Research paper

Prevention of Pine Wilt Disease by Soil Injection with Fosthiazate

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【Summary】

Pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Bührer 1986), has caused serious pine disease in Taiwan since 1985. Soil injection with 75% fosthiazate to control pine wilt disease was conducted at 3 locations in northern Taiwan. Twenty healthy pine trees with diameter breast height (DBH) ranging 20~40 cm were selected by oleoresin exudation for the inoculation test. Ten trees were treated with 75% fosthiazate by soil-injection application in May 2004 according to the manufacturer's protocol and a DBH table; another 10 pine trees were used as controls. One month after the soil injection, all trees at 3 locations were artificially inoculated with 30,000 pinewood nematodes (*B. xylophilus*). Symptoms of pine wilt disease were first observed 3 mo after inoculation; the disease progressed rapidly from September 2004. Chemical protection efficacy was evaluated 9 mo after inoculation. For chemical-treated trees, disease incidences at Zhongli, Daxi, and Zhudong were 40, 30, and 20%, and values of the disease severity index were 2, 1.1, and 1, respectively. However, for the non-treated group, disease incidences were 80, 80, and 70%, and values of the disease severity index were 4.0, 4.0, and 3.5, respectively. Our results demonstrated that fosthiazate not only decreased the incidence of pine wilt, but also retarded the disease progression.

Key words: pine wood nematode (PWN), fosthiazate, soil injection, disease control.

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研究報告

福賽絕土壤注射法於松材線蟲病之預防

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摘 要

自1985年起由松材線蟲所引起的松樹萎凋病一直是台灣松樹嚴重的病害。2004年5月至2005年3月間，分別在台灣北部三個地點，進行土壤灌注系統性殺線蟲劑的試驗，以評估藥劑對松材線蟲萎凋病的防治效果。試驗採完全逢機設計，每一試驗區包括20棵松樹，10棵灌注藥劑當為處理組，另10棵不灌注藥劑當為對照組。這些松樹皆經流脂診斷法確定未遭松材線蟲(*Bursaphelenchus xylophilus*)感染，處理組的松樹，每棵以氣體加壓方式，將75%福賽絕灌注入土壤，藥劑用量依樹之胸徑大小而定，對照組則未做任何處理。藥劑灌注一個月後，將人工培養的三萬隻松材線蟲以鑽孔接種法接種到所有供試松樹，每棵松樹以鑽孔接種法接種三萬隻松材線蟲。接種後，病徵於9月間開始陸續出現，經過9個月後，以藥劑保護的松樹在中壢、大溪、竹東三個試驗地點，其發病率分別為40、30和20%，罹病程度指數則為2、1.1和1；而未處理組的松樹，其發病率分別為80、80和70%，罹病程度指數則為4、4和3.5，試驗結果顯示75%福賽絕具有良好的保護效果，不僅降低松材線蟲萎凋病的發病率，也可延緩病勢的進展。

關鍵詞：松材線蟲、土壤注射法、福賽絕、疾病控制。

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INTRODUCTION

Pine wilt disease (PWD) is caused by the pine wood nematode (PWN), *Bursaphelenchus xylophilus* Nickle (Steiner and Bührer 1986) and was demonstrated to induce irreparable damage to forested ecosystems. The disease was first reported in North America and is now distributed in Japan, Korea, China, Taiwan, and Portugal (Togashi and Matsunaga 2003). PWD has been a serious pine disease in Taiwan since 1985 (Kosaka et al. 2001).

The PWN gains access to woody tissues through wounds on twigs made by the Japanese pine sawyer beetle (JPS, *Monochamus alternatus*) (Kobayashi et al. 1984). Some fungi were considered nutrient sources of PWN-vectoring beetles (Hyun et al. 2007). Beetles carry nematodes and fly to healthy pine trees (genus *Pinus*; subgenus *Pinus*) thereby infecting them. Most infected trees display a reduction or cessation of resin exudation on the trunk, reddish-brown needles, and death in less than a year (Togashi and Shigesada 2006, Roriz et al. 2011). There is no effective treatment for PWD once a susceptible tree becomes infested with the pinewood nematode (Gaofu et al. 2008). Current methods are based on killing the JPS by spraying an insecticide, burning or chipping wilted pines, silvicultural control, biological control, or controlling the nematode by injection of tree trunks or fumigation with an anti-nematodal compound (Kosaka et al. 2001, James et al. 2006, Kim et al. 2011, Kwon et al. 2011). Some traditional methods

of controlling the disease have some limitations, such as time, considerable labor, and easy loss of desirable economic characters (Gaofu et al. 2008). Therefore, developing new technologies to prevent PWD is needed. Our goal was to develop an effective high-pressure soil-injection technique for pine wilt prevention. We determined the effectiveness of a fosthiazate injection in the soil to prevent PWD in field trials. The second objective was to assess whether fosthiazate could be successfully absorbed into pines using a high-pressure injection technique.

MATERIALS AND METHODS

Isolation of PWN

The PWN, *B. xylophilus*, was kindly obtained from Dr. Tung-Tsuan Tsay at the Plant Nematology Laboratory, Department of Plant Pathology, National Chung Hsing Univ. (Taichung, Taiwan). The nematodes were reared on the fungus, *Botrytis cinerea* (Pers:Fx), cultured on potato dextrose agar in Petri dishes at room temperature for 21 d, and then extracted using the Baermann funnel technique (Yang et al. 2003) at room temperature for 48 h. A nematode suspension was concentrated by low-speed centrifugation (1000 x*g*, 3 min). Isolated PWNs were adjusted to 10,000 PWNs ml^{-1} in water and used in the inoculation experiments.

Field studies

Experiments were carried out on pines (*Pinus elliottii*) at the Longgang 6th Army (Zhongli, Taiwan), Chung Cheng Institute of Technology (CCIT), National Defense Univ. (Daxi, Taiwan), and Industrial Technology Research Institute (ITRI; Zhudong, Taiwan) on May 2004 to May 2005 due to their history as locations of PWD. Twenty health pine trees at each location were selected by pine oleoresion exudation, visual symptoms of PWD, and the Baermann funnel technique.

Fosthiazate application

The injection was performed according to the diameter at breast height (DBH) at a 130-cm height from the ground (Table 1). Fosthiazate (O-ethyl-S-(1-methylpropyl) (2-oxo-3thiazolidinyl) phosphonothioate) (ISK, Taipei, Taiwan) at 75% was diluted 125 times according with manufacturer's protocol. The diluted fosthiazate was injected into the soil at a distance of 1~1.5 m from the tree base at 30~35 cm in depth in May 2004. Water was injected as the control group.

Inoculation experiments and symptom detection

One month after soil injection, nematodes were injected into the pine trees to assess the effect of fosthiazate. Inoculation involved drilling a 6.5-mm-diameter hole to a depth of 5 cm at 130 cm in height from the ground, and 3 ml of the nematode suspension (30,000 nematodes) was pipetted into the hole. The wound was then capped with a wood bung. After inoculation, symptoms were observed and recorded every 2~4 wk. The efficacy of chemical prevention was evaluated

Table 1. Tree diameter breast height (DBH) and the amount of fosthiazate usage

DBH (cm)	Amount of 75% of fosthiazate (L)	DBH (cm)	Amount of 75% of fosthiazate (L)
< 15	3.2	31~35	15
16~20	4.8	36~40	20
21~25	7.2	41~45	25
26~30	11	45~50	32

9 mo after inoculation. Visual symptom ratings for disease severity were assigned on the following rating scale: 0 healthy, (1) 1~20% dieback, (2) 21~40% dieback, (3) 41~60% dieback, (4) 61~80% dieback, and (5) 81% to complete mortality. The disease severity = Σ (disease index × the number of pine trees with the disease index)/(total number of pine trees investigated × 5) × 100.

PWNs were extracted from infected branches with the Baermann funnel technique to check if the prevention process was effective and if the nematodes had survived. The disease incidence (%) was calculated by the formula: (infected pine trees/total pine trees inoculated) × 100.

Statistical methods

The data obtained were first analyzed with an analysis of variance (ANOVA) in the SAS program (SAS, Cary NC, USA). Treatment differences were tested by Duncan's multiple-range comparison ($p < 0.05$).

RESULTS AND DISCUSSION

Before the inoculation experiments, PWNs were not isolated from branches, and symptoms were not observed in the selected pine trees. After inoculation, symptoms and PWNs were found in the fosthiazate-treatment groups and control groups (Tables 2, 3). The disease progressed rapidly from September 2004. In fosthiazate-treated trees, the range of disease incidence was 20~40%. The range in the control groups was 70~80%. Values of the disease severity index in the chemical-treated groups at the 6th Army, CCIT, and ITRI were 2, 1.1, and 1 and were 4, 4, and 3.5 in the control groups, respectively. Duncan's analysis showed that significant differences were observed in the fosthiazate group.

In developing a soil-injection technique to control PWD, it is necessary to identify a chemical material that is widely available to formulate a water-soluble injection. Fosthiazate is a relatively novel organophosphorus nematicide which is registered in Europe and the US as a fumigant for controlling and protecting against nematode-induced diseases in crops. Hence, we used a dilution of 75% fosthiazate to diffuse to every part of the tree from root uptake at an effective concentration and kill the nematodes. However, fosthiazate

Table 2. Results of pine wilt disease incidence after the inoculation experiment

Treatment	Pine wilt disease incidence (%)			
	6th Army	CCIT	ITRI	Duncan's test (5%)
75% fosthiazate	40	30	20	[a]
Control group: water	80	80	70	[b]

[a)] Means in a column significantly differ according to Duncan's multiple range test ($p \leq 0.05$).
[b)] Means in a column do not significantly differ according to Duncan's multiple range test ($p \leq 0.05$).

Table 3. Results of pine wilt disease severity after the inoculation experiment

Treatment	Pine wilt disease severity			
	6th Army	CCIT	ITRI	Duncan's test (5%)
75% fosthiazate	2.0	1.1	1.0	[a]
Control group: water	4.0	4.0	3.5	[b]

[a)] Means in a column significantly differ according to Duncan's multiple-range test ($p \leq 0.05$).
[b)] Means in a column do not significantly differ according to Duncan's multiple-range test ($p \leq 0.05$).

is generally weakly degraded and adsorbed in the soil, so it may contaminate groundwater resources or agricultural products under certain soil conditions (Karpouzas et al. 2007, Lee et al. 2011). It should be noted that fosthiazate residues in some well-managed pine forests such as those used for producing Matsutake mushrooms or those growing in tourist areas could possibly cause harm to humans.

We also found that infected pine trees were detected after the soil injection. This can be explained by the selected "healthy" trees being infected. A previous study indicated that in newly infested trees, the population of PWN is very low and difficult to detect using the traditional Baermann funnel extraction of wood from discs cut out of trees at breast height (Zhao et al. 2009). Fosthiazate may be a good candidate for PWD prevention, but it is not a good cure for PWD. Another factor which may affect the efficacy of fosthiazate concerns the installation points. We recommend that with a large DBH, multiple points be used for fosthiazate soil-injection in order to obtain good diffusion in the pine tree.

In summary, the 21st century is the age of a green environment. "Eco" is becoming a key point around the world. Most pine trees are valuable and highly coveted in Taiwan. We provide a high-throughput, simple, and safe soil-injection method to prevent PWD. This method gave reliable results and should greatly assist in protecting healthy pine forests.

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