Effects of Plant Age, Leaf Position, Inoculum Density, and Wetness Period on *Bipolaris coicis* Infection in Adlays of Differing Resistance

Seog Won Chang, Northern Agricultural Research Station, Kyeonggido Agricultural Research and Extension Services, Yonchon 486-833, Korea, and Byung Kook Hwang, Laboratory of Molecular Plant Pathology, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea

ABSTRACT

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The effects of plant age, leaf position, inoculum density, and wetness period on the development of adlay leaf blight caused by *Bipolaris coicis* were evaluated using six adlay cultivars or lines of differing resistance in field plots and in the greenhouse. Levels of resistance to leaf blight among the six cultivars or lines were consistent between the 1998 and 1999 field experiments. With the aging of adlay plants, leaf blight development gradually increased in all cultivars or lines tested. Leaf blight severity was significantly greater on the lower (older) leaves than on the upper (younger) leaves. As inoculum density increased from 10^3 to 10^6 conidia per ml, the development of leaf blight increased. Disease severity also increased as the time of leaf wetness duration increased from 0 to 60 h. Wetness duration above 48 h and a high inoculum density at 10^6 conidia per ml caused severe leaf blight symptoms in adlay seedlings. The data from the greenhouse experiments consistently revealed the same trends in relative levels of susceptibility of adlay cultivars to leaf blight as those seen at late plant growth stages under field conditions.

Bipolaris coicis (Nisikadoi) Shoem. (teleomorph: *Cochliobolus nisikadoi*) causes leaf blight of adlay (*Coix lacryma-jobi* L. var. *ma-yuen* (Romanet) Stapf.) (10,22). Seeds of adlay perennial grasses are used ornamentally and as a medicinal and cereal food in many countries (11). Leaf blight of adlay plants was first found in 1977 in Korea's Kyeonggi Province. During the past decade, leaf blight has become one of the most destructive diseases throughout most of the adlay-growing regions of Korea. Increased severity of adlay leaf blight has been associated with infested crop residues that remain on the soil surface (2,15).

Leaf blight is extremely severe in adlay plants at the ripening stage (2,3). Brown lesions are linear and delimited by the major veins of leaves. Eventually, the entire leaf becomes blighted (2,22). Significant differences in disease severity among adlay cultivars or lines have been documented in the field (3,10). Reactions of adlay plants to leaf blight appear to differ quantitatively rather than qualitatively (3). Leaf blight causes significant losses in adlay production under environments conducive to severe epidemics. Total yield, kernel weight, percent kernel ripeness, and

the number of grains per plant are reduced (3). Resistance to leaf blight in adlay cultivars profoundly influences the amount of yield loss.

Improving resistance to *B. coicis* is an important objective of adlay breeding programs, such as the program at the Northern Agricultural Research Station (NARS) in Korea (2,14). Greenhouse and field screening may provide breeders with additional cycles of evaluation necessary to determine whether an adlay breeding line is a potential source of resistance to *B. coicis*. Developing appropriate screening techniques, including defining the appropriate inoculum concentration, duration of wetness after inoculation, and stage of development at which plants are inoculated, is essential to differentiate reactions of adlay lines. Extensive studies have been conducted to examine the effects of diverse environmental variables on infection development of *Helminthosporium* and related genera in grasses (1,5-7,12,16,19,20).

Infection of adlay by *B. coicis* was shown to be influenced by cultural practices and host genotypes (2,15). No quantitative information is available on the development of leaf blight in relation to the combined effects of wetness period and inoculum concentration.

The objectives of this study were to determine the effects of growth stage and leaf position of adlay on development of leaf blight in the greenhouse and the field, and to evaluate the effects of inoculum density and wetness duration on infection of adlay seedlings by *B. coicis*.

MATERIALS AND METHODS

Plant and pathogen. The six adlay cultivars or lines used in this study were selected based on results of preliminary field tests of 30 cultivars or lines at Yonchon, Korea (3). The adlay lines Tsukuba B15 and Muzu were used as resistant checks; the cultivars Yulmu 1 and Yonchon had moderate resistance; and the lines Limgae and Huksuk 2 were included as susceptible checks. Heading dates of all adlay cultivars or lines were within 2 weeks of each other.

Isolate BC-97006 of *B. coicis* was obtained from naturally infected adlay leaves at Yonchon. After the isolate was grown on cornmeal agar at $25 \pm 1^\circ\text{C}$ for 7 days, the cultures were induced to sporulate by placing them under continuous fluorescent light at $25 \pm 1^\circ\text{C}$ for 48 h. Inocula were prepared by flooding the plates with sterile water and gently scraping the media surface with a sterilized spatula. Mycelial and conidial suspensions then were filtered through two layers of cheesecloth. The conidial suspension was used as inoculum in the greenhouse study.

Field study. Tests were conducted in the field at the experimental farm of the Northern Agricultural Research Station (NARS) of Kyeonggido Agricultural Research and Extension Services (KARES), Yonchon, Korea. The environment at the station is favorable for leaf blight development in adlay plants. Since natural infection levels were considered sufficient in the experimental plots, artificial inoculation with *B. coicis* was unnecessary.

To evaluate disease reactions on individual leaves or whole plants of adlay at different stages of growth, the six cultivars or lines were sown in 42 m² plots with 11 rows and 0.6-m row spacing on 17 April, 5 and 25 May, 15 June, and 5 July in 1998 and 1999. The plots were spaced 1 m apart and arranged in a randomized complete block design with three replicates. Fertilizer was applied before sowing at the rate of 180, 60, and 60 kg of actual N, P, K per hectare in the forms of (NH₄)₂SO₄, P₂O₅, and K₂O, respectively. Additional N fertilizer was applied at 72 kg/ha after heading. The field was hand-weeded. The positions of leaves on the culms of 10 plants randomly selected within each plot were marked by small plastic clips of different colors to permit disease assessments of the same leaves. The leaves of an adlay plant were numbered to flag leaf for each leaf

Corresponding author: B. K. Hwang
E-mail: bkhwang@korea.ac.kr

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position from the bottom to the top of the plant.

Greenhouse study. Ten adlay seeds of each cultivar for the experiments in the

greenhouse were sown in plastic pots (5 × 15 × 10 cm) containing a steam-sterilized soil mix of a commercial compost soil (peat moss:perlite:vermiculite, 5:3:2, vol/

vol/vol), sand, and loam soil (1:1:1, vol/vol/vol). The adlay plants were grown in a greenhouse at 25 ± 3°C. The basal fertilizer was applied at 0.16, 0.16, and 0.1 g of actual N, P, K per pot. The additional 0.1 g of nitrogen fertilizer per pot was applied to increase susceptibility to the leaf blight infection before inoculation.

To evaluate the effect of inoculum density on disease development at the two-leaf stage, conidia of *B. coicis* isolate BC-97006 were counted using a hemacytometer, and suspensions were adjusted to 10³, 10⁴, 10⁵, and 10⁶ conidia per ml with sterile tap water. Ten plants per treatment were inoculated by spraying conidial suspensions containing 0.03% Tween 20 on the leaves of the adlay plants with an atomizer, until the beginning of runoff. The experiment was conducted twice in a randomized complete design.

To evaluate the effect of wetness duration on infection by *B. coicis*, 10 adlay plants per treatment at the two-leaf stage were sprayed with a conidial suspension of approximately 10⁵ conidia per ml of isolate BC-97006 amended with 0.03% Tween 20, and then incubated in a moist chamber at 25 ± 1°C for 0, 12, 24, 36, 48, or 60 h. The experiment was conducted twice in a completely randomized design.

To evaluate effects of growth stage, leaf age, and leaf position on disease development, leaves of 10 potted adlay plants of different ages were uniformly sprayed with a conidial suspension (10⁵ conidia per ml) of *B. coicis* isolate BC-97006 using an atomizer. The whole plants of the three adlay cultivars or lines Huksuk 2, Yulmu 1, and Tsukuba B15 that had differed in susceptibility to leaf blight in the field were inoculated with *B. coicis* at different leaf stages.

The second and third leaves of the three adlay cultivars or lines also were inoculated with *B. coicis* at different days after leaf expansion. To determine disease severity on the different leaf positions of adlay plants, different leaves of the three cultivars or lines were spray-inoculated with *B. coicis* at the six-leaf stage. The inoculated plants were placed in a moist chamber at 100% relative humidity and 25 ± 1°C for 24 h. After incubation in the moist chamber, the plastic pots were transferred to a greenhouse at 25 ± 3°C for disease evaluation. This experiment was conducted twice in a completely randomized design with similar results.

Leaf blight assessment. Disease development on each of the marked leaves in the field was rated weekly from 15 June to 23 September in 1998 and 1999 after the first appearance of leaf blight lesions in the field. Final disease severity ratings on 23 September in the two experimental years are presented in Tables 1, 2, and 3. The mean of 10 plants per plot for each leaf position was the basic datum used in all subsequent analyses. Leaf blight severity

Table 1. Disease severity (% diseased leaf area) for the sixth leaves of six cultivars or lines of adlay at five growth stages in an evaluation of *Bipolaris coicis* in field plots in 1998 and 1999 at Yonchon, Korea^a

Cultivar or line	Planting date				
	17 April	5 May	25 May	15 June	5 July
1998					
Huksuk 2	97.8 ^b	78.6	89.4	88.9	12.9
Limgae	100.0	100.0	75.7	86.1	20.7
Yonchon	99.4	81.9	70.8	93.5	12.6
Yulmu 1	86.1	74.0	80.7	70.7	11.8
Muzu	100.0	77.1	83.2	61.4	11.1
Tsukuba B15	77.8	56.9	48.1	65.3	13.2
1999					
Huksuk 2	100.0	98.9	100.0	85.8	8.3
Limgae	100.0	98.9	92.2	59.4	9.4
Yonchon	100.0	100.0	97.1	51.8	7.0
Yulmu 1	100.0	100.0	93.6	69.4	8.4
Muzu	100.0	52.9	67.9	46.3	12.9
Tsukuba B15	99.0	64.2	58.1	35.7	2.6

^a Rated 23 September.

^b Values are means of 10 plants in each of three replicate plots per genotype and planting date.

Table 2. Disease severity (% diseased leaf area) for the ninth leaves of six cultivars or lines of adlay at five growth stages in an evaluation of *Bipolaris coicis* in field plots in 1998 and 1999 at Yonchon, Korea^a

Cultivar or line	Planting date				
	17 April	5 May	25 May	15 June	5 July
1998					
Huksuk 2	68.6 ^b	42.3	22.7	16.0	0.3
Limgae	46.3	35.0	25.5	11.3	0.4
Yonchon	34.0	27.4	22.8	13.5	0.2
Yulmu 1	31.0	27.8	12.7	12.0	0.1
Muzu	30.4	28.5	11.5	12.7	0.0
Tsukuba B15	10.5	18.0	10.4	5.5	0.3
1999					
Huksuk 2	84.4	44.0	42.8	24.7	3.4
Limgae	85.4	39.7	45.8	10.5	3.8
Yonchon	64.9	48.8	43.5	4.9	3.4
Yulmu 1	87.4	64.6	40.8	5.4	4.6
Muzu	78.3	21.9	29.3	12.7	3.8
Tsukuba B15	44.4	21.1	26.8	6.5	1.4

^a Rated 23 September.

^b Values are means of 10 plants in each of three replicate plots per genotype and planting date.

Table 3. Disease severity (% diseased leaf area) for the whole plants of six cultivars or lines of adlay at five growth stages in an evaluation of leaf blight caused by *Bipolaris coicis* in field plots in 1998 and 1999 at Yonchon, Korea^a

Cultivar or line	Planting date				
	17 April	5 May	25 May	15 June	5 July
1998					
Huksuk 2	83.2 ^b	72.4	68.2	65.7	41.0
Limgae	66.4	68.0	55.6	52.4	36.4
Yonchon	68.0	64.3	57.9	57.4	35.2
Yulmu 1	66.1	61.8	57.3	53.6	32.3
Muzu	65.1	60.7	56.9	54.9	31.0
Tsukuba B15	52.3	53.6	48.8	46.7	31.4
1999					
Huksuk 2	23.9	15.2	15.8	2.9	3.0
Limgae	24.9	10.4	11.0	1.0	3.0
Yonchon	9.4	12.1	11.6	1.0	3.0
Yulmu 1	12.0	16.0	8.6	1.4	1.0
Muzu	8.4	7.7	8.9	1.0	3.0
Tsukuba B15	6.7	5.1	6.0	1.0	1.0

^a Rated 23 September.

^b Values are means of 10 plants in each of three replicate plots per genotype and planting date.

was recorded as the percentage of leaf area diseased. The disease severity of whole plants was calculated as the mean of the leaf blight severity of individual leaves using the same number of related leaves per plant.

In the greenhouse study, disease severity of cultivars or lines was rated 10 days after inoculation. Leaf blight severity was recorded as the percentage of diseased leaf area of each leaf. The disease severity of whole plants was calculated as the mean of the leaf blight severity of individual leaves. All data are the means of 10 plants. All experiments were conducted twice with similar results. The data presented are from two experiments conducted subsequently.

Differences between cultivars or lines in the field or greenhouse were compared using Fisher's protected least significant differences (LSD) test at $P = 0.05$. An analysis of variance was performed on the effects of genotype or planting date, with data expressed as leaf blight severities for the sixth and ninth leaves, and the whole plants of six cultivars or lines of adlay planted at five different dates. Leaf blight severities for plant leaf stages, leaf ages, and leaf positions of three cultivars or lines of adlay inoculated with *B. coicis* in the greenhouse also were analyzed statistically by the analysis of variance procedures. Linear regression analysis was used to evaluate relationships between leaf wetness duration and inoculum density for disease development. The Statistical Analysis System for personal computers (SAS Institute Inc., Cary, NC) was used for all analyses.

RESULTS

Effect of plant growth stage on disease development in field plots. Disease severity in the 1998 experiment was in general lower than in 1999 (Tables 1 to 3). Disease severity was greatest for the earliest-sowed plants, decreasing for plants sowed later. Lower leaves were more severely infected than were upper leaves. The sixth leaves of all cultivars or lines tested were highly susceptible to leaf blight, except for those sowed on 5 July (Table 1), which had considerably less disease in all cultivars or lines tested. The ninth leaves of all cultivars or lines tested were less diseased than the sixth leaves (Tables 1 and 2), as shown by the analysis of variance (Table 4). For plants sowed 5 July, all cultivars or lines showed a slight infection on the ninth leaves, indicating no significant differences in disease severity among the six cultivars or lines. Disease severity was lowest on Tsukuba B15 throughout all sowings, but usually highest on Huksuk 2. Lines Muzu, Yonchon, Yulmu 1, and Limgae were intermediate in resistance levels. There were some significant differences among these four cultivars or lines in disease severity values, and there were significant interactions between host genotype and planting date.

The sowings in 1999 showed results similar to 1998, although slightly lower disease severity was observed in 1999 (Tables 1 to 3). The relative levels of leaf

blight severity among adlay cultivars or lines also were significantly different in all sowings. The line Tsukuba B15 was the most resistant to leaf blight throughout all

Table 4. Analysis of variance for leaf blight severities for the sixth and ninth leaves and for the whole plants of six cultivars or lines of adlay planted at five different dates in field plots at Yonchon, Korea

Source of variation	df	Mean square	F value	P
1998				
Sixth leaf				
Cultivar	5	1,160.7	49.1	<0.01
Planting date	4	17,189.3	727.9	<0.01
Cultivar $\times$ planting date	20	232.5	9.9	<0.01
Ninth leaf				
Cultivar	5	895.7	30.8	<0.01
Planting date	4	2,277.5	78.3	<0.01
Cultivar $\times$ planting date	20	160.2	5.5	<0.01
Whole plant				
Cultivar	5	594.9	33.3	<0.01
Planting date	4	2,856.0	159.7	<0.01
Leaf position (6th leaf $\times$ 9th leaf)	1	13,676.0	519.1	<0.01
Cultivar $\times$ planting date	20	25.1	1.4	<0.05
Cultivar $\times$ planting date $\times$ leaf position	20	226.1	8.6	<0.01
1999				
Sixth leaf				
Cultivar	5	1,731.8	107.4	<0.01
Planting date	4	23,841.6	1,478.9	<0.01
Cultivar $\times$ planting date	20	7,662.1	23.8	<0.01
Ninth leaf				
Cultivar	5	894.4	39.5	<0.01
Planting date	4	14,115.1	623.7	<0.01
Cultivar $\times$ planting date	20	272.2	12.0	<0.01
Whole plant				
Cultivar	5	459.5	133.5	<0.01
Planting date	4	6,474.6	1,880.8	<0.01
Leaf position (6th leaf $\times$ 9th leaf)	1	52,282.1	2,698.2	<0.01
Cultivar $\times$ planting date	20	43.7	12.7	<0.01
Cultivar $\times$ planting date $\times$ leaf position	20	222.4	11.5	<0.01

Fig. 1. Effect of inoculum concentration on disease development in three adlay cultivars or lines inoculated with 10^5 conidia of *Bipolaris coicis* isolate BC-97006 per milliliter by foliar spraying at the two-leaf stage. All values were evaluated 10 days after inoculation on 10 plants each.

sowings, while Huksuk 2 was the most susceptible. Differences in disease severity among the cultivars or lines were more distinguishable in the sixth leaves of the 15 June sowing plants than in those of other sowings. Based on the levels of disease severity, the relative levels of resistance to leaf blight were, in descending order, Tsukuba B15,

Muzu, Yulmu 1, Yonchon, Limgae, and Huksuk 2.

Similar trends were found in the disease severity values of whole plants for both experiment years (Table 3). The disease severity values were greatest in the earliest sowing and decreased in the successively later sowings. The disease severity value was lowest on Tsukuba B15 throughout all

sowings, and highest on Huksuk 2. The levels of disease severity varied among the other four adlay cultivars or lines tested. Based on the differences in disease severity values of the whole plants, the relative ranks of cultivars or lines in levels of resistance were in descending order as follows: Tsukuba B15, Muzu, Yulmu 1, Yonchon, Limgae, and Huksuk 2.

The analysis of variance on leaf blight severities for the sixth and ninth leaves and for the whole plants of six cultivars or lines planted at five different dates in this experiment showed highly significant differences among cultivars, planting dates, leaf position, and cultivar $\times$ planting date $\times$ leaf position interactions (Table 4). The adlay cultivars or lines differed in resistance to leaf blight, and also in their reaction to planting date. The different leaf positions affected individual expressions of disease.

Effect of inoculum density and wetness period on disease development. When rated 10 days after inoculation, disease severity increased as inoculum density increased from 10^3 to 10^6 conidia per ml (Fig. 1). A linear relationship was found between inoculum density and disease severity at the two-leaf stage. Conidial concentration used for the inoculation of three cultivars or lines explained an average of 87, 86, and 93% of the variation in disease severity in Huksuk 2, Yulmu 1, and Tsukuba B15, respectively. Few symptoms were induced on the cultivar or lines inoculated with 10^3 conidia per ml. Disease severity was intermediate at 10^4 to 10^5 conidia per ml. At 10^6 conidia per ml, the most plants of Huksuk 2 and Yulmu 1, but not Tsukuba B15, were dead within 7 days after inoculation. Disease severity was lowest on Tsukuba B15 and highest on Huksuk 2.

Disease severity increased in adlay plants as wetness duration increased (Fig. 2). A linear relationship was observed between the leaf wetness duration and disease severity at the two-leaf stage. There was little difference among the cultivars or lines in the slopes of the leaf wetness–disease severity lines. However, the cultivars or lines differed in their disease reaction to leaf wetness, because disease developed slightly on leaves of resistant plants (Tsukuba B15) that were not kept wet after inoculation. After exposure to continuous wetness for 48 or 60 h, susceptible plants (line Huksuk 2) were dead within 7 days after inoculation (data not shown). At all wetness durations, disease severity development on Tsukuba B15 was less severe than on Huksuk 2 or Yulmu 1.

Effect of plant growth stage on disease development. Leaf blight severity was greatest at the 11-leaf stage, and much less at the earlier stages (Fig. 3). Significant differences in disease severity among the three cultivars or lines tested were observed at all growth stages. Disease severity of whole plants was lower on Tsukuba

Fig. 2. Effect of wetness duration on disease development in three adlay cultivars or lines inoculated with 10^5 conidia of *Bipolaris coicis* isolate BC-97006 per milliliter by foliar spraying at the two-leaf stage. All values were evaluated 10 days after inoculation on 10 plants each.

Fig. 3. Disease severity on the whole plants of three adlay cultivars or lines inoculated with 10^5 conidia of *Bipolaris coicis* isolate BC-97006 per milliliter at different leaf stages in the greenhouse. All the values were determined 10 days after inoculation on 10 plants each. Values in figure with different letters show significant differences at $P = 0.05$ according to the Fisher's protected least significant difference (LSD) test.

B15 than on Yulmu 1 and Huksuk 2 at 7-, 9-, and 11-leaf stages.

With the aging of the second and third leaves after leaf expansion, leaf blight increased (Fig. 4). Disease development in the third leaves at the different ages was significantly lower than in the second leaves. Disease severity in the second and third leaves at the different ages was greatest in Huksuk 2, intermediate in Yulmu 1, and least in Tsukuba B15. The line Tsukuba B15 was highly resistant to leaf blight in the third leaves. Data obtained from experiments 1 and 2 in the greenhouse were similar to each other.

As leaves grew older, they became more susceptible to *B. coicis* infection (Fig. 5), indicating that leaf positions affect disease development. Lower (older) leaves were more severely infected than upper (younger) leaves. For all leaf positions, Tsukuba B15 was significantly more resistant than Yulmu 1 and Huksuk 2, as indicated by lower disease severity values. The two experiments showed similar trends in disease severities for all the adlay cultivars at different leaf positions.

The analysis of variance on leaf blight severities for plant leaf stages, leaf ages, and leaf positions of three cultivars or lines of adlay artificially inoculated in the greenhouse showed highly significant differences among cultivars, plant leaf stages, leaf ages, leaf positions, and the interactions of cultivars with leaf stages, leaf ages, or leaf positions (Table 5). The adlay cultivars or lines differed in resistance to *B. coicis* infection. The different leaf stages, leaf ages, and leaf positions affected individual expressions of disease on adlay plants in the greenhouse.

DISCUSSION

The 2-year field study enabled us to evaluate the quantitative resistance of adlay during moderate (1998) and severe (1999) epidemics of leaf blight (Tables 1 to 3). The disease levels in six adlay cultivars or lines varied greatly between the two years of the experiment, possibly due to the changes in environmental conditions and amount of inoculum in the field. The disease severity values (percent diseased leaf area) on whole plants of Huksuk 2 for sowings of 17 April, 5 and 25 May, 15 June, and 5 July in 1998 were 83.2, 72.4, 68.2, 65.7, and 41.0%, respectively, which suggests that younger plants at later planting dates are more resistant to leaf blight than older ones at earlier planting dates. To determine whether the greater disease for earlier planting dates may be due to plant age, we have conducted further greenhouse experiments for the effects of plant age on *B. coicis* infection. Leaf blight severity was much greater at late growth stages than at earlier stages when inoculated simultaneously in the greenhouse (Fig. 3). Therefore, the possibility of increased susceptibility of older adlay plants to leaf blight under field

conditions is well supported by these data from the greenhouse experiments. The greater disease for earlier planting dates could also result from different inoculum

levels during the adlay growing season, because the older plants were exposed to inoculum for a longer period of time. These findings were very similar to the

Fig. 4. Disease development on the second and third leaves of three adlay cultivars or lines inoculated with 10^5 conidia of *Bipolaris coicis* isolate BC-97006 per milliliter at different ages in the greenhouse. All the values were determined 10 days after inoculation on 10 plants each. Values in figure with different letters show significant differences at $P = 0.05$ according to the Fisher's protected least significant difference (LSD) test.

Fig. 5. Disease severity on different leaves of three adlay cultivars or lines inoculated with 10^5 conidia of *Bipolaris coicis* isolate BC-97006 per milliliter at the six-leaf stage in the greenhouse. All values were determined 10 days after inoculation on 10 plants each. Values in figure with different letters show significant differences at $P = 0.05$ according to the Fisher's protected least significant difference (LSD) test.

Table 5. Analysis of variance for leaf blight severities for plant leaf stages, leaf ages, and leaf positions of three cultivars or lines of adlay inoculated with *Bipolaris coicis* isolate BC-97006 in the greenhouse

Source of variation	df	Mean square	F value	P
Leaf stage (Fig. 3)				
Leaf stage	3	1,858.9	50.2	<0.01
Cultivar	2	1,605.8	43.4	<0.01
Leaf stage × cultivar	6	133.6	3.6	<0.05
Leaf age (Fig. 4)				
Leaf 2				
Leaf age	3	9,367.8	86.2	<0.01
Cultivar	2	10,607.5	97.6	<0.01
Leaf age × cultivar	6	545.2	5.0	<0.05
Leaf 3				
Leaf age	3	3,057.5	164.8	<0.01
Cultivar	2	6,807.8	367.0	<0.01
Leaf age × cultivar	6	938.6	50.6	<0.01
Leaf position (Fig. 5)				
Leaf position	4	642.0	71.3	<0.01
Cultivar	2	1,418.9	157.5	<0.01
Leaf position × cultivar	8	87.9	9.8	<0.01

results of tan spot ratings (0 to 5 scale) of 2.3, 3.0, and 4.1 on flag, flag-1, and flag-2 leaves of wheat genotype 147, respectively, in the *Drechslera tritici-repentis*-wheat combination (8).

The leaf position or age of adlay plant had a significant influence on leaf blight severity, which was higher on the lower (older) leaves (leaf no. 6) and lower on upper (younger) leaves (leaf no. 9) (Tables 1 to 4). Leaf position appeared to affect quantitative levels of leaf blight infection in adlay cultivars or lines. Our data have demonstrated that lower leaves were more severely infected by leaf blight than upper leaves, irrespective of growth stage and cultivar or line (Table 4). This phenomenon has been recognized in other plant-pathogen systems (4,8,9,13,17,18,21). The increased susceptibility of wheat to tan spot, caused by *Pyrenophora tritici-repentis*, with leaf aging was found by Raymond et al. (17), who showed that older leaves of wheat had higher disease ratings than younger leaves, despite being inoculated at the same time.

Inoculum density of *B. coicis* affected leaf blight development in adlay seedlings (Fig. 1). Generally, as inoculum density increased, symptom development increased. With a high level of inoculum density (10^6 conidia per ml), initial lesions appeared 2 days after inoculation, suggesting the significance of higher inoculum density to rapid development of leaf blight. To compare response of adlay to leaf blight at the two-leaf stage, inoculum concentration of over 10^5 conidia per ml of *B. coicis* seemed to be required for disease development.

Duration of leaf wetness period was directly proportional to disease severity (Fig. 2). At least 24 to 36 h of leaf wetness was

necessary to induce leaf blight in the greenhouse, whereas plants receiving 48 and 60 h of leaf wetness were severely blighted. Similarly, Hosford et al. (8) observed that wetness duration significantly affected tan spot severity in wheat. Disease reactions with a 24-h dew period allowed us to evaluate the degrees of resistance of six adlay cultivars or lines that resembled those on adult plants in the field.

In the greenhouse experiment, growth stage and leaf position significantly affected leaf blight severity (Figs. 3 to 5, Table 5). Young leaves (plants) of all cultivars or lines had a lower disease severity than older leaves and plants, despite being inoculated at the same time. These trends were similar to those observed in the field experiments. A similar phenomenon has been noted by the observations of Riaz et al. (18) that the tan spot severity of wheat caused by *Pyrenophora tritici-repentis* was enhanced on old tissues. Overall, our study strongly suggests that plant maturity may be an important factor in development of leaf blight of adlay. Older leaves, being nearer to natural senescence, may consequently be easily infected by *B. coicis*.

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