Efficiency of Ascospores of *Venturia inaequalis* in Producing Scab Lesions on Apple Leaves

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ABSTRACT

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McIntosh apple trees at three early stages of leaf expansion were exposed to freshly discharged ascospores of *Venturia inaequalis* in a dew chamber at 18–19 C. Spores deposited on glass coverslips positioned next to the leaves at each node were counted. Spores on some coverslips were also tested for viability. Lesions were noted after the trees had been kept in a growth chamber (12 hr of light at 25 C followed by 12 hr of darkness at 20 C) for 11–27 days. The efficiency of lesion production was calculated as the ratio of the number of lesions per unit of leaf surface area at the time of inoculation to the number of spores per unit of coverslip area. Lesion-forming efficiency averaged 5, 6, and 14% for the three groups of trees.

Apple scab is a serious disease of apple (*Malus domestica* Borkh.) caused by the ascomycete *Venturia inaequalis* (Cke.) Wint. The severity of an apple scab epidemic is determined largely by weather conditions, inoculum pressure, and the number of primary lesions produced by the ascospores discharged in the spring (2,6).

Keitt and Jones (6) investigated the infection of small apple trees (cultivars Wealthy and Fameuse) by mature ascospores from source leaves (cultivar unknown). Moore (8) used ascospores from cultivar Bramley on cuttings of MM109. Previous work, however, has not included information on the efficiency of lesion formation by ascospores of *V. inaequalis*. To estimate lesion-forming efficiency, we designed an experiment to compare the number of scab lesions formed on leaves to the number of spores deposited on nearby coverslips.

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MATERIALS AND METHODS

Apple leaves with scab lesions were collected on 23 and 26 April 1990 from an orchard planted with the cultivar McIntosh (pollinator trees were in a nearby orchard). Leaves were dry when they were collected and were stored in plastic bags at 4 C. Squash mounts (5) of pseudothecia confirmed that many mature ascospores were present.

We assumed that a high proportion of the *V. inaequalis* strains present on the infected leaves would be virulent to McIntosh apple trees, because this trait is controlled by alleles at a single locus (2,3,7). That is, because the ascospores came from lesions on McIntosh leaves, the female parents had to have a virulence allele at *pl*. The male parent genotypes might have included the virulence allele at *pl*, if they came from McIntosh leaves, or the virulence or avirulence allele at *pl*, if they came from other apple cultivars outside the orchard. Therefore, because half of the haploid ascospores would have had the allele carried by their female parent, at least 50% of the ascospores should have had the virulence allele at *pl*.

Ten dormant McIntosh (Summerland) trees on Mark rootstock were obtained from the New York State Fruit Testing Cooperative Association, Inc., in Geneva, NY. They were potted in ProMix BX and maintained in a greenhouse until leaf budbreak. All were about 1 m tall and had about the same number

of nodes. Nine to 26 nodes on each tree had produced leaves by the time of the experiment.

Nine of the trees were randomly assigned to three groups of three trees each for inoculation. (One tree remained in the greenhouse as a control.) The groups were exposed to ascospores of *V. inaequalis* 3 days apart.

Because ascospores were deposited by sedimentation, only the top surface of unfurled leaves was exposed. We calculated the exposed area of unfurled leaves as $A = (0.7 \times \text{length} \times \text{width}) + 0.36$; this equation was derived from linear regression of length × width on area measured on 25 McIntosh apple leaves ($r^2 = 0.99$). The exposed area of furled leaves was estimated as length × diameter. The average (top surface) area per leaf at the three stages of leaf expansion was 1.23 (stage 1), 2.88 (stage 2), and 4.4 (stage 3) cm^2. The average number of unfurled leaves per node was 2 at stage 1, 4.6 at stage 2, and 5.3 at stage 3 (Fig. 1). Because new leaves continued to form on the extension shoots at each node, the trees at stage 3 had more leaves (six per node) than those at stage 1 (2.6 per node) (Table 1).

Plastic-coated wire twist-ties were attached to coverslips (22 × 22 mm) with small pieces of transparent plastic tape. The free ends of the ties were twisted around the stems of the apple trees at the nodes to position the coverslips at the approximate angle of the expanded leaves (Fig. 2). The node positions and the length and width of all of the leaves were recorded.

The trees were placed on the lower shelf of a dew chamber (Percival model I-35D). The temperature of the air inside the chamber was maintained at 18–19 C, and the wall temperature was set to produce fine-sized dewdrops on the leaves and coverslips with no dew runoff.

The source leaves with mature ascospores were dipped in demineralized water and placed on a screen shelf above the trees (Fig. 2). The side of each leaf showing obvious scab lesions was placed against the screen. The leaves were misted lightly with demineralized water, and the dew chamber was closed. A 40-W incandescent bulb provided light over the leaves. The trees were left in the dew chamber with the source leaves for 24 hr.

At the end of this time, the trees were moved to the room to dry (room temperature about 25 C), and the cover glasses were carefully removed. Fifteen cover glasses were placed upside down on water agar (2%), and the plates were incubated at 20 C to determine the percentage of ascospores of *V. inaequalis* that were able to germinate. The remaining 72 cover glasses were placed upside down on drops of Hoyer's mounting medium (4) on glass slides and allowed to dry. These were then examined at ×250, and ascospores of *V. inaequalis* present were counted.

After drying for 12–15 hr (overnight) in the room, the trees were placed in a growth chamber (Percival model PT-80) set at 25 C for 12 hr of light, followed by 12 hr of darkness at 20 C. Trees were examined daily for signs of leaf lesions.

RESULTS

No lesions developed on the tree kept in the greenhouse as a control. Faint chlorotic spots on the leaves of the trees exposed to ascospores of *V. inaequalis* developed into faint olivaceous spots. Sporulating conidiophores were present 11–27 days after the trees were exposed to the source leaves, which confirmed that the spots were apple scab lesions. Lesions developed on all trees at about the same time, but sporulation was delayed on the trees in the first (youngest) group. The number of lesions may have been underestimated slightly because of lesion coalescence.

We calculated the ratio of the number of lesions at a node divided by the leaf area at that node to the number of spores deposited on the cover glass adjacent to that node divided by the area of the cover glass. The average value of this ratio for each group of trees was our estimate of lesion-forming efficiency (efficiency = lesions/cm^2 of leaf area ÷ ascospores/cm^2 of cover glass area) (Table 1). Average lesion-forming efficiency was 4.6 and 5.7% at the two earliest stages of leaf expansion and 14.4% at the last stage of leaf development. Ascospores in the three groups differed significantly in lesion-forming efficiency ($F = 13.8$; df = 2,69). Ascospores in the first two groups did not differ significantly in efficiency

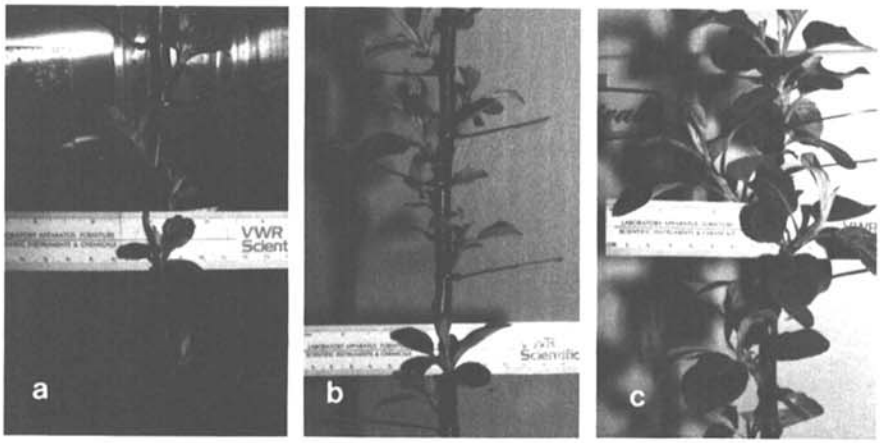

Fig. 1. Leaves on McIntosh apple trees at stages 1 **(A)**, 2 **(B)**, and 3 **(C)** of leaf development when the trees were exposed to ascospores of *Venturia inaequalis*.

Table 1. Lesion-forming efficiency of ascospores of *Venturia inaequalis* released from McIntosh source leaves above McIntosh apple trees in a dew chamber

Group[a]	Lesion-forming efficiency[b]	Total leaves	Total lesions	Total spores	Total leaf area (cm^2)	Total nodes
1	0.046 ± 0.016	65	57	2,699	80	25
2	0.057 ± 0.006	149	404	2,430	430	27
3	0.144 ± 0.019	120	960	1,660	528	20
Overall	0.078 ± 0.009	334	1,421	6,789	1,038	72

[a]Trees were exposed to ascospores of *V. inaequalis* at three stages of leaf development, with average areas per leaf of 1.23, 2.88, and 4.4 cm^2 and an average of 2, 4.6, and 5.3 unfurled leaves per node in stages 1, 2, and 3, respectively.

[b]Lesion-forming efficiency was calculated as the ratio of the density of scab lesions (lesions per square centimeter of leaf area) to the density of ascospores deposited on coverslips (ascospores per square centimeter of coverslip area) placed next to the leaves. Data are means plus or minus standard errors of the means.

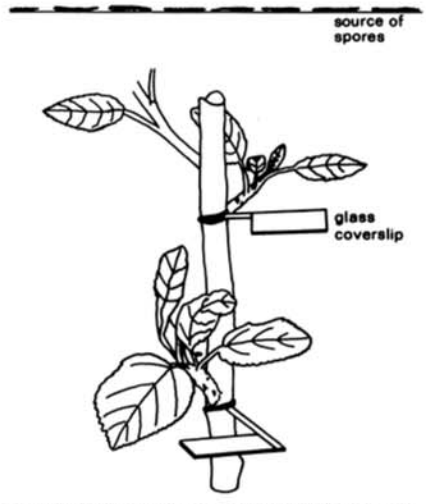

Fig. 2. Sketch of a stem of a McIntosh apple tree from group 1 (average leaf surface area 1.23 cm^2, with an average of two unfurled leaves per node). Note coverslips attached to stem with twist-ties. Care was taken to orient the coverslips at the approximate angle of the fully unfurled leaves beside them.

($P > 0.05$), whereas those in the third group were significantly ($P < 0.001$) more efficient at forming lesions than those in the first two groups.

On average, 82% of ascospores tested for viability germinated on water agar over the course of the experiment.

DISCUSSION

Aderhold (1) reported that ascospores in water applied to a leaf produced about 33% infection, but he noted that unfortunately no numbers were written down. Turner et al (9) noted that ascospores from McIntosh leaves germinated and formed appressoria on leaves of open-pollinated McIntosh seedlings (pollinator cultivar not reported), but they did not calculate the efficiency with which ascospores formed lesions. Keitt and Jones (6) exposed plates in "representative situations" in their inoculation chamber and checked (with a microscope) that ascospore inoculum was "abundant," but they did not report the relationship between number of spores and number of lesions. Their Wealthy apple leaves developed 13–40 lesions per leaf after 11–14 days under conditions similar to ours. Fameuse apple leaves had six and eight lesions per leaf after 12 days.

Moore (8) used air-sedimentation of ascospores from Bramley leaves to infect leaves on rooted cuttings of MM109. He reported one experiment in which ascospores were counted (four to six ascospores per 2-mm microscope field). If we assume that the MM109 apple leaves (+1 to 3) measured about 5 × 7 cm, then the data in Moore's Table 2 imply that 0.051 lesions were produced per square centimeter of leaf area (mean of 1.8 lesions per leaf), corresponding to 0.0003 lesions per ascospore. This level of efficiency (0.03%) is much lower than the level we found, perhaps because of a lack of MM109 virulence alleles in the Bramley ascospores. Because our ascospore source leaves came from a McIntosh orchard, at least 50% of the spores should have had McIntosh virulence alleles.

Although the efficiency of lesion formation by ascospores may vary with climate and genetics, our data suggest an upper limit of scab potential under nearly ideal conditions. This upper limit will improve the accuracy of models of apple scab epidemics in predicting the potential number of scab lesions from the number of ascospores of *V. inaequalis* in the air.

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