STEM CANKER OF ONE-YEAR-OLD *PINUS RADIATA*

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ABSTRACT

After misting for 24 hours, hardened and dehardened 1-year-old plants of Pinus radiata *D. Don were inoculated with* Pseudomonas syringae *pv.* syringae *van Hall and exposed to frosts 2°C warmer than the lethal frost temperatures. Plants which were both inoculated and frosted developed significantly more stem cankers than those either inoculated only or frosted only.*

INTRODUCTION

In the past decade there have been several reports of a canker disease causing mortality of 1-year-old *Pinus radiata* plants in the central North Island during the winter months. A survey of two sites in the winter of 1977 showed that, over an area of approximately 200 ha, 35% of the plants had cankers and 50% of these were dead because the canker had girdled the stem.

The cankers were commonly located on the lower stem of the plants, usually between 10 and 30 cm above the ground. Cankers varied in size from very small sunken necrotic patches to large resinous lesions completely girdling the stem. In the latter, foliage discoloration, wilting, and finally death of the plant occurred. Frequently the foliage showed some signs of frost damage (needle scorching, dead buds). Older trees on the same sites were usually unaffected, although occasionally cankered 2-year-old trees were found. Those plants not completely girdled which survived the winter months seldom showed any further deterioration. Callus tissue began to cover the cankered area with the onset of spring growth and, after 3 or 4 growing seasons, even trees which had been 75% girdled showed no sign of the previous damage, although stem breakage sometimes occurred at the weakened point.

Isolations from cankers in an early stage of development consistently yielded *Pseudomonas syringae* pv. *syringae* van Hall 1902, an organism reported to cause dieback of the terminal

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shoot in nursery seedlings of *P. radiata* (Langridge and Dye, 1982). When 6-month-old seedlings of *P. radiata* were sprayed with a suspension of the organism isolated from the cankers and subjected to –5°C for 8 hours, symptoms of terminal dieback, identical with those described by Langridge and Dye (1982) developed. Sunken necrotic patches developed on stems of 1-year-old *P. radiata* when lightly pricked with a fine needle and sprayed with a suspension of *P. syringae.*

A relationship between *P. syringae* and low temperatures has frequently been observed and frost was considered to play an important part in the canker and dieback diseases of poplar, peach and apricot (Sabet, 1956; Davis and English, 1969; Klement *et al.*, 1974; Weaver, 1978; de Kam, 1982). Langridge and Dye (1982) reported that a minimum of 24 hours' wetness, followed by a frost, appeared to be the optimal conditions for the infection of *P. radiata* seedlings by *P. syringae.* Field observations from 1977 to 1980 indicated that bacterial cankering is usually found on grassy, fertile sites (frequently converted farmland) and affected trees were concentrated in frost hollows or on frost flats. The work reported in this paper was undertaken to investigate the relationship between low temperatures, *P. syringae,* and the formation of cankers.

MATERIALS AND METHOD

Plant Material

The *P. radiata* seedlings were prepared 12 months in advance to simulate as closely as possible the physiological condition of 1-year-old plants in the field. Two hundred seedlings were lifted from the nursery bed in July and potted into 22 cm pails which were buried up to the rim in the ground and left for 12 months. Six days before the experiment began, half of the total number of plants were dehardened by placing them in a controlled environment room with day/night temperatures of 16/8°C and photoperiod of 8 hours. The remaining plants were left outside (winter conditions — day/night temperatures of the order of 10/4°C). Forty hardened and 40 dehardened plants were tested, 10 at a time, in a frost chamber at different temperatures (–8, –10, –12 and –14°C for hardened plants, and –6, –8, –10 and –12°C for dehardened plants) to determine the lethal frost temperatures. The leachate electroconductivity test (Aronsson and Eliasson, 1970) was used to estimate the frost damage. Plants averaged 1 metre in height when treatments were applied.

Inoculum and Inoculation Procedure

A strain of *P. syringae* pv. *syringae* known to be pathogenic to *P. radiata* (Plant Diseases Division Culture Collection 5766) was grown on fresh glucose-yeast-chalk-agar (GYCA) slants and incubated for 48 hours at 27°C. Colonies were washed from the agar surface with sterile water to form a suspension with approximately 10^9 bacteria/ml. Each inoculated plant was sprayed with 75 ml of suspension, sufficient to give a film over the entire stem and the foliage. For 24 hours prior to inoculation plants were placed under a spray system regulated to keep the foliage moist without runoff. Non-inoculated plants were also misted for 24 hours. Frost treatments were applied immediately after misting and inoculation.

Frost Treatments

Plants were frosted at temperatures 2°C warmer than the lethal frost temperature as determined by the test frosts. The temperature was lowered from 10°C to the test temperature over 8 hours, held at the test temperature for 8 hours and then raised back to 10°C at a rate of 5°C/hour. There were 15 plants/treatment and the treatments applied are given in Table 1.

TABLE 1: EFFECT OF INOCULATION WITH *P. SYRINGAE* pv. *SYRINGAE* AND FROSTING ON DEVELOPMENT OF STEM CANKERS

Treatment	*Inoc.*	*Frosted (°C)*	*Condition of Plants*	*No. of Plants at End of Expmt.*	*No. of Plants With Cankers*†	*Ave. No. of Cankers**	*Ave. Lgh. of Cankers (mm)*
1	+	–10.5	hardened	9	8a	3.8c	29.5
2	+	– 8.3	dehardened	9	8a	2.5c	28.5
3	—	–10.5	hardened	13	2b	0.15d	10.0
4	—	– 8.3	dehardened	11	1b	0.27d	16.5
5	+	no	hardened	15	1b	0.33d	30.0
6	+	no	dehardened	15	3b	0.26d	10.0
7	—	no	hardened	15	0b	0d	0

* Figures with a common letter (c, d) not significantly different at $P=0.05$ (Scheffé contrast) between treatments.

† Figures with a common letter (a, b) not significantly different at $P=0.01$ (χ^2 test) between treatments.

Growth Conditions after Treatment

After the frost treatments, half the plants from each treatment were placed in a growth room maintained at a day/night temperature of 16/8°C, vpd of 2/2 mb and photoperiod 8 hours, and the remaining plants were placed in another growth room maintained at a day/night temperature of 10/6°C, vpd of 1/2 mb and photoperiod of 8 hours.

Observations and Measurements

Twelve weeks after treatment plants were removed from the growth rooms, examined for canker development and assessed for frost damage on a 0-5 scale (Menzies and Holden, 1981) as follows:

0 No damage
1 Some needles reddening
2 10-30% needles killed
3 40-60% needles killed
4 70-90% needles killed, buds dead
5 dead

Examination of canker development entailed careful stripping of the bark to expose the cambium. Measurements were made of the length of necrotic lesions and the distance of the centre of the lesion above the soil.

RESULTS

Lethal frost temperatures could not be assessed accurately: as a result the treatment frosts were a little too severe and a number of plants died within a week of treatment. These showed typical frost damage symptoms: buds killed, needles scorched and dead, no cankers. Plants scoring 4 or 5 were discarded. The experiment was therefore completed with a different number of trees in each treatment. Growth conditions after frosting had no significant effect on the number or length of cankers within each treatment. The results from the two growth rooms were therefore combined for each treatment and are in Table 1.

In treatments 1 and 2 where plants were both inoculated and frosted, more trees died (6 in each treatment) with typical frost damage symptoms than in treatments 3 and 4 (a total of 6 deaths) where plants were frosted but not inoculated. No deaths occurred in the unfrosted treatments 5-7 and these plants all scored 0.

Cankers developed on 8 of the 9 plants in treatments 1 and 2 (inoculated and frosted), though only on 2 plants in treatment 3 and 1 in treatment 4 (not inoculated). Of the unfrosted plants 1 in treatment 5 (hardened) and 3 in treatment 6 (dehardened) developed cankers. Plants which were neither inoculated nor frosted (treatment 7) did not develop any cankers. The number of cankers per plant was significantly greater in treatments 1 and 2 than in any other treatment, and average length of cankers (with the exception of 1 plant in treatment 5) was also significantly greater in these treatments. No girdling cankers developed in any treatment and there was no mortality which could be attributed to canker formation.

DISCUSSION

Although disease development did not exactly parallel what has been observed in the field, a clear relationship between frosting and canker formation in the presence of *P. syringae* pv. *syringae* was established in this trial. In the absence of the bacterium there was virtually no injury when plants were frosted at temperatures warmer than the lethal frost temperature.

Views on the nature of the relationship between frost and infection by *P. syringae* have varied. Sabet (1956) stated that, although freezing increased the damage caused by a well-established infection of poplar, freezing prior to infection had no appreciable effect on the establishment of the disease. However, Davis and English (1969) concluded that frost damage probably preceded infection. Klement *et al.* (1974) found that more damage to apricots occurred during cold winters and that necrosis of phloem and cambian only occurred when the population of *P. syringae* had time to increase at warmer temperatures before the frosts.

The potential for involvement of *P. syringae* in promoting the freeze injury of plant cells was demonstrated by Arny *et al.* (1976) with their work on corn leaves. *P. syringae* is active as an ice nucleus; this initiates ice crystallisation and thus frost damage. They found that leaf tissue sprayed with a suspension of *P. syringae* was injured at temperatures not low enough to injure non-inoculated plants. The ability of bacteria to act as ice nuclei appears to be restricted to some species of the genera *Pseudomonas* and *Erwinia* (Paulin and Luisetti, 1978). This phenomenon may have been at least partly responsible for some of the frost damage incurred in this trial.

Cankered plants frequently occur in hollows and on flats which are prone to the ponding of cold air, and on grassed sites rather than cleared ground. The presence of ground cover can have the effect of lowering the minimum temperature by as much as 4°C in comparison with a site where all vegetation has been removed (Menzies and Chavasse, 1982). Menzies and Chavasse (1982) also found that, although on cultivated ground there was not much change in temperature with increasing height above the ground, on uncultivated sites with ground cover the minimum temperature occurred at 20 cm above the ground. Field observations indicate that cankers normally occur between 10-30 cm above ground.

Although no significant difference in results from the two post-treatment temperature regimes was found, the differences in temperatures applied were not very great. Further experimentation with a wider range of temperatures may show a positive effect of temperature on disease development.

It is possible that the sequence of weather conditions is very important in the development of this disease. Langridge and Dye (1982) observed that a minimum of 24 hours foliage wetness prior to frost was optimal for the development of terminal die-back of *P. radiata* seedlings caused by *P. syringae* pv. *syringae.* Shaw (1935) showed that flooding of the intercellular spaces of apples and pears with water increases the growth rate of the bacterial pathogen, causing fire-blight disease. Also Ivory and Whiteman (1978) have shown that plants with high turgor pressure are less resistant to frost damage than those with low turgor pressure, and Arny *et al.* (1976) found that corn plants held under mist for 24 hours before freezing had significantly more frost damage than those left dry.

These reports considered in conjunction with field observations and results of this work suggest that rain followed by a frost or frosts are the conditions most likely to result in cankering of *P. radiata.*

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REFERENCES

Arny, D. E.; Lindow, A. E.; Upper, C. D., 1976. Frost sensitivity of *Zea mays* increased by application of *Pseudomonas syringae. Nature, 262*: 282-4.

Aronsson, A.; Eliasson, L., 1970. Frost hardiness in Scots pine (*Pinus sylvestris* L.) 1. Conditions for test on hardy plant tissues and for evaluation of injuries by conductivity measurements. *Studia Forestalia Sueccia,* Nr 77.

Davis, J. R.; English, H., 1969. Factors related to the development of bacterial canker in peach. *Phytopathol., 59*: 588-95.

de Kam, M., 1982. Damage to poplar caused by *Pseudomonas syringae* in combination with frost and fluctuating temperatures. *Europ. J. For. Pathol., 12*: 203-9.

Ivory, P. A.; Whiteman, P. C., 1978. Effects of environmental and plant factors on foliar freezing resistance in tropical grasses. 1. Precondition factors and conditions during freezing. *Aust. J. Agric. Res., 29*: 243-59.

Klement, Z.; Rozsnyay, D. S.; Arsenyevic, C. M., 1974. Apoplexy of apricots. III. Relationship of winter frost and the bacterial dieback and canker of apricots. *Acta Phytopathologica Academiae Scientarum Hungaricae, 9*: 35-45.

Langridge, Y. N.; Dye, D. W., 1982. A bacterial disease of *Pinus radiata* seedlings caused by *Pseudomonas syringae. N.Z. Jl Agric. Res., 25*: 273-6.

Menzies, M. I.; Chavasse, C. G. R., 1982. Establishment trials on frost-prone sites. *N.Z. Jl For., 27*: 23-49.

Menzies, M. I.; Holden, D. G., 1981. Seasonal frost-tolerance of *Pinus radiata, Pinus muricata* and *Pseudotsuga menziesii. N.Z. Jl For. Sci., 11*: 92-9.

Paulin, J.-P.; Luisetti, J., 1978. Ice nucleation activity among phytopathogenic bacteria. *Proc. 4th Int. Conf. Plant Pathogenic Bacteria, Angers*: 725-31.

Sabet, K. A., 1956. Studies on the bacterial dieback and canker disease of poplar. *Ann. Appl. Biol., 40*: 645-50

Shaw, L., 1935. Intercellular humidity in relation to fire-blight susceptibility in apple and pear. *Cornell Univ. Agric. Exper. Sta., Memoir 181*.

Weaver, D. J., 1978. Interaction of *Pseudomonas syringae* and freezing in bacterial canker on excised peach twigs. *Phytopathol. 68*: 1460-3.