

## Colonization of Bentgrass Turf by *Curvularia lunata* After Leaf Clipping and Heat Stress

J. J. MUCHOVEJ, Departamento de Fitopatologia, Universidade Federal de Viçosa, 36570 Viçosa, MG, Brazil, and H. B. COUCH, Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg 24061

### ABSTRACT

Muchovej, J. J., and Couch, H. B. 1987. Colonization of bentgrass turf by *Curvularia lunata* after leaf clipping and heat stress. *Plant Disease* 71:873-875.

Creeping bentgrass turfs were grown, clipped, and maintained at 2 cm in 7-cm-diameter cups and grown until 30 or 120 days old. Turfs either did or did not receive high air temperature stress and were clipped 128, 64, 32, 16, 8, 2, 1, or 0 hr before inoculation with *Curvularia lunata*. Results showed that *C. lunata* could colonize heat-stressed and/or old clipped leaves but could not infect and colonize juvenile or mature leaves. The amount of turf foliage that was susceptible to thinning by *C. lunata* depended on the physiologic age of the leaf tissue. As stresses of high air temperatures are placed on leaf tissues, a greater percentage of the leaf blades are forced into advanced senescence, thereby increasing their susceptibility to infection and colonization.

Additional key words: *Agrostis palustris*

The nature of the symbiosis of *Curvularia lunata* (Wakk.) Boedijn and *Agrostis palustris* Huds. has been debated during the past 40 yr (12). Reports of pathogenicity of *C. lunata* to turfgrass species are usually accompanied by elevated ambient air temperatures, which are unfavorable to the growth of the host (3,7,8,14). Foliar symptoms are a yellow-green dapple color. Couch (4) questioned pathogenicity of *Curvularia* spp. to turfgrasses because of "an inability to produce leaf lesions with various *Curvularia* isolates when these were tested as primary parasites." Muchovej (11) demonstrated that *C. lunata* only induced accelerated senescence of mature, intact bentgrass leaf tissue.

Because clipping turfgrass leaves is an absolute practice on golf greens, where *C. lunata* is reported to be most damaging (13), and because clipping may increase disease severity (1), the relationship of leaf clipping and heat stress for the entry of *C. lunata* was studied.

### MATERIALS AND METHODS

*C. lunata* was isolated from symptomatic Penneagle bentgrass leaves growing at the Virginia Turfgrass Research Center in Blacksburg. The isolate was single-spored, lyophilized and sealed in glass ampules, and stored at 5 C. As needed, the fungus was cultured in petri

plates on a basal medium with 2 g of xylose added (2) and incubated under fluorescent lights at 24 C (11).

Stands of bentgrass plants were established from seed planted in washed river sand in 7-cm-diameter plastic cups. Turfs were irrigated with normal Hoagland's solution on alternate days and were illuminated with GE cool-white fluorescent lights (11). The growth chamber remained at  $22 \pm 2$  C and  $80 \pm 5\%$  relative humidity. Clipping was initiated when the plants reached a height of 2 cm, and that height was maintained by clipping every other day with a pair of shears. By sowing on two dates, two age groups of these small turf areas were established for the beginning of treatments: 1) those that contained mature and senescent leaves about 120 days old and 2) those that contained mostly

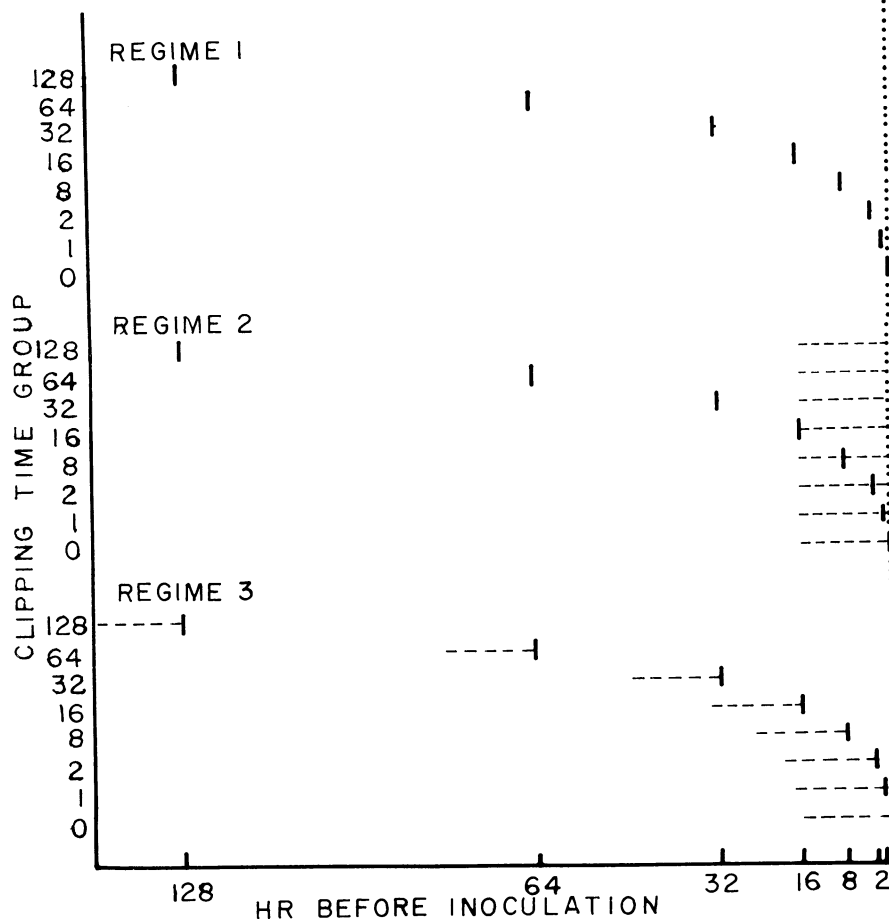


Fig. 1. Schematic of the time when plants were clipped (bar) and high air temperature stressed (dashed line) before inoculation with *Curvularia lunata* at time 0 (vertical dotted line).

juvenile leaves about 30 days old. Within each age group, three clipping/high air temperature induced stress (HATS) regimes were established (Fig. 1) by subjecting the turfs to air temperatures of 38 C for 18 hr in a moisture-saturated atmosphere in the dark. Within these regimes, the turf areas were clipped 128, 64, 32, 16, 8, 2, 1, or 0 hr before inoculation. In regime 1, turfs received no HATS; in regime 2, HATS was applied preclipping; and in regime 3, HATS was applied preinoculation. Inoculation was done by spraying each 7-cm-diameter cup with 3 ml of a suspension containing  $1 \times 10^3$  spores of *C. lunata* per milliliter. The spore suspension was filtered through double cheesecloth. Distilled water was applied to uninoculated controls for each time reference within each clipping regime. The experiment was done with four

replicates and was repeated once on a different date.

After inoculation, all turfs, including uninoculated controls, were placed in a mist chamber at  $22 \pm 2$  C for 48 hr and observed daily for the presence of fungal mycelium growing from the cut end of the leaf blade. Fragments of mycelium present were lifted from the leaf blade and were plated on agar medium (11) for later identification. Plant samples were also taken from all treatments every 8 hr for 96 hr, cleared in glacial acetic acid:95% ethanol (1:1, v/v), then stained and mounted in 0.25% aniline blue in lactophenol to observe for the presence of mycelium (9).

Turfs were also rated for the incidence of leaf tip chlorosis 5 days after inoculation. A rating scale was established by counting the leaf tips showing chlorosis within four randomly selected

1-cm areas of grass. Turfs that rated 0, 1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, and 80% of the leaves with tip chlorosis were used as standards against which the rest of the turfs could be compared and rated. Ratings were taken for both inoculated and uninoculated cups within each clipping-HATS regime.

Values obtained were compared statistically using the Wilcoxon rank sum test (6).

## RESULTS

Fungal mycelium was found growing from the chlorotic tips of 120-day-old plants that had received HATS. Plants that had been inoculated with *C. lunata* yielded *C. lunata*, whereas plants that were not inoculated yielded mostly *Cladosporium oxysporum* Berk. & Curt. and *Epicoccum purpurescens* Ehrenb. ex Schlect. as determined by illustrated keys (5). Similarly, fungal mycelium was encountered growing from chlorotic leaf tips of 30-day-old plants, and again, these yielded *C. lunata* and *Cladosporium oxysporum* plus *E. purpurescens*, respectively.

After clearing and staining, fungal mycelium was encountered in the chlorotic leaf tips of 30- and 120-day-old plants that were heat-stressed. There was no evidence of fungal mycelium in the leaf tips that were not chlorotic.

The symptom appearing on leaves was a tip chlorosis that began as a change in the shade of green to lighter and yellowish. This color change became more intense and progressed down the leaf blade. There was a gradual change in the shades of the leaf tissue between the tip and the unaffected areas below. At no point was a distinct separation found between affected and unaffected areas. As time progressed, the chlorosis progressed down the blade until the entire leaf was affected; however, no further progression occurred.

The presence of chlorosis at the clipped ends of the leaves varied among the time intervals when plants received or did not receive HATS immediately before inoculation. The young (30-day-old) turf showed little leaf tip chlorosis independent of HATS; only plants that had received HATS at least 32 hr before inoculation showed more than 5% leaf tip chlorosis (Table 1). All of the older (120-day-old) inoculated turfs showed leaf tip chlorosis, again independent of whether the plants had received HATS, with uninoculated plants showing much lower levels of leaf tip chlorosis. The leaves that showed chlorosis in the older turf were those that had not elongated after clipping. Leaves that had elongated showed no leaf tip chlorosis. In general, the youngest leaf elongated in all treatments to a length of as much as 2 cm by 128 hr postclipping, with only the youngest leaf on any plant elongating.

Plants that had been clipped immedi-

**Table 1.** Median rating of tip chlorosis of bentgrass turf inoculated (I) or not inoculated (NI) with *Curvularia lunata* at the respective times after turf clipping<sup>w</sup>

Hours after clipping when inoculated	30-Day-old plants					
	Regime 1		Regime 2		Regime 3	
	NI	I	NI	I	NI	I
0	0	1 <sup>x</sup>	0	5	0	1 a <sup>y</sup>
1	0	1	0	5	0	1 a
2	0	1	0	5	0	1 a
8	0	1	0	5	0	5 a
16	0	1	0	5	0	5 a
32	0	1	0	5	0	10 ab
64	0	1	0	5	0	15 b
128	0	1	0	5	0	15 b
	120-Day-old plants					
0	1	5 a	1	5 a	1	5 a
1	1	5 a	1	5 a	1	5 a
2	1	10 ab	1	10 ab	1	5 a
8	1	10 ab	1	20 b	1	10 ab
16	1	20 bc	1	40 c	1	20 b
32	1	25 c	1	40 c	1	40 c
64	1	25 c	1	40 c	1	70 d <sup>z</sup>
128	1	25 c	1	40 c	1	80 d <sup>z</sup>

<sup>w</sup>Plants were about 30 or 120 days old and were either non-heat-stressed (regime 1) or heat-stressed for 18 hr at 38 C immediately before clipping (regime 2) or inoculation (regime 3).

<sup>x</sup>Values are the median of eight 1-cm areas of turf compared against areas known to have 0, 1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 or 80% leaf tip chlorosis.

<sup>y</sup>Values in each column within each plant age group followed by the same letter are not significantly different ( $\alpha = 0.05$ ) according to the Wilcoxon rank sum test.

<sup>z</sup>Turf blighting present.

**Table 2.** Descriptions of symptoms that have been reported on grasses infected by *Curvularia lunata*

Symptom	Symptom description	Reference
Chlorotic striations	White, 1–2 mm enlarging to 3–4 cm long, no more than 1 mm wide and rectangular; first appearing near leaf tip, then midleaf and leaf base	10
Curvularia blight	Yellow-green dapple, found on greenhouse-grown material; similar to coloration that occurs on old leaf tissue that has been extracted with 95% ethanol	7,11
Leaf tip dieback	Chlorosis that becomes necrosis, starts at the leaf tip, then progresses down leaf blade to collar; resembles high air temperature induced stress	3,11
Leaf tip chlorosis	Chlorosis beginning at clipped end of leaf blade, then progressing downward toward collar, becoming necrosis only after chlorosis of entire leaf blade	Current study

ately after HATS and then returned to 22 C for the various time periods before being inoculated (regime 3) showed increased levels of leaf tip chlorosis and also plant blighting at the longer time periods (64 and 128 hr) for the 120-day-old turf (Table 1). Plants from shorter time intervals showed little difference from the plants that had been clipped just before the end of the heat stress period. When plant blighting occurred, the youngest leaf on the plant remained asymptomatic.

Non-HATS 120-day-old plants showed leaf tip chlorosis. Affected leaves were the third, fourth, and older leaves from the top of the plant.

## DISCUSSION

### Symptom development in plants.

Several types of symptoms have been described throughout this and other studies for the "disease" incited by *C. lunata* (Table 2). From this study, the only symptom that appears to be genuine to a pathogenic relationship between *C. lunata* and Penneagle bentgrass is the leaf tip chlorosis that was present when leaf tips were clipped.

**Infection.** Results agreed with other reports (3,7,8,11,13) in that high air temperature stressing of the leaves into senescence or old physiological age of leaf tissue was necessary before *C. lunata* could infect and colonize. Muchovej (11) has shown that the life of an intact bentgrass leaf was no more than 30 days, and that by that time, the leaf had begun to show a leaf tip dieback indicative of senescence. Also subjecting plants to heat stressing caused a rapid shift of the leaf into senescence (11). Leaf senescence may not be important to the plant as a whole, because the plant will recover and

produce more leaves given time. However, senescence is important to the aesthetic qualities of the turf, because loss of the older leaves will thin the turf and give an appearance that is not aesthetically pleasant. On the grass plants that were clipped, there were three to five older leaves present to only one new leaf. If all of the older leaves were removed, the turf could be thinned by 75–87%. Therefore, measures that would either reduce stand thinning or increase leaf replacement are necessary to reduce the visual impact of senescent leaf removal.

Symptom development on the plant is chlorosis beginning at the clipped end of physiologically older leaves and eventually progressing down the leaf blade. New leaves on 120-day-old plants were unaffected as were most leaves on younger plants regardless of heat stressing. *C. lunata* has not been implicated in the pathogenicity of intact leaves (11), and apparently, leaf clipping allowed entry of *C. lunata* into leaf tissue. *C. lunata* was then able to provoke chlorosis of the leaf tissue that had already entered into senescence because of stresses applied. This accelerated the change of the leaf tissue from green to yellow, which is aesthetically unpleasant. The amount of turf foliage that is susceptible to thinning by *C. lunata* depends on the physiologic age of the leaf tissue. As stresses of high air temperatures are placed on the leaf tissue, a greater percentage of the leaf blades are forced into advanced senescence, thereby increasing their susceptibility to infection and colonization by *C. lunata*. High rates of colonization of physiologically older leaves by *C. lunata* are dependent on stresses that have been placed on the grass days before germination of spores

of *C. lunata*. It is also possible that other stresses such as soil moisture, pollution, or adverse fertility may enhance infection of bentgrass plants by *C. lunata*.

## ACKNOWLEDGMENT

This research was supported in part by CNPq-Brazil.

## LITERATURE CITED

1. Beard, J. B. 1973. Turfgrass: Science and Culture. Prentice Hall, Englewood Cliffs, NJ.
2. Bischoff, T. W., and Garraway, M. O. 1985. The effects of xylose on the growth and sporulation of an isolate of *Bipolaris maydis* race T and its relation to pH and ammonium levels. *Phytopathology* 75:138-141.
3. Brown, G. E., Cole, H., Jr., and Nelson, R. R. 1972. Pathogenicity of *Curvularia* spp. to turfgrasses. *Plant Dis. Rep.* 56:59-63.
4. Couch, H. B. 1973. Diseases of Turfgrasses. 2nd ed. R. E. Kreiger Publ. Co., Huntington, NY.
5. Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.
6. Hollander, M., and Wolffe, D. A. 1973. Nonparametrical Statistical Methods. John Wiley & Sons, New York.
7. Howard, F. L. 1953. Helminthosporium-Curvularia blights of turf and their cure. *Golf Course Rep.* 21(2):5-9.
8. Howard, F. L., Rowell, J. B., and Keil, H. L. 1951. Fungus diseases of turfgrasses. *AES Bull.* 308, Univ. R. I.
9. Kunoh, H., and Akai, S. 1969. Histochemical observation of the halo on the epidermal cell wall of barley leaves attacked by *Erysiphe graminis hordei*. *Mycopathol. Mycol. Appl.* 37:113-118.
10. Muchovej, J. J. 1984. Definition of *Agrostis palustris* leaf health at the time of infection and colonization by *Curvularia lunata*. Ph.D. dissertation. Virginia Polytechnic Institute and State University, Blacksburg.
11. Muchovej, J. J. 1986. Definition of leaf health in *Agrostis palustris* at the time of infection and colonization by *Curvularia lunata*. *Ann. Appl. Biol.* 109:249-258.
12. Muchovej, J. J., and Couch, H. B. 1985. *Curvularia* blight of turfgrasses: A historical perspective. *Razen-Turf-Gazon* 16:88-92.
13. Smiley, R. W. 1983. Compendium of Turfgrass Diseases. American Phytopathological Society, St. Paul, MN.
14. Wernham, C. C., and Kirby, R. S. 1941. A new turf disease. (Abstr.) *Phytopathology* 31:24.