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Seasonal Pattern in Production of Conidia of *Godronia cassandrae* f. sp. *vaccinii* in Highbush Blueberry in Norway

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Summary

Godronia canker (*Godronia cassandrae* f. sp. *vaccinii*) is a severe disease in young plantings of highbush blueberry (*Vaccinium corymbosum* L.) in Norway. In Europe, only the imperfect stage (*Topospora myrtillii*) has been reported, and thus conidia produced in pycnidia are assumed to be important for dissemination of the fungus. The seasonal pattern of production of conidia was investigated in a commercial planting of 'Jersey' in 1998 and a research planting of 'Bluecrop' in 1998 to 2000. The disease became apparent in March on shoots infected the preceding growing season, and lesions started to form pycnidia containing conidia in April. Samples of stems with distinct lesions of Godronia canker were collected from the bushes every two to three weeks from early spring to autumn. Stem pieces with lesions were shaken in water to release conidia from the pycnidia, and the numbers of conidia were determined by microscopy. Conidia were present throughout the growing season, but the highest numbers occurred during May, with 10^6 to more than 10^7 conidia per lesion. The numbers generally decreased during July to 20 % or less of that found in May, and decreased further during September to around 4 %. Ability to germinate remained high throughout the period conidia were trapped, from April to November/December. Options to manage the disease are discussed.

Key words. epidemiology – *Fusicoccum* canker

Introduction

Godronia canker and the causing organism

Godronia canker (*Godronia cassandrae* (Peck) f. sp. *vaccinii* Groves) causes dieback of stems and young bushes of highbush blueberry (*Vaccinium corymbosum* L.) and is most prevalent in cool, humid climates in Europe and North America (Ramsdell 1995). In Norway, the disease was first recognised in 1995 (Stensvand and Langnes 1995), and it has since caused severe damage in highbush blueberry in all parts of the country where plantings have been established (Strømeng and Stensvand 2001). The perfect stage of the fungus has been found in Canada (Creelman 1958; McKeen 1958) and USA (Weingartner and Klos 1975a), but has never been reported from Europe, and it is thus assumed that the imperfect stage, *Topospora myrtillii* Feltg. (syn. *Fusicoccum putrefaciens* Shear), is the most important for dissemination of the disease (Parker and Ramsdell 1977; Melzer and Hoffmann 1980). *G. cassandrae* f. sp. *vaccinii* overwinters as mycelium in infection sites on the stems (Creelman 1958; Lockhart and Craig 1967; Weingartner and Klos 1975a). In spring, symptoms appear on the stems, often on the lower part, as circular to elliptical, grey to dark brown lesions. Complete girdling of the stems causes wilting above the infection site. Conidia are produced in pycnidia in the lesions and are spread by splashing water (Parker and Ramsdell 1977). One- and two-year-old stems are infected through stomata, petioles, leaf scars or wounds (Weingartner and Klos 1969, 1975b; Parker and Ramsdell 1977), and infections may occur in both spring/early summer (May and June) and in the autumn (from August) (McKeen 1958; Parker and Ramsdell 1977).

Time of formation of pycnidia and conidia

Previous reports indicate that production of conidia may occur at different times in different geographical regions. A study during two growing seasons in Michigan revealed that conidia were most numerous in late May (Parker and Ramsdell 1977), numbers decreased as the season progressed, and after August there were very few conidia left. These results are supported by observations on presence of pycnidia in Massachusetts, USA (Zuckerman 1959) and in British Columbia, Canada (McKeen 1958). In contrast, pycnidia first appeared in the stem lesions at the end of July and were visible for a period of five weeks in Nova Scotia, Canada (Creelman 1958). In Germany, pycnidia were formed throughout the year, and although they were most numerous in early spring, viable conidia were found throughout the year (Menzinger and Krupp 1977). However, no further investigations on time of

conidia formation were carried out in any of these regions.

Since there is some disagreement between previous reports on when conidia might be most abundant, and since a season-long investigation of conidia formation have not been thoroughly carried out outside Michigan, we wanted to study the seasonal pattern of conidia production more closely under climatic conditions occurring in Norway. A preliminary part of this study was included in a diploma-thesis (Strømeng 1999).

Materials and Methods

Time and location of sampling

Infected plant material was collected from two highbush blueberry fields in Norway; a commercial planting of 'Jersey' established in 1994 at Rygge in Østfold county, and a research planting of 'Bluecrop' established in 1990 at the Norwegian University of Life Sciences at Ås in Akershus county. Ås is located approximately 30 km south of Oslo, and Rygge is located an additional 30 km further south. The plant material had been propagated by cuttings and imported from Germany or The Netherlands as 3- to 4-year old plants. 'Bluecrop' was established with drip irrigation, and bark was used as mulching material. 'Jersey' was established without mulching and supplied with overhead irrigation. Sampling was done at Rygge in 1998 and Ås in 1998–2000. Stem pieces of 1-year old stems with distinct lesions containing *G. cassandrae* f. sp. *vaccinii* were collected at regular intervals during the growing season. At Rygge, samples were collected approximately every three weeks from the end of April to the end of September (9 times). At Ås in 1998, samples were collected approximately every three weeks from the end of April to the end of October (10 times). The following two years, samples were collected every two to three weeks at Ås; in 1999 from the end of April to early December (16 times), and in 2000 from early April to early November (12 times).

Sample preparation and spore counting

For each sampling time, stems with distinct lesions of Godronia canker, but still with green, healthy looking leaves, were collected at random from the bushes to obtain nine separate stem pieces, each with one lesion. The lesions were 1.5 to 4 cm long. The nine stem pieces were randomly divided into three replicates, each put in a 250 ml bottle with 25 ml distilled water added, and placed on a shaker (VKS, Edmund Bühler, Hechingen, Germany) for 60 min at a speed of 100 rotations min^{-1}. The stem pieces were removed from the conidial suspensions immediately after shaking to avoid further release of conidia. Droplets of the spore suspensions were placed on a haemacytometer (Bürker, Brand, Wertheim, Germany), and the conidia were counted in a light microscope. The number of conidia for each replicate was adjusted to numbers ml^{-1} of the conidial suspension, and the mean of the three replicates were calculated for each sampling time. The numbers per lesion were estimated by multiplying the mean number of conidia per ml by 25 and then dividing by three (there were three stem pieces in each replicate).

Germination of conidia

Ability to germinate was investigated in 1999, from April 30 to November 20 (12 times). Two droplets (approx. 50 µl) of spore suspension from one of the replicates at each sampling date were placed on a microscope slide. Blueberry juice was added to one of the droplets (2 µl) each time. The slides were incubated in sealed Petri dishes with moist filter paper in the bottom (to keep the humidity high) at room temperature (21±1 °C) for 24 h. Germination of 100 conidia in each of the two droplets was recorded each time. Conidia with a germinating tube longer than the width of the conidium were considered germinated.

Time of lesion appearance

In all years at Ås, new stems produced in the preceding growing season were examined in the autumn, winter and following spring to determine when symptoms of Godronia canker started to appear on the stems.

Weather data and statistics

Data on precipitation and temperature were provided from local weather stations (LMT, Bioforsk, Norway) at Rygge and Ås. Times of flowering and fruiting were recorded for 'Bluecrop' at Ås, but not for 'Jersey' at Rygge. Analysis of variance on numbers of conidia found at different times during the season within each field was performed using the GLM-procedure of Minitab 15 statistical software (Minitab Inc., USA), with sampling times as treatment factor. Data were log-transformed (Ås 1999) or arcsin square root-transformed (Rygge 1998, Ås 1998, Ås 2000) prior to the analyses to meet the assumption of normal distribution. The method of transformation that gave the best result in a normality test was chosen for the different data sets. Transformed treatment means were compared using Tukey's test (P=0.05). Non-transformed data are presented.

Results

Phenological development

In all years and both fields, bud swell occurred in mid April, and bud burst was in late April to early May. Dates of bloom and harvest in 'Bluecrop' are shown in Table 1. Full bloom occurred in early to mid June, and harvest lasted from mid August to early to mid September. Flowering and harvest was a few days later for 'Jersey' than for 'Bluecrop'. In September or October the leaves turned red, and by mid November most leaves were abscised.

Disease development

Examination of the current season's growth revealed that no symptoms were visible on young stems in the autumn and winter (data not shown). In March, small, red lesions started to appear on many of the stems. The lesions could not be visually determined as Godronia canker at that time, but were marked and determined later. Distinct lesions generally developed during April and the first part of May. Pycnidia started to appear in April, and were

Table 1. Dates of full bloom and harvest during 1998 to 2000 in highbush blueberry 'Bluecrop' at Ås, Norway.

Year	Full bloom	Harvest period
1998	June 19	August 12 – September 18
1999	June 18	August 18 – September 08
2000	June 05	August 15 – September 05

usually easy to detect in early May. Stems infected close to the base developed normally in the spring, but started to wilt in June. Yet, there were infected, living stems with visible symptoms of Godronia canker present in the field throughout the growing season.

Conidial production over the seasons

The highest numbers of conidia occurred in spring and early summer at both locations and in all three years. In 1998, conidia were most abundant in the samples collected in mid May at both Rygge and Ås, and there was a marked increase in conidial numbers from the first sample collected in late April to the second in mid May (Fig. 1 and 2). At Rygge, the number of conidia in the May sample (9×10^6 conidia ml^{-1}) was significantly higher than in all the other samples collected that season ($P<0.0001$) (Fig. 1). Although the number of conidia in the third sample collected at Rygge in early June was reduced to less than a third of the number in May, it was still significantly higher than what were found in the samples collected in April, and in all samples collected from late July and throughout the season ($P<0.0001$). The number of conidia in the May sample at Rygge corresponded to 7×10^7 conidia from each single lesion. This was more than 50 times higher than in July, and more than 600 times higher than in September. A similar seasonal pattern was found at Ås, but the peak in conidial production in May was lower compared to the rest of the season (Fig. 2). There were greater variations between the replicates of each sampling date than at Rygge, and the number of conidia in the May sample at Ås was only significantly higher than the sample collected in October ($P=0.01$). At Ås, the mid May sample had an average of 1×10^6 conidia ml^{-1}, corresponding to approximately 1×10^7 conidia per lesion. This was almost four times higher than in July, and more than 20 times higher than in September. The 1998 season was characterised by dry weather in May, but more abundant precipitation during the summer (Fig. 1 and 2).

The seasonal pattern of conidia production at Ås in 1999 was similar to the previous year. The highest number occurred at the end of May (6×10^5 conidia ml^{-1}, corresponding to approximately 5×10^6 conidia per lesion) (Fig. 3). This sample contained five times more conidia than what was found in July, and 15 times more than in September. Conidia were detected as late as in December (1×10^3 conidia ml^{-1}, corresponding to approximately 8×10^3 conidia per lesion). There were few significant differences between conidial numbers in the samples, but

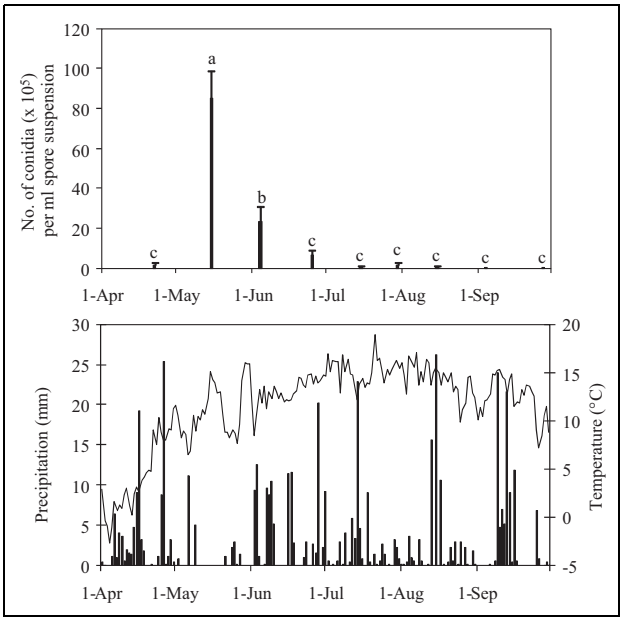


Fig. 1. Production of conidia of *Godronia cassandrae* f. sp. *vaccinii* in a commercial highbush blueberry field ('Jersey'), at Rygge, Norway 1998. Upper graph: Numbers of conidia in stem cankers (mean of three replicates, each with three cankers washed in 25 ml water). Error bars indicate standard error of the mean. Columns with different letters are significantly different (Tukey's test, P=0.05). Lower graph: Daily accumulated precipitation (bars) and daily mean temperatures (solid line).

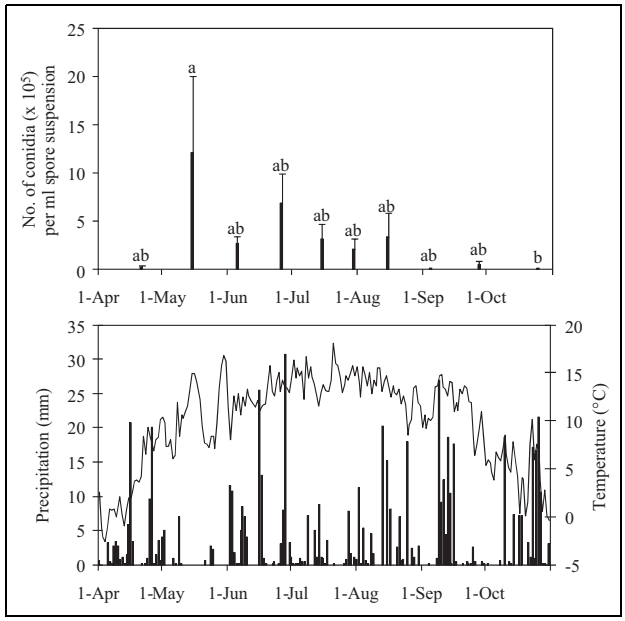


Fig. 2. Production of conidia of *Godronia cassandrae* f. sp. *vaccinii* in a highbush blueberry research field ('Bluecrop'), at Ås, Norway 1998. Upper graph: Numbers of conidia in stem cankers (mean of three replicates, each with three cankers washed in 25 ml water). Error bars indicate standard error of the mean. Columns with different letters are significantly different (Tukey's test, P=0.05). Lower graph: Daily accumulated precipitation (bars) and daily mean temperatures (solid line).

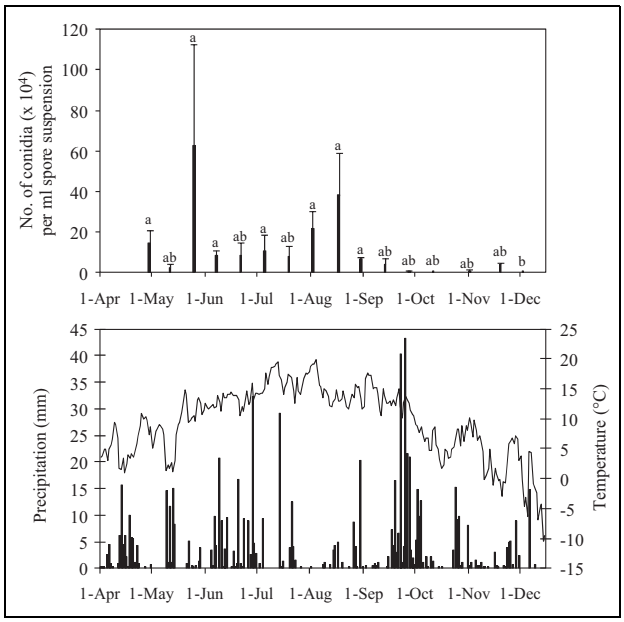


Fig. 3. Production of conidia of *Godronia cassandrae* f. sp. *vaccinii* in a highbush blueberry research field ('Bluecrop'), at Ås, Norway 1999. Upper graph: Numbers of conidia in stem cankers (mean of three replicates, each with three cankers washed in 25 ml water). Error bars indicate standard error of the mean. Columns with different letters are significantly different (Tukey's test, P=0.05). Lower graph: Daily accumulated precipitation (bars) and daily mean temperatures (solid line).

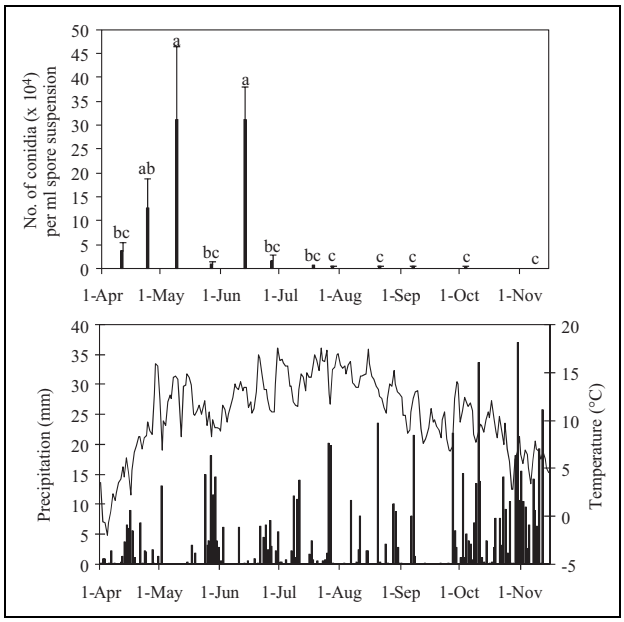


Fig. 4. Production of conidia of *Godronia cassandrae* f. sp. *vaccinii* in a highbush blueberry research field ('Bluecrop'), at Ås, Norway 2000. Upper graph: Numbers of conidia in stem cankers (mean of three replicates, each with three cankers washed in 25 ml water). Error bars indicate standard error of the mean. Columns with different letters are significantly different (Tukey's test, P=0.05). Lower graph: Daily accumulated precipitation (bars) and daily mean temperatures (solid line).

samples collected in late April and May, early June and July, and all samples collected in August had significantly more conidia than the sample collected in early December (P=0.003). In 1999, May was cooler than in the other years, and June and September had heavy rain (Fig. 3).

In 2000, the number of conidia increased from early April to early May, but in late May there was a distinct drop in the numbers, followed by an increase in mid June to the same level as in early May (Fig. 4). From July to October, numbers were low, and no conidia were found in the last sample collected in November. The highest number of conidia during the season occurred in early May and mid June (3×10^5 conidia ml^{-1}, corresponding to approximately 3×10^6 conidia per lesion). These samples contained 45 times more conidia than in July, and more than 90 times higher than in September. Except for the sample collected in late April, there were significantly more conidia in the early May and mid June samples than in the other samples in that season (P<0.0001) (Fig. 4). The late April sample contained significantly more conidia than the ones collected from late July and throughout the season. Precipitation during the season was mostly moderate, but the autumn was mild with abundant rain (Fig. 4).

Germination

Conidia germinated at all sampling dates during 1999 (Table 2). On average, germination was slightly higher (not statistically different) in spore suspensions containing blueberry juice than in spore suspensions without (88 and 81 %, respectively). Germination was generally higher than 70 % during spring, early summer and autumn, but in the sample collected on July 20, the germination was low in both spores suspended in pure water (55 %) and in spores suspended in water with blueberry juice added (24 %).

Discussion

Most conidia formed in spring and early summer

Conidia of *G. cassandrae* f. sp. *vaccinii* were most numerous in spring and early summer, and although delayed one month compared with Michigan, this correlates well with findings there (Parker and Ramsdell 1977). We observed abundant pycnidia formation in spring, which was also observed in British Columbia (McKeen 1958), Massachusetts (Zuckerman 1959) and Germany (Menzinger and Krupp 1977). Our observations did not agree with those in Nova Scotia where pycnidia appeared in lesions from late July (Creelman 1958). Furthermore, we found that conidia were present in lesions all through the growing season, which was also observed in Germany and Michigan (Menzinger and Krupp 1977; Parker and Ramsdell 1977). Zuckerman (1960) suggested that climatic differences possibly contributed to variations in time and duration of pycnidia formation. However, if comparing normals for monthly mean temperatures and monthly accumulated precipitation (for the periods 1971–2000 for North-America and 1961–1990 for

Table 2. Germination of conidia (100 conidia in each sample) of *Godronia cassandrae* f. sp. *vaccinii* from highbush blueberry 'Bluecrop' at Ås, Norway in 1999.

Date of registration	Percent germination	
	Pure	Juice added
April 30	100	–[+]
May 12	97	97
May 26	69	100
June 8	94	100
June 22	100	100
July 6	87	97
July 20	55	24
August 3	83	94
August 18	32	81
August 31	87	97
September 14	–	96
November 20	90	78

[+] Germination not investigated

Europe, available online) for Michigan (East Lansing), Massachusetts (East Wareham), Germany (Osnabrück), Nova Scotia (Kentville), and British Columbia (Pitt Meadows) to those of Rygge and Ås, this indicates that the climate of Nova Scotia is the most similar to that of the two locations in Norway. Climatic differences in the spring will most likely influence the start of pycnidia formation, but do not seem to explain the difference between Nova Scotia and other areas. In Germany, conidia were present all year in 1974 and 1975 (Menzinger and Krupp 1977), but the numbers were not quantified. The authors suggested that the mild winters made conidia present also at that time of the year. Under conditions in the present study, new lesions never started to develop before March.

Effect of weather on availability of conidia

Precipitation patterns during the season most likely influenced the number of conidia in the samples. Rain prior to or during sample collection in the field presumably washed out mature conidia, contributing to lower numbers after rainy periods. This seemed to be the case at Ås in May 1999 (Fig. 3) and 2000 (Fig. 4); we expected to find high numbers of conidia in the samples, but after periods of heavy rain the numbers were low. Correspondingly, dry periods prior to sample collection likely caused accumulation of mature conidia in the lesions, resulting in high numbers of conidia in the samples. The mid May counts at Ås and Rygge in 1998 were carried out after four to five days without rain, coinciding with increasing temperatures, both factors contributing to high numbers of conidia in the samples (Fig. 1 and 2). Similarly, the late May sample at Ås in 1999 (Fig. 3) and the early May and mid June samples at Ås in 2000 (Fig. 4) were collected in periods of dry weather, and all of these contained high numbers of conidia. However, despite protracted dry periods in both fields in September 1998, numbers were low. This was also the case for Ås in September 1999 (Fig. 3) and the first part of July 2000 (Fig. 4), which indicates that fewer conidia were produced in the summer and autumn than earlier in the season. This corresponds with findings in Michigan, where few conidia were trapped in rainwater in the autumn, despite heavy rainfall at that time (Parker and Ramsdell 1977).

Conidia formation and viability late in the season

Conidia were present over the entire growing season and were detected as late as December in 1999 (Fig. 3), after the bushes had entered the dormant period. Unfortunately, viability of the conidia was not investigated at that time, but up to 90 % of the conidia germinated in November that year. Earlier reports have shown that conidia of *G. cassandrae* f. sp. *vaccinii* were able to germinate at 2 °C (Melzer and Hoffmann 1980), and that the mycelium grew on potato dextrose agar at 0 °C (Lockhart 1975). These results suggest that if inoculum is present, infections may occur at times during the year when temperatures are low. This is in agreement with findings that natural infections occurred in both spring and autumn (Weingartner and Klos 1969; Parker and Ramsdell 1977). In this study, the conidial production was much lower in the autumn than in spring and early summer, and this may indicate that the risk of infection is much higher early in the growing season. However, October – November is the period for leaf abscission, and exposure of leaf scars and thus an abundance of possible infection sites in late autumn may counteract some of the strong reduction in conidia formation during that period.

Management

Godronia canker evidently thrives in a Nordic climate and if out of control, it is a limiting factor of highbush blueberry production in Norway (Strømeng and Stensvand 2001). There are great differences in susceptibility to Godronia canker among cultivars (Lockhart and Craig 1967; Liebster 1979; Menzinger and Krupp 1977; Strømeng and Stensvand 2001), but some of the most valuable cultivars are among the most susceptible. When establishing new plantings of highbush blueberry, it is important to use disease free planting material and cultural practices that allow good aeration in the plantings. Chemical control of *G. cassandrae* f. sp. *vaccinii* is difficult because there are no available fungicides with high efficacy, although some broad spectre, preventive fungicides seem to reduce disease incidence (Stensvand, unpublished data, Szmagara 2008). Fungicides used to control Godronia canker have also failed because they have not been applied frequently enough throughout the season (Creelman 1958; Zuckerman 1960; Sprau 1969; Menzinger and Krupp 1977). However, based on the present and other findings, broad spectre fungicides, such as mancozeb (Szmagara 2008), could be applied on a regular basis during the period of highest release of conidia in May to mid summer, and from after harvest throughout leaf fall in autumn. To avoid a build-up of conidial inoculum and to reduce the number of new infections in already infested plantings, stems showing symptoms of Godronia canker should be removed as soon as symptoms appear in early spring and prior to maturation of the pycnidia.

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