province and in other areas of the MRD. The decline in the importance of Tiem Dot Sam appears to be related to the reduction




2. Effect of duration and time of deep flooding on numbers of stems per hill in the presence or absence of *Ditylenchus angustus* and on the number of diseased stems in the presence of the nematode: A, no deep flooding; B, deep flooding from 5 to 75 d after transplanting (DAT); C, deep flooding from 15 to 75 DAT; D, deep flooding from 35 to 75 DAT.

in area of rice grown during the rainy season (Cuc and Prot 1992b). This reduction has come mainly from floating and deepwater areas. Many farmers who previously grew deepwater rice now maintain their fields fallow during the flood period and grow two irrigated crops during the W–S (December–March) and S–A (May–August) cropping seasons (Catling 1992). The nematode is still present in the fields and high-yielding modern cultivars grown during the W–S and S–A seasons are susceptible to the disease but, especially during the W–S season, only a few plants are damaged and yield is not affected. It has also been observed that Tiem Dot Sam disease is less severe in deepwater rice in areas where a W–S rice crop has been grown.

Direct seeding into water reduces seedling infestation by *D. angustus*. A similar observation has been made with *A. besseyi*, the white tip nematode, that parasitizes rice leaves (Cralley 1956). *Ditylenchus angustus*, which swims in the water, may be unable to infest the seedlings when they are totally submerged and may starve and lose its ability to invade the seedlings before they emerge from the water. Under conditions prevailing during our experiments, transplantation did not reduce the infestation and the water depth had no effect on seedling infestation by the nematode when seedlings were transplanted.

In our experiments, the multiplication of *D. angustus* was not reduced in the absence of deep flooding. At the same time, however, deep flooding favored the expression of Tiem Dot Sam disease. In these experiments, the expression of the disease and the reduction in yield it caused were not directly related to the ability of the nematode to multiply but deep flooding, especially early flooding, seemed to increase the susceptibility of the rice plant to Tiem Dot Sam disease.

The results obtained suggest: first, that direct seeding in water may reduce or prevent the infestation of the seedlings by the nematode *D. angustus*, the causal agent of Tiem Dot Sam disease. This mode of seeding, used by many farmers during the W–S cropping season, may be partly responsible for the decrease in occurrence of the disease in the MRD. Second, the absence of deep flooding does not result in a reduction of the multiplication of the nematode but in a reduction in the severity of the disease it causes. The absence of deep flooding during the W–S crop may partly explain why the yield loss caused by the nematodes on this crop is minimal in fields where it is present.

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# Vietnam and IRRI: A Partnership in Rice Research

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