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A brief survey of research on Pine Wilt Disease under various climatic conditions around the globe

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ABSTRACT

Pine wilt disease (PWD), caused by the pinewood nematode (PWN) Bursaphelenchus xylophilus, causes significant losses in coniferous forests in eastern Asia, including Japan, China, and South Korea, as well as western Europe, including Portugal. The results of the research papers given at the International Symposium on Pine Wilt Disease (IUFRO Working Party Meeting 4.04.03) in Nanjing, China, in July 2009 are summarised in this article. The basic themes discussed included pine wilt disease (PWD), the pinewood nematode (PWN) Bursaphelenchus xylophilus, and other PWN-associated microorganisms that play a significant role in PWD, such as bacteria (e.g., Pseudomonas fluorescens). The majority of the papers are based on PWD-PWN research in East Asia and Russia. The following are some of the specific topics covered: 1) fundamental concepts of PWD development, 2) pathogenicity, 3) host-parasite relationships, including histopathology of diseased conifers and the role of toxins from bacteria-nematode ecto-symbionts, 4) PWN life cycle and transmission, 5) B. xylophilus dissemination models, 6) associations (with other nematodes), 7) diagnostics, 8) quarantine and control of the PWN and 9) biocontrol of the PWN.

Keywords: Review; A brief survey of research on Pine Wilt Disease

1. Introduction

Pine wilt disease (PWD), caused by the pinewood nematode (PWN) Bursaphelenchus xylophilus, causes significant losses in coniferous forests in eastern Asia, including Japan, China, and South Korea (1), as well as western Europe, including Portugal (2). As a result, the PWN is one of the most important pests on many countries' quarantine lists around the world.

An International Pinewood Disease Symposium was organised in Nanjing Forestry University on July 20–23, 2009, under the auspices of the International Union of Forest Study Organizations (IUFRO), as a result of the relevance of PWD and ongoing research on the topic. This symposium included over 130 researchers from 12 nations, who presented their most recent research findings on both the pathogen and PWD. We evaluate the most recent research on the issues covered in the symposium, as well as other relevant papers, such as surveys being conducted in different countries, PWD pathogenicity, PWN taxonomy, and pine wilt control.

Longhorn beetles of the genus Monochamus vector the PWN's juvenile transmissive stage. The PWN interacts with a variety of wood-inhabiting microorganisms during its life cycle, including fungus on which it feeds and reproduces, as well as ecto-symbiotic bacteria from the genus Pseudomonas. PWNs eat on plant tissue in living trees and the mycelium of xylobiotic fungi in dead trees, and they live in resin channels within the wood. The PWN was introduced into southwestern Europe from East Asia in the 1990s, causing significant forest damage (3). Because of the devastating Pan-European spread of pine wilt disease in Portugal, the National Eradication Program to Control the Pinewood Nematode (PROLUNP) was established to develop protocols for controlling the PWN, as existing EU measures were ineffective (4,5). As a result of global warming, the PWN may invade Russia and parts of central and northern Europe. This, combined with the possibility of hybridization between the harmful species B. xylophilus and the widely distributed but weakly pathogenic B. mucronatus, could result in new pathogenic races, as seen in Japan (6).

2: Fundamental conceptions of PWD development:

PWD is thought to be caused by the interaction of three factors, including the presence of: 1) a susceptible pine host whose death is a prerequisite for vector beetle oviposition, 2) specific fungi upon which the PWNs feed and multiply, and 3) certain mycorhizas, which together promote plant immunity and water uptake, both of which decrease host mortality while favouring PWD spread, according to various studies (7,8). Zhao (1,9,10) believes that a combination of these two factors, namely 1) B. xylophilus nematodes and 2) bacteria of the Pseudomonas fluorescens species group (these bacteria are ecto-symbyonts on the nematode's mucous body sheath), is required for PWD development. In an infected tree, as well as on pine callus tissue, nematodes and bacteria boost each other's multiplication. PWN proliferation is stimulated, DNA is destroyed, and pine phloem cells are killed by a protein that is similar to the flagellin of P. fluorescens. The mucous body sheath extract reduces the plant's immunological response and promotes Pseudomonas proliferation. When bacteria from B. xylophilus species are added to plant tissue cultures, axenic B. mucronatus nematodes (non-pathogenic species of the B. xylophilus group) become pathogenic, whereas surface-sterilized Bursaphelenchus nematodes do not cause wilt symptoms. Because dying trees attract the beetle that vectors the nematodes and associated ecto-symbiotic bacteria, tree death (i.e. death caused by PWD) is an essential condition for the PWN-Pseudomonas life cycle to be completed.

3 Pathogenicity:

Only the Japanese strain was harmful to Cedrous deodara in a pathogenicity test with two PWN isolates from China and Japan, although both isolates were pathogenic to Pinus thundergii and Pinus massoniana (11). Pinus k.comoraiensis and Larix olgensis seedlings were destroyed by the B. mucronatus isolate (BmRFE) from Pinus koraiensis (Russian Far East), however Pinus sylvestris and Pinus densiflora seedlings survived. Furthermore, only the French strain of B. mucronatus induced P. sylvestris to wilt out of two B. mucronatus isolates (12). Three B. mucronatus isolates from different parts of Russia were studied (Grant RFBR 100401644), and it was discovered that B. mucronatus (BmRFE) harboured the most phytotoxic bacterium (10).

The authors investigated (Fig 1) the possibility of invasive species B. xylophilus genetic introgression into native populations of B. mucronatus (BM) and its impact on pathogenicity (6). The F1 BM PWN males were backcrossed with virgin females from the parent populations of BM and PWN. The findings show that the virgin female is solely responsible for cytoplasmic heredity. In addition, investigations have shown that hybrids containing the nuclear PWN genome are more likely to be successful. Virulent to P. thunbergii and have a higher population in plant tissue, the nuclear BM hybrids outperformed the nuclear BM hybrids, whereas for the purposes of this study, cytoplasm heredity was irrelevant Pathogenicity. Two PWN isolates were used in different tests. Three inbred lines were used to create the inbred lines. For P. thunbergii, two were virulent and two were avirulent. The results revealed that the virulence features of all lines differed from each other and from the parent isolates (the gene). AFLP was used to discover pathogenicity indicators); On cultures, pathogenic lines had low multiplication rates. Botrytis is a fungus (13). Pathogenicity is determined by the nematode's ectosymbionts rather than the nematode's multiplication rate on the fungus. Also, if the inoculum consists of PWN cultivated for many generations on Botrytis or if pine seedlings are inoculated with transmissive PWN juveniles from beetles, wilt mortality rates are reduced (14).

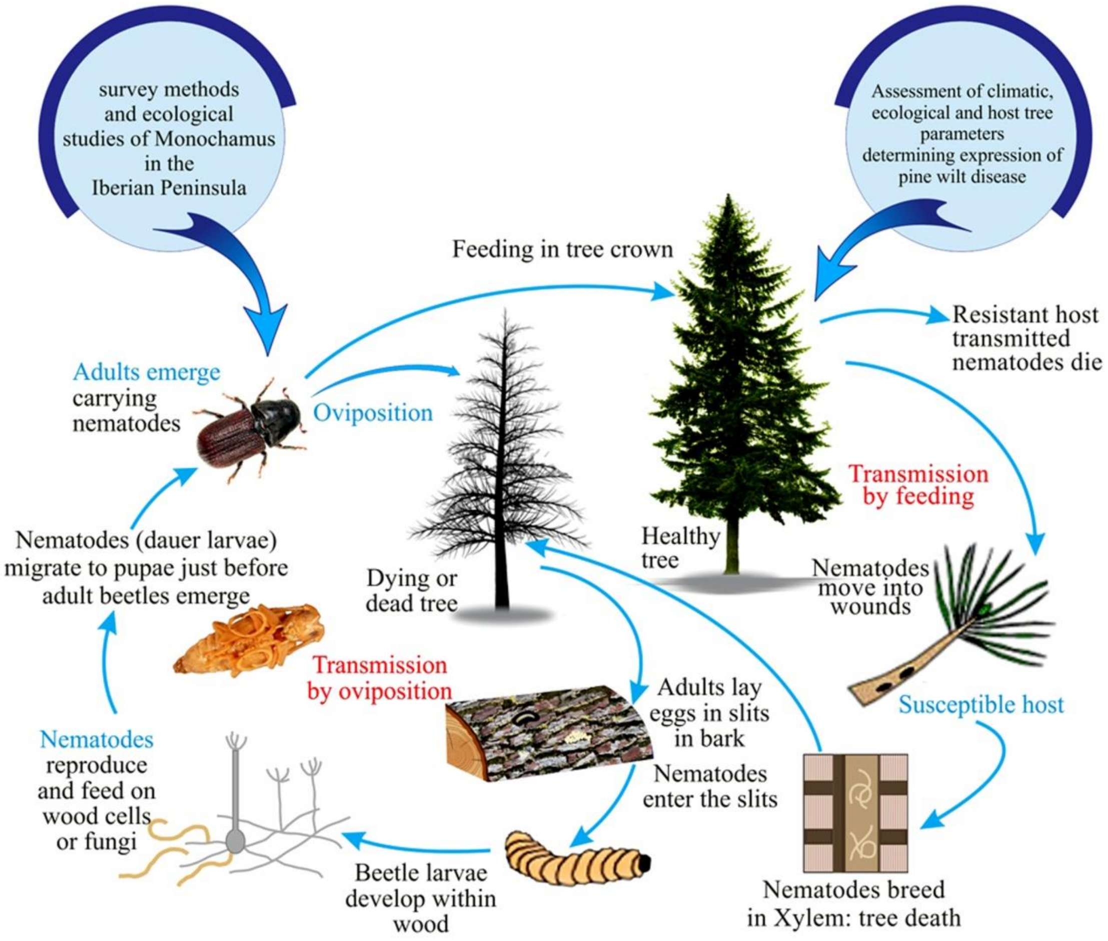

Fig 1. Pine wilt disease model showing assessment of climatic and ecological parameters expression under various environmental condition.

4. The histopathology of conifers and the significance of toxins from bacteria-nematode ectosymbionts in host-parasite relationships.

A histological analysis of 1-year P. thunbergii seedlings revealed that the nematode migrates slowly and only downward during the early stages of wilt disease development; however, as the population grows, nematode movement accelerates and becomes bidirectional (up and down). The cambium, then the cortex, phloem, and xylem parenchyma, show necrotic brown colour and tissue deterioration as the wilt progresses. In susceptible pine variants, this is followed by an initial increase in the water potential of the plant cells, which then decreases and then increases again; however, in susceptible pine varieties, all of these activities occur 2 days earlier than in resistant pine species (15). The quantity of PWNs and bacteria increases throughout PWD growth, particularly P. fluorescens, Pantoea sp., and Sphimgomenas pancimobilis (16).

PWN and bacteria have been linked in two ways, both of which are related to PWN presence in wood samples: Endophytic and true nematode bacterial partners (Actinobacteria and Firmicutes), and endophytic bacteria Janthinobacterium agaricidamnosum and Dyella yeojuens (17).

Such bacterial symbionts are connected to PWNs via a protein and carbohydrate-rich surface coating found on all parasitic and invasive stages of the PWN (18). The body surface of the worms must first be surface sterilised before examining the involvement of such bacteria in PWD pathogenicity. The following is a list of the efficacy of several sterilisation solutions, in order of effectiveness: > 0.1 percent mercuric chloride > 4 percent sodium hypochlorite > 3 percent hydrogen peroxide The optimal treatment time is 25 minutes at 25 degrees Celsius, which produces axenic nematodes. Longer nematode treatment times result in higher nematode mortality (19).

Using axenic callus tissue collected from mature P. thunbergii embryos, protocols have been established to assess the involvement of PWN and bacterium interactions in PWD formation. Such tissue is grown in the dark on a culture medium containing the required hormones. The cell browning response does not occur under these conditions (20). It is possible to test the harmful effects of bacterial secretions on pine cells using this approach. As a result, it has been demonstrated that a cell-free filtrate obtained from the growth of a P. fluorescens strain isolated from PWNs is multi-component, containing proteins and tiny organic compounds. The original non-dialyzed filtrate or the mixture of dialyzed components, rather than any single chemical, has the most harmful effect on P. thunbergii cell cultures (21). The P. fluorescens filtrates yielded a flagellin and two cyclic peptides, all of which cause the pine cells' DNA, nucleus, and cytoplasm to degrade, resulting in an increase in cell conductivity (22). The extracellular lignin peroxidase produced by P. fluorescens bacteria isolated from PWNs is involved in the biodegradation of lignin. P. thunbergii cell suspensions have been demonstrated to be toxic to this enzyme (23). Furthermore, the toxic effect of bacterial filtrates has been shown to be genetically determined, with culture filtrates of two Pseudomonas strains obtained using artificial mutagens being non-toxic to P. thunbergii cells (24).

5 The life cycle of PWN and how it is transmitted

Entomologists and nematologists from Europe and the Far East have recently discovered vital new information about the PWN-vector beetle relationship. Previously, only the connections between the PWN-Bursaphelenchus xylophilus and its beetle vector Monochamus alternatus in Asia and B. xylophilus and its vector M. caronensis in North America had been examined in depth. The beetle vector M. galloprovincialis, on the other hand, is quite abundant throughout southern Europe. On Pinus pinaster, it is a secondary xylobiont with a one-year life cycle. An investigation of the M. The transmission to the galloprovincialis cycle demonstrated that primarily occurs during the maturity of the conifer host tree six weeks after the adult beetle emerges) and During beetle oviposition, to a lesser extent (25). Juveniles in the transmission stage congregate in the beetle's metathorax once they've started, they’re in the pupal chambers, there was a relationship with a beetle. The skin of the mature callow beetle is extremely delicate (26). In a study conducted by South Korean academics in 2006, The beetle M. has a symbiotic relationship with the beetle M. pine and saltuarius Pinus koraiensis is a species of pine native to Korea. M. was found to be the winner. Saltuarius Beetles are vectors of PWN transmissive juveniles. Bursaphelenchus mucronatus and Bursaphelenchus mucronatus (European type) Han and colleagues (27). B. xylophilus is attracted to wood that has already been afflicted with nematodes, according to research on PWN migration. The fungal infection When compared to other fungus, Botrytis cinerea, which is used to raise PWN in the lab, is the most appealing. while the non-fractioned extract's attraction impact is bigger than any of its fractions (28). PWN transfer from roots to a 7-year-old pine (2009). seedlings have been observed and confirmed by PWNs are found in the roots of native forest plants. (29).

6. Models of Bursaphelenchus xylophilus dispersal;

At the Nanjing Symposium, several talks focused on simulation modelling of PWN's past spread and loss forecasts from PWD. For the evolution of the genus Bursaphelenchus, Ryss (2009) devised an allopatric model. According to the model, 1) the genus B. xylophilus arose in the north of the paleocontinent Pangea, 2) the B. xylophilus species group arose in East Asia, and 3) B. xylophilus arose in the American continent and was later returned to the initial area of its species group, namely East Asia, via an anthropogenic mode. The order Aphelenchida as a whole is thought to have originated in the Gondwana paleocontinent (as evidenced by the locations of the most basic taxa within the order), and that the aphelenchids then travelled north in Pangea. Anhydrobiotic and entomophilic stages evolved into various lines of aphelenchids throughout the historic spread to the north. PWN distribution models in China (30) are based on PWN occurrence records, particularly in economically important regions in the Yangtze and Pearl river drainage zones. The latter leads to the conclusion that PWD diffusion in China is primarily due to internal and international trade. For forest plantations in a group of Chinese provinces, mathematical prognostic models on PWN distribution were constructed. Elevation, annual precipitation, precipitation seasonality, annual temperature range, tree height, and crown diameter are all used in these models (31,32).

7 Associations:

A survey of the pines Pinus massoniana, Pinus thunbergii, Pinus elliottii, Pinus densifl ora, and Pinus armendii revealed that the PWN B. xylophilus co-inhabits with 10 other nematode species for the dominant species: B. mucronatus, B. aberrans, B. hofmanni, B. sp., Aphelenchoides macronucle(33). The Aphelenchoides nematodes from the Japanese black pine, P. thunbergii, are characterised as a new species with molecular and morphological differences from A. macronucleatus (34). The occurrence of morphologically and taxonomically identical aphelenchid species and genera highlights the need for molecular diagnostics for PWN.

8. Diagnostics:

Compared morphological and molecular data to determine Bursaphelenchus species identity, establishing 13 species groups based on six morphological characteristics and creating an illustrated tabular key using the most important morphological characteristics such as the number of lateral fields incisures, male caudal papillae patterns, and male spicule shapes.

Seventy-three of the nominal Bursaphelenchus species were successfully categorised, whereas twenty-four were not. For 39 species, a phylogenetic SSU r-DNA tree is provided (35).

Other presentations in Nanjing focused on the PWN's molecular diagnostics. In South Korea, 15 Bursaphelenchus isolates were analysed using PCRITS and D2D3 rDNA (sequencing and RFLP), resulting in the identification of Bursaphelenchus xylophilus, B. mucronatus (European subspecies on Pinus koraiensis and Asiatic subspecies on Pinus thunbergii), P. tusciae, P. lini, P. Because of the unique zipcode that hybridises on the carrier with oligonucleotides-primers and thus eliminates the accumulation of inhibitors of the PCR process (36) developed a padlock probe and HRCA technique to identify PWNs; the technique detection sensitivity was 10 times higher than that of conventional PCR. Because of its simplicity, a new diagnostic approach based on isothermal specific amplification of PWN DNA taken directly from tiny wood chips (37) is anticipated to become common in the future. Another presentation at the Nanjing meeting focused on another easy express approach for identifying the early stages of the PWN by revealing cellulase activity using a colour reagent. The premise for this approach is that dead wood containing B. mucronatus turns white, and real-time fluorescent molecular diagnostics (ATF-PCR ITS-2) are employed to validate PWN identification (38).

9: Control and quarantine of the PWN:

Quarantine methods contain a set of detection and control strategies for PWN, as well as approaches for specialist timber treatment. PWD is a standard process used in quarantine work. Epidemiological surveys, PWN diagnostics, and beetle plotting traps, vertical wood traps, and the disposal of dying animal’s branches on damaged trees or dead and dying trees, nematode-infested lumber fumigation, and biological control command (39). Using aerial photography is also a viable option. Near-infrared colour film photography enables for PWD-affected plants can be identified by their leaves. discolouration. The original recognises the latter. Infected trees can be recognised using GIS software. using a palmtop computer with a built-in camera Receiver for GPS (40).

The European Union's (EU) action plan for PWD calls for the creation of a three-kilometer-wide clear-cut zone encircled by a six-kilometer quarantine zone surrounding the affected area. Only after rigorous treatment and control can logs from the quarantine zone be transported out of the zone. In addition, the quarantine zone must be maintained for a period of 15 years following the last PWN report (41). Cutting, burning, or fumigation of wood, limits on transporting wood from infested areas, and a mandatory 3-year control in areas where the

PWN has been identified before the territory can be proclaimed a "clean area" are all part of South Korea's post-PWD management plans after detection (41). Heat treatment, methylbromide fumigation, and removal and burning of infected trees are the three forms of quarantine treatments used in the EU. Because to methyl bromide limits (due to environmental concerns), three alternative chemicals are now recommended: the fumigant sulphur fluoride, the trunk-injection drugs Milbemectin, and Emamectine benzoate (42). For wood goods and wood packaging material destined for international trade, the International Plant Protection Convention (IPPC) mandates a 30-minute heat treatment at 56°C (wood core temperature) (43). Because both conventional and real-time PCR rely on the detection of DNA, they are unable to distinguish between dead and live PWNs. As a result, a new reverse transcriptase PCR assay based on the utilisation of the heat shock protein 70 A mRNA as a viability marker has been created to evaluate the heat treatment efficiency. This marker degrades rapidly in dead PWNs in comparison to stable DNA (44).

10. The PWN's Biocontrol:

The vector beetles, PWNs, or their ectosymbiotic bacteria can all be targets for biocontrol. The PWN vector Monochamus alternatus has been infected with a pathogenic strain of the entomophilic fungus Metarhizium, which was originally obtained from the ichneumon Scleroderma. Mass release of the fungus carried on laboratory reared Scleroderma provides effective biocontrol in areas with severe PWD (46). Under field conditions in China the release of \sScleroderma guani (natural enemy of Monochamus \sbeetles) near PWD-affected trees leads to a 20 percent parasitism rate of the vector beetle M. alternatus (47). The regular release of radiation-treated Monochamus alternatus males into PWN-infested forests can effectively reduce the number of beetles; for example, after six years of regular release, the results are comparable to those obtained using chemical control against Monochamus, but at a much lower cost (48). In addition, wood-rotting fungus and certain plant compounds can be employed to combat the PWN. A search was conducted in China for a fungus that effectively decomposes the stumps of PWD-killed Pinus massoniana trees. One stipulation for such fungi is that they cannot be used as food by the PWN. For testing, a strain of the fungus Laetiporus sulphureus was chosen (49). The fraction with the highest antinematode activity was isolated by high-speed countercurrent chromatography (HSCCC) from five strains of the actinomycete Streptomyces with nematicide filtrates (50). In South Korea, the hypomycete Esteya vermicola is the first endoparasitic fungus recovered from fungus-infected PWN. The fungus's sticky lunate conidia connect to the nematode cuticle during its life cycle, and the mycelia penetrate and eat the nematode's body. During the next infection cycle, Esteya vermicola produces fresh conidia, which infect intact nematodes. Several aggressive Esteya strains were isolated, all of which killed PWN isolates in 4 to 5 days (51). Using a commercial biocontrol agent containing Esteya strains against the PWN and several application methods (suspension spraying, trunk injection into trees, and applying infected nematodes onto trees), researchers found that E. vermicola could help pine trees survive PWN infection in greenhouse and field tests (52). To manage PWD in Portugal, oil components from several aromatic plants were studied, and four of 27 oils were identified as anti-PWN. At 1 mg mL, their nematicidal activity was Thymbra capitata > Thymus caespititius > Satureja montana and > Cymbopogum citratus in order of efficiency. Carvacrol and geranial were discovered to be nematicidal compounds (53). The strong nematicidal activity of Cynanchum komarovii extracts has been determined in China, and the activity has been linked to succinate dehydrogenase inhibition in the PWN (54).

The strong efficacy of oxolinic acid against five bacterial strains isolated from the PWN has been identified in laboratory experiments employing six antibiotics. After injecting 3-mg oxolinic acid into 3-year-old seedlings, pine wilt symptoms in P. densiflora decreased by 70%. A mixture of oxolinic acid and the nematicidal agent abamectin showed better disease control of PWD in field experiments with 20-year-old pines than either oxolinic acid or abamectin alone (55).

PWD control has been proposed in Hunan Province, China, using local P. densiflora seedlings.

The second generation of resistant and susceptible pine clones hybrids that survived the experiments show increased resistance to PWN and a significant reduction in PWN-induced mortality.

The International Symposium in Nanjing outlined the essential concepts of parasite-caused conifer wilt, including newly collected knowledge on the function of symbionts and PWN vectors in the disease's pathogenesis and transmission. Biocontrol and contemporary quarantine tactics are examples of new technology tools in diagnostics and pest control.

4. Conclusion

We have successfully developed a numerical scheme known as (NSFDM) for the consider model. Through the mentioned method, we have computed the solution of the proposed model for different values of n, m. Hence (NSFDM) will excellently use in future for dealing non complicated problems.

Author contribution

Mr. Abdul Ghaffar Khan, conducted this experiment, collected the data, analyze the data and wrote the manuscript, while Dr. Imran revised and reviewed the manuscript. Dr. Tariq Mahmood and Dr. Hayat Zada identified disease and pathogens of the pine wilt study under various climatic conditions.

Conflict of interest

The authors declared that they have no any interest of conflict in publishing these findings.

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