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# Uniform Root Layer Application at Optimal Timing Can Effectively Improve Root-Knot Nematode Disease Control in Rui Yam

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**Abstract:** Root-knot nematode disease seriously threatens the production of Rui yams, making it important to explore effective management strategies, including the optimal time for disease control and efficient application techniques. In this study, we monitored the dynamics of a root-knot nematode J2 population in the soil using field sampling; moreover, we investigated the dynamics of root-knot nematode disease using a field sampling and visual in situ device based on identifying species of root-knot nematodes in Rui yams. Additionally, experiments pertaining to optimal application time and techniques were conducted in Ruichang and Nanchang. This is the first study to propose that chemical control should be optimally timed, with one application administered at the time of yam seedling flush, and another given approximately 60 days later. Applications of a 41.7% fluopyram suspension (1426 g.a.i./hm$^2$) and a 30% fosthiazate microencapsulated suspension (2925 g.a.i./hm$^2$) achieved disease control effects of 81.56–83.15% and 75.95–78.42%, respectively. Additionally, the comparative analysis demonstrated that using uniform root layer application technology at the optimal time produces a control effect exceeding 80%, which is significantly higher than conventional techniques such as drip irrigation and root irrigation. These results provide theoretical and technical support for the efficient control of root-knot nematode disease in Rui yams.

**Keywords:** rui yam; root-knot nematode disease; occurrence pattern; chemical control; optimal period; application techniques



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## 1. Introduction

Yam is a monocotyledonous herbaceous vine of the genus *Dioscorea*. It is widely grown in tropical and subtropical regions, and is an important food and economic crop [1–3]. Yam tubers are used for both medicine and food. They are rich in carbohydrates, minerals, vitamins, and steroidal saponins, making yams a staple food for nearly 100 million people in African, Asian, and Latin American countries [4,5]. Yams are also used by African and Asian people to treat diabetes, increase coronary circulation, and prevent hypercholesterolemia [5–7].

Although yams are an important crop, their production is limited by biological factors such as fungi, nematodes, and bacteria, among which plant-parasitic nematodes are the most devastating [8]. In 2013, root-knot nematodes (*Meloidogyne* spp.) ranked first in a survey of the top ten plant-parasitic nematodes reported by the journal *Molecular Plant Pathology* [9], and are widespread in yam-growing areas around the world [10,11]. Root-knot nematodes are mainly represented by *M. incognita*, *M. javanica*, *M. arenaria*, and *M. enterolobii* [10], among which *M. incognita* and *M. arenaria* are the main species found in Chinese yams [12,13]. Yam plants infested with root-knot nematodes showed stunted growth, yellow leaves, and reduced yields; galls on the surface of tubers seriously affected

their marketability [10,14]. Yam yields reportedly decreased by more than 80% after severe infestation by *M. arenaria*, and significantly decreased after moderate or low infestation by *M. incognita* and *M. javanica* [12,15,16]. As China's yam industry has increased in recent years, yam root-knot nematode disease has become increasingly serious [17]. According to Sulaiman's survey, the detection rate of root-knot nematodes in rhizosphere soil and tubers in the main yam-producing areas of Jiangxi and Shandong both reached 65% [17]. Our preliminary survey showed that root-knot nematode disease was widespread in the main yam-producing areas of Jiangxi, with an incidence rate exceeding 30% in typical fields, and reaching 100% in heavily diseased fields, causing huge economic losses to growers. Therefore, yam root-knot nematode disease has become a bottleneck that is restricting the healthy development of the yam industry [17].

Yam root-knot nematode disease is a soil-borne disease, and nematodes mainly overwinter in the soil and in yam residues as eggs or as second-stage juveniles (J2) [18]. Therefore, soil treatment with chemicals is a common and easy method for controlling root-knot nematodes. The reproductive period of Rui yams is long, with 2–3 months between planting and seedling emergence; this means that the control effect will be greatly reduced if soil treatment is performed before planting. Therefore, soil treatment must be performed at the onset of the disease (optimal control timing) to ensure the control effect is optimal. However, it is difficult to observe the disease incidence in situ in the absorbing root and tubers of yams, making it hard to accurately identify the optimal time for disease control. Therefore, a visualization device is urgently needed to observe the onset of disease in the underground parts of plants. Additionally, the dynamics of the J2 population in the soil and the occurrence dynamics of yam root-knot nematode disease must be monitored with field sampling. No study has systematically reported on these topics.

The tubers of Rui yams can reach approximately 80 cm in depth, and the root-knot nematode is found in soil from 0 to 80 cm. The planting areas of Rui yam in Jiangxi are dominated by sticky and heavy soil, and conventional techniques such as drip irrigation, micro-spraying, and root irrigation often lead to unsatisfactory control effects due to the loss of the chemical solution [19].

To solve these problems, we investigated the dynamics of the root-knot nematode J2 population in the soil, as well as the occurrence of root-knot nematode disease in yams by field sampling; we also observed the onset of yam root-knot nematode disease using an underground visualization device. This allowed us to determine the optimal time for controlling yam root-knot nematode disease. Additionally, we conducted a comparative analysis between the uniform root layer application technology and conventional application technology, in order to assess their ability to control yam root-knot nematode disease.

## 2. Materials and Methods

### *2.1. Materials*

Rui yam varieties were obtained from the Yam Germplasm Preservation Center of Ruichang Agricultural and Rural Bureau. The experimental nematicides included a 30% fosthiazate microencapsulated suspension (Hebei Sannong Agrochemical Co., Shijiazhuang, China), a 41.7% fluopyram suspension (Bayer Co., Beijing, China), and 10 billion spores/g of *Bacillus firmus* wettable powder (Jiangxi Shunquan Biotechnology Co., Fuzhou, China). Sampling tools included a shovel and a drill (purchased from local market); application tools included uniform applicators (self-made), a drip irrigation device, and a measuring cup (purchased from local market). Root-knot nematode samples were collected from the experimental field in the core planting yam area in Ruichang City, Jiangxi Province (Fanzhen Village, Fan Town, 29°59′ N, 115°58′ E; Lefeng Village, Gaofeng Town, 29°64′ N, 115°56′ E) and the experimental field of the "Rui Yam" planting base in Nanchang County (Tujia Village, Xiaolan Township, Nanchang County, 28°38′ N, 115°97′ E). All of the field trials were performed at these locations, from March 2019 to November 2021.

### 2.2. Pathogen Identification

In 2019, diseased root samples were collected from nine representative test plots at three experimental sites in Ruichang City and Nanchang County, Jiangxi Province. Five plants were collected from each plot. After rinsing with clean water, the oocysts were obtained from the diseased root systems under a dissecting microscope, surface sterilized with 1% NaClO solution, and J2 was incubated in an incubator at 25 °C. The DNA of a single nematode was extracted according to methods described by Subbotin [20], and PCR amplification was performed using primers that were specific to common root-knot nematodes (Table 1) [21–25]. The reaction program administered was as follows: 95 °C for 3 min, followed by 35 cycles including 95 °C for 15 s, annealing for 15 s (*M. incognita* at 58 °C, *M. javanica* at 55 °C, *M. arenaria* at 53 °C, *M. enterolobii* at 56 °C, *M. hapla* at 58 °C), 72 °C for 15 s, and a final step at 72 °C for 5 min. Rui yams were sown in 20 cm diameter plastic pots containing sterilized soil, and were then placed in a greenhouse. When the yam plants grew to the 2-leaf to 3-leaf stage, each yam seedling root was inoculated with 4 mL of root-knot nematode suspension (250/mL). Sterile water inoculation was used as a control. The experiment was repeated three times, and yam incidence was observed 60 days later.

**Table 1.** Specific primers for the five major root-knot nematodes.

| Species | Specific Primers | Fragment Length |
|---|---|---|
| *M. incogita* | 5′-GTGAGGATTCAGCTCCCCAG-3′<br>5′-ACGAGGAACATACTTCTCCGTCC-3′ | 995 bp |
| *M. javanica* | 5′-ACGCTAGAATTCGACCCTGG-3′<br>5′-GGTACCAGAAGCAGCCATGC-3′ | 517 bp |
| *M. arenaria* | 5′-TCGGCGATAGAGGTAAATGAC-3′<br>5′-TCGGCGATAGACACTACAACT-3′ | 420 bp |
| *M. enterolobii* | 5′-AACTTTTGTGAAAGTGCCGCTG -3′<br>5′-TCAGTTCAGGCAGGATCAACC-3′ | 236 bp |
| *M. hapla* | 5′-TGACGGCGGTGAGTGCGA-3′<br>5′-TGACGGCGGTACCTCATAG-3′ | 610 bp |

### 2.3. Dynamic Monitoring of Root-Knot Nematode J2 Population in Soil

From 2019 to 2021, a nursery was established to observe the dynamics of the root-knot nematode J2 population. The nursery was situated on a 2-year continuous cropping field located at the experimental site, with an area of 667 $m^2$ that was equally divided into 3 plots. Yams were sown around mid-January at a density of 60,000 plants/$hm^2$, with protective rows around the experimental field. The crops were managed by following routine field practices without nematicide. Yam rhizosphere soil samples were collected from the monitoring nursery at approximately 30-day intervals from post-sowing (late January) to harvest (late November). Each plot was sampled at 5 points diagonally, with 3 plants per point. Soil samples were obtained at a depth of 0–80 cm by drilling 5–10 cm apart from the vine base, with one subsample from 4 directions of each plant. Twelve subsamples from each point were mixed as one soil sample. A volume of 100 mL of each soil sample was taken, and the nematodes were isolated using a modified shallow disk immersion method [26]; the number of root-knot nematode J2s were counted under an inverted microscope. Each soil sample was repeated three times.

### 2.4. Dynamic Investigation of Disease Occurrence in the Field

Dynamic monitoring of disease occurrence in the field was conducted in the observation nursery, as described in Section 2.3. After the yam emergence rate exceeded 50%, each plot was sampled using 5 points on the diagonal, and 3 plants were collected from each point to investigate the incidence of yam root and tuber disease. The survey period was from mid-May to mid-June for observing root disease, and from the end of May to the pre-harvest period for observing tuber disease. During these periods, the survey was

conducted every 5 days in May, every 10 days in June, and every 30 days after July. The grading survey was performed according to the grading criteria of Mao [27], and the disease index was calculated according to the following formula:

$$DI = [\Sigma (N_i \times i)/(N \times 9)] \times 100 \quad (1)$$

where DI is the disease index, $N_i$ is the number of diseased plants at each level, $i$ is the corresponding grade value, and $N$ is the total number of investigated plants.

### *2.5. Visual In Situ Observation of Absorbing Root Disease Incidence*

Plant roots are "positively geotropic", and will yield to obstructions when their downward growth is blocked. Therefore, when the obstruction is a transparent and hard material, changes in the underground part of the plant can be observed. As such, we designed an in situ observation device (Figure 1) to observe root-knot nematode disease in the root. In 2020 and 2021, soil samples were obtained from the same observation nursery (Nanchang) as described in Section 2.3, and 5-point samples were mixed and placed in the observation device. The sown time was the same as that in the observation nursery. After yam root germination, root changes were observed every 2 days.

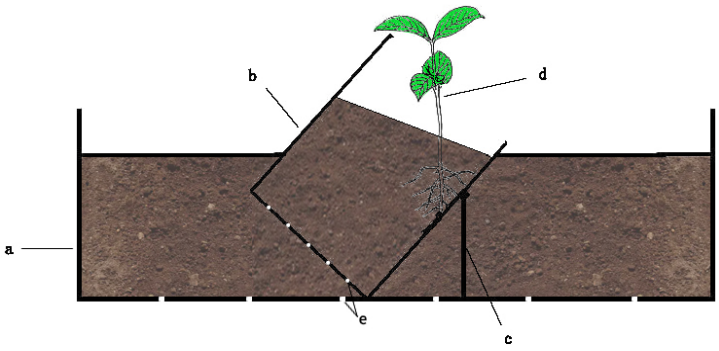



**Figure 1.** Schematic diagram of the in situ observation device used to visualize the underground part of plants. (a) is a large container used to contain soil, and provides a culture with a light-proof environment, and a constant temperature and humidity for the observation device. (b) is a transparent container for planting seeds and seedlings; the soil contained is the same as that in (a). (c) is a holder to keep the transparent container at an inclined angle. (d) are seeds or seedlings, planted against the beveled side of the support side so that the roots grow tightly against the transparent bevel. (e) are outlet holes near the bottom surface of containers a and b that allow excess water to flow out. When necessary, the transparent container (b) could be taken out to observe the roots from this side.

### *2.6. Trials on the Optimal Time of Chemical Control*

We set up 8 treatments with 3 replicates for each treatment. In total, 24 plots were randomly arranged in blocks. Table 2 displays the application site, time, test nematicides, and dosage. After being soaked with 5% abamectin emulsifiable concentrate of 50 mg available ingredient/kg, the yams were planted by furrowing in strips at a density of 60,000 plants/hm$^2$, with protective rows placed around the test field. The quantitative application was performed with a crop root layer uniform applicator [28], with a flow rate of 100 mL/s, and a volume of 1000 mL/plant at a soil depth of 60 cm. The disease incidence of each treatment was investigated via grading during the harvest period. The grading criteria and disease index were calculated as outlined in Section 2.4, and the control effect was calculated according to the following formula:

$$P(\%) = (DI_{CK} - DI_{PT})/DI_{CK} \times 100 \quad (2)$$

where P is the control effect, $DI_{CK}$ is the blank control disease index, and $DI_{PT}$ is the treatment disease index.

**Table 2.** Application time and dosage during 2019–2021.

| Treatment | Nematicide | Dosage (g.a.i./hm$^2$) | 2019 Ruichang | | 2020 Ruichang | | 2020 Nanchang | | 2021 Ruichang | | 2021 Nanchang | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Application Date | | Application Date | | Application Date | | Application Date | | Application Date | |
| | | | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| A | 41.7% Fluopyram suspension | 1426 | 4–26 * | 6–23 | 4–24 | 6–26 | 4–25 | 6–21 | 4–27 | 6–29 | 4–23 | 6–23 |
| B | | | 5–22 | — | 5–24 | — | 5–22 | — | 5–28 | — | 5–25 | — |
| C | | | 5–22 | 7–29 | 5–24 | 7–31 | 5–22 | 7–26 | 5–28 | 7–30 | 5–25 | 7–31 |
| D | | | 6–23 | 8–30 | 6–26 | 8–29 | 6–21 | 8–27 | 6–29 | 8–31 | 6–23 | 8–30 |
| E | 30% Fosthiazate microencapsulated suspension | 2925 | 4–26 | 6–23 | 4–24 | 6–26 | 4–25 | 6–21 | 4–27 | 6–29 | 4–23 | 6–23 |
| F | | | 5–22 | — | 5–24 | — | 5–22 | — | 5–28 | — | 5–25 | — |
| G | | | 5–22 | 7–29 | 5–24 | 7–31 | 5–22 | 7–26 | 5–28 | 7–30 | 5–25 | 7–31 |
| H | | | 6–23 | 8–30 | 6–26 | 8–29 | 6–21 | 8–27 | 6–29 | 8–31 | 6–23 | 8–30 |
| CK | — | | — | — | — | — | — | — | — | — | — | — |

* The date format is represented by "Month-Day". The first spraying time occurred before yam germination for treatments A and E, at the yam seedlings flush for treatments B, C, F, and G, and approximately 30 days after yam seedlings flush for treatments D and H. The same parameters were used for the below experiments.

### *2.7. Application Method Test*

The yams were disinfected and sown using the same method as described in Section 2.6. Five treatments were set up, with three replicates for each treatment. A total of 15 plots were arranged in random blocks. Treatment I was the first and second applications performed with uniform application in the root layer. Treatment II involved the first application via drip irrigation, and the second was with uniform application in the root layer. For treatment III, both applications used drip irrigation. In treatment IV, both applications used conventional root irrigation. CK was the blank control. The two applications were made when the yams were in seedling flush (late May), and approximately 60 days later (late July). The first application was a mixture of 30% fosthiazate microencapsulated suspension (2925 g.a.i./hm$^2$) and 10 billion spores/g *Bacillus firmus* wettable powder (12,000 g/hm$^2$). The second application was a 41.7% fluopyram suspension (1426 g.a.i./hm$^2$). The root layer uniform application method and dosage were the same as described in Section 2.6, with drip irrigation under a hydraulic pressure of 0.1 MPa, a volume of 1500 mL/plant, and root irrigation with a volume of 500 mL/plant. The disease incidence in each treatment was investigated during the harvesting period, using the same method described in Section 2.6.

## 3. Results

### *3.1. Identification of Root-Knot Nematode Species*

A total of 45 samples of root-knot nematode were obtained in 9 experimental plots, and were used as templates for amplification with specific primers for five main root-knot nematodes. A single fragment (955 bp) was obtained in all 45 samples using species-specific primer for *M. incognita* (Figure 2), but nothing was amplified with the other four primer sets. During re-inoculation, the roots of inoculated yams showed typical root-knot symptoms (Figure 3a), and a large number of oocysts and females of *M. incognita* were found in their infested roots; meanwhile, the blank control yam had no disease (Figure 3b). Therefore, the root-knot nematodes of the nine experimental fields in three sites were all *M. incognita*.

### *3.2. Dynamics of the M. incognita J2 Population in Soil*

Results from monitoring the *M. incognita* J2 population showed that it was low from January to March, followed by a substantial increase in April, little change in May, an initial decrease followed by a continuous increase in June, a peak in October, and a decrease in November (Figure 4).

As seen from the relationship between the dynamics of changes in the J2 population and the monthly average temperatures (Figure 4), when the monthly average temperature from January to February was low, the base of the J2 population was small; when the average temperature increased in March, the J2 population also increased; when the average temperature rose to nearly 20 °C in April, a large number of overwintered eggs in

the soil hatched, and a small peak in the J2 population appeared; when the monthly average temperature further rose from May to June, the J2 population barely changed in May, and the J2 population decreased in June; from July to August, the monthly average temperature ranged from 27.26 to 31.24 °C, and the J2 population continued to increase; in November, the average temperature dropped to about 15 °C, and the J2 population decreased.

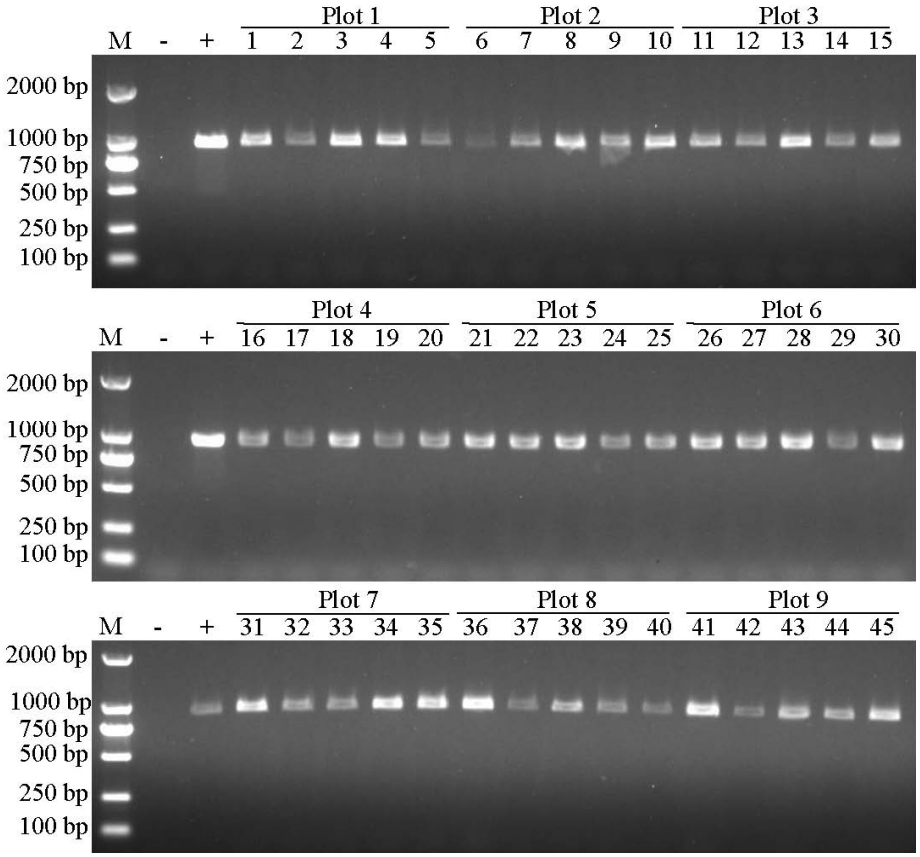



**Figure 2.** A single band of 955 bp was amplified from 45 nematode samples using specific primers for *M. incognita*. "M" indicates DNA Mark (2000 bp). "−"and "+" indicate negative control and positive control, respectively. (Samples 1–45 from 9 experimental plots).

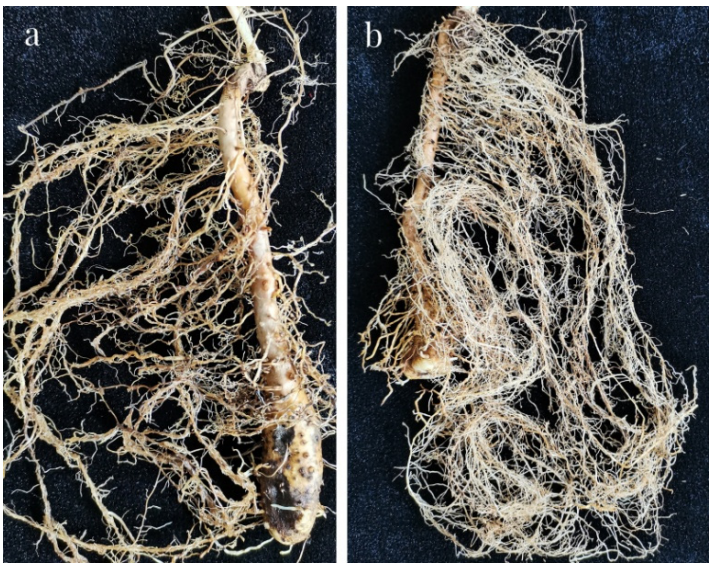



**Figure 3.** Pathogenicity identification of yam root-knot nematode. (**a**) Root symptoms of yam seedlings after root-knot nematode re-inoculation. (**b**) Blank control.

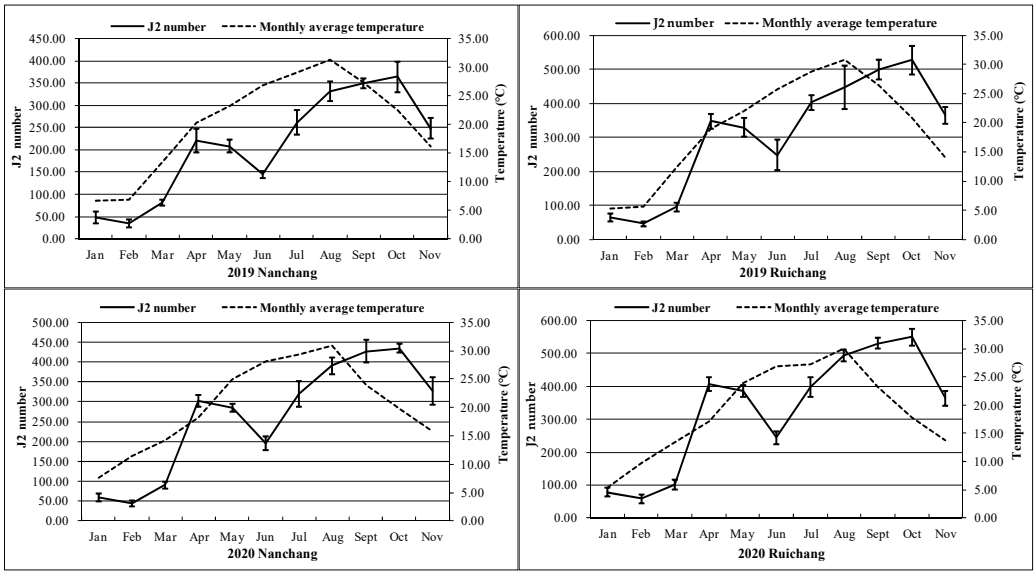
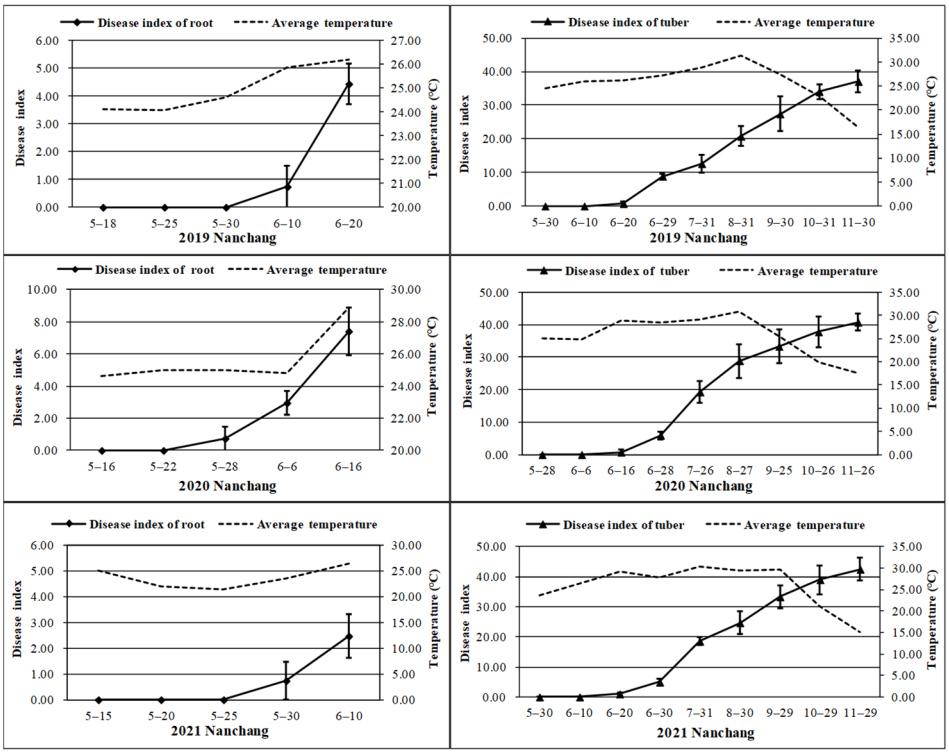



**Figure 4.** Dynamics of the *M. incognita* J2 population in soil (2019–2020, Nanchang and Ruichang).

### *3.3. Dynamics of Yam Root-Knot Nematode Disease in the Field*

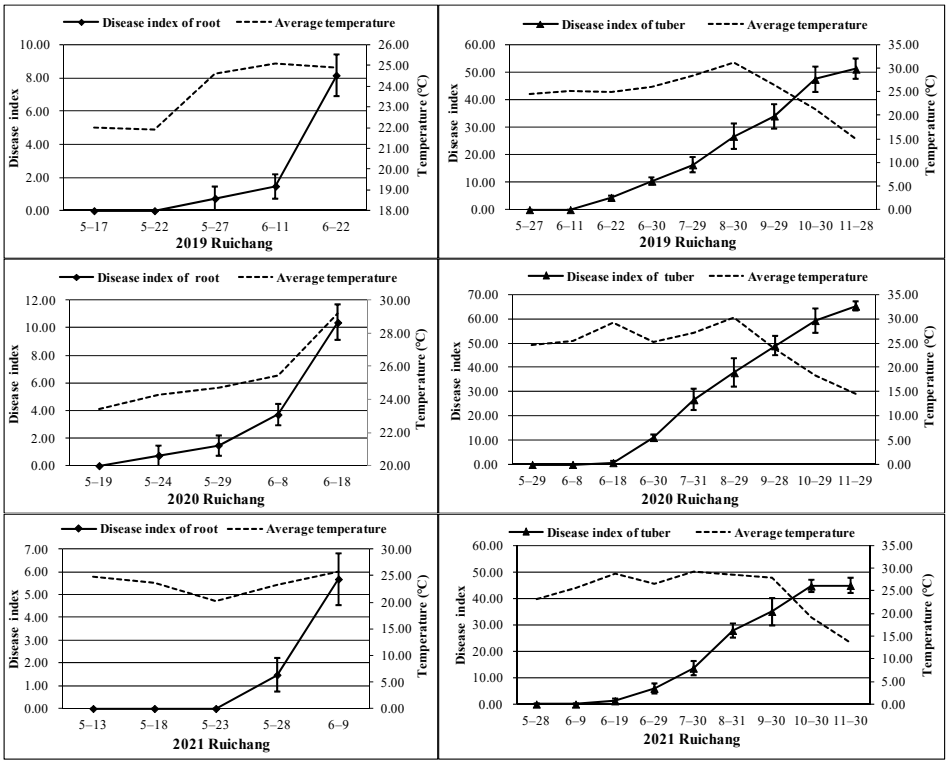
A dynamic survey of the disease occurrence showed that it first afflicted the absorbent roots in late May (around the seedling flush), and afflicted the tubers in mid- to late June. The tuber disease continued to grow thereafter, with the index reaching 37.04–65.19 at the harvesting stage (Figure 5a,b).

As shown by the relationship between temperature and the disease (Figure 5a,b), with the temperature increasing from May to August, the disease index increased continually. However, when the temperature declined from August to November, the disease index still increased steadily.




(**a**)

**Figure 5.** *Cont.*




(**b**)

**Figure 5.** Dynamics of disease index growth of yam root-knot nematode disease (2019–2021, Nanchang (**a**) and Ruichang (**b**)).

### *3.4. Visual In Situ Observation of Root-Knot Nematode Diseasee*

After the yam was sown in the visualization monitoring device, its root disease was periodically observed. The disease was first observed in absorbing roots, with obvious root-knot symptoms on 20 May 2020 (Figure 6a), after which the number of root knots increased, and the disease worsened. Obvious root-knot symptoms in absorbing roots were observed on 23 May 2021 (Figure 6b), after which the number of root knots increased over time. This indicates that visualization observations are typically consistent with the results from monitoring the dynamics of yam root-knot nematode disease in the field.

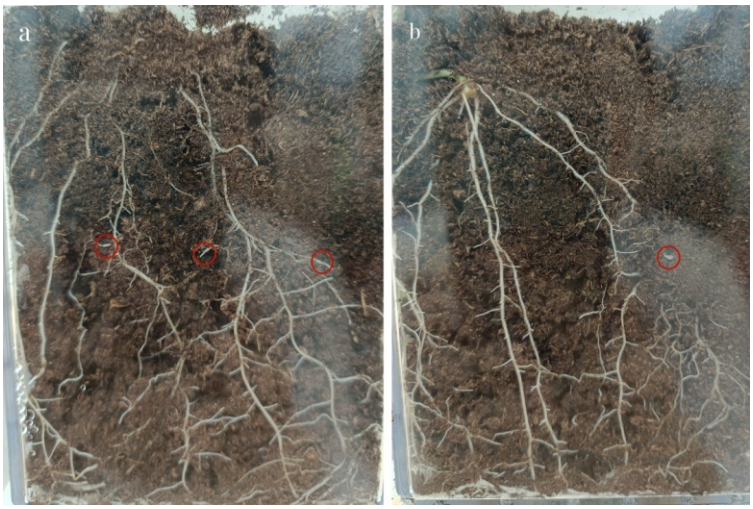



**Figure 6.** Visual in situ observations of yam root-knot nematode disease. Symptoms of disease onset in the absorbing roots on 20 May 2020 (**a**) and 23 May 2021 (**b**). Red circles show the root knots produced by nematode infestation.

### *3.5. Control Effect of Chemical Application at Different Periods on Root-Knot Nematode Disease of Yam*

As shown in Table 3, applying the 41.7% fluopyram suspension (1426 g.a.i./hm$^2$) only once in late May (yam seedling flush) produced poor control effect on the root-knot nematode of yam; that is, 27.56–38.75% in treatment B. the three treatments of two applications, once in late May (yam seedling flush) and once approximately 60 days later (late July), produced the best control effect, reaching 81.56–84.15% (C). Similar results also were seen when applying the 30% fosthiazate microencapsulated suspension (2925 g.a.i./hm$^2$). It produced a good control effect of 75.95–78.42% in treatment G. Thus, the control effect of applying nematicide once in late May (yam seedling flush) and once approximately 60 days later (late July) was significantly higher than the effects of the other three treatments.

**Table 3.** Control effects of different periods of applications on root-knot nematode disease of yam.

| Treatment | 2019 Ruichang | | 2020 Ruichang | | 2020 Nanchang | | 2021 Ruichang | | 2021 Nanchang | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Disease Index | Control Effect (%) | Disease Index | Control Effect (%) | Disease Index | Control Effect (%) | Disease Index | Control Effect (%) | Disease Index | Control Effect (%) |
| A | 13.45 ± 0.90 | 70.56 ± 1.97 b | 16.70 ± 1.13 | 70.16 ± 2.01 b | 13.92 ± 0.79 | 71.17 ± 1.64 b | 11.44 ± 0.55 | 72.84 ± 1.31 b | 9.67 ± 0.91 | 74.42 ± 2.40 b |
| B | 30.26 ± 1.20 | 33.76 ± 2.62 d | 40.53 ± 1.60 | 27.56 ± 2.86 d | 32.98 ± 1.26 | 31.68 ± 2.61 d | 27.53 ± 0.91 | 34.62 ± 2.15 d | 23.15 ± 0.92 | 38.75 ± 2.42 d |
| C | 7.70 ± 0.97 | 83.15 ± 2.14 a | 10.31 ± 0.97 | 81.56 ± 1.72 a | 8.00 ± 0.27 | 83.43 ± 0.55 a | 7.35 ± 0.84 | 82.54 ± 1.98 a | 5.99 ± 0.34 | 84.15 ± 0.89 a |
| D | 20.13 ± 1.09 | 55.95 ± 2.40 c | 26.33 ± 0.98 | 52.95 ± 1.75 c | 21.31 ± 0.94 | 55.85 ± 1.95 c | 18.28 ± 1.03 | 56.58 ± 2.46 c | 16.13 ± 0.70 | 57.30 ± 1.86 c |
| E | 19.57 ± 1.10 | 57.18 ± 2.40 b | 25.47 ± 1.43 | 54.49 ± 2.56 b | 19.72 ± 0.87 | 59.15 ± 1.81 b | 17.29 ± 0.62 | 58.94 ± 1.48 b | 14.54 ± 0.77 | 61.52 ± 2.03 b |
| F | 35.01 ± 1.54 | 23.36 ± 3.25 d | 45.07 ± 1.31 | 19.44 ± 2.33 d | 37.39 ± 1.37 | 22.53 ± 2.85 d | 30.90 ± 1.03 | 26.62 ± 2.44 d | 26.61 ± 0.70 | 29.59 ± 1.84 d |
| G | 10.84 ± 0.84 | 76.27 ± 1.85 a | 13.45 ± 1.08 | 75.95 ± 1.92 a | 11.03 ± 1.11 | 77.16 ± 2.31 a | 9.33 ± 0.55 | 77.85 ± 1.31 a | 8.25 ± 0.60 | 78.42 ± 1.73 a |
| H | 22.76 ± 1.02 | 50.19 ± 2.23 c | 30.19 ± 1.20 | 46.03 ± 2.11 c | 23.25 ± 1.17 | 51.83 ± 2.43 c | 21.46 ± 0.96 | 49.03 ± 2.29 c | 17.77 ± 0.88 | 52.98 ± 2.31 c |
| CK | 45.69 ± 1.91 | — | 55.95 ± 1.38 | — | 48.27 ± 1.55 | — | 42.11 ± 0.95 | — | 37.79 ± 1.78 | — |

The data in the table are shown as mean ± standard error. Different lowercase letters after the same column indicate significant differences at the $p < 0.05$ level, by Duncan's new multiple-step test.

### *3.6. Control Effect of Different Application Methods on Root-Knot Nematode Disease in Yam*

Our analysis demonstrated that the control effect of uniform root layer application twice on yam root-knot nematode disease was 81.47–83.69%, which was significantly higher than those of the other three treatments. The control effect of the "drip irrigation at yam seedling flush and root layer uniform application at about 60 days later" treatment was the second most effective, ranging from 75.39% to 77.44%. The control effect of drip irrigation twice was relatively poor (less than 70%), and the control effect of root irrigation twice was the worst (about 50%) (Table 4).

**Table 4.** Control effects of different combinations of application methods on root-knot nematode disease of yam.

| Treatment | Spraying Method of Nematicide | | 2020 Ruichang | | 2020 Nanchang | | 2021 Ruichang | | 2021 Nanchang | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Yam Seedling Flush | about 60 Days Later | Disease Index | Control Effect (%) | Disease Index | Control Effect (%) | Disease Index | Control Effect (%) | Disease Index | Control Effect (%) |
| I | Uniform application in the root layer | Uniform application in the root layer | 9.69 ± 0.58 | 81.47 ± 1.11 a | 7.72 ± 0.68 | 82.80 ± 1.52 a | 7.44 ± 0.36 | 82.94± 0.82 a | 6.02 ± 0.48 | 83.69 ± 1.31 a |
| II | Drip irrigation | Uniform application in the root layer | 12.87 ± 0.85 | 75.39 ± 1.62 b | 10.12 ± 0.55 | 77.44 ± 1.23 b | 10.38 ± 0.74 | 76.22 ± 1.71 b | 8.70 ±0.66 | 76.42 ± 1.79 b |
| III | Drip irrigation | Drip irrigation | 16.92 ± 0.73 | 67.65 ± 1.40 c | 14.01 ± 0.75 | 68.76 ± 1.68 c | 13.95 ± 0.74 | 68.01 ± 1.69 c | 11.30 ± 0.85 | 69.37 ± 2.30 c |
| IV | Root irrigation | Root irrigation | 29.21 ± 1.28 | 44.16 ± 2.44 d | 23.01 ± 0.57 | 48.71 ± 1.27 d | 22.88 ± 1.05 | 47.54 ± 2.41 d | 18.67 ± 0.78 | 49.41 ± 2.12 d |
| CK | — | — | 52.31 ± 0.98 | — | 44.86 ± 1.29 | — | 43.62 ± 2.03 | — | 36.90 ± 1.64 | — |

The data in the table are shown as mean ± standard error. Different lowercase letters after the same column indicate significant differences at the $p < 0.05$ level, by Duncan's new multiple-step test.

## 4. Discussion

As a medicinal and food crop, yams are a staple food in many regions. In some regions, yams are being grown to supplement staple foods [29]. In short, its planting area is increasing. The reproductive period of yams is generally long, and can reach 200 days. During this developmental period, the underground parts (absorbing roots and tubers) are susceptible to infestation and damage by a variety of pathogens. Among them, the

root-knot nematode is one of the most serious factors affecting the growth and development of yam plants, and is the most important factor that affects continuous cropping. "Rui yam" is an excellent local variety in Jiangxi Province, and is a product of China's National Geographical Indication [30]. However, the species identification of root-knot nematodes, its occurrence dynamics, and efficient and precise methods for chemically controlling Rui yam root-knot nematode disease have not yet been reported.

*M. incognita* is the most common root-knot nematode species in global yam production areas [10,11]. The identification of root-knot nematode species in Nigerian yams by Kolombia [31] showed that in addition to *M. incognita*, *M. javanica*, and *M. enterolobii*, *M. arenaria* is another species that infests yams. In this study, the root-knot nematode species of "Rui yam" in Fan Town, Gaofeng Town, and Xiaolan Township, Nanchang County, Jiangxi Province, were all identified as *M. incognita*, which is consistent with the results of previous research [17,32]. No other root-knot nematode species that affect yams have been reported in Jiangxi Province. In the future, root-knot nematode species identification should be performed in multiple locations, in order to develop targeted disease control measures.

Research showed that the optimal temperature for *M. incognita* is 15–30 °C. Egg hatching and the J2 population are inhibited when the temperature is above 40 °C or below 5 °C [33]. This is the first study to systematically monitor the dynamics of the *M. incognita* J2 population in yam soil and root-knot nematode disease in the field. Root-knot nematodes were found to primarily overwinter in the soil as eggs and J2s. As the air temperature increased in spring, the soil temperature increased, and the eggs that successfully overwintered began to continuously hatch the J2s [18]. This explains the substantial increase in the J2 population observed in April. After the emergence of yams, J2s gradually invaded absorbing roots and tubers, decreasing the J2 population in the soil in June. Although the monthly average temperature in July and August reached 27.6–31.24 °C, the tendency of yam tubers to grow downward protected some nematodes from the adverse effects of high temperatures. On the other hand, the continuously expanding tubers provided abundant nutrients for nematode reproduction. These factors all facilitated the continuous expansion of J2 populations in the soil. The average temperature in November dropped to about 15 °C, and the low temperature dropped below 10 °C in mid- to late November, which hindered nematode growth and development, and decreased the J2 population. Additionally, many other factors can influence the nematode population, such as humidity, pH, and oxygen [34]. These factors should be considered in future research.

Sensory neurons of root-knot nematode J2s in the soil drove them towards the root system when they sensed $CO_2$ and the other exudates produced by the sprouting root system [34,35]. This also resulted in the rapid upregulation of several genes, such as *MiCTL1*, *P66E1*, *P64A1*, and *MAP-1*, which helped the J2 population complete the colonization and infestation [36–38]. Yam is a typical resource-acquiring plant [39], and the $CO_2$ and the other exudates released from its roots and tubers are extremely attractive to fast-growing nematodes such as root-knot nematodes [40,41]. When Rui yam absorbing roots started to germinate in early May, $CO_2$ and the other exudates produced by root germination induced the J2 population to colonize and infect the yams. After monitoring the yam root-knot nematode disease in the field for three consecutive years, we found that the disease began in late May (yam seedling flush) for the roots, and in mid- to late June for the tubers.

Usually, frequent digging and replanting yam roots and tubers is needed to monitor the dynamics of root-knot nematode disease on the same yam plant. However, this approach results in a high mortality rate of the replanted yams, and is a time-consuming method. Therefore, we developed a visual in situ observation device to achieve in situ and real-time monitoring of yam root disease. Two-year trials, conducted in 2020 and 2021, showed that the results of our in situ monitoring were consistent with those from field sampling surveys.

The critical period for disease control is during the initial stage of disease occurrence. The root-knot nematode disease on Rui yam began to develop in late May. By comparing the control effects of different application periods on root-knot nematode disease of yams, we found that the control effect of a single application was significantly lower than that

of two applications. The reason for this is because the development period of yam plants can exceed 200 days, and the disease lasts for a long time; thus, even a single application during early disease stages cannot control the root-knot nematode [42]. The best control was achieved using one application in late May (yam seedling flush), and one application approximately 60 days later. Yam seedling flush is the disease's initial stage, so applying a control at this time can minimize the root-knot nematode base in the soil. At the same time, the systemic agents can effectively kill nematodes that are infesting the yam. After 60 days, the second application can keep the nematode population in the soil at a low level, which can ultimately control root-knot nematode disease.

Numerous studies have demonstrated that rhizosphere soil nematodes are primarily distributed in the soil layer at a depth of 0–30 cm [43,44]. The depth of the root system can affect nematode distribution in the soil [45–47]. In contrast to conventional tillage for shallow-rooted crops, yams typically require deep digging with an excavator before planting. This mixes the upper and lower soil layers, causing a large number of root-knot nematodes that were originally distributed in the upper soil layer to be turned over to the lower soil layer. Yam tubers can reach 80 cm underground, providing a rich food source for nematodes in the soil layer at a depth of 0–80 cm [48]. The penetration depth of the chemical solution is limited with conventional root irrigation and drip irrigation application methods, leading to poor control effects. In addition, a large loss of the chemical solution during root irrigation application can harm the environment. In this study, we independently developed a root layer uniform application technology. The applicator can reach a depth of more than 60 cm; combined with the downward penetration of the agents, the control agent can be distributed throughout the soil layer where yam roots and tubers grow, effectively controlling this disease.

This study monitored the dynamics of the *M. incognita* J2 population in yam soil and root-knot nematode disease in the field. Combined with the results of visual in situ observations of yam root disease, we first proposed an optimal period for the chemical control of yam root-knot nematode disease, and developed a uniform application technique in the root layer. This provides technical support for the efficient and targeted control of the disease. Follow-up experiments will further monitor the dynamics of the root-knot nematode population in the soil after different control periods, and under different application methods.

## 5. Conclusions

In this study, *M. incognita* was found to be the root-knot nematode species in the experimental sites of Ruichang City and Nanchang County, Jiangxi Province. The occurrence pattern of root-knot nematode disease in Rui yams was investigated via field sampling and visual in situ monitoring, and an application strategy known as "one application at yam seedling flush and one at approximately 60 days later" was proposed. In addition, a root layer uniform application technique with efficient control for yam root-knot nematode disease was developed. This provides theoretical and technical support for the effective control of yam root-knot nematode disease.

## 6. Patents

A medicine applicator device for plant protection (ZL201921477225.0) resulted from the research reported in this manuscript.

**Author Contributions:** Conceptualization, R.Z. and J.H.; methodology, R.Z. and W.F.; data curation, X.L.; writing—original draft preparation, R.Z.; visualization, Y.S.; investigation, Q.Z. and X.W.; supervision, Y.S., J.H., Q.W., and S.H.; writing—review and editing, R.Z. and A.C. All authors have read and agreed to the published version of the manuscript.

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