Agricultural Science & Technology, 2017, 18(2): 332–343




# Theory and Practice of Controlling and Extinguishing Pine Wilt Disease

**Yanxue LAI***

Ningbo Forest Pest Control and Quarantine Station, Zhejiang 315012, China

**Abstract** **[Objective]** This study was aimed to review the controlling experience of pine wilt disease in the past 25 years, explore the theories and methods of controlling pine wilt disease, and improve the scientific level of controlling techniques and the protection capacity of healthy pine trees. **[Method]** Eleven items of effects were used to refine the theory of clearing dead pine trees affected by pine wilt disease, namely, "1 priority", "2 objections", "3 principles", "4 measures", and "5 managements". On the basis of comprehensive control and complete removal of the infected pine trees, a variety of comprehensive and efficient controlling methods were developed to carry out targeted chemical ecology trapping, bionic pesticide killing and releasing natural enemies of *Sclerodermus guani*, *Dastarcus helophoroides*. High efficient emamectin benzoate immune injection was developed to inject the healthy pine trees for prevention, so as to extinguish the pine wilt disease. **[Result]** The pine wilt disease dropped from the peak of 3.5 million dead trees with an infecting area of 28 273 hectares in 1999 to 0.068 million with an area of 4 333 hectares in 2012 gradually, reducing by 98.06% in number and 84.84% in area, respectively. On the basis of removal, *Dastarcus helophoroides* was also released, which could make the number of dead pines decrease more significantly than the control, and after releasing for 5 consecutive years, the dead pine trees dropped to 0.511 plant/hm$^2$ in 2012, with a mortality rate of 0.022 7%, which achieved the control effect, reaching extremely significant level. "Forest land removal+infected trees isolation+natural enemy release" could extinguish the pine wilt disease. The test of isolating 24 heaps of infected pine trees showed that there were 9 heaps of pine trees extinguished the pine wilt disease, which controlled the occurrence of pine wilt disease for 100%, accounting for 37.5% of the total, in which the number of those isolated using iron netting and nylon net were 4 for each, accounting for 88.9%, and there was one heap using polypropylene net, accounting for 11.1%. The invention of emamectin benzoate immune injection laid the foundation for extinguishing pine wilt disease. The follow checking of the effects of emamectin benzoate immune injection on pine wilt disease found that the number of dead trees caused by pine wilt disease decreased significantly after injecting, and became very small in October of the next year, and the disease was completely extinguished in the third year. **[Conclusion]** Pine wilt disease could be controlled and extinguished with positive control by using "comprehensive cleaning+industrialized removal", "comprehensive cleaning+natural enemy release", "comprehensive cleaning+infected trees isolation+natural enemy release" and "comprehensive cleaning+emamectin benzoate immune".

**Key words** Pine wilt disease; Pine wood resources; Control; Extinguish

There is no need to give unnecessary details of the harmfulness of pine wilt disease and the importance of pine resources. It is imperative to formulate countermeasures and take measures to control the increasingly serious harm caused by pine wilt disease. However, pine wilt disease was considered incurable, even known as the "cancer" or "AIDS" for pine, causing a lot of damage. And the damaged area saw a large number of pines dying of wilt, severely destroying the stability of forest ecological system, the magnificence of pine natural scenery and the development of human's economic life, but there is a lack of effective countermeasures and control methods. Pine wilt disease was first found

Supported by the Scientific Research Project of National Level of YANG Zhongqi of Chinese Academy of Forestry (2012AA101503).

* Corresponding author. E-mail: 87169312@163.com

Received: December 9, 2016 Accepted: January 25, 2017

## 松材线虫病控制与扑灭的理论与实践

来燕学* （浙江省宁波市森林病虫防治检疫站，浙江宁波 315012）

**摘 要** [目的]回顾25年松材线虫病发生防治经历，探索松材线虫病防控理论与方法，提高防控科学技术水平与健康松树保护能力。[方法]用"双松效应"和"分担效应"等11项效应，提炼"1优先"、"2反对"、"3原则"、"4措施"、"5管理"松材线虫病死树清除理论，在全面清理与彻底除害松疫木的基础上，开发多种简便有效的综合防治方法，对带疫松褐天牛作针对性的化学生态诱杀、仿生农药扑杀和释放天敌昆虫管氏肿腿蜂、花绒寄甲寄生或捕杀；开发高效甲维盐免疫针剂对健康松树注射预防，扑灭松材线虫病危害。[结果]松材线虫病从1999年28 273公顷350万株病死树的高峰期，逐年下降到2012年的4 333公顷6.8万株病树最低期，分别下降87.84%与98.06%；在清理基础上，同时施放花绒寄甲，能使枯死松材数量比对照大幅度下降，连续施放5年，松林平枯死量到2012年降到0.511株/hm$^{-2}$，枯死率为0.022 7%，达到控制效果，达极显著水平；林地清理+疫木隔离+天敌释放，能达到扑灭松材线虫病。24堆松疫木隔离试验结果：扑灭松材线虫病，即100%遏制松材线虫病发生的，有9堆，占37.5%，其中铁丝网、尼龙网各4堆，占88.9%，编织袋1堆，占11.1%；甲维盐免疫针剂的发明为扑灭松材线虫病奠定了基础。2008年和2009年对北仑区甲维盐注射扑灭松材线虫病的效果作跟踪检查，发现注射当年的松材线虫病死树大幅减少；次年10月接近扑灭，第三年彻底扑灭了松材线虫病。[结论]松材线虫病只要积极防治，采用"全面清理+工业化化除害"，"全面清理+天敌昆虫"，"全面清理+疫木隔离+天敌昆虫"，"全面清理+甲维盐免疫"，完全有可能达到控制与扑灭目标。

**关键词** 松材线虫病；松林资源；控制；扑灭

**基金项目** 中国林科院杨忠岐先生的国家级科研项目(2012AA101503)。

**作者简介** 来燕学(1956–)，男，浙江宁波人，教授级高级工程师，主要从事森林病虫害防治方面研究，E-mail：87169312@163.com。* 通讯作者。

**收稿日期** 2016-12-09

**修回日期** 2017-01-25

in Ningbo, Zhejiang Province in August, 1991[1]. In September 27, 1991, the Ministry of Forestry issued the "Emergency Notice on Blocking the Pine Wilt Disease in Xiangshan County", putting forward the guideline of "blocking, controlling, extinguishing"; on October 7, the Ningbo Municipal People's Government Office forwarded the "Notice on the Rapid Control of the Spread and Diffusion of Pine Wilt Disease" from the Forestry Department of Ningbo City, and on October 18, the Ningbo Municipal People's Government invited related leaders, specialists and experts to make discussion on the "Emergency Control Scheme for the Pine Wilt Disease in Xiangshan County", putting forward the management strategy of "outside first then inside, lightly infected area first then severely". Since then, Ningbo City opened the practice of controlling pine wilt disease.

The time when Ningbo saw the occurrence of pine wilt disease was the 20th anniversary of the finding of pine wilt disease in Japan. At that time, it was generally believed that pine wilt disease, like cancer, was incurable, and pine tree was doomed to die out. Such kind of thinking seriously affected the control of pine wilt disease. Because of such thinking, the promoted controlling methods were "clear cutting" for "better to kill all than to miss one"; transforming the pinewood, and changing to plant non-host species; or no cutting but blocking the infected area to make the pine go through natural selection. The practical results of such thinking were that the pine wilt disease was failed to be controlled and extinguished, the harm became more and more severe, and the covering area became larger and larger. Therefore, we believed that the ideas and methods of "outside first then inside, lightly infected area first then severely", "clear cutting", and cutting off the pine and building the isolation belt failed to purposefully eliminate the effective strength of the spread and diffusion of pine wilt disease, the vector insects——*Monochamus alternatus* Hope, and the plant parasitic nematode——*Bursaphelenchus xylophilus*, getting half the result with twice the effort[2-5].

Through the long-term personal experience in practice, I realized that it should break through the traditional thinking, make breakthrough in scientific technology, and combine with management innovation and long-term unremittingly efforts, so as to form a new controlling idea, to solve the conflict in the game relationship, to completely control or extinguish the pine wilt disease. To this end, in 25 years, I made long-term tracking research on the occurrence and epidemic regularity of pine wilt disease, made multilayer innovations and development in multiple aspects for the control technology, and achieved the innovation and breakthrough in the controlling theory and technology of pine wilt disease, creating the controlling effects of overall control and partial distinguishing pine wilt disease. This paper focused on the control and extinguishing techniques, in order to provide some theoretical basis.

## Theory and Methods

### Theoretical exploration

Eleven items of effects, namely, "Two pines effect", "Sharing effect", "Retention effect", "Core effect", "Schedule effect", "Spot spreading effect", "Surface spreading effect", "Transferred increase effect", "Mutagenic effect", "Syndrome effect", "Assets effect", were the theoretical basis to guide the control and extinguishment of pine wilt disease[2-5].

Two pines effect refers to narrow the whole pinewood down to 2 pine trees-Pine A and Pine B. When Pine A dies from the infection of pine wilt disease, what measures should be taken to protect the only Pine B? There are 4 combinations of strategies to be selected: one, clearing Pine A and retaining Pine B; two, clearing Pine A and clearing Pine B, or clear cutting; three, retaining Pine A and retaining Pine B, or giving up the saving; four, retaining Pine A and clearing Pine B, or transforming the pinewood in advance. The first strategy proved to right, and could achieve the desired results, while the other 3 strategies failed after the trial[4].

Sharing effect refers to the competition between the number of pines affected with *M. alternatus* and the number of pines. When the number of pine is a constant, the occurrence amount of pine wilt disease is in direct proportion to the number of introduced *M. alternatus*; when the number of *M. alternatus* is a constant, the occurrence amount of pine wilt disease is inversely proportional to the number of pines. If there are 100 *M. alternatus* to invade to a pinewood with 10 000 pine trees, and there are 100 rare pine trees listed in the key protection list in this pinewood, the strategies that can be taken faced with the invasion of pine wilt disease are as follows: one is to cut down the 9 900 non-protected pine trees in advance, to form an isolation belt with no pines; two is to protect the 10 000 pine trees, with no one cutting down, and then deal with the invasion according to the "Two pines effect" after the invasion. The latter strategy should be taken. The reason is that the introduction of *M. alternatus* could only lead to 100 pine trees dying from affecting the wilt disease at most, in which there might be 1 rare pine tree. However, if cutting down 9 900 pine trees, then the 100 rare pine trees are in the bare state, which is difficult to protect[2].

Retention effect refers to the control strategies taken when pine wilt disease has occurred in Pinewood A, and Pinewood B and c are under the stress of Pinewood A: one is to clear Pinewood A completely; two is to remove all the withered pine trees in Pinewood A, and retain all the living pines. The second strategy is right, because neither clear cutting nor removing cannot distinguish *M. alternatus* and *B. xylophilus* for 100% . The remaining pathogens and vector insects will do harm to the retained living pines in the following year through diet supplement after emergence. And it is impossible for them to migrate and flight away because of no food, making the pine wilt disease retain in the original place of occurrence[2].

Core effect refers to control strategy of "light first and severe latter" or "severe first and light later" after the invasion of pine wilt disease for many years, when the number of withered pines forms the distribution infection state of "light, medium and severe".

According to the theory of the cardinal number of pathogens determining the occurrence amount, the only measure to be taken is the strategy of severe first and light later. The reason is that in the area with severe infection of pine wilt disease, the number of withered pines is large, so is the number of stored vector insects and nematodes, making it the base for the occurrence and spread of pine wilt disease, and it can achieve the effects of half the work with double results by focusing on clearing the occurrence base[2, 4–5].

Spot spreading effect refers to the occurrence effect of pine wilt disease caused by the first invasion of *M. alternatus* of small numbers into healthy pines. Spot spreading effect usually takes place in the area far from the occurrence area of pine wilt disease, where there are usually few plants died from the affection at the beginning of the occurrence, and it is caused by natural spreading and human transmission. Human transmission refers to the spreading caused by the transportation of pine products without quarantine by man; natural spreading refers to the individual *M. alternatus* spread to the pinewood with air or wind randomly. The spot spreading effect of natural spreading usually happens in the distant mountains, where it is hard to find; when found, it usually has become a large occurrence base. It is an important reason for the pine wilt disease occurring one after another. Human transmission is often in the vicinity of industrial or residential areas, and it is easier to find and easier to manage[2, 4–5].

Surface spreading effect refers to pine wilt disease occurrence effect caused by the invasion of large quantity of *M. alternatus* to healthy pinewood. Surface spreading effect usually takes place around the core occurrence area of pine wilt disease, and once happening, it will lead to a large area of dead pines. In the mid-1990s, after Zhoushan and Xiangshan gave up the control of pine wilt disease (so-called blocking and isolation), a large number of *M. alternatus* crossed Xiangshan Harbor and Jintang Port, leading to a large area of pinewood covered with pine wilt disease on the North Shore of Xiangshan Harbor and West Coast of Jintang Port[4–5].

Transferred increase effect refers to the increase of *M. alternatus* and *B. xylophilus* populations by transferring to other places. The pine wilt disease of light degree could transfer to the burnt pinewood, the logging residues of pines, and the pinewoods with the occurrence of *Dendrolimus punctatus* walker, bark beetles, *Lecanosticta acicola* in the next year so as to hide and reproduce the later populations, and in the year after, these hiding and reproduced *M. alternatus* could have massive emergence, thereby infecting and damaging new pinewood, resulting in the outbreak of pine wilt disease. Transferred increase effect is elusive, and it is usually ignored by the appearance of fire burning or the withering caused by other pests and diseases. Therefore, in the occurrence area of pine wilt disease, all withered pine varieties should be removed and inspected, and it could not only remove the withered pines affected by pine wilt disease only[6–10].

Mutagenic effect refers to the mutation of *B. xylophilus* into *B. mucronatus. B. xylophilus* and *B. mucronatus* are the different ecotypes of the same species, and *B. mucronatus* has stress resistance. It has strong resistance to the adverse environment. For example, when affecting the masson pine, slash pine and pinaceae plants of non-Pinus, *B. xylophilus* often differentiate the terminal mucro, becoming the so-called *B. mucronatus.* These two kinds of nematodes have the same morphology of dauerlarvae ($L_{IV}$), and when $L_{IV}$ leaves the vector insect and enters into the pines, it can change into *B. xylophilus* or *B. mucronatus* according to the resistance of the invaded pine tree. Such effect indicates that it is a major risk of ignoring *B. mucronatus* in our practical work[10].

Syndrome effect means that pine wilt disease is not a kind of disease with nematode *B. xylophilus* as the only pathogen, but a syndrome of pine under the combining effects of *M. alternatus* and the nematodes, fungi, bacteria and other pathogens it carried[1, 8, 11–14]. *M. alternatus* adults could carry nematodes and microbes into the endodermis of pine trees and then lay eggs inside. After the eggs hatching, *M. alternatus* larvae began taking nutrients, from the cambium tissue. At the same time, nematodes carrying with bacteria enter into the deep vascular system of pines. After then the bacteria begin the proliferation in large amount in vascular, and the proliferation quickens with the increase of temperature, which can also produce toxins and cellulase, leading to the wide infection of vascular cells and then the occurrence of pine wilt disease. In the meantime, the fungi also

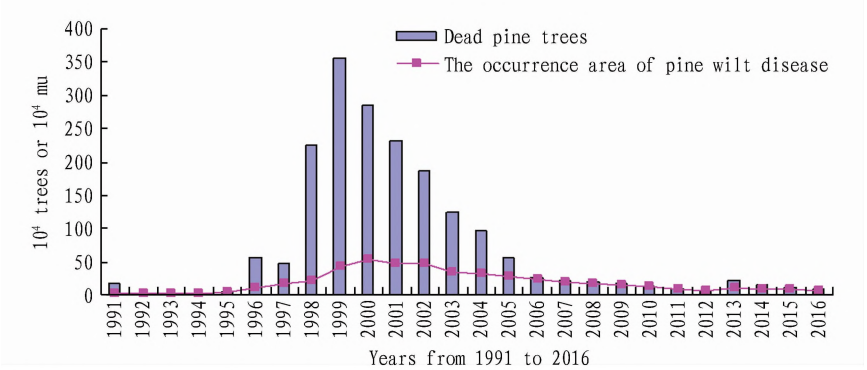


**Fig. 1** The occurrence of pine wilt disease in Ningbo over the years


a is wire netting isolation; b nylon net isolation; c polypropylene net isolation.

**Fig. 2** Isolation materials and isolation ways

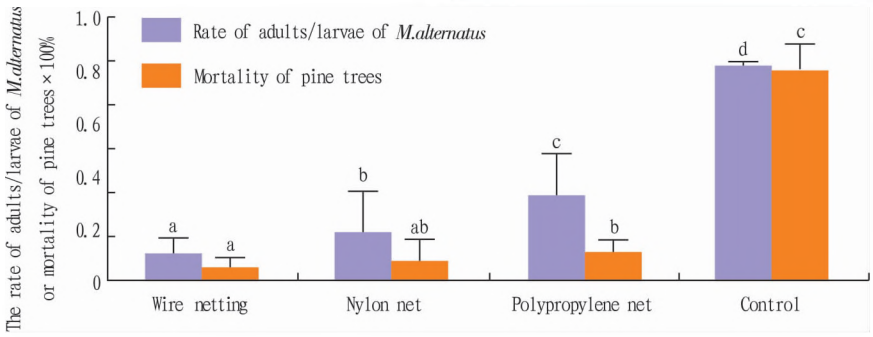


**Fig. 3** Relations between the rate of adults/larvae of *M. alternatus* and mortality of pine trees based on different isolator materials

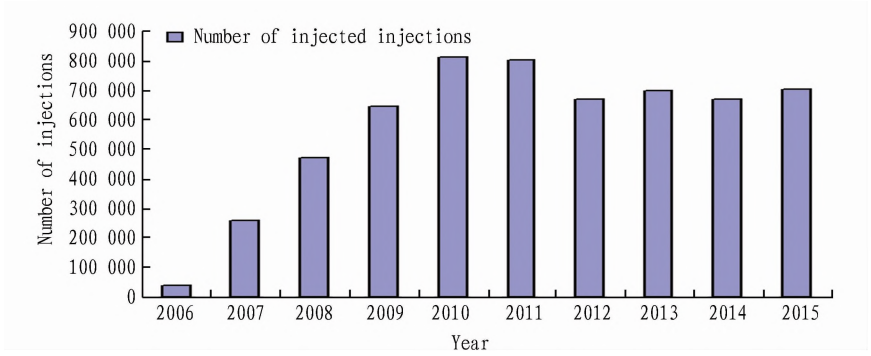


**Fig. 4** The usage amount of immune injections for pine wilt disease over the years

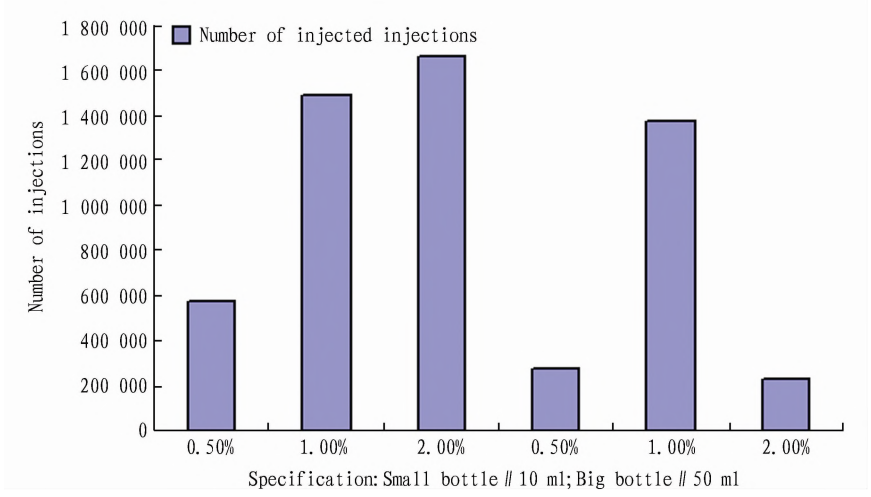


**Fig. 5** The number of injections of different specifications

proliferates in large amount, and the mycelia spread to the parenchyma cells in the pine xylem, resulting in the destruction and discoloration (such as blue stain) of a large number of xylem cells, thereby leading to the withered pines which are called comprehensive syndrome medically. Knowing that pine wilt disease is a kind of comprehensive syndrome can make people get rid of the shackles of the single factor of pine wood nematode, and pay attention to the dominant role of *M. alternatus*, thereby getting the initiative role in the control.

Schedule effect refers to the procedure arrangement of controlling the pinewood with pine wilt disease occurrence. Schedule effect is closely related with the life history of *M. alternatus*. The clearing, transportation and removal of pine trees affected with *M. alternatus* must be completed before the late April every year. The macro layout should give first priority to the removal of withered pine trees, and then the protection of living trees. During the clearing, it should begin with the core and then the outside, the area with serious infection first and then light infection[2-5]. When making the control goals, it prefers the long term continuous effect rather than the extinguishing with one action, and even with an annual occurrence of 5%, the clearing control can continue 20 years [5]. Such kind of timing can not be reversed. If effort is given to the so-called pines in the protection zone without clearing the dead pine trees dying from the infection in advance, it is to fail just like the passive defense without actively attacking the effective strength of "enemies" and only the active actions can protect the safety of pines in the protection zone.

Assets effect means that the control of pine wilt disease is a large forestry ecological project, which needs to invest a lot of manpower, materials and financial resources to drive the system project. Without financial guarantee, the dead pine trees in the mountains cannot get treatment. It is not practical for the government or operators (according to the principle of "who operate who control" in the "Forest Pest Control Regulations") to take the responsibility of the huge control funds, and it is a deadlock. The only solution is to treat the dead pine trees as "assets", which can make these trees play the effect of assets, promoting the development of the control [15-16]. Actions have been taken in Ningbo to carry out the safe use of the infected pine trees. Factories are built to use the dead pine trees by providing funds to transport the trees going downhill to the factories as the raw materials, and killing the vector insects, nematodes, bacteria and other pathogens in all links of factory production.

The above effects can be summarized as the pine wilt disease control theory of "1 priority, 2 objections, 3 principles, 4 measures, and 5 managements". "1 priority": priority is given to the clearing of infected withered pine trees, which is "the one and only way" to control pine wilt disease, and any other way is to fail. "2 objections": (1) resolutely object the clear cutting of pine trees in advance under the excuse of transforming the pine forest; (2) firmly object giving up clearing the withered dead pine trees under the excuse of blocking and isolation. "3 principles": (1) comprehensive clearing-up principle, all pine trees affected by *M. alternatus* in the pine wilt disease occurrence one should be

cleared up with no "dead end" left, including the burnt pine trees, wind-falls, trees crushed by snow, trees affected by other pests and diseases as well as the pine trees that have already been transported to downhill; (2) complete removal principle, all cleared pine trees and products should go through the treatments of fumigation, heat drying, smashing or burning within the stipulated time, so as to completely wipe out the containing *M. alternatus* or *B. xylophilu*; (3) key protection principle, prevention and protection should be taken to the pine trees without infection according to the location and function importance, and pine tree inventory should be listed into the assessment of the control results of pine wilt disease that after the control, the inventory of healthy pine trees over 90% is A; 80%–90% is B; 60%–70% is C, and less than 60% is D. "4 measures": (1) winter clearing measure; (2) time-limited removal measure; (3) spring protection measure; (4) immunization measure. "5 managements": (1) quarantine management, pine wilt disease is a plant disease, which must take comprehensive management to the pathogens, media, hosts, removal, transportation according to "National Phytosanitary Regulation"; (2) goal management, it should make a feasible goal, but not pursue the prompt military decision to put out the disease in one action, and it should pursue the aim of compressing the cardinal number of occurrence every year under the conditions of controlling, to realize the goal of "no disaster of the disease" until the disappearance of the disease, to maximize the protection of forest resources and sustainable development; (3) project management, the control of pine wilt disease is a system project, and the clearing, removal, extinguishing adults *M. alternatus* as well as the prophylactic immunization involve a lot of manpower and material resources as well as instruments and equipment and facilities, which are required to organize the implementation and management according to the engineering principle and method; (4) ecosystem management, pine forest is the most important forest vegetation, and it is the basis of forest ecology. *B. xylophilus* and *M. alternatus* are destructive harmful ecological factors, which cannot be destroyed by eliminating the hosts, because such kind of method is to help the spread of the disease and the destruction of the basis of forest ecology, for example, clear cutting pine trees prompts the reverse succession of pine forests; (5) achievement and cost management, pine wilt disease is a natural disaster, and its control involves the investment and use of a large number of national financial fund, so it must adopt the performance management method to optimize the use efficiency and effect of national funds, and it will not allow the occurrence of the irresponsible incident of "no pine tree left with money using up".

**Method innovation**

**Removal project**

The removal project of pine wilt disease refers to the two aspects of clearing and removal. Clearing is to clear the affected dead pine trees and the pine forest; removal is to kill all the vector insects and nematodes hiding in the pine trees, like *M. alternatus* and pathogenic *B. xylophilus*.

**Winter clearing**

Clearing the dead pine trees affected with pine wilt disease is similar to the sanitation cutting in forestry production, but there are also some differences between the two. The technical requirements for cleaning up dead trees are higher, so that it is comprehensive, thorough and timely. The purpose of cleaning is actually the control of *M. alternatus* larvae and *B. xylophilus*, known as winter clearing. Every year, the beginning of November to the end of March of the following year every year is the time for the *M. alternatus* larvae to over-winter in pine trees, during which time, theoretically, *M. alternatus* and *B. xylophilus* will not run away, and hiding in the withered tree makes it easy to find. The comprehensive, thorough and timely clearing of withered pine trees is the first step in the control of pine wilt disease. There are various withered pine tree varieties distributing mainly in high mountains and lofty hills with huge quantity, so it is never easy to make a comprehensive clearing. Clearing is s huge forest project, and the steps are as follows: forest survey, job design, project bidding, clearing treatment, project supervision, inspection schedule, quality inspection, rework makeup, project acceptance.

**Removal**

*M. alternatus* larvae and *B. xylophilus* have hard wood barrier, so it is a very difficult job to kill the vector and pathogenic nematode in the pine trees. For a small amount of withered dead trees, the commonly used methods are burning, artificial tree cutting; for large quantity, the only way is to carry out the safe use of infected pine trees, namely, the comprehensive forestry industry system, so as to use the industrialized mechanical cutting, knife-cutting, milling, rotary-cut method, and hot pressing and heating methods to kill *M. alternatus* and *B. xylophilus*, such as chipping removal, heating removal, microwave removal.

**Combining clearing and removal**

Clearing and removal can be separated, and can also be combined with each other. In separate operation, it should also need to do the downhill transportation, admission, weighing, banking after clearing the infected pine trees. Combing clearing and removal means, first, the instantly burning dead pine at cutting spots [17–18], that is, after cutting in the forest, the dead pines are burnt in the same place; second, the instantly fumigating dead pine at cutting spots, that is, after the dead pines are cut, the forest is blocked, and then aluminum phosphide or methyl bromide in small cans are put in the forest for fumigation; third, isolation on the spot, that is, simple sheds are built in the affected forest at logging operation to put all the things related with the source of the disease from the clearing inside, or using metal net to isolate the source of the disease; fourth, peeling off the bark to remove the pests in the stump[19], or together with artificial spraying and natural enemy release to block the *M. alternatus* from further damaging the pines or spreading the disease.

**Protection project**

The pine forest protection project also has two aspects of blocking infection and disease-resistance for pine trees. Blocking infection is to stop the M. alternatus for mature feed and laying eggs to spread the pine wilt dis-

ease by killing *M. alternatus* beetles; disease-resistance is to inject the immunization drugs to the healthy pine trees to improve the nematode-resistance.

**Spring prevention**

Contact killing or trap killing *M. alternatus* adults, or the traditional chemical protection method for pest and diseases, is called the spring prevention. Every year, from the late April to the late July, after emergence, *M. alternatus* adults bite through the xylem and leave the withered pine trees carrying nematodes, and fly to healthy pine trees for mature feed and spread nematode *B. xylophilus*. This is the only period when *M. alternatus* and *B. xylophilus* are exposed to the open space, and it is also most likely to use drug killing and trapping to kill the beetles. It is obviously can achieve the aim of killing the beetles by using contact killing and spraying pesticide. However, it should develop special pesticide to achieve the purpose killing of *M. alternatus* before the spraying, and in the meantime, pesticide spreading method and instruments should also be developed so as to shoot the pesticide to the highest crowns and thick pine forest; it should exploit trapping methods and instruments for the trap killing by studying the chemical ecology of *M. alternatus*. We have made innovations in contact killing and trap killing beetles: the technology of Sea swallow 650C airplane spraying contacted-breaking release microcapsules, PEM-pesticide and Bt bio-pesticide powder with ultra low volume to control pine wilt disease[20-22]; the development of the technology of "trapping *Monochamus altermatus* adults with chemically-treated pine logs and branches" by using chemical ecological theory and technology, which has suspended 20 000 sets of traps to trap *M. altermatus* in total [23]; the development of the technology of 3WF–14G+ Bt bio-pesticide powder and 3W–14G+ new PEM of ultra low volume for ground powder injection and mist spraying to control pine wilt disease in Xiangshan County, Beilun District and Yuyao City[24-26]; the development of "pesticide gun" to control pine wilt disease getting the national invention patent, which was used in the control of pine wilt disease in Chashan mountain, Ninghai County. In order to coordinate with the comprehensive control in spring, the following new pesticide and new instruments are also developed and applied: new pesticides mainly include fenitrothion + buprofezin mixture (generation 1 PEM), Luseweilei, *Beauveria bassiana*, *Verticillium chalamydosportium* prepartion, *Sophra flavescens* +avermectin emulsion, *Bacillus thuringiensis*+avermectin (Songyike) powder, avermectin and Emamectin immune injection; the new instruments are ultra high lift sprayer (special equipment for old tall pine trees protection), drug powder firecracker, log microwave pest control machine, panel microwave pest control, tress punching and injecting gun; new technical methods such as light aircraft ultra low volume spraying method (powder), *Beauveria bassiana*, *Verticillium chalamydosportium*, Songyike powder + 3WF –14G ultra low volume ground dusting method.

**Immunoprophylaxis**

Like the control of all diseases, the injection of immune agents is the best way to prevent or block the occurrence of infectious diseases. The development of a kind of injection to the pine trees to prevent the occurrence of pine wilt disease is the prerequisite to completely extinguish pine wilt disease[27]. In order to break through the key technology, 22 commonly used pesticides were collected, and after screening and toxicity determination in laboratory, the drug with high sensitivity to pine wilt disease was obtained[28]; at the same time, formulations were made to the selected drug, obtaining the emamectin benzoate twain compound injection, which was gone through the mould pressing to the injection bottle for the resistance test in the forest with pathogen inoculation and natural infection, achieving breakthrough progress[29]. Since 2006, this kind of injection has an annual input of 1 million, which has extinguished the pine wilt disease of a large area.

**Isolation of pine wood in situ and release of natural enemies**

This also can effectively protect the healthy pine trees from pine wilt disease. When clearing the forest infected with the disease, it can achieve good protection effect by releasing Dastarcus helophoroides or Sclerodermus guani after isolating the beetle M. alternatus of source of the diseases using the metal net (70 mesh)[30-31].

According to these new theories and new methods, we have defined the connotation and denotation of the concept of pine wilt disease control, and developed implementation objectives and progresses. The idea of the control is control first, followed by extinguishment, that is, first control and then extinguishing. The control method is to reduce the death rate of withered pine trees at 0.1 –0.03% through the overall clearing and integrated control under the premise of protecting healthy pines to the maximum. The extinguishing method is to control the death rate of withered pine trees in the next year at 0 –0.01% through the isolation of infected branches and twigs and release of natural enemies of *Dastarcus helophoroides* or *Sclerodermus guani*, or through the injection under the premise of controlling the damage of pine wilt disease.

## Results and Analysis

Both the area of pine wilt disease occurrence and the number of dead pine trees decreased for successive years. According to the record of the occurrence area and the number of withered trees, Xiangshan County saw a number of 174 400 trees with an area of 2 106 hectares dying from pine wilt disease in 1991, experienced the of a number of 3 500 000 trees of an area of 28 273 hectares dying from pine wilt disease in 1999, and then witnessed a gradual decrease to the 68 000 trees with an area of 4 333 hectares in 2012, showing that the control effect was significant. The single peak curve, which spanned 25 years, clearly reflected the influence of the change of control ideas and methods on the control effect, which could be divided into 5 stages.

The first stage-explore stage (1991–1994): the characteristic of this stage were that it was a blank about how to control the pine wilt disease,

and the only way was to explore while controlling. When the Ministry of Forestry put forward the control strategy and method "outside first and then inside", "gradual clear cutting from the outside to inside", the local government considered it was hard to carry out for the work amount of this plan was too big, and the available labor force could not meet the requirements of the work amount in limited time at all. Moreover, these pine trees were planted by them, so they were willing to cut them when they finally became the forest. Therefore, the final decision was to cut more dead pine trees and fewer living trees, use methyl bromide fumigation to disinfect the pine trees downhill with the branches and twigs burning, and smear methamidophos diesel mixture at the stumps. In the spring of 1992, the clearing and removal work was almost finished. In this year, there were 174 370 trees cut with a total clearing area of 2 109 hectares, 1 456.7 $m^3$ infected wood processed with methyl bromide fumigation, 6 322 small trees and 778 000 kg branches and twigs burnt. The investigation in October 1992 showed that there were only 7 031 newly added withered pine trees, and the occurrence area was 903 hectares, with the decreases of respectively 95.97% and 57.16%, indicating significant effect. The effect of this approach extended to 1994. In 1993 and 1994, the number of withered pine trees was 5 680 and 1 1900, respectively, and the disease occurrence area was 1 040 hectares and 1 367 hectares, respectively.

The second stage-diffusing stage (1994–1997): this stage carried on the control effect of the first stage. In 1993, the State Forestry Department held the on-the-spot meeting in Xiangshan County about the control of pine wilt disease, which affirmed the achievements, pointed out the problems that the control of pine wilt disease did not carry out according to the plan of the experts, and the there was not enough clear cutting, so only the number decreased but the extinguished quality of three years did not achieve the goal, and the number of villages and counties affected by the disease showed no decrease. Therefore, after the meeting, together with the rising trend of the disease in 1994, the local decision-making departments began to doubt the original methods of cleaning without clear cutting, requiring change. It was the time when Xiaotang village of Xiangshan County began the pine wilt disease with an area of 27.13 hectares and 79 trees withered, and the disease occurrence place was an isolated pine forest. The decision-making level thought it was a chance to extinguish the disease place, so financial resources and manpower were centralized to implement the clear cutting to this place, but loosened the control of the original places which were considered as the previously forest of pine wilt disease. As a result, the investigation in 1995 showed that the pine wilt disease showed a substantial expansion, and the number of villages and towns in the county increased to 13 with the area increasing to 2 373 hectares (+67.2%) and the number of withered trees increasing to 43 613 (+ 277.6% ). It was the time when the sudden break of the disease aroused public panic, the decision-making level of the State Forestry Department issued the blocking order, which prohibited cutting pine trees and prohibited the transportation and use of pine trees. It was actually to give up the control. The results was a large outbreak of epidemic in 1996, which added 2 more disease occurrence area, namely Beilun District (occurrence area of 1 009 hectares, number of infected withered trees of 90 853), Daxie Development Zone (occurrence area of 467 hectares, number of infected withered trees of 36 203); the occurrence area in Xiangshan was 6 057 hectares, with the number of infected withered trees of 149 820. By 1997, the occurrence area in Xiangshan had risen up to 10 131 hectares, with the number of affected withered trees rising to 440 175; Beilun and Daxie adopted the method of "instantly burning dead pine at cutting spots" to control the pine wilt disease, making the epidemic situation decreased (Beilun: occurrence area of 1 172 hectares, the number of affected withered trees of 11 225; Daxie: occurrence area of 467 hectares, he number of affected withered trees of 25 003). The control work at the Ningbo City level turned to set the non-pine tree isolation belt with the width of 4 km at the junction of Ninghang County and Xiangshan County. However, it proved that the isolation belt had no effect on blocking the pine wilt tree at all.

The third stage-outbreak stage (1997–1999): this stage carried on the disease diffusion of the second stage. By 1997, Xiangshan had been unable to organize the control, and officially announced to replace clearing and controlling affected pine trees with "blocking and isolation". Zhoushan archipelago, close of Ningbo, saw an outbreak of pine wilt disease, which fully verified the power of "Core effect" and "Surface spreading effect", leading to the further disease outbreak in Ningbo. To 1998, three occurrence areas added in Ningbo District, namely, Zhenhai District, Fenghua City, Ninghai County, with the total occurrence area of 146 33 hectares, number of affected withered pine trees of 2 254 600, which increased by 21.94% , 373.26%, respectively. The further development of the epidemic situation in 1999 made the other cities in Ningbo (Yinzhou, Yuyao, Cixi, Jiangbei) all have the occurrence of pine wilt disease, and the occurrence area of the whole Ningbo City reached up to 28 273 hectares, increasing by 93.21% ; the number of withered pine trees reached 3 548 800, increasing by 57.40%. At this time, the epidemic situation made the decision-making level of Ningbo City realized that it was impossible to block and isolate the pine wilt disease, and only the vigorous control and decrease the cardinal number of occurrence was the only way out. We proposed the safe application of affected pine trees, which created conditions for clearing the affected withered pine trees in large area, and developed the airplane spraying PEM with ultra low volume to kill the vector beetle of *M. alternatus* in large area. It happened that the delegation of the academicians of the China Association for Science and Technology, for field study of pine wilt disease, passed by Ningbo, and they affirmed the new strategy and method for the control of pine wilt disease in Ningbo. It was in that year that Ningbo organized airplane spraying in Beilun

District, killing a great number of vector beetles, and the number of withered pine trees in 1999 increased by only 57.40% than 1998, which was far lower than the increase rate of 373.26% from 1997 to 1998. Thus, the trend of disease occurrence got controlled. However, the occurrence area still presented huge growth, and what was worth mentioning was that it was thought that the natural barrier in the north of Xiangshan County, Xiangshan gulf with an average width of 6 km, could block the diffusion of *M. alternatus*, as a matter of fact, pine wilt disease was found in a large area of pine forest in Fenghua, Yinhou to Yuyao, Cixi, Jiangbei on the north shore of Xiangshan gulf.

The fourth stage-control stage (1999–2010): this stage carried on the disease outbreak in the third stage. From 1999 on, Ningbo City restarted the active and positive control of pine wilt disease, clearly pointed out to change the unwilling and passive attitude to active and positive attitude towards the control of pine wilt disease, change the useless affected pine trees to useful materials, change the disorderly circulation into orderly circulation, so as to combine dredging and blocking to carry out the safe application of affected pine trees, to promote the control of pine wilt disease. During this period, 18 factories were built for the safe application of affected pine trees, providing industrialized basis for the timely consumption of affected pine trees in the affected area every year. The building and operation of these factories promoted the comprehensive clearing of dead pine trees with the labor force of 4 000–5 000 every year, leaving no pine tree. All the cleared affected pine trees were purchased by the quarantine department, and sent to the appointed factory for disinfection and utilization, decreasing the loss of affected pine trees (branches and twigs) to the maximum, promoting the resource utilization and economic development. An average of 60 000 tons of dead wood was reused, and the economic benefit was 100 million Yuan. At the same time, the development and application of comprehensive prevention and control technology opened up a new situation for the spring and summer control of pine wilt disease. Spring and summer is the time for *M. alternatus* to spread pine wilt disease, and it is also the weakest time for *M. alternatus* and *B. xylophilus* because they are exposed to the open air. The comprehensive control of *M. alternatus* during this period can always have the effects of half the work with double results[15–32]. In 2006, the trial of the immune prevention technology of pine wilt disease succeeded, further laying the foundation for the fight against pine wilt disease. These control practices made the occurrence area of pine wilt disease and number of withered trees reduced and decreased year by year. To 2010, the occurrence area of the whole Ningbo City was 8 547 hectares with the number of dead pine trees of 131 100, which decreased by 69.78% and 96.31% respectively to 1999. Combining the thoroughly cleaning and complete killing pest with chemical control, physical control and biological control can make the occurrence of pine wilt disease reduce to 0.03%.

In the 5 years from 2008 to 2012, the number of dead pines trees was recorded every year in the 4 areas with a total area of 462.8 hm2 of 62 subcompartments, in which the control test area had an area of 397.4 $hm^2$, and the control had an area of 65.4 $hm^2$ (Table 1). As shown in Table 1, the number of average dead pine trees in the test area was 13.56/$hm^2$, while the control had an average of 37.69/$hm^2$, reducing by 54.21%, and the student's t test showed that $t=4.704 > t_{0.001}=3.46$ ($f=n_1+n_2-2=60$), indicating significant difference. *Dastarcus helophoroides* was first released in 2008 to control the pine wilt disease together with cleaning, and the number of average dead pine trees in the test area was 5.27/$hm^2$, while the control had an average of 9.82/$hm^2$. Therefore, compared with simple cleaning, cleaning +*D. helophoroides* release had the dead pine trees decreased by 46.64% per hectare, and *t* test showed that $t=1.687>t_{0.1}=1.687$ ($f=n_1+n_2-2=60$), which did not reach the significant difference level. To 2012, the number of average dead pine trees in the test area was 0.51/$hm^2$, while the control had an average of 6.18/$hm^2$, and the number of the test area decreased over 91.74% to that of the control per hectare. Moreover, *t* test showed that $t=6.296>t_{0.001}=3.46$ ($f=n_1+n_2-2=60$), indicating that the difference reached the extremely significant level. Therefore, on the basis of cleaning, the release of *D. helophoroides* can make the number of dead pine trees decrease significantly than the control, and after continuous release for 5 years, the decrease of dead pine trees reached the extremely significant difference level at $P>99.99\%$.

Table 1 also showed that it was

**Table 1** The number of dead pine trees in the control area and the prevention area released *D. helophoroides*

| Region | Sample plot | Area//$hm^2$ | Number of plots | Number of healthy pine trees before test (spring of 2012) | Number of healthy pine trees after test (winter of 2012) | Total number of dead pine trees | Number of dead pine trees//$hm^2$ | Number of dead pine trees//$hm^2$ | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 2008 | 2012 |
| Yuyao | Prevention area | 110.6 | 22 | 116 008 | 113 111 | 2 897 | 26.19 | 9.57 | 0.52 |
| | CK | 23.5 | 4 | 25 103 | 23 115 | 1 948 | 82.89 | 21.02 | 14.21 |
| Zhenhai | Prevention area | 57.1 | 7 | 102 997 | 102 113 | 884 | 15.48 | 6.90 | 0.11 |
| | CK | 14.4 | 3 | 27 804 | 27 541 | 263 | 18.26 | 5.69 | 2.5 |
| Cixi | Prevention area | 109.6 | 12 | 174 974 | 173 823 | 1 151 | 10.46 | 2.99 | 0.97 |
| | CK | 16.4 | 1 | 11 798 | 11 625 | 173 | 10.55 | 2.43 | 1.28 |
| Fenghua | Prevention area | 120.1 | 12 | 91 138 | 90 680 | 458 | 3.81 | 1.61 | 0.27 |
| | CK | 11.1 | 1 | 2 508 | 2 427 | 81 | 7.29 | 2.34 | 1.17 |
| Total | Prevention area | 397.4 | 53 | 485 117 | 479 727 | 5 390 | 13.56 a | 5.27 a | 0.51 a |
| | CK | 65.4 | 9 | 67 213 | 64 748 | 2 465 | 37.69 b | 9.82 a | 6.18 b |

very difficult to achieve the control target of the disease by cleaning the infected trees. To reduce the dead pine trees to 0.03% (0.675 tree/hm$^2$), 0.1% (2.25 trees/hm$^2$) was the control target of pine wilt disease. But the tracking observation for 5 consecutive years showed that the number of dead pine trees did not reach the extinguishing requirements, and the observation for 3 consecutive years showed that the number of dead pine trees was 6.18/hm$^2$, far more than the standard of 2.25 trees/hm$^2$. On the other hand, the prevention area released with *D. helophoroides* showed that the number of dead pine trees dropped to 0.511 tree/hm$^2$ in 2012, and the dead rate was 0.022 7%, achieving the control effect.

The fifth stage-extinguishing stage (2010–2016): this stage carried on the fourth stage. After the pine wilt disease was controlled and reduced, the control of pine wilt disease could come to the stage of extinguishing the disease in some places to protect the pine trees in key area and the important pine trees. During this period, Ningbo City required all counties (cities) to carry out the "peak cutting and disease removing" project of pine wilt disease, in which the control of pine wilt disease was carried out with the basic unit of villages and towns, and those with relative severe occurrence still adopted the peak cutting method to reduce the cardinal number of occurrence, while those with relative low cardinal number of occurrence adopted the extinguishing methods to remove the disease from the affected area. This project achieved significant effects that after the implementation, 49 villages and towns had completely got rid of the disease, and Zhenhai District and Jiangbei District reached the standard to remove. In 2012, the occurrence area of pine wilt disease in the whole Ningbo City reduced to 4 333 hectares, and the number of dead pine trees decreased to 68 100, indicating that the pinewood restored its vitality. Two technical measures were taken to remove the disease from the affected areas:

First, comprehensive cleaning+infected trees isolation +natural enemy release could extinguish the pine wilt disease. Isolation is the most common and effective method for the spread of the epidemic. Theoretically, *M. alternatus* in the pine trees can be isolated, so the pine wilt disease can be extinguished. However, the dead pine trees in the forest were tall and straight, making it impossible to isolate. Therefore, the cleaning method was used by covering the dead pine trees which were centralized together with wire netting, and then *D. helophoroides* was released, which could then achieve the effect of extinguishing the pine wilt diseases. The isolation test results of 24 heaps of infected pine trees showed that there were no pine wilt disease around 9 heaps, which extinguished the occurrence of pine wilt disease for 100% , accounting for 37.5% of the total, in which the number of those isolated using iron netting and nylon net were 4 heaps for each, accounting for 88.9%, and there was one pile of polypropylene net, accounting for 11.1%. There were one dead pine tree around 6 heaps of infected pine trees, which meant the inhibition rate of the occurrence reached 95% , accounting for 25%, in which the number of those isolated using iron netting, nylon net and polypropylene net was two for each, each accounting for 33.3%. Three heaps of infected pine trees had 2 dead pine trees, accounting for 12.5% of the total, in which there were 2 heaps isolated using iron netting, 1 using polypropylene net. The analysis on the isolation effect showed that the material used for the isolation had a profound effect on the mortality rate of pine trees.

Whether it was the polypropylene net or nylon net or iron netting to isolate the infected pine trees, the mortality rate of the pine trees around these isolated heaps decreased significantly compared with the control with the results of iron netting: nylon net: polypropylene net: CK =1 :2.25 :4 :32 control, indicating that the mortality rate of pine trees without isolation was 32 times of those isolated using iron netting. Statistical analysis showed that the test value of the groups isolated using iron netting, nylon net, polypropylene net with the CK was, respectively, $u_{\text{iron-CK}}$=10.993, $u_{\text{nylon-CK}}$= 9.864 , $u_{\text{polypropylene-CK}}$=8.631>$u_{0.001}$=3.291, and all isolated groups showed extremely significant difference with the CK, fully indicating that the isolation of infected pine trees could effectively control the pine wilt disease. The test value of iron netting with nylon net $u_{\text{iron-nylon}}$=1.414<$u_{0.09}$=1.695 had no significant difference, and the test value with polypropylene net $u_{\text{iron-polypropylene}}$ = 2.768>$u_{001}$=2.576 showed significant difference, and the test value of nylon net with polypropylene net $u_{\text{nylon-polypropylene}}$ = 1.459 <$u_{0.09}$ =1.695 had no significant difference, indicating that iron netting had good isolation effect on *M. alternatus.* Further analysis showed that the isolation effect as not totally determined by the isolation material, and the rate of adult/larvae of *M. alternatus* also had some effect on the results. As shown in Fig. 3, the rate of adults/larvae of *M. alternatus* presented a proportional relation with the mortality of pine trees, and a high rate of adult/larvae of *M. alternatus* usually had high mortality rate of pine trees. For the CK group, the rate of adult/larvae of *M. alternatus* was close to the mortality rate of pine trees. According to this standard, the rate of adult/larvae of *M. alternatus* in the group isolated using iron netting was the lowest, and the mortality rate of pine trees was also low. Moreover, the low rate of adult/larvae of *M. alternatus* in the group isolated using iron netting was the results of releasing the natural enemy-*D helophoroides*, which had high parasitic rate of *M. alternatu.* Therefore, the isolation of infected pine trees together with the release of *D. helophoroides* could effectively extinguish the spread and damage of pine wilt disease.

Second, forest land cleaning+immunoprophylaxis could completely distinguish the pine wilt disease. After years of study, the immune technology for pine wilt disease which was convenient and had low cost was finally developed, laying the foundation for the accurate control of individual trees. The trial in the forest from 2005 to 2006 showed that the pine trees injected with the newly developed drug had the survival rate of *B. xylophilus* of 100%; while for the control group, 75% of pine trees trunk-injected with the Japan-specific injection drugs survived from inoculation of *B. xylophilus*[29].

**Table 2** emamectinbenzoate injection effect of pine wilt disease was put out

| Year | Location | Forest category | Area $hm^2$ | Number of injected plants | Check time and result (Number of wilted pine trees) | | |
|---|---|---|---|---|---|---|---|
| | | | | | October of the first year | October of the following year | October of the third year |
| 2008 | Jiufeng Mountain | Pine forest | 24.2 | 16 222 | 3 | 0 | 0 |
| | Road sides | Pine forest | 111.5 | 8 500 | 37 | 12 | 0 |
| 2009 | Dagan | Pine forest | 172.3 | 159 802 | 53 | 4 | 0 |
| | Small ports | Pine forest | 23.3 | 19 778 | 0 | 0 | 0 |
| | New roads | Pine forest | 57.4 | 53 017 | 21 | 0 | 0 |
| Total | | | 388.7 | 257 319 | 114 | 16 | 0 |

The promotion test achieved significant effect, which protected the pine trees in the scenic spots from pine wilt disease. From December 2006 to February 2015, a total number of 576 4595 injections were promoted, protecting over 5 700 000 pine trees from pine wilt disease (Fig. 4). The promotion first began in 2006, when the application amount was 40 000, mainly in Zhongshan Park, Tashan Park, of Fenghua City and Dongqianhu Yuewangmiao Park, and the immunization rate reached up to 99.9%; in 2007, 260 816 injections were applied, greatly enlarging the promotion range; to 2010, the injected amount of the immune injections was 813 175, and the immunization rate reached 99.6%. In 2006, there was only one specification for the injection, namely 2% 50ml/injection, and after the accurate determination, the effective dose of emamectin benzoate for each cubic of pine log volume was only 1 g. Therefore, the specifications of the injection were redeveloped, so as to adapt to the injection demand of pine trees with different diameter at breast height (DBH) in the forest. As shown in Fig. 5, the maximum amount was 2% 10 ml of emamectin benzoate injection with the cumulative application amount of 1 668 098, indicating that the diameters of the pine trees in this area were 14–16 cm. The pine trees with the diameter of this size only need to inject one 2% 10 ml of emamectin benzoate, which can make the pine tree immune pine wilt disease. Followed by the 1% 10 ml emamectin benzoate injection with the application amount of 1 485 014, the pine trees with the DBH of 10–14 cm could immune the pine wilt disease by injecting 1 injection for each tree. And then the 1% 50ml emamectin benzoate injection with the application amount of 1 371 073, it is suitable for the pine trees with the DBH of 22–30 cm, and can make the pine tree immune the pine wilt disease by injecting 1 injection for each tree. The injection of small capacity is easy for artificial injection and does little damage to pine trees, so it has been popularized and applied very well (Fig. 5).

Following checking was made on the effect of emamectin benzoate injection on pine wilt disease in Beilun District in 2008 and 2009, finding that after injecting the emamectin benzoate injection, the number of dead pine trees of the year decreased significantly to only 114, accounting for 0.044% of the injected pine trees, and 0.013% of the total pine tress (no injection to the pine tree with the DBH of less than 9 cm). And in October of the next year, the proportion was 0.002 2% and 0.000 9%, respectively, close to completely extinguish, and the check in the third year showed that the pine wilt disease had been completely extinguished (Table 2).

## Conclusions and Discussion

After 25 consecutive years of control in Ningbo City, the pine wilt disease finally got controlled, and it was extinguished in some areas, proving that pine wilt disease was not incurable, but could be prevented, controlled and extinguished. For 25 years, the core method for the control of pine wilt disease was combining comprehensive cleaning with integrated control to reduce the cardinal numbers of pathogens and vector insects-*M. alternatus*[29-37] to gradually reduce the harmful effects of pine wilt disease and ensure the health and safety of the pinewood. In 1999, the delegation of the academicians of the China Association for Science and Technology affirmed the basic ideas and methods of the pine wilt disease control in Ningbo. After that, the decision-making level of the State Forestry Department piloted new technical indicators to measure the control effect of pine wilt disease, that is, the pine wilt disease was considered to be extinguished or almost extinguished when the number of dead pine trees reduced to ≤0.01%, remained stable at >0.01% and ≤0.03% for 3 years. The indicator seemed simple, but the effect was great, which completely changed the ideological restraint that pine wilt disease was incurable. Especially in 1999, the State Forestry Department made agreement on transporting the dead pine trees from Ningbo for safe application in the artificial board factory of Shanghai, making the disinfection of affected pine trees able to be completely removed by the industrialization. The infected pine trees can be used safely under the quarantine management made the whole pine wilt disease control system operate smoothly. It was from 1999 that large-scale cleaning control and infected pine tree safe application project were carried about in Ningbo, leading to the gradual decrease in both the occurrence area and occurrence amount of pine wilt disease in the whole city. However, only the simple cleaning and removal could not reach the indicators issued by the State Forestry Department, and it was still hard to reach the control indicator of the number of dead pine trees of > 0.01% and ≤0.03% by combining the comprehensive control method with the complete cleaning and removal, such as the chemical trap killing, chemical spray killing, natural enemy parasitoids killing, which were carried out to kill *M. alternatus* adults purposefully. Therefore, after years of exploration, we came up with the mode of "forest clearing+infected pine trees isolation +*Dastarcus helophoroides release*" and the mode of "emamectin benzoate immunization

injection to healthy pine trees" before the pine wilt disease was controlled and extinguished completely. Ningbo was heavily-stricken region of pine wilt disease, with a long history, large area and extremely severe damage of occurrence, and the 2 indicators of occurrence area and number of dead pine trees once accounted for 70% of the total in China. It was once believed that if the pine wilt disease in Ningbo could be controlled, then it would be much easier to control the pine wilt disease in other regions. In retrospect, the reason why the pine wilt disease could be controlled in Ningbo was that it clarified the mechanism of "11 effects" of pine wilt disease. Two pine trees effect was the basis, revealing the control rule of clearing all infected pine trees and protecting healthy pine trees. Spot spreading effect, Surface spreading effect, Core effect were all the derivative effects of dead pine trees, all of which could be inhibited from the source by clearing and removing the dead pine trees. Sharing effect, Retention effect, schedule effect suggested that healthy pine trees were the natural barrier to control the spread and diffusion of pine wilt disease, which should be vigorously protected, so it should resolutely oppose the practice of clear cutting in advance. The "Assets effect" of infected pine trees revealed that the control of pine wilt disease not only had ecological attribute but also had social attribute and economic attribute, making the control of pine wilt disease able to carry out by combining with the social production activities. According to the theory of these effects, the pine wilt disease control theory of 1 priority, 2 objections, 3 principles, 4 measures, and 5 managements was summarized, which achieved the "control, decrease, extinguishment" of pine wilt disease through practice.

The finding of Transferred increase effect provided a new idea for the control of pine wilt disease. *M. alternatus* had the habits of preferring to do damage to burnt pine wood[6-9], pine logging residues [7], pine trees affected with *Dendrolimus punctatus* Walker [8] and pine trees crushing by snow. When M. alternatus laid eggs on these trees, the weak resistance of these trees made it favorable for the expansion of *M. alternatus* and *B. xylophilus*, resulting in the big outbreak of pine wilt disease in the following year. The effect showed that *M. alternatus* and *B. xylophilus* could hide in all kinds of weakened pine trees, and as a result of the relay, the disease can be diffused, spread and broke out[6, 9]. Therefore, it was of important function and significance for the pine wilt disease control to clear all kinds of dead pine trees. The Syndrome effect showed that pine wilt disease was the comprehensive forest diseases and insect pest syndrome, the exact name of which should be pine wilt disease, and *M. alternatus* was the cause of pine wilt. Inoculating the eggs and larvae of *M. alternatus* to healthy pine trees could result in the death of the tree, indicating that *M. alternatus* could kill the pine tree alone without *B. xylophilus*[14]. Moreover, the parasites, fungi, bacteria and other microorganisms carried by *M. alternatus* improved the lethal efficiency of *M. alternatus* to pine trees[13, 33-36]. The anatomy of *M. alternatus* adults found that *M. alternatus* adult could not only carry *B. xylophilus* with it, but also can carry *B. mucronatus*, *Rhabditis axei*, protozoa, fungi, bacteria, and so on. These parasites and microbes could enter into the pine trees when *M. alternatus* got the mature feed and laid eggs. Although there was no conclusion on the pathogenicity of these parasites and microbes, the author believed that the pathogenicity of them was not strong, yet the microorganisms could affect the parenchyma cells after nematode with bacteria and fungi passed through the xylem, which could result in pine wilt[13]. Mutagenic effect further revealed that *B. xylophilus* and *B. mucronatus* could transform to each other under certain conditions. The study on the molting and morphology variation of *B. xylophilus* showed that *B. xylophilus* and *B. mucronatus* were actually the two phenotypes of the same species [10]. Transferred increase effect, Mutagenic effect and Syndrome effect further proved that the control of *M. alternatus* had important significance. In fact, *M. alternatus* was much more harmful than *B. xylophilus*, and could cause more dead pine trees [12]. Therefore, only by getting hold of *M. alternatus* could it be able to protect the pinewood health[2, 37].

## References


[1] LAI YX (来燕学). Preliminary report on eelworm type by microscopy in hardromestome of flaccid pine in Ningbo (宁波萎蔫松树木质部内线虫类型镜检初报)[J]. Journal of Zhejiang Forestry Science and Technology, 1993, 13(1): 40–44.

[2] LAI YX (来燕学). Flight behavior of *Monochamns alternatus* and strategic thoughts to control pine wilt disease (松墨天牛飞行特性与防治松材线虫病的指导思想)[J]. Journal of Zhejiang Forestry College, 1998, 15(3): 320–323.

[3] LAI YX (来燕学), *et al*. Relationship between flight behavior and shape variation of *Monochamns alternatus* [C]. Proc. of China-US Workshop on Asian Longhorn Beetle, Yinchuan, China, 2002, 215–216.

[4] LAI YX (来燕学), ZHOU YP (周永平), YU LX (俞林祥), *et al*. Cause of pine wilt disease infecting healthy pine forest (松材线虫病新疫点的成因机制初探)[J]. Journal of Zhejiang Forestry College, 1999, 16(4):425–429.

[5] LAI YX (来燕学). A tactic for controlling pine wilt disease based on its distribution and spread (松材线虫病自然扩散特性及防治策略)[J]. Journal of Zhejiang Forestry College, 2000, 17(2): 170–176.

[6] LAI YX, ZHANG SY, YU LX, *et al*. Study on the relations between the fire of pine forest and the epidemic spread of pine wilt disease [C]. Proc. of the Regional Workshop of IUFRD, Seoul, Korea, 1997, 259– 269.

[7] LAI YX (来燕学), CHEN XL (陈小龙), LI GP (李国平), *et al*. Study on relations between spread of *Bursaphilenchus xylophilus* and slashes (松材线虫病流行与松树采伐剩余物关系研究)[J]. Journal of Zhejiang Forestry Science and Technology, 2001, 21(4): 30–33.

[8] LAI YX (来燕学), XU QY (徐企尧), CHEN XL (陈小龙), *et al*. Study on the relations between the epidemic spread of pine wilt disease and pest of pine caterpillars *Denrolimus punctatus* Walker (松材线虫病流行与松毛虫危害关系研究)[J]. Journal of Jiangsu Forestry Science and Technology, 2002, 29(1): 16–18.

[9] LAI YX (来燕学), ZHOU YP (周永平), YU LX (俞林祥), *et al*. The relations between the fire of pine forest and the epidemic of pine wilt disease (松林火灾对松材线虫病流行关系研究)[J]. Forest Research, 2000, 13(2): 182–187.

[10] LAI YX (来燕学). The morphology and differential variation of dauerlarvae (LIV) of *Bursaphelenchus xylophilus*

and B. *mucronatus* ((拟)松材线虫耐久型幼虫(LIV)的形态及其分化变异)[J]. Forest Research 2009, 22 (2): 294–299.

[11] LAI YX. Study on the Types and Symptoms of Pine Wilt Disease[C]. Proc. of Int. Sym. Beijing, China, 1995, 59–63.

[12] LAI YX (来燕学), ZHANG SY (张世渊), HUANG HZ (黄华正), *et al*. *Monochamus alternatus* withered pine (松墨天牛在松树枯萎中作用)[J]. Journal of Zhejiang Forestry College, 1996, 13(1): 75–82.

[13] LAI YX (来燕学). Detecting of parasites and microbes in the pine dying twigs (松树枯萎病枝条内的寄生虫和微生物群落)[J]. Journal of Nanjing Agricultural University, 2006, 29(1): 49–53.

[14] LAI YX (来燕学). Characteristic damage of *Monochamus alternatus* inoculated under the bark of pine tress (皮下接种松墨天牛卵及幼虫致死松树的研究)[J]. Chinese Bulletin of Entomology, 2005, 42(2): 193–195.

[15] LAI YX (来燕学), MA LF (马灵飞), CHI SY (池树友), *et al*. Comparing physical and mechanical properties of dead wood resulting from pine wilt disease and healthy wood (松材线虫病病死木木材物理力学性质测定 [J]. Journal of Zhejiang Forestry College, 2008, 25 (1):7– 11.

[16] LAI YX (来燕学), WANG YH (王亚红), ZHANG YF ((张毅丰), *et al*. Microwave equipment for killing *Bursaphelenchus xylophilus* and *Monochamus alternatus* in pine wood and its technical parameters (松材线虫病疫木除害的微波设备与技术参数)[J]. Journal of Northeast Forestry University, 2010, 38(9): 96–101.

[17] LAI YX (来燕学), ZHOU YP (周永平), YU LX (俞林祥), *et al*. Study on industry burning dead pines at cutting spots in pine forest to control pine wilt disease (林内就地火烧病死木防治松材线虫病试验) [J]. Journal of Jiangsu Forestry Science and Technology, 2000, 27(6): 28–32.

[18] LAI YX (来燕学), ZHOU YP (周永平), YU LX (俞林祥), *et al*. Effect of controlling *Bursaphelenchus mucronatus* by means of two-stage eradication in origin place in the east part of Ningbo (宁波市东部用2阶原点除害法防治松材线虫病效果评价)[J]. Journal of Zhejiang Forestry Science and Technology, 2001, 21(1): 51–53.

[19] LAI YX (来燕学), ZHOU YP (周永平), YU LX (俞林祥), *et al*. Techniques for eliminating of pests in files of trees endangered by *Bursapheienchus mucronatus* (松材线虫病树伐桩除害技术) [J]. Journal of Zhejiang Forestry Science and Technology, 1999, 19 (4): 52–55.

[20] LAI YX (来燕学), ZHOU YS (周岳松), ZHOU YP (周永平), *et al*. Application of contacted-breaking release microcapsules by aerial spraying for pine wilt disease controlling (触破式微胶囊剂在飞防松材线虫病中的应用)[J]. Journal of Nanjing Forestry University (Natural Science Edition), 2000, 24(2): 75–77.

[21] LAI YX (来燕学), ZHOU YP (周永平), YU LX (俞林祥), *et al*. An experiment report on controlling pine wilt disease by spraying pesticides named PEM (保松灵(PEM)农药防治松材线虫病试验) [J]. Journal of Jiangsu Forestry Science and Technology, 2000, 27(5): 44–47.

[22] LAI YX (来燕学), ZHOU YP (周永平). Controlling pine wilt disease by means of aircraft spray of PEM with ultra-low-volume (飞机超低量喷洒保松灵防治松材线虫病) [J]. Journal of Zhejiang Forestry College, 2002, 19 (3): 282–287.

[23] LAI YX (来燕学), YU LX (俞林祥), ZHOU YP (周永平), *et al*. Trapping adults of *Monochamus alternatus* with chemically-treated pine logs and branches (用双环诱杀松墨天牛成虫控制松材线虫病)[J]. Journal of Zhejiang Forestry College, 2001, 18(1):60–65.

[24] LAI YX (来燕学), *et al*. Trails on the parasitism of *Beauveria bassiana* or *Verticillium lecaniion* on larvae of *Monochamus alternatus* Hope (白僵菌×蜡蚧轮枝孢菌(BV制剂)对松墨天牛幼虫寄生试验初报)[J]. Journal of Jiangsu Forestry Science and Technology, 2003, 30(4): 7–9.

[25] LAI YX (来燕学), *et al*. Trials on killing beetles of *Monochamus alternatus* and controlling pine wilt disease with Manic-EC and mixtures of *Beauveria bassiana* × *Verticillium lecaniion* 苦烟制剂、BV制剂扑灭松墨天牛成虫及防治松材线虫病试验 [J]. Journal of Jiangsu Forestry Science and Technology, 2005, 32(1): 4–7.

[26] LAI YX (来燕学), LU G (陆高), WANG YH (王亚红), *et al*. Practice and effectiveness of integrated control to pine wilt disease in the scenic pine forest (松兰山景区松材线虫病综合治理研究) [J]. Journal of Jiangsu Forestry Science and Technology, 2003, 30 (6): 13–16。

[27] LAI YX (来燕学), YU LX (俞林祥), ZHOU YP (周永平), *et al*. First aid techniques and mechanism for dying pine trees damaged by pine wilt disease (松材线虫病濒死急救技术与救活机理)[J]. Journal of Zhejiang Forestry College, 2000, 17(4): 404–409.

[28] LAI YX (来燕学), *et al*. Pesticide toxicity and selection for the pine wood nematode (杀灭松材线虫的高效药物筛选与毒性测定)[J]. Journal of Zhejiang Forestry College, 2011, 28 (3): 479–485.

[29] LAI YX (来燕学), 何月秋 (HE YQ), 池树友 (CHI SY), *et al*. Concoction of effect anti-nematode drugs injected into pine tree for prevention of pine wilt disease and their effect in forest (高效松材线虫病预防注射药剂配制与林间药效研究)[J]. Acta Agricultureae Universitatis, 2016, 38(5): 871–878.

[30] LAI YX (来燕学), *et al*. Controlling pine wilt disease with PP-bags +*Sclerodrema* spp. and so on )用PP袋+肿腿蜂等防治松材线虫病技术试验)[J]. Journal of Jiangsu Forestry Science and Technology, 2002, 29(2): 18–20.

[31] LAI YX (来燕学), 王亚红 (WANG YH), 王小艺 (WANG XY), *et al*. A field test of controlling the pine wilt disease with the technique of isolating *Monochamus alternatus* on forest spot and releasing parasitoid (松褐天牛疫木隔离结合人工释放管氏肿腿蜂防治松材线虫病)[J]. Chinese Journal of Biological Control, 2012, 28(4): 460–466.

[32] LAI YX (来燕学), 杨忠岐 (YANG ZQ), 王小艺 (WANG XY), *et al*. Retarded development in *Monochamus alternatus* larva (Coleoptera Cerambycidae) by parasitoid *Sclerodermus guani* (Hymenoptera: Bethylidae) (管氏肿腿蜂诱导松褐天牛幼虫推迟发育现象的初步研究 [J]. Chinese Journal of Biological Control, 2015, 31(3): 305–311.

[33] LAI YX. Distribution of nematodes (*Bursaphelenchus xylophilus*) in the beetle *Monochamus alternatus* and its exiting transmission way [C]. PWD: A Worldwide Threat to Forest Ecosystems by Paulo Vieira, Manuel Moto; 2006, 243–254.

[34] LAI YX (来燕学). Distribution and monitor of nematode in adults body of pine sawyer (松墨天牛成虫解剖与松材线虫分布研究)[J]. Entomological Journal of East China, 2004,13(2): 21–26.

[35] LAI YX (来燕学), *et al*. Separation and detection of *Bursaphelenchus xylophilus* by methodofpne twigs +Petri-dish and microscope (松细枝+培养皿法分离镜检松材线虫试验)[J]. Journal of Jiangsu Forestry Science and Technology, 2003, 30(2): 13–15.

[36] LAI YX (来燕学). Quick detection of *Bursapheienchus xyiophilus* by method of dissecting pine twigs (用松枝解剖法快速检测松材线虫病原)[J]. Journal of Zhejiang Forestry College2005, 22(2): 188–192.

[37] LAI YX. The different effects of practicing two strategic ways to control the pine wilt disease[C]. Proc. of Int. Sym. Tokyo, Japan, 1998, 181–183.

**Responsible editor: Na LI**

**Responsible proofreader: Xiaoyan WU**