Negative Geotropism in *Venturia inaequalis*

David M. Gadoury and William E. MacHardy

Research associate and professor, respectively, Department of Botany and Plant Pathology, University of New Hampshire, Durham 03824.
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ABSTRACT

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Pseudothecia of *Venturia inaequalis* developed on both the adaxial and abaxial surfaces of scabbed apple leaves overwintered on the floor of a New Hampshire orchard in 1982. However, the ascocarps were usually not found on both surfaces simultaneously. In a controlled study, scabbed leaves were overwintered in an orchard with either the adaxial or abaxial surface exposed. In mid-February, prior to ascus formation, leaves from each group were collected and incubated in the dark for 30 days at 10 C and 90% RH. The leaves were either inverted upon incubation or were incubated with the original upward surface facing up. Leaves were then embedded, sectioned, and examined microscopically. The neck and ostiole of over 90% of the ascocarps were directed towards the surface that had faced upward during winter, except in the leaves that were inverted upon incubation. In the inverted leaves, ascocarps formed on both the adaxial and abaxial surfaces. Some ascocarps in the inverted leaves developed both adaxial and abaxial ostioles. A central hymenium gave rise to asci that were directed towards both leaf surfaces. The surface colonized by *V. inaequalis* during the parasitic phase did not affect the orientation of the ascocarps or the number of ascocarps that formed. Orientation of pseudothecia was negatively geotropic. This negative geotropism ensures that ascospores will be ejected toward the atmosphere and may provide a mechanism for conservation of inoculum in disturbed leaf litter.

Additional key words: apple scab.

Venturia inaequalis (Cke.) Wint., the apple scab fungus, overwinters as a saprophyte on fallen apple leaves. One month after leaf abscission, hyphae from the subcuticular stroma have grown into the palisade and mesophyll tissues of the leaf and have formed the small stromatic spheres that eventually become pseudothecia (6). Pseudothecia enter the winter in this early stage of development in the northern U.S., and further development is delayed by low winter temperatures. In spring, the development of pseudothecia accelerates as the contents of the locule differentiate to form ascogenous hyphae, asci, and pseudoparaphyses. The pseudothecium continues to increase in diameter, forms a neck and ostiole, and breaks through the leaf cuticle, sometimes accompanied by the formation of setae surrounding the ostiole. Many researchers have commented upon the relative abundance or scarcity of pseudothecia on either the adaxial or abaxial leaf surface. Curtis (3) and Cunningham (2) found that pseudothecia were most common on the abaxial surface, but Clinton (1) believed that pseudothecia formed on whatever surface was infected. Jehle and Hunter (10) and Holz (7) reported that pseudothecia were more abundant on whatever leaf surface was exposed to light (positive phototropism). Frey (4) and Wallace (12) found pseudothecia on both the adaxial and abaxial surfaces, but believed that the ascocarps were more numerous on the uppermost surface (negative geotropism). Wilson (13) reported that pseudothecia were more numerous on abaxial leaf surfaces because diffuse stromata found on the abaxial surfaces produced more ascocarps than the more discrete colonies on the adaxial surfaces. James et al (8) described an unusual form of biostiolate ascocarp that may have represented a geotropic response. Jeger (9), however, recently claimed that there is little evidence of significant geotropism in ascocarp development. Jeger (9) reported that the lesions on the adaxial leaf surface of cultivar Bramley's Seedling produced most of their ascocarps on the adaxial surface, irrespective of which surface was exposed during overwintering. However, lesions on the abaxial surface produced the largest number of pseudothecia on whichever surface was exposed during overwintering (9).

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We are currently developing a system to forecast population densities of *V. inaequalis* in commercial apple orchards (5,11), and the possibility of geotropism in ascocarp formation is one of many factors to be considered in the system. The absence of geotropism could mean that leaves in the matted litter of the orchard floor could only contribute to the airborne ascospore dose if an infected surface faced the atmosphere. Assuming that this event occurs randomly, absence of geotropism could result in a halving of the inoculum available for air dispersal. Therefore, our objective was to determine whether pseudothecia of *V. inaequalis* are geotropic.

MATERIALS AND METHODS

Orchard study. Scabbed leaves were collected from an unsprayed cultivar McIntosh apple tree at the Mast Road Research Orchard in Durham, NH, on 1 November 1981. The leaves were stored overwinter in wire mesh trays on the orchard floor until 16 April 1982, at which time the pseudothecia had begun to produce mature ascospores. A 1-cm disk was cut from each of 100 leaves, fixed in FAA, dehydrated in tertiary butyl alcohol, and embedded in paraffin. The disks were then serially sectioned at 13 μm, stained with acid fuchsin in lactophenol, mounted, and examined microscopically. The leaf surface that was colonized during the parasitic phase, the number of pseudothecia per disk, and the orientation of the pseudothecia with respect to the leaf surface were recorded on 34 disks that each bore at least 10 pseudothecia.

Laboratory study. Scabbed leaves were collected as before on 25 October 1983 and were overwintered under wire mesh on the orchard floor with either the adaxial or abaxial surface facing upward. On 15 February, prior to ascus formation, leaves from each group were collected and incubated in the dark at 10 C and 90% RH for 30 days, some with the adaxial surface facing upward and some with the adaxial surface facing down. Thus, there were four treatments: leaves overwintered and incubated with the adaxial surface upward, leaves overwintered and incubated with the abaxial surface upward, leaves overwintered with the adaxial surface upward and incubated with the adaxial surface down, and leaves overwintered with the abaxial surface upward and incubated with the abaxial surface down. Serial sections of 1-cm-diameter disks were prepared from 50 leaves from each treatment, and data were collected as above.

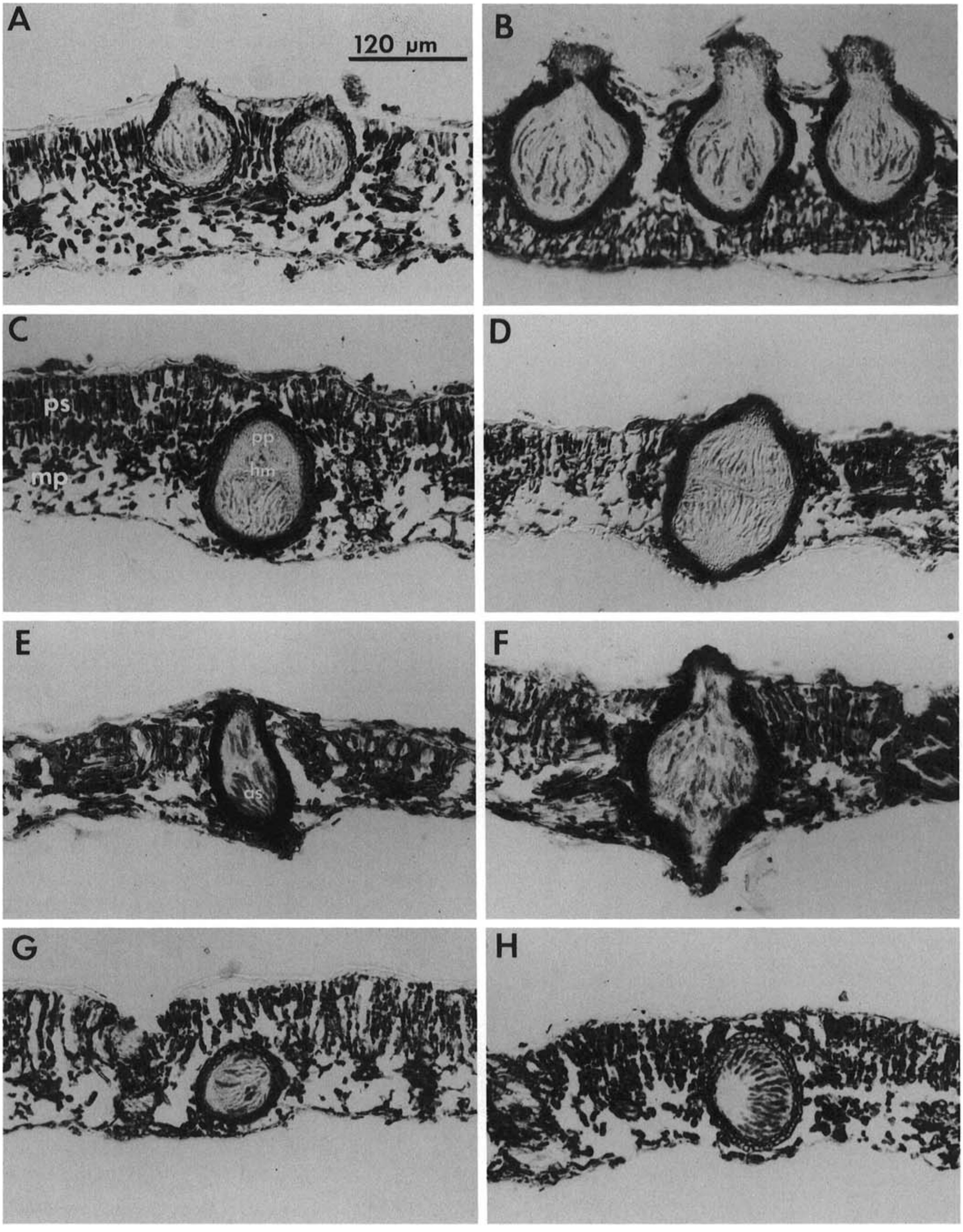

Fig. 1. Normal and aberrant pseudothecia of *Venturia inaequalis*. **A,** Adaxially directed ascocarps from leaf overwintered and incubated with the adaxial surface uppermost. **B,** Abaxially directed ascocarps from leaf overwintered and incubated with the abaxial surface uppermost. **C–F,** Stages of development of biostiolate ascocarps in leaves overwintered with the abaxial surface uppermost and then inverted. **G and H,** Stages in the development of ascocarps directed towards internal leaf tissues from field stored leaves. (ps = palisade tissue, mp = mesophyll tissue, pp = pseudoparaphyses, hm = hymenium, and as = asci.) Differential interference microscopy was used to prepare micrograph D. All other micrographs were prepared with brightfield illumination.

RESULTS AND DISCUSSION

Pseudothecia developed on both the adaxial and abaxial surfaces of scabbed leaves in the orchard study (Table 1). However, the ascocarps were usually not found on both surfaces simultaneously (Fig. 1A and B). Approximately 71% of the leaves examined bore pseudothecia only on one surface. Of the remaining 29% of the leaves, nearly half bore biostiolate pseudothecia as described by James et al (8) or pseudothecia whose ostioles were directed towards the internal tissues of the leaf (Fig. 1G and H). The adaxial leaf surface was colonized during the parasitic phase of development in all but two of the 34 leaf samples from the orchard study, i.e., exclusive abaxial colonization was seen in only two leaves (Table 1). Approximately 29% of the leaves bore a subcuticular stroma on both leaf surfaces. There was no pronounced influence of the leaf surface colonized on the orientation of the ascocarps: on 22 leaves with adaxial stromata, 10 leaves bore pseudothecia that were directed adaxially, seven bore pseudothecia directed abaxially, and five bore pseudothecia directed towards both surfaces (Table 1).

In the laboratory, there was a clear geotropic response. Leaves that were not inverted upon incubation formed over 90% of their pseudothecia directed towards the upper surface, regardless of whether this surface was adaxial or abaxial, and irrespective of the surface colonized during the parasitic phase (Table 2). When leaves were inverted upon incubation, pseudothecia were directed towards both leaf surfaces, and as many as 14% of the pseudothecia were biostiolate or otherwise deformed (Table 2 and Fig. 1C–H). Biostiolate ascocarps were only observed in leaves that were inverted during ascocarp development.

TABLE 1. Orientation of pseudothecia of *Venturia inaequalis* with respect to the adaxial and abaxial leaf surface and leaf surface colonized during the parasitic phase[a]

Leaf sample number	Pseudothecia (%) Directed adaxially	Directed abaxially	Biostiolate	Surface colonized during parasitic phase
1	100	0	0	Adaxial
2	100	0	0	
3	100	0	0	
4	100	0	0	
5	100	0	0	
6	100	0	0	
7	100	0	0	
8	100	0	0	
9	100	0	0	
10	100	0	0	
11	0	100	0	
12	0	100	0	
13	0	100	0	
14	0	100	0	
15	0	100	0	
16	0	100	0	
17	0	100	0	
18	0	33	67	
19	67	0	33	
20	67	33	0	
21	50	50	0	
22	83	17	0	
23	100	0	0	Abaxial
24	40	40	20	
25	100	0	0	Both
26	100	0	0	
27	100	0	0	
28	100	0	0	
29	100	0	0	
30	0	100	0	
31	33	50	17	
32	0	88	12	
33	12	88	0	
34	20	80	0	

[a]Leaves were overwintered at the Mast Road Research Orchard in Durham, NH, and were examined on 16 April 1982.

Neither the surface colonized during the parasitic phase nor the surface on which the pseudothecia were borne had a significant effect on the number of ascocarps that formed in either the orchard or laboratory. The mean and 95% confidence interval of the number of ascocarps on disks bearing adaxial, abaxial, or both adaxial and abaxial stromata was 16.6 ± 1.7, 21.1 ± 8.3, and 18.0 ± 2.1, respectively. The mean and 95% confidence interval of the number of ascocarps on disks bearing pseudothecia on only the adaxial or abaxial surface was 16.7 ± 2.4 and 17.0 ± 4.6, respectively. We noticed no consistent differences in the density of the stromata on adaxial and abaxial surfaces. Both appeared to be primarily the diffuse type described by Wilson (13). The uniformly dense groups of ascocarps may be reflective of a uniformity in the type of stromata. Concomitantly, differences in numbers of ascocarps formed on adaxial versus abaxial surfaces noted in earlier studies may have been due to differences in the type of stomatal colonization rather than any intrinsic property of adaxial or abaxial tissues.

We concluded that pseudothecial orientation of *V. inaequalis* was negatively geotropic and insensitive to the leaf surface colonized during the parasitic phase. The subject of phototropism was not addressed in our study, but pseudothecia did orient toward the uppermost surface when incubated in the dark.

Most earlier studies were not designed to detect geotropism (1–4,7,8,10,12,13) and some were based upon observations in the field rather than experimentation (1–4,10,12). Thus, differences reported in these studies may have resulted from the lack of an appropriate experiment designed to detect geotropism rather than variability in the response of *V. inaequalis* to gravity. Jeger (9) may not have found a geotropic response in ascocarp development for two reasons. First, the method used to examine the pseudothecia, i.e., low-power microscopic examination of leaf disks followed by removal and crushing of the ascocarps on glass slides for more detailed examination, would have obliterated the morphological features noted in our study. Secondly, four of the seven sampling dates were in late winter, and may have preceded any clear morphological expression of geotropism. Furthermore, the presence of an ascocarp in the mesophyll (abaxial) tissue rather than the palisade and upper epidermal (adaxial) tissues is not necessarily related to the orientation of the ostiole. Fig. 1C shows a pseudothecium that began to develop in the abaxial tissues, but later development was directed toward the adaxial surface.

Negative geotropism provides a mechanism for conservation of inoculum in disturbed leaf litter and ensures that ascospores will be ejected towards the atmosphere regardless of the orientation of the

TABLE 2. Orientation of pseudothecia of *Venturia inaequalis* with respect to orientation of the leaf surface during overwintering and during subsequent development

Uppermost surface during over-wintering	Uppermost surface during incubation	Surface colonized during parasitic phase	Pseudothecia (%) directed Adaxially	Abaxially	Biostiolate
Adaxial	Adaxial	Adaxial	...[z]	...	...
		Abaxial	...	...	...
		Both	97	3	0
Abaxial	Abaxial	Adaxial	7	93	0
		Abaxial	0	100	0
		Both	0	100	0
Adaxial	Abaxial	Adaxial	53	33	14
		Abaxial	36	58	6
		Both	59	37	4
Abaxial	Adaxial	Adaxial	17	75	8
		Abaxial	49	51	0
		Both	...	...	...

[z]... Leaf disks of this type were not found in the sample. Leaves were overwintered with either the adaxial or abaxial surface upward and were then incubated in the dark at 10 C and 90% RH for 30 days with the adaxial surface facing up or facing down.

leaf or subcuticular stroma on the orchard floor. The geotropic response was observed in later stages of ascocarp development, and thus followed the compression and stabilization of the matted litter following leaf fall (5). The original orientation of the ascocarps was reversible, as evidenced by the presence of biostiolate ascocarps and leaves that bore pseudothecia on both surfaces. The significance of this response in regard to forecasting ascospore dose is that a scab lesion can be expected to contribute to the airborne ascospore dose in an orchard, irrespective of its orientation on the orchard floor, so long as the leaf litter is not disturbed at a critical point in development. There may even be a potential for eradicating a portion of the overwintering population of *V. inaequalis* by manipulation of the leaf litter during ascocarp development.

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