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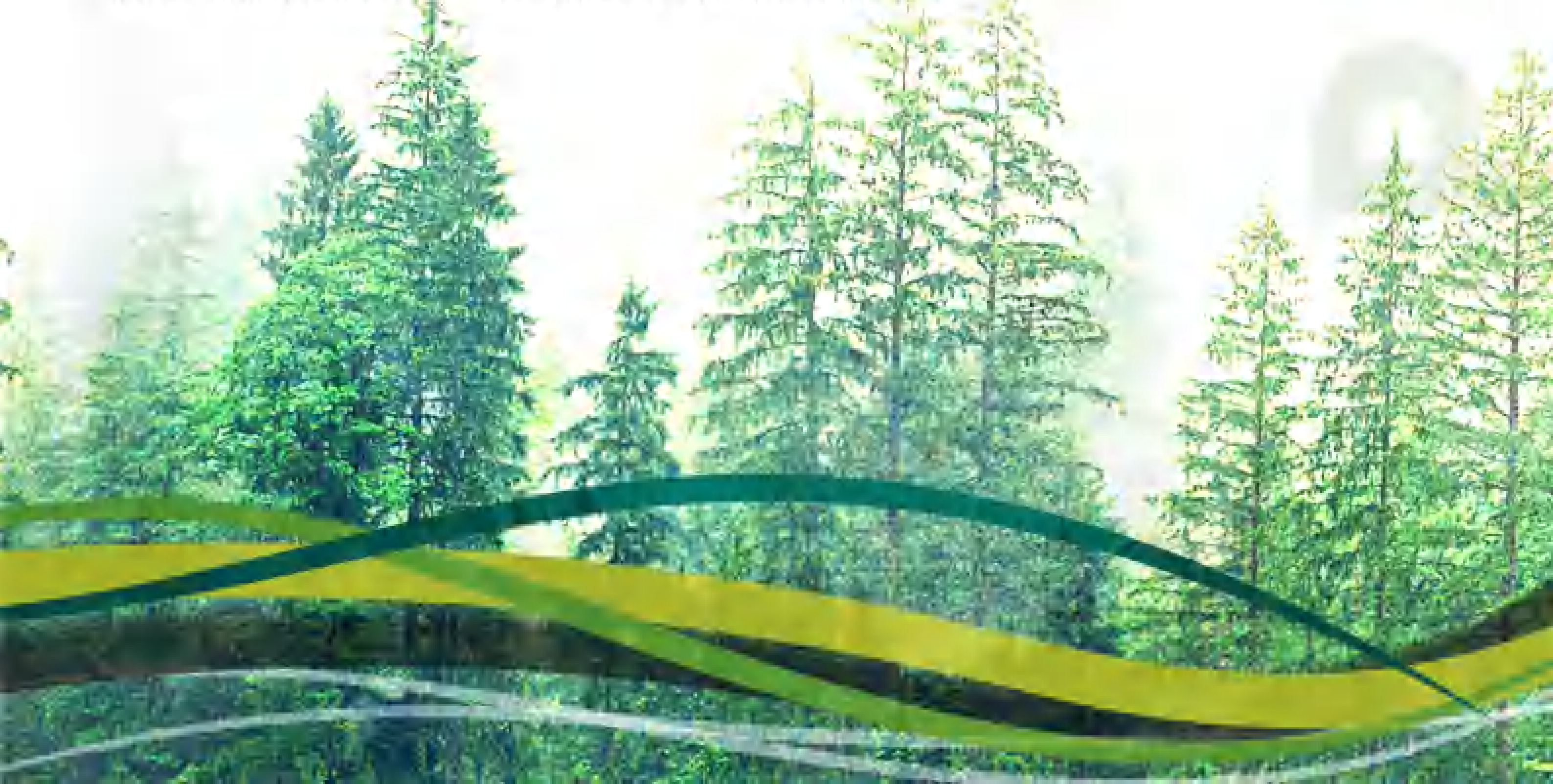

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Molecular identification of interspecific hybrids between the quarantine nematode *Bursaphelenchus xylophilus* and native, nonpathogenic *B. mucronatus*

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Keywords: crossbreeding, detection, hybrids, PCR technique, species-specific primers

The significant similarity of the morphology, biology and ecology of the quarantine nematode *Bursaphelenchus xylophilus* and the common, relatively harmless, species *B. mucronatus*, as well as ambiguous reports on the possibility of interbreeding between them, cause difficulties in the their correct identification and pose a potential threat that they may form interspecies hybrids presenting new combinations of adaptive features.

In the conducted study, the process of inter-specific hybridization between these two species has been examined with the aid of the *B. xylophilus* Roller *(Bxy-rol(tom3)* mutation as a morphological marker, in the breeding protocol, and then confirmed by examination of individual nematodes with the PCR technique with the designed species-specific primers.

PCR amplification of DNA from parental nematodes with the primers generated a single fragment of 767 bp for examined *B. xylophilus* populations or a single fragment of 305 bp for *B. mucronatus* populations. Our earlier study confirmed that PCR mixtures of these two primer sets and DNA of both *B. xylophilus* and *B. mucronatus* produced simultaneously two fragments specific for these species (i.e. 767 and 305 bp) in individual lane of agarose gel.

The use of the primer mixture in the PCR with DNA from individual hybrids (from the F1 to F16 generation offspring of *B. xylophilus* and *B. mucronatus*) resulted in simultaneous production of two easy-to-distinguish fragments of 767 bp and 305 bp in each lane of the agarose gel. That result indicated the presence of both *B. xylophilus* and *B. mucronatus* DNA fragments in individual nematodes. Since each PCR amplification was conducted with DNA extracted from a single specimen (i.e. female or male), the obtained electrophoretic separations clearly confirm the inter-specific hybrid status of nematodes pre-selected up to F16 generation offspring.

The results obtained revealed full species-specificity, high sensitivity of the primer pairs and optimal conditions for the PCR, which together allowed for a rapid and precise confirmation of the parental or inter-specific hybrid status, based on individual nematodes.

Integrated management and research advances of pine wilt disease in South Korea

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Keywords: Diagnosis, Monitoring, Management, Pine wood nematode, Pine wilt disease

The Pine Wilt Disease (PWD) is a fatal disease of pines caused by the pinewood nematode (PWN) *Bursaphelenchus xylophilus*. In Korea, most native species of pines are highly susceptible to PWN and there have been a lot of damages and losses in pine forest since it first occurred in 1988. Currently PWD has been reported in 140 administrative cities & counties from total 228 as of April 2023. The numbers of damaged pine trees have been fluctuated during 35 years and it peaked up to 2,180,000 in 2014. Then it declined continuously at 1,370,000(2016), 690,000(2018), and 390,000(2021), but the numbers of damaged pine rebounded to 784,903 in 2022. PWN is transferred from dead to healthy pines via insect vector, *Monochamus* species of beetle. Therefore, the control tactics are targeting on both PWN and vector insect. For the control of dead infected trees, cut & fumigation, cut & chipping, and cut & burning is common control methods to get rid of nematode and insect at the same time. A chipping of wood material is a highly recommended control method in South Korea because it is the most environmentally friendly method. Trunk injection is common way of prevention of PWD by chemical injection into a healthy tree by targeting on nematode inside of pine tissues. In Korea, recently, an aerial spray by manned helicopter is suspended because of potential of chemical residue of neonicotinoid on honey bee. Unarmed aerial spray method for the selected area is applied for the control of insect vector instead of large area spray by helicopter. The system of PWD investigation and the diagnostic techniques have been dramatically changed and developed. NFC electronic tags are in use to prevent the monitoring blind spots, and a record management system using QR code from the monitoring and diagnosis of dead pine trees to the control has been established. It allows that information entered in the field can be checked in real time, and record on the work process can be managed. In addition, for rapid diagnosis of PWD, a recombinase polymerase amplification (RPA) and a loop-mediated isothermal amplification (LAMP) are developed for field application.

DEVELOPMENT OF ON-SITE QUICK DIAGNOSIS SYSTEM FOR DETECTING PINE WILT DISEASE IN SOUTH KOREA

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Keywords: *Bursaphelenchus xylophilus*, diagnosis system, LAMP, pine wilt disease, RPA

Since the first occurrence of pine wilt disease (PWD) in South Korea in 1988, various diagnosis system and control methods have been developed and applied to the field, but the damage of PWD has now spread nationwide. The number of damaged trees was 890,000 as of 2023, the largest increase since 2.14 million in 2014, and accordingly, the demand for prompt and accurate diagnosis has also increased rapidly. Therefore, we developed loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) assays for PWD diagnosis, considering on-site performance and rapidity. We focused on supplementing the disadvantages of the conventional microscopy diagnostic method or PCR technique. LAMP and RPA assays can be intuitively confirmed by quantifying the amplification value of a marker gene. In addition, the process of separating the nematodes from the wood samples was omitted, and the reaction time and process were drastically shortened by amplifying the gene at isotherm. For LAMP assay, 6 Primer set sequences were designed from mitochondrial COX1 region (1-BX-F3, 1-BX-B3, 1-BX-FIP, 1-BX-BIP, 1-BX-LF, 1-BX-LB). After amplifying the target gene at 62°C for 20 minutes, the infection can be confirmed by adding the SYBR green I dye and reading the absorbance value on the device. For RPA assay, we designed primers from ITS2 region (Bx-ITS2-F1, Bx-ITS2-R1). It is identical to the LAMP method except for amplification at 37°C for 15 minutes. Both methods were able to specifically diagnose Bursaphelenchus xylophilus and other nematodes at the DNA level, and also stably diagnose the infection of PWD in wood samples collected from dead trees. And we have developed a portable mobile device that can perform 8 assays at once, and are promoting supplementary research to enhance on-site capability such as making a premixture without using a pipetting so that it can be directly used in the field.

Study of the possibility of transmission of the pinewood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle to deciduous trees by the white mottled sawyer of *Monochamus urussovi* Fisch

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Keywords: PWN, vector, *M. urussovi*, Betula, Populus

The pinewood nematode (PWN) *Bursaphelenchus xylophilus* causes wilting of coniferous forests in China, Japan, and South Korea but is absent in Russia. PWN vectors are the longhorn beetles of *Monochamus* spp. In Russia, *M. urussovi* is widespread, affecting conifers but can also infect deciduous species such as birch and aspen. The goal of our studies was to clarify whether PWN could survive and reproduce in the wood of these trees. In the experiment, we used logs of birch (*Betula pendula*), aspen (*Populus tremula*), and pine (*Pinus sylvestris*) as controls. Each trial included 20 logs infested with the PWN (BxAm), which were kept at 27 °C. Every six weeks, the logs were tested for the PWN presence. The total duration of the experiment was 190 days. In experiments, it was found that *M. urussovi* actively feeding on young birch twigs. In the PWN log infestation experiment, nematodes were present and multiplied in all trials until the end of the experiment. The maximum number of nematodes in aspen and pine logs was found in the third month (19668 and 2632 nematodes per 100 g of wet weight, respectively). By the end of the experiment, there was a decrease in nematode numbers in these logs, but their numbers were significantly higher (2348 and 1665 nematodes per 100 g of wet weight, respectively) than in the initial inoculum. The maximum number of PWN in birch logs was in the second month (143 nematodes per 100 g of wet weight), and their number at the end of the experiment (62) was actually the same as in the first month (51). *Trichoderma harzianum* (in birch) and *T. atroviride* (in aspen and pine) fungi were detected in PWN-infested logs. Experiments have shown the ability of PWN to reproduce in aspen and birch wood, with aspen being preferable.

PREDICTING POTENTIAL SPREAD OF THE PINE WILT DISEASE: WHICH PROGRESSES AND WHICH FUTURE DIRECTIONS

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Keywords: dispersal, establishment, insect vector, modelling, pine wood nematode

Predicting potential spread of the pine wilt disease (PWD) is very important to anticipate future infestations and optimize surveillance for early detection. Although good progress has been done so far to model potential spread (in Japan, China, Korea, Portugal/Spain - Europe), there is a need : 1) to review available models, 2) to point out their improvements and drawbacks, and 3) to identify future directions.

Spread of an invasive species results from the combination of dispersal in new environment, establishment and population growth processes. For the PWD, processes are more complex since the disease is caused by the invasive pine wood nematode (PWN), *Bursaphelenchus xylophilus*, itself carried and transmitted to pine trees by native longhorn beetles of the genus *Monochamus*. Furthermore, PWN-infested trees show wilting symptoms only under specific conditions, notably high summer temperatures. Spread of the disease thus results from an interaction among at least three actors (PWN, insect vectors and host trees) in specific environmental conditions. Therefore, modelling potential spread of PWD could consider the overall process, or only parts of it. Here, I review the main types of models used to assess the potential spread of the PWD, pointing out the main results, and highlighting future directions to refine the spread predictions. Models used so far to describe potential spread of PWD are mainly: species distribution models, dispersal models (including least-cost path analyses), disease expression models based on thermal conditions, and integrated spread models. The development of (semi-) mechanistical models describing the key processes would allow refining the assessment of the PWD potential spread in various or changing conditions. Despite the knowledge accumulated so far, many unknowns remain even about the biology, the ecology or the behaviour of the PWN and the insect vectors. Collecting such data is important to assess more accurately the PWD potential spread.

MANAGING THE PINE WOOD NEMATODE WITHOUT CLEAR-CUTTING; A SCENARIO ANALYSIS

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Keywords: cost-effectiveness, eradication, individual-based model, *Monochamus galloprovincialis*, pine wood nematode

The pine wood nematode, *Bursaphelenchus xylophilus* (PWN) is an invasive nematode causing heavy mortality to the pine trees in its invaded range. In Europe, *Monochamus galloprovincialis* is so far the only recognized vector of PWN. To eradicate PWN, the European Union regulations require a 500-m-radius clear-cutting around individual infested trees to prevent the spread of the disease. However, this radius is likely not sufficient to stop the spread of the disease, while eradication costs are high. Here, we investigate whether intensive surveillance along with only cutting infested trees could be a more cost-effective control alternative. Thus, we compare the cost-effectiveness of the two control strategies: 1) 500-m-radius clear-cutting around the detected PWN-infested trees, and 2) only cutting the detected PWN-infested trees, assuming that wilting symptoms are likely to occur after PWN inoculation. Both strategies were combined with different surveillance methods: ground visual surveys, aerial surveillance, and trapping networks targeting the insect vector. We used an individual-based model to describe the dispersal of *M. galloprovincialis* and transmission of PWN from a single source in a 20 km × 20 km homogenous pine forest. Sub-models were built to assess the cost and effectiveness of surveillance and cutting strategies. Effectiveness was assessed by net reproductive numbers of beetles ($R0_{vector}$) and infested trees with eggs ($R0_{cases}$), and the proportion of healthy trees remaining. Total costs incorporated the costs of surveillance, wood sampling, chipping and logging, and the losses in wood production value due to cutting healthy trees. With intensive aerial surveillance, tree-by-tree selective cutting could eradicate PWN at 140 times less costs than clear-cutting. Costs were mainly saved by preserving healthy trees. Our results highlight the importance of exploring alternative strategies to eradicate pests in a more cost-effectiveness, and environmentally friendly way.

REASSESSING THE THREAT POSED BY PINEWOOD NEMATODE (*BURSAPHELENCHUS XYLOPHILUS*) TO UK FORESTRY: EXPLORING ALTERNATIVE VECTORS AND NOVEL DETECTION TOOLS

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Keywords: alternative vectors, arena experiments, introduction risk, PWN, Volatile organic cuticular compounds (VOCC)

Pine wilt disease (PWD) is one of the most serious threats to conifer forestry worldwide. The disease is caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, which has formed novel phoretic associations with different species of cerambycid beetles, in the genus *Monochamus*, in each new country in which it has become established. Despite extensive border surveillance and containment efforts in the affected countries, PWD continues to spread. Pinewood nematode has been intercepted on infested material entering UK ports and processors, and this is expected to increase in frequency in the coming years. In contrast to continental Europe where PWN has already established, the UK has no indigenous *Monochamus* species, but it is unclear as to whether these absences preclude the establishment of PWN. The UK has genera of beetles (incl. *Tomicus, Hylobius,* and *Pissodes*) which, like *Monochamus*, carry out maturation feeding on healthy plant tissues, and could enable infection via primary transmission of the nematode in the absence of *Monochamus*. These genera and other cerambycids are known to vector related *Bursaphelenchus* spp. PWN aggregates toward their *Monochamus* vectors in the pupal stage using volatile organic cuticular compounds (VOCCs) from the beetle cuticle. We present plans for a new PhD project that will examine the fidelity of the *Monochamus*-PWN relationship, and the potential of alternative vector species to facilitate the spread of PWN in the UK. The project will characterise and compare the specific cuticular profiles of *Monochamus* species to candidate vector species. In addition, PWN will be exposed to a range of beetle life stages from putative vector species, both with and without the co-presence of *Monochamus* in choice/no choice arena experiments. This research will investigate the potential to develop a PWN VOCC lure to offer an efficient in-field surveying method.

In vitro propagation and field evaluation of pine materials resistant to pine wilt disease

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Keywords: *Pinus*, *Bursaphelenchus xylophilus*, nematode resistance

To accelerate the breeding and selection of pines resistance to pine wilt disease (PWD), caused by the pinewood nematode (PWN), Bursaphelenchus xylophilus, a micropropagation system was established and nematode resistance was evaluated. Cotyledon-hypocotyl explants from 28-day-old seedlings were first cultured on Gresshoff and Doy medium supplemented with 4.0 mg L^{-1} 6-benzyladenine (6-BA) and 0.2 mg L^{-1} α-naphthaleneacetic acid (NAA) to stimulate the formation of buds. Induced buds were subsequently subcultured on Gupta and Durzan medium supplemented with 0.1% (w/v) activated charcoal for elongation. Stem sections derived from shoots were used as explants for the further multiplication. Rooting percentage of micropropagated shoots were influenced by the concentration of sucrose and NAA in the rooting medium, shoot height, and shoot generation. The nematode resistance test in vitro showed that symptoms in micropropagated shoots after infection with PWN were similar to those in plants infected in the field. The wilting rate varied from 20 to 100% among different clones 18 days after inoculation. The most susceptible clone was Clone 6-4 with a 100% wilting rate, while clone 1-A showed a relatively high resistance with a 20% wilting rate. The plantlets which grew in a nursery for 4-years were planted in the field in May 2012. In the 10-year field trial, clone 1-A remain healthy, while between 10 to 50% of control pines were wilted. This work contributes to the breeding of PWN resistance in P. densiflora and provides an effective method to study the host/pathogen interaction between PWN and pines.

Study on the resistance of Larix spp. to Pine Wood nematode

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Keywords: Larix spp.; lipid; overwintering survival rate; pine wood nematode; resistance breeding

Pine wood nematode (PWN, Bursaphelenchus xylophilus) is a devastating disease that has damaged conifer forests in China for nearly half a century. In 2016, PWN was found in Dalian, Liaoning Province, the first discovery of PWN in Northeast China. In 2018, the new hosts of Larix spp. (L. olgensis, L. kaempferi, L. principis-rupprechtii) with natural infection of PWN were found in Fushun, Liaoning Province. This means that the vast Larix spp. forests in Northeast China will be seriously threatened. It is urgent to identify the resistance of Larix spp. to PWN and reveal the mechanism, which provides a theoretical basis for the prevention and control of PWN.

The resistance of Larix spp. and Pinus spp. to PWN was identified. The study was conduct3ed with L. olgensis and Pinus koraiensis to reveal the resistance difference. The results of the artificial inoculation test of adult trees showed that the L. olgensis was more resistant than the P. koraiensis. The number of PWN isolated from L. olgensis was significantly lower than that isolated from P. koraiensis. In the third year after artificial inoculation, the rate of showing symptoms was 0 in 100 L. olgensis and 83% in 100 P. koraiensis. Metabolomics analysis and verification showed that the significant up-regulation of caffeic acid in L. olgensis inhibited the lipid accumulation of PWN. The lipid content of PWN in L.olgensis was significantly lower than that of PWN in P. koraiensis, which led to the low overwintering survival rate of PWN in L. olgensis. This ultimately leads to a low rate of showing symptoms in L. olgensis, which is shown to be resistant to PWN.

Intraspecific resistance differences in Larix spp. have been studied. One resistant family was screened out of 38 families and one resistant clone was screened out of 30 clones, respectively. There was a significant variation in the resistance of Larix spp., which can be used to control PWN through breeding the resistance.

TRUNK INJECTION FOR PINE WILT DISEASE CONTROL AND ITS BIOSAFETY ASSESSMENT IN CHINA

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Keywords: *Bursaphelenchus xylophilus*, emamectin benzoate, residue, trunk injection

The pine wood nematode (PWN) *Bursaphelenchus xylophilus* is the causative agent of pine wilt disease (PWD). It has caused disastrous damage to forests and gardens worldwide. Trunk injection with emamectin benzoate (EB) has been proved effective for PWD control. The 24 h LC50 and LC90 were 0.23 mg/L and 0.75 mg/L, respectively, applying dipping method. In greenhouse trial, the disease prevention rate was 100% at 10 days post nematode inoculation. The EB residue in pine tissue was determined by HPLC(UVD), and the results showed that the EB concentration remained 38.29 mg/kg, 8.46 mg/kg, 2.73 mg/kg, and 1.02 mg/kg at 0. 5a, 1a, 2a, 3a after injection, respectively. The residue concentrations were higher than LC90 against B. xylophilus, indicating that the prevention efficacy would maintain at least 3 years. In field trials, disease prevention rates have been assayed since 2014. EB trunk injection could control the pine death rate low to 0.19-0.58% for three consecutive years, comparing to 16.38-35.19% deaths in the mock area. Analysis on residues of EB in trunk, branch and needle of pine tissue after 1 year of trunk injection disclosed that the highest residue of EB was 29.95 mg/kg in the trunk and 8.46 mg/kg in needle. Though EB residue decreased three years later, it remained over 1.00 mg/kg. Noticeably, the emamectin benzoate was prepared in the micro-emulsion formulation at the concentration of 2.3% (W/V), resulting in the trunk injection-ready pesticide product. Further, it was shown that trunk injection with EB has no significant effect on the community diversity indices of total soil arthropods and flying insects (Hymenoptera). In summary, trunk injection with EB is an environment-safe and efficient method for PWD control.

PATTERNS OF BELOWGROUND MICROBIOME AND NEMATODE COMMUNITIES IN RESPONSE TO PIINE WILT DISEASE

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Keywords: Pine wilt disease, Root endophytes, Rhizosphere, Soil microbiome, Soil nematodes

We investigated patterns of belowground microbial and nematode communities of *Pinus koraiensis* following pine wilt disease (PWD), to estimate the ecosystem consequence of this disease for pine forests. Overall, bacterial diversity within the roots was significantly lower than that in bulk or rhizosphere soils of the pines, regardless of the PWD occurrence. Fungal diversities decreased with the distance of compartments from the roots, showing the lowest value within the roots, and the highest in bulk soil for healthy pines. Such pattern disappeared for infected pines which had a higher fungal diversity in rhizosphere soil but lower in root endophytes than healthy pines. Fungal and bacterial community compositions within the roots differed from those in rhizosphere or bulk soils for both healthy and infected pines. In contrast, the compositions in soils differed between healthy and infected pines, e.g. with a decrease in the relative abundance of bacterial genera *Acidocella* and *Dokdonella*, as well as fungal genus *Umbelopsis*. However, within the roots only the bacterial community composition differed. Besides, PWD appeared to decrease the number of unique genera of bacteria but increase the number of unique fungi in the soils, in line with a lower number of positive interactions but a higher number of negative ones among microbial species. The PWD did not change the diversity of the soil nematode community but altered the community composition by resulting in a decreased abundance of fungal-feeding nematodes and accordingly an increased proportion of bacterial feeders and carnivores. Our study helps to understand the belowground biotic consequence of PWD, providing pivotal implications for biological control of this disease using soil microbiota.

Chromosome-level genome assembly of *Monochamus saltuarius* and its interaction mechanism with pine wood nematode

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Keywords: chromosome-level genome, adaptive strategy, interspecific relationship

We investigated and evaluated the vectors and potential vectors of pine wood nematode (PWN, *Bursaphelenchus xylophilus*) in northeast and northwest China. *Monochamus saltuarius* (Coleoptera: Cerambycidae) was reported as the vector beetle of the PWN in Japan and Europe. It was first reported to transmit PWN to *Pinus* species of China in 2018 in Liaoning Province. However, the lack of genomic resources has limited the in-depth understanding of its interspecific relationship with PWN.

We assembled the scaffolds into ten chromosomes (including an X chromosome) and obtained a 682.23 Mb chromosome-level genome with a N50 of 73.69 Mb. In total, 427.67 Mb (62.69 %) repeat sequences were identified and 14,492 protein coding genes were predicted, of which 93.06 % were annotated. Chr 10 of *M. saltuarius* had high synteny with chromosomes of the other eight insects. Among these species, *M. saltuarius* had high genome synteny with *Pyrochroa serraticornis* and low synteny with *Pogonus chalceus*. The phylogenetic tree showed that *M. saltuarius* clustered with other seven Chrysomeloidea. *M. saltuarius* and *Anoplophora glabripennis* formed a sister lineage to Cerambycidae. We described the *mth/mthl*, *P450*, *OBP* and *OR* gene families associated with the vector beetle's development and resistance, as well as the host selection and adaptation, which serve as a valuable resource for understanding the host adaptation in insects during evolution.

We also revealed the adaptive strategies of *M. saltuarius* to PWN from the perspective of growth and development. After inoculating by PWN, the average developmental duration of larva to adult shortened by approximately four days. The average life span of *M. saltuarius* adult after emergence shortened by approximately five days. The ecdysone titer increased at the pupal stage. Based on the genome information, genes associated with ecdysone, juvenile hormone, and epidermal synthesis pathways were changed significantly in the larval and pupal stages.

Antimicrobial peptides from Japanese Pine Sawyer and the inhibitory of pine wood nematode

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Keywords: Antimicrobial peptides, *Bursaphelenchus xylophilus*, *Monochamus alternatus,* RNA interference

Monochamus alternatus is the main vector of pine wilt disease (PWD) in China. Controlling *M. alternatus* can effectively curb the spread of pine wilt disease. In the *M. alternatus-Bursaphelenchus xylophilus* complex, overcoming the innate immunity of *M. alternatus* to invading pathogens is the key to improving the efficiency of biological control. Antimicrobial peptides (AMPs), as an important effector of insect innate immunity, mediate the resistance of insects to pathogenic microorganisms through Toll and IMD signaling pathways, and have important application value in interference regulation. This study investigated the effect of key immune genes *MaltSpz* and *MaltRelish* on upstream immune recognition receptor (GNBP3) and downstream effectors (AMPs) by RNAi. The combined *MaltSpz* and *MaltRelish* knockdown increased the infection rate of *M. alternatus* larvae from 20.69% to 83.93%, achieving the best efficiency in synergistic *Beauveria bassiana* infection. Furthermore, we also found the antibacterial peptide S-defensin was synthesized from *M. alternatus*. The result of its nematicidal activity test showed that S-defensin significantly inhibited the survival, motility, oviposition, reproduction and intracellular biological oxidative stress of PWN by cell membrane damage and regulating the ERK/MAPK signaling pathway. Knockdown of the *Let-23*, *Let-60*, *Mek-2* and *Lin-1* genes involved in the ERK/MAPK signaling pathway significantly affected the reproduction and longevity of *B. xylophilus*. *Mek-2* is an important RNAi target gene for the control of *B. xylophilus*, and its dysfunction can affect reproduction and longevity in PWN. These could represent candidate gene targets for the biological control of PWD by RNAi.

NEW DERIVATIVES OF TENVERMECTINS AGAINST PINE WOOD NEMATODE, *BURSAPHELENCHUS XYLOPHILUS* AS POTENTIAL NEMATICIDE

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Keywords: tenvermectin derivatives, *Bursaphelenchus xylophilus*, nematicidal activity

The pine wood nematode (PWN), *Bursaphelenchus xylophilus*, causes significant damage to pine trees in China since from 1982. And now it is still the most serious forest disaster in China， which posed a serious threat to China's ecological safety. Because this disease involves very complicated interactions between a pathogenic nematode, its vector beetle, host pine species, and fungi in dead hosts. There are few effective control measures available for PWD .The injection of chemical agent exerted a preventative effect against the pine wilt disease, which brings about a direct effect on the nematode and is environmentally friendly, the authors aimed to develop a novel trunk-injection agent against the disease. Sixteen-membered macrocyclic lactone antibiotics are used extensively in veterinary to control parasitic infections, emamectin benzoate (EB)， avermectins(AVMs) and ivermectins have been considered as the typical representatives， and have recently been successfully applied as a trunk injection for preventative control of the pine wilt disease (PWD).In previous papers, the authors reported that many insecticidal 16-membered macrocyclic lactone compounds, were potent nematicidal activity and thus acandidate for the ingredient of a trunk-injection agent. In further work to explore the trunk-injection agent, using domain swapping of avermectin PKS, two hybrid compounds named tenvermectins A and B, which possess partial structural features of avermectin and milbemycin, were obtained from two genetically engineered strains *Streptomyces avermitilis* MHJ1011 and *Streptomyces avermitilis* AVE-H39. TVMs A and B showed enhanced insecticidal activity against *Bursaphelenchus xylophilus*. In order to discover more potent TVM derivatives, microbial transformation of TVM-A and TVM-B by *mycolicibacterium* sp. Huz021 and Bacillus subtilis were investigated and offered us eight metabolites with regioselectivity, especially rare glycosuccinylated products. Their chemical structures were well characterized via HR-ESI-MS and 1D/2D NMR spectra. All these metabolites exhibited good nematicidal activities against *Bursaphelenchus xylophilus*. These results indicated that the metabolites can serve as potential trunk-injection agents for future.

Predicting Potential distributions of *Monochamus alternatus* and *Monochamus salturius* under Current and Future Climatic Scenarios Based on Optimized MaxEnt model

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Keywords: climate change, *Monochamus alternatus*, *Monochamus salturius*, suitable area, the optimized MaxEnt model

Monochamus alternatus and *Monochamus salturius* are considered the most important and efficient insect vectors for the spread of the important international forest quarantine pest, *Bursaphelenchus xylophilus*. And monitoring of *M. alternatus* and *M. saltuarius* have become a key strategy to prevent and control the disease in China. This is the first study to use the optimized maximum entropy model and ArcGIS to accurately predict suitable geographical areas for *M. alternatus* and *M. saltuarius* based on different climatic conditions in China. In this study, the optimized MaxEnt model parameters were set as FC = LQHP. Under the current climate conditions, the suitable areas of *M. alternatus* covered about a quarter of China land area, and the predicted suitable areas for *M. saltuarius* were mainly at latitudes north of 33° in China, and larger suitable areas were mainly distributed in Northeast China and North China. Using future climatic scenarios SSP126 and SSP585, the suitable areas of *M. alternatus* has a tendency to spread northward in China; the predicted suitable areas of *M. saltuarius* will continue to expand from that of 2040, 2070, and 2100, with highly and moderately suitable areas showing larger increasing trends but low suitable distribution areas will decrease to varying degrees. The results of this study can provide a theoretical basis for the risk analysis and the precise prevention and control of *M. alternatus* and *M. saltuarius* in China.

DESIGN AND CULTIVATION OF NEW PINE VARIETIES RESISTANT TO PWD

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Keywords: disease resistance genes, genetic transformation, genome selection, pine wood nematode, *Pinus*

Regarding resistance to pine wood nematode disease, the stilbene substances pinosylvin and pinosylvin monomethyl ether were clarified to respond to nematode infection specifically. Three genes (*PmPMT*, *PmSTS*, and *PmWIRK*) involved in regulating stilbenoid synthesis were cloned and characterized in *Pinus massoniana*. In addition, four receptor kinase genes, *PmNBS-LRR16/64/97* and *PmHs1*$^{pro\text{-}1}$ were identified. Structural variations were discovered in the *PmNBS-LRR16* gene, with an 11-fold increase in expression observed in highly resistant *P. massoniana*. This finding holds potential for developing molecular markers for disease resistance in *P. massoniana*. Furthermore, approximately 1.6 billion SNPs were identified in *P. massoniana*, laying a solid foundation for developing disease-specific microarrays.

The embryogenic cell suspension system of *P. massoniana* was optimized, and it was discovered that the phytosulfokine peptide promotes the embryogenic maintenance and enhancement of callus tissues in *P. massoniana*. Breakthrough was achieved in overcoming the bottleneck technique of maintaining embryogenic cell lines and achieving efficient decontamination after co-cultivation in the transgenic system of *P. massoniana*. A novel non-tissue culture-mediated genetic transformation technique mediated by K599 was developed for *P. massoniana*. Additionally, gene editing vectors and multi-gene co-expression vectors were constructed for *P. massoniana*, and five transgenic embryogenic callus-expressing disease-resistant genes were induced. The research mentioned above provides a fundamental material basis for effectively controlling pine wood nematode disease, ensuring the development of China's pine tree industry, and securing seed safety.

Remote sensing monitoring of pine wilt disease in China

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Keywords: pine wilt disease, unmanned aerial vehicle, satellite, hyperspectral, monitor

Pine wilt disease (PWD) is a global threat to forests and has caused significant damage in China. Accurate and efficient monitoring is the basis of controlling PWD. Therefore, it is essential to establish "Satellite-Unmanned aerial vehicle (UAV)-Ground" technology to monitor PWD accurately. At the ground level, we constructed hyperspectral databases of various host species at different infection stages, and identified the spectral responses of physiological indices at needle levels. At the UAV scale, we developed accurate identification technology for dead trees using visible images and deep learning algorithms, with an accuracy above 85%. Additionally, we developed discriminant techniques for identifying infested trees at different stages (green, early, middle, heavy, and grey) based on UAV-based hyperspectral images and light detection and ranging (Lidar), particularly for early monitoring. Furthermore, we used time series of UAV-based hyperspectral images to monitor temporal changes of disease distribution and determine the optimal detection period. At the satellite scale, we created a feasible and straightforward large-scale inversion tool for infested forests by combining the remote sensing mechanism model (ESRT) with medium-resolution satellite imagery, which can be used for the inversion mapping of the PWD disaster levels. We integrated Sentinel-1 microwave and Sentinel-2 optical time series data, and used the remote sensing cloud platform GEE (Google Earth Engine) to construct a time series monitoring model of infested forests. The overall accuracy was 80.62%, and the Kappa coefficient was 0.61. We also explored the sensitivity of surface temperature (LST) to PWD. The results showed that the combined vegetation index (DVI) and LST could improve the accuracy of inversion results for infested forests, and advance the monitoring and warning time for infested forests. Accurate and intelligent monitoring technology has been applied in many provinces and cities, providing technical support for scientific prevention and control of PWD.

Epidemiological model including spatial connection features improves prediction of the spread of pine wilt disease

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Keywords: forest pests; infectious disease dynamics; prediction; pine wilt disease; timber transportationn

In recent decades, tree killing pine wilt disease (PWD) has been spreading globally and has caused substantia damage especially in China, causing annual losses worth of nearly 15 $billion. This disease is caused by pine wood nematode (*Bursaphelenchus xylophilus*) and its *Monochamus spp.* vector insect, but little is known about the factors affecting of transmission of PWD. According to reports PWD, more than 3/4 of the transmission is linked to anthropogenic activities such as wood transportation. For the first time, we utilized a dataset categorizing several anthropogenic transmission factors and used an improved infectious disease dynamics method of COVID-19 to quantitatively predict the odds of future PWD infections for the entire pine tree distribution range of China. The validation results show that PWD will spread even faster in China, with the average risk of infection exceeding 30% in 2023 and 50% by 2025 in the study samples. Meanwhile, the spread showing a significant trend from infected areas to adjacent areas, high infection risk is still mainly caused by natural bordering. However, 3.8% of the samples at high infect risk were caused by the transportation of pine wood, requiring extra vigilance. By adding spatial links between the samples to the features for PWD prediction, the AUC of the model reaches 0.90, which achieves an accurate prediction of the key areas of PWD propagation, provides guidance for more accurate development of prevention and control measures, and provides new ideas for similar studies.

TRANSCRIPTOMICS AND COEXPRESSION NETWORK PROFILING OF THE EFFECTS OF LEVAMISOLE HYDROCHLORIDE ON *BURSAPHELENCHUS XYLOPHILUS*

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Keywords: *Bursaphelenchus xylophilus*, RNA-Seq, Levamisole hydrochloride, Short Time-series Expression Mine, Spearman

Bursaphelenchus xylophilus is one of the most dangerous forest pathogens in the world, causing devastating pine forest deaths with considerable economic losses. We investigated the *B. xylophilus* responses of two different concentrations of levamisole hydrochloride (LH). The body-wall muscle twitching, paralysis and, ultimately, death. 2.5 mg/ml and 3.5 mg/ml LH have toxicological effects on *B. xylophilus*, with mortality increasing significantly with concentration ($p < 0.05$). Gene Ontology annotation and Kyoto Encyclopedia of Genes and Genomes pathway analyses of the 12 intersecting genes revealed that these genes are mostly involved in metabolism of xenobiotics and have essential roles in drug sensitivity. Through the trend analysis of DEGs, it was divided into 8 modules, and the significant modules were selected to construct the co-expression network as the central genes of the drug metabolism-cytochrome P450 pathway (ko00982) and metabolism of xenobiotics by cytochrome P450 (ko00980). Eight highly related genes were identified, including cuticle collagen, cystathionine beta-synthase, endochitinase, pyruvate dehydrogenase E1 component subunit beta, aldehyde dehydrogenase, lipase, and zinc metalloproteinase. The expression levels of these genes were upregulated significantly at low concentrations and were significantly related to the resistance of *B. xylophilus* to LH. This study shows that *B. xylophilus* gene family expansions occurred in xenobiotic detoxification pathways through gene expression and potential horizontal correlated gene transfer with LH and helps to elucidate LH lethality and the evolutionary mechanisms underlying the adaptations of *B. xylophilus* to the environment.

MOLECULAR DEFENSE RESPONSE OF *BURSAPHELENCHUS XYLOPHILUS* TO THE NEMATOPHAGOUS FUNGUS *ARTHROBOTRYS ROBUSTA*

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Keywords: *Arthrobotrys robusta*, *Bursaphelenchus xylophilus*, molecular response mechanism

Bursaphelenchus xylophilus causes pine wilt disease, which poses a seriously jeopardizing to forestry ecology. We investigated the endophytic microbial community in *Pinus massoniana* field with and without *B. xylophilus*. An increased abundance of pathogenic fungus was witnessed in infected pines. Furthermore, *Ophiostoma* and saprophytic fungus were exclusively prominent in infected pines. Otherwise, the NADH/NADPH and carbon-nitrogen bonds were more in healthy pines, but acid anhydrides and transferring phosphorus-containing groups were more in infected pines. Then, we isolated a nematophagous fungus-*Arthrobotrys robusta*-from the xylem of infected pines and analyzed the molecular response mechanism of *B. xylophilus* interacted with *A. robusta*. The neuromodulators genes and immune response genes were significantly up-regulated when *B. xylophilus* were captured by *A. robusta* for 24h; *B. xylophilus* were obviously constricted when *B. xylophilus* interacted with *A. robusta* for 30h, lipid and xenobiotic metabolism genes of *B. xylophilus* were significantly up-regulated; lysosome and autophagy-related genes were activated, and the nematode body wall was lysed when *B. xylophilus* interacted with *A. robusta* for 36h. We constructed a network of gene co-expression patterns by WGCNA to characterized the process of *B. xylophilus* interacted with *A. robusta*. The network suggested that metabolic processes, developmental processes, detoxification, biological regulation, and signaling were influential when the *B. xylophilus* interacted with *A. robusta*. We proposed a model in which mobility improved whenever *B. xylophilus* entered the traps of *A. robusta*. The model will provide a solid foundation to understand the molecular and evolutionary mechanisms underlying interactions between nematodes and nematophagous fungi.

Detection of *Bursaphelenchus xylophilus* based on polarization imaging

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Keywords: Bursaphelenchus xylophilus; Depolarization parameters; Mueller matrix; Polarization imaging; Water content

Currently, timely detection of pine wood nematodes in forestry practices poses a challenge. A rapid and real-time detection method is needed to identify early-stage infection of pine trees by pine wood nematodes. Polarization imaging, which separates information not obtainable in regular optical images, offers deeper insights into tissue structures. Polarization detection has found applications in various fields such as military, industrial inspection, and biomedical research, proving advantageous in terms of speed and accuracy for biological tissue analysis.

Pine wood nematode disease disrupts pine tree health by affecting water transport, leading to its deterioration. Hence, we propose polarization detection of water content variations in plants as a means to detect pine wood nematode infection. By constructing a custom polarization detection system, we capture the Mueller matrix of the target object. Utilizing non-physically meaningful elements within the Mueller matrix, we derive the depolarization parameters of the target object. Subsequently, we establish a mathematical model correlating these depolarization parameters with plant water content, enabling water content prediction.

In the preliminary stages of pine tree water content detection, we have successfully detected water content in holly leaves. Presently, our focus is on evaluating water content in pine trees infected with pine wood nematodes. Ongoing research is dedicated to refining disease detection of pine wood nematodes through water content analysis.

Breeding for Resistance to Pine wilt Disease in the Pine Breeding Programme in Guangdong

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Keywords: resistance breeding, pot trial, genetic materials, *Pinus elliottii* × *P. caribaea*

Various pine species were previously dominant tree species in Guangdong, which were planted for harvest both resin and timber. However, Guangdong's pine plantations were seriously threatened by the pine wilt disease (PWD). In the natural environment, local *Pinus massoniana* seem to be more susceptible to PWD than the exotic pines introduced from US. But more and more PWD affected trees were also detected in slash pine plantation. To understand the mechanism underlying the resistance performance in resistant species and susceptible species, resistance breeding has been conducted in Guangdong since 2022. The 1-year-old genetic materials from *P. massoniana*, *P. elliottii* and *P. elliottii* × *P. caribaea* were cultured in greenhouse and inouclated by 3000 individuals of pine wood nematodes (*Bursaphelenchus xylophilus*). Preliminary test indicated the higher resistance of *P. elliottii* × *P. caribaea* at seedling stages. One month after inoculation, only 16.7% of the seedlings showed slight symptom and only a few nematodes were propagated in the stem near the inoculation position. The higher resistance of the hybrid than its female parent (*P. elliottii*) was inconsistent with their field performance. Adopting older seedlings and inoculating on the older trees in the stands at affected area are necessary.

TECHNIQUE ON MIXED FOREST OF CAMELLIA OLEIFERA WITH PINUS MASSONIANA CLONES FOR RESISTANCE TO BURSAPHE-LENCHUS XYLOPHILUS

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Keywords: *Bursaphelenchus xylophilus*, clone, Camellia oleifera, mixed forest, *Pinus massoniana*

Resistance of *Pinus massoniana* from different provenans to *Bursaphelenchus xylophilus* were screened by artifical inoculation. Clone these selected ones, and screened out resistant clones of *Pinus massoniana* through congducting two artifical inoculations again. Cultivate the *Pinus massoniana* clones for forestation by using spikes from these clones as mother trees.In order to make full use of soil fertility, improve the yield per unit area of forest land, and enhance the resistance of forest to pests and diseases deeply， experiment on mixed foristation of *Pinus massoniana* clones resistant to *Bursaphelenchus xylophilus* and *Camellia oleifera* were developped. This paper summarized the technical points of this mixed afforestation experiment in order to lay the foundation for further analysis of production benefits of mixed foresstation of *Pinus massoniana* and *Camellia oleifera* and provide a new reference for afforestation management mode of timber tree species and economic tree species.

Identification of Discolored Pine Trees Affected by Pine Wilt Disease Based on ResNet Model

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Keywords: pine wilt disease；UAV；monitoring；ResNet

In the contemporary landscape of research, this investigation endeavors to scrutinize the feasibility of deploying multiple deep learning-based methodologies for the precise identification of trees that have been discolored due to Pine Wood Nematode (PWD) disease. To fulfill this objective, an Unmanned Aerial Vehicle (UAV) fitted with a high-resolution RGB digital camera was deployed to capture aerial images with high spatial resolution. Following this, the acquired visible light orthophoto images were subjected to preprocessing procedures that take into account elevation and terrain features, subsequent to which texture information was extracted. Thereafter, we employed a ResNet neural network for classification and recognition training.

In the specialized area of pest and disease identification, the ResNet model has been proven to have high accuracy and stable performance, thereby establishing itself as an ideal computational algorithm for the identification of PWD-affected standing trees.

In summary, this research endeavor involved the collection of low-altitude remote sensing images from three distinct test regions using a UAV fitted with a specialized visible light camera. This facilitated the implementation of a deep learning-based tree identification method, following which the vegetation was segmented and individual plants were isolated. Ultimately, a deep convolutional network-trained model was applied for the intelligent identification of discolored, PWD-affected wood. The algorithm showcased an average accuracy rate of 92.29%, with peak performance metrics reaching up to 96.51%.

A COLEOPTERAN PARASITOID ON PINE SAWYER BEETLE

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Keywords: *Dastarcus helophoroides*, immune response, *Monochamus alternatus*, olfactory, parasitic mechanism

Dastarcus helophoroides Fairmaire (Coleoptera: Bothrideridae) is a major natural ecto-parasitoid of pine sawyer beetle *Monochamus alternatus* Hope (Coleoptera: Cerambycidae). To illustrate the parasitic mechanism between *D. helophoroides* and *M. alternatus*, we investigated the host foraging behavior of *D. helophoroides* adults, the volatile chemicals emitted from host pine trees and the immune responses of *M. alternatus* to the parasitism of *D. helophoroides*, and identified the olfactory genes and the venom proteins from *D. helophoroides* larvae. The results indicated that: 1. The volatile chemicals emitted from both of uninfested *Pinus massoniana* logs and infested logs with *M. alternatus* larvae were equally attractive to *D. helophoroides*, which were probably D-Limonene and α-Pinene. 2. *D. helophoroides* larvae could regulate *M. alternatus* immune system and resulted in the changes in host immune responses, and the expression levels of 108 immunity-related genes in *M. alternatus* larvae were remarkably altered in response to the parasitization by *D. helophoroides*. 3. 62 putative olfactory genes and 50 putative venom proteins were identified from the transcriptome and the proteome of *D. helophoroides* larvae, separately. 4.Temporal expression of parasitism-related olfactory genes in larvae of *D. helophoroides* showed that the genes encoding chemosensory protein 2 (*DhelCSP2*), odorant receptor 2 (*DhelOR2*), and ionotropic receptor 2 (*DhelIR2*) were significantly upregulated before parasitization was initiated, whereas the genes encoding odorant binding protein 8 (*DhelOBP8*), gustatory receptor 5 (*DhelGR5*), and sensory neuron membrane protein 1 (*DhelSNMP1*) were significantly upregulated 4-5 d after initiation of parasitism.

OPTIMIZATION OF FACTORS AFFECTING THE ROOTING OF PINE WILT DISEASE RESISTANT MASSON PINE (*PINUS MASSONIANA*) STEM CUTTINGS

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Keywords: Pine wilt disease resistance, non-juvenile, Masson pine, cutting propagation, rooting

Pine wilt disease (PWD) is a devastating disease affecting trees belonging to the genus Pinus. To control the spread of PWD in the Masson pine forest in China, PWD resistant Masson pine clones have been selected by the Anhui Academy of Forestry. However producing cutting seedlings of 9-year-old PWD resistant Masson pine clones is challenging, because Masson pine is a difficult-to-root species, especially for trees older than 5 years of age, which impedes the application of PWD resistant clones. In this study, we investigated the factors affecting rooting of PWD resistant clones and established a cheap, reliable, and simple method that promotes rooting. Rooting was observed only in stem cuttings treated with the full-light automatic spray management method. Additionally, stem cuttings showed a significantly higher rooting rate and root quality than needles cuttings. Compared with other substrates, stem cuttings planted in perlite produced the longest adventitious root and the highest total root length and lateral root number. Moreover, stem cuttings of PWD resistant clones collected in May showed a significantly higher rooting rate and root quality than those collected in June and July. Moreover, stem cuttings prepared with a horizontal cut while retaining the needles showed significantly higher rooting rate and root quality than those prepared with a diagonal cut while partly removing the needles. This study promotes the reproduction of seedlings of PWD-resistant Masson pine clones which helps control the spread of PWD, meanwhile, provides a technical reference for the propagation of non-juvenile pine trees via cuttings.

Chitosan oligosaccharide induces plant resistance gene expression in *Pinus massoniana*

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Keywords: chitosan oligosaccharide; plant resistance gene; *Pinus massoniana*; pine wilt disease

Chitosan oligosaccharides are the main degradation products from chitosan or chitin and have been reported to induce resistance to diseases in herbaceous plants like cucumber and *Arabidopsis*. Concomitantly, pine wilt disease (PWD) is a devastating disease of conifer tree species. Here, we hypothesized that chitosan oligosaccharides induce plant resistance gene (PRG) expression in the woody plant Masson pine, *Pinus massoniana*. Chitosan oligosaccharides were inoculated into *P*. *massoniana* seedlings and the BGISEQ-500 platform was used to generate transcriptomes from chitosan oligosaccharide-treated *P*. *massoniana* and control seedlings. A total of 501 differentially expressed genes (DEGs) were identified by comparing the treatment and control groups. A total of 251 (50.1%) DEGs were up-regulated in the treatment relative to the control seedlings and 250 (49.9%) were down-regulated. Inoculation of chitosan oligosaccharide induced the expression of 31 PRGs in *P*. *massoniana* seedlings and the relative expression levels of six of the PRGs were verified by RT-qPCR. This is the first study to demonstrate that chitosan oligosaccharide induces the expression of PRGs in a tree species. These results provide important insights into the function of chitosan oligosaccharides and furthers the prospects of developing a chitosan oligosaccharide-based immune inducer for controlling PWD.

THE EVALUATION ON THE EFFECT OF CONTROL PWD BY IPM PERFORMANCE CONTRACTING MANAGEMENT IN XINGYE COUNTY, GUANGXI, CHINA

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Keywords: Evaluation, Integrated pest management (IPM), Performance contracting management, Pine wilt disease (PWD)

This paper defines the concept of performance contracting management and its assessment indicators for control of PWD. Comparative analyzing the control effects of PWD before and after IPM performance contracting management in Xingye country. The results show that before 2018, the traditional control mode was used, including clearing up dead pine trees, trapping and killing *Monochamus alternatus*, spraying chemical and releasing natural enemies controlling of *M. alternatus*, but the epidemic continues to spread and the damage was increasing year by year. The number of epidemic towns, villages, forest subcompartment, and dead pine trees have increased by double, 6, 30, and 2 times respectively compared to the early epidemic stages. The population density of *M. alternatus* has increased from 78/traps to 154/traps. After implemented the IPM performance contracting management in 2019, focusing on cleaning up of dead pine trees, trapping and killing adults of *M. alternatu*s and setting up pine wood pile to lure and prevent the escape of *M. alternatu*s female adults, standardizing its spawning sites. The continuous spread trend of PWD has been effectively controlled. By 2022, The number of epidemic towns, villages, forest subcompartment, and dead pine trees have decreased by 50.00%, 42.86%, 33.33%, and 80.52% respectively. The PWD epidemic in Kuiyang Town and Shinan Town were successfully cleaned up. The population density of *M. alternatu*s declined gradually, and the average annual decline rate in 2020-2022 was 5.82%, 74.88% and 43.58%, respectively. IPM performance contracting management has achieved remarkable results in the control of PWD.

Prevention and control of pine wilt disease in Nanjing -- Review and reflection of forty years

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Keywords: Pine wilt disease (*Bursaphelenchus xylophilus*); The course; Experience; countermeasure; Nanjing City

Pine wilt disease (*Bursaphelenchus xylophilus*) has become the most devastating forest disaster in Nanjing even the world, since it was detected in the eastern suburbs of Nanjing in 1982. This paper reviewed the development and control course of pine wilt disease in Nanjing in the past 40 years, objectively analyzed the prevention and control experiences and effects obtained in the past 40 years, and reflect on the prevention and control countermeasures of pine wilt disease in the future, so as to provide efficient models and methods for the prevention and control of pine wilt disease in other areas of our country and the world.

Rapid and Accurate Identification of Pine Wilt Disease with a Portable Nucleic Acid Detection System

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Keywords: *Bursaphelenchus xylophilus*, detection, EmDEA,

A portable nucleic acid detection system, based on enzyme mediated duplex exponential amplification（EmDEA） platform, has been developed and applied in the field detection of *Bursaphelenchus xylophilus*. To reduce the risk of carry-over contamination, enzyme mediated programmed DNA hydrolysis (EmPDH) technique is incorporated into the system. The sawdust from packing wood sample is treated with 95°C for 5 minutes and the resultant supernatant could be transferred into lyophilized reagent in PCR tube for DNA amplification and detection. The on-site detection process of *Bursaphelenchus xylophilus*, combined with sample treatment, takes less than 30 minutes and detection sensitivity is ~ 5 copies/test. Our portable nucleic acid detection system of *Bursaphelenchus xylophilus* has been validated in Shanghai Customs Technology Center and Ningbo Customs Technology Center with packing wood samples.

An Efficient Method for Mass Rearing *Dastarcus helophoroides* (Coleoptera: Bothrideridae): an Important Natural Enemy of *Monochamus alternatus*

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Keywords: *Dastarcus helophoroides*, infestation ratio, offspring number, rearing cost, rearing efficiency

Dastarcus helophoroides (Coleoptera: Bothrideridae) is an important biocontrol agent against wood-boring longhorned beetles. In this study, to improve the mass-rearing efficiency and reduce rearing costs of this parasitoid, a rearing tube was designed, and two new methods, the natural infestation method without Tube (NnT) and the natural infestation method with Tube (NwT), were developed based on the rearing tube to address the limitations of the traditional artificial infestation method (AM) for mass rearing the parasitoid. Parameters of the fitness of this parasitoid were compared, including the successful parasitism rate, the emergence rate, the total numbers of cocoons and emergences per rearing box, the duration, and the time consumption using these three methods under four different parasitoid-to-host ratios. The cost of producing 10,000 parasitoid adults under different methods was calculated. The results showed that compared with AM and NnT, the cocoon and emergence numbers per rearing box, and emergence rate were significantly increased, and the duration was significantly shortened in NwT. The successful parasitism rate in NwT was significantly increased compared to that in AM. The time consumption in NwT and NnT was significantly shortened compared to that in AM. Compared with that in AM, the total cost of producing 10,000 adults in NwT was reduced by 75.21%~87.32%, while in NnT, it was reduced by 35.17%~76.79%. The study indicates that using NwT for mass rearing of *D. helophoroides* enhanced the efficiency and reduced costs, and the highest input-output could be achieved at a parasitoid-to-host ratio of 10:1.

Biological characteristics of Cyanopterus ninghais (Hymenoptera: Braconidae), a newly discovered larval parasitoid of *Monochamus alternatus* (Coleoptera: Cerambycidae)

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Keywords: Biological control, Developmental threshold temperature, Effective accumulated temperature, Natural enemy, Reproductive capacity

Cyanopterus ninghais (Hymenoptera: Braconidae), a newly discovered gregarious ectoparasitoid, is a promising biological control agent against the 3rd-5th instar larvae of the Japanese pine sawyer, *Monochamus alternatus* (Coleoptera: Cerambycidae). Effects of constant ambient temperatures (17, 20, 23, 26, 29, and 32 °C) on the reproduction and development of the parasitoid were determined in the laboratory. Results showed that the pre-oviposition periods of *C. ninghais* decreased gradually with increasing temperatures, ranging from 8.10 d (at 17 °C) to 3.60 d (at 32 °C). Both the parasitism rates and brood size exhibited a parabolic trend in relation to increasing temperatures, with the highest parasitism rate of 80% (at 29 °C) and the maximum brood size of 7.19 (at 26 °C). While temperature did not significantly affect the emergence rate and sex ratio of progeny. The duration of each developmental stage displayed an inverse correlation with temperature within the range of 20 to 32 °C. The egg-to-adult development time recorded was 32.25 days at 20 °C and 12.50 days at 32 °C. The developmental threshold temperatures for egg, larva, pupa, and the entire generation were 8.64, 12.59, 12.83, and 12.10 °C, respectively, and the effective accumulated temperatures were 34.29, 51.32, 148.91, and 240.01 day-degree, respectively. The optimal and upper developmental temperatures estimated by the Logan I model for the entire generation of *C. ninghais* were 32.36 and 38.79 °C, respectively. These findings provide important implications for improving the artificial rearing efficiency and field release of this parasitoid under different temperature conditions.

Cradle for the newborn *Monochamus saltuarius*: Microbial associates to ward off entomopathogens and disarm plant defense

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Keywords: community structure; entomopathogenic bacteria; host plant adaptation; plant secondary metabolites; symbiotic bacteria

The Japanese pine sawyer, *Monochamus saltuarius*, as a beetle vector of *Bursaphelenchus xylophilus* (pine wood nematode), is an economically important forest pest in Eurasia. To feed on the phloem and xylem of conifers, *M. saltuarius* needs to overcome various stress factors, including coping with entomopathogenic bacteria and also various plant secondary compounds (PSCs). As an important adaptation strategy to colonize host trees, *M. saltuarius* deposit eggs in oviposition pits to shield their progeny. These pits harbor bacterial communities that are involved in the host adaptation of *M. saltuarius* to the conifers. However, the composition, origin, and functions of these oviposition pit bacteria are rarely understood. In this study, we investigated the bacterial community associated with *M. saltuarius* oviposition pits and their ability to degrade PSCs. Results showed that the bacterial community structure of *M. saltuarius* oviposition pits significantly differed from that of uninfected phloem. Also, the oviposition pit bacteria were predicted to be enriched in PSC degradation pathways. The microbial community also harbored a lethal strain of *Serratia*, which was significantly inhibited. Meanwhile, metatranscriptome analysis indicated that genes involved in PSCs degradation were expressed complementarily among the microbial communities of oviposition pits and secretions. In vitro degradation showed that bacteria cultured from oviposition pits degraded more monoterpenes and flavonoids than bacteria cultured from uninfected phloem isolates. Disinfection of oviposition pits increased the mortality of newly hatched larvae and resulted in a significant decrease in body weight in the early stages. Overall, our results reveal that *M. saltuarius* construct oviposition pits that harbor a diverse microbial community, with stronger PSCs degradation abilities and a low abundance of entomopathogenic bacteria, resulting in the increased fitness of newly hatched larvae.

Transcriptome analysis reveals adaptive strategies of immunity and metabolism in *Monochamus saltuarius* to *Bursaphelenchus xylophilus*

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Keywords: *Bursaphelenchus xylophilus*, Fatty acid metabolism, *Monochamus saltuarius*, Reference genes, Transcriptome

The transmission of pine wilt disease (PWD) involves complex relationship on chemical ecology, development synchronization, and immunoreaction, etc. between pine wood nematode (PWN) and vector beetles. *Monochamus saltuarius* (Coleoptera: Cerambycidae) is one of the main vectors of PWN in the middle temperate zone of China. However, the effect of PWN to *M. saltuarius* is rarely studied so far. We studied the lipid metabolic and immune responses of *M. saltuarius* inoculated with PWN in the different developmental stages and tissues, through transcriptome analyses. The stability of reference genes for gene expression analysis was Identificated and evaluated. We found that the lipid metabolism pathways of *M. saltuarius* was significantly up-regulated revealing by weighted gene co-expression network analysis (WGCNA). Hub genes involved in fatty acid synthesis, very long chain fatty acids proteins genes (*ELO*), one acyl-CoA desaturase gene (*FAD*), and one fatty acyl-CoA reductase gene (*FAR*) were significantly upregulated, especially in the gonads and trachea of adults. Most of the immune responses dominated by IMD signaling pathway were activated at the pupal stage. While at the adult stage, most of the immune genes showed no significant change. In addition, 60S ribosomal protein L7 (*RPL7)* and 40S ribosomal protein S5 (*RPS5)* genes were considered the most stable reference genes in the pupae inoculated with PWN. *RPS5* and sorting nexin 6 (*SNX6)* genes could be used as reference genes in the adults inoculated with PWN. This study advances our understanding of the phoretic relationship between nematodes and its vectors.

PINE WILT DISEASE IN NORTHEAST AND NORTHWEST CHINA: A COMPREHENSIVE RISK REVIEW

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Keywords: altitude, host tree, *Monochamus* vector, risk analysis, temperature

Pine wilt disease (PWD) caused by the pine wood nematode (PWN) is China's most serious forest disease in recent decades. Its complex disease system with unclear pathogenesis and differentiated pathogenicity makes it challenging to control. PWD has spread rapidly in Northeast and Northwest China in recent years, exceeding previous predictions and the traditional understanding of its potential distribution. We reviewed the comprehensive risk of PWD in Northeast and Northwest China through influencing factors. Regarding PWN's temperature resistance, all regions of China are suitable for PWD. In terms of altitude, the PWD system is mainly distributed below 1000 m. In China, *Pinus* and *Larix* species are confirmed host trees of PWN, while *Monochamus alternatus* and *M. saltuarius* are confirmed vector insects. In this case, PWD may occur in all low-altitude pine forests in China. Northeast China has a higher risk than Northwest China due to its distribution of suitable host trees and vector beetles at low altitudes. To confront the threat, two scientific bottlenecks are required to be demonstrated. The first is the pathogenicity of Chinese PWN strains to Chinese *Picea* and *Abies* species. The other is the geographical distribution and highest altitude distribution of other *Monochamus* species in the Palaearctic region of China and their PWN transmission ability. In conclusion, this review provides a new perspective and reference for the management and research of PWD in China. Moreover, the occurrence and continuous spread of PWD in Northeast China provide a warning for low-latitude regions around the world.

EFFECTS OF PINE WILT DISEASE ON RHIZOSPHERE MICROBIOTA AND FINE ROOT FUNGI: INSIGHTS INTO ENZYME ACTIVITY, ECTOMYCORRHIZAL INFECTION, AND MICROBIAL COMPOSITION

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Keywords: *Bursaphelenchus xylophilus*, community and functional structure, enzyme activity, microbe-host interaction, pine wilt disease

Pine wilt disease (PWD), caused by the pine wood nematode (PWN) *Bursaphelenchus xylophilus*, poses a significant threat to pine forests worldwide. However, the understanding of the impact of PWD on the host microbiome remains insufficient. This study aimed to investigate the structure and function of the fungal community associated with *Pinus thunbergii* fine roots, as well as the rhizosphere fungi and bacteria of the tree naturally infected by PWN. We employed high-throughput sequencing in conjunction with functional prediction tools, and soil enzyme activity measurement. The results showed that PWN infection significantly decreased the activity of β- cellobiosidase and β-glucosidase enzymes involved in carbon cycling in the rhizosphere ($P < 0.05$). Interestingly, PWN infection did not alter the diversity of rhizosphere bacteria and fine root fungi, but it did cause a significant decrease in the richness of rhizosphere fungi ($P < 0.05$). In terms of function, PWN infection resulted in higher abundance of intracellular parasites in bacterial functions in the rhizosphere, while ureolysis exhibited a lower abundance ($P < 0.05$). In fungal functions, saprotroph-symbiotroph exhibited a higher abundance in the rhizosphere after PWN infection, whereas symbiotroph showed a lower abundance ($P < 0.05$). Additionally, it led to a significant reduction in the infection rate of ectomycorrhizal fungi ($P < 0.05$). Infected host fine root exhibited higher abundance of pathotroph-symbiotroph, while symbiotroph had a lower abundance ($P < 0.05$). The study sheds light on the role of the fungal community and rhizosphere bacteria in PWN-affected trees and contributes to our comprehension of the overall impact of PWD on the pine forest ecosystem.

Unveiling the microbial community shifts triggered by pine wilt disease in *Pinus thunbergii* forests

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Keywords: enzyme activity, forest succession, microbial community, nutrient cycling, pine wilt disease

Pine forests worldwide are threatened by pine wilt disease (PWD), caused by the pine wood nematode *Bursaphelenchus xylophilus*. Despite its devastating impact, the influence of PWD on the various aspects of host microbial communities remains understudied. This research investigated the effects of PWD on the microbiome associated with *Pinus thunbergii* trees, the bulk soil, as well as the forest litters. High-throughput sequencing techniques and functional prediction tools were employed to unravel changes in microbial structures and functions.

The findings revealed that PWD significantly affected the composition and functional potential of microbial communities in different section of the host. Both bacterial and fungal communities in the soil displayed alterations in response to PWD, with certain enzymes involved in carbon cycling and nutrient degradation experiencing decreased activity. Furthermore, PWD appeared to influence the presence of specific bacterial and fungal taxa, influencing their abundances and thus potentially affecting ecosystem functions.The study emphasized the significance of considering anatomical regions within the host, as the impact of PWD was often region-specific. Notably, the influence of PWD extended to ectomycorrhizal fungi, which play a pivotal for nutrient uptake in trees. Such changes in the microbial community could consequently impact the dynamics of litter degradation and nutrient cycling across the forest succession caused by PWD.

In summary, this research provides insights into the impact of PWD on host microbiomes and ecosystem functions. The findings highlight the need for a comprehensive understanding of the microbial intricacies within trees and their surroundings, especially in the context of disease-induced changes. Such knowledge is important for both forest management strategies and the broader understanding of plant-microbe interactions in changing ecosystems.

Early diagnosis of pine wood nematode disease based on chlorophyll fluorescence parameters and organic acids

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Keywords: *Bursapherenchus xylophilus*; Organic acids; Chlorophyll fluorescence parameters; Early diagnosis; *Pinus hwangshanensis*

Pine wood nematode disease is a destructive disease to ecological environment and forest resources. The onset of the disease is extremely rapid and the pathogenesis is unclear. The monitoring of this disease is still important in production, so it is of great significance to explore its early diagnosis. In this study, the changes of chlorophyll fluorescence dynamic induction parameters, non-photochemical quenching parameters and organic acid content in needles of 7-year old infected *Pinus hwangshanensis* were studied by chlorophyll fluorescence technology and organic acid mass combined technology. The results showed that in chlorophyll fluorescence dynamic induction group, Fm/Fo, Fv/Fo, Fm, Psi_o and Pi_Abs showed a significant downward trend, while Phi_Do, ABS/RC, TRO/RC and DIO/RC showed a significant uptrend. In non-photochemical quenching, Fm showed a downward trend, while Qp_L1, Qp_L2, QY_L1, QY_L2 and QY_ Lss showed a significant upward trend. In the change of organic acid content, ferulic acid, syringic acid, gallic acid and jasmonic acid showed a significant increasing trend, while benzoic acid and salicylic acid showed a first increasing trend and then decreasing trend. Experimental results show that pine wood nematode had significant effects on photosynthesis and organic acid content of pine before it showed symptoms of disease. After comprehensive analysis, chlorophyll fluorescence dynamic induction parameters Pi_Abs、Fm、Phi_Do、TRO/RC、Psio, non-photochemical quenching parametersQY_L2、Qp_L1、QY_Lss、QY_L1, and organic acids ferulic acid, syringic acid, benzoic acid and gallic acid contents are ideal indicators for early diagnosis of pine wood nematodiasis.

Study on the genetic differentiation of *Bursaphelenchus xylophilus* in East China based on SNP markers

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Keywords: *Bursaphelenchus xylophilus;* SNP; whole-genome resequencing; genetic differentiation

Studying the genetic differentiation of *B. xylophilus* population in East China regions will provide important basic genetic information for the establishment of *B. xylophilus* epidemic source tracing system in China. The samples were collected from pine trees affected by pine wilt disease in East China, and the strains were isolated and purified. The genomic DNA of the strains was extracted and sequenced. The number and types of SNP loci and genotypes in each region were analyzed by using bioinformatics software. Cluster analysis was used to compare the genetic differentiation among different strains based on SNP markers, and Treemix was used to detect the gene penetration routes. A total of 67 *B. xylophilus* strains were isolated and collected in 60 county-level administrative regions in East China. A total of 6 531 684 SNPs loci were obtained, and the number of SNP were different among different strains. While the number of SNP counts, homozygotes, missing SNPs and private SNPs showed no significant differences in regional among the 6 provinces, and there was no significant correlation with the invasion time. In general, the *B. xylophilus* strains in East China can be divided into 3 groups, and there was a certain correlation between different groups and geographical areas. In the area where the 1-AH and 2-AH groups were located, there may be re-invaded by strains from other groups in other areas.

EXPLORING THE ROLE OF DETOXIFICATION GENES IN THE RESISTANCE OF *BURSAPHELENCHUS XYLOPHILUS* TO DIFFERENT EXOGENOUS NEMATICIDAL SUBSTANCES USING TRANSCRIPTOMIC ANALYSES

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Keywords: *Bursaphelenchus xylophilus*, comparing transcriptomic, detoxification genes, gene family analysis, RNAi

Bursaphelenchus xylophilus has become a worldwide forest disease due to its rapid infection ability, high lethality and difficulty in control. The main means of countering *B. xylophilus* is currently chemical control, but nematicides can present problems such as environmental pollution and drug resistance. The development of novel environmentally-friendly nematicides has thus become a focus of recent research. In this study, *BxUGT3* and *BxUGT34,* which might be related to detoxification, were investigated by comparing transcriptomic and WGCNA approaches. Three other genes with a similar expression pattern, *BxUGT13, BxUGT14,* and *BxUGT16*, were found by gene family analysis. Further bioassays and qPCR assays confirmed that these five genes showed significant changes in transcript levels upon exposure to α-pinene and carvone, demonstrating that they respond to exogenous nematicidal substances. Finally, RNAi and bioassays showed that *B. xylophilus* with silenced *BxUGT16* had increased mortality in the face of α-pinene and carvone stress, suggesting that *BxUGT16* plays an important role in detoxification. Taken together, this study used novel molecular research methods, explored the detoxification mechanism of *B. xylophilus* at a transcriptomic level, and revealed a molecular target for the development of novel biopesticides.

Functional analysis of 3 genes in xenobiotic detoxification pathway of *Bursaphelenchus xylophilus* against matrine

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Keywords: *Bursaphelenchus xylophilus*, Matrine, RNA interference, Xenobiotic detoxification pathways genes

Bursaphelenchus xylophilus is the causative agent of pine wilt disease. It has caused devastating damage to ecosystems worldwide, owing to the characteristic of being widely spread and uncontrollable. However, the current methods of control are mainly based on pesticides, which can cause irreversible damage to the ecosystem. Therefore, the search for new drug targets and the development of environmentally friendly nematicides is especially valuable. In this study, three key genes of the xenobiotic detoxification pathways were cloned from *B. xylophilus*, which were subsequently subjected to bioinformatic analysis. The bioassay experiment was carried out to determine the concentration of matrine required for further tests. Subsequently, enzyme activity detection and three gene expression pattern analysis were performed on matrine treated nematodes. Finally, RNA interference was conducted to verify the functions carried out by the three genes in combating matrine. The results indicated that cytochrome P450 and glutathione S-transferase of *B. xylophilus* were activated by matrine, which induced high expression of *BxCYP33C4*, *BxGST1*, and *BxGST3*. After RNA interference of three genes of *B. xylophilus*, the sensitivity of *B. xylophilus* to matrine was increased and the survival rate of nematodes was reduced to various degrees in comparison to the control group. Overall, the results fully demonstrated that *BxCYP33C4*, *BxGST1*, and *BxGST3* are valuable drug targets for *B. xylophilus*. Furthermore, the results suggested that matrine has value for development and exploitation in the prevention and treatment of *B. xylophilus*.

Enterobacter ludwigii AA4 exhibiting a powerful pine wood nematode–killing effect

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Keywords: *Enterobacter ludwigii*, nematocidal activity, *Bursaphelenchus xylophilus*, pine wilt disease, colonization.

Essentially, no efficient methods for controlling *Bursaphelenchus xylophilus* and pine wilt disease (PWD) have yet been developed. By using a high-throughput screening system based on a fluorescent dye-exclusion viability test, we identified a powerful pine wood nematode (PWN)-killing bacterial strain, *Enterobacter ludwigii* AA4. The corrected mortality of the *B. xylophilus* treated by *E. ludwigii* AA4 was more than 98%. Morphological changes in *B. xylophilus* treated with strain AA4 suggested that the death of *B. xylophilus* might be caused by an increased number of vacuoles in non-apoptotic cell death and the damage to tissues of the nematodes. The disease index of the seedlings of Scots pine treated with strain AA4 plus *B. xylophilus* was less than 39, significantly lower than 92.5 in the control plants treated with distilled water and *B. xylophilus*. We created a *sdaB* gene knockout in strain AA4 by deleting the gene that was putatively encoding the beta-subunit of L-serine dehydratase through Red homologous recombination. The nematocidal and disease-suppressing activities of the knockout strain were remarkably impaired. We also found that the deletion of gene *ecpE* encoding a subunit of the ECP fimbriae of AA4 notably reduced its colonization on *B. xylophilus*. Furthermore, the application of the biological nematicide developed from AA4 on the *Pinus thunbergii* grown in the coastal area in Yantai remarkably decreased the incidence rate of PWD from 4.5% to 1.5%. Therefore, *E. ludwigii* AA4 has significant potential to serve as an agent for the biological control of pine wilt disease caused by *B. xylophilus*.

Screening and identification of microbial diversity and saprophytic bacteria in inter-rooted soil of Pinus sylvestris with different disease levels

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Keywords: defence enzyme activity, Lysinibacillus capsici, Lelliottia jeotgali, nematicidal bacteria, pine wood nematode disease

Pine wilt disease (PWD), caused by *Bursaphelenchus xylophilus*, is a systemic infectious disease and has been called the cancer of pine trees. The microbial community in plant ecosystems is an important functional component that stimulates the development of natural resistance to pathogens in plant growth or acts as a biological control agent for pathogenic microorganisms and is one of the main sources of biological control. In this study, we first showed by high-throughput sequencing of inter-root soils of diseased and healthy Sargasso pine that the community structure of each group was different at different levels. After performing inter-root soil isolation and purification, four strains *Lysinibacillus capsic*, *Bacillus Paramycoides*, *Delftia tsuruhatensis*, and *Lelliottia jeotgali* were screened for optimal nematicidal effect.The inoculation of the roots of 2-year-old pinus sylvestris seedlings with four strains of mycorrhizal suspension and fermentation solution for 4 d was followed by inoculation with pine wood nematodes, and the changes in the activities of defence-related enzymes in pinus sylvestris within 4 d after treatment and 28 d after inoculation with pine wood nematodes were measured. The results showed that the activities of PAL, POD and CAT were increased within 4 d after treatment, while the activities of PAL, POD and CAT were significantly increased within 28 d after inoculation with pine nematodes, which greatly reduced the incidence of nematode disease in pine trees. Lysinibacillus capsic strain fermentation and Lelliottia jeotgali fermentation were screened on the basis of changes in defence enzyme activity and showed better nematicidal effect and induced resistance and lasted up to 28 days.

Green pesticide screening and prevention determination of pine wood nematodes

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Keywords: pine material nematode, pine material nematode disease, natural substances, green nematode pesticide

Pine wood nematode disease (PWD) is a devastating forest disease caused by Bursaphelenchus xylophilus, threatening the ecological health of pine forests worldwide. At present, the main control method is the use of chemical agents, because long-term use is easy to cause pine wood nematode drug resistance, and its residue to the ecological environment to form pollution, in nature to find green and safe line killers has become a research hotspot. In this study, the soil metabolites of pine trees with different degrees of susceptibility were investigated and analyzed in the same underground ground, and it was found that there were differences in soil metabolites in the rhizosphere of pine trees with different degrees of disease. Based on this, the green substances with high virulence against pine wood nematodes were ginkgo flavonoids, 2,3-dichloro-5-(trifluoromethyl)pyridine, sodium silicate, arecine and amygdalin, among which 2,3-dichloro-5-(trifluoromethyl)pyridine had the best effect and stability. In the prevention experiment, the effect of elemental agent 2,3-dichloro-5-(trifluoromethyl) was the best, and the control rate of Pinus massoniana seedlings under the condition of 28 days was 87.82%. In the efficacy experiment, the effect of the compound green line-killing agent was better than that of the elemental agent, and the effect was relatively average, and the control rate of Pinus massoniana seedlings after 28 days of treatment was about 70%, which had the application potential of alternating use with each other, which could effectively avoid the emergence of drug resistance.

ESTABLISHMENT OF MOLECULAR-TARGETED NEMATOCIDE DISCOVERYING PLATFORM FOR THE CONTROL OF *BURSAPHELENCHUS XYLOPHILUS*

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Keywords: *Bursaphelenchus xylophilus*, nematocide, target screening, lead compound

In the present study, an novel molecular-targeted nematocide discoverying platform was developed for finding efficient agents against *Bursaphelenchus xylophilus*. The molecular targets for nematocide discovery were investigated according to multi-omics strategy, including genomics, transcriptomics, phenotypic databases, and the targeted genes were verified according to the results of RNAi. The structure of corresponding protein and potential nematocides were predicted based on AlphaFold2 and Schrödinger. In this platform, target discovery, validation and lead compound screening were integrated, providing a holistic approach to innovative drug discovery. We have successfully identified the molecular mode of action and structural basis of several drugs, including Abamectin, Emamectin and Fluopyram, on their respective targets in *B. xylophilus*. Furthermore, based on the functional investigation of GPCR in *B. xylophilus*, five potential molecular targets were screened by multi-omics analysis, including *npr-5*, *npr-11*, *fshr-1*, F59D12.1 and *pdfr-1*. Structural prediction, virtual screening, and druggability prediction were performed on these five molecular targets, and three lead compounds were found. The LC_{50} values of these 3 compounds against *B. xylophilus* were 9.30 mg/L, 8.76 mg/L and 9.19 mg/L, respectively, showing high nematicidal activity. This platform can not only accelerate the process of nematocide discovery but also provide a new perspective for the control of pine wilt disease.

Unravelling Epigenetic Factors in Sex Determination of *Bursaphelenchus xylophilus*: Transcriptome Analysis of Temperature-Sensitive Embryo Stages

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Keywords: *Bursaphelenchus xylophilus*, Transcriptome, Sex-determination, Epigenetics

The expansion of pine wilt disease has been observed, manifesting a trend of northward and westward movement in China. Field surveys have unveiled a decline in the female-to-male ratio of *Bursaphelenchus xylophilus* with decreasing of temperature or increasing latitude, ranging from Zhejiang (♀/♂: 2.37) to Liaoning (♀/♂: 0.86) provinces. However, the mechanisms governing sex determination undergo remarkably rapid evolution in numerous taxa, yet the understanding of such processes remains obscure within the vast nematode phylum. Notably, *B. xylophilus* lacks sex chromosomes, thus suggesting the likelihood of epigenetic regulation triggering sex determination. To unveil this intriguing epigenetic phenomenon, the temperature-sensitive window for sex determination is identified during the lima beans and J1 stages of embryo development through temperature-shift experiments. Subsequently, transcriptome sequencing was performed on *B. xylophilus* embryos during the lima beans and J1 stages, at two distinct temperatures (15°C and 25°C). The analysis revealed a total of 657 differentially expressed genes during lima beans and 3775 during the J1 stage. Functional annotation of these genes allowed us to identify 21 genes (e.g., *mab-3*, *sma-2*, *ptr-1*) exhibiting higher expression at 15°C and 17 genes (e.g., *mog-4*, *rnt-1*, *hda-1*) displaying higher expression at 25°C. Notably, these genes are associated with sex determination and differentiation. Overall, this study has unveiled novel epigenetic factors through RNA-seq data analysis of *B. xylophilus* embryo, contributing to the understanding of the nematode's sex-determining mechanism.

Scenario-Based Design of "Digital Forest Protection" and Application for Monitoring & Tracking on Pine wilt Disease in Zhejiang Province, China

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Keywords: cloud-processing framework, digital forest protection, epidemic, monitoring and tracking, pine wilt disease, scoring-metrics method

The rapid-spreading pine wilt disease (PWD) has brought global forest health crisis. High-efficient and precise monitoring & tracking on PWD could provide the detailed epidemic information and the hint of development trend, which should be given priority in the integrated management strategy. In this work, we proposed a closed-loop monitoring & tracking (CMT) system and an on-line remote management (ORM) platform based on the disease cycle of PWD. The CMT system is built on the concept of scenario design, in which every essential link of PWD management chain from monitoring to eradication of diseased pine tree were presented visually, playing and exploring potential “what-if” and “scoring-metrics” settings. Six scenarios, including epidemic survey, eradication of dead tree, real-time eradication of wilting tree in early stage, random sampling and checking on quality of eradication measures, trunk injection with nematocide and quarantine of pine, were proposed and designed. Each scenario was constructed as an individual module. All the modules were integrated into one system based on a unique data collection and cloud-processing framework. This system was developed into a visualized mobile App ‘Digital Forest Protection’ (DFP version 1. 96) (or Web vision: https://szsf.lyj.zj.gov.cn). The App serves over 20, 000 clients who worked for monitoring and control of PWD in Zhejiang province. A total of more than 38.21 million data and 11.77 million images were collected since its first launch on Nov. 2021. All the diseased pine trees caused by PWD have been located in longitude and latitude, and the entire processes of dead-tree treatment were precisely tracked by CMT system. As a powerful management tool, DFP App serves the integrated management of PWD successfully, which contributes largely to substantial disease reduction in Zhejiang province of China.

Potentially Suitable Geographical Areas for *Monochamus alternatus* under Current and Future Climatic Scenarios Based on Optimized MaxEnt model

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Keywords: climate change, *Monochamus alternatus,* suitable area, the optimized MaxEnt model

Monochamus alternatus is an important wood boring pest, and it is also considered the most important and efficient insect vector for the transmission of the pine wood nematode. The precise determination of potential suitable areas of *M. alternatus* is important for monitoring, prevention, and control of *M. alternatus*. Based on the latest occurrence data and climate data of *M. alternatus*, the optimized MaxEnt model and ArcGIS 10.7 software were used to predict the current and future potentially suitable areas of *M. alternatus* in the world and in China under different climatic conditions. In this study, the optimized MaxEnt model parameters were set as FC = LQHP and RM = 1.5, which were determined by the values of AUC_{diff}, OR_{10}, and ΔAICc. The monthly mean temperature difference (Bio2), minimum temperature of the coldest month (Bio6), mean temperature of warmest quarte (Bio10), annual precipitation (Bio12), and precipitation of driest month (Bio14) were the dominant bioclimatic variables affecting the distribution of *M. alternatus*.Under the current climate conditions, the potentially suitable habitats of *M. alternatus* were distributed across all continents except Antarctica, accounting for 4.17% of the Earth’s total land area. Under future climate scenarios, the potentially suitable areas of *M. alternatus* increased significantly, spreading to a global scale. Our findings can be used to provide a theoretical basis for the risk analysis of the spread of *M. alternatus* worldwide and the precise monitoring and prevention of this beetle.

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杭州费洛蒙生物科技有限公司是一家高科技企业，总部位于浙江省杭州市。我们的愿景是牢记“守护每一片绿水青山”初心使命，秉持“因为专注，所以卓越”的理念，专注于有害生物绿色防控技术领域，打造一个在国内具有一定影响的科技型公司。已经形成诱捕器、引诱剂、天敌昆虫、菌剂、取样管等10余类产品，销售覆盖全国27个省份，并出口韩国等东南亚地区。拥有浙江农林大学、中科院动物所、法国农科院等专家团队技术支持，获得国家梁希科技进步奖等多个奖项，授权10余项专利，相关技术达国际同类领域领先水平。

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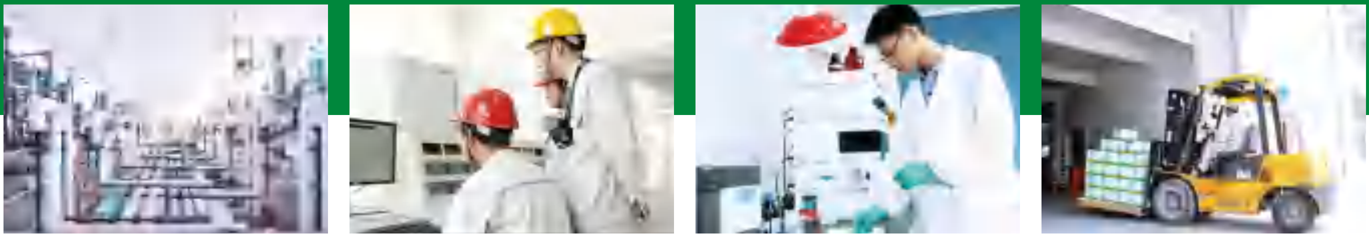
功成科技®

江苏功成生物科技有限公司始建于1999年，坐落于美丽的南黄海之滨、江苏省南通市洋口化工园内，占地面积34000平方米。公司注册资本5000万元，是国家定点的农药生产企业。主要从事农药原药及制剂，特别是林业杀虫剂、红火蚁防治药剂、白蚁防治药剂的研发、生产、销售、技术服务和进出口贸易。是同行业较早通过ISO9001质量体系认证的企业，江苏省高新技术企业。

竭诚欢迎海内外朋友与我们携手合作，共谋发展！

Jiangsu Gongcheng Bio-tech Co., Ltd. was founded in 1999, located in the beautiful seashore of South Yellow Sea, Nantong Yangkou Chemical Industry Park, Jiangsu Province, covers an area of 34,000 square meters. With a registered capital of 50 million yuan, the company is a nation-designated pesticide manufacturer. Mainly engaged in the development, production, sales, technical services, and import & export trade of pesticides, especially forestry pesticides, red imported fire ant pesticides, termicides. Gongcheng has got the ISO9001 certification earlier in the industry., also Gongcheng is the Jiangsu high-tech enterprises.

We sincerely welcome friends domestic and abroad to cooperate with us and seek common development!



主营林业有害生物防治产品 Main forestry pesticides

绿色焦点®10%甲维·吡虫啉可溶液剂 Emamectin benzoate 1% + Imidacloprid 9% SL	术翠®200克/升氯虫苯甲酰胺悬浮剂 200g/L Chlorantraniliprole SC
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绿色焦点®3%噻虫啉微囊悬浮剂 3% Thiacloprid CS	绿色焦点®1%苦参碱水剂 1% Matrine AS
绿色焦点®1%噻虫啉微囊粉剂 1% Thiacloprid Microcapsule powder	大功达®10%高效氯氟氰菊酯可湿性粉剂 10% Lambda-cyhalothrin WP

江苏功成生物科技有限公司
JIANGSU GONGCHENG BIO-TECH CO., LTD.

企业简介

江苏擎宇化工科技有限公司创建于2008年，坐落于扬州化学工业园区，是一家集高分子表面活性剂研发、生产、销售为一体的国家高新技术企业，现有25000吨高分子分散剂和功能聚醚产能，先后被相关部门认定为江苏省专精特新中小企业、江苏省农业科技型企业、扬州市瞪羚企业等，被仪征市委、市政府授予“仪征市优秀企业”称号。

公司建有“江苏省梳型结构聚羧酸盐高分子表面活性剂工程技术研究中心”、“江苏省作物保护剂减施增效功能材料工程研究中心”、“江苏省企业技术中心”，研发团队实力雄厚，参与了“十四五”国家重点研发计划中“重大病虫害防控”、江苏省碳达峰碳中和科技创新等项目，获得“中国农药行业优秀助剂供应商”、“全国农牧渔业丰收奖”等多项荣誉。

公司秉持“为客户提供绿色助剂和精准服务，让药效更好表达”的初心，致力于成为具有国际影响力的专业农化助剂综合服务商。

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保绿丰是一家集农业技术、林业技术、环保技术、生物技术、信息技术和智能航空技术融于体的高新科技型企业。

践行理念:绿水青山就是金山银山!企业宗旨:保护绿色,护航丰收!

企业文化:诚实做人, 踏实做事!

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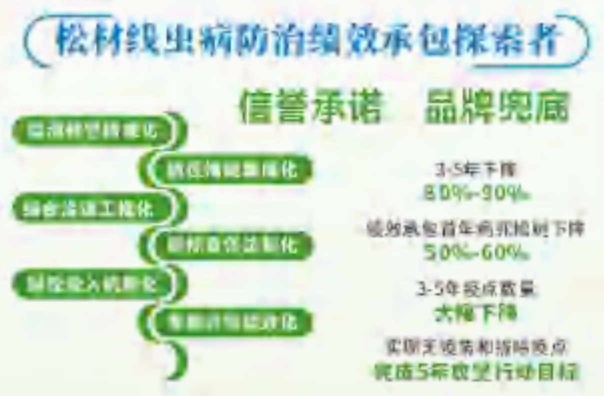


松材线虫病变色木智能分析系统

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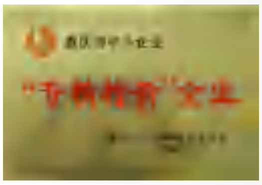
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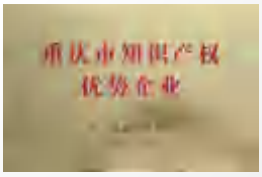
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