4
Pine Wilt Disease in China

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4.1 Introduction and Spread of the Disease

Pine wilt disease was first discovered in People's Republic of China in 1982 in Nanjing City, Jiangsu Province (Cheng et al. 1986). That year only 256 dead trees were found in the city. Subsequently, the disease has spread to 10 provinces and a city: Jiangsu, Zhejiang, Anhui, Guangdong, Shandong, Jiangxi, Hubei, Hunan, Yunnan, Guizhou Provinces and Chongqing City (Fig. I.7). The affected areas have reached nearly 80,000 ha and 50,000,000 trees have been killed by the disease. In recent years, the disease has spread quickly. The infected forest areas and the number of dead trees per year since 1982 are shown in Figs. I.8 and I.9. The disease threatens Huangshang and many other famous scenic spots and places of World Natural or Cultural Heritages; or both, and so, the disease has become a serious forest problem affecting local economies.

Since 1982, when the disease was identified in Jiangsu Province, it has spread in the next 10 years to several locations in Nanjing and Zhenjiang City and Wuxi City. In 1996, the disease was found in Suzhou City and, in 1998, in Changzhou City and Yangzhou City. Up to now, the disease has infested forests in 23 prefectures or cities in Jiangsu Province.

In Anhui Province, the disease was first found in 1988 in Maanshan City, He Prefecture and Mingguang City. Now occurs in the forests of 22 prefectures or cities, including Dangto, Chaqozhou, Xuancheng, Ningguo, Guangde, Chuzhou, Mingguang, Dingyuan, Laian, Quanjiao and Huainan Prefectures or cities in Anhui Province. At present damage occurs on 5,000 ha.

In Zhejiang Province the disease first appeared in a forest in Xiangshan Prefecture. Since then it has spread to 21 prefectures or cities, such as Dinghai, Diemu,

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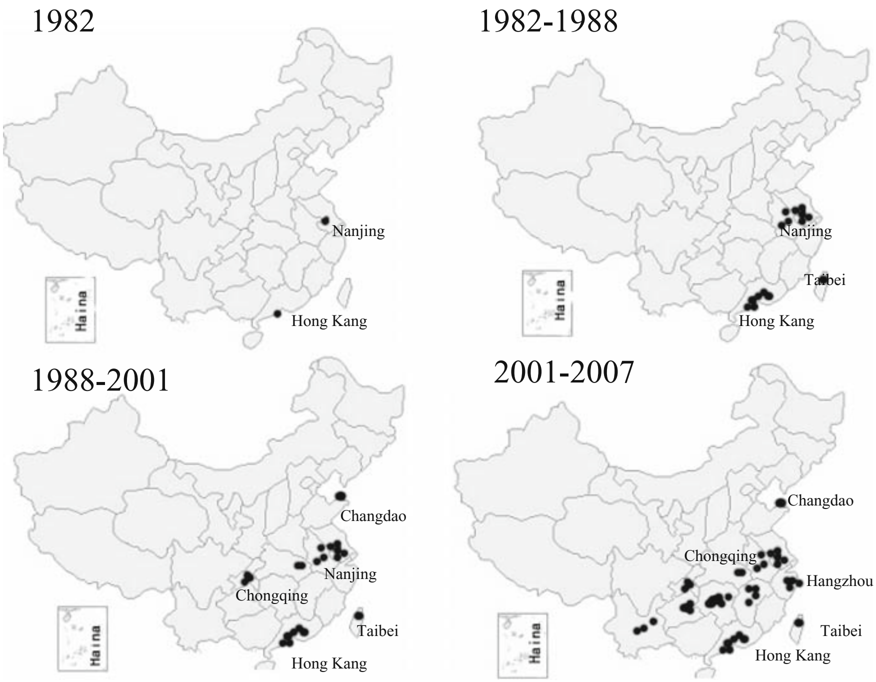

Fig. I.7 Distribution changes of pine wilt disease in China

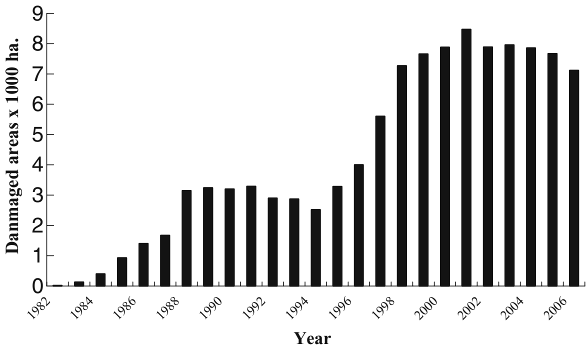

Fig. I.8 The annual areas damaged by pine wilt disease in mainland China (based on information from the Chinese Ministry of Forestry)

Ningbo, Ninghai and Hangzhou. In 2006, the total damaged areas reached 23,000 ha of forest and 53,540,000 trees have died of the disease in Zhejiang Province.

Pine wilt was first discovered in Guangdong Province in 1988. Up to now, damaged forests have been found in 15 prefectures, including Huizhou City, Dongguan City, Shenzhen City and its prefectures. The total affected area has now increased to 1,600,000 ha.

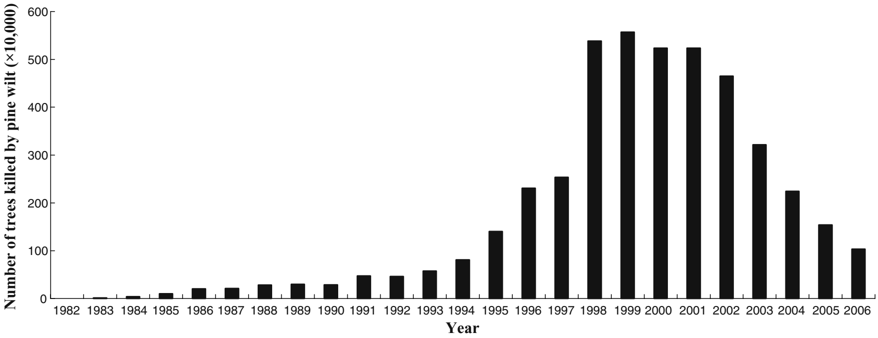

Fig. I.9 The annual number of trees killed by pine wilt disease in mainland China (based on information from the Chinese Ministry of Forestry)

In recent years, the disease has been found in several other provinces, Hubei Province in 2000, Chungqing in 2001, Jiangxi Province, Hunan Province, Guizhou Province in 2003, and Yunnan Province in 2004. The total damaged areas amount to 11,800 ha (all of these data have been provided by the Chinese Ministry of Forestry).

From the 1970s in Taiwan province, China, in the Northern Forest of the island, pines began to show wilt symptoms. In 1983, pine wilt disease was finally identified in Shimen Village of Taibei City. The damaged area has now reached 3,263 ha.

Five key factors determine occurrence of pine wilt disease: (1) yearly mean temperature, (2) the mean temperature in July, June and August, (3) latitude, (4) days above 25°C and (5) precipitation. Based on these criteria the southeast part of mainland China, is a potentially suitable area for occurrence of the pine wood nematodes (PWN), *Bursaphelenchus xylophilus* (Lu et al. 2005; Zhang et al. 2006). If a line were drawn from Beijing to Yunnan Province the southeast part of China below that line is potentially suitable for pine wilt occurrence.

4.2 Strategies to Control Pine Wilt Spread

The main control measures taken after the finding of pine wilt in China in 1982 are described below.

4.2.1 Quarantine Measures

After the disease was found in Nanjing in 1982, the Chinese government strengthened quarantine measures at Chinese ports of entry and established quarantine stations within China to prevent the movement of infected logs, lumber and wood products from infected to uninfested areas. These measures played an important role in stopping and delaying the spread of the disease (Li and Zhao 2006).

4.2.2 Monitoring the Disease

Traps with artificial attractant lures for monitoring the pine sawyer were developed and used in the disease management program. Several Synthetic formulations of lures for *Monochamus alternatus* have been developed and are used for controlling the disease (Liu et al. 2003; Wang et al. 2006).

4.2.3 Chemicals Used to Control the Disease

In some provinces, insecticide sprays or trunk injection for trees in affected forests has resulted in some success; however, such insecticides have resulted in harmful side effects to the environment. For example, the populations of certain birds and some beneficial insect parasites and predators have been reduced by the sprays. These side effects led to the government decision in the 1980s to stop spraying chemical insecticides for controlling pine wilt in Nanjing. Several new formulations of insecticides were developed, such as the spray formulation, Baosongling, and a microcapsule formula of insecticides, Green Mines, and the injection formulation Chongxianqing (Huang 2001). Because they are more environment friendly and high efficiency, they are used operationally in some severely affected forests to control pine sawyers.

4.2.4 Removal of Dead and Weakened Trees in the Affected Forests and Establishment of Isolation Belts Around Such Forests

Upon entry of the disease into China the main control measure was to remove dead trees from infested forests. In 1999, the Chinese government launched a project to manage pine wilt disease. The measures taken were mainly to treat and remove the dead trees in the infested forests and to monitor the pine sawyer populations. In Guandong Province 449,286 trees were killed in 1997 before these measures were implemented, however use of these practices resulted in fewer trees being killed by 35.2% in 2003 (Ye et al. 2005). Removing dead trees from affected forests and fumigation or burning the dead lumber to kill the pine sawyer larvae have been also very effective. In some severely damaged forests all the pine trees were clear cut. Those measures resulted in the reduction of both dead trees and infested forest areas in the two provinces (Pan 2000). By the turn of the century, the Chinese

Ministry of Forestry had taken strong measures to remove and treat dead trees in wilt-affected forests. These measures have led to a substantial reduction in numbers of diseased trees (Fig. I.9).

To protect parks, areas around historic buildings and so forth the government decided that cultural sites, such as Huangshan, a World Natural and Cultural Heritage site, to establish a 4-km wide, 100-km long pine tree-free strip around the mountain. Within this belt, all the pine trees were felled (Zhang 2005). The belt has had a major effect in protecting the forests inside the area from the disease; however, it could not stop PWN resulting from human activity.

There are 33,330,000 ha of forests in South-east of China of which 80,000 has been damaged, that is, 0.24% of the total forest area, the task of protecting the remaining healthy forest is still tough.

4.3 Research on PWN and its Vector Insect, *Monochamus alternatus*

After pine wilt was discovered in China, considerable time and research has been devoted to the pathogen and its insect vector. Over 400 papers on pine wilt disease have been published in Chinese (Ning et al. 2005). That information is summarized below.

There are 24 species or subspecies of the genus *Monochamus* in China, the most common being *M. alternatus*, *M. saltuarius* and *M. sutor* (Ning et al. 2004).

Research on the control *M. alternatus* by using biological agents (Table I.2) focused on, the parasite wasp, *Scleroderma guani*, and this parasite has been used operationally (Song et al. 1998; He et al. 2006; see Part VI). Techniques such as artificial rearing in forests have been studied and protocols established (Yang et al. 2005; Zhou et al. 2005). In the subtropical forests, the wasp can be established locally and it has long-lasting benefits (Song et al. 1998; Zhou et al. 2005).

Other biological control agents, which have been studied in detail include *Beauveria bassiana*, and *Metarrhizium anisopliae*, *Syncephalastrum racemosum* and *Hypomyces* sp., which produces metabolites that inhibit the development of plant nematodes including PWN (Mo et al. 2002; Ning et al. 2005). Strains of *B. bassiana* have been selected for their pathogenicity to *M. alternatus* (Zhang et al. 2000). The fungus in mixture with other agents has been used for a long time to control forest insect pests in China (Zhang et al. 2000; Lai et al. 2003).

Plant secondary metabolites were also examined for nematicidal chemicals for use against the PWN. *Sophora alopecuroides*, a desert shrub that grows in north-west China, contains high amounts of quinolizidine alkaloids. Zhao (1996) reported that aloperine, one of the alkaloids from the plant, was very toxicity to the PWN at LC50, 2.63×10^5 g. The molecular mechanism of the high toxicity of aloperine was also hypothesized (Zhao 1999) and later demonstrated experimentally (Li et al. 2000). Tests of the chemical showed that treated pines could be effectively protected (Zhao et al. 1998).

Table I.2 New control measures against *Monochamus alternatus* in China

	Species	Effects	Application	References
Parasite insects	*Scleroderma guani*	Wasps parasitize larvae of beetles	In the first year, 15,000–18,000 wasps hm^{-2} were released in Guangdong, and mortality was reduced upto 85.16–92.24% the next year.	Song (1998) and Zhang and Yang (2006)
	Dastarcus longulus	Pupae and larvae of *M. alternatus* can parasitize beetle	Large-scale field tests have not been done yet.	Yang (2004) and Zhang and Yang (2006)
Fungi	*Beauveria bassiana* *B. brongniatii* *B. tenella* *Metarhizium* sp. *Isaria farinose* *Aspergillus flavus* *Verticillium* spp. *Acremonium* sp.	Both adults and larvae can be infected with them, causing death	Spray the spores in forests	Zhang et al. (2000)
Insecticide	Green mine	Contact-breaking release microcapsules of pesticides	Spray	Tang et al. (1999) and Yan et al. (1999)

Other extracts from certain plants were also studied (Table I.3); however, no other active anti-PWN compounds have not been isolated or identified before 2003 (Liu et al. 2003).

The mechanism of pine wilt disease has been one of the main research projects supported by the Natural Science Foundation of China. Although some scientists still think PWN is the only pathogen causing wilting and tree death, this one-pathogen theory has been challenged by a new hypothesis that the disease is caused by PWN and the symbiotic pathogenic bacteria that it carries (Zhao et al. 2003). The new theory was proposed followings a series of inoculation experiments (Zhao et al. 2003). According to the new hypothesis bacteria carried by the nematode play an important role in the disease; therefore, control of those bacteria should result in reducing PWN pathogenicity. Experiments in which Japanese black pine or Masson pine seedlings were treated with antibiotics showed that such treatments delayed wilting (Zhao et al. 2000a; Chen et al. 2003).

Table I.3 Natural compounds in scientific research to control pine wood nematode (PWN) in China

	Species	Compounds	Toxicity	References
Fungi	*Syncephalastrum racemosum*	Water soluble	All PWNs died when cultured with the fungus	Sun (1997), and Zhou et al. (2005)
	Lampteromyces japonicu *Pleurotus spodoleucus* *P. corticatus* *P. ferulae* *P. memberancens*	Unknown toxins	>90% PWNs died when cultured with these fungi	Dong et al. 2000
	Streptomyces	Ramification of peptide acylcytosine nucleotides	PWN mortality 100% within 4 h at 144 μg ml^{-1}	Song et al. (2000)
	Basidiomycetes YL14	Unknown toxins	>90% PWNs died when cultured with the fungus	Li and Zhang (2001)
Antibiotics		Mixture of antibiotics	Inhibitory effects on symptoms of the disease	Zhao et al. 2000 Chen et al. 2003
Plants	*Sophora alopecuroide*	Aloperine	LC50 = 2.63×10^{-5} g ml^{-1}	Zhao (1996, 1999), and Zhao et al. (1998)
	Derris elliptic *Cephalotaxus fortune* *C. sinensis* *Paeonia suffruticosa* *Sophora viciifolia* *Dendranthema indicum*	Unknown toxins	Inhibitory effects on reproduction of PWN	Wen et al. (2001)

To identify dead trees or lumber from pine wilt killed trees a method has been devised for checking the pH of wood. The technique based on the observation that the pH of tissue from trees that are killed by the disease is significantly lower than that of healthy trees (You et al. 1994; Wang et al. 2001). Also several methods have been studied for identifying PWN. These include use of DNA probes, however, other techniques including PCR identification methods, including PCR–RAPD, PCR–RFLP and PCR–SSCP techniques and satellite DNA techniques have been developed (Wang 2004), but some problems still exist in using these techniques on a large scale, for example, their cost and accuracy are among the main obstacles to use in disease management.

Many fungi have been tested for PWN control in China, including some that produce nematicidal chemicals (Liu et al. 2003); however, most of their active components have not been isolated and identified. More research is needed in this area.

4.4 Research on Resistance of *Pinus massoniana* to Pine Wilt Disease

Because Masson pine, *Pinus massoniana*, grows well in the arid, sandy soil and the dry climate of certain areas of southeast China it is a major forest species in those areas. Before 1982, when pine wilt was first found in China some scientists thought that Masson pine was resistant to the disease; however, subsequently it has been shown to be only moderately resistant, and many Masson pines have been killed by the disease. Research showed that when Masson pines reached age older than 16 years that they begin to gradually lose their resistance to the disease (Wang et al. 2006). Recently, trees younger than 10 years old were also found to be killed by pine wilt. This indicates that the resistance of Masson pine or virulence of the pathogens are changing. *P. massoniana* resistance to the disease has been related to the tree growth rate, some metabolites, like phenolic compounds and less content of free amino acids in the resistant tree species (Xu et al. 2000).

Plant hormone, calcium, salicylic acid and ammonium were tested to increase *P. massoniana* wilt resistance. Applying calcium alone or combined with ammonium were shown to be the best treatments for increasing resistance to the disease (Ge et al. 1999).

Inoculation of trees form 40 Masson pine strains collected from South-east China showed that all of three resistant strains originated from Guangdong and Guangxi Provinces along China's-southern coast (Xu et al. 2000). Interestingly, the most virulent PWN strains were also reported to come from Guangdong Province (Gao et al. 2005).