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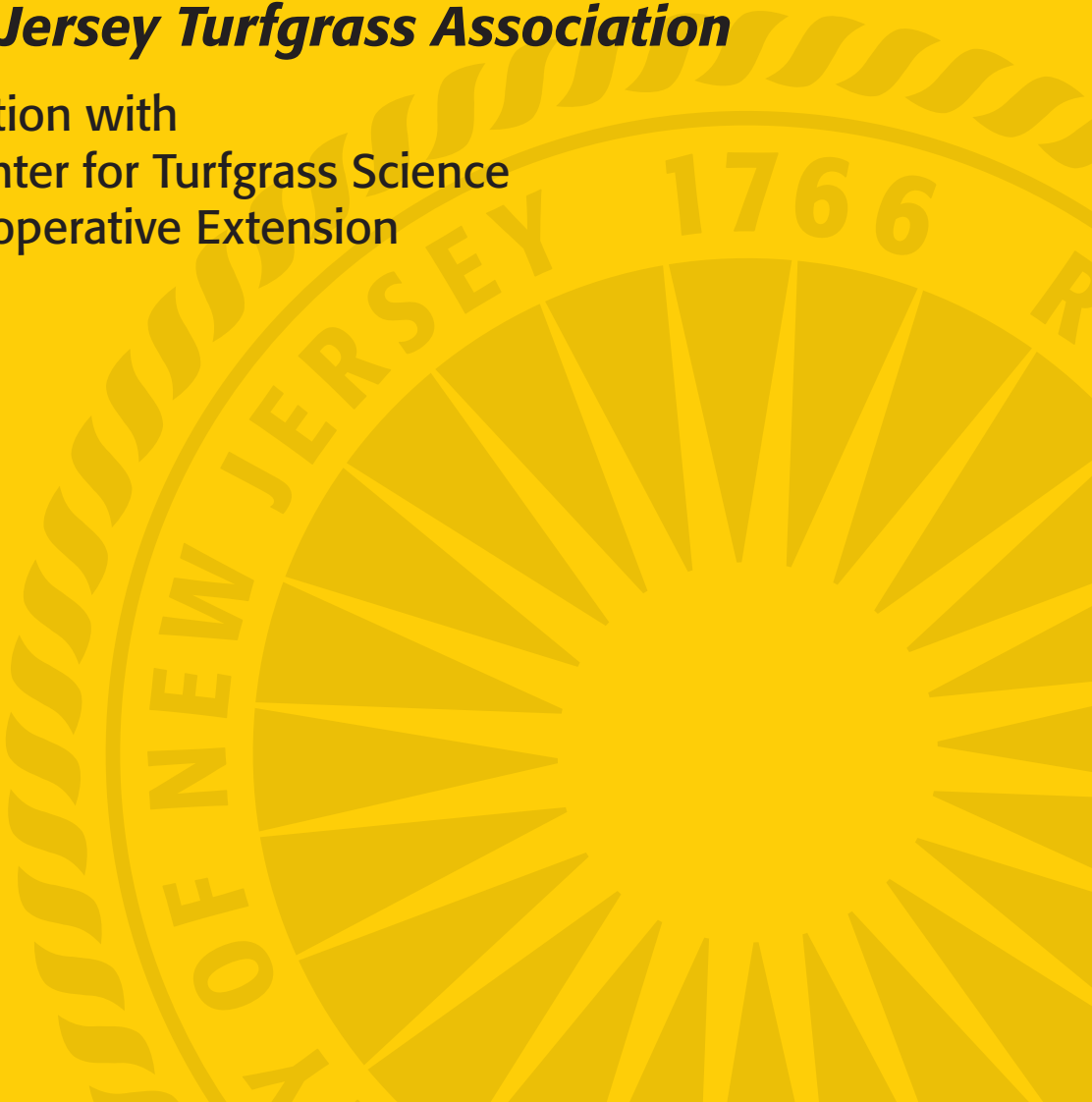

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The Rutgers Turfgrass Proceedings is published yearly by the Rutgers Center for Turfgrass Science, Rutgers Cooperative Extension, and the New Jersey Agricultural Experiment Station, School of Environmental and Biological Sciences, Rutgers, The State 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 2012 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.

Special thanks are given to those who have submitted papers for this proceedings, to the New Jersey Turfgrass Association for financial assistance, and to Barbara Fitzgerald, Anne Diglio, and Ann Jenkins for administrative and secretarial support.

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

IMMUNOBLOT SCREENING FOR PRESENCE OF *NEOTYPHODIUM* SPP. IN TALL FESCUE (*FESTUCA ARUNDINACEA*)

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Tall fescue (*Festuca arundinacea* Schreb.) is a cool-season, bunch type grass that performs well in a wide variety of soil conditions. Tall fescue is a non-native grass that is the most heat tolerant of the cool season grasses. It is well adapted for use in the "transition zone" of the southeast and the mid-Atlantic regions of the United States. It is used for forage and for lawns as it is low maintenance and will tolerate moderate traffic and infrequent mowing.

Most tall fescue contains an endophyte that is a naturally occurring fungus (*Neotyphodium coenophialum*) that lives within the leaf, sheath, and stem tissues of certain grasses. Tall fescues infected with the *Neotyphodium* endophyte have enhanced insect resistance and stress tolerance, but this fungus also produces alkaloids that can cause toxicosis in livestock. As a result, tall fescues intended for pasture use must be screened for endophyte. This can done microscopically, but immunoblot screening is a more rapid and accurate technique (Koh et al., 2006).

Seed from the National Turfgrass Evaluation Program (NTEP) Tall Fescue Trials established in 2012 was screened for the presence of endophyte using a solid phase stacked immunoblot assay in which monoclonal antibodies generated to cell wall proteins of the endophyte will react to *Neotyphodium* proteins present in tall fescue seeds. The limit of detection of *Neotyphodium* in seed is 50 ng *Neotyphodium* protein/seed and in tiller it is 50 ng *Neotyphodium* protein/1.6 mm tiller cross section.

PROCEDURES

Seed from 116 entries established at the Plant Biology and Pathology Research and Extension Farm in Adelphia, NJ was screened 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, 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* 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 was added; color develops wherever membrane-bound *Neotyphodium* protein is present. The presence of chromogen is usually in the shape of the seed.

RESULTS AND DISCUSSION

Results are shown in Table 1. Endophyte infection in the entries ranged from a high of 100% to a low of 0%. Endophyte was not detected (0%) in two cultivars, and infection in another eight cultivars was less than 20%. A majority of the cultivars (70%) tested had *Neotyphodium* infection levels that were $\geq$ 90%; infection in the remaining 25 cultivars was between 20 and 90%. Selections with $\geq$ 90% infection levels

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meet the legal requirements for breeding and for use at airports attempting to reduce geese and migratory bird populations in take off and landing zones.

Endophyte-infected tall fescue cultivars are useful in certain stress situations but must be avoided for pasture. The results indicate that immunoblot screening for *Neotyphodium* in tall fescue can be used as a tool for determining which cultivars to use in recreational/residential or pasture seed mixes.

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 tall fescue entries in a turf trial established in 2012 at Adelphia, NJ.

Entry Number		*Neotyphodium* (%)
1	Terranova	0
2	KY-31	86
3	Regenerate	88
4	Fesnova	86
5	ZW-44	97
6	W45	96
7	U43	100
8	LSD	100
9	Aquaduct	86
10	Catalyst	94
11	Marauder	52
12	Warhawk	97
13	Annihalator	100
14	Comp. Res. SST	100
15	204 Res. BLK4	98
16	JS819	27
17	JS818	100
18	JS809	100
19	JS916	98
20	JS825	41
21	MET1	100
22	F711	91
23	IS-TF 291	95
24	IS-TF 276 M2	74
25	IS-TF 305SEL	92
26	IS-TF 269SEL	84
27	IS-TF 282 M2	89
28	IS-TF 284 M2	93
29	OR21	19
30	TY10	40
31	Gxp-TF-09	12
32	TPC	96
33	WEI	100
34	W43	99
35	Grade 3	96

(Continued)

Table 1. *Neotyphodium* infection in tall fescue, 2012 (continued).

Entry Number		*Neotyphodium* (%)
36	POI	93
37	U45	88
38	B23	100
39	ATF 1612	95
40	ATF 1704	95
41	BURL TF-2	98
42	BURL TF-136	100
43	LTP-FSD	98
44	LTP-TW U6	100
45	LTP-FSDPDR	98
46	IS-TF- 289	90
47	MET6 SEL	95
48	IS-TF 330	56
49	TF-287	78
50	IS-TF 307 SEL	62
51	IS-TF 308 SEL	91
52	IS-TF 311	91
53	IS- TF 285	97
54	IS TF 310 SEL	93
55	IS TF 272	54
56	ATF 1736	91
57	ATF 1754	97
58	HEM1	100
59	Firebird 2	95
60	Bullseye	97
61	PST-5EV2	99
62	5GRB	97
63	5 SALT	94
64	PST-5SDT	74
65	PST-5DZP	0
66	5R05	95
67	PST-5BPO	96
68	PST-5BRK	100
69	DB1	100
70	RZ2	98
71	TD1	99
72	DZ1	94
73	T31	92
74	PSGGSD	3
75	PSG 8BP2	4

(Continued)

Table 1. *Neotyphodium* infection in tall fescue, 2012 (continued).

Entry	Number	*Neotyphodium* (%)
76	PSGTT4	92
77	FAITH	68
78	K12-13	82
79	K12-05	98
80	PPG-TF156	96
81	PPG-TF 157	97
82	PPG-TF 169	92
83	PPG-TF 170	100
84	PPG-TF 137	92
85	PPG-TF 135	98
86	PPG-TF 115	88
87	PPG-TF 105	98
88	PPG-TF 172	94
89	PPG-TF 151	96
90	PPG-TF 152	85
91	PPG-TF 148	92
92	PPG-TF 150	97
93	Bizem	93
94	CCR2	98
95	MET-3	92
96	W41	94
97	PPG TF-145	6
98	PPG TF-138	56
99	PPG-TF 139	84
100	PPF-TF 142	20
101	RAD TF 89	89
102	RAD TF 92	96
103	GO-DF12	70
104	K12-MCD	93
105	PST-5EX2	94
106	SMVD	94
107	RAD TF 83	97
108	RAD TF 88	90
109	BAR Fa 12078	81
110	BAR Fa 121089	96
111	BAR Fa 121091	100
112	BAR Fa 121095	98
113	PST-R5NW	5
114	BURL TF 69	97
115	Falcon IV	0

(Continued)

Table 1. *Neotyphodium* infection in tall fescue, 2012 (continued).

Entry Number		*Neotyphodium* (%)
116	Falcon V	96