Vertical Migration of the Rice White-Tip Nematode, Aphelenchoides besseyi[1]

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Abstract: In the laboratory, the vertical migration of *Aphelenchoides besseyi* was favored by rough surfaces and an inverse water gradient. In relation to gravity, the nematode migrated down or up equally, a circumstance suggesting that a geotaxis was not involved. Stems of rice seedlings were effective surfaces for vertical migration of nematodes only when the stems were continuously supplied with moisture. *Key Words:* behavior, geotaxis.

The literature dealing with the rice white-tip disease and its causal organism, *Aphelenchoides besseyi* Christie, was recently reviewed by Ou (11) and Ichinohe (10). Although no work on vertical migration has been reported, a number of observations indicate the importance of the nematode's migratory behavior to survival, dissemination, and its general life pattern.

Several factors which affect orientation and vertical migration of above-ground, plant-parasitic nematodes have been investigated (1, 2, 3, 9, 18). Some parasitologists have studied the nature of plant surfaces as related to the movement of animal-parasitic nematodes and the abiotic ecological factors involved in vertical migration (4, 6, 12, 13). Studies on the vertical migration of plant-parasitic forms have concentrated primarily on *Aphelenchoides ritzemabosi* (Schwartz) Steiner and Buhrer and *Ditylenchus dipsaci* (Kühn) Filipjev.

In the case of *A. besseyi,* the literature clearly suggests a relationship between migratory behavior and the overall biology of the animal. Apparently, infection of rice plants by the nematode from soil is minimal, whereas the organism was found to spread via irrigation water (19). Tamara and Kegasawa (14) reported that *A. besseyi* did not attack the young roots of rice seedlings. However, once on the rice plant, the animal was found to move from one part to another (8). Todd (15) could not find the nematode in any tissues and concluded that it was an ectoparasite on rice. It has been suggested that the nematode does not overwinter in soil (5) and is disseminated primarily through infested seed (7). Fukano (7) indicated that irrigation water containing infested seeds was a secondary means of spread. A chemical attraction for *A. besseyi* to certain cultivars of young growing rice plants or aqueous extracts of germinating seed has been demonstrated (9). The nematode migrates upward in moisture that forms on the walls of flasks when it is reared on fungi growing on steamed rice (16). We have also observed that worms aggregate in droplets on both covers of Petri plates and on walls of culture tubes.

Since *A. besseyi* must migrate vertically to reach developing seed, we initiated investigations to determine the nature of vertical migration and the factors that influence this behavior. Experiments were undertaken to determine the effects of moisture, texture, surface area, and gravity on migration of *A. besseyi.*

MATERIALS AND METHODS

Nematodes used were from cultures maintained in the laboratory on *Alternaria brassicae* (Berk.) Sacc. grown in potato-dextrose-agar (PDA). The nematodes were originally isolated from rice grown in Potatan, Iloilo, the Philippines. Studies were conducted during December, January, and February when the normal laboratory temperatures were 25-30 C. All experiments were conducted in the dark. The test structures were removed after 18-24 h of incubation, soaked in water, and the water examined for numbers of nematodes present.

Upward migration in a moist environment: Upward migration was determined by using capillary tubing (0.8 mm diam), glass microscope slides (13 x 38 mm),

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wooden match sticks (2 x 2 mm), and rice seedling (*Oryza sativa* Linn., 'Taichung Native I') stems about 4 mm wide. All the materials were 38 mm long. Each structure was placed individually and separately in an upright position in a Syracuse dish previously filled with melted paraffin wax (Fig. 1 A-C). One ml of sterile distilled water was placed around each structure, and 10 handpicked, freshly isolated, active worms were added. Each treatment was replicated 10 times. The Syracuse dishes were placed in enamel pans which in turn were kept in large moistened plastic bags. After 20 h, the structures were removed and soaked in water. Rice stems and match sticks were teased into small pieces and soaked overnight. Nematodes isolated from each structure were counted.

Upward migration in a film of moisture: In addition to the vertical structures utilized in the first experiment, three-ply cotton string (0.7 mm diam) was also included in the next series of tests. A film of moisture was provided to each surface by a string serving as a wick drawing water from a small well. A pair of capillary tubes, match sticks, rice stems, or strings was placed in an upright position in a dish containing minced nematode-fungus culture. Treatments were replicated 10 times. Each structure was individually provided with a small well (Fig. 1-E, 2-B). One of the wells of each pair contained 3 ml of water, and the other was empty.

Two other structures, glass slides and capillary tubes without wells, were also included in the second experiment. Three 13 x 38 mm glass slides, provided with wells, were placed in a dish of minced culture. Two of these slides had smooth surfaces, and the other was roughened with a diamond pencil (Fig. 1-D). One of the smooth slides was allowed to remain dry. A pair of capillary tubes, without wells, was placed in a dish of culture. One of the tubes was half-filled with water, and the other was dry. Each structure was removed after 24 h, soaked, and the number of nematodes on each was determined as previously described.

Surface area: A third experiment was conducted to determine the relationship of surface area to numbers of nematodes climbing glass slides. Three glass slides of the same length were used (Fig. 1-F). Two were of the same width (14-15 mm), but one had a rough surface and the other was smooth. The third glass slide had a rough surface and a narrowed width (7-8 mm). A film of moisture was provided for each slide by a string drawing water from a well. The slides were removed from the culture dishes, and the worms were counted 18 h after initiation. The experiment was replicated 5 times.

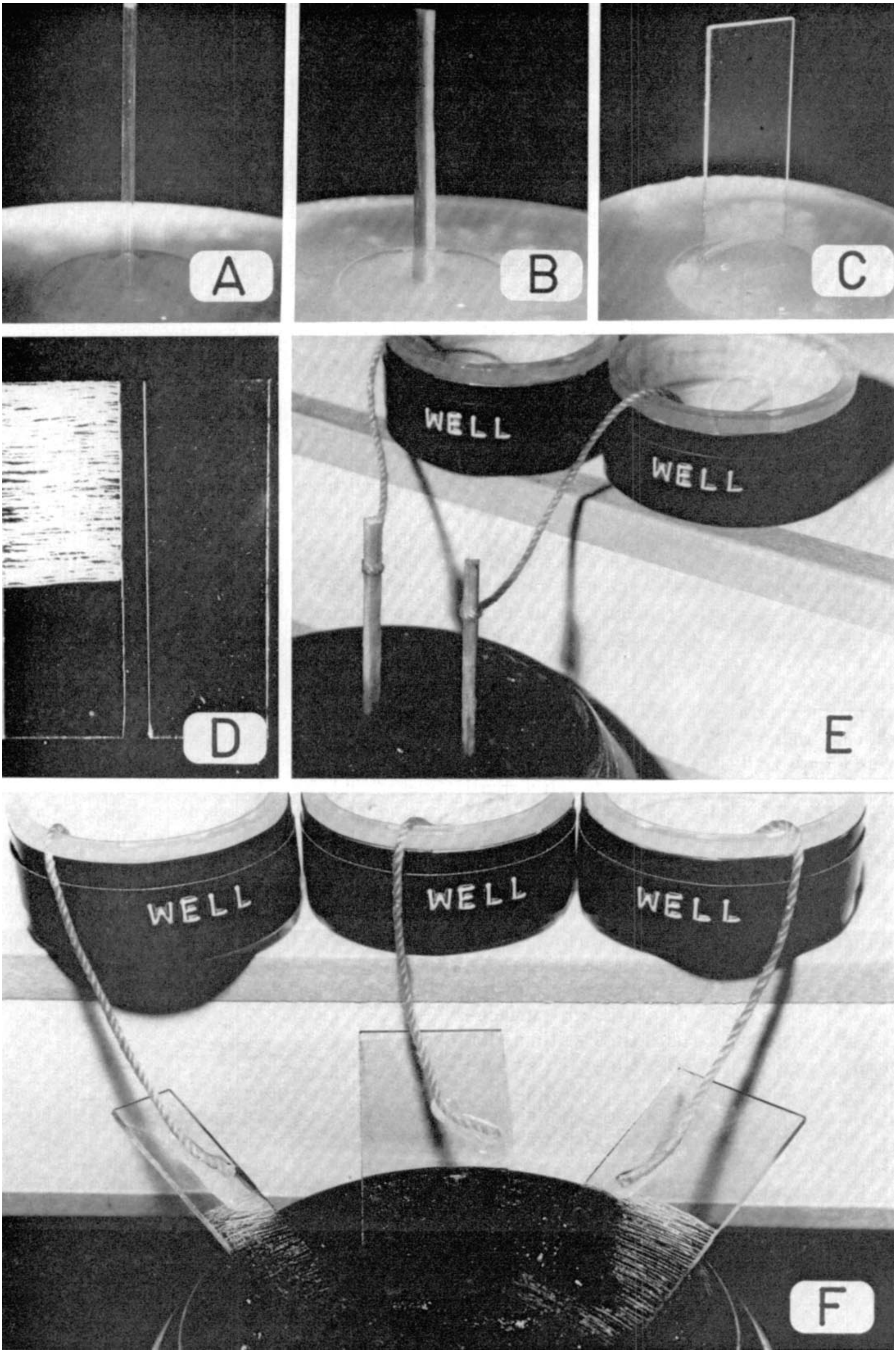
Downward migration in a film of moisture: The descending behavior of the nematode was determined by using a set-up similar to that previously described. Two cotton strings were connected to each of five dishes of minced culture and extended downward into small wells below (Fig. 2-A). One well of each pair of strings was provided with water. Each string was removed after 24 h and soaked before the nematodes were counted.

Vertical migration in relation to gravity: A pair of wells with accompanying cotton strings was attached directly to each culture dish containing the nematodes. One well of each pair was placed above the culture dish, and the other was placed below (Fig. 2-C). In the first treatment, only the upper wells were filled with water, whereas in the second treatment, the lower wells were filled. In the third treatment, both the upper and lower wells were supplied with water. No water was supplied in any well in the last treatment. Instead, water was applied on the culture dishes. All treatments were replicated 5 times. The wells and strings were removed after 20 h and the numbers of nematodes present were determined.

RESULTS

Upward migration in a moist environment: The upward migration of *A. besseyi* on vertical structures from a "pool" of water and in a moist environment was found to vary with surface conditions. The ascent on nontextured, nonabsorbent, surfaces of capillary tubes or glass slides was nil. The rate of climb on rough, absorbent match sticks was high although it was not so on rice seedling stems exhibiting textured surfaces.

Upward migration in a film of moisture: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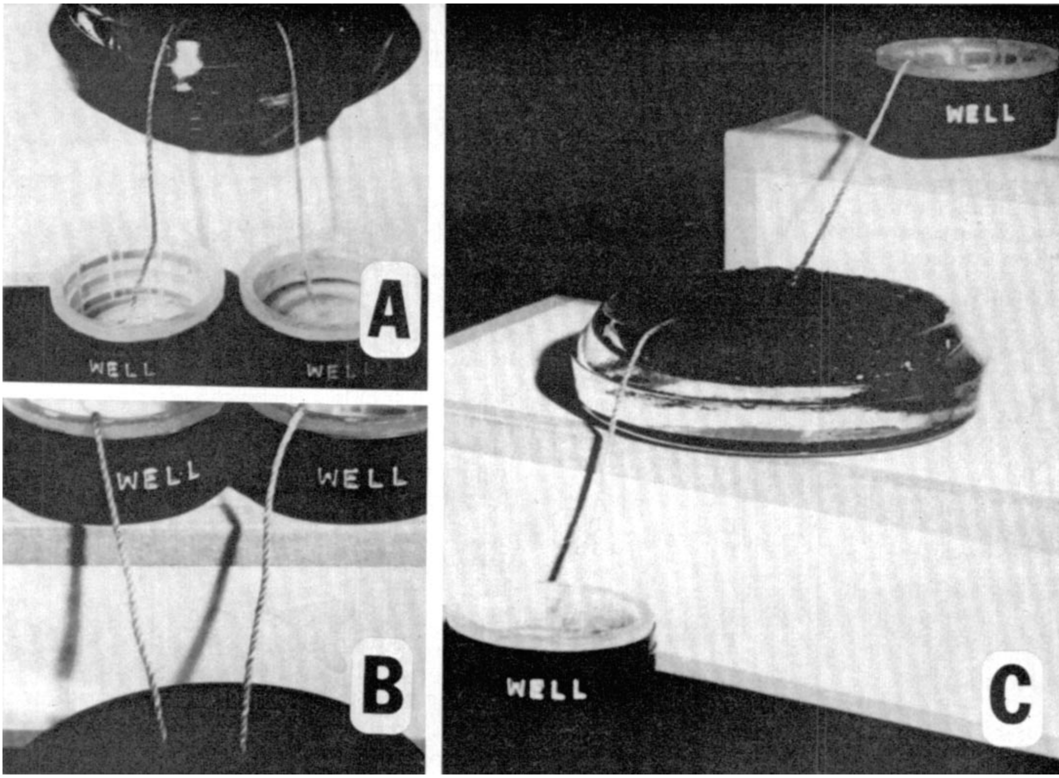

FIG. 2 (A-C). Arrangement of strings, wells, and cultures for vertical migratory studies on the nematode, *Aphelenchoides besseyi. A)* Set-up for descending studies. *B)* Position of material for ascending studies. *C)* Arrangement of materials for tests involving vertical migration in relation to gravity.

The vertical ascent of nematodes in a film of moisture was found to be texture dependent (Table 1). The upward climb of *A. besseyi* on smooth surfaces, regardless of moisture conditions, was almost nil. The number of worms climbing wet and dry capillary tubes did not differ. Likewise, no differences were found in the numbers of nematodes moving up wet and dry smooth-surfaced glass slides. All textured surfaces supplied with a continuous film of moisture (from a source located above the dish) encouraged an upward movement of nematodes. A few worms were observed to move upward on strings connected to dry wells. The string became wet when moisture was imbibed from the culture dish by capillary action.

Surface area: The number of nematodes ascending glass slides was found to vary with the area of the structure (Table 2). The results show that nematodes climbed rough-surfaced glass slides of wider width more effectively than smooth surfaces. More worms climbed roughened 14.5-mm wide slides than those with a 7.5 mm width.

Downward migration: The descending movement of nematodes on string was observed when moisture was initiated from a source located below the culture dish. As in the earlier experiment, strings connected to dry wells became wet as a result of capillary action.

Vertical migration in relation to gravity: The vertical migration of *A. besseyi* was not directly affected by gravity (Table

FIG. 1 (A-F). Structures and arrangements of materials used in ascending migratory studies on the nematode, *Aphelenchoides besseyi. A-C)* Capillary tube, match stick, and "cut" glass slide set-up in wax-filled dishes with "pools" of water. *D)* Rough and smooth slides. *E)* Match sticks—showing the arrangement of vertical structures, strings and wells for migration studies in a film of moisture. *F)* Modified glass slides—showing the position of three different slides (rough thin, smooth wide, and rough wide), strings, and wells for upward migratory studies.

TABLE 1. Upward migration of *Aphelenchoides besseyi* in a film of moisture on various structures.

Structure and treatment	Surface texture[x]	No. nematodes ascending[y]
Capillary tube without well		
A dry surface (control)	NT	0 a
B wet surface	NT	0 a
Capillary tube with well		
A dry well (control)	NT	0 a
B wet well	NT	0 a
Glass slide with well		
A dry well (control)	NT	0 a
B wet well	NT	6 a
C wet well	T	111 b
Match stick with well		
A dry well (control)	T	1 a
B wet well	T	86 b
Rice seedling with well		
A dry well (control)	T	6 a
B wet well	T	90 b
String with well		
A dry well (control)[z]	T	4 a
B wet well	T	113 b

[x]T = Textured; NT = nontextured.
[y]Mean of 10 replications each. Treatment means followed by the same letter do not differ from one another according to Duncan's multiple range test (P = .05).
[z]Strings attached to dry wells became wet due to capillary action.

3). There was significant upward movement of the nematode when only the wells above the culture initially contained water. On the other hand, the direction of movement shifted downward markedly when only the wells below had water. In cases where both upper and lower wells were provided with water, migration in both directions was observed. However, the mean number of nematodes moving upward was greater than that migrating downward. The flow of water from the wells in this treatment was appreciably influenced by gravity. After incubation, the upper wells were nearly empty, but the lower wells were almost full. When all wells were initially dry, migration was comparatively less, but there was significant downward movement of the nematode. Like those in all previous tests, strings attached to dry wells were wet upon removal and tinted with pigment which apparently came from the fungi in the culture dish.

TABLE 2. Upward migration of *Aphelenchoides besseyi* as affected by the surface area of moistened glass slides.

Treatment	No. nematodes ascending[z]
Smooth slide with ca. area of 145 mm^2/cm (control)	2 a
Rough slide with ca. area of 75 mm^2/cm	75 b
Rough slide with ca. area of 145 mm^2/cm	165 c

[z]Mean of five replications each. Treatment means followed by the same letter do not differ from one another according to Duncan's multiple range test (P = .05).

DISCUSSION

The results of the present work showed that *A. besseyi* climbed any roughened surface continuously supplied with moisture or which had the ability to hold moisture. Effectiveness of surfaces for vertical migration was positively related to structure width. *Aphelenchoides besseyi* descended under conditions of a water gradient initiated from below, and in most cases the animal migrated against an opposite gentle

TABLE 3. Vertical migration of *Aphelenchoides besseyi* in a film of moisture on string.

Treatment (position of moisture supply)	No. nematodes migrating (ascending/descending)[a]
Above culture dish[b]	212/3**
Below culture dish	3/210**
Both above and below culture dish	400/99*
Neither above nor below culture dish (in culture dish only)	20/60*

[a]Mean of five replications each. Mean numbers of nematodes ascending and descending were analyzed for each treatment by the paired t-test, (*, ** = significant at 5 and 1% levels, respectively).
[b]In all cases strings attached to dry wells became wet due to capillary action.

flow of water. The data also indicated that the movement was not directional with respect to gravity; *A. besseyi* migrated up or down equally well, a circumstance suggesting that a geotaxis was not involved.

Stems of rice seedlings were found to be relatively ineffective structures for nematode migration when they were wetted from below. However, when moisture was continuously supplied, *A. besseyi* could readily migrate on rice stems.

Most of the data obtained were in general agreement with the concept of greater migration toward the wetter end of a water gradient. In two experiments conducted, the effect of gravity on the flow of water influenced nematode migration. When moisture was applied to the surface of the cultures but not supplied in the wells, it was found that water migrated up and down strings as indicated by its physical presence. In this case, more water and more worms were found below, although generally low numbers of nematodes were encountered. These events can be explained by way of a water gradient hypothesis and gravity. However, when upper and lower wells were initially wet and the cultures relatively dry, it was found that the upper wells were nearly dry while the lower wells were full and pigmented at the end of the experiment. These results suggested that water initially and continuously migrated down from upper wells, while at some point the upward migration of water from the lower wells stopped and reversed its direction or trend of movement. If a gradient was the operating factor, equal or higher numbers of nematodes should have been found descending. In this case, more worms migrated upward. The data suggested that the worms were affected by another factor.

There is little evidence for rheotactic responses of plant-parasitic nematodes. The findings of Wallace (17), and of Barraclough and French (1) showed this was not the case with *A. ritzemabosi*. A form of flow orientation, perhaps as a tactile stimulation, would help to explain the present observation and would be in agreement with the data. Whether this observation was a result of the nature of the experiment or a form of flow orientation remains to be shown.

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