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BIONOMICS OF WHITE-TIP NEMATODE, *APHELENCHOIDES BESSEYI* IN RICE FLORETS AND DEVELOPING GRAINS[1]

C. S. HUANG and S. P. HUANG[2]

Abstract

An apicular "tunnel" of about 30 μ in diameter, circumscribed by the apiculi of lemma and palea, was the only natural opening found to serve as floral infection court except during anthesis. More nematodes were found accumulated around the basal portions of florets or grains. Fleshy tissues such as ovary, stamens, lodicules and embryo therefore were suspected as preferred feeding sites. No nematode was found penetrated into any plant tissue. Population analyses indicated active nematode multiplication in florets before heading. After anthesis, however, nematode reproduction declined with age of the infested grains and was nil about three weeks after heading. The most drastic curtailment in reproduction took place in the beginning of second week after heading, corresponding to "soft dough" stage of grain development. The decline in nematode reproduction was highly correlated to grain dehydration. Development of the second stage larvae (to third stage), however, continued in developing grains up to the 18th day after heading and that of third stage larvae (to adults) lasted to the 30th day when the grains were ripen. In the ripen grains, adult nematodes dominated the population and all of them became extremely sluggish.

Introduction

Floral infections of *Aphelenchoides besseyi* Christie, 1942, the rice white tip nematode, are known to result in distorted glumes, ill-developed grains and in severe casses sterilized florets of susceptible varieties (Yoshii and Yamamoto, 1950; Fukano, 1962; Todd and Atkins, 1958). The nematodes are found coiled under the hulls of ripen grains (Yoshii, 1944; Tamura, 1959; Fukano, 1962) and remained viable in dry grains for up to 23 months (Todd and Atkins, 1958).

How does the nematode penetrate into florets, however, is not yet clear. Yoshii (1944) suggested that the apicular end of floret was the position of nematode penetration. The photographic evidence presented, however, was obscure. Equally unclear are the subsequent activities of the parasite in florets

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(2) Associate Research Fellow and Research Assistant, respectively, Inst. of Bot., Academia Sinica, Nankang, Taipei, Taiwan.

after penetration. Fukano (1962) reported that the nematode multiplied in rice florets much faster than on seedlings where only somatic organs of the host were available for the parasitization. The reproduction and activity of the nematode related to the developmental stages of the infested grains are not known.

This paper reports our investigation on the infection court and activity of the nematodes in developing grains.

Materials and methods

The test nematodes, *Aphelenchoides besseyi*, were initially isolated from infested seeds and reared on *Fusarium solani* in laboratory. To prepare inoculum, nematodes in the fungal cultures were isolated by briefly shaken with small amount of distilled water and the suspension thus obtained placed on Baermann funnels under which glass vials were attached to collect the migrated nematodes. Conidia and mycelial fragments, when present, in the nematode preparations were removed by placing the latters once more onto Baermann funnels using nylon cloth which has porosity of about 150 mesh/ in^2 as filter. Fairly "clean" nematode inoculum, practically free from fungal propagules, could be obtained in this manner.

A *japonica* rice (*Oryza sativa*) Taita No. 1, known to be susceptible, was chosen as test plant. The plants were grown in 23×20 cm clay pots (without bottom drainage) and remained in a green house.

To obtain floral infections, about 3,000 nematodes were poured onto each pots in early tillering stage. The inoculation resulted in floral infection, though the number of nematodes infected varied between florets and the number of infected florets differed with panicles.

From the day of heading, panicles were harvested periodically and the spikelets dissected in small dishes of water to examine the developmental stages of the nematodes. Body lengths reported by Huang *et al.* (1972) were employed to approximate the various larval stages. From each batch of harvests, some of the florets were processed through paraffin sections using TBA procedures (Jensen, 1962) to investigate the infection court and pathogenic effect on the floral parts. After hard dough stage, sectioning of the grains became extremely difficult. To overcome the difficulties, the FAA fixed grains were treated with 15% HF solution (46% stock: H_2O=2:1) for 2–4 days, washed in running water for two days, followed by TBA procedures. The treatments effectively removed silica content in the husk which was the main source of the sectioning difficulties. A much longer treatment with the HF solution caused intolerable damage to the endosperm. The aforementioned treatments, however, did not seem to render serious damages on the general morphology

of aleurone layer, husks and the nematodes between them.

Results

1. *Infection court*

A rice floret consists of a 5-nerved lemma, a 3-nerved palea and floral parts between them (Fig. 1 A and B). Lemma is a larger brack whose two margins respectively bends inwardly to look like a hook in cross sections. The edges of a palea then join the lemma by wedging their outwardly projected ridges under the "hooks" (Fig. 1 E, F and G). Serial sections indicated that the edges of lemma enveloped their palea counterparts in the aforementioned manner along the entire length of a floret (Fig. 1 E, F and G). A "tunnel" of about 30 μ or more in diameter, circumscribed by the apiculi of lemma and palea, is the only opening detectable for a floret before anthesis (Fig. 1 A, B, C and D). During anthesis, lemma and palea dissociate from each other but reunite themselves after pollination.

Nematodes were observed in the apicular "tunnels" (Fig. 1 C and D) in the sections of florets before anthesis but not in the similar stuctures of developing grains. The observations suggested that the apicular "tunnels" were the court of floral penetration and the penetrations did not seem to take place on the developing grains.

As previously indicated, lemma and palea joined each other longitudinally, with their edges curled somewhat spirally, resulted in small spaces on the interior as well as exterior of a floret (Fig. 1 E, F and G). Though nematodes were located in the spaces on both sides of the florets before anthesis (Fig. 1 F and G), serial sections of the latters indicated, however, that the "sutures" of lemma-palea joint was physically inhibitory to nematode penetrations.

2. *Locations of nematodes in florets and developing grains*

Nematodes were distributed in the space in the infested florets, mostly around the ovaries and lower portion of stamens (Fig. 1 E). None of the parasites, however, was found penetrated into any tissue. In the developing grains, nematodes were found only in the space between the inner surface of glumes (lemma and palea) and epicarp. Again most of the parasites colonized in the basal portion of a grain (Fig. 1 H and I), without any indication of tissue penetration.

3. *Reproduction and developments of the nematodes in developing florets and grains.*

For practical purpose, members of an *A. besseyi* population were classified

into second stage larvae (L_2), third stage larvae (L_3) and adults using body length measurements (Huang *et al.* 1972). The adults here included females after 4th stage and apparent males. Males constituted less than 6% of the population (unpublished data). As diagrammed in Fig. 2, a life cycle thus involves reproduction (R), which includes oviposition and hatching, developments of L_2 to L_3 (D_1) and L_3 to adults (D_2).

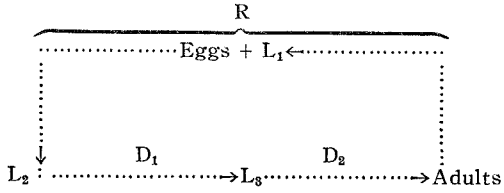
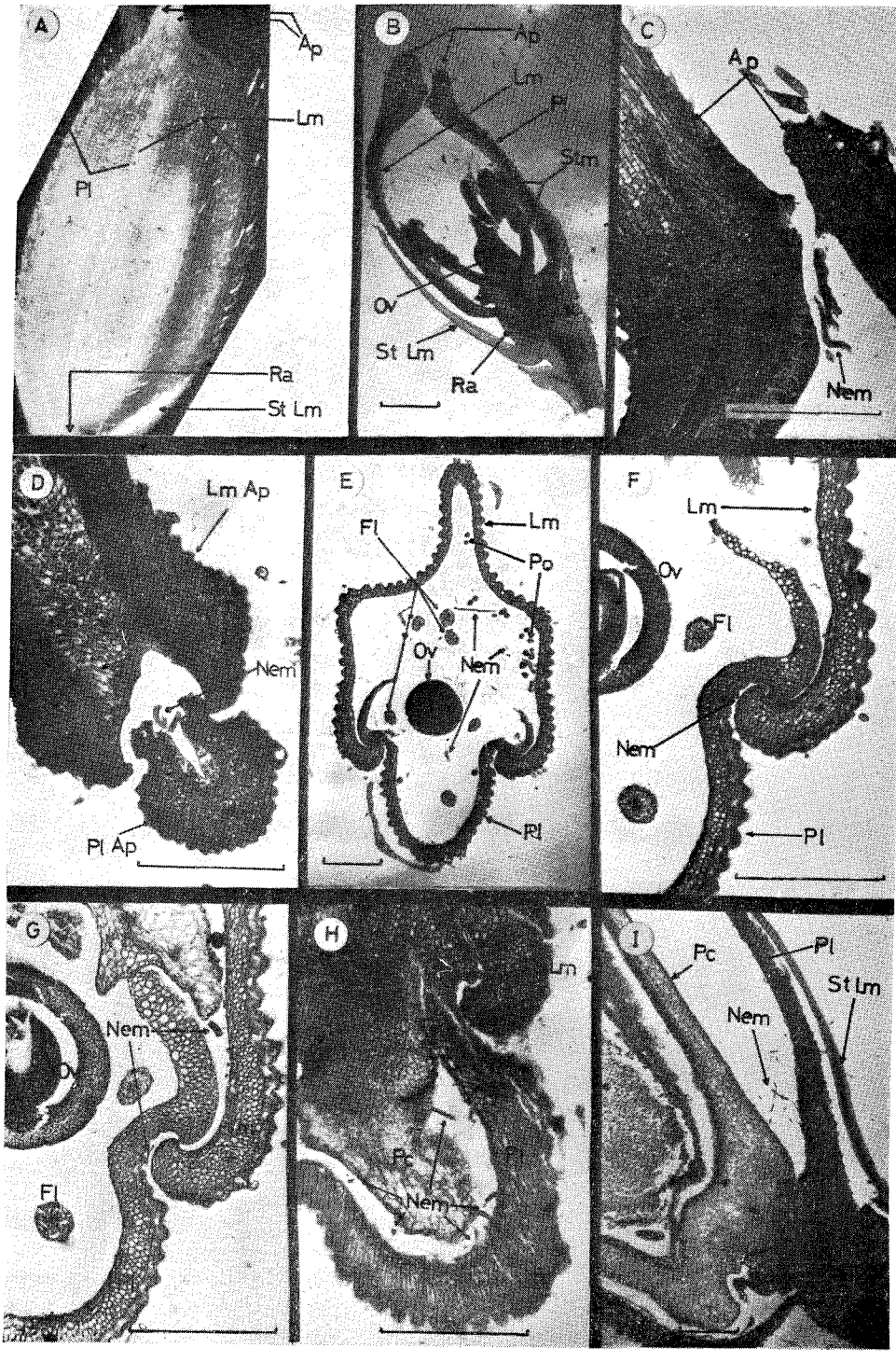


Fig. 2. Diagram of life cycle of *A. besseyi*. L_1=first stage larvae in eggs. See text for explanations for other symbols.

A nematode population should have constant percents L_2, L_3 and adult during the period when it is multiplying at a constant pace, i. e., with constant rates of R, D_1 and D_2. Changes in the rate of R, D_1 or D_2 will invariably alter the L_2, L_3 or adult proportionalities. Thus changes in larval or adult constituencies serve as indicators for the modes of reproduction and development.

To evaluate the efficiency of nematode multiplication in developing florets and grains, panicles from the inoculated plants were harvested at 3-day

Fig. 1 ***Aphelenchoides besseyi*** infection in the florets and developing grains of a japonica rice ***Oryza sativa*** var. Taita No. 1. (A). External morphology of a floret. The palea (Pl) is partially enveloped by the lemma (Lm) along the margins except at the terminal where the apiculi (Ap) circumscribe a "tunnel". A floret is rested on rachilla (Ra). One of the two sterile lemmas (St Lm) is also visible. (B). Longitudinal section of a young floret. Ov=ovary. Stm=stamens. Note the apicular "tunnel" between the apiculi (Ap). (C). Longitudinal section of the apicular portion of a floret just before heading. Note a nematode (Nem) in the "tunnel" between two apiculi (Ap). (D). Cross section of the apicular portion of a floret in the similar developmental stage as that of (C). Note again the penetrating nematodes (Nem) in the apicular "tunnel". (E), (F) and (G). Serial cross sections of a "mature" floret (immediately after heading) from an inoculated plant. Positions of the sections are respectively about 700 μ, 600 μ and 400 μ from the base of palea. Note that numerous nematodes (Nem) are intermingled with ovary (Ov), filaments (Fl) and fallen pollen grains (Po). The spiral manner with which lemma (Lm) and palea (pl) join at the margins is clearly shown. Note the nematodes (Nem) on the exterior side of the floret whose further penetration is "blocked" by the lemma-palea joint. (H) and (I) are respectively the cross and longitudinal sections of the basal portions of grains at soft dough stage. Note nematode (Nem) accumulations on the basal portions of the grains. All nematodes are located only in the space between pericarp (Pc) and hull (Lm and Pl).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interval, and the developmental stages of the nematodes determined. Ten panicles were obtained from each harvesting for replication. In order to minimize the variation of panicle ages, only those emerged from sheaths on the same day were used throughout the investigation.

As shown in Figs. 3 and 4, L_2 and adults dominated the nematode populations in florets on the day of heading, suggesting that during booting stage, the parasites underwent active reproduction.

Percent constituencies of L_2. L_3 and adults went through series of changes after heading (Figs. 3 and 4), indicating that the rates of nematode reproduction and development altered with the age of grains. Following reproduction and developmental alterations can be deduced for the nematode population from Figs. 3 and 4. (1). Percent L_2 declined with concurrent increase in that of L_3 while the adult constituencies maintained roughly on the same level in the first week of heading, indicating that the rate of reproduction decreased, that of D_1 increased, or both, in the period. (2). The rate of nematode reproduction declined even more sharply in the second week after heading, as suggested by the drastic descent in percent L_2 and rapid ascent in adult constituencies during the period. Two weeks after heading, percent L_3 essentially levelled off. (3). 18 days after heading, percent L_3 declined with grain age, that

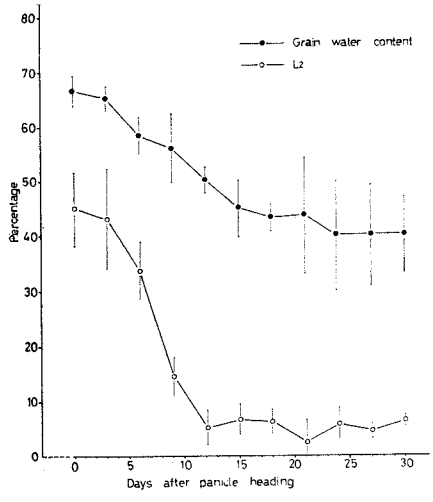

Fig. 3. Grain water contents (w/w) and second stage larval (L_2) constituencies of *Aphelenchoides besseyi* populations in developing grains. Correlation coefficient between the two: $\gamma = 0.865^{**}$

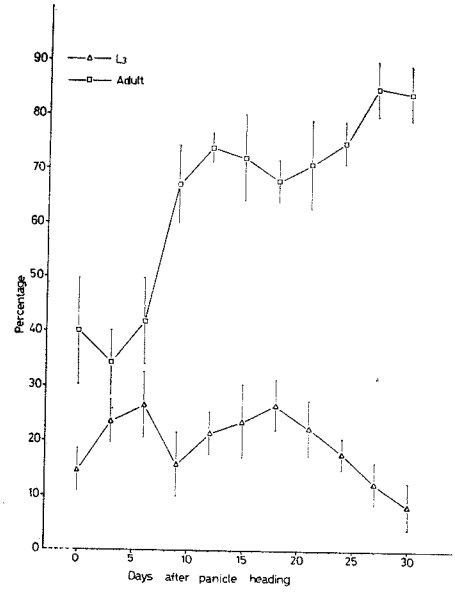

Fig. 4. Third stage larval (L_3) and adult constituencies of *Aphelenchoides besseyi* in developing grains. Adults include females after 4th stage and apparent males.

of adults continued to increase slightly whereas the L_2 constituency remained at very low level. The population changes therefore suggested that in developing grains about 18 days after anthesis, the nematodes experienced obvious D_1 inhibition but D_2 continued throughout the entire period of present investigation. They also indicated that nematode reproduction was nil in the maturing grains. Since the rate of reproduction diminished in grains 18 days after heading and yet percent L_2 still remained at certain level, second stage larvae must be able to survive without further development in the ripening grains.

In order to investigate if nematode reproductions in the developing grains were related to dehydration in grain maturation, a portion of rice from each harvesting was used to determine its water contents. Water contents of developing grains were plotted in Fig. 3. A correlation coefficient, $r=0.856^{**}$, was obtained between grain water contents and percent L_2 in the grains, indicating that the decline in second stage larval constituency was highly correlated with dehydration.

In ripen grains 30 days after heading, adults dominated the nematode populations and all parasites were extremely sluggish immediately after dissected out in water.

Discussion

Apicular "tunnel" (Fig. 1 B, C and D) was the only detectable natural opening for nematode penetration into a floret before anthesis. During anthesis, nematodes on the surface of a floret (Fig. 1 F and G) are likely to continue penetrations. Fukano (1962) demonstrated that on the 14th day after heading, a small number of nematodes were still detectable on grain exterior, but on the 30th day, none was observed. Since the apicular tunnels also exist in developing grains according to our observation, penetration of nematodes after anthesis is also likely, supposedly at the presence of water film. Attempt was not made in the present investigation, however, to verify Fukano's observation.

Accumulation of nematodes around the basal portion of a floret or a grain (Fig. 1 H and I) suggests that fleshy tissues such as ovary, stamens, lodicules and embryo are probably the major sites of feeding. However, no nematode was found penetrated into any plant tissue. No evidence for histological aberrations due to the infection was detected. Mechanisms with which the nematodes render ill-development of grains, distorted glumes and sterility (Yoshii and Yamamoto, 1950; Todd and Atkins, 1958; Fukano, 1962) are therefore problems deserve further investigations.

The nematode is known to parasitize young folded leaves and inner sides

of leaf sheaths only externally (Yoshii and Yamamoto, 1950; Todd and Atkins, 1958 and Fukano, 1962). In mature grains, it is reported only in the space between hulls and pericarp (Yoshii and Yamamoto, 1950; Tamura, 1959; Todd and Atkins, 1958 and Fukano, 1962). Our observations confirmed these reports. In addition, the present investigation further indicated that in the florets and developing grains, ectoparasitism was also the case.

Population analyses showed high second stage larval constituency in the florets before anthesis (Figs. 3 and 4), indicating active reprduction of nematode during booting stage. Eggs were found frequently in florets before heading (unpublished data), suggesting that at least portion of the multiplication took place in the florets. Inasmuch as only nematodes in florets and grains were employed in the population analyses, the data (Figs. 3 and 4) do not exclude the possibility of nematode reproduction prior to floral penetration. Fukano (1962), whose publication was acquired while this paper was being prepared, also reported rapid increase in nematode counts in florets just before heading. The Japanese worker concluded that late booting stage was the time when the nematode reproduced most rapidly, though exact sites of the multiplication were not discussed. Thus the extent of nematode multiplication prior to floral penetration remains to be further studied.

In contrast to florets, our results demonstrate that the environment in developing grains are inhibitory to the nematode reproduction. The most drastic decline in the rate of reproduction takes place in the second week after anthesis (Figs. 3 and 4), corresponding to the "soft dough" stage of grain development. Nematode reproduction continued to diminish with grain age and was nil in the "hard dough" stage. High correlation between percent L_2 and dehydration in grain maturation (Fig. 3) suggests that the latter is a limiting factor in the nematode reproduction. It is not known, however, if other physiological changes in developing grains are also the contributing factors. Nor is it clear whether the reproduction decline is due to decreased oviposition, lessen hatching or both.

A floret (or a grain) is essentially a closed system. Sudakova and Stoyakov (1967) reported that the life span of individual *A. besseyi* is as long as 35–50 days under "optimal conditions". Thus if the initial number of penetrated nematodes is known, subsequent increase in the nematode count should indicate the extent of reproduction and the rate of nematode reproduction in the developing florets (or grains) can therefore be estimated. With the inoculation method employed in this investigation, however, the amount of nematode penetrated is highly variable with florets, even among those of the same panicle. Total nematode count therefore was not considered a valid criterion for the population analyses. Several other methods such as injecting the

nematode suspension into booting leaf sheaths or submerging the latters in the nematode inocula were also tried in an attempt to obtain uniform floral penetration but all yielded poor result. An urgent need is felt, therefore, for an inoculation method which gives controllable amount of floral penetration.

It is interesting to note that whereas the reproduction declines, development of the nematode juvenile nevertheless continue in the developing grains (Figs. 3 and 4). Worth pointing out further is the fact that the development of second stage larvae (to third stage) is terminated in the hard dough stage while that of third stage (to adults) continues. The results clearly indicate that the processes of reproduction (oviposition and hatching) and juvenile developments have different physiological requirements.

The juveniles which stopped developing apparently were able to survive in the developing grains. In the ripen grains, however, adults dominate the nematode populations (Figs. 3 and 4). The results disagree with Tamura (1959) who stated that the nematodes survived in rice seed in larval stages.

水稻白尖病病原線蟲在花器及穀粒內的生態

黃 炤 雄　黃 修 斌

水稻花器的稃尖具有約 30 μ 直徑的天然孔道。病理解剖發現此一天然孔道爲白尖病病原線蟲侵入花器的唯一途徑。侵入後，線蟲大多積聚在花器的基部，顯示柔軟組織如子房、花絲、鱗片 (lodicules) 和胚等皆爲其可能之刺食對象。不過切片從未顯示線蟲穿入任何寄主之組織，證實此一線蟲確營組織外寄生。線蟲在花器內的繁殖率以出穗前爲最高；受粉後則因穀粒的成熟而遞減。線蟲繁殖率的減退，以出穗後第二週即乳熟期最爲急劇。到了第三週即黃熟期已無繁殖可言。線蟲繁殖率的遞減與穀粒成熟過程中含水量的下降有極顯著的正相關。第二齡幼蟲的發育於出穗 18 天以後變得緩慢並漸趨停止狀態，惟第三齡幼蟲之發育顯然可以延續至出穗後第 30 天。因此，成熟的穀粒內部大部份爲成蟲。此時所有的線蟲皆極不活躍。

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