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Developmental differences between a Chinese and a North American isolate of the pinewood nematode *Bursaphelenchus xylophilus* (Tylenchida: Aphelenchoididae) under laboratory conditions

Shuai Zhang[1,2†], Jing Ning[1,3†], Wei Zhang[1], Christian Salcedo[1] & Lilin Zhao[1*]

[1]*State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China;*
[2]*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China;*
[3]*College of Life Science, Hebei University, Baoding 071002, China*

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Dear Editor,

The pinewood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970, the causal agent of Pine Wilt Disease (PWD), is an invasive species known to have originated from North America, where it was not reported to cause large-scale outbreaks (Wingfield et al., 1984). Ecological conditions encountered by *B. xylophilus* in China are different from conditions in its native North American range. Ecological differences, together with short generation times, improve the chances that changes in developmental traits may occur during lengthy invasive processes. One of the most important adaptations that nematodes have evolved for survival is the modulation of survival stages when facing unfavorable or favorable conditions. Under favorable conditions, *B. xylophilus* develops through four propagative juvenile stages (designated as J_1 to J_4) to the reproductive adult. Continuous reproduction leads to high population density and causes death of the host pine trees. When the nematode density is high, it cannot continuously develop to adults but develop into a growth-arrested alternative third stage juvenile which is named dispersal third stage juvenile (J_{III}) along with the decaying of the dead trees.

It is common for invasive species to express reproductive and physiological adaptations which are not present in populations from native areas. The pinewood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970 is an invasive plant pathogen native to North America, and has caused devastating damage to pine forests in large parts of the world including China. However, *B. xylophilus* did not cause large scale damage in North America. Here we showed that *B. xylophilus* isolates from China and North America presented differences in developmental traits. We found that: (i) the Chinese isolate reproduces faster and had a higher female to male ratio than the North American isolate when grown on artificial medium; (ii) the Chinese isolate propagated a larger population than the North American isolate when inoculated to pine seedlings in a greenhouse ex-

†Contributed equally to this work
*Corresponding author (email: zhaoll@ioz.ac.cn)

periment; (iii) the North American isolate tends to form more dispersal third stage juveniles (J_{III}) than the Chinese isolate on agar plates. These results suggest the pinewood nematode may have evolved reproductive adaptations that differ from its originating population. The relatively faster propagation rate, higher female to male ratio and higher percentage of J_{III} juvenile formation, may facilitate the successful invasion of this alien invasive species.

Changes caused by natural selection (*i.e.* ecological conditions) in the modulation mechanisms of essential survival stages in nematodes can influence the success of invasive species. For instance, detecting harsh or favorable environmental conditions more readily can trigger faster formation of arrested stages or faster propagation of reproductive stages. Since its introduction to China in the 1980s, the PWN has reproduced for thousands of generations under Chinese unique conditions that clearly differ from conditions in the place of origin. Here, we revealed differences in population growth, propagation, and in formation of dispersal third stage juvenile J_{III} between the Chinese isolate and the North American isolate of *B. xylophilus*. Our analyses include population dynamics on culture medium, multiplication on pine seedlings, and variation in natural and dafadine-induced J_{III} formation.

The nematode number of *B. xylophilus* CN strain is significantly higher than that of the US strain, indicating that the CN strain has a higher fecundity or shorter life cycle (Figure S1 in Supporting Information). CN population was roughly six times larger than the US population at day 7. Sex ratio for both populations at the seventh day showed that the CN isolate had higher female:male ratio than that of the US isolates (F=0.113, t=2.514, df=16) (Figure 1A).

After 20 days of inoculation, infested seedlings showed typical wilting symptoms (Figure S2 in Supporting Information), while the control seedlings did not show any symptoms. There was no obvious difference in progress of the pine wilt symptom between the Chinese and North American strains. However, the population of the CN isolate was significantly larger than that of the US isolate (F=0.866, t=2.750, df=18) (Figure 1B). CN isolate population increased up to five times more compared with the initial inoculation, while the US isolate population only doubled its size.

The US isolate formed significantly more J_{III} than the CN isolate (F=4.768, t=−12.826, df=8) (Figure 1C), although in general the percentage of natural J_{III} formation was relatively low as <1% under normal culture conditions.

When treated with dafadine, propagative J_2 nematodes formed dispersal J_{III} juvenile with increased lipid storage and dark body. Similar to naturally occurring J_{III} formation, the US isolate formed significantly more J_{III} juveniles than the CN isolate (F=0.372, t=−2.490, df=8) (Figure 1D). However, in the controls of both CN and US isolates, no J_{III} juveniles were formed (data not shown).

Previous studies showed that different isolates of pinewood nematode have variation in some traits, such as pathogenicity or virulence (Aikawa et al., 2003; Mota et al., 2006). These variations may contribute to the different extent of damage in

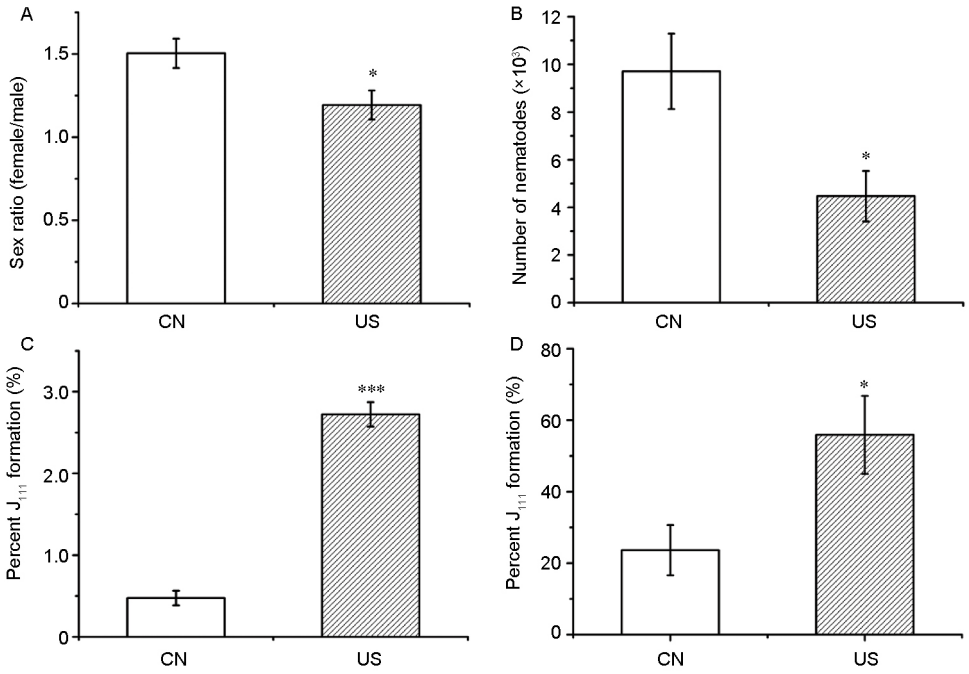

Figure 1 A, Sex ratio of the population of Chinese (CN) and North American (US) isolates of *B. xylophilus* on day 7 grown on PDA plates. B, Propagation of CN and US isolates of *B. xylophilus* on pine seedlings (*P. thunbergii*). C, Variation in natural J_{III} formation in CN and US isolates of *B. xylophilus*. D, Variation in dafadine-induced J_{III} juvenile formation in CN and US isolates of *B. xylophilus*. Error bars are SE and *, *** stand for significant difference under P<0.05 and P<0.001 respectively.

different pinewood nematode-infested regions.

In this study, we showed that a Chinese and a North American isolates of the PWN had different developmental traits. Not only one isolate from each country showed such difference, when we got more isolates form China and North America, similar trends were also found (Zhang et al., personal communication). In general, the US isolates reproduce slower than the CN isolates. The two initial isolates were used to do further research including seedling assay, natural and dafadine-induced J_{III} formation assay.

The ability of the CN isolate to generate higher female:male ratio than the US isolate is an important difference that has direct consequences in reproductive success. More females will increase reproductive potential and, under the right conditions, population growth will be faster (Triantaphyllou, 1973). Reproductive variation on the CN isolate was further confirmed by the seedling inoculation assay. The CN isolate reproduced more within pine seedlings compared to the US isolate. This shows that the CN isolate has higher reproductive potential and success which may make it more efficient in causing the pine wilt disease once it is transferred to the right host.

Following the trend observed on non-dafadine induced experiment, the US isolate formed significantly more J_{III} juveniles than the CN isolate. These results suggest that the US isolate is naturally more sensitive to J_{III}-inducing pheromone or dafadine, which may be caused by receptors sensitivity or penetration capacity of the molecule(s). The observed higher reproductive potential of the CN isolate would favor formation of larger populations, which will produce more J_{III} upon high density and at lower temperature. This would confer the CN isolate more chances to be carried by the vector beetle to new hosts after becoming J_{IV}. The mechanisms underlying natural differences in sensitivity to environmental conditions of *B. xylophilus* isolates deserves further studies.

Our results provided basic information about natural differences in environmental sensitivity, reproductive and developmental trends among US and Chinese isolates of *B. xylophilus*. Whether or not these traits confer adaptive advantages that favor invasive success is an open question that can only be answered by studying several isolates covering the involved geographic areas, as well as doing a comprehensive analysis of the ecological factors that affect invasion.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Figure S1 Population dynamics of the Chinese (CN) and North American (US) isolates of *B. xylophilus*. Error bars stand for standard errors (SE) and *, ** and *** stand for significant differences at $P<0.05$, $P<0.01$ and $P<0.001$ respectively.

Figure S2 Pine seedling (*P. thunbergii*) phenotypes after 20 days' inoculation with *B. xylophilus*. CK, healthy seedlings without nematode inoculation; CN, seedlings inoculated with Chinese isolate of *B. xylophilus*; US, seedlings inoculated with North American isolate of *B. xylophilus*.

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