Anthracnose of *Stylosanthes scabra*: Effect of Leaf Surface Wetness on Disease Severity

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ABSTRACT

Leaf surface wetness periods (SWPs) most commonly lasted less than 2 hr during the day or night at Samford, Queensland, Australia. In controlled environment and glasshouse studies, the severity of anthracnose, caused by *Colletotrichum gloeosporioides*, on two accessions of the tropical pasture legume *Stylosanthes scabra* increased with increasing duration of SWP. An SWP of 12 hr or longer favored disease development in the susceptible cultivar Fitzroy, and maximum severity was reached after 36 hr. Increases in severity were generally associated with improved infection efficiency, although this association was modified by defoliation and coalescence of lesions. Anthracnose was not significantly less severe when SWP was preceded by 6 hr of low or 12 hr of high relative humidity. However, longer periods of low relative humidity before the onset of wetting resulted in lower disease severity and infection efficiency. Severity was relatively unaffected by brief interruptions in SWP during the initial 12 hr after inoculation, provided a continuous SWP or relative humidity above 85% was maintained for the next 24 hr. We concluded that anthracnose can develop over a range of durations of leaf surface wetness.

*Stylosanthes scabra* Vog. ‘Seca’ and *S. hamata* (L.) Taub. ‘Verano’ are among the major herbaceous legumes used in improving native pastures in tropical and subtropical Australia. Two types of anthracnose, A and B, caused by variants of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., cause severe reductions in dry matter yield (4) and seriously limit the persistence and expansion of *Stylosanthes*-based pastures. Type A anthracnose, which is economically more important than type B, causes discrete light brown to gray lesions with a dark margin on all aerial parts of all species of *Stylosanthes* (10). Pathogenic specialization has been recorded in both types (3,10,11), and the usefulness of race-nonspecific resistance (2) and genotypic mixtures in anthracnose management is currently being studied.

Conidia of *C. gloeosporioides* are produced in acervuli in a mucilaginous matrix (17). A period of leaf surface wetness is necessary for suspending conidia and thereby making them available for splash dispersal and is also necessary for infection (6). Relationships between the minimum length of a leaf surface wetness period (SWP) and infection form the basis of many disease forecasting models (14,22). In a previous study (12), severe type A anthracnose developed at 20-34 C after a 24-hr SWP. No information is available on the duration of SWPs in the field or on what durations are adequate for anthracnose infection. In this study, we monitored the duration of SWPs and the time of their onset in the field for two seasons. We determined the influence of duration of SWP on the severity of anthracnose in controlled environment and glasshouse experiments and examined the effect of interruptions in SWP and of its delayed onset under different relative humidity (RH) conditions.

MATERIALS AND METHODS
Monitoring surface wetness in the field. We monitored the time of onset and the duration of each SWP during the 1987–1988 and 1988–1989 summers at the Samford Pasture Research Station (27°22’S, 152°53’E) in an area that has been used as a *Stylosanthes* nursery. An SWP that began between 5 a.m. and 6 p.m. was considered a daytime SWP; if it began between 6 p.m. and 5 a.m., it was considered a nighttime SWP. Anthracnose severity on the susceptible *S. scabra* cultivar Fitzroy during the two seasons was high (50–60% of tissue damaged) to very high (more than 80% of tissue damaged).

We used an electronic sensor similar to the leaf wetness sensor of Weiss and Lukens (25), with a modified general-purpose data logger. The sensor surface was a gold-plated printed circuit grid measuring 35 x 35 mm, with a spacing of 0.3 mm between lines. The sensor was mounted horizontally 20 cm above ground level in the canopy, where it was out of direct sunlight for most of the day. The sensor and the data logger were both designed and manufactured at the CSIRO electronic workshop, and the sensor is currently available commercially (Monitor Sensors, Queensland 4556, Australia).

Effect of duration of SWP on infection. Fitzroy and a moderately resistant *S. scabra* accession (Q10042) from the CSIRO collection were used in this experiment. Seca was not used in this study because despite its susceptibility to a type A race, it maintains a high level of resistance in the field. Single seedlings were raised in a loamy riverbed soil in pots measuring 5 x 5 cm in controlled environment cabinets (CECs) at the Cunningham laboratory of CSIRO. They were given a 14-hr photoperiod at a light intensity of 250–300 μE·m⁻²·s⁻¹ and were maintained at a temperature of 30 C during the day and 25 C at night for the initial 8 wk.

Four CECs were used to impose day-night temperature regimes of 30–25 or 25–20 C, with the SWP starting during the day or the night. RH in the CECs was not controlled and ranged from 40 to 60% during the day and from 80 to 95% at night. Seedlings were fertilized at 2-wk intervals with a 0.8 g/L solution of a mineral fertilizer (Aquadol; Hortico, Sydney, Australia) containing 23% N, 4% P, and 18% K as well as trace elements.

Two isolates of *C. gloeosporioides*, UQ14 and WRS20, were used to inoculate Fitzroy and Q10042, respectively. Both isolates are virulent on the two host accessions, but UQ14 is more virulent on Fitzroy, whereas WRS20 is more virulent on Q10042. The isolates were grown on oatmeal agar with 12 hr of near-ultraviolet light and 12 hr of darkness for 5–7 days at 25 C. Conidia were harvested to prepare an inoculum containing 10⁵ conidia/ml for each isolate as described previously (3).

The three youngest fully expanded leaves on 8-wk-old seedlings were tagged and numbered according to their age. Seedlings were sprayed to incipient runoff with one of the isolates of *C. gloeosporioides*. Three petri dishes containing 2% distilled water agar amended with 100 μg of streptomycin and 10 μg of tetracycline per milliliter were exposed during the inoculation of seedlings with each isolate. These dishes were incubated at 25

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C for 24 hr, and the average number of germinated conidia from five randomly selected microscopic fields (×100) per dish was used to estimate the density of spore deposition on the leaf surface.

Seedlings were incubated in a saturated atmosphere inside a plastic tent (35 × 77 × 40 cm) on a galvanized metal tray (37 × 80 × 5 cm) to impose SWPs of 2, 4, 6, 8, 12, 16, 24, and 36 hr. Air temperatures inside the tents were 1–1.5 C higher than in the CECs. Three replicate seedlings were maintained for each SWP in each CEC, and the entire experiment was repeated once. For a control, inoculated seedlings were incubated for 36 hr in a saturated atmosphere at a constant temperature of 25 C in darkness (12).

Ten days after inoculation, the percentage of diseased leaf tissue was assessed on whole seedlings using a 10-point scale based on the Horsfall and Barratt grading system (8). The three numbered leaves and all subsequent leaves that emerged during the 10 days following inoculation were rated individually for disease severity (SEV) on a 10-point scale (Fig. 1) and were scored for the number and type of lesions. Lesion type 1 was minute brown or dark brown specks similar to a hypersensitive reaction (highly resistant). Type 2 lesions were less than 0.5 mm in diameter, with a dark margin and gray center (susceptible). Type 3 lesions were 0.5–1 mm in diameter, with a dark brown margin and gray center (susceptible). Type 4 lesions were more than 1 mm in diameter, with a dark brown margin and gray center (highly susceptible).

Because both accessions produced a mixture of lesion types on a single leaf, weighted lesion types (WLTs) were calculated by ranking lesion types according to their relative frequency (23). The most and least prevalent lesion types were assigned the highest and lowest ranks, respectively, and WLT was calculated for each of the three replicates as follows: $\text{WLT} = \Sigma(\text{rank} \times \text{lesion type}) / \Sigma\text{rank}$.

Surface area of infected leaves was measured with a Delta-T system (Delta-T Devices Ltd., Burwell, Cambridge, England). Infection efficiency (IE) was calculated as 100 × (number of lesions on each leaf / estimated number of conidia deposited on each leaf), assuming that a single lesion resulted from a single infection.

Leaves were divided into two groups based on their age; the three fully emerged leaves at inoculation were the “older” leaves, and leaves that expanded after inoculation were the “younger” leaves.

**Fig. 1.** Disease assessment key used to estimate the severity of anthracnose on individual leaves of *Stylosanthes scabra.* Each category represents a range in the percentage of leaf area damaged by the disease.
leaves. Data on SEV and WLT were used to examine the influence of leaf age on infection. Valid estimates of IE could not be obtained for younger leaves because they expanded in area during the 10-day period between inoculation and lesion scoring.

Data were analyzed by analysis of variance using the Genstat 5 statistical package (20). A square-root transformation was used for percentage of diseased leaf tissue and a fourth-root transformation was used for IE to make the variances more homogeneous. No transformation was needed for SEV and WLT.

Effect of delayed onset of SWP on infection. Eight-week-old seedlings of Fitzroy, grown in a glasshouse (14-hr photoperiod, 34.4±24.3°C day-night temperatures, 50±20-80±5% day-night RH), were inoculated with isolate UQ14. Inoculated seedlings were held in the glasshouse or in one of two CECs (14-hr photoperiod, 28±0.5-22±0.5°C day-night temperatures) for 0, 2, 4, 6, 12, or 24 hr before being placed in a saturated atmosphere inside a tent for the remainder of a 36-hr period. All delays were started in the daytime. The day-night RH in the CECs was maintained at 85±5-97±2% (high RH) or 65±5-80±5% (low RH). A tent was maintained in each of the three environments.

All seedlings were returned to the glasshouse 36 hr after inoculation. Plant growth conditions, inoculation methods, and disease and other assessments were the same as in the SWP duration experiment. Five replicate seedlings were used for each delay treatment in each of the three environments, and the experiment was repeated once. Analysis of variance was performed on the square root of the percentage of diseased leaf area and the fourth root of IE.

Effect of interrupted wetting on infection. Eight-week-old seedlings of Fitzroy were inoculated with isolate UQ14, placed in the glasshouse or in one of the above two CECs with high or low RH, and subjected either to one of two dry-wet cycles (2 hr dry-2 hr wet or 4 hr dry-4 hr wet-4 hr dry) or to one of two wet-dry cycles (2 hr wet-2 hr dry or 4 hr wet-4 hr dry-4 hr wet) for the first 12 hr. Thereafter, the seedlings were maintained either inside or outside the tent for a further 24 hr. Following a dry period, seedlings were lightly atomized with sterile distilled water and were placed inside the tent for the onset of a subsequent wet period. Seedlings maintained in a saturated atmosphere inside a tent for the entire 36-hr period served as controls.

All seedlings were returned to the glasshouse 36 hr after inoculation, and disease and other assessments were made 10 days after inoculation. Five replicate seedlings were used for each treatment (e.g., 2 hr wet-2 hr dry cycle for the first 12 hr, followed by a 24-hr wet period) at each of the three environments, and the experiment was repeated once.

RESULTS

Surface wetness in the field. SWPs ranged in duration from less than 15 min to more than 60 hr. In both seasons, the most frequent duration of SWP was less than 2 hr during either day or night (Table 1). The next most frequent SWPs were those that lasted 10-15 hr. The mean daily duration of wetness caused by rainfall and/or dew ranged from 7.7 hr (December 1987) to 16.1 hr (April 1988).

Effect of duration of SWP on infection. After leaves were removed from a tent, the water on their surfaces evaporated within 30-45 min during the day and 1-1.5 hr during the night. The percentage of diseased leaf area increased significantly with increased duration of SWP (Table 2). Overall, similarly low levels of anthracnose severity were recorded at SWPs of 2, 4, and 6 hr. Each increase in duration between 8 and 36 hr resulted in an increase in disease severity, although the difference between 24 and 36 hr was not significant (P < 0.05).

The percentage of diseased leaf area exceeded 5% on Fitzroy at all day-night temperatures following SWP of 16 hr or longer (Fig. 2A). The percentage of damaged leaf area on Q10042 exceeded 5% on only three occasions following an SWP of 24 hr or longer (Fig. 2B). Disease was most severe when plants were exposed to a 36-hr SWP; in Fitzroy, 70.2% of leaf area was diseased at a constant temperature of 25°C (control), and in Q10042, 10.7% of leaf area was diseased in both the control and at 30-25°C.

The four CECs used to impose the SWPs did not differ significantly (P < 0.05) in leaf disease severity (Table 2). Whether an SWP was started in light or darkness at 25°C had no effect on severity. In Fitzroy, leaf disease severity rose rapidly at SWPs lasting more than 12 hr, whereas in Q10042, severity increased gradually over the entire range of duration of SWP (Fig. 2); this resulted in the significant accession × SWP interaction (Table 2). Disease for both accessions was significantly more severe in the glasshouse than in the CECs; the magnitude of this difference was larger for Fitzroy than for Q10042.

Lengthening the SWP increased IE, WLT, and SEV, but the four CECs did not significantly affect the three variables. In Fitzroy, WLT was significantly (P < 0.001) higher on younger leaves than on older leaves, whereas SEV was significantly (P < 0.001) lower on younger leaves than on older leaves. Lengthening SWP from 0 to 36 hr resulted in a 73-fold increase in SEV on older leaves but only a 59-fold increase in IE. Older leaves of

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**Table 1. Duration and time of onset of wetness periods at the Samford Pasture Research Station during two summer seasons (November–April)**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day (%)</td>
<td>Night (%)</td>
</tr>
<tr>
<td>&lt;2</td>
<td>30.9</td>
<td>13.2</td>
</tr>
<tr>
<td>2-4</td>
<td>1.7</td>
<td>4.5</td>
</tr>
<tr>
<td>4-6</td>
<td>1.0</td>
<td>5.6</td>
</tr>
<tr>
<td>6-8</td>
<td>0.5</td>
<td>7.9</td>
</tr>
<tr>
<td>8-10</td>
<td>0.0</td>
<td>7.6</td>
</tr>
<tr>
<td>10-15</td>
<td>1.4</td>
<td>16.0</td>
</tr>
<tr>
<td>15-20</td>
<td>3.6</td>
<td>2.2</td>
</tr>
<tr>
<td>&gt;20</td>
<td>2.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Percentage of total number of wetness periods.

†Between 5 a.m. and 6 p.m.

‡Between 6 p.m. and 5 a.m.

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**Table 2. Analysis of variance for leaf disease severity (square root) of two Stylosanthes scabra accessions inoculated with Colletotrichum gloeosporioides and incubated under four sets of controlled environment conditions (CEC) for nine durations of surface wetness (SWP)**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>F value</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>2.04</td>
<td>62.94</td>
<td>0.001</td>
</tr>
<tr>
<td>CEC</td>
<td>3</td>
<td>0.15</td>
<td>1.54</td>
<td>0.346</td>
</tr>
<tr>
<td>SWP</td>
<td>8</td>
<td>6.65</td>
<td>23.26</td>
<td>0.001</td>
</tr>
<tr>
<td>Accession</td>
<td>1</td>
<td>2.17</td>
<td>98.47</td>
<td>0.001</td>
</tr>
<tr>
<td>CEC × SWP</td>
<td>24</td>
<td>0.52</td>
<td>0.60</td>
<td>0.898</td>
</tr>
<tr>
<td>Accession × CEC</td>
<td>3</td>
<td>0.08</td>
<td>1.27</td>
<td>0.298</td>
</tr>
<tr>
<td>Accession × SWP</td>
<td>8</td>
<td>1.31</td>
<td>7.45</td>
<td>0.001</td>
</tr>
<tr>
<td>Accession × control</td>
<td>1</td>
<td>0.43</td>
<td>19.66</td>
<td>0.001</td>
</tr>
<tr>
<td>Accession × SWP × CEC</td>
<td>24</td>
<td>0.15</td>
<td>0.29</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Difference between the control (plants given a 36-hr SWP at a constant temperature of 25°C) and the other treatments.
control plants had the maximum SEV (84.9%), but their IE (0.1) was not the highest.

Means across the four CECs for each of the three variables were regressed on SWP using a four-parameter logistic model (20). Detailed results are presented only for Fitzroy (Fig. 3). The logistic model was a good fit for all three variables. It gave R² values of 99.4, 86.8, and 98% for SEV, WLT, and IE, respectively, for the older leaf group, and 95.1 and 98.7% for SEV and WLT, respectively, for the younger leaf group. In Q10042, however, WLT and SEV were both significantly (P < 0.05) higher in younger leaves than in older leaves (data not shown).

Effect of delayed onset of SWP on infection. The percentage of diseased leaf area on inoculated plants declined consistently after 6 hr for plants held in the glasshouse and after 12 hr for plants held in one of the two CECs (Fig. 4). The effect of delayed onset of SWP was less pronounced in plants maintained in the high-RH environment. Compared with IE on plants that received wetness immediately after inoculation, IE in the glasshouse and the low-RH environment was reduced by 21 and 98% and by 47 and 96%, respectively, after 12- and 24-hr delays, respectively. In the high-RH environment, IE increased by 12% following a 12-hr delay and decreased by 60% after a 24-hr delay. At this RH, severe anthracnose developed when plants were maintained outside the tent for the entire 36-hr period.

Effect of interrupted wetting on infection. When wet-dry sequences started with a wet period, disease severity was almost always higher in 4-hr cycles (Table 3), whereas with a dry start, 2-hr cycles always produced significantly (P < 0.05) higher severities. The start × wet-dry sequence interaction was statistically significant (P < 0.001). Disease severity was also significantly higher when interruptions in SWP occurred under high-RH conditions than under low-RH conditions. When the initial 12-hr period was followed by a 24-hr wet incubation, the cumulative duration of SWP was 30 hr for the 2-hr cycles and 28 and 32 hr for the 4-hr cycles with a dry or a wet start, respectively. In all three environments, severities at these cumulative durations were similar to or significantly (P < 0.05) greater than severity at the uninterrupted SWP of 36 hr (control). The percentage of diseased leaf area in plants maintained inside a plastic tent for 24 hr after the first 12 hr was significantly (P < 0.01) higher than in plants kept outside the tent, although the difference was less pronounced in the high-RH environment (Table 3).

DISCUSSION

Anthracnose caused by C. gloeosporioides in the susceptible S. scabra cultivar Fitzroy was favored by an SWP of 16 hr or longer and was most severe after 36 hr within a temperature range of 20–30 C. This is consistent with the results of an earlier study (12), where wetness lasting 24 hr or longer was necessary for maximum disease expression in Fitzroy at constant temperatures of 20, 25, 30, and 34 C. These results are similar to findings for C. lagernarium (Pass.) Ell. & Halst. (16) and C. orbiculare (Berk. & Mont.) Arx (19). The influence of duration of SWP was much less pronounced in the moderately resistant accession Q10042.

In the present study, a constant temperature was maintained for the entire 36-hr period only for the control, which was held at 25 C. Disease severity of control plants was significantly higher than that of plants held at average daily temperatures of 27.9 C (30–25 C day-night) or 22.9 C (25–20 C day-night). The finding that the two day-night temperature regimes did not influence the level of susceptibility in Fitzroy is consistent with the results of an earlier study involving four day-night temperature combinations and a 36-hr SWP (1).

Colletotrichum spp. vary in their response to light during the infection period. Darkness promotes appressorium formation in C. orbiculare (19) and disease development in C. truncatum (Schwein.) Andrus & W. D. Moore (18), whereas both conidium germination and appressorium formation in C. corchori are greater under light (21). In our studies, whether SWP began in light or in darkness did not influence anthracnose severity.

Increased IE with longer duration of wetness has been demonstrated for Plasmopara viticola (Berk. & Curt.) Berl. & De Toni in Sac. on American grape (15). In our study, with the exception of control plants, increases in SEV with longer SWP were generally associated with improvements in IE. However, for the older leaf group, increases in IE were not proportional to increases in SEV. We assigned the maximum severity rating of nine to leaf loss as a result of anthracnose; however, defoliation often results

Fig. 2. Severity of anthracnose on susceptible cultivar Fitzroy (A) and moderately resistant accession Q10042 (B) of Stylosanthes scabra. Plants were incubated at one of two day-night temperature combinations with increasing duration of surface wetness starting at 30 C day (●), 25 C day (▲), 25 C night (△), or 20 C night (○). The vertical bar represents the standard error of the difference between means.
from a single lesion on the petiole, and older leaves with their petioles exposed at the time of inoculation are more prone to abscission than younger leaves. In estimating IE, we assumed that a single lesion results from a single infection. Because coalescence of lesions is common in anthracnose, especially when disease is severe (10), IE is underestimated at high levels of severity. Counting the number of infections before lesions start to coalesce and using less concentrated inoculum would yield more accurate estimates of IE.

Our results highlight the importance of free water or high RH during the first 12 hr after inoculation in initiating infection. Severity was relatively unaffected by brief interruptions in SWP during this period, provided a continuous wet period or RH above 85% prevailed for the next 24 hr.

When free water is present on Fitzroy leaves at favorable temperatures, conidia of *C. gloeosporioides* germinate to produce sessile appressoria by 3 hr (13). These appressoria start to become melanized 6 hr after inoculation, and subcuticular hyphae are visible 12 hr after inoculation. Both conidial germination and penetration increase with time, and up to 58% of conidia germinate by 72 hr after inoculation (24). Increased IE with increasing SWP is likely a result of increased conidial germination and penetration.

Little difference in the amount of disease was noted when the onset of SWP was delayed by up to 6 hr. Although surface water dried within 1 hr of inoculation in the low-RH environments, high RH of the phylloplane probably allowed sufficient time for a substantial number of conidia to form melanized appressoria, which withstand drying. Reduced viability of *C. gloeosporioides* conidia on citrus after a dry period of 6 hr has been noted (5). When RH was high, delays of up to 12 hr in the onset of SWP did not influence anthracnose severity.

Severe disease developed during the two seasons of field monitoring of SWP (S. Chakraborty, unpublished) despite the fact that continuous SWPs of 24 hr or longer were rare. This shows the importance of SWPs of less than optimum duration in field infections. Although brief wetting followed by drying reduced anthracnose severity in our studies, the role of very short SWPs under field conditions depends on the length and number of dry interruptions between such SWPs. The cumulative effects of discrete SWPs were similar to the effects of continuous SWPs of comparable duration, which suggests that different combinations of SWPs may result in similar disease levels.

The printed circuit board type of sensors used to monitor SWP in the field differ in radiation and surface character-istics from *Stylosanthes* leaves. Sensors similar to the one used in this study have been shown to give different results in different weather conditions (9), so caution is warranted in the interpretation of field data. These sensors also do not record guttation water, which may be a source of free water for fungi (7). Results from our glasshouse and controlled environment studies show that both duration of wetness and RH of the environment influence anthracnose; detailed field studies are needed to verify these findings.

![Graph A](image1)
\[
\text{SEV} = 0.54 + 0.47(1 + \exp(-0.29(\text{SWP} - 19)))
\]
\[
\text{SEV} = 0.57 + 0.20(1 + \exp(-0.35(\text{SWP} - 16)))
\]

![Graph B](image2)
\[
\text{WLT} = 0.24 + 1.1(1 + \exp(-0.54(\text{SWP} - 7.7)))
\]
\[
\text{WLT} = 0.53 + 2.2(1 + \exp(-0.46(\text{SWP} - 10)))
\]

![Graph C](image3)
\[
\text{IE} = 0.04 + 0.16(1 + \exp(-0.15(\text{SWP} - 14)))
\]

**Fig. 3.** Mean anthracnose severity (SEV) (A), weighted lesion type (WLT) (B), and infection efficiency (IE) (C) on older (O) and younger (∆) leaf groups of cultivar Fitzroy of *Stylosanthes scabra*. Plants were inoculated with *Colletotrichum gloeosporioides* and incubated at nine durations of leaf surface wetness. Means across the four controlled environment cabinets for each of the three variables were regressed on the duration of leaf surface wetness (SWP) using a four-parameter logistic model. WLT was calculated as the sum of the ranks of lesion types × lesion types, divided by the sum of the ranks. Lesion type 1 was minute brown or dark brown specks (highly resistant); type 2 lesions were less than 0.5 mm in diameter (susceptible); type 3 lesions were 0.5–1 mm in diameter (susceptible); and type 4 lesions were more than 1 mm in diameter (highly susceptible). The most and least prevalent lesion types were assigned the highest and lowest ranks, respectively. IE was calculated as the number of lesions on each leaf divided by the estimated number of viable conidia deposited on each leaf, multiplied by 100. IE was estimated for the older leaf group only.
Fig. 4. Anthracnose severity on cultivar Fitzroy of Stylosanthes scabra held in a glasshouse (●) or in a controlled environment cabinet with low (○) or high (△) relative humidity before the onset of surface wetness. The vertical bar represents the standard error of the difference between means.

Table 3. Percentage of leaf area (square root) of Stylosanthes scabra ‘Fitzroy’ affected by anthracnose after selected wet-dry sequences for the first 12 hr after inoculation followed by dry or wet incubation for a further 24 hr in three environments

<table>
<thead>
<tr>
<th>Initial sequence</th>
<th>Wet-dry sequence (hr)</th>
<th>Glasshouse</th>
<th>Controlled environment cabinet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet</td>
<td>Dry</td>
<td>Low RH</td>
</tr>
<tr>
<td>Wet</td>
<td>2-2</td>
<td>5.85</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>4-4</td>
<td>6.42</td>
<td>0.93</td>
</tr>
<tr>
<td>Dry</td>
<td>2-2</td>
<td>7.33</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>4-4</td>
<td>5.62</td>
<td>0.64</td>
</tr>
<tr>
<td>Controlb</td>
<td></td>
<td>4.48</td>
<td>5.49</td>
</tr>
</tbody>
</table>

aData are the square roots of the percentage of diseased leaf area. The standard error of the difference between means was 1.04.

bControl plants were maintained inside a tent in each of the three environments for the entire 36-hr period.

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LITERATURE CITED