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Identification, Characterization, and Chemical Management of *Fusarium asiaticum* Causing Soybean Root Rot in Northeast China

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Abstract: Soybean root rot, a soil-borne fungal disease, is caused by multiple pathogens that seriously affect soybean production. During spring 2021, 92 pathogenic fungal strains were isolated from soybean plants with root rot in Hailun City, Heilongjiang Province, China. Through morphological and molecular identification, these strains were identified as *Fusarium oxysporum* (39.1%), *F. asiaticum* (30.4%), *F. graminearum* (13.0%), *Pythium macrosporum* (8.7%), and *Rhizoctonia solani* (8.7%). Among them, *F. oxysporum* was the dominant species, and *F. asiaticum*, not previously reported as a soybean root rot pathogen in Northeast China. Approximately 50% of the *F. asiaticum* isolates were moderately pathogenic. In addition, *F. asiaticum* had a wide host range, infecting black soybean, French bean, white hyacinth bean, mung bean, and adzuki bean but not corn, peanut, rice, and oat roots. Regarding field management, fludioxonil and pyraclostrobin had the best control effects of 73.8% and 69.4%, with EC_{50} values of 0.0029–0.0071 μg·mL^{-1} and 0.0045–0.0076 μg·mL^{-1}, respectively. The study reported that *F. asiaticum* is a pathogen causing soybean root rot in northeast China. The application of chemical fungicides and non-host crop rotation can effectively control the disease caused by *F. asiaticum*.

Keywords: soybean; root rot; *Fusarium asiaticum*; host range; identification; fungicide efficacy

Academic Editor: Maria Céu Lavado da Silva

Received: 9 January 2025
Revised: 29 January 2025
Accepted: 30 January 2025
Published: 31 January 2025

Citation: Liu, J.; Cui, W.; Zhao, Q.; Ren, Z.; Li, L.; Li, Y.; Sun, L.; Ding, J. Identification, Characterization, and Chemical Management of *Fusarium asiaticum* Causing Soybean Root Rot in Northeast China. *Agronomy* **2025**, *15*, 388. https://doi.org/10.3390/agronomy15020388

1. Introduction

Soybeans [*Glycine max* (Linn.) Merr.] hold the highest economic value among food and oil crops worldwide. They are abundant in protein, oil, vitamins, and various mineral nutrients. Moreover, they can be easily adapted to different environments and are widely cultivated globally for human consumption, animal feed, and biodiesel production [1]. With the adjustment of the supply side structure in China, soybean planting area in this country is increasing every year [2]. Soybean planting area will increase by 667,000 hectares to 45,700,000 hectares from 2021 to 2022 in Heilongjiang Province, accounting for nearly 50% of China's total soybean planting area. Located in one of the three major black soil zones in the world, Heilongjiang has fertile soil, suitable climatic conditions, and a high-quality ecological environment, providing a suitable production environment for soybeans. However, soybean root rot has become a major obstacle to soybean production [3].

Pathogenic fungi can infect root cells, causing serious damage to soybean roots. Infected plants often exhibit growth retardation due to the weakened ability of their roots to absorb water and nutrients [4]. In severe cases, it can lead to plant death, significantly reducing the yield and quality of soybeans [5]. The economic loss caused by the impact of various pathogenic fungi on soybeans has increased in recent years [6].

The community structure of fungal pathogens that cause soybean root rot is complex. At present, 64 fungal pathogens causing soybean root rot have been reported internationally, including species from the genus *Fusarium* such as *Fusarium pseudograminearum*, *F. proliferatum*, *F. sporotrichioides*, *F. fujikuroi*, *F. graminearum*, *F. armeniacum*, *F. commune*, *F. tricinctum*, *and F. asiaticum* [7–15]; species from the genus *Pythium,* including *P. oopapillum*, *P. macrosporum*, *P. aphanidermatum*, and *P. deliense* [4,16–18]; *Rhizoctonia solani*; *Helicobasidium mompa*; *Thielaviopsis basicola*; *Stachybotrys chartarum*; *Sclerotium rolfsii*; *Mycoleptodiscus terrestris*; and *Phymatotrichopsis omnivora* [19–25]. Thirty species have been reported domestically, including *F. oxysporum* var. rendolens, *F. oxysporum*, *F. graminearum*, *F. chlamydosporum*, *F. merismoidescorda*, *F. episphaeria*, *F. camptoceras*, '*Candidatus* Pythium huanghuaiense', *Phytophthora sojae*, *Phytophthora sansomeana*, *Rhizoctonia solani*, *Phomopsis longicolla*, and *Pratylenchus coffeae* [26–36]. Sixteen species have been reported in Heilongjiang Province, e.g., Fusarium *graminearum*, *Phytophthora sojae* [28,37]. In addition, *Rhizoctonia solani*, *Phomopsis longicolla* have also been reported. The above results indicate that the species diversity of fungal pathogens that cause soybean root rot in the Heilongjiang Province, the main soybean production area, is potentially very complex. However, *F. asiaticum,* as a pathogenic fungus of soybean root rot, has not been reported in Heilongjiang province.

Soybean root rot caused by *Fusarium* spp. is a major disease, mainly transmitted in the soil [38,39]. Fusarium root rot of soybeans can endanger any stage of soybean development, resulting in water-soaked decay after sowing and before germination, which affects the germination rate of seeds after infection. Seedling infection leads to the decay of the root epidermis, browning of vascular bundles, withered yellow leaves, and plant death in serious cases, along with shriveled grains and serious economic costs [40].

Reducing the impact of diseases during soybean cultivation is crucial for increasing yield [41,42]. Currently, the most cost-effective way to control soybean root rot is to cultivate disease-resistant soybean varieties [43]. However, there are few specially bred Fusarium-resistant cultivars [44]. Therefore, fungicide treatment is one of the most effective disease management strategies for controlling soybean root rot [45]. The fungicides commonly used to control Fusarium root rot are pyraclostrobin, fludioxonil, and azoxystrobin [46,47]. However, *F. asiaticum* has unique genetic variations that may make it resistant to commonly used fungicides, and existing control methods for other *Fusarium* species may no longer be effective [48]. Therefore, it is necessary to screen fungicides and determine the sensitivity of target pathogens causing soybean root rot in the field.

In 2020 and 2021, soybean root rot occurred in Hailun city, Heilongjiang Province, with a diseased seedling rate of 20–30% in general plots and over 60% in severely infected plots. The objectives of this study were to identify the pathogenic microorganisms causing soybean root rot, analyze their pathogenicity, and determine the sensitivity and efficacy of common fungicides against these pathogens, providing a basis for formulating control strategies.

2. Materials and Methods

2.1. Pathogen Isolation and Assessment of Their Pathogenicity

Field investigation of soybean root rot at the seedling stage was conducted at 3 sites (5 hectares per field) in an important soybean planting area in Hailun city. The local soil, mainly black sandy clay with 4–5% organic matter, had a disease incidence of 10–20% in the surveyed fields (about 5 ha each). Soybean plants (n = 182) with root rot symptoms

were collected using five-spot sampling (Figure 1). The roots were thoroughly rinsed under running tap water for 10 min to remove soil and debris. Pathogens were isolated from symptomatic root tissues following a published method and cultured on potato dextrose agar (PDA) at 28 °C in the dark [49]. After three days, hyphal tips were transferred to isolate and purify the fungal cultures. One diseased tissue sample from each infected seedling was selected and isolated. The number of isolated and purified pathogens was recorded and the percentage isolated to each species was calculated.

Figure 1. Soybean plants with root rot symptoms in the field.

The pathogenicity of isolated and purified strains was evaluated according to the Koch hypothesis [50]. A method of inoculating soybeans with fungus by embedding roots of sorghum grain [51]. All strains were re-isolated from diseased soybean seedlings and observed. The specific method was as follows: A total of 1/3 of the volume of the sterile soil was inserted into a flowerpot (diameter of 15 cm), and 20 g of sorghum grains that are already overgrown with pathogenic fungi were evenly sprinkled. Then, 12 soybean seeds (the variety used was HeNong 511) were evenly placed on the surface, covered with a layer of culture soil (1 cm), and after the seedlings emerged, 10 were kept in each flowerpot [2]. Seedlings cultured without isolation strains were used as the control group. Each treatment had three replicates, and the experiment was conducted twice. After 20 days, the incidence of root rot was investigated, and the infected plants were re-isolated and morphologically identified according to Koch's postulates; the disease index was calculated according to the classification standard of root rot by Wang et al. [52]. Ten highly pathogenic strains were selected for subsequent experiments.

Disease severity was visually scored on a scale of 0–7 based on the growth status of soybean seedlings: 0 = no symptoms; 1 = the taproot was basically unchanged or slightly browned, the fibrous root was not long, the growth point was browned, and the plant growth was normal; 3 = the taproot turned black but continued to grow through the infection point, the fibrous root tip turned black, and the plant grew normally; 5 = the taproot was seriously blackened and could not continue growing through the infection point, the fibrous root was obviously reduced or absent, the aboveground growth was poor, and the plant growth was short; and 7 = root rot, failure of normal growth or emergence, partial cotyledon rot, or plant death [52].

The percentage of disease index (PDI) for soybean root rot was calculated using the following formula: PDI = ∑(the number of diseased plants at each level × the corresponding relative ratings)/(the total number of surveyed plants × the highest disease level rating) × 100.

The pathogenicity of the isolates was classified based on the average disease index of the two repeated experiments. Isolates with a disease index less than 50 were classified as having weak pathogenicity (designated as W), those with a disease index between 50 (inclusive) and 60 (exclusive) were classified as having moderate pathogenicity (designated as M), and those with a disease index of 60 or greater were classified as having high pathogenicity (designated as H).

2.2. Identification of the Pathogen

Isolates responsible for soybean root rot were identified through their morphological characteristics and molecular methods [53]. For molecular identification, genomic DNA was extracted from the mycelia of representative isolates using a Tiangen Genome Extraction Kit (Tiangen Biotech, Beijing, China). The internal transcribed spacer region (ITS), translation elongation factor 1-α (*Tef1*), and β-tubulin (*Tub2*) genes were amplified and sequenced using primers ITS1/ITS4, EF1-728F/EF1-986R, and Bt2a/Bt2b, respectively [54–56]. Subsequently, the obtained sequences were submitted to the GenBank database (Table A1). Polymerase chain reaction (PCR) was carried out in a 50 µL reaction system containing 10 µM of each primer, 2 × Taq Master Mix, and 10 ng of template DNA. The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles, each cycle including 1 min denaturation at 94 °C, 1 min annealing at 52 °C, 1.5 min extension at 72 °C, and finally a 10 min final extension at 72 °C. The PCR products were purified and sequenced by GENEWIZ (Azenta Life Sciences, Suzhou, China).

Phylogenetic trees of representative isolates were constructed using PhyloSuite v1.2.2 (http://phylosuite.jushengwu.com/, accessed on 2 June 2023) following the MrBayes method and were further edited in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 2 June 2023) [57,58].

2.3. Biological Characteristics of Fusarium asiaticum

To determine the optimum pH and temperature for the isolates, the mycelium growth rate method was employed [59,60]. Ten isolates were cultured on PDA at different pH levels (5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0) and temperatures (10 °C, 20 °C, 25 °C, 28 °C, 30 °C, and 35 °C). A 0.5 cm diameter mycelial plug from a 96 h PDA-grown isolate was transferred to different treated PDA plates and incubated under the corresponding conditions. Each treatment had three replicates, and the experiment was conducted twice. The colony diameters of the isolates were measured after 72 h.

2.4. Host Range Determination of Fusarium asiaticum

The *F. asiaticum* isolates causing soybean root rot were inoculated on crops commonly grown in Heilongjiang, which were black soybean (*Glycine max* Linn.), French bean (*Phaseolus vulgaris* Linn.), white hyacinth bean (*Dolicho Lablab* Linn.), mung bean (*P. radiatus* Linn.), corn (*Zea mays* Linn.), peanut (*Arachis hypogaea* Linn.), rice (*Oryza sativa* Linn.), adzuki bean (*Vigna umbellata* Thunb.), and oat (*Avena sativa* Linn.) seedlings. The inoculation method was consistent with that used to determine the pathogenicity of the above isolates. Each treatment was replicated thrice. Approximately 20 days after inoculation, the pathogenicity of the isolate in each crop was investigated and evaluated. The pathogen was re-isolated and identified from the inoculated seedlings to complete Koch's postulates. All experiments were performed twice.

2.5. Sensitivity of Fusarium asiaticum to Fungicides

The mycelial growth rate method was employed to evaluate the sensitivity of the isolates to the following fungicides that are commonly utilized for controlling *Fusarium* root rot [46,47]: pyraclostrobin [25% flowable concentrate (Jiangsu Tuoqiu Agrochemicals Co., Ltd., Yancheng, China)], prochloraz (450 g·L^{-1} EW) [Shanghai Hulian Biopharmaceutical (Xiayi) Co., Ltd., Shanghai, China], fludioxonil (25% FSB) [Syngenta (Nantong) Crop Protection Co., Ltd., Nantong, China], and a mixture of 11.7% propiconazole + 7% azoxystrobin [18.7% suspoemulsion (Syngenta Nantong Crop Protection Co., Ltd., Nantong, China)] [61].

The fungicides were dissolved in 1000 mL of sterile distilled water. Stock solutions of the four fungicides were then added to PDA at concentrations of 0 (control), 0.001, 0.002,

0.005, 0.01, and 0.02 μg·mL^{-1}. The PDA plates were incubated at 26 °C for five days. Subsequently, a 0.7 cm diameter mycelial plug of the isolate was placed at the center of each fungicide-amended PDA plate and incubated in the dark at 26 °C for seven days. Each treatment was replicated three times, and the entire experiment was conducted twice. After the incubation period, the colony diameters were measured. The effective concentration resulting in 50% mycelial growth reduction (EC_{50}) of the four fungicides was calculated according to the method described by Lehner et al. [62]. Data from the two replicate experiments were pooled, and EC_{50} values were calculated. The inhibitory effect was expressed as a percentage relative to the control, using the formula: 1 − [(diameter of treated colony − 0.5)/(diameter of control colony − 0.5) × 100] [63].

2.6. Efficacy of Pyraclostrobine and Fludioxonil Against Soybean Root Rot Caused by Fusarium asiaticum

Pot experiments were conducted in 20 cm diameter plastic pots in a greenhouse at the experimental station of Northeast Agricultural University, Harbin, China. The greenhouse conditions were set at 25 ± 3 °C with a 12 h/12 h (light/dark) photoperiod. The specific treatments applied were as follows: (1) pyraclostrobine at effective doses of 62.5, 125, and 250 μg·mL^{-1}; (2) fludioxonil at effective doses of 62.5, 125, and 250 μg·mL^{-1}; (3) controls treated with sterile water. The sorghum seed inoculation method was once again applied for pathogen inoculation to determine pathogenicity. Ten soybean seedlings were kept in each bowl, with three replicates for each treatment. The experiment was carried out by soaking seeds, first washing the seeds with sterile water, and then soaking with different concentrations of fungicide liquids configured for 20–30 min. Control seeds are soaked in equal amounts of sterile distilled water. After 20 days, the disease severity was determined using the same method as the pathogenicity determination of the isolates. The disease index was calculated as described above. Seedling height, mass, and root length were measured using a graduated ruler (1 mm) and balance (1 mg). The control efficacy was calculated using the following formula:

The control efficacy was calculated using the formula: Control efficacy = (Disease index of the control group − Disease index of the fungicide − treatment group)/Disease index of the control group × 100%.

2.7. Data Analysis

Analysis of variance (ANOVA) was conducted using SPSS Statistics 17.0 (IBM/SPSS, Armonk, NY, USA). The treatment means were compared and separated by applying the least significant difference (LSD) test at a significance level of $p = 0.05$. The EC_{50} values were estimated with the assistance of GraphPad Prism 8 (GraphPad Software Inc., USA).

3. Results

3.1. Disease Symptoms and Identification of Causal Organisms

In May 2021, diseased soybean seedlings were detected in Hailun City, Heilongjiang Province, China (47.46093° N, 126.9682° E). Lesion plaques were evident at the base of the stem and were initially reddish-brown and then gradually enlarged, followed by blackening of the cortex, decay, and necrosis. The above-ground parts of infected plants were dwarfed by healthy plants, with the green leaves fading. According to the Koch postulates, 92 pathogenic isolates were isolated from 182 symptomatic seedlings. Based on morphological and molecular identification, these isolates were classified into five species (Table 1, Figure 2): *F. oxysporum* (39.1%), *Fusarium asiaticum* (30.4%), *F. graminearum* (13.0%), *Pythium macrosporum* (8.7%), and *Rhizoctonia solani* (8.7%). In addition to *F. asiaticum*, other pathogenic fungi that cause soybean root rot have been reported in China. Therefore, further systematic identification of *F. asiaticum* was performed in this study. Twenty-eight

isolates from infected soybean roots (Figure 3B,C) were white in color, flocculent, luxuriant, and dense with a rose red pigment (Figure 3A). The average growth rate of mycelium was 20.7 mm·d^{-1} on PDA at 28 °C. The macroconidia were thick, with curved apical and basal cells, usually having 4–6 septa, and measuring 44.9 to 44.2 × 3.4 to 5.4 µm on carnation leaf agar. The apical cells were beak-shaped and slightly curved, and the podocydia were not obvious (Figure 3D–F). The chlamydospores were globose to subglobose. Based on these characteristics, the isolates were identified as *F. asiaticum* [64–66].

Table 1. Frequency of pathogens isolated from soybean root rot samples in Hailun, Heilongjiang province, China.

Pathogens	Number of Isolates	Frequency (%)
Fusarium oxysporum	36	39.1
F. asiaticum	28	30.4
F. graminearum	12	13.0
Pythium spp.	8	8.7
Rhizoctonia solani	8	8.7

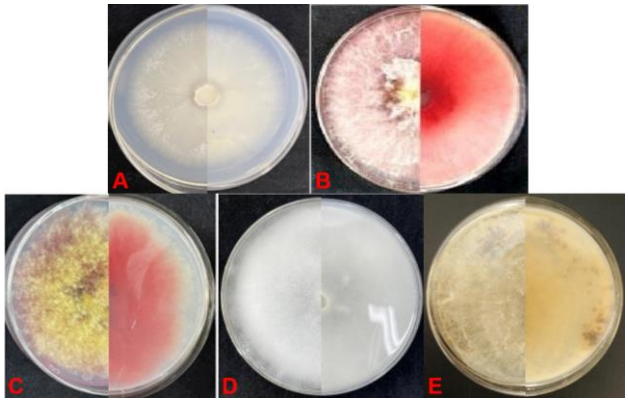


Figure 2. Colony of pathogenic fungi causing soybean root rot on PDA. (**A**) *Fusarium oxysporum*; (**B**) *F. asiaticum*; (**C**) *F. graminearum*; (**D**) *Pythium* spp.; (**E**) *Rhizoctonia solani*.

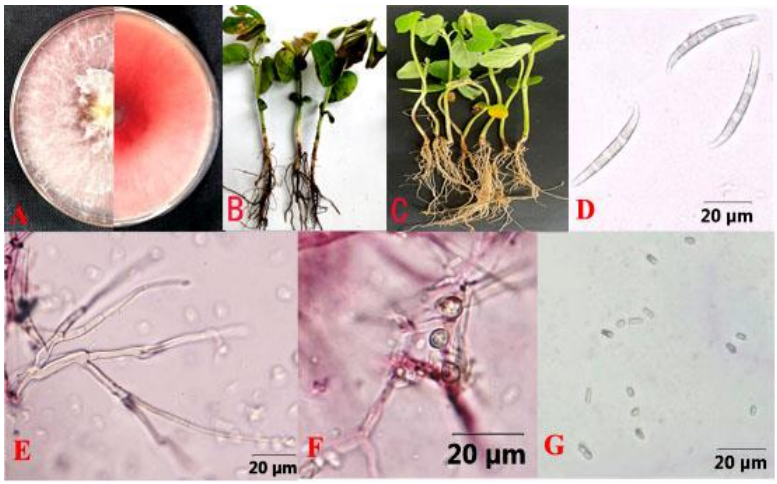


Figure 3. *Fusarium asiaticum* causing soybean root rot. (**A**) Colony of *F. asiaticum* isolate HL35 on PDA; (**B**) Field symptoms of soybean root rot caused by *F. asiaticum*; (**C**) Indoor potting symptoms of soybean root rot caused by *F. asiaticum*; (**D**) Macroconidia; (**E**) Conidiophores; (**F**) Chlamydospore; (**G**) Microconidia.

Genomic DNA from four single-conidium cultures (HL9, HL15, HL25, and HL35) was extracted and amplified using fungal universal primers ITS, *Tef1*, and *Tub2*. The

obtained sequences were deposited in the GenBank (accession numbers are shown in Table A1). BLAST analysis revealed that the ITS1/4, EF1-728F/986R, and Bt2a/Bt2b sequence amplicons of HL9, HL15, HL25, and HL35 shared high similarity with those of *F. asiaticum* strain MTLYB02 (OM100564.1), strain RTH17 (LC500693.1), and strain HBTS484 (KM062027.1), respectively. In addition, the phylogenetic analysis showed that isolates HL9, HL15, HL25, and HL35 belonged to the same evolutionary branch as *F. asiaticum*, with high similarity (Figure 4). The combination of molecular and morphological methods confirmed the twenty-eight isolates were *F. asiaticum*.

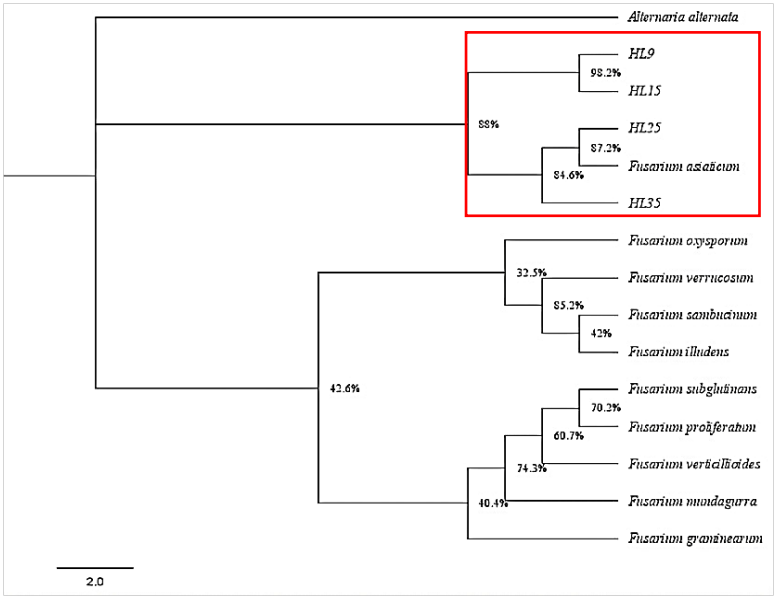


Figure 4. A phylogenetic tree of *Fusarium asiaticum* isolates HL9, HL15, HL25, and HL35, along with members of *Fusarium* spp., was constructed based on Bayesian inference. The analysis was performed on the combined dataset of internal transcribed spacer region (ITS), translation elongation factor 1-α (*Tef1*), and β-tubulin (*Tub2*) gene sequences. The tree-sampling frequency was set at 1000 generations. Branches with Bayesian posterior probabilities of 0.997 were considered significantly supported. *F. asiaticum* was designated as the outgroup.

3.2. Pathogenicity of Fusarium asiaticum on Soybean Roots

Differences in pathogenicity were detected among the 28 isolates of *F. asiaticum*. Among these were four highly pathogenic isolates, 10 moderately pathogenic isolates, and 14 weakly pathogenic isolates (Table 2). The four highly pathogenic isolates and six moderately pathogenic isolates were selected for subsequent tests.

Table 2. Disease index and pathogenicity of representative *Fusarium asiaticum* isolates isolated from soybean root rot samples in Hailun, Heilongjiang province, China.

No.	Isolates	Disease Index	Pathogenicity [1]	No.	Isolates	Disease Index	Pathogenicity [1]
1	HL7	51.4	M	15	HL40	27.1	W
2	HL9	27.1	W	16	HL42	47.1	W
3	HL12	54.3	M	17	HL43	58.6	M
4	HL15	62.9	H	18	HL45	57.1	M

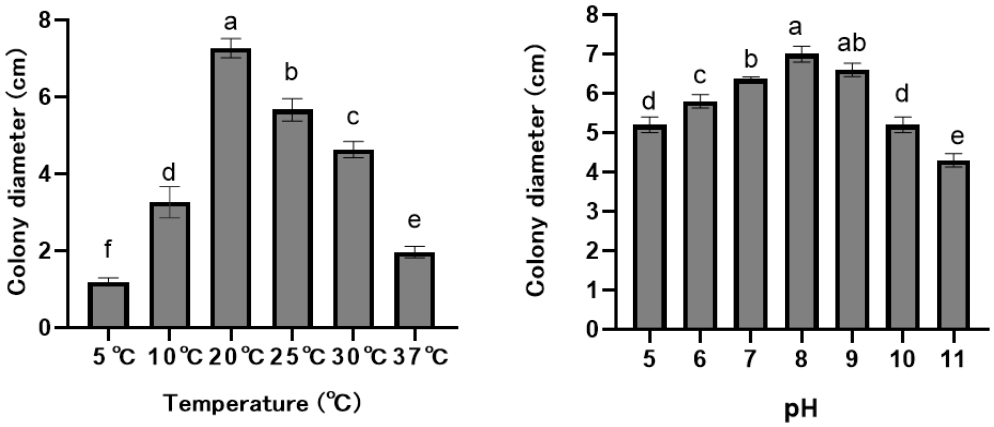
Table 2. *Cont.*

No.	Isolates	Disease Index	Pathogenicity [1]	No.	Isolates	Disease Index	Pathogenicity [1]
5	HL18	30.0	W	19	HL48	31.4	W
6	HL19	54.3	M	20	HL51	48.6	W
7	HL20	30.0	W	21	HL55	65.6	H
8	HL24	21.4	W	22	HL56	54.3	M
9	HL25	57.1	M	23	HL58	47.1	W
10	HL28	35.7	W	24	HL59	51.4	M
11	HL32	54.3	M	25	HL62	31.4	W
12	HL35	71.4	H	26	HL66	45.7	W
13	HL37	57.1	M	27	HL73	62.9	H
14	HL38	32.8	W	28	HL81	42.6	W

[1] Isolates with a disease index of less than 50 were classified as having weak pathogenicity (designated as W), those with a disease index between 50 (inclusive) and 60 (exclusive) were classified as having moderate pathogenicity (designated as M), and those with a disease index of 60 or greater were classified as having high pathogenicity (designated as H).

3.3. Biological Characteristics of *Fusarium asiaticum*

The *F. asiaticum* isolates grew in the pH range 5.0–11.0, but mycelial growth varied significantly at different pH values ($p < 0.05$), with an optimal pH of 8.0 (Figure 5A). The ten isolates could grow in the temperatures of 10–30 °C and did not grow at 5 °C and 37 °C, with an optimal temperature of 20 °C (Figure 5B).



(**A**) (**B**)

Figure 5. Colony diameters of *Fusarium asiaticum* isolates at different pHs and temperatures. (**A**) Temperature. (**B**) pH. According to the least significant difference test ($p = 0.05$), the different letters above the bar indicate significant differences for each isolate.

3.4. Host Range Determination of *Fusarium asiaticum*

The pathogenicity tests of ten *F. asiaticum* isolates on different crops showed that they were pathogenic to the roots of black soybean, French bean, white hyacinth bean, mung bean, and adzuki bean roots, but not peanut, corn, adzuki bean, and oat roots (Figure 6). No symptoms were observed in the control seedlings of each crop that were treated with sterile water. *Fusarium asiaticum* isolates inoculated on seedlings of different crops were successfully re-isolated from the diseased parts of inoculated black soybean, French bean, white hyacinth bean, mung bean, and adzuki bean roots but could not be isolated from the inoculated parts of corn, peanut, rice, and oat roots.

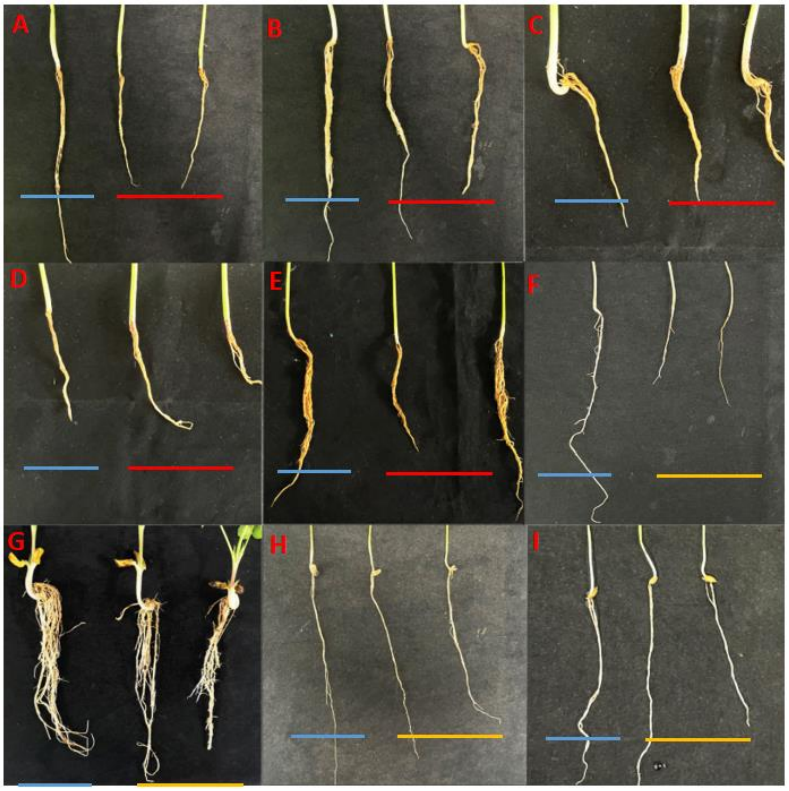


Figure 6. Host range of *Fusarium asiaticum* determined. (**A**) black soybean (*Glycine max* L.) roots. (**B**) French bean (*Phaseolus vulgaris* L.) roots. (**C**) white hyacinth bean (*Dolicho Lablab* L.) roots. (**D**) mung bean (*P. radiatus* L.) roots. (**E**) adzuki bean (*Vigna umbellata* T.) roots. (**F**) rice (*Oryza sativa* L.) roots. (**G**) peanut (*Arachis hypogaea* L.) roots. (**H**) corn (*Zea mays* L.) roots. (**I**) oats (*Avena sativa* L.) roots. In each picture, the blue line represents the control plant; the red line represents the plants inoculated with *F. asiaticum*, whose roots showed significant underdevelopment and even decay compared to the control; the yellow line represents the plants inoculated with *F. asiaticum* but showed no significant difference in root morphology compared to the control.

3.5. Sensitivity to Fungicides

The ten tested *F. asiaticum* isolates showed consistent sensitivity to pyraclostrobine, prochloraz, fludioxonil, and propiconazole·azoxystrobin. Fludioxoni had the strongest inhibitory effect on *F. asiaticum*, with an EC_{50} value of 0.0029–0.0071 μg·mL^{-1}, followed by pyraclostrobine and prochloraz, with EC_{50} values of 0.0045–0.0076 and 0.0059–0.0126 μg·mL^{-1}, respectively. In addition, propiconazole·azoxystrobin had the weakest inhibitory effect, with an EC_{50} value of 0.0101–0.0187 μg·mL^{-1} (Table 3, Figure 7).

Table 3. Sensitivity of the ten tested *Fusarium asiaticum* isolates to frequently used fungicides for the control of soybean root rot in northeast China.

Fungicides	EC_{50} (μg·mL^{-1})	Fungal Sensitivity to Fungicides [1]
Fludioxonil	0.0029–0.0071	S
Pyraclostrobine	0.0045–0.0076	MR
Prochloraz	0.0059–0.0126	MR
Propiconazole·azoxystrobin	0.0101–0.0187	R

[1] S (sensitive): EC_{50} < 0.0050 μg·mL^{-1}; MR (moderately resistant): EC_{50} > 0.0050–0.01 μg·mL^{-1}; R (resistant): EC_{50} > 0.01 μg·mL^{-1}.

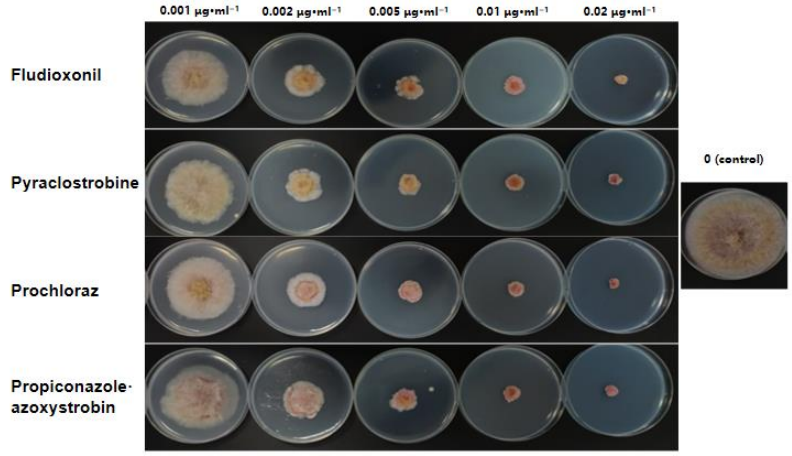


Figure 7. Sensitivity of *Fusarium asiaticum* isolate HL35 to fungicides.

3.6. Efficacy of Fungicides on Soybean Root Rot Caused by Fusarium asiaticum

Based on the result of the sensitivity of *F. asiaticum* to fungicides, fludioxonil and pyraclostrobin were selected as field control agents. In the tested doses, the higher the effective dose of fludioxonil and pyraclostrobin, the better the pot control effects (Table 4). As shown in Table 4, fludioxonil at 250 μg·mL^{-1} markedly reduced the severity of soybean root rot caused by *F. asiaticum* and had the best control efficacy of 73.8%. Pyraclostrobin at 250 μg·mL^{-1} also had good control efficacy of 69.4%. Overall, fludioxonil at 250 μg·mL^{-1} was the most effective dose for controlling soybean root rot. Moreover, the average plant height, root length, and fresh weight of all treated plants were significantly greater than those of the control group ($p < 0.05$).

Table 4. Control effect of fludioxonil and pyraclostrobin on soybean root rot through pot experiments in a greenhouse.

Fungicide	Effective Dose (μg·mL^{-1})	Control Efficacy (%) [1]	Plant Height (cm) [1]	Root Length (cm) [1]	Fresh Weight (g) [1]
fludioxonil	250	73.8 ± 2.2 a	33.4 ± 0.6 a	12.2 ± 0.6 a	8.0 ± 0.1 a
	125	55.6 ± 1.7 b	27.3 ± 0.4 b	9.6 ± 0.2 b	6.7 ± 0.1 b
	62.5	23.8 ± 2.5 c	11.4 ± 0.4 c	7.3 ± 0.4 c	5.3 ± 0.4 c
[2] Control	-	-	3.9 ± 0.2 d	4.3 ± 0.3 d	3.6 ± 0.2 d
pyraclostrobin	250	69.4 ± 1.7 a	25.8 ± 0.93 a	10.4 ± 0.3 a	7.6 ± 0.2 a
	125	43.1 ± 5.4 b	21.6 ± 0.95 b	7.8 ± 0.2 a	6.5 ± 0.2 b
	62.5	18.8 ± 2.2 c	7.9 ± 0.12 c	6.4 ± 0.6 b	4.2 ± 0.1 c
[2] Control	-	-	3.9 ± 0.2d	4.3 ± 0.3 c	3.6 ± 0.1d

[1] Values in the column represent the mean ± standard error (SE) of three repeated experiments. Values followed by different letters are significantly different according to the least significant difference test at $p = 0.05$. [2] Control: Not treated with fungicide.

4. Discussion

Soybean is a pivotal food crop and oil crop in China, of which Heilongjiang Province is the main soybean-producing area [67]. Soybean root rot, a soil-borne ailment, affects the entire soybean production lifecycle and severely curtails soybean yields globally [68]. However, distinguishing *F. asiaticum* from other *Fusarium* species using traditional morphological inspections or molecular analysis relying on rDNA-ITS sequencing proves challenging [69]. To ensure the accuracy of identification, the translation elongation factor 1-α (*Tef1*) and β-tubulin (*Tub2*) genes of representative isolates can be amplified and sequenced, as was performed in the current study. *Fusarium asiaticum* has been reported to infect soybeans and cause root rot in southwest China [15], but it is the first identified pathogen of soybean root rot in northeastern China. There are significant differences in

climate and soil between the two places. Thus, characterizing *F. asiaticum* is crucial for understanding the etiology of the disease, including its occurrence and prevalence, as well as developing more scientific and appropriate prevention strategies.

There are many inoculation methods for determining the pathogenicity of *Fusarium* spp., including the root injury inoculation method, root-dipping inoculation method, and root-burying method using sorghum grains with fungal hyphae [70–72]. The inoculation amount, investigation time, and investigation standards also differ among these methods. Because the root embedding method is simple, fast, and more closely reflects natural disease infection, this inoculation method was selected in the current study.

A previous study showed that the optimum temperature and pH for *F. asiaticum* were 20–25 °C and 7.0–9.0, respectively. Soybean root rot occurred during the entire soybean growth period. The optimum growth conditions for *F. asiaticum* causing soybean root rot were temperatures of 20–25 °C and pH between 7.0 and 9.0, which was consistent with temperature and soil alkalinity conditions in Northeast China. Our results differed from other studies to some extent, which may be due to the different environmental conditions in which the disease occurs. Therefore, this may be one of the reasons why these isolates may seriously harm soybean production in Northeast China.

In the host range determination, *F. asiaticum* isolates infected black soybean, French bean, white hyacinth bean, mung bean, and adzuki bean roots, but did not infect rice, peanut, corn, and oat roots. It has been reported in China that *F. asiaticum* infection caused panicle rot in foxtail millet [*Setaria italica* (L.) P. Beauv.], stem rot in Ligusticum chuanxiong, seedling blight in maize, fruit rot in melon (*Cucumis melo* L.), and Fusarium head blight (FHB) in wheat (*Triticum aestivum* L.) [73–76]. *Fusarium asiaticum* has a relatively wide geographical distribution and host range, which can lead to significant yield losses. The non-host crops can disrupt the disease cycle by reducing the pathogen's population in the soil. By alternating soybean cultivation with non-host crops, the accumulation of *F. asiaticum* in the soil can be minimized, thus reducing the risk of root rot in subsequent soybean crops. However, the selection of non-host crops depends on various factors, such as soil type, climate, and local agricultural practices. Further research is still being conducted to optimize the combination of non-host crops in different regions to achieve the best disease-control and yield-improvement effects.

Currently, the most efficient way to mitigate soybean root rot caused by *Fusarium* spp. is soybean seed coating with appropriate fungicides [77]. In this study, four chemical fungicides—pyraclostrobin, prochloraz, fludioxonil, and pyraclostrobine—were selected. Results indicated that fludioxonil exerted the strongest inhibitory effect on the growth of *F. asiaticum*, followed by pyraclostrobin, based on their sensitivities to the selected fungi. In the greenhouse pot experiment, 250 $\mu g \cdot mL^{-1}$ fludioxonil reduced disease incidence by 73.8% and improved soybean-seedling quality. However, Qiu et al. (2018) found that four field strains of *F. asiaticum* were highly resistant to fludioxonil, the EC_{50} values ranging from 80 to > 400 $\mu g \cdot mL^{-1}$ [78]. In the present study, *F. asiaticum* isolates from diseased soybean roots showed high sensitivity to fludioxonil. *F. asiaticum* is a newly emerged pathogen causing soybean root rot in northeast China; the isolates have not developed resistance to fludioxonil. Thus, fludioxonil holds potential for controlling soybean root rot caused by *F. asiaticum* in northeast China. Nevertheless, further research is advisable to precisely determine the appropriate application method and timing.

5. Conclusions

To the best of our knowledge, this study is the first to delve into the effects of *Fusarium asiaticum* on soybean root rot in northeast China. Our results show that *F. asiaticum* has a broad host range and can cause root rot, thus posing a potential risk to regional crop

production. Employing intercropping with non-host plants, combined with the application of fludioxonil, can effectively control the soybean root rot caused by *F. asiaticum*. Therefore, when devising advanced strategies for soybean disease management, it is crucial to conduct in-depth investigations into the occurrence of this disease. This not only aids in better understanding the disease mechanism but also facilitates the development of more targeted and effective control measures. By comprehensively examining disease occurrence and considering factors such as soil conditions, climate, and crop growth cycles, we can optimize the application of control methods like intercropping and fungicide use. This comprehensive approach will contribute to the sustainable development of soybean production in northeast China, ensuring both high yields and good quality.

Author Contributions: Conceptualization, Y.L. and J.L.; methodology, Y.L. and J.D.; software, J.L., W.C., Q.Z., Z.R., L.L., and L.S.; validation, J.L., W.C., Q.Z., Z.R., L.L., and L.S.; formal analysis, Y.L. and J.D.; investigation, J.L., W.C., Q.Z., Z.R., L.L., and L.S.; resources, Y.L. and J.D.; data curation, J.L., W.C., Q.Z., Z.R., L.L., and L.S.; writing—original draft preparation, J.L., W.C., and Q.Z.; writing—review and editing, Y.L. and J.L.; funding acquisition, Y.L. and J.D. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Key Project of the Heilongjiang Provincial Natural Science Foundation (ZD2023C001), the National Natural Science Foundation of China (32372048), the China Agriculture Research System of MOF and MARA (CARS04), the Heilongjiang Province Seed Industry Innovation and Development Project, the Heilongjiang Collaborative Innovation and Extension System of Modern Agricultural Industry Technology of Forage and Feed, and the Talent Introduction Project of Northeast Agricultural University (54960312).

Data Availability Statement: The datasets generated and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Sequences used for concatenated alignment.

Strains	ITS Regions	*TEF* Gene	*GPD* Gene
Fusarium asiaticum HL9	OQ061210.1	OQ378361.1	OQ378358.1
F. asiaticum HL15	OQ061466.1	OQ378360.1	OQ378363.1
F. asiaticum HL25	OQ061472.1	OQ378362.1	OQ378359.1
F. asiaticum HL35	OM967192.1	ON011079.1	ON011080.1
F. asiaticum	OM100564.1	LC500693.1	KM062027.1
F. oxysporum	MH221085.1	KY123890.1	LC592361.1
F. subglutinans	KY318486.1	KF467375.1	OK000516.1
F. verticillioides	KX385055.1	KF467376.1	OK000520.1
F. mundagurra	MZ379241.1	MZ399212.1	MZ399215.1
F. verrucosum	KM231812.1	KM231940.1	KM232077.1
F. proliferatum	GU074010.1	KF467371.1	GU338455.1
F. sambucinum	DQ132833.1	KM231941.1	KF896804.1
F. illudens	KM231806.1	KM231934.1	KM232068.1
F. graminearum	JX162395.1	MW620072.1	OM048104.1
Alternaria alternata	MK351431.1	MT178330.1	MN607983.1

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