Etiology, Symptomology, Epidemiology, and Control of Naemacyclus Needlecast of Scotch Pine

B. R. Kistler and W. Merrill

Research Assistant and Professor, respectively, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Contribution No. 956, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication 27 May 1977 as Journal Series Paper No. 5305.

Accepted for publication 29 August 1977.

ABSTRACT

KISTLER, B. R. and W. MERRILL. 1978. Etiology, symptomology, epidemiology, and control of Naemacyclus needlecast of Scotch pine. Phytopathology 68: 267-271.

Naemacyclus minor is a primary parasite of Scotch pine; it causes needlecast in Pennsylvania Christmas tree plantations. The current year's needles are infected in mid-July and August, and again in November. Viable ascospores are present throughout the year and infection may occur at any time environmental conditions are favorable. Symptoms develop in September and October after a 10- to 15-mo incubation period. Infected needles become yellow with prominent, transverse brown bars. These needles are cast during October and November, and the characteristic waxy-tan apothecia then form on them. Optimum temperatures for mycelial growth and development of apothecia in vitro are 25 C and 21 C, respectively. Infection was significantly reduced by Manzate 200 applied [1.7 kg/ha in 939 liters of water (= 1.5 lb/100 gal/acre)] in October and November.

Additional key words: *Pinus sylvestris*, *Eulachnus agilis*, conifer foliage diseases.

In 1973, studies of needlecast of Scotch pine (*Pinus Sylvestris* L.) Christmas trees in Pennsylvania (PA) showed that much of the damage previously attributed to *Lophodermium pinastri* (Schrad. ex Fr.) Chev. was in fact due to another pathogen. This unknown pathogen caused a yellowing and casting of the previous year's foliage in the fall, whereas *L. pinastri* caused a reddening and casting of the previous year's foliage in the spring. Yellowing and casting of the foliage in the fall had also been attributed previously to premature needle senescence or to feeding damage caused by the two-spotted aphid, *Eulachnus agilis* (Kaltenbach) (Homoptera: Aphididae).

In some PA plantations, 40% or more of the trees were infected by the unknown pathogen. The loss of the previous year's foliage resulted in trees that were either of low quality or completely unmerchantable. Furthermore, cast needles left hanging within the tree had to be removed by hand or by mechanical means, thereby increasing production costs. One grower estimated losses in wholesale value due to this disease at $1,135/ha, with uninfected, high quality trees valued at about $9,880/ha. Thus, this disease posed a serious threat to PA Christmas tree growers, who normally harvest between 1 and 1.5 million Scotch pines/yr, and who grow their stock 8 to 12 yr before harvesting. In addition, the disease was widespread in nursery seedbeds, causing possible reduction of tree vigor and death of out-planted seedlings.

Preliminary studies showed that a fungus resembling *Naemacyclus niveus* (Pers. ex Fr.) Sacc. (Ascomycetes: Hemiphacidiaceae) (6) was associated with the disease syndrome. This fungus previously had been reported to cause, or be associated with, a yellowing and casting of needles on 19 species of two-, three-, and five-needled pines in North America, Africa, Europe, and Oceania (1, 2, 4, 5, 7, 8, 9, 10, 11, 13). Some workers considered it to be a primary pathogen (4, 5, 8, 9, 11, 13); others considered it to be secondary (7, 8, 10). Attempts to inoculate healthy pines with it were unsuccessful (7). Although ascospore release was reported to occur after rains during June and July in England (12), other details of the life cycle and disease cycle were unknown. Attempts to control this pathogen with Dithane M-45, Curitan, and Orthocide did not prevent the renewed yellowing of needles (7).

Butin (3) recently differentiated the monotypic genus *Naemacyclus* into two species, *N. niveus* and *N. minor* Butin. They were delimited primarily on the basis of host ranges and size of the pycnidiospores. The pycnidiospores are thought to be spermatia and do not play an active role in infection. The species associated with Scotch pine was reported to be *N. minor* (3).

Studies were made to determine the etiology, symptomology, epidemiology, and control of Naemacyclus needlecast of Scotch pine in PA.

MATERIALS AND METHODS

Etiology.—To identify the species of *Naemacyclus* responsible for needlecast of Scotch pine, symptomatic needles and needles bearing mature apothecia were collected from affected plantations in Bradford, Clearfield, Indiana, Lackawanna, McKean, Sullivan, and Tioga Counties of PA. Diseased needles were surface sterilized in a 0.52% aqueous solution of sodium hypochlorite for 90 sec, cut into three pieces and placed on 2% malt extract agar (MA = 20 g malt extract, 15 g powdered agar/liter of distilled water) acidified with 1.0 ml of concentrated lactic acid/liter of autoclaved medium

00032-949X/78/000 045$03.00/0

(AMA). These isolates were incubated in diffuse light at 21 C for 10 days. Fungal colonies matching the reported description of *N. niveus* and *N. minor* were transferred to MA and incubated in diffuse light at 21 C for 14 to 21 days. Pycnidia formed under tufts of hyphae in the mycelial mat; apothecia later formed on the surface of the mycelium. The size of the pycnidiospores, asci, ascospores, and apothecia were determined with an ocular micrometer. Measurements of apothecia, asci, and ascospores produced on needles were made also.

To determine if the isolates of *N. minor* were pathogenic, 4- and 5-year-old healthy Scotch pine (French and Spanish provenances) growing in a peat-perlite mixture in 20-cm diameter pots were used. Needles bearing sporulating apothecia of *N. minor* were collected and placed upon the newly formed needles of five trees. The trees then were misted with distilled water until runoff occurred, sealed in plastic bags, and placed in the shade on a greenhouse bench for 72 hr. The bags and apothecia-bearing needles were removed, and the trees placed out of doors. This procedure was repeated at 2- to 3-wk intervals during July and August of 1974 and May to July of 1975. Trees were observed periodically until symptom development occurred.

Following symptom development, needles bearing apothecia were collected, washed in running tap water for 15 min, taped inside the lids of glass petri dishes and suspended over water agar (WA = 15 g powdered agar per liter of distilled water) for 24 hr. Single germinated ascospores were removed from the agar surface, transferred to MA, incubated in diffuse light at 21 C, and the resulting colonies were compared with known isolates of *N. minor*.

Symptomology.—Three-year-old and older Scotch pine growing in plantations throughout the state and known to be infected by *N. minor* were observed over a 3-yr period. Symptoms of Naemacyclus needlecast, natural needle senescence, and attack by *E. agilis* were observed and compared.

Epidemiology.—To determine the time of year when viable ascospores are present in affected plantations, cast needles bearing apothecia were collected periodically from October 1974 through July 1976. These needles were washed under running tap water for 15 min and taped inside the lids of glass petri dishes containing WA, which then were incubated at 21 C for 24 hr.

To determine the infection period of *N. minor* on Scotch pine, 6-to 8-year-old trees (French and Spanish provenances) located in a commercial plantation in Sullivan County, PA, and affected by *N. minor* the previous year were used. At 14-to 21-day intervals from July 1975 to July 1976, one shoot containing needles formed in 1975 was removed from the lower portion of the crown from each of 20 randomly selected, susceptible trees. Ten randomly selected needles were removed from each shoot and isolations were made as described previously. These isolates were incubated at 21 C under diffuse light for 3 wk. For each isolation period, the percentage of shoots containing needles infected by *N. minor* as well as the percentage of infected needles per shoot were determined.

To determine the relation of temperature to growth of *N. minor* in vitro, 11 different isolates were transferred to potato-dextrose agar (PDA) and incubated at 21 C for 14 days. Plugs of agar with mycelium, 5 mm in diameter were cut from the colony margins and placed mycelium side down in the center of 90-mm diameter glass petri dishes containing PDA. Replicate cultures were incubated at 2, 6, 10, 12, 16, 19, 22, 25, 30, and 35 C. At 7, 14, and 21 days, colony diameters were measured to the nearest millimeter.

Isolates of *N. minor* grown on MA at 21 C form apothecia in 21 days. To determine the optimum temperature for formation of apothecia in vitro, six randomly selected isolates were grown on MA for 14 days. Agar plugs then were transferred from the margins of the colonies to petri dishes containing MA, two plugs per dish, and incubated in diffuse light at 21 C for 10 days. Five replications of each of the six isolates then were placed in the dark at 2, 6, 10, 15, 21, 24, and 30 C. The number of apothecia formed 7, 14, 21, 29, and 35 days after being placed at the various temperatures was recorded for each isolate.

Chemical control.—The effects of Manzate 200 (zinc-maganese ethylene-bisdithiocarbamate) (E. I. duPont de Nemours and Co., Inc., Biochemicals Department, Wilmington, DE 19898) and benomyl [methyl 1-(butylcarbamoyl) 2-benzimidazolecarbamate] (E. I. du-Pont de Nemours Co., Wilmington, DE) on the growth of *N. minor* were determined in vitro. The PDA medium was amended with benomyl at concentrations of 1,000, 100, 10, 1, 0.5, 0.1, 0.01, and 0.001 μg active ingredient/ml of agar and with Manzate 200 at 1,000, 100, 10, and 1 μg active ingredient/ml of agar. Four isolates of *N. minor* were grown on PDA for 10 days at 21 C. Plugs of agar and mycelium, 5 mm in diameter were cut from the colony margins; each was placed mycelium-side-downward in the center of a 90-mm diameter glass petri dish containing one of the amended media. Similar plugs were placed on nonamended PDA as controls. The cultures were incubated at 21 C in diffuse light for 14 days. Each treatment was replicated 10 times. Colony diameters were measured to the nearest mm at 7 and 14 days.

The effect of Manzate 200 and benomyl on in vitro ascospore germination of *N. minor* was determined on WA amended with Manzate 200 at 1,000, 100, 10, 1, 0.1, 0.01, and 0.001 μg active ingredient per milliliter of agar, and benomyl at 1,000, 100, 10, and 1 μg active ingredient per milliliter of agar. Ascospores were released onto the agar surface as before at 21 C for 18 hr. The needles then were removed from the lids and the dishes incubated at 21 C for 24 hr to allow the ascospores to germinate. For each concentration of each fungicide, at least 1,000 spores were observed and the percentage germination and the average germ-tube length were recorded.

To determine the effectiveness of Manzate 200 preventing Naemacyclus needlecast in a commercial plantation of 6- to 8-yr-old trees, five adjacent blocks, each containing three rows of at least 20 trees each and bordered on each side by two buffer rows of nonsprayed trees, were selected for four randomly assigned spray applications (Table 1). On 3 October apothecia had not yet developed on the nontreated, susceptible 1973 needles. On 17 October apothecia were present on the 1973 needles just adjacent to the 1974 growth. On 31 October apothecia were in about the same stage as on 17

October, but more apothecia were present on the 1973 needles. On 19 November most of the 1973 needles had been cast and bore apothecia.

Manzate 200 was applied at the rate of 1.7 kg of formulated product/939 liters of water/ha (1.5 lbs/100 gal/acre) with a backpack mist blower. The effects were evaluated 30 September 1975, after needle yellowing but prior to needle casting.

RESULTS

Etiology.—The general symptoms of needlecast on Scotch pine Christmas trees in PA were similar to those attributed by Darker to *N. niveus* (4). The average measurements of the apothecia (226 μm × 493 μm), the asci (10.5 μm × 108.5 μm), and ascospores (3.2 μm × 87.0 μm) fell within the ranges of those described by Darker (230-280 μm × 300-500 μm, 8-10 μm × 92-115 μm, and 2-3 μm × 75-98 μm, respectively). This proved the presence of *N. niveus* on Scotch pine in PA. However, Butin (3) reported that pycnidiospores of *N. niveus* produced in vitro ranged from 9.0 to 15.5 μm long and those of *N. minor* to range from 6.5 to 8.0 μm long. The average length of pycnidiospores from 10 different isolates of the fungus from PA was 7.7 μm (range 4.8 μm to 9.6 μm). Therefore, based on host range and size of the pycnidiospores and accepting Butin's concept of species (3) the pathogen responsible for this previously unidentified needlecast of PA Scotch pine is *N. minor*.

When 4- and 5-yr-old potted Scotch pine were inoculated with this fungus, five of 20 trees developed apothecia of *N. minor* on their needles 14 mo after inoculation. One of these five trees was inoculated 30 July, one was inoculated 13 August, and three were inoculated 29 August. Ascospores collected from these apothecia developed into colonies identical to known colonies of *N. minor*. Needlecast did not develop on noninoculated check trees.

Symptomology.—In general, the first symptoms were small, light-green spots which developed in September. They enlarged and lightened in color; eventually the whole needle became yellow with prominent, transverse brown bars (Fig. 1-B). Symptoms developed first on the second-year needles at the branch whorl between the previous year's and current year's shoot growth, and on the third year needles not infected the previous year. During October most of the remaining second-year needles developed these symptoms and began to be cast. A severely infected tree appeared distinctly yellow. At this time, apothecia began to develop on the needles, first on the brown bars and then over the entire needle surface (Fig. 2). Symptom expression, development of apothecia and casting of needles continued until late November. Some diseased needles remained attached to the tree throughout the winter; these may bear apothecia throughout the winter, or form apothecia the following spring. Severely diseased trees retain only the current year's needles.

These symptoms are sometimes confused with those of natural needle senescence. However, naturally senescing Scotch pine needles turn tan to brown without first turning a distinct yellow, brown bars do not develop on the needles, and normally only fourth- and fifth-year needles are affected.

More commonly, symptoms of Naemacyclus needlecast have been confused with those caused by feeding of Eulachnus aphids. High aphid populations will cause a general yellowing and casting of second-year needles at the same time that Naemacyclus needlecast symptoms are visible. However, aphid-damaged needles turn yellow but lack the transverse brown bars (Fig. 1-A).

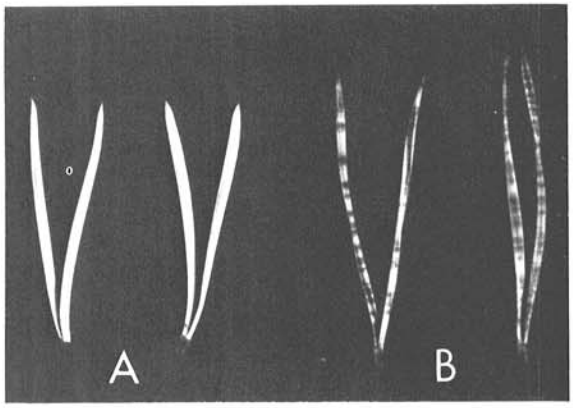

Fig. 1-(A, B). Yellowed Scotch pine needles showing **A)** lack of bars on needles injured by *Eulachnus agilis* feeding and **B)** barring caused by *Naemacyclus minor*.

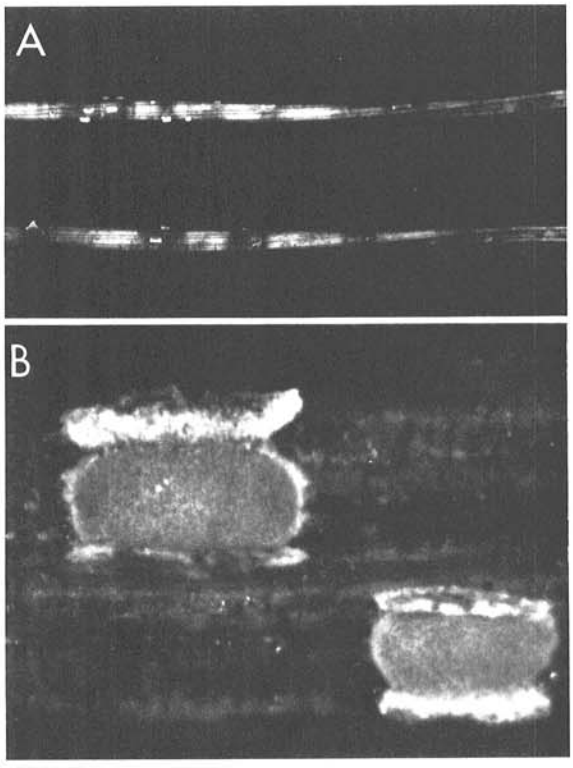

Fig. 2-(A, B). Scotch pine needles bearing apothecia of *Naemacyclus minor* **A)** × 2 magnification, and **B)** × 60 magnification.

Furthermore, most of the aphid feeding activity is confined to the upper portion of the tree, whereas Naemacyclus needlecast is more evenly distributed throughout the tree.

Epidemiology.—Ascospore release of *N. minor* was continuous throughout the year. Newly formed apothecia on the 1973 needles collected in October 1974 readily released ascospores which germinated on WA within 12 hr. This also was true of apothecia and ascospores from the same year's needles collected periodically until late July, 1975. From late July to 9 September 1975, ascospore release and germination were sporadic. Thereafter, only a few ascospores were released; a few of them germinated but most of them lysed.

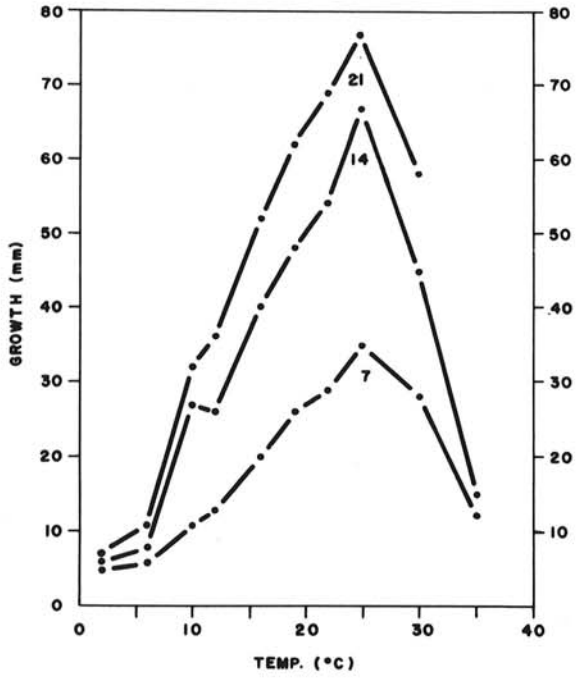

Fig. 3. Average colony diameters of 11 different isolates of *Naemacyclus minor* incubated for 7, 14, and 21 days at various temperatures.

TABLE 1. Effect of Manzate 200 spray[a] applied in the fall of 1974 on the incidence of Naemacyclus needlecast of Scotch pine

Spray date	Trees Infected[b] (%)
Nonsprayed Check	14 x
3 Oct	11 xy
3, 17 Oct	6 xyz
3, 17, 31 Oct	3 yz
17, 31 Oct; 19 Nov	2 z

[a]Spray formulation: 1.7 kg of formulated product/939 liters of water/ha (1.5 lb/100 gal/acre).

[b]Averages followed by the same letter are not significantly different from each other, $P = 0.05$.

Naemacyclus minor was first isolated on 16 July 1975 from 19% of the current year's needles on 70% of the shoots. The frequency of isolation from the 1975 shoots averaged 60% (range 30 to 90%) from July 1975 to July 1976. However, the percentage of infected needles on these same diseased shoots increased markedly after 3 November 1975. The percentage of infected needles averaged 20% (range 13 to 25%) from 16 July 1975 to 3 November 1975. From 19 November 1975 to 19 July 1976, the percentage of infected needles averaged 31% (range 20 to 41%). The difference between the isolation percentage to 3 November and the isolation percentage after 3 November was significant at $P = 0.01$. There were no significant differences in the percentages of shoots infected for those same two periods. These results indicate that there were two infection periods, one in mid-July and August and another in November.

The optimum temperature for growth of *N. minor* in vitro over a 3-wk period was 25 C (Fig. 3), but that for development of apothecia was 21 C.

Chemical control.—Manzate 200 and benomyl were effective against *N. minor* in vitro. Manzate 200 and benomyl slightly inhibited mycelial growth at 10 μg/ml and 0.001 μg/ml, respectively, but completely inhibited growth at 100 μg/ml and 0.01 μg/ml, respectively. In each case where no growth occurred, *N. minor* grew when transferred to amended PDA.

In the ascospore germination study, benomyl concentrations of 10 and 100 μg/ml were necessary to significantly inhibit ascospore germination; 1,000 μg/ml were necessary to completely inhibit germination. Manzate 200 completely inhibited ascospore germination at 1 μg/ml; it reduced germination by 35% and relative germ-tube length by 50% at 0.1 μg/ml.

Manzate 200 was effective in the fall spray trial (Table 1). Although in the check plot 14% of the trees were infected, repeated sprays reduced the percentage of diseased trees to 3 and 2%.

DISCUSSION

Inability to infect healthy Scotch pine with *N. minor* and the common association of this fungus with other foliar pathogens led some investigators to conclude that *N. minor* was saprophytic (7, 8, 10). However, the association of *N. minor* with severe needlecasting in the absence of other pathogens was accepted as circumstantial evidence of the pathogenicity of this fungus (4, 5, 8, 11, 13). Isolation of *N. minor* from newly developed, 1-mo-old needles in the absence of any other known pathogens, plus the successful artificial inoculation of healthy, 4- to 5-yr-old potted Scotch pine in these studies proves the pathogenicity of *N. minor*.

The complete disease cycle still is not known. Most infection of the current year's needles occurs in mid- to late July and early August. Any susceptible second- and third-year needles that escaped infection the previous year also can become infected at this time. This infection level remains constant until early November when new apothecia are formed on the previous year's needles. During November and December spores from these apothecia cause a significant increase in the percentage of needles infected compared to the July-August infection period.

Preliminary studies indicate that if the current year's needles are not infected during the summer and fall, they may be infected during the spring and summer of the following year. Symptoms develop when the infected needles are 15 to 16 mo old.

Since apothecia developed in cultures stored at 2 C for several months and mycelial growth in vitro also occurred at this temperature, development of apothecia, ascospore release, and infection may occur during the winter months. In fact, needles bearing apothecia collected periodically from October to September of the following year released viable ascospores in vitro, 10 to 11 mo after the first apothecia were formed on these needles.

Benomyl and Manzate 200 show promise for field control of *N. minor*. Good control of Naemacyclus needlecast should be possible if sprays are applied at 14- to 21-day intervals from mid-July through August and mid-October through November. Spray trials with benomyl in commercial Scotch pine plantations should be carried out to determine efficacy.

LITERATURE CITED

1. ANONYMOUS. 1960. Forest pathology. Pages 28-32 *in* Rep. For. Res. Inst. N.Z. 1958-1959. Rotorua, N.Z.
2. ANONYMOUS. 1963. Annual report of the Department of Agriculture, Nyasaland, for the year 1961-1962. Part II. 196 p.
3. BUTIN, H. 1973. Morphologische und taxonomische Untersuchungen an Naemacyclus niveus (Pers. ex Fr.) Fuck. ex Sacc. und verwandten Arten. Europ. J. For. Pathol. 3:146-163.
4. DARKER, G. D. 1932. The Hypodermataceae of conifers. Contrib. Arnold Arboretum, 1. 131 p.
5. GILMOUR, J. W. 1965. Mycology. Pages 63-65 *in* Rep. For. Res. Inst. N.Z. 1964. Rotorua, N.Z.
6. KORF, R. P. 1962. A synopsis of the Hemiphacidiaceae, a family of the Helotiales (Discomycetes) causing needle-blights of conifers. Mycologia 54:12-33.
7. MAGNANI, G. 1972. Sulla presenze di Naemacyclua niveus su aghi di Pinus radiata. Publ. Centro Sper. Agric. For. 11:315-320.
8. MARTINEZ, B. J. 1942. Las micosis del Pinus insignis en Guipuzcoa. Inst. For. Exp. Madrid 13. 72 p.
9. MC KEE, R. K. 1968. Plant Pathology research division. Pages 88-93 *in* Rep. Res. and Tech. Work, Minist. Agric. N. Ireland, 1967.
10. MILLAR, C. S. 1970. Role of Lophodermella species in premature death of pine needles in Scotland. Pages 176-178 *in* Rep. for For. Res. for the year ending March 1970. Her Magesty's Stationery Office, London.
11. NATTRASS, R. M. 1948. Annual report of the senior plant pathologist, 1946. Pages 149-152 *in* Rep. Dept. Agric., Kenya, 1946.
12. PAWSEY, R. G. 1967. Spore discharge of Naemacyclus niveus following rainfall. Trans. Br. Mycol. Soc. 50:341-347.
13. STAHL, W. 1966. Needlecast fungi on conifers in the Australian Capital Territory. Austr. For. 30:20-32.