# CHAPTER TWO

# INVESTIGATING CAUSES AND CURES FOR LOCALIZED DRY SPOTS ON CREEPING BENTGRASS PUTTING GREENS

## ABSTRACT

Localized dry spots (LDS) on creeping bentgrass (*Agrostis palustris* Huds.) putting greens are characterized by irregular patches of wilting turf that resist wetting by irrigation or rainfall. Turf managers around the world have attempted to control LDS, rarely with long term success. Previous research has demonstrated the causal agent of LDS to be of fungal origin, possibly a basidiomycete fairy-ring causing pathogen. Water injection cultivation (WIC), a non-ionic surfactant blend soil wetting agent (SWA), and flutolanil fungicide have all shown limited control of LDS. Combinations of these treatments may provide enhanced control of LDS. Additionally, if the causal agent of LDS is identified, better strategies to control this problem may be developed. The objectives of this research were (i) to evaluate the effects and interactions of WIC, SWA, and flutolanil on the control of LDS on a sand based creeping bentgrass putting green, and (ii) to more thoroughly define the spatial distribution of the most affected area by LDS and (iii) to further evaluate the relationship of fungal biomass and LDS activity. A preventative LDS study was conducted in 1998 and 1999 on a creeping bentgrass putting green with a coarse-textured root zone. Treatments were arranged in a 2 x 2 x 2 factorial, with the factors consisting of: WIC (tri-weekly or none), SWA (1.3 ml $m^{-2}$ tri-weekly or none), and flutolanil (0.9 g a.i. $m^{-2}$ tri-weekly or none). Treatment effects on turf quality, wilt, soil moisture,

and water drop penetration times varied between years. Wilting was reduced with WIC in 1999, while flutolanil improved general turf quality during summer stress periods in 1998. Flutolanil and SWA improved turf quality when applied as curative treatments in 1998. The depth and surface distribution of water repellant characteristics in randomly selected LDS patches were evaluated by direct visualization using stereophotmicrography to locate where LDS symptoms were most prominent. These studies indicated that water drop penetration times were greatest at the inside and edge of dry spots, just beneath the thatch layer. Buried slides examined by quantitative brightfield microscopy and computer assisted image analysis revealed a significantly greater amount of growing hyphae at the edges of the dry spots, consistent with the proposal that the cause of LDS may be of fungal origin.

## INTRODUCTION

Localized dry spots (LDS) routinely confound turfgrass management because of unpredictable occurrence and difficulty of control. Localized dry spots are defined as dry spots of turf and soil surrounded by more moist turf conditions, which resist rewetting by normal irrigation or rainfall (Beard, 1982). Most turf areas afflicted with LDS share common characteristics (Henry and Paul, 1978; Wilkinson and Miller, 1978) including: 1) coarse-textured soils, 2) only the surface centimeters of the soil are hydrophobic, and 3) water repellent coatings on soil particles. Since the United States Golf Association introduced putting green specifications (USGA, 1960), most new greens have been constructed with sand based root zones, increasing the incidence of LDS (Karnok et al., 1993). Continued use of sand topdressing as a putting green management practice also contributes to the LDS problem.

Localized dry spot is not confined to any single geographic region. In a survey by York (1993) 86% of the greenskeepers in the United Kingdom encountered LDS on their golf courses and 61% had LDS problems for at least five years. In addition to reducing available water, LDS may contribute to other poor soil physical properties. Dry putting green soils in Michigan became hard and reduced the penetration of cultivation units (Rieke, 1974). Creeping bentgrass putting greens (*Agrostis palustris* Huds.) with LDS in Ohio had 20% lower infiltration rates than did healthy areas (Wilkinson and Miller, 1978).

Once LDS occurs on turf, complete eradication is difficult in a short period of time. Only 3% of those experiencing LDS in the York (1993) survey achieved complete control with wetting agent applications. Hydrophobic soils in northern Michigan were improved only temporarily with application of high rates of a wetting agent (Rieke and Beard, 1975). Management practices that completely prevent LDS have not been firmly established. A survey of 10 Georgia golf course superintendents experiencing LDS on creeping bentgrass putting greens showed no correlation between standard management practices and LDS severity (Tucker et al., 1990). Additionally, the survey showed no relationship between LDS severity and chemical soil tests.

A specific causal agent of LDS has not been discovered, but the literature suggests it may be of fungal origin (Bond and Harris, 1964). Organic coatings on sand particles taken from LDS areas in Ohio had the presence of fungal mycelia. The coatings were determined to be primarily fulvic acid compounds (Miller and Wilkinson, 1977). Some basidiomycete fungi that cause fairy rings are known to induce hydrophobic conditions that stress turf within the rings. (Smith et al., 1988). Soil samples taken from within several fairy rings caused by *Marasmius oreades* on golf course fairways in Norway had significantly low moisture content (Smith, 1975). Similarly, inner zones of *M. oreades* fairy rings on Kentucky bluegrass (*Poa pratensis* L.) turf in Saskatoon, Canada had significantly low soil moisture contents (Smith and Rupps, 1978).

We hypothesize that the presence of fungi may contribute to LDS formation by depositing organic coatings on sand particles. Flutolanil (Prostar® fungicide manufactured by AgrEvo, Montvale, NJ) controls many fairy ring causing fungi (Elliot and Hickman, 1998) and may be effective in preventing LDS.

Two other management practices that have reduced LDS severity are water injection cultivation (WIC) with a Toro HydroJect 3000® (Karcher, 1997) and the application of an effective soil wetting agent (SWA) (Gelernter and Stowell, 1998). Additionally, if we can identify the properties of the causal agent of LDS and locate its growing front, better recommendations for effective control are possible. The objectives of this research were (i) to evaluate the effects and interactions of flutolanil, WIC, and SWA wetting agent applications on the control of LDS on a sand based creeping bentgrass putting green, (ii) locate the zone of maximum soil hydrophobicity in characteristic LDS, and (iii) further examine the role of soil fungi in development of LDS.

## MATERIALS AND METHODS

### Experimental Area

Three separate experiments were designed to address research objectives: A preventative study to compare the effects of WIC, SWA, and flutolanil on the prevention of LDS, a curative study to compare the above effects on curing turf that was severely afflicted with LDS, and an isolation study to determine the precise location of activity and identify the characteristics of the LDS causal agent.

Plots were established for the preventative study on a 'Penncross' creeping bentgrass putting green with a modified sand root zone (96% sand, 3% silt, and 1% sand) in early April 1998. Plots were mowed at 4.0 mm five days per week throughout the study. Nitrogen was applied at 30 g $m^{-2}$ $year^{-1}$ and irrigation was applied only at severe drought to provide reasonable growth, but to encourage the onset of LDS. Other nutrients were applied according to soil test recommendations. In April 1999 the experiment was repeated on a 'Penncross' creeping bentgrass putting green with a modified loamy sand soil (modified fine-loamy, mixed, mesic, Typic Hapludalf). The experiment was repeated in this area because it had frequent occurrences of LDS in 1998.

For the isolation experiment, three individual patches showing pronounced LDS symptoms adjacent to the preventative study were selected in 1998. Plots were mowed five times per week at a 5.0 mm height and fertilized with 20 g N $m^{-2}$ $year^{-1}$. Formation of LDS occurred despite this area being irrigated daily at rates approximating evapotranspiration water loss.

All experimental areas were topdressed lightly with 100% sand approximately every six weeks. The topdressing sand layer depth remained less than 1.0 cm throughout the experiments. Each fall, all plots were core cultivated at a 7.5 by 5.0 cm spacing to an approximate depth of 8 cm using 1.0 cm diameter hollow tines. Cores were brushed, returning the soil to the plot area, and the remaining thatch was removed. Fungicides were applied on a curative basis.

## Treatment Design

### *PREVENTATIVE STUDY*

The preventative study had three treatment factors: WIC, SWA, and flutolanil. Each factor had two levels, applied or not applied, yielding eight treatment combinations (2 x 2 x 2 factorial). In both years, treatments were applied on 21-day intervals beginning in late April and ending in mid October. Plots were drenched with 2.5 cm of water several hours prior to all treatments, in accordance with the flutolanil label. Water injection cultivation treatments were made first with a Toro HydroJect 3000® set at the closest hole spacing (7.5 by 2.5 cm). Flutolanil and SWA were applied next at rates of 0.9 g a.i. $m^{-2}$ (WP) and 1.3 ml $m^{-2}$, respectively, with a $CO_2$ powered plot sprayer. Following treatments, plots were irrigated with 1 cm of water. Treatments were replicated 3 times in a randomized complete block design.

### *CURATIVE STUDY*

In August 1998, a turf area adjacent to the preventative LDS study, but with separate irrigation control, had severe wilt symptoms with random dry patches characteristic of LDS. A curative study was established with treatments identical to the preventative study on this area. Treatments were applied tri-weekly from August to October in 1998 for this study.

### *ISOLATION STUDY*

The treatment factors in the isolation study consisted of surface location and depth, relative to the dry spots. Surface location was classified as the center, edge, or outside of the dry spot. The edge of the dry spot was identified as the border between turf with visual wilting symptoms from LDS and turf with no visible LDS symptoms. Sampling of the outside of the dry patch was made 15 cm from the outside perimeter. Depth was classified in one cm increments from just beneath the thatch layer to a 5 cm depth. Location and depth factors were arranged in a randomized complete block design (blocked by dry spots) with three replications.

## Treatment Evaluations

### *PREVENTATIVE AND CURATIVE STUDIES*

Treatments in the preventative and curative studies were evaluated for turf quality and soil moisture content. Additionally, wilt and soil wettability were evaluated in the preventative study. Quality and wilt were evaluated weekly, and when symptoms were visible, respectively. The rating scale for quality was from 1 to 9 (1=dead, 2=mostly dead, 3=severely flawed, 4=flawed, 5=slightly flawed, 6=acceptable, 7=good, 8=excellent, 9=ideal), and for wilt was from 1 to 5 (1=no wilt, 2=slight wilt, 3=moderate wilt, 4=significant wilt, 5=severe wilt). Wilt ratings were typically done in the late afternoon when wilt symptoms were easiest to detect. Volumetric soil moisture to a 15 cm depth was measured weekly on three randomly selected locations per plot with a Trime®-FM portable time domain reflectometry unit (manufactured by IMKO, Ettlingen, Germany). Soil wettability was evaluated monthly by water drop penetration times. Three randomly selected soil cores per plot were pulled and sectioned by depth into five one cm increments. Within 48 hours of sampling, cores were sliced in half vertically with a razor blade. A 100 µl water droplet was then placed on the flat surface of each core section and the time elapsed until the droplet had completely penetrated the soil surface was recorded.

### *ISOLATION STUDY*

Treatment evaluations for the isolation study were designed to precisely locate where the LDS causal agent affected soil wettability. Water drop penetration times were measured on samples from the inside, edge, and outside of three separate dry patches using the methods described above. Rossi-Cholodny buried slides (Johnson and Curl, 1972) were inserted just beneath the thatch layer at the inside, edge, and outside of three individual dry patches and incubated for 21 days. The use of buried slides allowed for precise spatial analysis of alive, active fungi capable of colonizing the slide. Following incubation, slides were carefully extracted and stained with lactophenol-aniline blue-acid fuchsin. An AusJenaval brightfield microscope (25x objective) and Panasonic WV185 Neuvicon camera were used to output fungal images to a video monitor so that hyphae could be traced onto transparency overlays. All of the hyphae occurring on a one-cm$^2$ portion of the slide that was adjacent to the soil immediately below the thatch layer were recorded. The hyphae were digitized and analyzed by the cumulative hyphal length feature of the Image Tool option in the CMEIAS software package (Liu et al., 2000).

## Statistical Analysis

Quality and wilt rating were analyzed using the proportional odds model that is incorporated into the Rating Data Analysis File Package (Karcher, 2000). Treatment separation was done with pairwise chi-square tests of the treatment least squared estimates. Probability distributions were constructed to represent the odds of a treatment level to be rated in a particular category. These distributions were constructed by inserting the appropriate combination of least square estimates into the logit-link function.

Soil moisture, soil wettability, and hyphal length data were analyzed with ANOVA. If treatment effects were significant, means were separated using LSD at the 0.05 probability level. Where repeated measures were made on the same experimental units, time was analyzed as a sub-plot factor of the experiment. The best fitting covariance model among compound symmetry, first order auto-regressive, and spatial exponential was used to fit correlations among time points. The best fitting covariance model was determined by the highest Akaike's Information Criteron value (Littell et al., 1996). A log transform was used to normalize the water drop penetration data, which originally were highly right skewed because of several values near zero.

## RESULTS AND DISCUSSION

### Preventative Study

ANOVA results and the main effects of WIC, SWA, and flutolanil on turf quality, wilt, and soil moisture are summarized in Table 1. The main effects of WIC, SWA, and flutolanil on turf quality were not significant in either year. However, there was a significant flutolanil x time interaction in 1998 and a significant SWA x flutolanil interaction in 1999.

The flutolanil x time interaction resulted from a significant flutolanil effect on 5 out of the 20 rating dates in 1998 (Figure 11). On 7 May flutolanil slightly decreased the probability to be rated high in quality, but on 17 July, 11 August, 18 August, and 18 September flutolanil significantly increased the probability to be rated high in quality. The dates when flutolanil significantly increased quality were dates when the plots, averaged over all treatments, were rated significantly low in quality (Figure 12). In 1999, the probability of plots to be rated high in quality decreased when flutolanil and SWA were both applied compared to the application of either alone (Figure 13).

These results suggest that flutolanil may be effective in increasing turf quality when environmental conditions are particularly stressful. On 11 August and 18 September, 1998, the experimental area was drought stressed, resulting in low overall quality. However, flutolanil significantly improved turf quality on these rating dates. These results are to be expected if drought symptoms in turf are partially caused by a fungal species susceptible to flutolanil. Adams (1989) found that flutolanil had a high degree of fungicidal activity against *Marasmius oreades*, a fungus known to cause fairy ring often expressing hydrophobic soil conditions (Bayliss, 1911; Smith, 1975; Smith and Rupps, 1978).

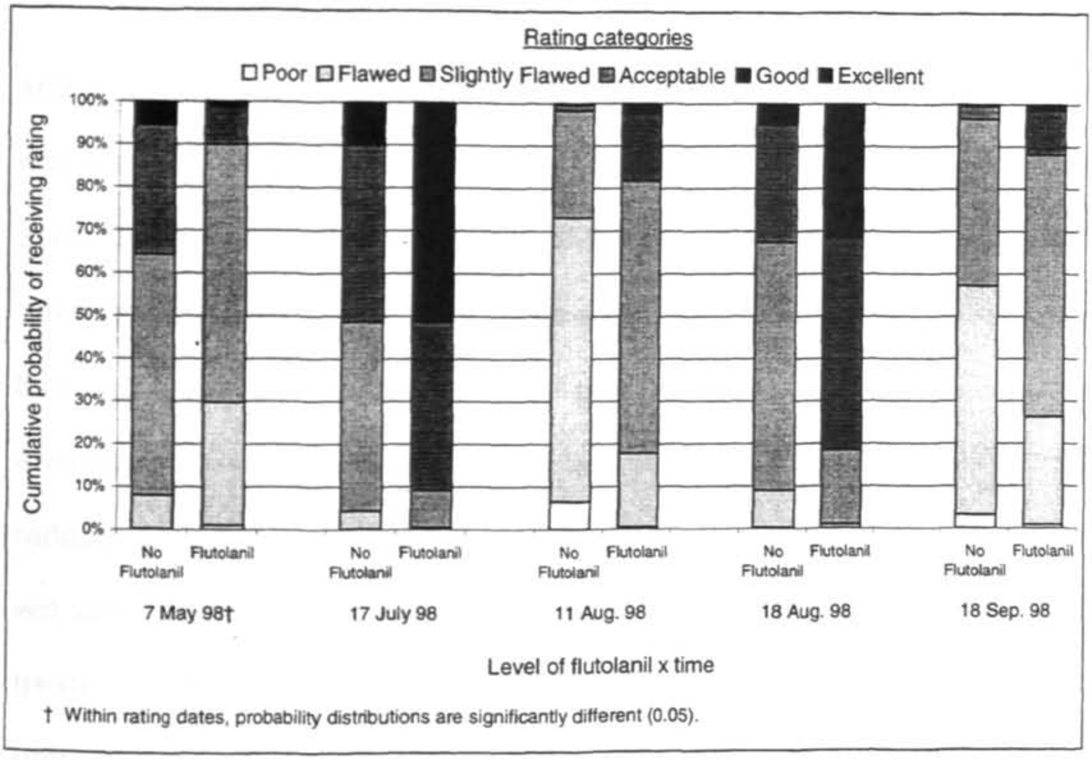
The flutolanil x SWA interaction suggests that these products may be slightly phytotoxic when applied together at the highest labeled rate of each. Flutolanil has been reported to cause phytotoxicity when mixed with other products (Gelernter and Stowell, 1997).

**Table 1. Effects of WIC, SWA, and flutolanil on quality, wilt, and soil moisture of a creeping bentgrass putting green.**

| | 1998 | | | | | | 1999 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Effect | df | Quality Parameter | df | Wilt Parameter | df | Soil Moisture | df | Quality Parameter | df | Wilt Parameter | df | Soil Moisture |
| | | likelihood estimate | | likelihood estimate | | $m^3 m^{-3}$ | | likelihood estimate | | likelihood estimate | | $m^3 m^{-3}$ |
| WIC | | | | | | | | | | | | |
| None | | -1.22 A† | | 5.22 A | | 26.6 A | | -1.03 A | | -0.95 A | | 26.6 A |
| Tri-Weekly | | -0.92 A | | 2.11 A | | 26.2 A | | -0.60 A | | 0.15 B | | 27.3 A |
| SWA | | | | | | | | | | | | |
| None | | -0.89 A | | 3.50 A | | 26.4 A | | -0.84 A | | -0.28 A | | 26.5 A |
| 1.3 ml $m^{-2}$ | | -1.24 A | | 3.83 A | | 26.5 A | | -0.79 A | | -0.52 A | | 27.4 A |
| Flutolanil | | | | | | | | | | | | |
| None | | -0.80 A | | 3.76 A | | 26.5 A | | -0.84 A | | -0.57 A | | 26.6 A |
| 0.9 g a.i. $m^{-2}$ | | -1.34 A | | 3.57 A | | 26.4 A | | -0.78 A | | -0.23 A | | 27.3 A |
| | | | | | | ANOVA | | | | | | |
| Source of variation | | | | | | | | | | | | |
| Block | 2 | *** | 2 | *** | 2 | ** | 2 | *** | 2 | *** | 2 | ** |
| WIC (w) | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | * | 1 | NS |
| SWA (s) | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS |
| w x s | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS |
| Flutolanil (f) | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS |
| w x f | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS |
| s x f | 1 | NS | 1 | NS | 1 | NS | 1 | ** | 1 | NS | 1 | NS |
| w x s x f | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS | 1 | NS |
| Time (t) | 19 | *** | 1 | NS | 16 | *** | 13 | *** | 2 | *** | 4 | *** |
| w x t | 19 | NS | 1 | NS | 16 | *** | 13 | NS | 2 | NS | 4 | NS |
| s x t | 19 | NS | 1 | NS | 16 | NS | 13 | NS | 2 | NS | 4 | NS |
| w x s x t | 19 | NS | 1 | NS | 16 | NS | 13 | NS | 2 | NS | 4 | NS |
| f x t | 19 | * | 1 | NS | 16 | NS | 13 | NS | 2 | NS | 4 | NS |
| w x f x t | 19 | NS | 1 | NS | 16 | NS | 13 | NS | 2 | NS | 4 | NS |
| s x f x t | 19 | NS | 1 | NS | 16 | NS | 13 | NS | 2 | NS | 4 | NS |
| w x s x f x t | 19 | NS | 1 | NS | 16 | NS | 13 | NS | 2 | NS | 4 | NS |

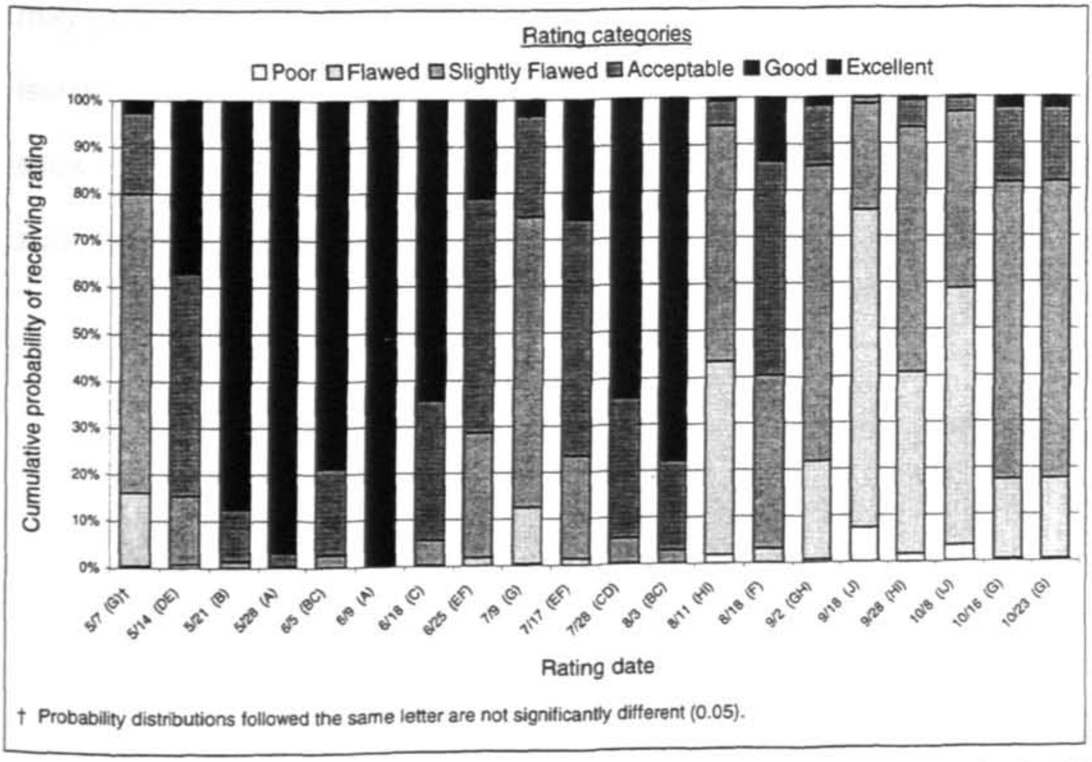
*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† Within effects, means sharing a letter are not significantly different



† Within rating dates, probability distributions are significantly different (0.05).

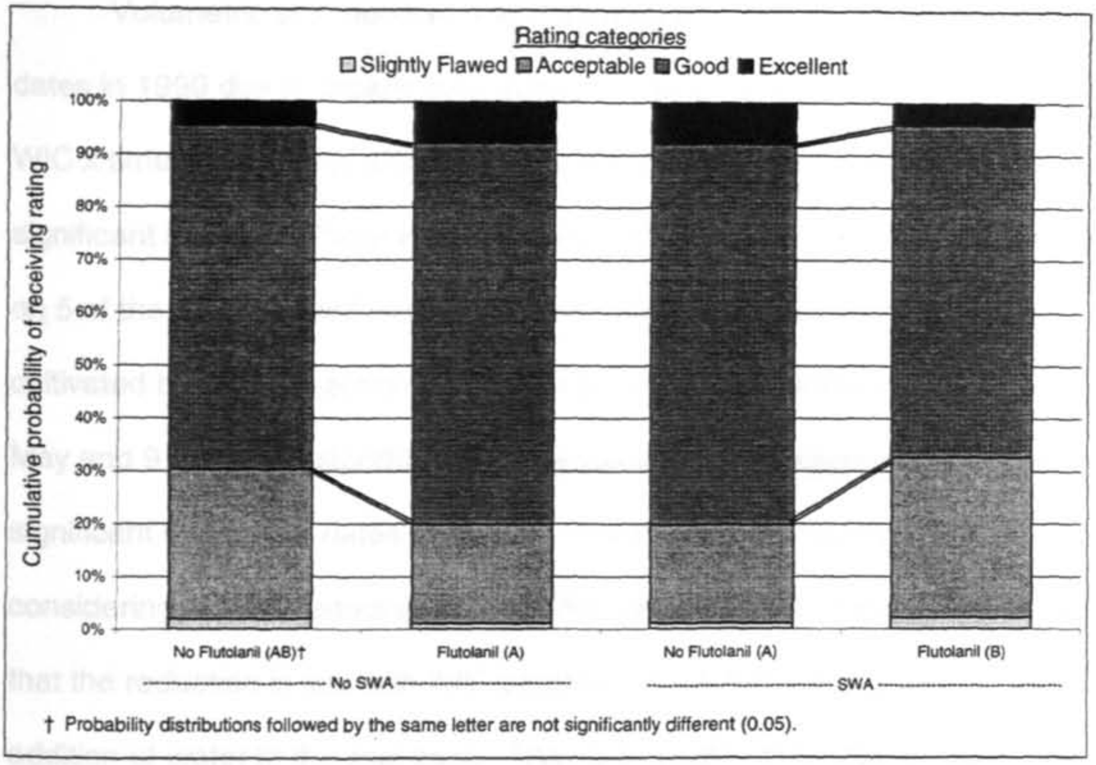
**Figure 11. Quality rating probability distributions as affected by flutolanil x time. Preventative Study, 1998.**



† Probability distributions followed the same letter are not significantly different (0.05).

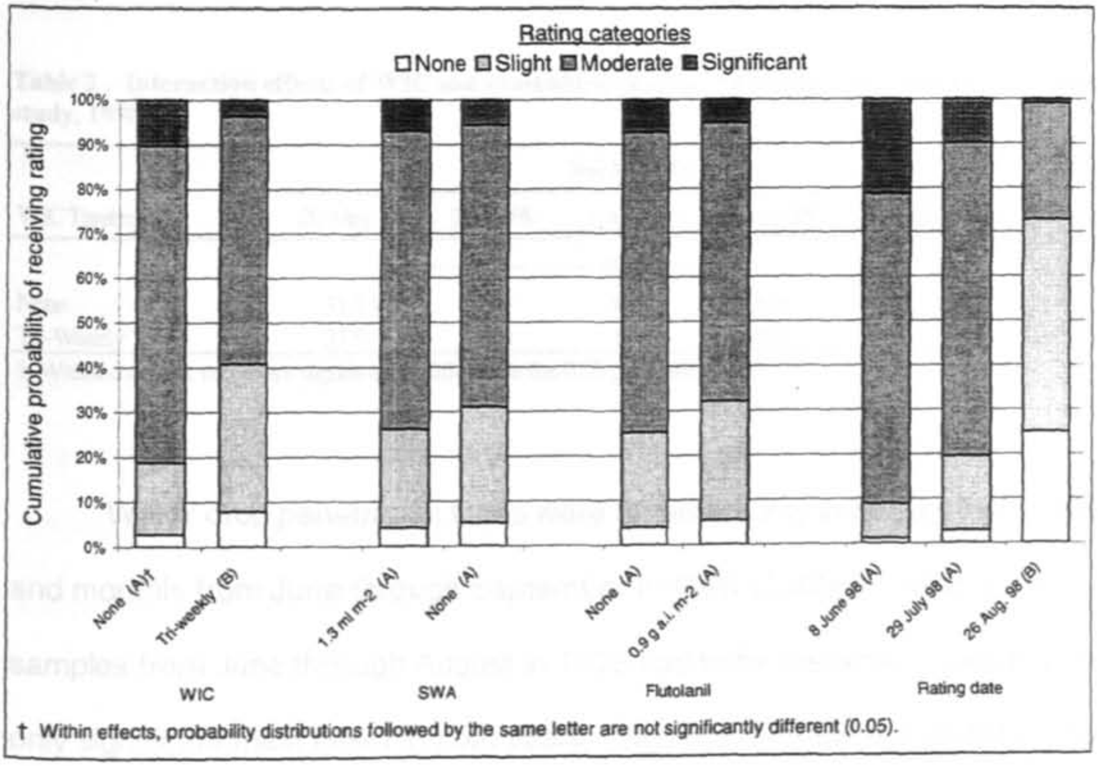
**Figure 12. Quality rating probability distributions as affected by time. Preventative Study, 1998.**

The experimental area wilted enough for ratings on only two dates in 1998 and three dates in 1999. The lack of LDS formation on the experimental area was probably due to the irrigation practices used to accommodate treatment application. A tri-weekly application of 3.5 cm of water during the study may have inhibited the onset of LDS, since a reported precursor to LDS formation is a thorough drying down of the soil (Paul and Henry, 1973). When the experimental area dried down, it usually did so in a uniform fashion rather than by forming random dry spots characteristic of LDS. The only significant treatment effect on wilt was WIC in 1999 (Figure 14). Averaged over all rating dates in 1999, WIC treated turf had significantly lower probability to be rated high in wilt than untreated turf. This result is consistent with results from previous WIC experiments (Karcher, 1997). The 20 MPa water blasts from the HydroJect® unit may partially remove hydrophobic coatings on sand particles. Additionally, isolated channels created by the cultivation blast (Murphy and Rieke, 1994) may allow for deeper rooting and subsequently, more total water available in the root zone for WIC treated turf.



† Probability distributions followed by the same letter are not significantly different (0.05).

**Figure 13. Quality rating probability distributions as affected by SWA x flutolanil. Preventative study, 1999.**



† Within effects, probability distributions followed by the same letter are not significantly different (0.05).

**Figure 14. Wilt rating probability distributions as affected by WIC, SWA, flutolanil, and time. Preventative *LDS* study, 1999.**

Volumetric soil moisture was measured on 17 dates in 1998, but only 5 dates in 1999 due to broken time domain reflectometry equipment. Time and WIC x time effects were significant in 1998, whereas only time effects were significant in 1999. There were significant differences between WIC treatments on 5 of the 17 dates soil moisture was measured in 1998 (Table 2). Turf cultivated by water injection had significantly greater soil moisture content on 20 May and 9 June, but significantly lower soil moisture content on the three significant evaluation dates in August. These data are surprising when considering the wilt reducing effect of WIC seen in 1999. These data suggest that the reduction in wilt with WIC treatment is probably not caused by the simple addition of water to the root zone. The HydroJect® adds approximately 1.5 mm water to the turf at the closest hole spacing and with the roller washers off.

**Table 2 . Interaction effects of WIC and evaluation date on volumetric soil moisture. Preventative study, 1998.**

| | Soil Moisture | | | | |
|---|---|---|---|---|---|
| WIC Treatement | 20 May. 98 | 9 Jun. 98 | 3 Aug. 98 | 6 Aug. 98 | 12 Aug. 98 |
| | $m^3 m^{-3}$ | | | | |
| None | 31.3 † | 34.4 | 16.4 | 20.6 | 23.7 |
| Tri-Weekly | 33.5 | 37.0 | 10.4 | 16.9 | 21.1 |

† Within columns, means are significantly different at the 0.05 probability level.

Water drop penetration times were recorded only in September in 1998 and monthly from June through September in 1999 (Table 3). Mishandled samples from June through August in 1998 had to be discarded. Depth was the only significant main effect in both years. Significant interaction effects included

WIC x depth in 1998, and flutolanil x depth, depth x time, WIC x flutolanil x time, and WIC x SWA x flutolanil x time in 1999.

In 1998, the 3 and 4 cm depths had significantly longer penetration times than depths 1, 2, and 5. Although the log(s) penetration times were statistically significant, there were no practical differences in the untransformed means among treatments. In 1999, the soil samples were significantly less wettable near the surface and became more wettable with depth. However, all soil depths showed some degree of non-wettability in both years. The 1999 data are fairly consistent with those of Wilkinson and Miller (1978), who found that hydrophobic conditions in sand based putting greens were restricted to the upper 2 cm of soil. However, the 1998 data do not show this trend. Wilkinson and Miller evaluated turf showing characteristic LDS symptoms, whereas the turf in this study dried down uniformly. Uniform dry down conditions may result in hydrophobic soil at depths greater than 2 cm. Bond (1968) reported hydrophobic conditions in sands at depths of up to 0.5 meter.

Where no WIC applications were made in 1998, the 3 and 4 cm depths were significantly less wettable than the 1 and 2 cm depth (Table 4). However, there were no differences in wettability among depths where WIC was applied. The 20 MPa blast of the WIC unit probably mixed the soil so that there were no differences in penetration times among soil depths. Previous studies have shown that WIC significantly mixes soil layers in putting green (Karcher, 1997).

**Table 3. Effects of WIC, SWA, flutolanil, and sample depth on water drop penetration time.**

| | Water drop penetration time | | | | | |
|---|---|---|---|---|---|---|
| Effect | ——— 1998 ——— | | | ——— 1999 ——— | | |
| | df | s | log(s)† | df | s | log(s) |
| WIC | | | | | | |
| None | | 5.7 | 0.22 A | | 30.2 | 2.69 A |
| Tri-Weekly | | 11.3 | 0.21 A | | 34.4 | 2.89 A |
| SWA | | | | | | |
| None | | 5.2 | 0.16 A | | 32.8 | 2.78 A |
| 1.3 ml m$^{-2}$ | | 11.8 | 0.28 A | | 31.8 | 2.80 A |
| Flutolanil | | | | | | |
| None | | 14.1 | 0.30 A | | 34.3 | 2.92 A |
| 0.9 g a.i. m$^{-2}$ | | 2.9 | 0.13 A | | 30.3 | 2.66 A |
| Depth | | | | | | |
| 1 | | 9.5 | 0.10 B | | 56.1 | 3.71 A |
| 2 | | 9.7 | 0.13 B | | 48.7 | 3.46 A |
| 3 | | 10.0 | 0.32 A | | 32.3 | 2.92 B |
| 4 | | 10.8 | 0.35 A | | 15.2 | 2.17 C |
| 5 | | 2.6 | 0.18 B | | 9.2 | 1.70 D |
| | | | | ANOVA | | |
| Source of variation | | | | | | |
| Block | 2 | | NS | 2 | | NS |
| WIC (w) | 1 | | NS | 1 | | NS |
| SWA (s) | 1 | | NS | 1 | | NS |
| w x s | 1 | | NS | 1 | | NS |
| Flutolanil (f) | 1 | | NS | 1 | | NS |
| w x f | 1 | | NS | 1 | | NS |
| s x f | 1 | | NS | 1 | | NS |
| w x s x f | 1 | | NS | 1 | | NS |
| Depth (d) | 4 | | ** | 4 | | *** |
| w x d | 4 | | * | 4 | | NS |
| s x d | 4 | | NS | 4 | | NS |
| w x s x d | 4 | | NS | 4 | | NS |
| f x d | 4 | | NS | 4 | | * |
| w x f x d | 4 | | NS | 4 | | NS |
| s x f x d | 4 | | NS | 4 | | NS |
| w x s x f x d | 4 | | NS | 4 | | NS |
| Time (t) | --- | | --- | 3 | | *** |
| w x t | --- | | --- | 3 | | NS |
| s x t | --- | | --- | 3 | | NS |
| w x s x t | --- | | --- | 3 | | NS |
| f x t | --- | | --- | 3 | | NS |
| w x f x t | --- | | --- | 3 | | *** |
| s x f x t | --- | | --- | 3 | | NS |
| w x s x f x t | --- | | --- | 3 | | * |
| d x t | --- | | --- | 12 | | ** |
| w x d x t | --- | | --- | 12 | | NS |
| s x d x t | --- | | --- | 12 | | NS |
| w x s x d x t | --- | | --- | 12 | | NS |
| f x d x t | --- | | --- | 12 | | NS |
| w x f x d x t | --- | | --- | 12 | | NS |
| s x f x d x t | --- | | --- | 12 | | NS |
| w x s x f x d x t | --- | | --- | 12 | | NS |

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† Data were normalized by a log(seconds) transformation for statistical analysis.

When no flutolanil was applied in 1999, soil became significantly more wettable with depth, except from 4 to 5 cm (Table 5). Flutolanil application increased the wettability of the 1 cm depth to where it was equal to the 2 cm depth. The 1999 data suggest that flutolanil application improves soil wettability near the turf surface, which would be expected if hydrophobic soil conditions were partially caused by a fungus susceptible to flutolanil.

The other high order interactions with time imply that the effects of depth, WIC x flutolanil, and WIC x SWA x flutolanil were inconsistent from month to month.

**Table 4. Water drop penetration times as affected by WIC x depth interaction. Preventative study, 1998.**

| | Depth (cm) | | | | |
|---|---|---|---|---|---|
| WIC Treatement | 1 | 2 | 3 | 4 | 5 |
| | log(s) | | | | |
| None | 0.02 | 0.05 | 0.36 | 0.43 | 0.23 |
| Tri-Weekly | 0.17 | 0.21 | 0.28 | 0.27 | 0.13 |

† Within columns LSD = 0.28, within rows LSD = 0.18, both at the 0.05 probability level.

**Table 5. Water drop penetration times as affected by flutolanil x depth interaction. Preventative study, 1999.**

| | Depth (cm) | | | | |
|---|---|---|---|---|---|
| Flutolanil Treatment | 1 | 2 | 3 | 4 | 5 |
| | log(s) | | | | |
| None | 3.97 | 3.46 | 2.81 | 2.03 | 1.63 |
| 0.9 g a.i. $m^{-2}$ | 3.45 | 3.47 | 3.02 | 2.31 | 1.76 |

† Within columns LSD = 0.56, within rows LSD = 0.49, both at the 0.05 probability level.

## Curative Study

For the 1998 curative study, ANOVA results and the main effects of WIC, flutolanil, and SWA on turf quality and soil moisture are summarized in Table 6.

The main effects of SWA and flutolanil on turf quality were significant in the curative study. There was also a WIC x SWA interaction with regard to quality. Time was the only significant effect with regard to soil moisture in the curative study.

Averaged over all rating dates, both SWA and flutolanil application significantly increased the probability of the turf to be rated high in quality compared to application of neither (Figure 15). There are no data in the refereed literature pertaining to initiating treatments on turfs that are already severely affected with LDS. These results indicate that turf affected by LDS can be improved by curative applications of SWA or flutolanil.

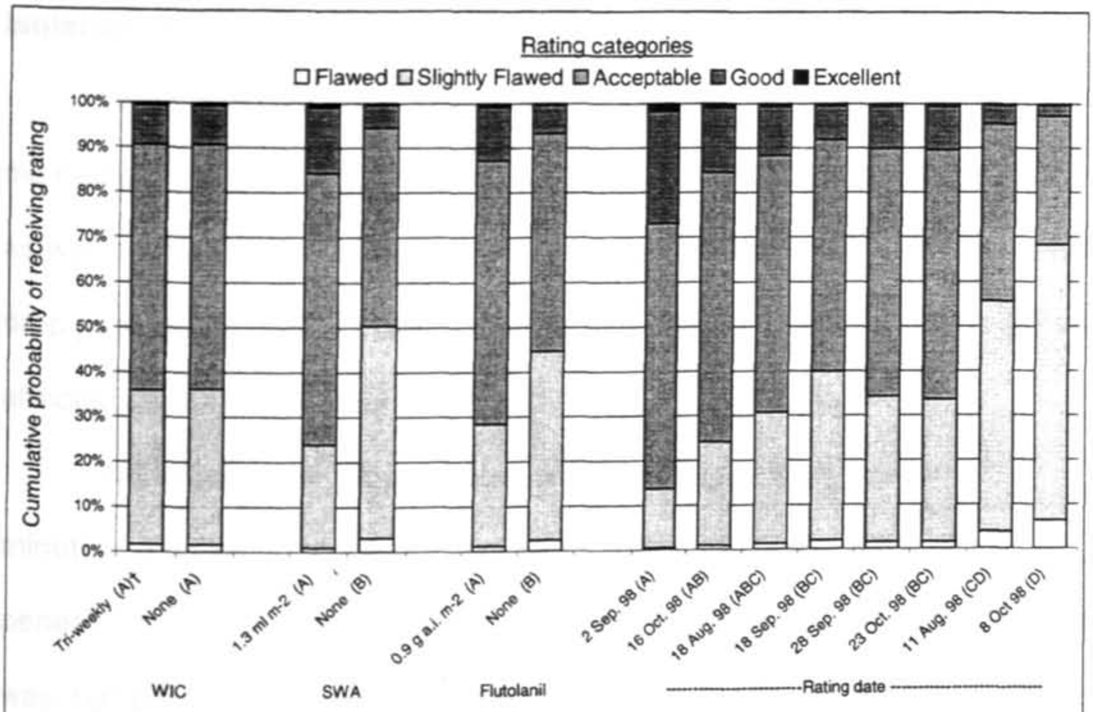
When plots received no WIC treatment in the curative study, quality probability distributions were not affected by the addition of SWA (Figure 16). However, plots receiving WIC treatment had a significantly greater probability of being rated high in quality when SWA was also applied. The creation of channels by WIC probably allowed better penetration of the wetting agent through the thatch layer into hydrophobic soil areas. This may have made soil more wettable and improved turf quality when WIC preceded SWA applications. Wilkinson and Miller (1978) showed that a combination of core cultivation plus wetting agent significantly improved turf quality over either alone.

**Table 6. ANOVA and main effects of WIC, SWA, and flutolanil on turf quality and soil moisture. Curative study, 1998.**

| Effect | df | Quality Parameter | df | Soil Moisture |
|---|---|---|---|---|
| | | likelihood estimate | | $m^3\ m^{-3}$ |
| WIC | | | | |
| None | | 0.75 A† | | 28.8 A |
| Tri-Weekly | | 0.73 A | | 29.2 A |
| SWA | | | | |
| None | | 1.33 B | | 29.0 A |
| 1.3 ml $m^{-2}$ | | 0.15 A | | 29.0 A |
| Flutolanil | | | | |
| None | | 1.09 B | | 29.3 A |
| 0.9 g a.i. $m^{-2}$ | | 0.38 A | | 28.8 A |
| | | | ANOVA | |
| Source of variation | | | | |
| Block | 2 | *** | 2 | NS |
| WIC (w) | 1 | NS | 1 | NS |
| SWA (s) | 1 | *** | 1 | NS |
| w x s | 1 | ** | 1 | NS |
| Flutolanil (f) | 1 | * | 1 | NS |
| w x f | 1 | NS | 1 | NS |
| s x f | 1 | NS | 1 | NS |
| w x s x f | 1 | NS | 1 | NS |
| Time (t) | 7 | ** | 2 | *** |
| w x t | 7 | NS | 2 | NS |
| s x t | 7 | NS | 2 | NS |
| w x s x t | 7 | NS | 2 | NS |
| f x t | 7 | NS | 2 | NS |
| w x f x t | 7 | NS | 2 | NS |
| s x f x t | 7 | NS | 2 | NS |
| w x s x f x t | 7 | NS | 2 | NS |

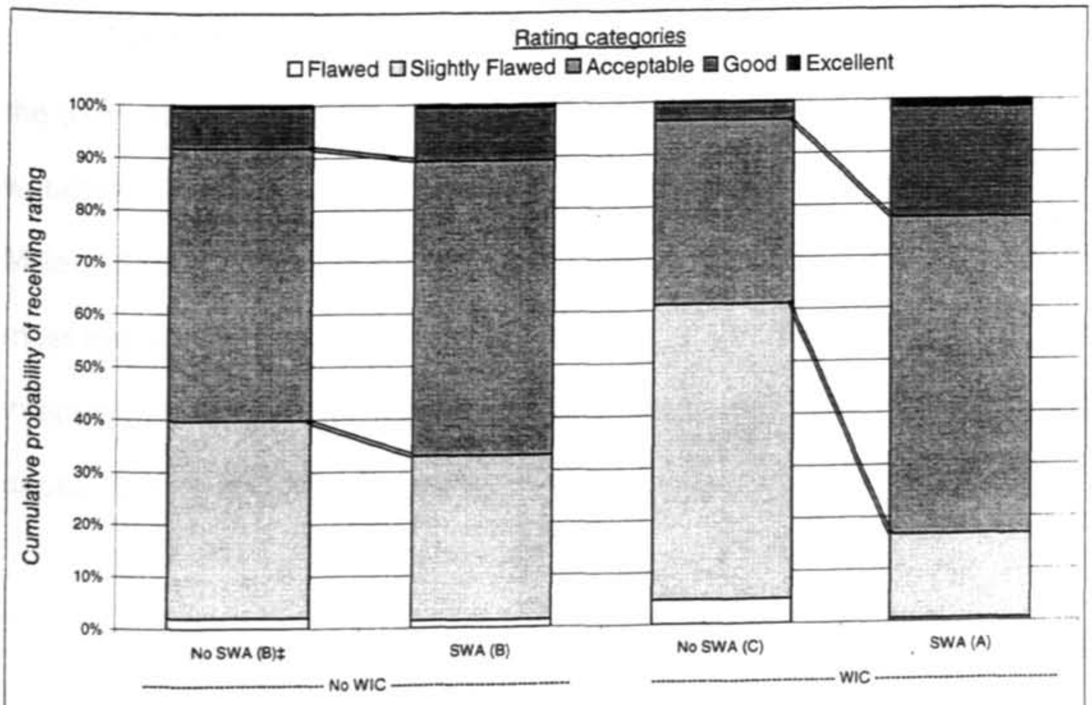
*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† Within effects, means sharing a letter are not significantly different.



† Within treatment factors, probability distributions followed the same letter are not significantly different (0.05).

Figure 15. Quality rating probability distributions as affected by WIC, SWA, flutolanil, and rating date. Curative study, 1998.



† Probability distributions followed the same letter are not significantly different (0.05).

Figure 16. Quality rating probability distributions as affected by WIC x SWA. Curative study, 1998.

**Isolation Study**

Water drop penetration time and cumulative hyphal length ANOVAs and main effect means are summarized in Table 7. Location and depth main effects, as well as the location x depth effect, were all significant with regard to water drop penetration time. Location relative to the center of the dry spot significantly affected cumulative hyphal length at the 0.06 probability level.

Individual water drop penetration times ranged from 1 second to 21 minutes. The inside of the dry spot had significantly longer water drop penetration times than the edge and outside. Additionally, the edge of the patch was significantly less wettable than outside (Figure 17). There is no mention in the refereed literature of evaluating differences in water drop penetration time among locations, relative to the center of a dry patch.

The 1 and 2 cm depths were less wettable than all other depths, whereas the 3 cm depth was intermediate, and the 4 and 5 cm depths were most wettable. These results are similar to those reported previously (Wilkinson and Miller, 1978; Tucker et al., 1990), where the upper few cm of the soil were the most hydrophobic in sand based putting greens. The significant location x depth interaction indicated that significant differences among locations only occurred at depths of 1, 2, and 3 cm (Table 9).

**Table 7. Water drop penetration times and cumulative hyphae length as affected by surface location and depth relative to LDS. Isolation study, 1998.**

| Effect | df | Water drop penetration time | | df | Cumulative hyphal length |
|---|---|---|---|---|---|
| | | s | log(s) | | µm hyphae $cm^{-2}$ |
| Location | | | | | |
| edge | | 56 | 2.5 B† | | 10295 A‡ |
| inside | | 284 | 3.5 A | | 3067 B |
| outside | | 3 | 0.8 C | | 7841 A |
| Depth (cm) | | | | | |
| 1 | | 416 | 4.4 A | | -- |
| 2 | | 128 | 3.6 A | | -- |
| 3 | | 24 | 2.4 B | | -- |
| 4 | | 3 | 0.6 C | | -- |
| 5 | | 2 | 0.3 C | | -- |
| ANOVA | | | | | |
| Source of variation | | | | | |
| Block | 2 | | ** | 2 | NS |
| Location (l) | 2 | | *** | 1 | § |
| Depth (d) | 4 | | *** | -- | -- |
| l x d | 8 | | *** | -- | -- |

§, **, *** Significant at the 0.1, 0.05, and 0.001 probability levels, respectively.

† Within effects, means sharing a letter are not significantly different ($P \leq 0.05$).

‡ Means sharing a letter are not significant at the 0.10 probability level.

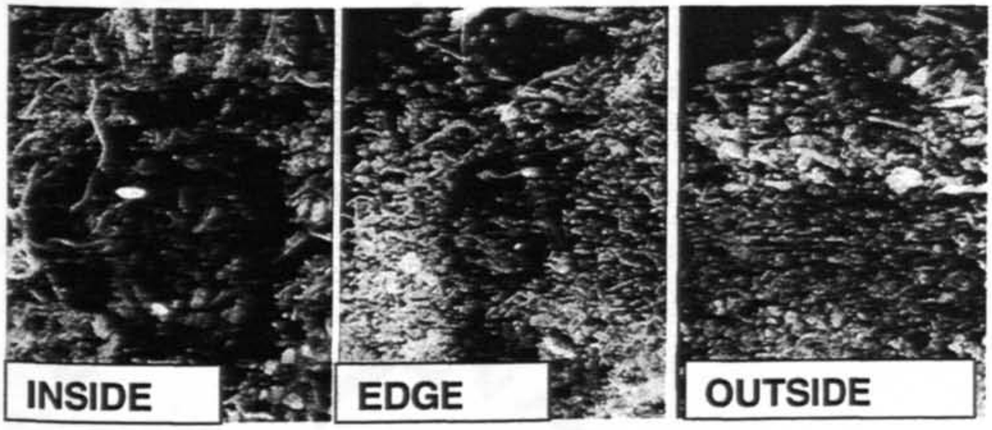


**Figure 17. Images taken 30 seconds after water drop placement just beneath the thatch layer at the inside, edge, and outside of a dry patch.**

Stained hyphae from the edge of a dry spot are shown in Figure 18. The edge of the dry spot had significantly ($P = 0.06$) greater fungal biomass (measured as the cumulative hyphal length) than the inside or outside. This is further evidence that LDS may be caused by a fungal organism. If the causal agent of LDS is of fungal origin, growth initiates at a central point and continues radially outward, with the highest concentration of active viable fungi biomass at the growing edge of a dry spot. This growth pattern is similar to many pathogenic fungal species that are known to cause fairy ring and patch diseases (Smiley et al., 1992). Although previous research has associated various fungal species with LDS (Miller and Wilkinson, 1978; York and Baldwin, 1992), no fungal species have been identified that are specific to LDS.

**Figure 18. Stained hyphae from a section of a buried slide at the edge of a dry patch, immediately beneath the thatch layer. Image is magnified 250 times.**

**Table 9. Effects of location x depth on water drop penetration time. Isolation study, 1998.**

| | Location relative to patch | | |
|---|---|---|---|
| Depth (cm) | Inside | Edge | Outside |
| | log(s) | | |
| 1 | 6.9† | 4.7 | 1.5 |
| 2 | 5.7 | 3.7 | 1.5 |
| 3 | 2.8 | 3.2 | 1.0 |
| 4 | 1.1 | 0.8 | 0.0 |
| 5 | 1.0 | 0.0 | 0.0 |

† Within columns LSD = 1.4, within rows LSD = 1.2, both at the 0.05 probability level.

## CONCLUSIONS

Results from the preventative study indicated that LDS occurrence and control were highly variable, as treatment effects were inconsistent between years. However, WIC, SWA, and flutolanil all showed some potential to alleviate LDS symptoms. Water injection cultivation seemed to decrease visual wilting symptoms, whereas flutolanil increased visual quality during general summer stress conditions. Soil moisture analyses established that the effect of WIC on wilt reduction was not simply by wetting the soil. Flutolanil and SWA appear to provide some curative control of LDS, however combining both at the highest labeled rate of each may result in phytotoxicity.

The causal agent of typical LDS symptoms appeared to affect the soil immediately beneath the thatch, although hydrophobic soil was measured at greater depths when dry down was uniform. A significantly greater accumulation of hyphae was measured at the edges of the dry spots than the inside. This is evidence that the causal agent of LDS may be of fungal origin.

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