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The Occurrence and Comprehensive Control Technology of Pine Wilt Disease

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Abstract: Pine wilt disease is known as one of the most harmful forestry diseases in the world. It is called the "cancer" of pine trees and seriously threatens forest resources and ecological environment. Economic globalization and regional economic integration have intensified the spread of exotic pests around the world. Therefore, it is of great significance to strengthen the prevention and control of pine wilt disease. This article introduces the pathogens and pathogenesis characteristics of pine wilt disease, summarizes the strategies adopted by Japan, the United States, South Korea and other countries to prevent and control pine wilt disease, and proposes specific comprehensive prevention and control technologies for pine wilt disease from the aspects of quarantine treatment, chemical control, biological control, and physical control. The management of pine wilt disease is a systematic project, which requires the cooperation of multiple measures and the implementation of comprehensive prevention and control strategies. This study is expected to provide a reference for the sustainable development of forest resources and the effective prevention and control of pine wilt disease.

Keywords: pine wilt disease,pine wood nematode,disease characteristics, control technology

Introduction

Pine wilt disease is a worldwide forestry disease caused by the pine wood nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle. It can cause devastating damage to pine forests. It has the characteristics of rapid spread, wide range of harm, rapid onset, and difficulty in prevention and treatment[1]. It has received great attention from countries around the world and has been legislated as a key quarantine object by more than 40 countries[2].

Pine wood nematode [*Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, 1970] was first isolated from longleaf pine (*Pinus palustris*) in Texas, USA in 1929 [3]. In 1972, it was confirmed that it causes pine wilt disease[4]. Pine wood nematode originated in North America and is commonly found in coniferous trees in the United States, Canada and other countries. However, local pine wilt disease only occurs sporadically and causes little damage [5,6]. When pine wood nematode was introduced to Asian and European countries through the timber trade, it caused huge harm due to changes in conditions such as hosts, vector insects, environment and natural enemies, and even symbiotic bacteria and fungi species. Countries where pine wood nematode occurrence has been clearly reported include the United States, Canada, Mexico, Japan, South Korea, Spain, Portugal, and China [7]. Economic globalization and regional economic integration have intensified the spread of exotic pests around the world, and global warming has intensified the spread speed and scope of pine wood nematode.

Pine trees infected with pine wood nematode are difficult to cure, and the mortality rate is extremely high. It is called "pine cancer". Pine wilt disease spreads very quickly, and can achieve large-scale spread and wide-area spread only through short-term accumulation. The density and proportion of pine trees are also positively related to the incidence trend and spread speed of pine wilt disease. In addition, the mortality rate of pine wilt disease is relatively high. After pine trees are infected with pine wood nematode, there are no symptoms in the early stage and they are in the incubation period, making it difficult to detect. Once an outbreak occurs, pine trees will wither in a short period of time. Therefore, understanding the etiology, symptoms and hazards of pine wilt disease, in-depth analysis and discussion of pine wilt disease prevention and control methods, and then taking necessary prevention and control methods and control countermeasures are of great practical significance for protecting the sustainable development of forestry.

Literature Review

Countries that are infested by pine wood nematode have adopted a variety of strategies and measures to control pine wilt disease. From a global perspective, countries and regions where pine wood nematode disease occurs seriously and require focused management are mainly concentrated in Asia and Europe, including China, Japan, South Korea, Portugal, etc.[8]. The management of pine wilt disease in these countries has its own characteristics and has made significant progress in many aspects.

Japan discovered pine wilt disease in 1905 and was the first country to be infested by pine wood nematode [9]. It was also the first country to carry out prevention and control. The prevention and control strategies for pine wood nematode are relatively complete and advanced. At present, Japan has three basic strategies to control pine wilt disease: (1)

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Reduce the pathogen - pine wood nematode. (2) Isolate the pathogenic pine wood nematode and healthy pine trees, mainly by controlling the transmission vector. (3) Improve the resistance of pine trees. These basic strategies are the basis for guiding various prevention and control measures in Japan.

Pine wood nematode invaded South Korea in 1988, causing the death of a large number of pine forests in South Korea. South Korea's pine wood nematode prevention and control strategy emphasizes continuous monitoring of pine forests to understand the health status of the forest; it emphasizes the detection of dead wood to achieve early detection of diseased plants; it emphasizes cutting down diseased plants to reduce the source of transmission; it emphasizes legal management and prohibits the transportation of infected wood. .

The United States is the origin of pine wood nematode, which was first reported in 1934. It was not until 1979 that it was reported that pine wood nematode can induce pine wood nematode disease in non-native pine trees in Missouri. The transmission vector of pine wood nematode in the United States is *Monochamus carolinensis*[10]. The most effective prevention strategy is to avoid planting non-native pine trees. Where non-native pine trees are already present, landowners can reduce susceptibility to exotic pine trees by increasing resistance.

So far, no pine wood nematode epidemic has been found in Canada [11]. Low temperature and highly resistant or even immune hosts are the reasons why pine wood nematode cannot cause disasters.

In Europe, pine wilt disease has occurred in two countries, namely Portugal (as early as 1999) and Spain (as early as 2011). The transmission vector in Europe is *Monochamu galloprovincialis*[12]. So far, Spain has exclusively utilized local eradication strategies. The most recent one was the detection of a dead pine tree infected with pine wood nematode in Lagunilla (Salamanca province, Castilla y Léon) in 2018, and an eradication strategy was immediately adopted, which was in line with the European Mediterranean Organization's pine wilt disease protocol[13]. Once discovered, all host trees within 50m of the susceptible trees will be cut down directly, and a 5km intensive investigation area and a 20km radius buffer zone will be established. In Portugal, pine wilt disease has spread to many places, and it is impossible to use a local eradication strategy. The strategy adopted is a regional approach. First, accurately delineate the pine wood nematode epidemic area through surveys to prevent the spread of the pathogen carried by vectors. The affected areas mainly adopt measures such as epidemic surveillance and census, cutting down and burning infected pine trees, preventing vector insects, and strictly controlling pine afforestation. The areas of infected areas and buffer zones are adjusted every year based on the occurrence of the epidemic. The second step is to establish clear-cutting zones and buffer zones. To avoid the spread of adult beetles from affected areas, it is necessary to develop strategies based on their flight behavior and the establishment of effective barriers. These barriers can be achieved through the use of traps, removal of suitable host trees, or through preventive chemical treatments. The size of these barriers depends on the distance the insect-carrying nematodes are able to fly. A 4km wide clear-cutting zone and a 20km buffer zone were established outside the epidemic area.

The strategies adopted by Japan, the United States, South Korea and other countries to prevent and control pine wilt disease mainly include enacting laws, rational management and protection, strengthening quarantine, and establishing buffer zones. The management of pine wilt disease is a systematic project that requires the cooperation of multiple measures. No country can achieve the goal of eradication by relying on only one measure. And no matter what kind of measure it is, it will be effective only if it is implemented properly. If we want to prevent and control pine wilt disease, we must not only prevent it from the transmission route, but also start from the causes and patterns of the disease, and introduce effective methods to prevent and treat it.

The Occurrence and Harm of Pine Wilt Disease

Characteristics of pine wilt disease:

Pine wilt disease consists of four factors: pine trees, pine wood nematodes, vector insects and suitable environmental conditions. Under these four factors, the disease forms and becomes prevalent. After pine wood nematode infects plants through wounds caused by feeding of vector insects, it reproduces in large numbers in the tree, causing blockage of resin canals [14]. A large amount of toxins excreted by nematodes during their growth accumulate in the tree, causing the tree to lose water, the wood to become lighter, and resin secretion to reduce or even stop.

Symptoms of pine wilt disease can be roughly divided into the following four stages: (1) Normal appearance, resin secretion decreases or stops, and transpiration decreases; (2) Needles gradually change color, resin secretion stops, and beetles can be observed. Damage and signs of egg laying; (3) Most of the needles turn light brown and wilted, and vector insect debris can be seen; (4) The needles are all yellowish brown or reddish brown and hang downward, and the entire diseased tree dies. The tree body is inhabited by a variety of secondary pests, and blue discoloration is often seen in the xylem of dead pine wood. Pine wilt disease generally causes symptoms of needle wilting and discoloration 15 to 30 days after the pathogenic nematode infects pine trees, and death occurs 30 to 45 days after external symptoms appear.

Vector insects:

In the natural environment, pine wood nematode generally cannot spread to other host plants through its own movement to cause disease. It mainly relies on the introduction of wounds caused by vector insects. Previous studies have shown [15] that insects of the genus *Monochamus* are the main vectors of pine wood nematode. In Asia, the main vector insect of pine wilt disease is *Monochamus alternatus*. The number of nematodes carried on the body surface of

adult beetles is mostly thousands or more, and can reach hundreds of thousands at most. *Monochamus alternatus* has nutritional supplement properties and is extremely efficient and harmful in transmitting diseases.
The pine wood nematode invades the tree through *Monochamus alternatus*, causing tree weakness and death. When the tree is weak, the *Monochamus alternatus* will carve grooves on the tree to lay eggs to breed offspring. In May and June of the next year, the pine wood nematode escapes from the diseased trees by the mature pine beetle after eclosion and spreads to other healthy trees, causing the disease to spread. Therefore, vector insects such as *Monochamus alternatus* can not only carry pine wood nematodes, but also transmit them to host plants, causing the disease.

The pattern of disease occurrenc:

There are some variations in the pine wood nematode infection cycle in different countries and regions, but the overall cycle is similar. Every spring, beetles emerge from the pine trees that died of disease the previous year and carry the pine wood nematodes in the dead trees. After *Monochamus alternatus*, the main vector insect in East Asia, emerges, it first searches for healthy pine trees around it and supplements nutrients on its young branches. When beetles feed on bark, they cause wounds on pine trees. The pine wood nematodes carried on the body surface are transferred to healthy pine trees and invade the host twigs from the wounds. Pine wood nematode grows and reproduces rapidly in pine trees. It reproduces for one generation in 4 to 5 days. Each female adult can lay about 80 eggs.
There are two developmental types in the life cycle of pine wood nematode. The first type is called the reproductive type. The nematode mainly grows in the body of pine trees, including eggs, 1st to 4th instar larvae and adults. The 1st instar occurs in the egg. The newly hatched larvae are in the 2nd instar stage. They grow rapidly through the 3rd and 4th instar stages at a suitable temperature. The life cycle from egg to adult usually takes 4 to 5 days to complete. Reproductive nematodes continue to reproduce and migrate in the vascular tissue and resin canals of healthy pine trees, and can cause the death of the entire pine tree in as quickly as 40 to 50 days. The second type is the spreading type, which includes 3rd to 4th instar spreading larvae. Pine wood nematode overwinters as 3rd instar spreading larvae in diseased and dead pine trees, and develops into 4th instar spreading larvae in the early spring of the next year. The 4th instar spreading larvae transfers and adheres to the body surface of the adult beetle, flies out as the adult beetle emerges, and invades healthy pine trees[16].
Since the optimal temperature for the growth and reproduction of pine wood nematode is 20°C, reproduction is inhibited or even stopped when it is lower than 10°C or higher than 28°C, and the disease is relatively serious when the soil moisture content is low. Therefore, the high temperature and dry climate are more suitable for the occurrence and spread of the disease.

Comprehensive Control Technology for Pine Wilt Disease

Inspection and quarantine treatment:

Establishing complete quarantine and control measures is the first line of defense in preventing and controlling pine wilt disease. Timber and its products from epidemic areas must be heat treated at 60°C or treated with nematicides before use or entry or exit. Once pine wood and its products are found to be infected with *Monochamus alternatus* or pine wood nematode during quarantine, they should be fumigated with methyl bromide immediately, or soaked in water for more than 5 months, or sent to the factory for treatment immediately to eliminate the artificial spread of *Monochamus alternatu*s and pine wilt disease[17]. The management and utilization of infected wood should be strictly implemented in accordance with the "Measures for the Management of Pine Nematode Infected Areas and Infected Wood". While adhering to eradication standards, zoning policies should be adopted to ensure the ecological security of key areas [18].
In the daily silviculture management of forest areas, we must also strictly pay attention to the treatment of diseased and dead wood in the forest area, and carry out centralized fumigation and burning of diseased wood. The number of diseased and dead trees is small and scattered, and when the dead tree rate is less than 10%, comprehensive cleaning measures should be adopted. When the occurrence area is large and the disease-killed plant rate is more than 10%, a one-time clear-cutting method should be used to completely eliminate the susceptible hosts in the diseased forest land. When cutting down dead pine trees, weak and disease-sensitive trees in the forest stand should be cleared, and all pine branches should be burned and cleaned.

Chemical control:

Currently, the main vector insect known for pine wilt disease is *Monochamus alternatus*. The prevention, control and eradication of vector insects such as *Monochamus alternatus* are the main means to eliminate and control the damage of pine wood nematode. During the emergence period of *Monochamus alternatus*, if the pine trees are treated with aldicarb once, the plant preservation rate can reach 100% in that year [17]. During the peak emergence period of beetles, two ultra-low-volume sprays(2% thiacloprid suspension) were carried out by airplane, which also had more than 90% control effect on adult beetles [19]. Forest spraying of fenthion, which has strong contact killing, stomach poisoning and penetrating properties, can also play a good control role [20]. Every year before October, fenitrothion emulsion (oil) agent (dosage 400-600 mL/m^2) is sprayed on the surface of the damaged trees. Basically, all beetle larvae in the trees are spared [21]. In addition, agents such as prothion, fenitrothion, MEP, and MP, and repellents such as eucalyptus essential oil can also be used for the control of pine wilt disease.

Biological control:
The rational use of biological control measures can eliminate vector insects while maintaining ecological balance, such as releasing *Beauveria bassiana* and *Scleroderma guani* in the forest[22]. During the young stage of the larvae of *Monochamus alternatus*, the natural enemy of the pine beetle, *Scleroderma guani*, is released in the forest. *Scleroderma guani* can also infect *Monochamus alternatus* larvae by carrying *Beauveria bassiana*. Natural enemies are released every year during the larval stage of *Monochamus alternatus*. It is best to do so on a sunny day with a temperature above 25°C. The releasing method of *Scleroderma guani* adopts single plant bee releasing method, center bee releasing method or divided bee releasing method. One bee releasing point is set up every 6.66 hm^2, and about 10,000 bees are released at each point.
Nematode-predating fungi are natural enemies of predatory nematodes that are widely distributed in nature. Spores of endoparasitic fungi can invade the nematode body when they germinate, reproduce and kill nematodes. More than 90 species of nematocidal toxin-producing fungi have been discovered, and researchers used *Esteya vermicola*, *Trichoderma sp.* and *Arthrobotrys sp.* and other fungi have conducted a large number of research on the prevention and treatment of pine wilt disease and achieved good experimental results. Compared with pathogenic fungi, research on the application of bacteria to control plant parasitic nematodes has also made great progress. *Bacillus thuringiensis* and others have shown strong poisoning effects on pine wood nematode; the new biocontrol bacterium *Stenotrophomnas maltophilia* also has the potential to control pine wood nematode.

Physical control:
Applying *Monochamus alternatus* attractants and setting up luring trees in the forest can trap and induce *Monochamus alternatus* to lay eggs and effectively control the number of *Monochamus alternatus*[23], reducing the spread and occurrence of pine wilt disease. In areas where the *Monochamus alternatus* has serious occurrences, special traps can be installed for prevention and control. You can also choose slender medium-diameter living trees for luring in airy places such as forest ridges or forest roads during the early stages of beetle emergence. However, the trapped beetles and luring trees need to undergo strict pest control treatment [24].
For epidemic forest farms with gentle mountains, when clearing infected trees in winter, the felled infected trees and branches can be promptly gathered in open areas or sheltered recesses. You can choose windless or breezy weather, and burn them uniformly while ensuring that fires are avoided. For epidemic forest farms with high and steep mountains, it is relatively difficult to transport infected wood down the mountain for burning. 8# wire mesh can be used to cover the infected wood to deal with it. Aluminum phosphide bagging and fumigation is also a relatively economical method, but this method becomes less effective when the minimum temperature is below 10°C. It is recommended to use it from May to November when the temperature is suitable [25]. In addition, slicing and sawing boards, water immersion method, light trapping method, drying method, water immersion of dead wood, etc. can also be used for the physical control of pine wood nematode.

Conclusion
It is of great significance to carry prevention and control analysis of pine wood nematode. The management of pine wilt disease is a systematic project that involves many factors. Attention must be paid to all processes and aspects of prevention, control and management. It requires the cooperation of multiple measures. No country can achieve the goal of radical cure by relying on only one measure. According to the characteristics of pine wood nematode infection, three key links should be paid attention to in the current disease prevention and control work, namely disease quarantine and epidemic monitoring, infected wood treatment and vector insect control. For some areas with more complex epidemics, measures such as clearing infected trees, aircraft spraying, tree trunk injections and biological control can be combined. There is an urgent need to further study the transmission pathways and pathogenic mechanisms of pine wilt disease, and formulate effective comprehensive prevention and control strategies to control the spread of pine wood nematode by *Monochamus alternatus*, reduce the damage to pine trees and prevent the further spread of pine wilt disease.
In addition, all measures to prevent and control pine wood nematode require manpower and material resources, and control will inevitably require high costs. Moreover, pine wilt disease cannot be eradicated in most cases, and when it has spread widely. Long-term containment strategies should be adopted. All regions should attach great importance to the prevention and control of pine wilt disease, combine local environmental characteristics and climate characteristics, pay attention to the prevention and control of pine wood nematodes, and flexibly adopt a variety of methods to effectively improve the efficiency of nematode prevention and control, and provide a safe environment for the growth of pine trees.

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