



Wilt of *Acer negundo* L. caused by *Fusarium nirenbergiae* in China

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Abstract Boxelder maple (*Acer negundo* L.) is widely grown as ornamental trees in China. In 2017 in Pizhou, Jiangsu Province, China, the boxelder maple trees suffered from a disease which caused wilting and yellowing leaves, rotted roots, and discolored xylem. The disease broke out from July to August. Seven fungal isolates were obtained from symptomatic tissue and tested for pathogenicity. Isolate An7 was isolated at high frequency from the discolored vascular tissues, which caused the wilt disease on healthy 2-year-old seedlings after inoculation and eventually killed all the seedlings. We conducted molecular analysis for An7, including the partial sequences of the rDNA internal transcribed spacer, calmodulin, RNA polymerase II second largest subunit and the translation elongation factor 1- α and evaluation of the variously sized conidia, chlamydospores and conidiophores and cultural traits. The pathogen was identified as *Fusarium nirenbergiae*, a member of the *Fusarium oxysporum* species

complex. To our knowledge, this is the first report of *F. nirenbergiae* causing wilt on *A. negundo* in China.

Keywords Boxelder maple · *Fusarium oxysporum* species complex · Identification · Pathogenicity test

Introduction

Native to North America, boxelder maple (*Acer negundo* L.), has a large crown, beautiful stature and colorful fall foliage. Because of its high ornamental value, rapid growth and valuable characteristics, such as tolerance of sun exposure and a wide range of soil conditions, including salt and alkali, it is widely used for urban greening in China, especially in northern China, where the soil is poor and highly saline (Li et al. 2014). Moreover, boxelder maple also has considerable food and industrial value. It blooms in early spring and is rich in nectar, making it a valuable food source for honey bees. Bark fiber from boxelder maple can be used for papermaking, and the quality of wood is so high that it can be used for furniture and joinery (Hu and Zhai 2017). However, in Pizhou, wilt has killed hundreds of boxelder seedlings during the past 2 years, affecting the local environment and causing serious economic losses.

Several pathogens of boxelder maple have been reported. In Seville, Spain, *Inonotus rickii* causes canker rot disease with stem bulge and bark necrosis, and golden-brown fungal tissue can be seen at the site of the disease, sometimes hidden under the bark (Intini 2002). In 2002 (Xinjiang Province, China) and 2006 (Turkey), pathogens associated with wilt were identified as *Verticillium dahliae* and *Fusarium solani*, respectively (Schmid and Momei 2003; Demirci and Maden 2006). Differences are obvious

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between the symptoms caused by two pathogens. *V. dahliae*, a well-known pathogen, causes rapid wilting and death, leaf drying and shedding, necrosis of twigs and discoloration of vascular tissue. Plants infected by *F. solani* show some withering of leaves and branches, and brown or red discolored areas are found in the diseased branches and sapwood, and cracks appear inside the branches of dead seedlings. Boxelder can also provide a suitable living environment for beetles, which may transmit fungi. For example, *Fusarium euwallaceae*, a member of the *F. solani* species complex, is transmitted by beetles and can infect and ultimately lead to tree death (Freeman et al. 2013).

F. oxysporum, one of the most infamous soil-borne fungal pathogens, can cause Fusarium wilt, a destructive vascular wilting in more than 100 species globally, including *Eucalyptus* (Wu et al. 2007), *Vernicia fordii* (Hua 1981), and *Populus × euarmiereana* (Xie et al. 1993) in China. It survives in the soil in the form of chlamydospores for several years, which germinate in response to root exudates from host plants, and forms conidia, new chlamydospores and hyphae, finally invading the hosts directly or through wounds in the roots. The hyphae then colonize the vascular tissues, and the pathogen spreads in the form of conidia within the stems. During this process, secondary fungal metabolites are secreted, which inhibit the innate immunity of the host, hampering water and nutrient transport, and causing leaf wilting and eventually death of the whole plant. As the dead plant decomposes, the pathogen returns to a dormant state in the soil until it encounters an appropriate host to infect (Nelson 1981; Goud and Hiemstra 1998).

In addition to the long survivability of the chlamydospores in the soil, the rapid speed and high mortality rates of Fusarium wilt make disease control difficult. The objectives of this study were: (1) to investigate the incidence and symptoms of the disease affecting *A. negundo* in Pizhou, Jiangsu Province; (2) to follow classical Koch's postulates (isolate the pathogen from diseased samples, reproduce symptom and disease progression, and recover the pathogen from inoculated plants); and (3) to identify the pathogen by morphological and molecular methods to better understand the epidemiology of the disease toward designing effective control measures.

Materials and methods

Fungal isolation, purification and preservation

From September 2017 to September 2018, planted plots of boxelder maple in Pizhou, Jiangsu Province, China were studied to understand the progression and incidence of wilt disease. Root and stem tissues were sampled from 10

symptomatic plants and washed with running tap water for about 30 min, then surface-sterilized with 75% (v/v) ethanol for 5 min, rinsed in sterilized water three times and dried with sterile filter paper. The discolored vascular tissues were cut into 5-mm-long segments and plated on potato dextrose agar (PDA) containing 0.1% w/v ampicillin to inhibit bacterial growth (Li et al. 2017), then cultured in the dark at 25 °C, and colony morphology, size, color and growth rate as differentiating variables, were monitored daily. Individual fungal colonies were purified by single-spore isolation, and spores were preserved and stored in 25% v/v glycerin at – 80 °C (Brady et al. 2011).

Preparing of ITS sequences and phylogenetic tree of *Fusarium* spp.

Except strains isolated from our study, other sequences were downloaded from GenBank (<ftp://ncbi.nlm.nih.gov/genbank/genomes/>) (Table S1). The software BioEdit version 7.05 (Ibis Therapeutics, Carlsbad, CA, USA) was used to align the sequences and manually correct any bases missing from these sequences. MEGA 5.0 was used to construct a phylogenetic tree with the neighbor-joining (NJ) approach (Tamura et al. 2011), which was validated using bootstrap analyses with 1000 repetitions (Saitou and Nei 1987) based on internal transcribed spacer (ITS) sequences.

Pathogenicity test

Conidia produced by the isolates from infected tissue were prepared in Czapek liquid medium (NaNO₃ 3.0 g, K₂HPO₃ 1.0 g, MgSO₄·7H₂O 0.5 g, KCl 0.5 g, sucrose 30 g in 1 L water) and incubated at 25 °C on a shaker at 250 rpm for at least 5 days. The fungal suspension was then passed through two layers of sterile filter cloth to obtain a spore suspension, which was adjusted to 1 × 10⁸/mL with sterile water. To fulfill Koch's postulates, 2-year-old boxelder maple seedlings were grown in non-woven bags used for pathogenicity tests. Shallow wounds were cut in the roots of seedlings using a sterile blade to provide entry sites for the fungus. Each plant was watered with 50 mL of the conidial suspension, control plants were watered with sterile water (Li et al. 2018). Each treatment and control group contained five replicates, one seedling per replicate. To replicate original growth conditions for seedlings, the plants were incubated outdoors.

After symptoms appeared on the inoculated plants, the pathogen was isolated from the roots and stems of the diseased plants, and its colony characteristics were compared with those of the original colony to verify pathogenicity.

Morphological observations

After 5 days on PDA in the dark, morphology, color and growth rate of the culture were observed and recorded (Garcia et al. 2018), and after 10 days, chlamydospores were observed and measured (Liu et al. 2015). Pigmentation production was tested on rice culture medium (10 g rice/conical flask). Conidial sporulation structures were observed when the isolates were grown on synthetic low-nutrient agar (KH₂PO₃ 1.0 g, KNO₃ 1.0 g, MgSO₄·7H₂O 0.5 g, KCl 0.5 g, sucrose 0.2 g, glucose 0.2 g, agar 15 g in 1-L water) for 2 days, while macroconidia and microconidia could be observed after 4 days (Krnjaja et al. 2011). The dimensions of 90 spores of each spore type were measured using a Zeiss microscope (Zeiss, Jena, Germany). The pathogen was identified according to the Booth classification system (Booth 1971).

Molecular identification

The test pathogen was cultured on PDA for 3 days at 25 °C, then genomic DNA was isolated using the cetyltrimethylammonium bromide (CTAB) method (Möller et al. 1992). Briefly, hyphae were cut up and placed in a sterile Eppendorf tube with 500 µL each of chloroform and 2% CTAB, then shaken for 1.5 h at 200 rpm, before being centrifuged at 4 °C, 13,000×g for 10 min. The supernatant (300 µL) was transferred to another tube with 600 µL ethanol, then centrifuged at 4 °C and 13,000×g for 5 min. The supernatant was discarded, and the pellet was washed twice with 75% (v/v) ethanol. After the ethanol was volatilized, 50 µL sterile deionized water was added to the pellet to obtain a crude DNA suspension.

For identifying the species of the *Fusarium* isolate, the ITS and the calmodulin (*cmdA*), RNA polymerase II second largest subunit (*RBP2*), and translation elongation factor 1- α (*TEF-1 α*) genes were amplified using the primer pairs in Table 1. The 50- μ L polymerase chain reaction (PCR) volume contained 25 µL Mix (Vazyme, Nanjing, China), 17 µL ddH₂O, 4 µL DNA and 2 µL of each primer.

The PCR reaction conditions for the ITS, *cmdA*, and *TEF-1 α* were 95 °C for 5 min; 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 45 s; and 72 °C for 10 min. For *RBP2*, the conditions were 95 °C for 5 min; 35 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1.5 min; and 72 °C for 10 min. For each sequence, the amplified product was detected by gel electrophoresis on 1% agarose. Primer synthesis and nucleic acid sequencing of PCR products were carried out by Shanghai Jieli Biotechnology Corporation (Shanghai, China).

The ITS sequence was used in a BLAST search of the GenBank database (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA; <http://www.ncbi.nlm.nih.gov>) to initially determine its identity. The *cmdA*, *RBP2* and *TEF-1 α* sequences of other species in the *Fusarium oxysporum* species complex (FOSC) were downloaded from NCBI to use in a cluster analysis (Table 2). The software BioEdit version 7.05 (Ibis Therapeutics, Carlsbad, CA, USA) was used to align the sequences and manually correct any bases missing from these sequences. MEGA 5.0 was used to construct a phylogenetic tree with the neighbor-joining (NJ) approach (Tamura et al. 2011), which was validated using bootstrap analyses with 1000 repetitions (Saitou and Nei 1987) based on the *cmdA*, *RBP2* and *TEF-1 α* sequences.

Results

Field studies on progression and characteristics of the disease

In recent years, as a result of poor management, low soil permeability, high planting density and other factors, diseases of boxelder maple, especially wilt, have become increasingly serious. In July 2017, some of the trees in Pizhou, Jiangsu Province showed the symptoms of wilt. Wilt symptoms first occurred in early May, reached their peak in July and August, and lasted until September. Disease progression was rapid. The pathogen infected seedlings of different ages, resulting in death within a short

Table 1 Primer sequences used for molecular identification of fungus causing wilt disease of boxelder

| Primer name | Sequence (5' to 3') | References |
|-------------|-------------------------|--|
| ITS1 | TCCGTAGGTGAACCTGCGG | White et al. (1994) |
| ITS4 | TCCTCCGCTTATTGATATGC | |
| Cal228F | GAGTTC AAGGAGGCCTTCTCCC | Carbone and Kohn (1999) |
| CAL2Rd | TGRTCNGCCTCDCGGATCATCTC | Groenewald et al. (2013) |
| 5f2 | GGGGAGATCAGAAGAAGGC | Liu et al. (1999) and Sung et al. (2007) |
| 7er | CCCATAGCTTGTTACCCAT | |
| EF-1Ha | ATGGGTAAGGAAGACAAGAC | O'Donnell et al. (1998) |
| EF-2 Tb | GGAAGTACCAGTGATCATGTT | |

Table 2 Related reference strains of *Fusarium* species used for phylogenetic analysis

| Strain | Species name | GenBank accessions | | |
|--|-----------------------------|--------------------|-------------|-------------|
| | | <i>cmdA</i> | <i>RPB2</i> | <i>TEF1</i> |
| CBS 187.53 | <i>Fusarium callistephi</i> | MH484693 | MH484875 | MH484966 |
| CBS 115423 | <i>F. callistephi</i> | MH484723 | MH484905 | MH484996 |
| CBS 144739 = CPC 25792 | <i>F. carminascens</i> | MH484752 | MH484934 | MH485025 |
| CBS 144740 = CPC 25793 | <i>F. carminascens</i> | MH484753 | MH484935 | MH485026 |
| CBS 144741 = CPC 25795 | <i>F. carminascens</i> | MH484754 | MH484936 | MH485027 |
| CBS 144738 = CPC 25800 | <i>F. carminascens</i> | MH484755 | MH484937 | MH485028 |
| CBS 111552 | <i>F. contaminatum</i> | MH484718 | MH484900 | MH484991 |
| CBS 114899 | <i>F. contaminatum</i> | MH484719 | MH484901 | MH484992 |
| CBS 117461 | <i>F. contaminatum</i> | MH484729 | MH484911 | MH485002 |
| CBS 620.72 = DSM 11271 = NRRL 36520 | <i>F. cugenangense</i> | MH484697 | MH484879 | MH484970 |
| CBS 130304 = BBA 69050 = NRRL 25433 | <i>F. cugenangense</i> | MH484739 | MH484921 | MH485012 |
| CBS 130308 = ATCC 26225 = NRRL 25387 | <i>F. cugenangense</i> | MH484738 | MH484920 | MH485011 |
| CBS 131393 | <i>F. cugenangense</i> | MH484746 | MH484928 | MH485019 |
| CBS 247.61 = BBA 8398 = DSM 62308 = NRRL 22545 | <i>F. curvatum</i> | MH484694 | MH484876 | MH484967 |
| CBS 141.95 = NRRL 36251 = PD 94/1518 | <i>F. curvatum</i> | MH484712 | MH484894 | MH484985 |
| CBS 102026 = NRRL 36115 | <i>F. duoseptatum</i> | MH484714 | MH484896 | MH484987 |
| CBS 217.49 = NRRL 36358 | <i>F. elaeidis</i> | MH484688 | MH484870 | MH484961 |
| CBS 218.49 = NRRL 36359 | <i>F. elaeidis</i> | MH484689 | MH484871 | MH484962 |
| CBS 255.52 = NRRL 36386 | <i>F. elaeidis</i> | MH484692 | MH484874 | MH484965 |
| CBS 144743 = CPC 25802 | <i>F. fabacearum</i> | MH484757 | MH484939 | MH485030 |
| CBS 144744 = CPC 25803 | <i>F. fabacearum</i> | MH484758 | MH484940 | MH485031 |
| CBS 120665 | <i>F. foetens</i> | MH484736 | MH484918 | MH484918 |
| CBS 176.33 = NRRL 36286 | <i>F. glycines</i> | MH484686 | MH484868 | MH484959 |
| CBS 200.89 | <i>F. glycines</i> | MH484706 | MH484888 | MH484979 |
| CBS 144746 = CPC 25808 | <i>F. glycines</i> | MH484760 | MH484942 | MH485033 |
| CBS 116611 | <i>F. gossypinum</i> | MH484725 | MH484907 | MH484998 |
| CBS 116613 | <i>F. gossypinum</i> | MH484727 | MH484909 | MH485000 |
| CBS 132476 | <i>F. hoodiae</i> | MH484748 | MH484930 | MH485021 |
| CBS 132477 | <i>F. hoodiae</i> | MH484749 | MH484931 | MH485022 |
| CBS 645.78 = NRRL 36531 | <i>F. languescens</i> | MH484698 | MH484880 | MH484971 |
| CBS 646.78 = NRRL 36532 | <i>F. languescens</i> | MH484699 | MH484881 | MH484972 |
| CBS 413.90 = ATCC 66046 = NRRL 36465 | <i>F. languescens</i> | MH484708 | MH484890 | MH484981 |
| CBS 300.91 = NRRL 36416 | <i>F. languescens</i> | MH484709 | MH484891 | MH484982 |
| CBS 872.95 = NRRL 36570 | <i>F. languescens</i> | MH484713 | MH484895 | MH484986 |
| CBS 119796 = MRC 8437 | <i>F. languescens</i> | MH484735 | MH484917 | MH485008 |
| CBS 144747 = CPC 25788 | <i>F. libertatis</i> | MH484751 | MH484933 | MH485024 |
| CBS 144749 = CPC 28465 | <i>F. libertatis</i> | MH484762 | MH484944 | MH485035 |
| CBS 127.81 = BBA 63924 = NRRL 36229 | <i>F. nirenbergiae</i> | MH484701 | MH484883 | MH484974 |
| CBS 115416 = CPC 5307 | <i>F. nirenbergiae</i> | MH484720 | MH484902 | MH484993 |
| CBS 115417 = CPC 5306 | <i>F. nirenbergiae</i> | MH484721 | MH484903 | MH484994 |
| CBS 123062 = GJS 91-17 | <i>F. nirenbergiae</i> | MH484737 | MH484919 | MH485010 |
| CBS 794.70 = BBA 11103 = NRRL 22550 | <i>F. odoratissimum</i> | MH484696 | MH484878 | MH484969 |
| CBS 102030 | <i>F. odoratissimum</i> | MH484716 | MH484898 | MH484989 |
| CBS 130310 = NRRL 25603 | <i>F. odoratissimum</i> | MH484740 | MH484922 | MH485013 |
| CBS 221.49 = IHEM 4508 = NRRL 22546 | <i>F. oxysporum</i> | MH484690 | MH484872 | MH484963 |
| CBS 144135 | <i>F. oxysporum</i> | MH484772 | MH484954 | MH485045 |
| CPC 25822 | <i>F. oxysporum</i> | MH484761 | MH484943 | MH485034 |

Table 2 continued

| Strain | Species name | GenBank accessions | | |
|-------------------------------------|-------------------------------|--------------------|-------------|-------------|
| | | <i>cmdA</i> | <i>RPB2</i> | <i>TEF1</i> |
| CBS 144750 = CPC 30822 | <i>F. pharetrum</i> | MH484769 | MH484951 | MH485042 |
| CBS 144751 = CPC 30824 | <i>F. pharetrum</i> | MH484770 | MH484952 | MH485043 |
| CBS 102028 = NRRL 36117 | <i>F. trachichlamyosporum</i> | MH484715 | MH484897 | MH484988 |
| CBS 116619 | <i>F. triseptatum</i> | MH484728 | MH484910 | MH485001 |
| CBS 119665 | <i>F. triseptatum</i> | MH484734 | MH484916 | MH485007 |
| CBS 177.31 | <i>F. udum</i> | MH484684 | MH484866 | MH484957 |
| NRRL 62542 | <i>F. veterinarium</i> | MH484765 | MH484947 | MH485038 |
| NRRL 62545 | <i>F. veterinarium</i> | MH484766 | MH484948 | MH485039 |
| NRRL 62547 | <i>F. veterinarium</i> | MH484767 | MH484949 | MH485040 |
| CBS 128.81 = BBA 63925 = NRRL 36233 | <i>Fusarium</i> sp. | MH484702 | MH484884 | MH484975 |
| CBS 680.89 = NRRL 26221 | <i>Fusarium</i> sp. | MH484707 | MH484889 | MH484980 |
| CBS 130323 | <i>Fusarium</i> sp. | MH484745 | MH484927 | MH485018 |

time. Normally, leaves senesce and fall in November. However, when the disease started, the leaves sporadically turned yellow (Fig. 1b). In later stages, most leaves became brown, withered and shrunken, falling off prematurely. The twigs became necrotic, and finally the seedling died

(Fig. 1c, d). The phloem turned light brown in the longitudinal direction (Fig. 1e), with longitudinal sections of xylem showing reddish-brown and lavender in color (Fig. 1f). The intact root was reddish brown or purple,

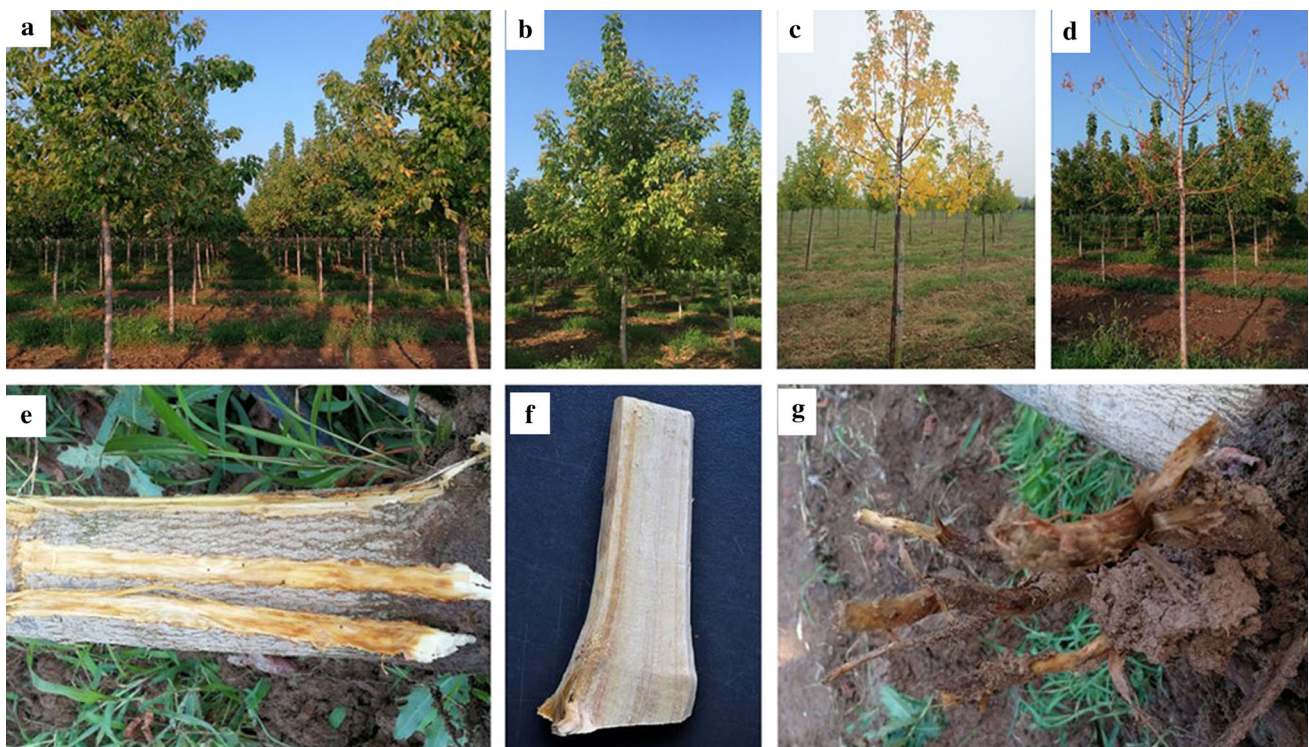


Fig. 1 Symptoms of fusarium wilt in *Acer negundo* L. **a** Planted plot; **b** early symptoms; **c** Later symptoms; **d** dead plant; **e** discolored phloem; **f** longitudinal section of xylem; **g** roots of diseased plant

epidermis was rotted and had exfoliated, and the root surface became fibrous and flocculent (Fig. 1g).

Fungal isolation and pathogenicity tests

Among the 80 single-spore isolates obtained, most were morphologically similar to *Fusarium* spp. Because of the diversity of *Fusarium* species, ITS sequencing and phylogenetic analysis (Fig. S1) of these strains performed, and the result showed that there were *Fusarium equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani* and *F. tricinctum*, others were *Bipolaris shoemakeri*, *Botryosphaeria dothidea*. These strains isolated at a frequency of 5, 10, 15, 5, 10, 10, and 45%, and named An1 to An7, respectively. (GenBank accessions of isolated *Fusarium* spp. and morphological characteristics were shown in Tables S2, S3–S6 and Figs. S2–S6.)

In the pathogenicity tests on healthy 2-year-old seedlings (5 each/fungal isolate), only isolate An7 caused symptoms, and controls were symptom-free (Fig. 2a). With An7, after 42 days, one seedling showed wilt symptoms (Fig. 2b) and died on day 46; the other four seedlings died on day 49, 52, 55 and 60, respectively. The roots of the dead plants were rotted and fibrous and easily exfoliated.

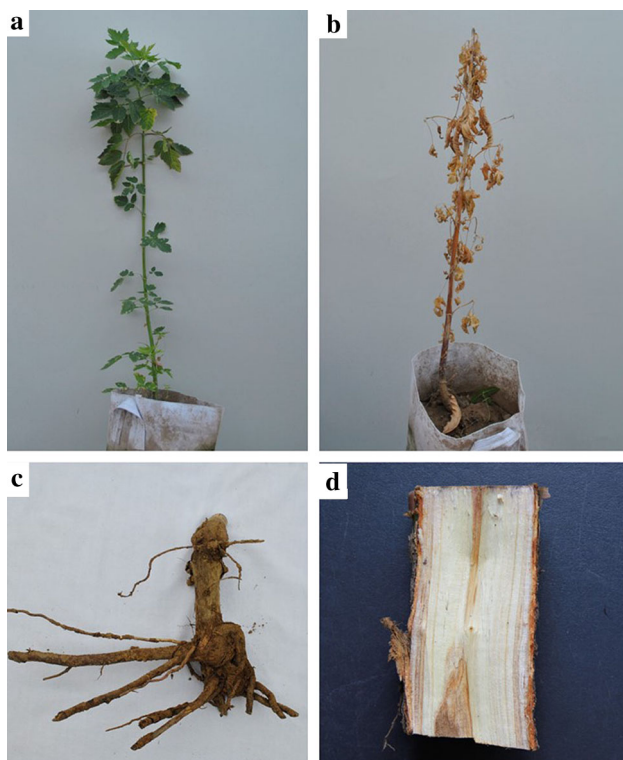


Fig. 2 Symptoms on 2-year-old boxelder maple saplings after inoculation with isolate An7. **a** Control treated with water. **b** Diseased sapling, **c** roots of diseased sapling, **d** longitudinal section from stem of diseased sapling 46 days after inoculation with An7

The cortex in some of the lateral roots was reddish-brown or lavender, and the stem had a distinct reddish-brown or lavender discoloration in longitudinal section (Fig. 2c, d). These symptoms resembled those on naturally infected diseased plants in the field. Isolate An7 was consistently reisolated from the roots and stems of all symptomatic seedlings and thus, determined to be the causative agent of *A. negundo* wilt.

Morphology

The pathogen grew moderately well on PDA; colony diameter was 6.4 cm after 5 days in the dark at 25 °C. At first, the colony was white with abundant and flocculent aerial hyphae, but later turned light purple (Fig. 3a, b). A purple pigment was also produced on rice culture medium. Microconidia were abundant, ovate, oval or kidney-shaped, 1–2-celled, with 0–2 septa (Fig. 4a–c). Macroconidia were less abundant, and in the shape of orange petal spindles, or sickles. The macroconidia had tapered, slightly curved apical cells and foot-shaped cells at the base (Fig. 4d–f). Macroconidia most commonly had three septa. Hyphae were septate, and spores were borne on long or short conidiophores, mostly with single branches (Fig. 4g). After 10 days on PDA, globose, thick- and smooth-walled chlamydospores were produced, intercalary or solitary, commonly single, but sometimes chained (Fig. 4h). Spores dimensions are given in Table 3.

On the basis of colony morphology and color, conidial characteristics including the pedicel and chlamydospore characteristics, the pathogen was identified as *F. oxysporum* (Booth 1971).

Molecular identification

After PCR amplification with primers ITS1/ITS4, Cal228F/CAL2Rd, 5f2/7cr and EF1H/EF2T, fragments of 529, 604, 936 and 576 bp were obtained, respectively (GenBank accessions MH990908, MK495366, MK495367, MK011886, respectively). The ITS sequence of the test pathogen 100% similarity with that of *F. oxysporum* in the BLAST search.

In the phylogenetic tree based on the *cmdA*, *RPB2* and *TEF-1 α* sequences of the test pathogen and other FOSSC strains (number of sites = 590, bootstrap replicates = 1000), the 60 strains of *Fusarium* species were clearly divided into several clades, representing different species within the FOSSC (Fig. 5). The test pathogen had highest similarity with *Fusarium nirenbergiae* rather than *Fusarium curvatum*. Therefore, the pathogen was confirmed to be *F. nirenbergiae*.

Fig. 3 Colony of *Fusarium oxysporum* isolated from *Acer negundo* cultured after 5 days on PDA at 25 °C. **a** Top and **b** reverse view

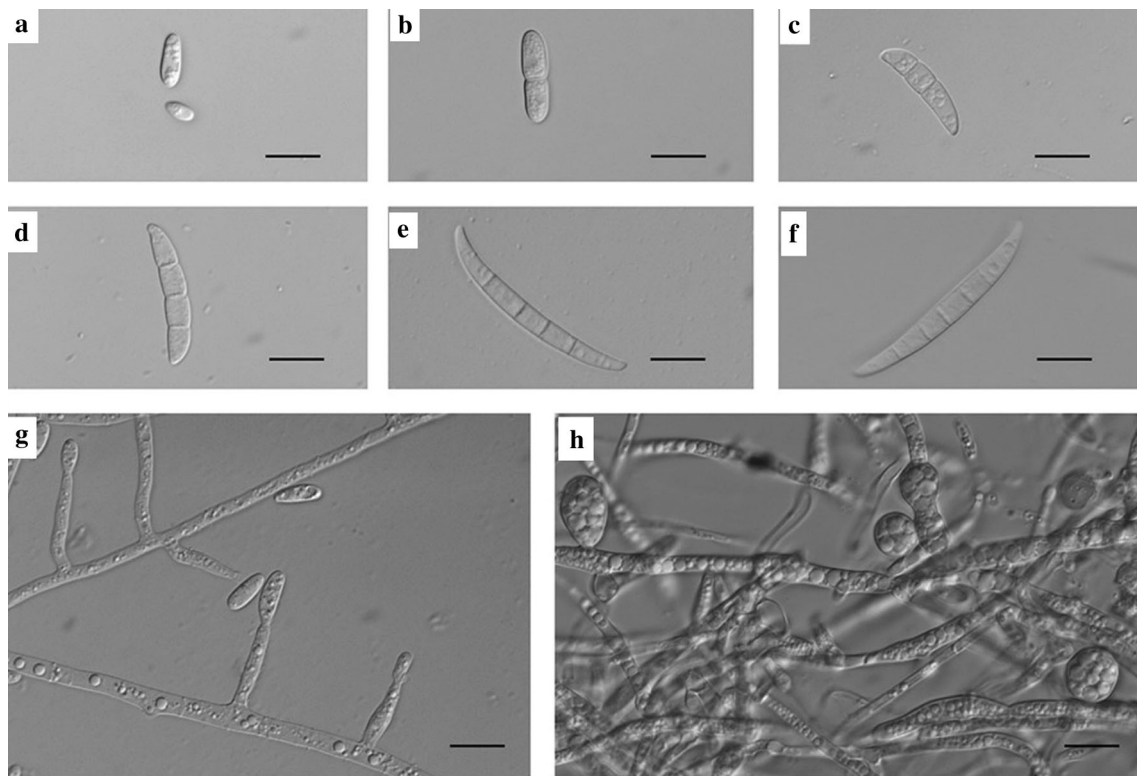
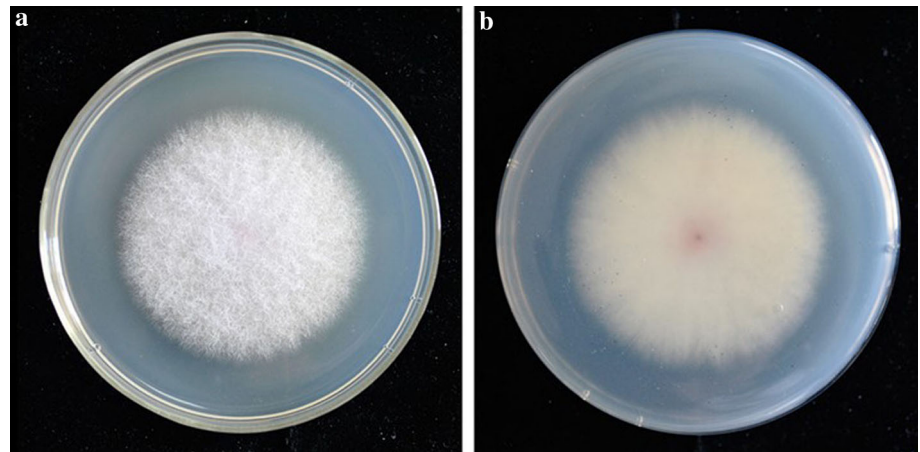


Fig. 4 Micro- and macroconidia, conidiophores and chlamydospores of *Fusarium oxysporum*. **a, b** Microconidia; **c–f** macroconidia with 2–5 cells; **g** conidiophores; **h** chlamydospores and hyphae. Scale bars, 10 μ m

Discussion

To the best of our knowledge, this is the first report of wilt on *A. negundo* caused by *F. nirenbergiae* in China. The FOSC has more than 150 putative plant host-specific formae speciales (Araújo et al. 2017), such as *F. oxysporum* f. sp. *elaedis*, the aetiological agent of Fusarium wilt of oil palm (Flood 2006). In India, *F. oxysporum* f. sp. *ciceri* can cause chickpea wilt (Barve et al. 2001). Zhou et al. (2010) found *F. oxysporum* f. sp. *niveum* race 3 was the high virulent race to cause the watermelon wilt.

Identifying *Fusarium* species is difficult because of their similar morphology and conservation of gene sequences. Identification on the basis of ITS sequence is not sufficiently accurate. Thus, 28S ribosomal RNA large subunit sequence, intergenic spacer region of the rDNA regions, the mitochondrial small subunit, and some protein-encoding genes, such as the *cmdA*, *RPB2*, *TEF-1 α* and *β -tubulin* have also been used. Although a single gene locus can sometimes identify a species, in other cases, multiple gene loci are often needed for identification (Yli-mattila et al. 2004). Correlation analysis of the above conserved genes

Table 3 Dimensions of the various spore types of isolate An7 that caused wilt disease of boxelder

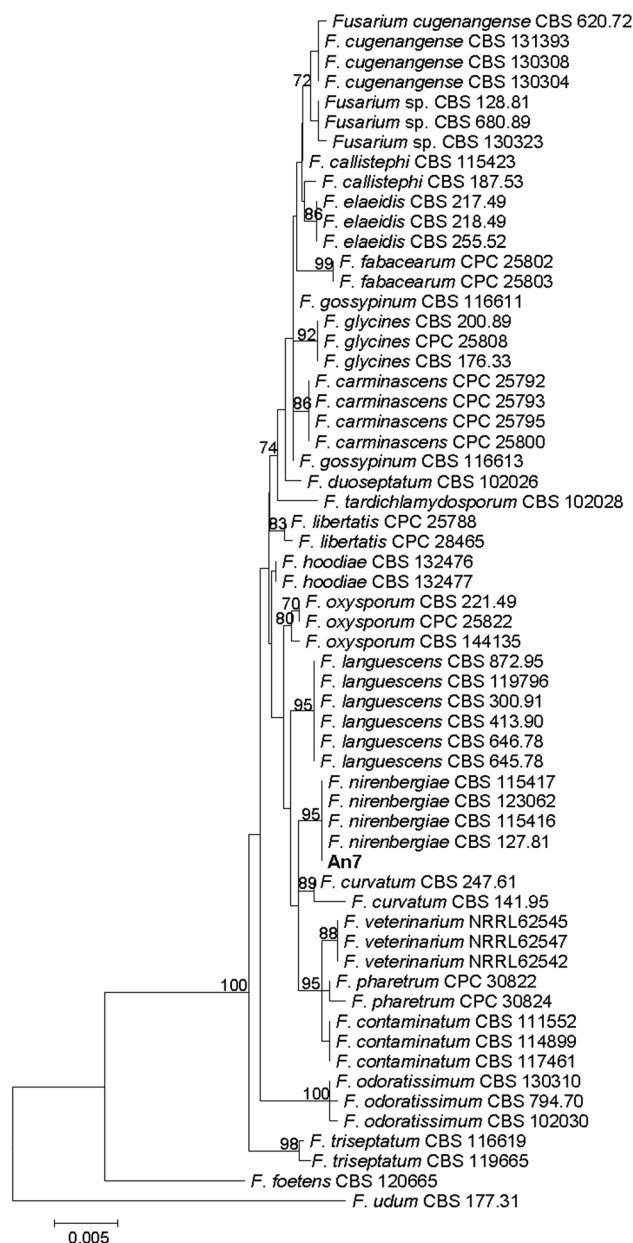
| Spore type | Length (range) × diameter (range), μm |
|---------------|--|
| 0 septum | 7.972 (4.372–16.413) × 3.748 (2.753–8.377) |
| 1 septum | 13.155 (5.966–20.857) × 4.245 (2.806–5.507) |
| 2 septa | 17.611 (10.075–24.587) × 5.133 (3.254–6.170) |
| 3 septa | 25.093 (16.442–35.575) × 4.503 (3.552–5.423) |
| 4 septa | 40.104 (35.417–45.744) × 4.421 (3.561–5.764) |
| 5 septa | 42.669 (38.672–46.553) × 4.358 (3.561–5.375) |
| Chlamydospore | 7.811 (4.196–14.196) × 7.556 (4.062–14.462) |

Microconidia: 0 or 1 septum; macroconidia: 2–5 septa

can effectively reduce errors caused by comparing single gene sequences. Lombard et al. (2019) clarified the taxonomy of *F. oxysporum* using four gene fragments, *cmdA*, *RPB2*, *TEF-1α* and *β-tubulin*. We thus used this method to identify the pathogen as *F. nirenbergiae*, a member of FOOSC. The 0-septate conidia, macroconidia and chlamydospore characteristics of pathogen are similar to the characteristics of *F. nirenbergiae*, but the colony morphology, other conidia, conidiophores differed slightly. Furthermore, according to Lombard et al. (2019) and the phylogenetic tree, *F. nirenbergiae* is closely related to *F. curvatum*, but we can distinguish them by morphology. *F. curvatum* produces strongly curved 3-septate macroconidia and aerial polyphialidic conidiogenous cells, whereas the aerial phialides of *F. nirenbergiae* are subcylindrical, and the macroconidia are slightly curving, tapering at both ends. On SNA, *F. curvatum* does not produce chlamydospores, but *F. nirenbergiae* produces nearly spherical chlamydospores.

For many pathogens, temperature plays a decisive role in growth. During the infection cycle of *V. dahliae*, high summertime temperatures can eliminate the pathogen from infected host plants, which then recover during the summer (Goud 2003). Unexpectedly, the outbreak period of the *A. negundo* Fusarium wilt was in mid to late summer, from July to August. However, in our later experiment, spores germinated below 58 °C. High temperature did not inhibit spore germination, but increases transpiration in plants, which is more conducive to the spread of spores in the vascular tissues, and the outbreak of Fusarium wilt is consequently serious.

Research into the pathogenesis of Fusarium wilt has mainly focused on cell-wall-degrading enzymes (Jonkers et al. 2009; King et al. 2011) and toxins (Bani et al. 2014). In boxelder, the root cortex was degraded, likely by cell-wall-degrading enzymes produced by the fungus. *F. oxysporum* can secrete extracellular metabolites during its growth, which can impair the permeability of the

**Fig. 5** Phylogenetic tree of isolate An7 of *Fusarium nirenbergiae* and *Fusarium* accessions from GenBank constructed based on *cmdA*, *RPB2*, *EF-1α* gene sequences

cytoplasmic membrane of the host and undermine the plant's immune response, thus causing metabolic disorders in the plant, which ultimately dies (Singh 1971; Jonkers et al. 2009). These metabolites include toxins such as fusaric acid and trichothecenes, which can destroy the membranes in the root system and inhibit the defense and functioning of the root system, resulting in rapid wilting of the plant (Pei et al. 2014). Deoxynivalenol exhibits strong phytotoxicity and accelerates the spread of the pathogen, while the trichothecenes can cause symptoms such as plant

wilting, chlorosis and blight, and inhibit protein synthesis (Shi et al. 1997).

Additional questions about the wilt of boxelder still need to be answered, including whether the pathogen is a host-specific pathogen of *A. negundo*, identifying the origin of the pathogen and its pathogenic mechanism. Maple, as a common ornamental tree species, is widely cultivated in China. The identification of this pathogen provides a reference for planting and management of this species. The identification of the causative pathogen of *A. negundo* Fusarium wilt will lead to improved detection of infested soils and sites for planting and the rationalization of fertilizer and fungicide applications, which may contribute to the reduction or prevention of the disease.

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