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The Rutgers Turfgrass Proceedings is published yearly by the Rutgers Center for Turfgrass Science, Rutgers Cooperative Extension, and the New Jersey Agricultural Experiment Station, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey in cooperation with the New Jersey Turfgrass Association. The purpose of this document is to provide a forum for the dissemination of information and the exchange of ideas and knowledge. The proceedings provide turfgrass managers, research scientists, extension specialists, and industry personnel with opportunities to communicate with co-workers. Through this forum, these professionals also reach a more general audience, which includes the public.

This publication includes lecture notes of papers presented at the 2010 GREEN EXPO Turf and Landscape Conference. Publication of these lectures provides a readily available source of information covering a wide range of topics and includes technical and popular presentations of importance to the turfgrass industry.

This proceedings also includes research papers that contain original research findings and reviews of selected subjects in turfgrass science. These papers are presented primarily to facilitate the timely dissemination of original turfgrass research for use by the turfgrass industry.

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Dr. Ann Brooks Gould, Editor
Dr. Bruce B. Clarke, Coordinator

# IMMUNOBLOT SCREENING FOR PRESENCE OF *NEOTYPHODIUM* SPP. IN PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.)

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Perennial ryegrass (*Lolium perenne* L.) is a cool-season, bunch type grass that performs well in a wide variety of soil conditions. It is often planted in mixtures with slower germinating grasses such as Kentucky bluegrass (*Poa pratensis* L.) and the fine fescues (*Festuca* spp.) to help prevent soil erosion during lawn establishment. Perennial ryegrass is also an important pasture and forage grass included in many pasture seed mixes and is also used in the southern United States for overseeding winter dormant lawns and athletic facilities.

Turfgrass breeders and researchers are continuing to research the beneficial role of endophytes in turfgrasses. Endophytes are naturally occurring fungi that live within the leaf, sheath, and stem tissues of certain grasses. Many perennial ryegrasses infected with the *Neotyphodium lolii* endophyte have enhanced insect resistance and stress tolerance. This endophyte also produces alkaloids which can cause toxicosis in livestock, thus perennial ryegrass es used for pasture need to be screened for endophyte. Perennial ryegrass infection with *N. lolii* can be determined either microscopically or immunologically.

We have screened seeds from perennial ryegrass trials established in 2010 for the presence of endophyte (*Neotyphodium* spp.) using a solid phase stacked immunoblot assay in which monoclonal antibodies generated to *Neotyphodium* spp. cell wall proteins will react to *Neotyphodium* spp. proteins present in perennial ryegrass seeds. The limit of detection of *Neotyphodium* spp. in seed is 50 ng/seed and in tillers it is 50 ng/1.6 mm tiller cross section. Immunoblot screening is a more rapid and accurate technique for *Neotyphodium* identification compared to microscopy (Koh, 2006).

## PROCEDURES

### Immuno Tissue Printing

Seeds were screened from 88 cultivars/selections established at the Plant Biology and Pathology Research and Extension Farm in Adelphia, NJ for endophyte using an immunoblot kit from Agrinostics, Ltd. Co. (Watkinsville, GA, USA). The seeds (100 per cultivar/selection) were surface sterilized in 5% (w/v) NaOH for 1 h and then rinsed with copious amounts of water and allowed to dry. A sponge was fitted into a container and wetted with extraction buffer solution. A piece of blotting paper was placed on the sponge followed by a nitrocellulose membrane. The surface sterilized seeds were placed on the nitrocellulose membrane and incubated at 45°C overnight.

The seeds were removed from the nitrocellulose membrane and blocking solution was added to the nitrocellulose membrane for 30 minutes while shaking. The blocking solution was decanted and the primary antibody consisting of the monoclonal antibody to *Neotyphodium* spp. cell wall protein was added to the membrane. The membrane was incubated for 1 h while shaking. The blot was rinsed in blocking solution and then incubated with goat anti-mouse antibody for 1 h while shaking. The secondary antibody has a color reactive enzyme conjugated to it. Excess antibody was removed by washing in blocking solution.

A chromogen solution is added and color develops wherever membrane-bound *Neotyphodium* spp. protein is present. The presence of chromogen is usually in the shape of the seed.

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## RESULTS AND DISCUSSION

Results are shown in Table 1. The range of endophyte infection in the cultivars/selections ranged from a high of 100% to a low of 1%. There were three cultivars/selections (PR-909, PST-2K9, and PRX-46GMI) with infection rates of 100% (100 seeds tested/cultivar). There were also three cultivars/selections (Linn, Sienna, and Pinnacle) that had infection rates less than 10% (100 seeds tested/cultivar). A majority of the of the cultivars/selections (58%) tested had *Neotyphodium* infection rates ≥80% (100 seeds/cultivar), while only 10% had an infection rate of ≤25%.

Endophyte infected perennial ryegrass cultivars are useful in certain stress situations but also need to be avoided for pasture. The results indicate that immunoblot screening for *Neotyphodium* spp. in perennial ryegrass can be used as a tool for determining which cultivar to use for either recreational/residential seed mix or for pasture seed mix.

## ACKNOWLEDGMENTS

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## REFERENCES

Koh, S., M. Vicari, J. P. Ball, T. Rakocevic, S. Zaheer, D. S. Hik, and D. R. Bazely. 2006. Rapid detection of fungal endophytes in grasses for large-scale studies. Func. Ecol. 20:736-742.

Table 1. Percent *Neotyphodium* infection (100 seeds per cultivar) in perennial ryegrass cultivars and selections in a turf trial established in 2010 at Adelphia, NJ.

| | Cultivar or Selection | *Neotyphodium* + (%) | *Neotyphodium* (-) (%) |
|---|---|---|---|
| NT-1 | Rinova | 95 | 5 |
| NT-2 | CL-11601 | 77 | 23 |
| NT-3 | PR-909 | 100 | 0 |
| NT-4 | CL-11701 | 75 | 25 |
| NT-5 | APR-2036 | 91 | 9 |
| NT-6 | Linn | 4 | 146 |
| NT-7 | Uno | 79 | 21 |
| NT-8 | DLF-LGD-3206 | 57 | 43 |
| NT-9 | DLF-LGD-3022 | 94 | 6 |
| NT-10 | PSRX-S84 | 96 | 7 |
| NT-11 | SRX-4RHD | 87 | 13 |
| NT-12 | PO2 | 70 | 30 |
| NT-13 | 585 | 89 | 11 |
| NT-14 | LTP-RAE | 87 | 13 |
| NT-15 | Allonte | 25 | 81 |
| NT-16 | Insight | 13 | 87 |
| NT-17 | Sienna | 3 | 98 |
| NT-18 | Brightstar SLT | 73 | 27 |
| NT-19 | CL-307 | 68 | 33 |
| NT-20 | APR-2320 | 92 | 7 |
| NT-21 | APR-2038 | 81 | 20 |
| NT-22 | PRG-PR121 | 99 | 1 |
| NT-23 | PDG-PR128 | 84 | 16 |
| NT-24 | PPG-PR133 | 93 | 7 |
| NT-25 | PPG-PR134 | 88 | 13 |
| NT-26 | LTP-PR135 | 83 | 17 |
| NT-27 | PRG-PR136 | 91 | 9 |
| NT-28 | PPG-PR137 | 86 | 14 |
| NT-29 | PPG-PR138 | 21 | 80 |
| NT-30 | PPG-PR140 | 86 | 14 |
| NT-31 | PPG-PR142 | 99 | 1 |
| NT-32 | PPG-PR143 | 87 | 13 |
| NT-33 | PPG-PR164 | 95 | 5 |
| NT-34 | PPG-PR165 | 81 | 19 |
| NT-35 | BAR Lp 10969 | 99 | 1 |

(Continued)

Table 1 (continued).

| Cultivar or Selection | | *Neotyphodium* + (%) | *Neotyphodium* (-) (%) |
|---|---|---|---|
| NT-36 | BAR Lp 10972 | 82 | 18 |
| NT-37 | BAR Lp 10970 | 78 | 22 |
| NT-38 | 2NJK | 97 | 3 |
| NT-39 | BAR Lp 7608 | 97 | 3 |
| NT-40 | Pinnacle | 1 | 99 |
| NT-41 | APR-2445 | 93 | 7 |
| NT-42 | Fiesta 4 | 84 | 16 |
| NT-43 | GO-G37 | 62 | 38 |
| NT-44 | CS-20 | 82 | 18 |
| NT-45 | ISG-36 | 20 | 80 |
| NT-46 | ISG-31 | 74 | 26 |
| NT-47 | A-35 | 89 | 13 |
| NT-48 | CS-PR66 | 68 | 34 |
| NT-49 | CST | 80 | 20 |
| NT-50 | JR-178 | 96 | 4 |
| NT-51 | JR-192 | 96 | 4 |
| NT-52 | PSRX-3701 | 94 | 6 |
| NT-53 | Pick 10401 | 94 | 6 |
| NT-54 | MachI | 92 | 8 |
| NT-55 | RAD-PR62 | 62 | 38 |
| NT-56 | RAD-PR55R | 65 | 33 |
| NT-57 | IS-PR409 | 53 | 47 |
| NT-58 | IS-PR463 | 86 | 14 |
| NT-59 | IS-PR469 | 66 | 34 |
| NT-60 | IS-PR479 | 62 | 38 |
| NT-61 | IS-PR487 | 97 | 3 |
| NT-62 | IS-PR488 | 66 | 34 |
| NT-63 | IS-PR489 | 83 | 17 |
| NT-64 | IS-PR491 | 91 | 8 |
| NT-65 | IS-PR492 | 74 | 26 |
| NT-66 | BLF-LGT 4182 | 25 | 75 |
| NT-67 | ISG-30 | 55 | 45 |
| NT-68 | PST-204D | 92 | 8 |
| NT-69 | PST-2NKM | 92 | 8 |
| NT-70 | PST-2DR9 | 74 | 26 |
| NT-71 | PST-2MG7 | 97 | 3 |
| NT-72 | PST-2TQL | 59 | 51 |
| NT-73 | PST-2AG4 | 22 | 78 |
| NT-74 | PST-2MAGS | 94 | 6 |
| NT-75 | PST-2K9 | 100 | 0 |

(Continued)

Table 1 (continued).

| Cultivar or Selection | | *Neotyphodium* + (%) | *Neotyphodium* (-) (%) |
|---|---|---|---|
| NT-76 | PST-2BNS | 89 | 11 |
| NT-77 | PST-2ACR | 72 | 28 |
| NT-78 | Rio Vista | 67 | 33 |
| NT-79 | CL-301 | 93 | 7 |
| NT-80 | Bonneville | 98 | 2 |
| NT-81 | PSRX-4CAGL | 53 | 47 |
| NT-82 | GO-PHS | 81 | 19 |
| NT-83 | GO-PR60 | 71 | 29 |
| NT-84 | GM3 | 99 | 1 |
| NT-85 | PRX-46GMI | 100 | 0 |
| NT-86 | SRX-45MH | 97 | 3 |
| NT-87 | Pick4DFHM | 66 | 34 |
| NT-88 | Palmer V | 67 | 33 |