Discharge and Dissemination of Ascospores by *Venturia inaequalis* During Dew

Arne Stensvand, Terje Amundsen, and Lars Semb, Norwegian Crop Research Institute, Plant Protection Centre, Department of Plant Pathology, Fellesbygget, 1432 Ås, Norway, and David M. Gadoury and Robert C. Seem, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, 14456

ABSTRACT

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Abundant airborne ascospores of the apple scab pathogen (*Venturia inaequalis*) have never before been observed during periods of dew. We studied ascospore release in *V. inaequalis* in two orchards in southeastern Norway using Burkard 7-day volumetric spore traps. At Ås in 1990, 1992, and 1997, and at Svelvik in 1992, a total of 14.8, 1.4, 0.27, and 26.9%, respectively, of the season's total spore release was trapped during periods of dew. Dew followed by spore release was observed 22 days at the two locations. During one night with dew at Ås in 1990 and two nights with dew at Svelvik in 1992, approximately 13 and 20%, respectively, of the season's total spore numbers were observed. High numbers of spores were trapped prior to sunrise, and on an average, 48.4% of the spores were trapped prior to 0400 in the morning. Episodes in which more than 1% of the season's inoculum was released during dew occurred around bloom of apple, which is the peak period for ascospore discharge, and followed more than 2 days of fair weather (clear, warm days and cool, humid nights). The ordinary suppression of ascospore release in *V. inaequalis* during darkness has been overcome in previous studies under laboratory conditions when protracted periods favorable for ascospore maturity occur without opportunity for ascospore discharge. This is the first confirmed report of relatively large (>10% of the season's total inoculum) numbers of airborne ascospores in orchards during dew. The sequential occurrence of specific weather conditions, for example (i) fair-weather days, (ii) cool nights with abundant dew formation, (iii) significant release and dispersal of airborne ascospores, and (iv) poor drying conditions or additional hours of leaf wetness due to fog or rain, would be required for dew-released ascospores to constitute a threat of infection. Absent the foregoing, release during dew is more likely to deplete the ascospore supply with no consequent increase in the overall risk of disease.

Additional keywords: aerobiology, epidemiology

Apple scab, caused by the fungal pathogen *Venturia inaequalis* (Cooke) G. Wint., is one of the most important diseases of apples worldwide. Ascospores released by rain from pseudothecia in leaf litter are the principal source of primary inoculum of *V. inaequalis* in most regions. Ascospore release in *V. inaequalis* is closely linked to host phenology, and most ascospores are released between the tight cluster stage of the apple flower bud and 1 to 2 weeks after petal fall (6–9, 12–14, 17–19, 22, 25, 27–29, 31, 32, 34).

Several authors have detected very few or no airborne ascospores in orchard air after dew formation (4, 8, 12, 14, 16–18, 21, 33). Miller and Waggoner (17) detected trace levels of airborne ascospores during one event between 5 and 11 A.M. during which dew may have been present (i.e.,

trapping followed a night during which relative humidity [RH] exceeded 95%). Moore (18) found high numbers of ascospores within a film of dew on overwintered leaves and stated that dew can release ascospores, but that they remain submerged in the relatively thick water film rather than becoming airborne. In this paper, we report on the capture of substantial numbers of airborne ascospores of *V. inaequalis* during and following their release by dew in Norway. Preliminary accounts of this study have been published elsewhere (24, 25).

MATERIALS AND METHODS

Spore traps. Burkard 7-day recording volumetric spore traps (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK) were installed immediately before budbreak in two orchards in southeastern Norway in 1990 (Ås and Svelvik) and at Ås in 1992 and 1997. The traps were powered by either a 12-V DC or a 230-V AC source and were adjusted to sample 10 liters/min. A plastic tape attached to the clock cylinder of the spore trap served as the trapping surface. The tape was prepared, removed, dissected, and

examined microscopically for ascospores of *V. inaequalis* as described by MacHardy and Gadoury (16). The number of ascospores of *V. inaequalis* recorded in each transect of the microscope tape was adjusted for the proportion of the tape examined and the volume of air sampled. Spore numbers were reported as spores per m³ air.

Weather stations. Electronic data-loggers with sensors (KMS-P from Anton Paar KG, Graz, Austria; or Instrumenttjenesten AS, Ås, Norway) provided hourly records of precipitation, temperature, RH, and leaf wetness (LW) at each site. Each device had two LW sensors, one of which was placed within the canopy in an orientation not exposed to direct sunlight or wind, at a height of 1 to 1.5 m aboveground. A second sensor was placed outside the canopy at approximately the same height. The exterior sensor was used to detect the initial rain or dew formation to define the starting time of wetting periods, while the interior sensor was used to define the end of the wetting period. Temperature and RH were measured outside the canopy at 2 m aboveground within weather shelters or radiation shields. The rain gauges measured rain at 0.2-mm increments. Additional precipitation data at Ås were obtained from a rain gauge with 0.1-mm accuracy at a meteorological station situated approximately 1 km from the orchard. Dew point at 2200 h was calculated based on relative humidity and temperature, using a Molliere diagram.

Inoculum source. A 2 to 3 m² area of the orchard floor at each site was covered with heavily infected apple leaves of the cultivars Summerred, Lobo, Gravenstein, or Vista Bella in the fall. Orchards were located on level to gently sloping terrain with full exposure to sun, and bore trees approximately 2 to 3 m in height on dwarf or semidwarf rootstocks. Overwintered leaves were held in place with a plastic screen and exposed to rain and snow. To prevent earthworms from consuming the leaf litter during winter and spring, the ground of the study area was drenched with endosulfan prior to overwintering the leaves.

RESULTS

The total numbers of ascospores trapped per m³ of air per season were 20,115, 33,772, and 87,085 at Ås in 1990, 1992, and 1997, respectively, and 6,639 at

Corresponding author: D. M. Gadoury
E-mail: dmg4@cornell.edu

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Svelvik in 1992. At Ås in 1990, the cultivar Gravenstein was in bloom on 5 May and at petal fall on 9 May. Bloom and petal fall at Ås and Svelvik in 1992 occurred on 24 May and 30 May, respectively. In 1997, Gravenstein reached bloom and petal fall at Ås on 30 May and 2 June, respectively.

Trapping of ascospores of *V. inaequalis* coincided with dew on 22 occasions (Table 1). Totals of 14.8, 1.4, 0.27, and 26.9% of the season's ascospores were trapped during dew at Ås in 1990, 1992, and 1997, and at Svelvik in 1992, respectively (Table 1). During one night with dew at Ås in 1990, approximately 13% of the season's total number of spores was trapped. At Svelvik, ascospores were trapped during six successive nights with dew from 19 to 24 May 1992. During two of these nights, approximately 20% of the season's ascospores was trapped.

Table 1 shows climatic conditions occurring during the episodes when spores were released during dew. The difference between maximum temperature the preceding day and minimum temperature during the night varied from 6 to 17°C. In 20 of the episodes, the minimum temperature during night was 1 to 6°C below the dew point. In two of the episodes, minimum temperature was not below the dew point, but dew was observed on the ground in the morning. Zero to 10 h of moisture was recorded by the leaf wetness sensors during dew periods in which airborne ascospores were trapped.

In episodes of dew where a total of more than 75 spores per m³ of air were trapped,

spore catches were distributed between 2200 and 0900 h (Fig. 1), often with significant captures before 0300 h (Fig. 1). During the episode 6 to 7 May 1990 at Ås, in which 2,606 ascospores were trapped, 77.3% of the spores were detected before 0300 h.

Table 2 shows spore releases during the last rainy day before and the first rainy day after the dew periods in Table 1. Each rain event occurred during daylight; thus the magnitude of ascospore release should not have been confounded by darkness (4,16). No consistent relationship was observed between the relative numbers of ascospores released during dew and the rain events that preceded or followed the dew period. At Ås in 1990 and at Svelvik in 1992, fewer ascospores were observed during the rain event preceding dew than during dew (Table 2). In the same years and locations, ascospore numbers during dew approximated those observed during rain that followed dew (Table 2). However, at Ås in 1992, more ascospores were trapped during the rain events preceding and following dew than during all dew periods combined. The total number of ascospores trapped at Ås in 1992 during all dew episodes was only 2.5% of the number trapped during the rain events before and after dew (Table 2), far less than was trapped during dew at Ås in 1990 or at Svelvik in 1992 (Table 2). At Ås in 1997, the percentages trapped during dew were even lower. Compared with the first rain event preceding and following dew episodes, only 0.1 to 2.5% of the ascospores were trapped during dew.

Fig. 1. Release of ascospores by *Venturia inaequalis* during dew at Ås in 1990 and 1992 and at Svelvik in 1992 in Norway. Ascospore release was measured by a Burkard volumetric spore sampler at each site. Indicated interval for sunrise is for the period 1 May to 1 June.

Table 1. Ascospores of *Venturia inaequalis* trapped in a Burkard 7-day volumetric spore trap during periods of dew: duration of leaf wetness, maximum temperature the day preceding spore release, minimum temperature during night of spore release, calculated dew point at 2200 h, number of ascospores trapped per m³ air, and the percentage of the season's total number of ascospores trapped for each episode

Location, date, and degree-day accumulation (Base = 0°C) ^a	Duration of leaf wetness (h)	Max. temp previous day (°C)	Min. temp during night (°C)	Dew point at 2200 h (°C)	No. of spores	Season's spores trapped (%)
Ås 1990						
6-7 May, 379 DD	2	22.8	5.7	10.6	2,606	12.96
9-10 May, 396 DD	7	22.9	9.0	10.8	176	0.87
10-11 May, 413 DD	8	22.3	7.6	11.8	203	1.01
Ås 1992						
14-15 May, 243 DD	5	18.9	9.0	10.3	8	0.02
20-21 May, 335 DD	5	22.3	9.1	12.5	153	0.45
23-24 May, 391 DD	3	24.4	11.6	14.7	77	0.23
24-25 May, 410 DD	1	27.0	11.8	15.0	100	0.30
26-27 May, 451 DD	1	23.8	11.1	13.5	84	0.25
27-28 May, 491 DD	0	26.7	12.3	11.5	15	0.04
30-31 May, 554 DD	0	27.7	11.8	11.5	8	0.02
6-7 June, 709 DD	3	28.3	12.5	15.0	31	0.09
Svelvik 1992						
18-19 May, 292 DD	6	20.7	6.8	10.0	184	2.77
19-20 May, 307 DD	5	21.8	9.0	11.4	222	3.33
20-21 May, 322 DD	10	19.5	7.0	11.0	621	9.35
21-22 May, 338 DD	3	23.9	9.2	11.9	713	10.74
22-23 May, 356 DD	4	23.8	11.3	14.2	23	0.23
23-24 May, 372 DD	9	20.0	9.9	12.8	21	0.46
Ås 1997						
17-18 May, 193 DD	3	13.1	6.9	9.1	46	0.05
5-6 June, 394 DD	2	15.1	5.9	7.8	31	0.04
9-10 June, 462 DD	8	21.4	7.8	13.5	61	0.07
11-12 June, 496 DD	4	23.0	9.6	10.2	8	0.01
13-14 June, 533 DD	8	22.6	11.9	12.0	84	0.10

^a Accumulated degree-days from date of bud break.

Capture of airborne ascospores during dew began during the exponential phase of ascospore maturation (9), approximately 250 to 375 degree-days (base = 0°C) after budbreak (Table 1), and was observed as late as 709 degree-days after budbreak (Table 1). Trapping also followed 2 or more days with no rain and sunny, relatively warm, dry days and cool, humid nights. At Ås in 1990, minimum night temperatures ranged from 5 to 10°C, followed by daytime maxima of 20 to 26°C before and during the days on which ascospores were released by dew. RH often exceeded 95% during the night, while the RH at midday was 20 to 40%. Similar conditions occurred in 1992 and 1997.

DISCUSSION

Dew alone has never before been reported to be associated with high numbers of airborne ascospores of *V. inaequalis*, although some ascospore release by *V. pirina* has been reported during dew (23). In a 4-year study in a high-inoculum orchard in New Hampshire (16), ascospores of *V. inaequalis* were not trapped during dew, except when dew was closely associated with rain. Of 27 episodes with dew, ascospores were only trapped during two. During these two episodes, rain preceded dew, and only a 2-h dry interval separated rain and dew (16). The number of spores trapped during dew was very low compared with the preceding rain events (16). In a similar study in England (12), a few airborne ascospores were detected during 5 nights of heavy dew without rain, but the number captured was very low compared with release during rain. It was concluded that dew alone had little importance in liberating spores (12,16) unless dew followed rain before the leaves had dried. Similar results and conclusions were reported from studies conducted in Connecticut (17), Wisconsin (8,14), and Switzerland (33).

Brook (4) stated that both low temperatures and darkness before dew formation might depress the rate of spore release during dew and concluded that few ascospores are trapped during dew because few ascospores are released, not because they fail to become airborne. However, Moore (18) found numerous ascospores trapped in a water film on overwintered scabbed

leaves and speculated that asci might remain submerged in thick water films, and thereby release ascospores into the dew film rather than into air. Brook (4) observed that asci protruded approximately 40 µm beyond the ostiole of the pseudothecium before releasing ascospores. Ascospores of *V. inaequalis* rapidly lose energy when discharged into water and travel relatively short distances compared with those discharged into air (1). Given theoretical maximum rates of dew formation of 70 µm/h (11) and the facts that measured dew films range from approximately 300 to 600 µm (11) and that still-air conditions are normally associated with dew formation (15), it is not surprising that ascospores are rarely detected during periods of dew. Nonetheless, we observed relatively large ascospore trappings during dew on 6 to 7 May 1990 at Ås, and on four successive nights at Svelvik in 1992 (Table 1).

Were night temperatures in our study during dew sufficiently low to suppress ascospore release during dew, as suggested by Brook (4)? Our minimum temperatures during nights with dew and high spore release varied from 5.7 to 12.5°C. Considerable ascospore release during rain has been observed at these temperatures in Norway (24) and other countries (12,16). Both Hirst and Stedman (12) and Stensvand et al. (26) reported that the rate of discharge of *V. inaequalis* ascospores is slowed at colder temperatures, but the effect is not great above 6°C. Thus, temperature seems an unlikely factor to either suppress or favor ascospore release during the period of our study.

Ascospore release in *V. inaequalis* is normally suppressed when rain occurs during darkness (2–5,8,12–14,16,20,24,25,30). At Ås and Svelvik, sunrise occurred between 0320 and 0430 h standard time between 1 May and 1 June, respectively (data of Institute of Theoretical Astrophysics, University of Oslo, Norway). Stensvand (24) examined light levels on a clear day in early May at Ås with a multi-spectral radiometer. At a wavelength of 720 nm, a light intensity of 0.03 and 0.65 µW/cm² was recorded at 0330 and 0400, respectively. Gadoury et al. (10) reported light intensity of 0.5 µW/cm² at 725 nm to be stimulatory for ascospore release in *V. inaequalis*. Thus, light inten-

sity sufficient for ascospore release may occur between 0330 and 0400 h in early May in southeastern Norway, in particular on clear evenings and mornings associated with the formation of dew. However, we often observed considerable spore release at Ås and Svelvik several hours before light reached this intensity (Fig. 1). Therefore, elevated light levels during predawn hours at extreme northern latitudes could only partially explain our capture of large numbers of airborne ascospores during dew.

Gadoury et al. (10) have been able to negate the normal suppression of ascospore release during darkness under laboratory conditions by promoting ascospore maturation over extended periods without opportunity for ascospore discharge. They have also reported that the normal diurnal periodicity of ascospore release in orchards may break down as populations senesce and the last 5 to 10% of the ascospores mature (10). Thus, the accumulation of mature ascospores during extended periods with abundant dew (but without rain) and/or the senescence of the pathogen population might occasionally lead to significant ascospore release during dew, given that temperature, thickness of the dew film, and absence of stimulatory light levels are not always limiting. Most of our observations of dew and high ascospore trapping occurred when degree-day accumulation (Table 1) would suggest that ascospore maturation was in an exponential phase (9). Additionally, 7 to 8 rain-free days preceded the two largest ascospore trappings (Tables 1 and 2). Some combination or interaction of these factors may have been responsible for the relatively large numbers of ascospores trapped during occasional dew periods.

This is the first confirmed report of relatively large (>10% of the season's total inoculum) numbers of airborne ascospores in orchards during dew. Obviously, the conditions that promote large releases of airborne ascospores during dew are complex, as well as very uncommon (4,8,12,14,16–18,33). In management of apple scab, long-term (e.g., >24 h) survival of ascospores on dry leaves is assumed to be nil (15). The sequential occurrence of specific weather conditions, for example (i) fair-weather days, (ii) cool nights with

Table 2. Last day of rain prior to and first day of rain after the dew periods in Table 1, mm precipitation per rain event, number of ascospores of *Venturia inaequalis* trapped, and percentage of the season's total number of ascospores trapped

Location/year	Date of preceding rain	Rain (mm)	No. of spores	Season's total spores (%)	Date of subsequent rain	Rain (mm)	No. of spores	Season's total spores (%)
Ås 1990	29 April	1.9	135	0.7	14 May	1.1	2,363	11.8
Ås 1992	12 May	16.4	4,753	14.1	8 June	12.6	14,421	42.7 ^y
Ås 1997	12 May ^y	9.2	4,899	5.6	22 May	1.0	4,171	4.8
Ås 1997	29 May	5.8	7,820	9.0	12 June	...	8,441	9.7
Ås 1997	13 June	... ^z	15,563	17.9	14 June	0.6	3,419	3.9
Svelvik 1992	12 May	12.6	238	3.6	8 June	1.0	1,947	29.2

^y Spore release over 2 days.

^z Sprinkler irrigation, quantity not recorded.

abundant dew formation, (iii) significant release and dispersal of airborne ascospores, and (iv) poor drying conditions or additional hours of leaf wetness due to fog or rain, may be required for dew-released ascospores to constitute a threat of infection. Absent the foregoing, release during dew may be more likely to deplete the ascospore supply without a consequent increase in the overall risk of disease.

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