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DISEASE NOTES

First Report of Root and Basal Stem Rot in Sacha Inchi (Plukenetia volubilis) Caused by Fusarium oxysporum in China

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Sacha Inchi (Plukenetia volubilis L.) is a perennial oilseed woody liana belonging to the Euphorbiaceae family, and it is native to the rainforests of South America (Gillespie 2007). Sacha Inchi seeds produce oil containing a high amount of alpha-linolenic acid, and it is considered one of the most promising new crops for the production of healthy vegetable oil (Chirinos et al. 2013). In recent years, a disease characterized by root and basal stem rot, growth retardation, wilt, leaf yellowing, and eventually plant death has been observed in Sacha Inchi fields in the Xishuangbanna Prefecture of Yunnan Province in southwestern China, where the plant was introduced from South America in 2006. The disease spreads rapidly with increasing plant age, and the incidence rate exceeds 50% in plants grown for more than 3 years. Typical diseased samples were collected from the discolored vascular tissues of stems and roots and then surface-sterilized with 75% ethanol for 30 s and 1% hypochlorite for 3 min, washed three times with sterile distilled water, transferred to potato dextrose agar (PDA) plates, and incubated at 30°C for 3 days in darkness. Hyphal tips from the leading edge of each colony were then transferred to new PDA plates and further incubated for 6 days to obtain pure cultures. The mycelia appear floccose and are initially white but change to pale violet with growth. Macroconidia are long (16.73 to 26.99 x 1.93 to 3.67 micrometers), straight or slightly curved, and usually 3-septate. Microconidia are oval- or kidney-shaped (3.02 to 9.83 x 1.22 to 3.68 micrometers), and usually 0- or 1-septate. The chlamydospores are smooth and nearly round (diameter 4.69 to 17.18 micrometers). DNA sequencing of the PCR products of the internal transcribed spacer (ITS) region (MF187550), which were amplified using primers ITS1/ITS4 (White et al. 1990), revealed 100% nucleotide identity with Fusarium oxysporum (KX823413). The sequence of the translation elongation factor 1-alpha (EF1-alpha) gene (MF197315), which was obtained using primers EF1-728F/EF1-986R (Carbone and Kohn 1999), was 99% identical to F. oxysporum (KU361427). Based on the above morphological characteristics and the nucleotide sequence analysis, the fungus was identified as F. oxysporum isolate FoPvo1. Subsequently, the pathogenicity of the FoPvo1 isolate was tested. One-month-old Sacha Inchi plants were chosen for inoculation. The roots were wounded by cutting off the root tips (one fifth of the roots) and then soaked in a spore suspension of 5 x 10^6 conidia/ml for 20 min. The inoculated plants were transplanted into 240-ml cups with sterile soil wetted with 10 ml of spore suspension per plant. The controls were inoculated with sterile water. Fourteen plants were used for each treatment. All inoculated plants were maintained in a growth chamber at 28°C, 60% relative humidity, and a 16/8-h light/dark photoperiod. At 2 weeks post inoculation, the internal roots and stems showed brown or black streaks in the vascular bundles, and the leaves gradually yellowed; these symptoms were similar to those observed during natural infection. No symptoms were observed in the control plants. The inoculation experiments were repeated three times with similar results. Furthermore, F. oxysporum was reisolated from inoculated plants but not from asymptomatic plants or noninoculated controls, satisfying Koch's postulates. To our knowledge, this is the first report of F. oxysporum causing root and basal stem rot of Sacha Inchi in China.

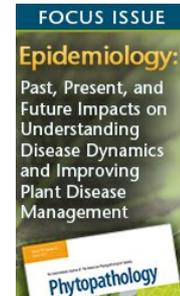
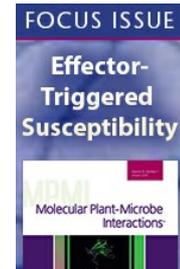
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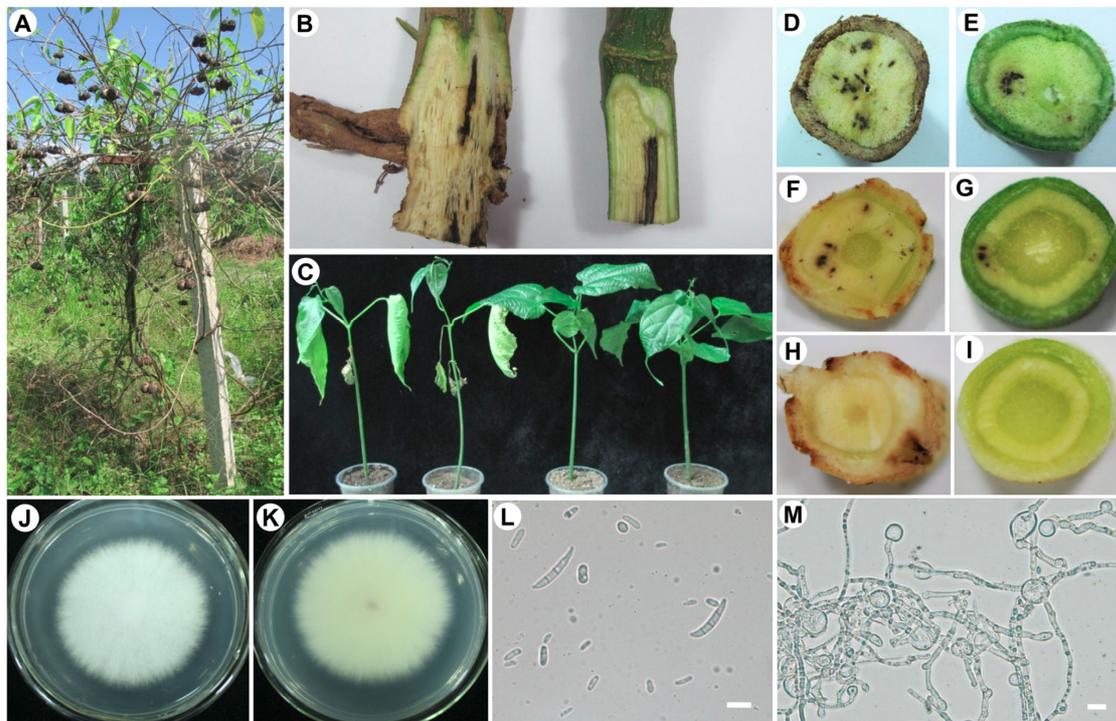
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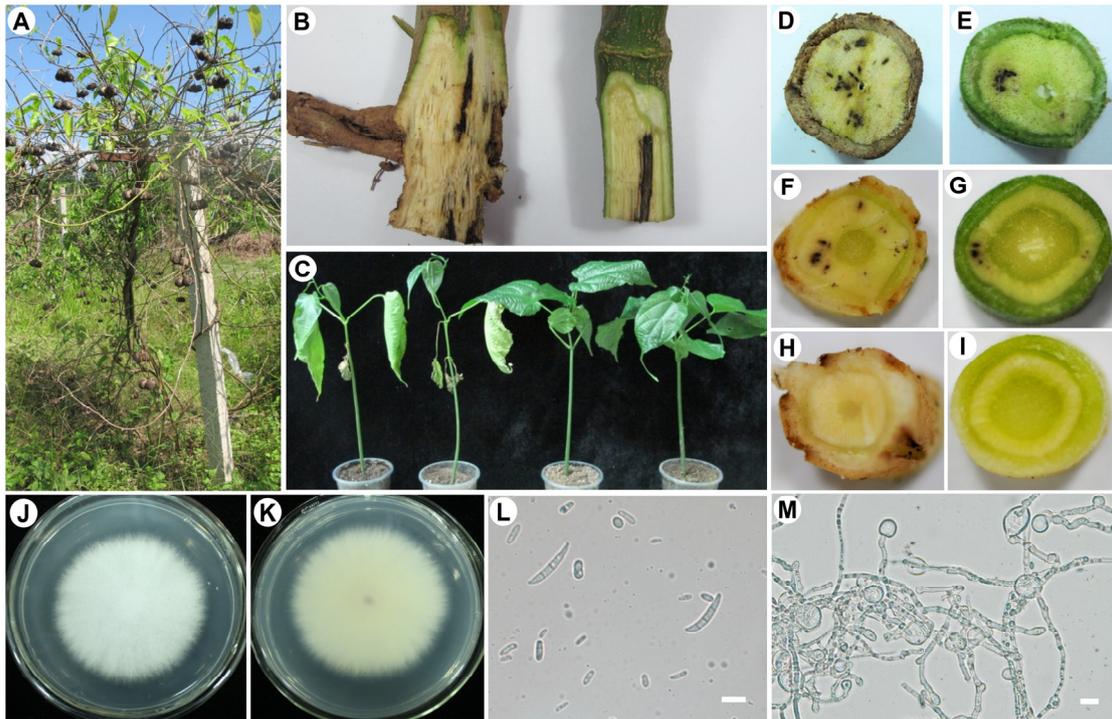
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Supplementary Figure S1 Disease symptoms of root and basal stem rot of Sacha Inchi (*Plukenetia volubilis*) and morphology of *Fusarium oxysporum* isolate FoPvo1. (A) Dead infected plants in the field. (B) Longitudinal section of the root (left) and stem (right) of infected plants in the field showing black streaks. (C) Plants showing leaf yellowing at 14 days post inoculation with *F. oxysporum* isolate FoPvo1 (left two plants), and control plants showing no symptoms (right two plants). Discolored vascular tissues were observed in transections of the root (D) and stem (E) of infected plants in the field and the root (F) and stem (G) of inoculated plants in the greenhouse, whereas no symptoms were observed in the root (H) and stem (I) of controls. Colony of *Fusarium oxysporum* isolate FoPvo1 observed from the top (J) and bottom (K) of a PDA Petri dish cultivated for 6 days. (L) Macroconidia and microconidia. (M) Chlamydospore. Scale bars = 10 micrometer.



Supplementary Figure S1 Disease symptoms of root and basal stem rot of Sacha Inchi (*Plukenetia volubilis*) and morphology of *Fusarium oxysporum* isolate FoPvo1. (A) Dead infected plants in the field. (B) Longitudinal section of the root (left) and stem (right) of infected plants in the field showing black streaks. (C) Plants showing leaf yellowing at 14 days post inoculation with *F. oxysporum* isolate FoPvo1 (left two plants), and control plants showing no symptoms (right two plants). Discolored vascular tissues were observed in transections of the root (D) and stem (E) of infected plants in the field and the root (F) and stem (G) of inoculated plants in the greenhouse, whereas no symptoms were observed in the root (H) and stem (I) of controls. Colony of *Fusarium oxysporum* isolate FoPvo1 observed from the top (J) and bottom (K) of a PDA Petri dish cultivated for 6 days. (L) Macroconidia and microconidia. (M) Chlamydospore. Scale bars = 10 micrometer.