



BIOTECHNOLOGY IN ACTION

By James S. Busse

Biotechnology, that snappy catchphrase spoken in biochemical and molecular biology circles, conjures several meanings among the plant science crowd. Nitrogen fixation by non-legumes, genetic transformation, and whole plant regeneration are all possibilities of biotechnology. One of the most interesting and applied aspects of biotechnology is the use of antigen — antibody reactions in turf disease detection. This process is successfully being utilized by the Agri-Diagnostics Corporation for Pythium Blight, Dollar Spot, and Brown Patch detection.

Each of these fungi have highly specific chemical entities associated with

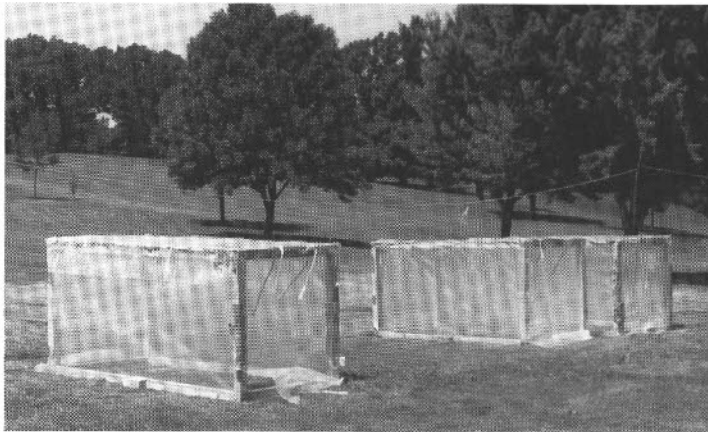
them known as antigens. Antigens are injected into laboratory animals which produce antibodies. The antibodies with their specific antigens, from a fungus causing a turf disease, produce a chemical reaction. The extent of this reaction can be assayed by the degree of color change of certain dyes. This process is known as Enzyme Linked Immunosorbent Assay (ELISA).

The Agri-Diagnostic test kits take roughly three hours to complete. The procedure is simple, and each kit has very thorough directions. A reflectance photometer is used to determine color change, and hence, the level of disease activity. This meter reading is

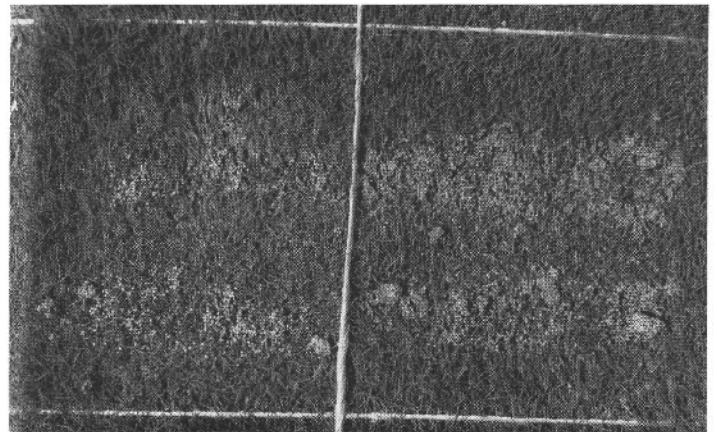
compared against a gradient scale of danger categories. One kit, capable of six tests, costs \$105.00. The meter is an additional \$200.00.

Testing was conducted this summer at the Blackhawk Country Club to determine the reliability of these kits for conclusively identifying a disease outbreak in situations where a reliable testing laboratory was not immediately available. This would bolster confidence of visual confirmation, and help a superintendent make an intelligent, accurate choice of fungicide(s) to be used for control.

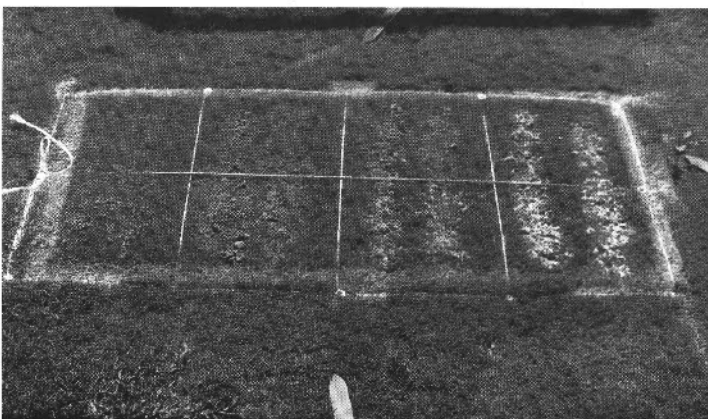
Unfortunately for our study, very little natural infection occurred this hot,



The three controlled environment structures were 4'x2'x2' and secured with tent stakes.



Pythium damage under environment structure.



Diseases promoted under environment tents left to right, check: no disease, Pythium, Brown Patch, and Dollar Spot.



Kits are currently available for testing Brown Patch, Dollar Spot, and Pythium Blight diseases.

dry summer. With the help of Dr. Worf and Monroe Miller, I was able to inoculate the three diseases (Pythium Blight, Dollar Spot, and Brown Patch) August 11, onto both open, exposed turf and turf covered by small, home-built greenhouse structures. The turf used was a practice tee consisting of roughly 85% bentgrass and 12% *Poa annua*; the rest was composed of Kentucky bluegrass and crabgrass. The turf was double cut at 1/2" with a triplex mower prior to inoculation. Misting of the plots was done at dusk.

Within four days, Pythium and Dollar Spot visible damage was appearing, especially on the covered plots. The Brown Patch fungus had actively growing hyphae, but was nonvirulent and caused no disease symptoms. This hyphae growth, however, provided the antigens for the antibodies in the test kit to combine with.

Testing was carried out with leaf samples collected, free of soil, from all areas. The level of disease corresponded well between the gradient of

meter readings, provided by Agri-Diagnostics, and that of visual observations. Badly diseased samples gave higher readings than turf which was not as diseased. This lends evidence to support the idea that these kits can be used to verify a suspected disease, and also to predict how serious the infection is. The limited testing of natural infection in Kentucky Bluegrass, bentgrass and *Poa annua* supported the same conclusion.

New, experimental, rapid test kits for the three diseases were also tested. These prototype kits from Agri-Diagnostics are not available to the public yet. They are still being researched and perfected.

The rapid test kits take approximately ten minutes to complete. They have the distinct advantage of telling the user if the test was performed correctly. This is done with false-positive and false-negative indicators.

The results of these tests were mixed. The Pythium kit performed well, while the Dollar Spot kit did not. The

test conditions allowed for few conclusions to be drawn about the Brown Patch kit. The rapid kits will have a position in the industry and will hopefully be refined and on the market soon.

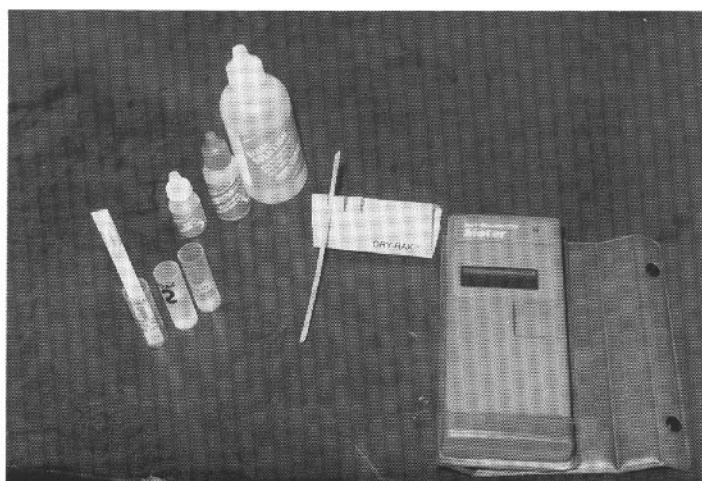
Even if the rapid testing kits are not available, the effectiveness of the long test kits should not be overlooked. Although three hours is a long time to wait for a test to be completed and \$500.00 is a lot of money for the kits and meter, this cost is small compared to the cost of one misapplication of fungicide to a wrongly diagnosed disease. The testing kits available currently are very capable of positively identifying diseases of high maintenance turf.

Editor's Note: Jim Busse is a senior at the UW-Madison. He is majoring in botany and horticulture and will graduate in May 1989. He is intending to go to graduate school studying aspects of biotechnology.

Jim worked this summer on the golf course staff at Blackhawk Country Club.



Inoculum used to create symptoms, a check, Pythium, Rhizoctonia, Sclerotinia (Dollar Spot).



Testing involves moving a "dipstick" between solutions and reading the color change with a reflectance photometer.

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