

CYCLANEUSMA (NAEMACYCLUS) NEEDLE-CAST OF PINUS RADIATA IN NEW ZEALAND

1: BIOLOGY OF CYCLANEUSMA MINUS

P. D. GADGIL

Forest Research Institute, New Zealand Forest Service,
Private Bag, Rotorua, New Zealand

(Received for publication 27 October 1983; revision 9 April 1984)

ABSTRACT

Rooted cuttings of three clones and seedlings of *Pinus radiata* D. Don were inoculated with ascospores or mycelium of *Cyclaneusma minus* (Butin) DiCosmo *et al.* and kept for 3 months in growth rooms at different temperatures. All cuttings belonging to two of the clones developed typical symptoms of *Cyclaneusma* needle-cast and *C. minus* was isolated from needles taken from these cuttings. Needles from cuttings of the third clone yielded very few isolates of *C. minus* and the cuttings did not exhibit any symptoms of ill health. Seedlings were not infected by the fungus. An estimate was made of the number of spore-bearing apothecia of *C. minus* present on the litter layer of a stand of 10-year-old *P. radiata*, subject to the needle-cast, by collecting needles shed over fortnightly periods and counting the number of spore-bearing apothecia present on a sub-sample. The collected needles were returned to the stand, placed in a mesh-lined frame, and examined every fortnight until no more apothecia were seen. The two peak periods of needle-cast were in spring and autumn, and the greatest number of apothecia was found in autumn-winter (May to August). Records from a Hirst spore trap set up in the same stand showed that the *C. minus* ascospores occurred most frequently in autumn-winter and that ascospore release was dependent on rainfall. Monthly isolations from needles showed that current season's needles were first colonised by *C. minus* in autumn-winter (May-June) when they were about 8-9 months old and by *Lophodermium* spp. about 2 months later. Most of the infected needles were shed when they were about a year old, but some needles were retained until the following winter.

FUNGAL NOMENCLATURE

The genus *Naemacyclus* was erected by Fuckel (1873-74) to accommodate *Propolis pinastri* de Lacroix in Desmazieres. Although he indicated that *Propolis nivea* (Persoon ex Fries) Fries (and *P. hysterooides* (Desmazieres) Fuckel) may belong to *Naemacyclus*, he did not make the transfers. Saccardo (1884) included two species - *Naemacyclus pinastri* (de Lacroix) Fuckel and *N. niveus* (Persoon ex Fries) Saccardo (as *N. niveus* (Persoon) Fuckel) - in his description of *Naemacyclus* and later he (Saccardo 1889) reduced *N. pinastri* to synonymy with *N. niveus*. As Korf (1962) pointed out, later

authors have identified the holotype of *Naemacyclus* with the older species *Stictis nivea* Persoon (= *Propolis nivea*), which was transferred to *Naemacyclus* by Saccardo (1884). Korf (1962) noted that *Naemacyclus* was a monotypic genus when proposed (based on *N. pinastri*) and none of the species added to it later appears to be congeneric. Butin (1973) and DiCosmo *et al.* (1983) have shown that the binomials *N. pinastri* and *N. niveus* referred to fundamentally different fungi. It is clear therefore that *N. niveus* (and the similar *N. minor*) cannot correctly remain in the genus *Naemacyclus* as they do not follow the type species *N. pinastri*.

A new generic name *Cyclaneusma* to accommodate these two species has been introduced by DiCosmo *et al.* (1983). These authors considered the possibility of proposing the conservation of *Naemacyclus* in its present sense, but decided that the proposal was unlikely to succeed. It is a pity that the name change affects such well-known fungi, but it is undoubtedly necessary. The accepted names for this series of papers are :

1. *Cyclaneusma niveum* (Persoon ex Fries) DiCosmo, Peredo & Minter (= *Naemacyclus niveus* (Persoon ex Fries) Saccardo) found on *Pinus nigra* Arnold, *P. halepensis* Miller, and *P. pinaster* Aiton.
2. *Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter (= *Naemacyclus minor* Butin) found on *P. radiata*, *P. sylvestris* Linnaeus, *P. ponderosa* P. & C. Lawson, *P. patula* Schlechtendal & Chamisso, *P. jeffreyi* Greville & Balfour, and *P. mugo* Turra.

Butin (1973) has shown that *C. minus* is the only species of *Cyclaneusma* found on *P. radiata*. Accordingly, all records of *C. niveum* (as *N. niveus*) on *P. radiata* in New Zealand are regarded as those of *C. minus*.

INTRODUCTION

Cyclaneusma minus is associated with needle-cast in many species of *Pinus* and has been reported from all continents (Millar & Minter 1980). Gilmour (1959) first recorded the fungus in New Zealand in 1959 (as *Naemacyclus niveus*) on *P. radiata* and *P. ponderosa*. According to Gilmour (1959), this fungus had previously been mis-determined as *Stictis* sp., so the time of its first appearance in New Zealand is not known.

In New Zealand, *C. minus* has been associated for many years with periodic abnormal casting of 1-year and older needles of *P. radiata*. The needles first turn a mottled yellow-green colour, then yellow-brown, and are cast prematurely. Severe needle-cast of this type was reported in 1952, 1956, and 1962 (Gilmour 1966) and, more recently, in 1974, 1975, 1981, and 1982 (FRI unpublished records). The only reported test of the pathogenicity of *C. minus* to *P. radiata* was inconclusive (Magnani 1972). Karadzic (1981) has recently shown it to be pathogenic to *P. sylvestris*. The work reported here was undertaken (a) to establish whether the fungus was pathogenic to *P. radiata*, (b) to determine the pattern of its inoculum build-up and spore dispersal, and (c) to gain information on infection periods.

PATHOGENICITY TRIAL

Materials and Methods

Plant material

Rooted cuttings of three clones of *P. radiata*, taken from 7-year-old trees and grown for 2 years in a glasshouse, and 2.5-year-old glasshouse-grown seedlings of *P. radiata* were used. The clonal material was not specially selected but was available as surplus from an unrelated trial, and the seedlings were grown from commercial seed.

Inoculum

The inoculum of *C. minus* was of three types:

- (1) Ascospores from apothecia produced naturally on needles of *P. radiata*;
- (2) Ascospores from 4-week-old cultures grown on 3% malt agar;
- (3) Macerated mycelium harvested from 4-week-old liquid shake cultures in 3% malt.

To prepare inoculum types 1 and 2, asci were picked with a needle from ascocarps and suspended in water. The ejected ascospores tended to clump and it was difficult to determine their concentration in the inoculum suspension. There were between 70 000 and 90 000 ascospores/ml in both type 1 and type 2 inocula.

Inoculation

In February 1976, 12 cuttings of each clone and 24 seedlings were each sprayed with 20 ml of one inoculum type (a total of 36 cuttings per clone and 72 seedlings were inoculated) and the plants were then placed in four growth rooms maintained at different temperatures and vapour pressure deficits (*see* Table 1 for details of room conditions) which were available for this work. Each growth room was assigned three cuttings per clone and six seedlings sprayed with each inoculum type, and an equal number of unsprayed cuttings and seedlings. The plants were misted for 4 days after inoculation and the misting intervals were so arranged that the foliage remained moist without appreciable run-off. After the misting period the plants were watered as required, usually once a week.

After 96 days in the growth rooms the plants were taken to the laboratory. Twenty, 1-year-old or older, apparently healthy needles were chosen randomly from each plant, dipped in 90% ethanol for about 30 s and then immersed in hydrogen peroxide (30 vols) for 5 min. They were then cut into approximately 1-cm segments, placed on 3% malt agar in petri dishes, and incubated at 20°C.

TABLE 1—Conditions in growth rooms

Growth room	Temperature (°C ± 0.5°C)		Vapour pressure deficit (mb)		Light intensity (W/m ²)	Photoperiod (hours)
	— — — — —		— — — — —			
	Day	Night	Day	Night		
1	20	12	2	2	140	16
2	25	15	8	5	150	14
3	15	10	8	5	146	14
4	25	20	8	5	132	14

Results

By the end of the experiment yellow-brown mottling, typical of the needle-cast, was apparent in many of the older needles belonging to two of the three clones. All needles on cuttings of the third clone, the seedlings, and the control plants showed no symptoms of ill health. There was no premature needle fall.

Needles of the two clones that showed yellowing of the older needles yielded cultures of *C. minus*, while only a few isolates of the fungus were obtained from the symptomless needles of the third clone (Table 2). The seedlings and the control plants did not yield any *C. minus* cultures. No differences attributable to inoculum type or growth room conditions were found. The results showed that *C. minus* was pathogenic to some adult plants of *P. radiata* but not to the 2.5-year-old seedlings tested.

TABLE 2—Number of isolates of *Cyclaneusma minus* per 10 cm needle length. Results from plants inoculated with different inocula have been pooled

	Growth room			
	1	2	3	4
Clone X	3.1	3.4	2.9	2.8
Clone Y	3.2	2.2	2.4	3.0
Clone Z	0.1	0.04	0.08	0.1
Seedlings	0	0	0	0
Controls	0	0	0	0

ASCOSPORE PRODUCTION AND DISPERSAL

Materials and Methods

Experimental site

A small plantation (0.4 ha) of trees of four clones (A, B, C, and D) of *P. radiata* was used for this study. This plantation occupied part of a large level area in Whakarewarewa State Forest (near Rotorua) devoted to clonal and provenance trials of a number of species. It was established in 1968 and had been thinned to about 500 stems/ha. The trees were 10 years old and about 15 m tall when the study began. Trees of all four clones were known to have shown symptoms of premature needle-cast and two of the clones (A and B) had been particularly susceptible to the disease in the past. There was little understorey vegetation and the litter layer contained numerous needles bearing apothecia of *C. minus*.

Estimation of numbers of apothecia

Apothecia of *Cyclaneusma* are rarely found on living needles, but they usually develop on fallen dead needles (Rehm 1896; Dennis 1978). Observations have shown that *C. minus* on *P. radiata* behaves similarly in New Zealand. The litter layer is therefore the main source of ascospores of *C. minus*. The following procedure was adopted to estimate the number of sporiferous apothecia present on the litter layer

throughout the year: three rectangular litter traps, each with a collecting area of 1 m², were placed about 20 m apart in the experimental plantation under trees of clones A and B on 11 October 1978. Trapped litter was collected fortnightly until 13 February 1980. Spore-bearing apothecia of *C. minus* on 10 randomly selected needles were counted and the fresh weight of the total catch was recorded. A small sample of litter (about 10 g fresh wt) was removed, weighed, oven-dried at 70°C to constant weight, and weighed again to determine the amount of water present. The number of needles in this sub-sample was counted. From these data the total number of needles trapped in that fortnight was calculated. The remaining litter was then taken back to the experimental site and placed in a wooden frame (35 × 20 cm) which rested on the litter layer but was separated from it by a layer of nylon mesh. The frame was covered with nylon mesh raised a few centimetres above it. The mesh and the frame prevented ingress of additional needles, but allowed rain to get through. After the first fortnight, in addition to collecting needles from the litter traps, the needles exposed in the preceding fortnight(s) were also collected and taken to the laboratory. The number of spore-bearing apothecia on 10 needles from each collection was counted and the samples were returned to the appropriate frames. Each collection was examined every fortnight until no sporulating apothecia of *C. minus* had been found for two consecutive fortnights.

The first fortnight for which an estimate of the total number of spore-bearing apothecia of *C. minus* present on the litter layer could be made was the last fortnight (31 January to 14 February 1979) in which apothecia were found on the first collection (made on 25 October 1978). It was assumed that needles which fell before the first litter collection was made would have ceased fruiting by the time the first collection had stopped bearing sporiferous apothecia. The number of apothecia in a collection for a fortnightly period ending on a particular date was estimated by multiplying the average number of apothecia per needle by the estimated number of needles per square metre in that particular collection. The total number of apothecia present was obtained by adding together the numbers of apothecia in all the collections still bearing apothecia on that particular date. For example, the total number of apothecia present between 31 January and 14 February 1979 was obtained by adding the numbers of apothecia in collections made on 25 October, 8 November, 22 November, 6 December, 3 January, 17 January, 31 January, and 14 February.

Spore trapping

A Hirst spore trap, its orifice 1 m above ground level, was located in the middle of the plantation. It was equipped with a drum driven by a clockwork motor and completed one revolution in 7 days. Airborne particles were trapped on an 18-mm-wide strip of transparent positive film which was wound round the drum and which was printed with divisions 2 mm apart and lightly smeared with petroleum jelly. A spare drum with the collecting strip in place was exchanged for the used drum every Wednesday at 2 p.m. The vacuum pump of the trap was driven by a 6-volt d.c. aircraft motor. Airflow through the orifice was adjusted to 10 litres/minute. The spore sampling period ran from May 1977 to October 1979 (30 months) with an unfortunate break of 9 weeks from 22 March to 25 May 1979 because of frequent motor failures.

Hourly temperature and rainfall data were obtained from an official Meteorological Service climatological station sited 1 km from the spore trap.

Each exposed collecting strip was cut in sections representing successive 24-hour periods (starting at 2 p.m. on Wednesday) and mounted on microscope slides in lacto-phenol-aniline blue. The entire surface was scanned under a low power (10×) objective and the number of ascospores of *Cyclaneusma* seen on 2-mm sections (corresponding to exposure for 1 hour) was recorded. As explained by Pawsey (1967), the size, shape, and appearance of the *Cyclaneusma* ascospores is distinctive enough to be recognised even under low power magnification.

Results

There were two peak periods of needle-cast – spring (October–November 1978, September–December 1979) and autumn–early winter (April–June 1979) (Fig. 1). The period over which the needles bore sporulating apothecia of *C. minus* varied from 24 weeks (needles collected on 14 March) to 6 weeks (collected on 15 August and 10 October) with a mean of 10 weeks; no major seasonal variation in the length of

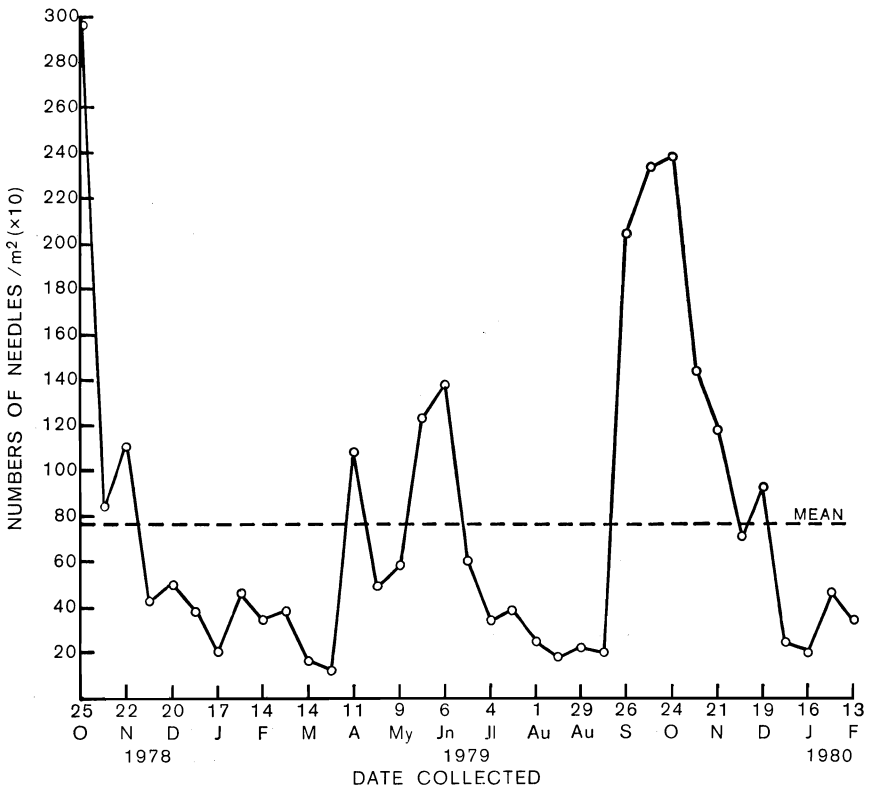


FIG. 1—Numbers of needles per square metre collected every fortnight from 25 October 1978 to 13 February 1980. (Only every other collection date is indicated.)

the fruiting period was found except that four collections made in the spring (two in 1978 and two in 1979) did not produce any apothecia for about a fortnight after collection (hatched areas, Fig. 2). All other collections contained needles which bore *C. minus* fructifications and, as the needles still on the trees were not seen to be bearing apothecia of *C. minus* when samples were collected for isolations (see "Infection Periods"), it is assumed that these fructifications were produced in the period which elapsed between needle fall and needle collection, a maximum of 14 days. A portion of the available data on the average number of spore-bearing apothecia per needle for

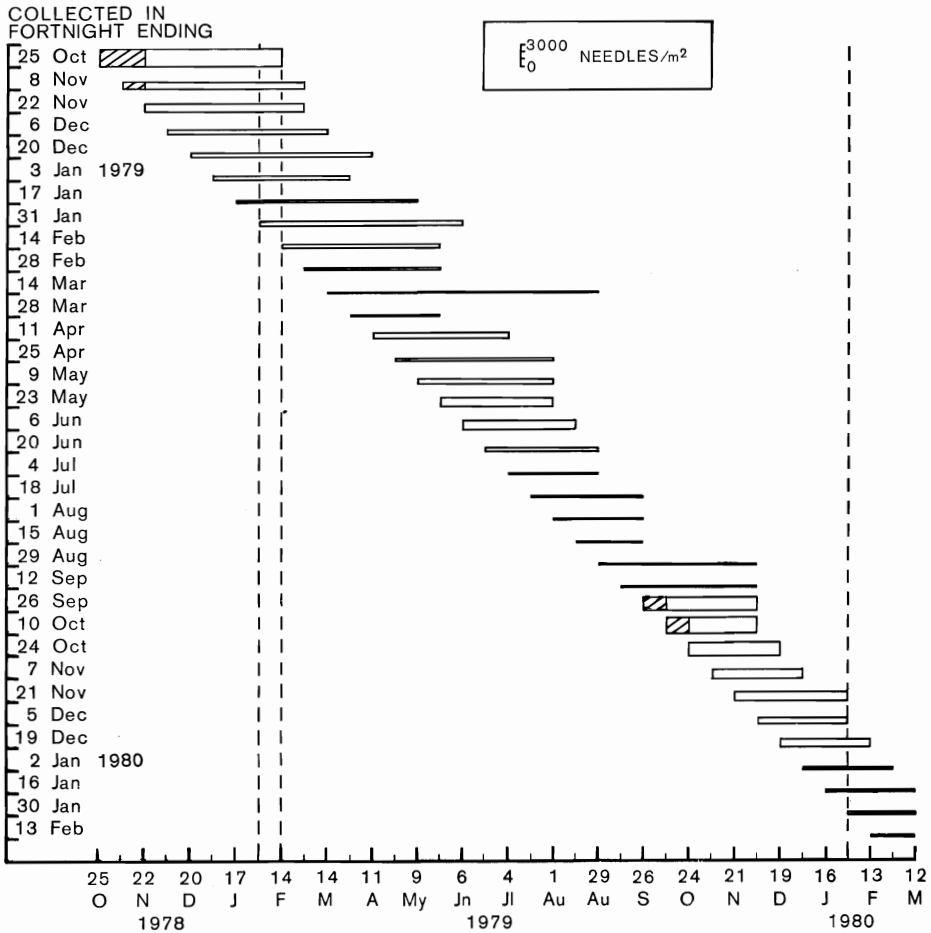


FIG. 2—Numbers of needles per square metre collected in fortnightly periods and the length of time for which they bore sporulating apothecia of *C. minus*. The hatched areas indicate the period over which no apothecia were found at the beginning of the exposure period. The pair of vertical broken lines indicates the first fortnight when the total number of apothecia was determined. The end of the counting period is indicated by a single vertical broken line.

individual fortnightly periods is given in Fig. 3. Usually, the number of apothecia per needle increased with time for two to three fortnights after needle cast and then decreased. There was a seasonal trend in the number of apothecia present – in decreasing order the ranking was autumn (most), winter, spring, summer (least) (*see* Fig. 3 for representative data).

Estimates of the total numbers of spore-bearing apothecia for fortnightly periods for a year are given in Fig. 4. The large numbers of apothecia present from 11 April to 1 August were the result of prolific apothecium production on the large number of needles cast in autumn–early winter (Fig. 1). The small November peak reflects the heavy spring needle fall with fewer apothecia per needle than on needles cast in autumn. Spore trap records for a part of this time were also available and are included in Fig. 4. There is a gap in the records, but the spore numbers peaked in winter as did the numbers of apothecia.

Cyclaneusma ascospores were trapped throughout the year but there was a marked seasonal variation in the frequency of occurrence of ascospores. In Fig. 5 the frequency of occurrence of trapped ascospores is expressed in terms of the proportion of the number of hours in a month in which spores were trapped to the total number of hours in that month. The major peaks in the frequency of occurrence of spores were in autumn and winter (May, June, July 1977; April–July 1978; June, July, August 1979). From September 1977 to March 1978 there were no hours in which more than 10 ascospores were caught and in the spring and summer of 1978–79 September, November, and December were the only months when some hours in which more than 10 spores were trapped were recorded (Fig. 5, open circles).

There was a significant correlation ($p < 0.01$) between frequency of occurrence of ascospores and frequency of rainfall (expressed as the proportion of rainy (>0.1 mm) to total hours) (Fig. 6). The relationship between spore release and rainfall was further explored and, when the lagged cross-correlations between hourly rainfall (in millimetres) and the number of spores caught in that particular hour were plotted against hours of rain (Fig. 7), it was seen that the maximum effect of rain on spore release occurred in the fifth hour after the commencement of rainfall. The effect of rainfall was modified by season and generally, although the same frequency of rainy hours yielded the same frequency of ascospore hours throughout the year (Fig. 6), a given amount of rainfall produced a greater spore count in autumn. The greater numbers of spore-bearing apothecia of *C. minus* during this period (Fig. 4) presumably account for this effect.

INFECTION PERIODS

Material and Methods

The experimental site described in the previous section was also used for this work. Five trees each of two clones (A and B) which were known to be particularly susceptible to premature needle-cast were selected. Clone A trees usually showed the symptoms about a month earlier than trees of clone B. Five branches in the lower crown of each tree which could be reached from the top rung of a 4.25-m ladder were tagged. In the first week of each month, one needle of the current season's growth and one needle of

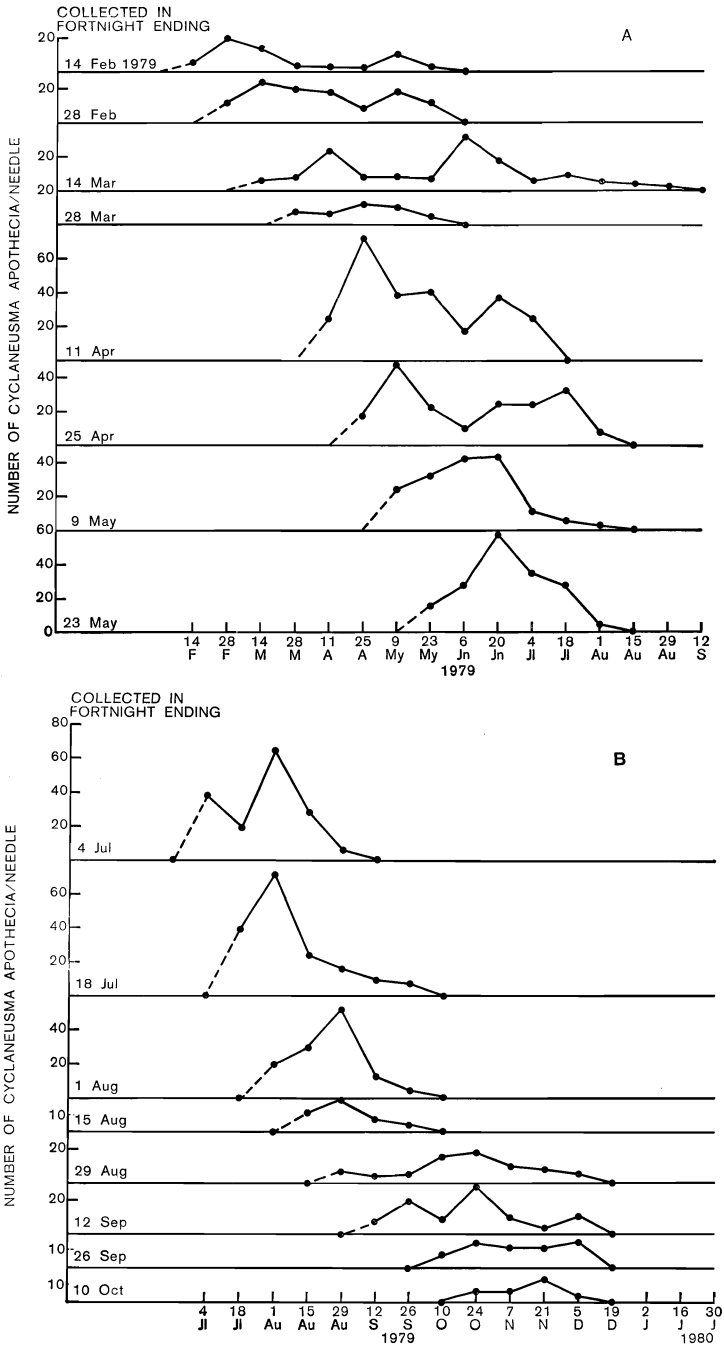


FIG. 3—Numbers of sporulating apothecia of *C. minus* per needle (mean of 10 needles per collection) in fortnightly collections made in different seasons.

A: Summer (February) and autumn (March, April, May)

B: Winter (July, August) and spring (September, October).

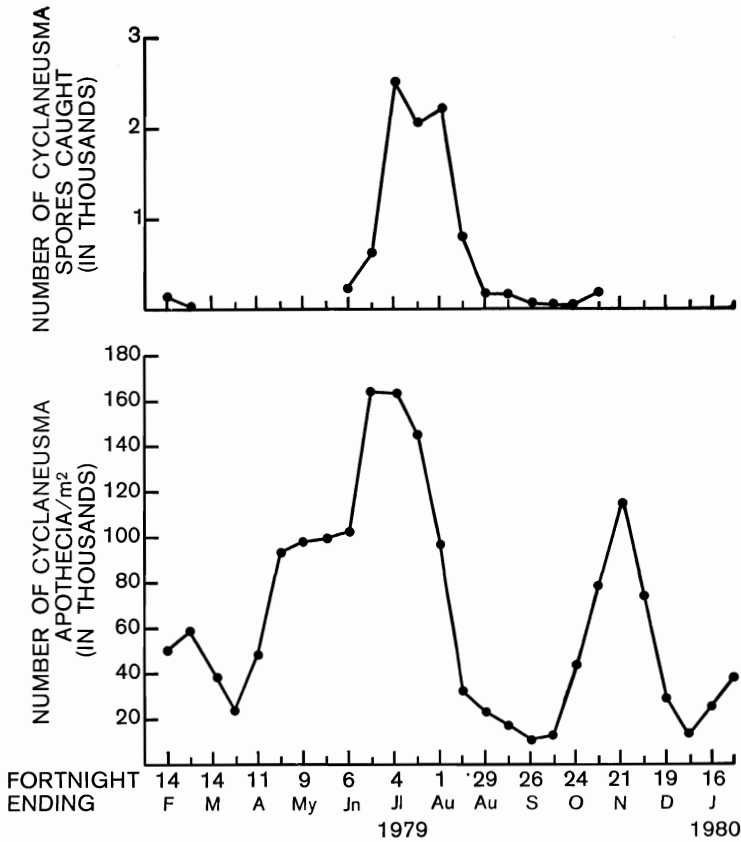


FIG. 4—Total numbers of sporulating apothecia of *C. minus* present on the litter layer for fortnightly periods and the numbers of *Cyclaneusma* ascospores caught in a Hirst spore trap over the same period. (The gap in the spore numbers data occurred because the trap was out of action.)

the previous season's growth were picked from each tagged branch. Collections were begun in November 1977 when the current season's needles were about 2 months old and the previous season's needles about 14 months old. Only living needles which had not formed an abscission layer were collected. The last collection was made in August 1979.

In the laboratory the length of each needle was recorded. Each needle was then immersed in 90% ethanol for 30 s, surface-sterilised by immersion in hydrogen peroxide (30 vols) for 5 min, and cut in approximately 1-cm segments; the segments were placed on 3% malt agar in petri dishes and incubated at 20°C. All fungi growing out from the needle segments were recorded and representative sub-cultures were made.

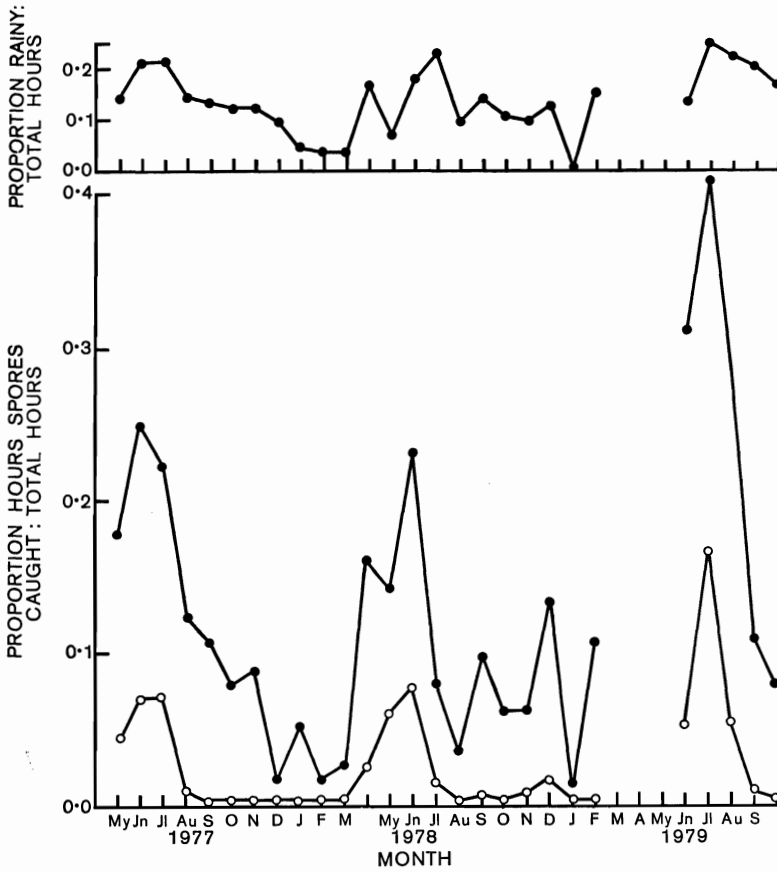


FIG. 5.—Frequency of occurrence of *Cyclaneusma* ascospores and the frequency of rainfall. An hour with more than 0.1 mm rain is regarded as rainy. Lower graph: solid circles = proportion spore hours : total hours; open circles = proportion hours >10 spores : total hours.

Results

Cyclaneusma minus colonies grew readily from the cut ends as well as from the undamaged sides of the needle segments. The appearance of *C. minus* colonies was easily recognisable, although there was considerable variation in colony texture and colour. Species of *Lophodermium* were also commonly isolated. These, unfortunately, could not be identified to species level. A study at present under way has indicated that at least six species of *Lophodermium* are present in New Zealand but they have not been definitely determined. *Lophodermium seditiosum* Minter *et al.*, the species shown to be pathogenic to *Pinus* spp. (Minter & Millar 1980) does not appear to occur here. Other fungi (species of *Neurospora*, *Sclerophoma*, *Cladosporium*, *Cephalosporium*, *Phomopsis*, *Chaetomium*, *Pestalotia*, and *Hendersonia* and unidentified species) were also isolated but no particular species appeared as frequently or as consistently as *C. minus* or *Lophodermium* spp.

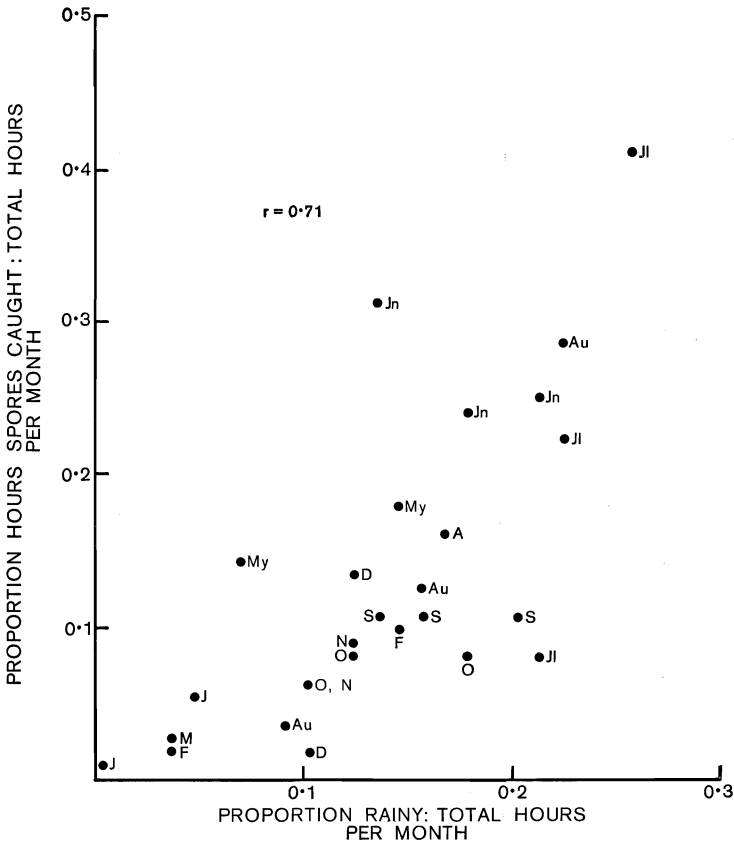


FIG. 6—Relationship between frequency of rainfall and frequency of occurrence of *Cyclaneusma* ascospores.

The pattern of colonisation of needles by *C. minus* and *Lophodermium* spp. was similar in both clones except that trees of clone B lagged 1–2 months behind clone A trees in the chronology of colonisation (Fig. 8). In 1977–78 neither fungus was isolated from current season's needles (flushed 1977) for the first 6 months. Isolates of *C. minus* first began to appear in late autumn–early winter (May–June). The number of isolates of *C. minus* per unit length of needle increased rapidly after this until September–October 1978 and then remained at a high level. Infection by *Lophodermium* spp. lagged behind that by *C. minus* by 1–2 months but showed a similar trend. Many needles showed the typical yellow-brown mottling associated with the needle-cast and were shed in September–October 1978 when they were about a year old, but some needles were retained and the last needles of the 1977 flush were cast in May–June 1979 (20–21 months old) by which time they were extensively colonised by both *C. minus* and *Lophodermium* spp. The pattern of colonisation of needles which flushed in 1978 was similar to those of the previous year, except that the *C. minus* numbers

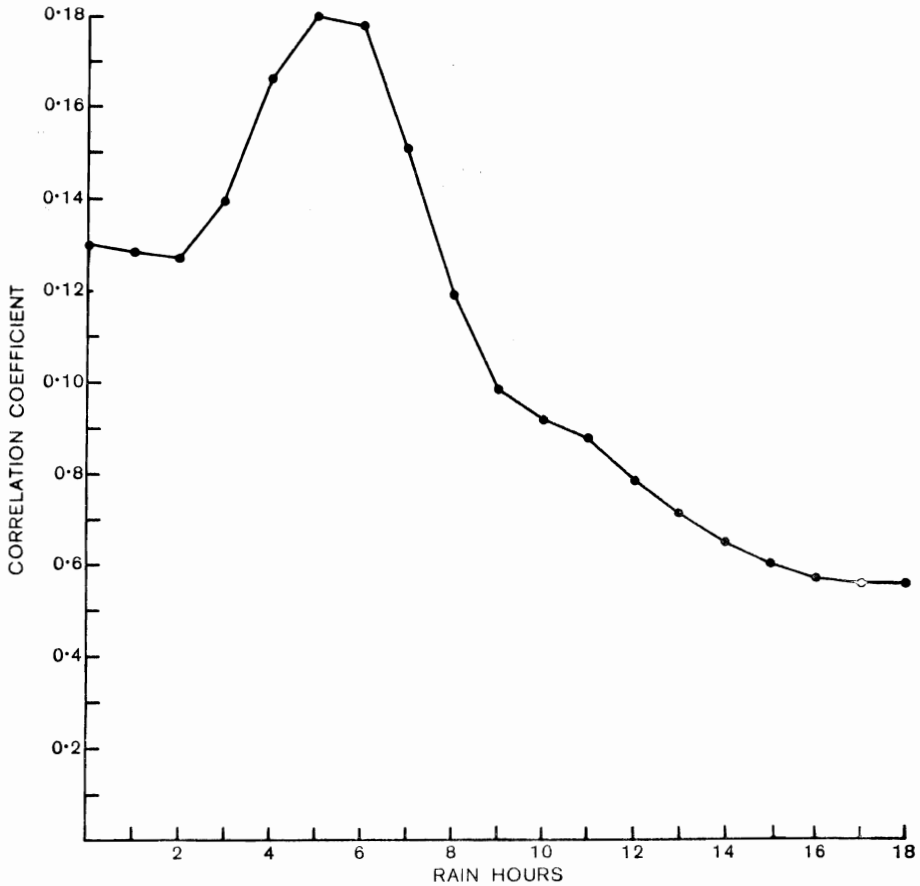


FIG. 7.—Correlation coefficients between hourly rainfall and the number of ascospores trapped in that hour plotted against hours of rain.

were lower. The needles which were already over a year old when sampling began in November 1977 were colonised by *C. minus* and *Lophodermium* spp. at that time and supported a fluctuating but moderately high *C. minus* population which showed a sudden increase in June–July 1978, just before the last needles were cast.

DISCUSSION

There have been conflicting reports about the pathogenicity of *Cyclaneusma* (*Naemacyclus*). Peace (1962) reported that, although *N. niveus* occurred on fallen needles of *P. sylvestris* in Britain, it had not been associated with defoliation. Benito Martinez & Torres Juan (1965) considered that *N. niveus* could be a parasite but was generally present as a saprophyte. According to Pawsey (1967), *N. niveus* was common on fallen needles of *P. radiata* in Australia and it was apparently largely saprophytic

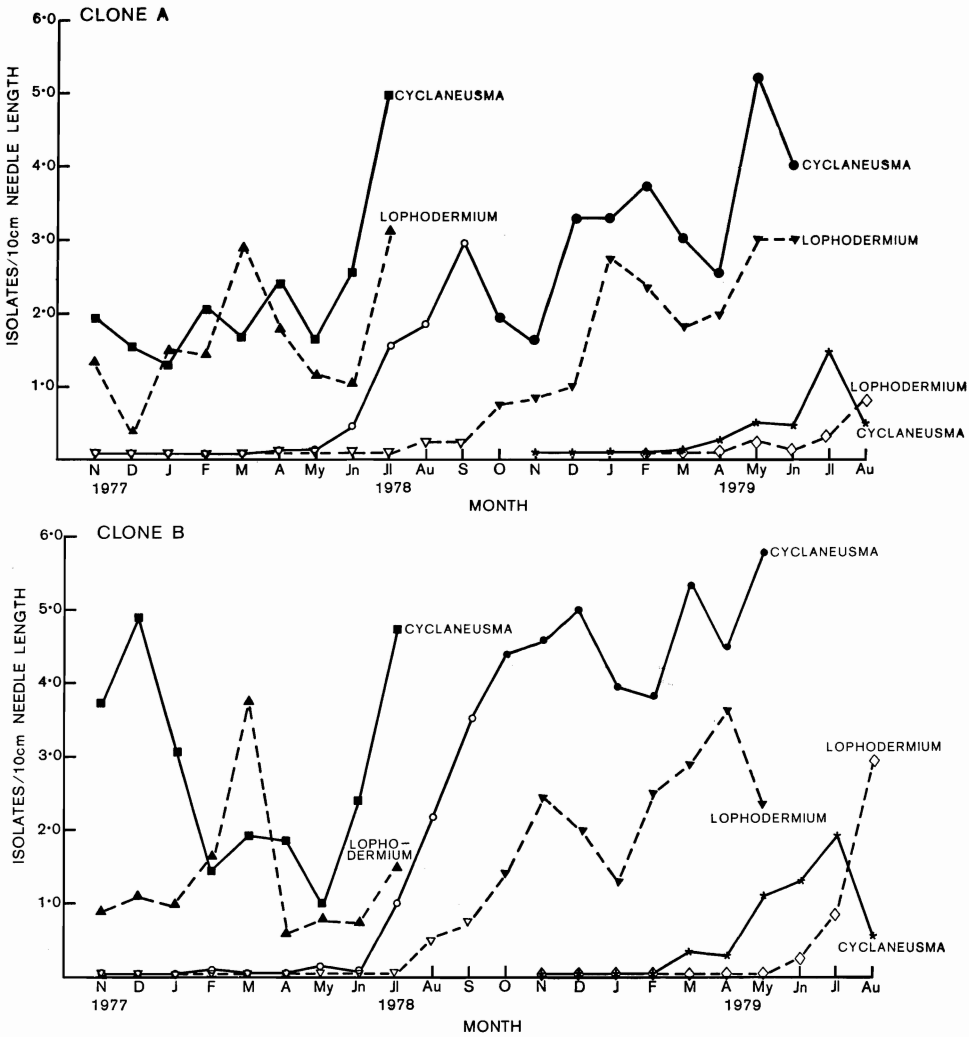


FIG. 8—Numbers of isolates of *C. minus* and *Lophodermium* spp. per 10 cm needle length. The data are for needles which flushed in August-September 1976, 1977, and 1978. Sampling began when the 1976 needles were 14 months old; it covered the full lifespan of the 1977 needles and the first 12 months of the 1978 needles. No data are presented for the first 2 months after needle-flush for the 1977 and 1978 needles as they were then too short to be sampled. Key to symbols:

Solid squares	=	<i>Cyclaneusma minus</i>	1976 needles	>1 year old
Solid triangles	=	<i>Lophodermium</i> spp.	1976 needles	<1 year old
Open circles	=	<i>C. minus</i>	1977 needles	<1 year old
Solid circles	=	<i>C. minus</i>	1977 needles	>1 year old
Open inverse triangles	=	<i>Lophodermium</i> spp.	1977 needles	<1 year old
Solid inverse triangles	=	<i>Lophodermium</i> spp.	1977 needles	>1 year old
Asterisks	=	<i>C. minus</i>	1978 needles	<1 year old
Open diamonds	=	<i>Lophodermium</i> spp.	1978 needles	<1 year old

in nature. Stahl (1966), however, thought that *N. niveus* might be the cause of a serious needle-cast in the Australian Capital Territory. In North America, Darker (1932) reported that *N. niveus* caused needle-casting in pines, and Hepting (1971) stated that, of the needle fungi on *P. radiata*, "*N. niveus* is probably the most widespread and damaging".

The first report of a pathogenicity test came from Magnani (1972) who inoculated 1-year-old seedlings of *P. radiata* with a suspension of ascospores of *N. niveus* and placed them in a glasshouse. The results of the test were negative and he concluded that *N. niveus* was a weak pathogen of debilitated needles of *P. radiata*. Kistler & Merrill (1978) inoculated 4- to 5-year-old *P. sylvestris* plants by placing needles bearing apothecia of *N. minor* on them, misted the plants for 72 hours, and placed them outside. Five out of 20 trees developed symptoms of needle-cast. Unfortunately, as it is not clear whether the uninoculated controls were also kept outside, one cannot be absolutely certain that the inoculum was responsible for the symptoms which developed. Although a semi-popular account of the pathogenicity trial detailed in this paper has been published (Forest Research Institute 1977), the first documented report which demonstrated that *N. minor* can act as a primary pathogen was that of Karadzic (1981). He inoculated 2-year-old transplants of *P. sylvestris* with a suspension of ascospores from culture and misted the plants for 10 days in a glasshouse where the chances of accidental contamination were low. After 2 months needles showed the first symptoms of the disease, and after 4 months apothecia began to develop on affected needles. Nearly half the needle segments from inoculated plants plated on 2% malt agar developed cultures of *N. minor*.

The pathogenicity trial described here showed that *C. minus* caused the symptoms held to be typical of *Cyclaneusma* (*Naemacyclus*) needle-cast on *P. radiata* in day/night temperatures varying from 25°/20°C to 15°/10°C with 4 days of leaf wetness, that all types of inocula were equally effective, and that the seedlings tested (<3 years old) and some adult plants of *P. radiata* were resistant to infection by *C. minus*. These results, the work of Karadzic (1981), and the supporting evidence of Kistler & Merrill (1978) show that *C. minus* is pathogenic to *P. radiata* and *P. sylvestris*. The finding that young seedlings used in this experiment and some individuals of *P. radiata* were resistant to infection by *C. minus* may serve to explain the inconclusive results of past pathogenicity tests (e.g., Magnani 1972). *Pinus sylvestris* seedlings were not resistant to infection by *C. minus* (Karadzic 1981).

In the central North Island of New Zealand infection by *C. minus* was first recorded in late autumn–early winter. Merrill *et al.* (1980), working in Pennsylvania, United States, with *P. sylvestris* recorded one major infection period in spring and two minor infection periods in summer and autumn–early winter. In New Zealand the needles were about 8–9 months old when isolates of *C. minus* were first obtained, whereas in Pennsylvania first infection occurred when the needles were fully expanded (about 5 months old) and the major infection took place when the needles were 12–13 months old. Rack (1981), working on the colonisation of *P. sylvestris* needles by *Cyclaneusma* and *Lophodermium seditiosum*, found that in Germany *Cyclaneusma* was first isolated from the current season's needles in late autumn when they were about 6 months old and the number of infections increased until the needles were about

1 year old, a pattern very similar to that found in this study. The isolation chronology with *L. seditiosum* was, however, the reverse of that of *Lophodermium* spp. in New Zealand. *Lophodermium seditiosum* was first isolated from 3-month-old needles in summer, well before *Cyclaneusma* made its first appearance. In New Zealand *Lophodermium* spp. lagged 1–2 months behind *C. minus*, which suggests that they are secondary colonisers rather than primary pathogens here, or that they sporulate later. Rack (1981) also reported that when needles were plated *Cyclaneusma* colonies nearly always grew out only from the cut ends. In the present study no such trend was found and the needle segments frequently had *Cyclaneusma* colonies growing out of the sides as well as the cut ends. These differences in behaviour may be accounted for by climatic differences, or they may indicate that either *P. sylvestris* needles are more susceptible to *Lophodermium* infection or that *L. seditiosum* is a more aggressive pathogen of *P. sylvestris* than the *Lophodermium* spp. present in New Zealand are of *P. radiata*. Minter & Millar (1980) considered *L. seditiosum* to be the most pathogenic *Lophodermium* species. Whatever the minor differences, all studies agree that *Cyclaneusma* is not isolated from young needles (<6 months old) and that the symptoms of the needle-cast do not appear until the needles are about 1 year old.

This study showed that the frequency of occurrence of *Cyclaneusma* ascospores in the air was related to periods of rainfall. This effect of rainfall is well known for many ascomycetes (Ingold 1971) and has been recorded for *Naemacyclus* (Pawsey 1967). Over the period of this study, rain was frequent and did not appear to be a factor which would limit ascospore release. The time of the year when the largest number of apothecia were present was, as expected, the time when ascospores were most frequently trapped and when most of the infection occurred. There were two peak periods of needle-cast and, of the two, the needle-cast in spring was the heavier. Most of the needles cast in the spring were just over a year old and were shed prematurely, almost certainly as a result of infection by *C. minus*. It might have been expected that the largest total number of apothecia would therefore be found in spring. But the needles cast in spring had fewer apothecia than the needles which fell in autumn–winter (Fig. 3) and although the total number of needles available for inoculum production was greater in spring, the total number of apothecia was less. A possible explanation of this relative paucity of apothecia is that the spring-cast needles had been exposed to only one infection and therefore had lower *C. minus* populations than the needles which were cast the following autumn–winter and had been exposed to two infection periods (Fig. 8).

The work described here supports and helps to explain the observation that although *C. minus* is common in the litter layer of *P. radiata* in New Zealand, the needle-cast caused by it is severe only in years with mild wet winters (Gilmour 1966). The weather in spring and summer when the current foliage is resistant to infection and the inoculum levels are generally low cannot affect the incidence of the needle-cast. In autumn or winter, when the needles are susceptible to infection, wet periods of about 5 hours duration and temperatures above 10°C would provide good conditions for infection. Severe needle-cast in the spring would be expected after an autumn or winter in which such conditions prevailed.

ACKNOWLEDGMENTS

The Director, Plant Physiology Division, DSIR, Palmerston North, kindly gave permission to use the growth rooms.

I am most grateful to Mrs A. D. Bawden for doing all the tedious work for the major part of this project and to Miss J. G. Somerville for taking over in the later stages. I thank Mr M. Kimberley for doing the statistical analysis of the data. The critical comments of my colleagues and of Dr Millar, Mr Strouts, and an anonymous referee were most helpful in revising the original manuscript.

REFERENCES

- BENITO MARTINEZ, J.; TORRES JUAN, J. 1965: Enfermedades de las coníferas Españolas. **Instituto Forestal de Investigaciones y Experiencias, Madrid, Boletina No. 88.**
- BUTIN, H. 1973: Morphologische und taxonomische Untersuchungen an *Naemacyclus niveus* (Pers. ex Fr.) Fuck. ex Sacc. und verwandten Arten. **European Journal of Forest Pathology 3:** 146-63.
- DARKER, G. D. 1932: The Hypodermataceae of conifers. **Contributions from the Arnold Arboretum No. 1:** 92.
- DENNIS, R. W. G. 1978: "British Ascomycetes". J. Cramer, Vaduz. p. 235.
- DiCOSMO, F.; PEREDO, H.; MINTER, D. W. 1983: *Cyclaneusma* gen. nov., *Naemacyclus* and *Lasiostictis*, a nomenclatural problem resolved. **European Journal of Forest Pathology 13:** 206-12.
- FOREST RESEARCH INSTITUTE 1977: How important is *Naemacyclus*? **New Zealand Forest Service, Forest Research Institute, What's New in Forest Research No. 56.**
- FUCKEL, L. 1873-4: Symbolae mycologicae, Beiträge zur Kenntniss der Rheinischen Pilze, 2nd supplement. **Jahrbücher des Nassauischen Vereins für Naturkunde 27-28:** 49-50.
- GILMOUR, J. W. 1959: Pathogenic needle-cast fungi. **New Zealand Forest Service, Forest Research Institute, Interim Research Release.**
- 1966: The pathology of forest trees in New Zealand. **New Zealand Forest Service, Forest Research Institute Technical Paper No. 48.**
- HEPTING, G. H. 1971: Diseases of the forest and shade trees of the United States. **U.S. Department of Agriculture Handbook No. 386.**
- INGOLD, C. T. 1971: "Fungal Spores". Clarendon Press, Oxford.
- KARADZIC, D. 1981: Infection of *Pinus sylvestris* by *Naemacyclus minor*. Pp. 99-101 in Millar, C. S. (Ed.) "Current Research on Conifer Needle Diseases". Aberdeen University, Aberdeen.
- KISTLER, B. R.; MERRILL, W. 1978: Etiology, symptomology and control of *Naemacyclus* needlecast of Scotch pine. **Phytopathology 68:** 267-71.
- KORF, R. P. 1962: A synopsis of the Hemiphacidiaceae, a family of the Helotiales (Discomycetes) causing needle-blight of conifers. **Mycologia 54:** 12-33.
- MAGNANI, G. 1972: Sulla presenza di *Naemacyclus niveus* su aghi di *Pinus radiata*. **Pubblicazioni del Centro di Sperimentazione Agricola e Forestale 11:** 315-9.
- MERRILL, W.; KISTLER, B. R.; ZANG, L.; BOWEN, K. 1980: Infection periods in *Naemacyclus* needlecast of Scots pine. **Plant Disease 64:** 759-61.
- MILLAR, C. S.; MINTER, D. W. 1980: *Naemacyclus minor*. **CMI Descriptions of Pathogenic Fungi and Bacteria No. 659.**
- MINTER, D. W.; MILLAR, C. S. 1980: Ecology and biology of three *Lophodermium* species on secondary needles of *Pinus sylvestris*. **European Journal of Forest Pathology 10:** 169-81.

- PAWSEY, R. G. 1967: Spore discharge of *Naemacyclus niveus* following rainfall. **Transactions of the British Mycological Society** 50: 341-7.
- PEACE, T. R. 1962: "Pathology of Trees and Shrubs". Oxford University Press, London.
- RACK, K. 1981: Interactions between *Naemacyclus minor* and *Lophodermium pinastri* during 'pine needle cast'. Pp. 103-11 in Millar, C. S. (Ed.) "Current Research on Conifer Needle Diseases". Aberdeen University, Aberdeen.
- REHM, H. 1896: Ascomyceten: Hysteriaceen und Discomyceeten. P. 174 in "Rabenhorst's Krypogamen-flora von Deutschland, Oesterreich und der Schweiz", Vol. 1, Part 3. E. Kummer, Leipzig.
- SACCARDO, P. A. 1884: Conspectus generum Discomycetum hucusque cognitorum. **Botanisches Centralblatt** 18: 247-56.
- 1889: "Sylloge Fungorum", Vol. 8. Padua. Pp. 701-2.
- STAHL, W. 1966: Needle-cast fungi on conifers in the Australian Capital Territory. **Australian Forestry** 30: 20-32.