Evaluation of Resistance to Stem Rust in Perennial Ryegrass Grown in Controlled and Field Conditions

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ABSTRACT

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Six cultivars of perennial ryegrass were evaluated for reaction to *Puccinia graminis* subsp. *graminicola* in controlled conditions when plants were 8 or 14 wk old and in the field as mature plants. Plants in controlled conditions were rated for rust infection type (0–4 scale) after inoculation with urediniospores, and an average stem rust infection index (ASRII) was used to compare cultivars. Plants in the field were assessed for percent incidence of infection and severity (percent modified Cobb scale). The area under the disease progress curve (AUDPC) was used to compare cultivars. Eight-week-old plants of Birdie II and Linn were significantly more resistant to stem rust than were Ovation, Delray, Palmer, and Yorktown II. ASRII were smaller for 14-wk-old plants than for 8-wk-old plants, but cultivars retained the same ranking for infection type. Field assessments of AUDPC showed Birdie II to be the most resistant. Linn was found to be intermediate, followed by Ovation, Yorktown II, Palmer, and Delray. Birdie II and Linn were slow-rusting cultivars. Cultivars ranked similarly in the field and in controlled-inoculation studies. Generally, plants that were resistant at 14 wk also were resistant as adults in the field. However, some plants rated susceptible in the greenhouse varied widely in stem rust reaction and were rated from 0 or trace to 100% susceptible in the field. Based on our results, cultivars should be tested by a double-screen procedure as 14-wk-old plants in controlled conditions and as adult plants in the field. This system would reduce disease escapes in young plants and retain slow-rusting characteristics expressed in the field by adult plants.

Additional keywords: grass seed production, *Lolium perenne*, slow rusting

Stem rust, caused by *Puccinia graminis* Pers.:Pers. subsp. *graminicola* Z. Urban, is among the most serious diseases of perennial ryegrass (*Lolium perenne* L.) grown for seed in western Oregon. It is distributed throughout the Willamette Valley and occurs worldwide on perennial ryegrass used for forage (27) and turfgrass (30,31). Perennial ryegrass is cross-pollinated, and cultivars are developed by recurrent phenotypic selection with single-plant progeny tests for specific traits or by backcrossing selected traits into an existing cultivar. In 1990, certified seed of 161 private, public, or experimental perennial ryegrass cultivars (R. L. Cook, *personal communication*) was produced in Oregon on 37,034 ha, with an estimated farm value of $61.4 million (W. C. Young III, *personal communication*).

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Fungicides are commonly used by seed producers to control stem rust, and repeated applications are needed from May to July (15). Their use for rust control has been studied extensively since the 1940s (10,11). Reduction in seed yield of perennial ryegrass attributable to stem rust was estimated to be 93% (17). A tank mix of chlorothalonil and propiconazole applied biweekly between 19 April and 16 June 1989 to control stem rust in perennial ryegrass cultivars Delray and Linn resulted in seed yields 45 and 42% larger, respectively, than those of nontreated controls (34).

Other than using fungicides, few other cultural practices are available to reduce the incidence of rusts. Rust resistance is an important alternative. Stem rust resistance has been reported in *Poa pratensis* L. (2,6,14,22,26,33) and *Phleum pratense* L. (1,20). The use of cultivars resistant to rust diseases in other open-pollinated grass hosts has been successful for cereal rye (4), corn, and sorghum (12).

Resistance to stem rust has been evaluated in field plots of turf-type perennial ryegrass (27), and cultivars resistant to stem rust have been released (18,19,23). Most evaluation and selection work for breeding disease-resistant, cool-season turfgrass cultivars in the United States is done in field and turf plots where diseases occur naturally (17).

More rapid progress can be made in selecting for rust resistance if dependable inoculation methods in controlled environmental conditions are available. These methods have been developed for infection by *P. graminis* in cereal grains (28), for infection by *P. coronata* Corda in perennial ryegrass (16), and for studying the genetics of pathogenicity in *P. coronata* (7,8). Developing controlled-environment inoculation methods for stem rust in perennial ryegrass would aid in plant breeding efforts, allow studies on the genetics of stem rust resistance, and expand research on host-pathogen-environment interactions. However, young plants evaluated in controlled conditions for resistance or susceptibility to stem rust also should be evaluated as adult plants under field conditions. Young plant reactions are not always related to adult plant reactions to stem rust. Temperature is known to affect the expression of genes for resistance (5), and adult plant resistance cannot be identified in the seedling stage (21). Seedlings of corn and sorghum rated susceptible to common rust varied widely in rust reactions as adults, some scoring from 0 to 100% susceptible (12).

The purpose of this study was to 1) evaluate the infection response of 8- and 14-wk-old plants of perennial ryegrass inoculated with *P. g. graminicola* in controlled conditions, 2) evaluate stem rust incidence and severity in the same plants in field plots, and 3) compare plant responses to *P. g. graminicola* in controlled conditions with plant responses in field plots.

MATERIALS AND METHODS

A forage-type perennial ryegrass cultivar (Linn [32]) and five turf-type cultivars (Delray, Birdie II [18], Ovation, Palmer [13], and Yorktown II [9]) were used in all experiments. These cultivars represented diverse germ plasm from several sources. Birdie II was previously selected for stem rust resistance in field test plots by backcrossing two sources of stem rust resistance into Birdie. Stem rust resistance in the six cultivars has not been evaluated in controlled conditions.

Plant growth procedures. Foundation seed of each cultivar was stored at 4 C in sealed containers until used. On each of five consecutive days, a replicate set of seed of each cultivar was placed on

blotter paper moistened with 0.17% KNO_3 and incubated in a germination chamber with alternating cycles of 25 C with 16 hr of light (50 $\mu E \cdot m^{-2} \cdot s^{-1}$) and 15 C with 8 hr of dark. After 2 wk, up to 20 individual young plants of each cultivar were transplanted to single cone-shaped plastic containers (3.8 × 21 cm) containing fine-grade vermiculite; the five consecutive plantings provided five replicate sets of cultivars. The experiment was a randomized complete block with five replications with up to 20 plants of each cultivar. These plants were used for each of the three experiments (8- and 14-wk-old plants and field test). Cultivar location in a replication and within each of the three experiments was rerandomized among experiments, but the sequence of order of plant location within a planting was not varied so disease scores for a plant could be traced throughout the study. Because of low germination, the study contained 93 plants of Linn and 98 plants of Palmer.

Cones containing 2-wk-old plants were placed in racks holding 98 cones, and racks were placed in an open-top mist chamber on a bench in a greenhouse at 20 ± 5 C for 7 days until plants were well-rooted. The racks were then moved to an incubation chamber at 20 C with 16 hr of light (489–501 $\mu E \cdot m^{-2} \cdot s^{-1}$) and 15 C with 8 hr of dark for 5 wk. Plants were watered daily and fertilized weekly with 0.81 g/L of 20-20-20 (N-P-K) liquid fertilizer (Peters) supplemented with Peters soluble trace element mix at the rate of 5.3 ml/kg of Peters 20:20:20 to maintain vigorous growth.

In the first experiment, each replication of 8-wk-old plants was inoculated with urediniospores once during five consecutive days and scored 14 days later for stem rust infection types. For the second experiment, plants were returned to a greenhouse at 20 ± 5 C for 14 days, cultivar location within a replication was rerandomized, foliage of each plant was cut 2–3 cm above the crown and discarded, and plants were incubated for 14 days in the growth chamber as described previously. A replication of plants, now 14 wk old, was inoculated with urediniospores once during five consecutive days and scored for rust infection types 14 days later.

After scoring, plants were returned to a greenhouse for 14 days, vernalized at 7 C with 8 hr of light (489–501 $\mu E \cdot s^{-1} \cdot m^{-1}$) for 28 days, "hardened-off" on the north side of a headhouse (fertilized two times per week with 20-20-20) for 19 days, and transplanted into field plots near Corvallis, OR.

Controlled-inoculation procedure. Urediniospores of stem rust were collected in June 1990 from plants of perennial ryegrass (cv. Delray) in a field near Corvallis, OR. The culture was maintained or increased on 5- to 7-wk-old plants of Delray growing in a greenhouse at 20 ± 5 C.

Fresh urediniospores were collected from 14- to 21-day-old sporulating pustules into a 00 gel capsule with a vacuum cyclone microcollector (3) and suspended in Soltrol 170, a highly refined, nonphytotoxic oil frequently used in rust inoculation of cereal grasses. Soltrol 170 does not influence the percentage of germination of urediniospores, host penetration, or number of infections (28). For each of the five daily inoculations (corresponding to the replicate planting sequence), the germinability of urediniospores was determined before inoculation by spreading a Soltrol 170 suspension on the surface of water agar (2%) and allowing Soltrol 170 to evaporate. Urediniospores were incubated in the dark for 16–18 hr at 18 C. The percentage of germination (based on two groups of 200–400 urediniospores each) of inoculum in replications one through five for 8-wk-old plants was 31, 35, 36, 77, and 91%, respectively, and for 14-wk-old plants was 35, 33, 83, 87, and 80%, respectively.

Urediniospore concentrations in the inoculum for replications one through five were 2.0, 8.0, 8.3, 6.0, and 6.8 × 10^6 per milliliter, respectively, for 8-wk-old plants and 7.5, 7.5, 7.0, 8.3, and 8.3 × 10^6 per milliliter, respectively, for 14-wk-old plants. The urediniospore suspension was applied uniformly from four directions to upper and lower leaf surfaces of individual plants with a spore-oil atomizer (3). Each rack of plants received 2.0–2.5 ml of the urediniospore suspension. Glass slides were attached to plant label stakes placed in the plant canopy. After inoculation, the glass slides were examined at ×100, and urediniospores deposited on the slide were counted in a 1 × 25 mm band (10 bands per slide) on each of four slides per plant rack in each of three racks. Urediniospore counts per 25 mm^2 averaged for replications one through five were 2.7, 3.7, 3.3, 3.4, and 3.2, respectively, for 8-wk-old plants and 2.8, 2.3, 2.8, 2.9, and 1.2, respectively, for 14-wk-old plants.

After the oil residue evaporated, plants were placed in a dew chamber without light at 18 C for 16–18 hr followed by light (intensity front to back of a dew chamber ranged from 110 to 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ at 25 C for 4–6 hr). Halfway through the light cycle, plant racks were rotated 180° to provide light to two sides of plants. After the 20- to 24-hr infection period, racks of plants were placed in plastic-lined wooden boxes 5 cm high and returned to the growth chamber. Plants were watered daily from below to avoid wetting the foliage. Plant growth was vigorous during the incubation period.

Beginning 5 days after inoculation, each plant was examined near midday for stem rust development. The infection type for each plant was rated 14 days after inoculation by a 0–4 system used to rate stem rust infection type in cereal grains, except that the "fleck" and mesothetic reaction classes were not used (24). An average stem rust infection index (ASRII) was calculated for each cultivar. ASRII = sum$(R + 1)N_R/N$, which was the sum of the rating (R) plus 1, multiplied by the number of plants at that rating (N_R), divided by the number of plants rated (N) in each replication.

Field experiment. Five replications of 20 plants of each cultivar (except where noted for Linn and Palmer) were transplanted on 3 April 1991 on 1-m centers in 20-m rows spaced 1 m apart. A replication consisted of a single row of each cultivar. Each plant received about 30 cm^3 of 18-18-18-2 (N-P-K-Fe) at transplanting, and irrigation was applied to establish the plants. The field was limed and fertilized according to soil test recommendations, and glyphosate isopropylamine salt was applied (1.12 kg a.i./ha) to control weeds 3 wk before transplanting. Thereafter, weeds were controlled by mechanical cultivation. The plants were not treated with other pesticides.

Stem rust inoculum was provided from natural sources or from latent infection in the plants from the controlled-environment studies. Pustules were not observed on plants at transplanting.

Beginning in mid-May, plants were examined once or twice a week for the

Table 1. Disease reactions of 8- and 14-wk-old plants of perennial ryegrass inoculated with *Puccinia graminis* subsp. *graminicola* in controlled conditions and disease reactions in the field

Cultivar	Controlled conditions[a]		Field[b]
	8 wk old	14 wk old	
Birdie II	3.99	1.72	116
Linn	4.15	3.06	348
Ovation	4.67	3.22	490
Delray	4.95	4.11	637
Palmer	4.98	4.60	1,004
Yorktown II	4.99	4.64	879
LSD = 0.05	0.03	0.12	234

[a]Rated by average stem rust infection index: sum $(R + 1)N_R/N$, where R = pustule infection type 0, 1, 2, 3, or 4 and N = the number of plants rated.
[b]Rated by area under the disease progress curve (Shaner and Finney [30]).

appearance of stem rust. On 7 June (calendar year [CY] day 158), plants were free of stem rust. Stem rust was first observed on 12 June (CY day 163); thereafter, plants were examined for stem rust at 4- or 5-day intervals until 15 July (CY day 196). Disease was assessed as the incidence of plants infected with stem rust in a cultivar, and the percentage of stem rust severity was assessed by the modified Cobb scale (28). Incidence was determined by a cumulative total of plants infected with stem rust between CY day 158 and CY day 196, divided by the number of plants of the cultivar in the study (maximum 100 plants). Disease severity (modified Cobb Scale) between CY day 158 and CY 196 was used to calculate the area under the disease progress curve (AUDPC) (29) for each cultivar in each replication. In the calculations, trace infection was recorded as 1%.

Comparison of inoculation methods for stem rust development. Because plants survived stem rust inoculations, each plant of each cultivar could be used to compare stem rust reactions as resistant or susceptible when they were 8 or 14 wk old and as field plants. For these comparisons, plants in the controlled-inoculation experiments with infection type 0 or 1 were considered resistant (R), and plants with infection type 2, 3, or 4 were considered susceptible (S). In the field experiment on the final day of disease assessment, plants with 10% or less stem rust severity were considered R, and plants with more than 10% stem rust severity were considered S. Combined stem rust reactions (S or R) in an individual plant would be one of the following eight combinations: RRR, RRS, RSR, RSS, SRR, SRS, SSR, or SSS (i.e., stem rust reaction [S or R] as 8 or 14 wk old and field plants).

Data analysis. In the controlled-inoculation studies, the significance of ASRII was tested by analysis of variance (ANOVA). In the field experiment, the AUDPC was tested by ANOVA. Fisher's protected LSD was used to compare cultivar means.

RESULTS

Controlled inoculations. In both experiments, uredinial blisters developed about 6 days after inoculation, and pustules erupted 1–2 days later. Pustules were evenly distributed on leaves, with no secondary spread of inoculum to new leaf growth. Stem rust infection types for plants within a cultivar are presented as ASRII (Table 1). There was a significant difference ($P = 0.001$) for ASRII among cultivars. Eight-week-old plants of Birdie II and Linn were significantly ($LSD_{P=0.05}$) more resistant to stem rust than Delray, Palmer, and Yorktown II; Ovation was significantly less resistant than Birdie II and Linn but more resistant than Delray, Palmer, and Yorktown II. ASRII values were smaller for 14-wk-old than for 8-wk-old plants; cultivars retained the same ASRII position rankings with more separation among scores.

The percentage of plants that scored 0 and 1 for each cultivar was always smaller for 8-wk-old than for 14-wk-old plants. At either age, Birdie II, Linn, and Ovation had more resistant plants than Delray, Palmer, and Yorktown II. By the evaluation criteria used in this study, Birdie II was more resistant to stem rust than the other five cultivars.

Field assessments. Differences ($P = 0.05$) in AUDPC for stem rust were observed among cultivars (Table 1). Field assessments of AUDPC indicated that Birdie II had the most stem rust resistance. Linn was intermediate, followed by Ovation, Yorktown II, Palmer, and Delray. Cultivars reacted similarly in field and controlled-inoculation studies.

Twenty-nine percent of Birdie II plants and 1% each of Linn and Ovation plants remained free of stem rust by CY day 196. All plants in the remaining cultivars

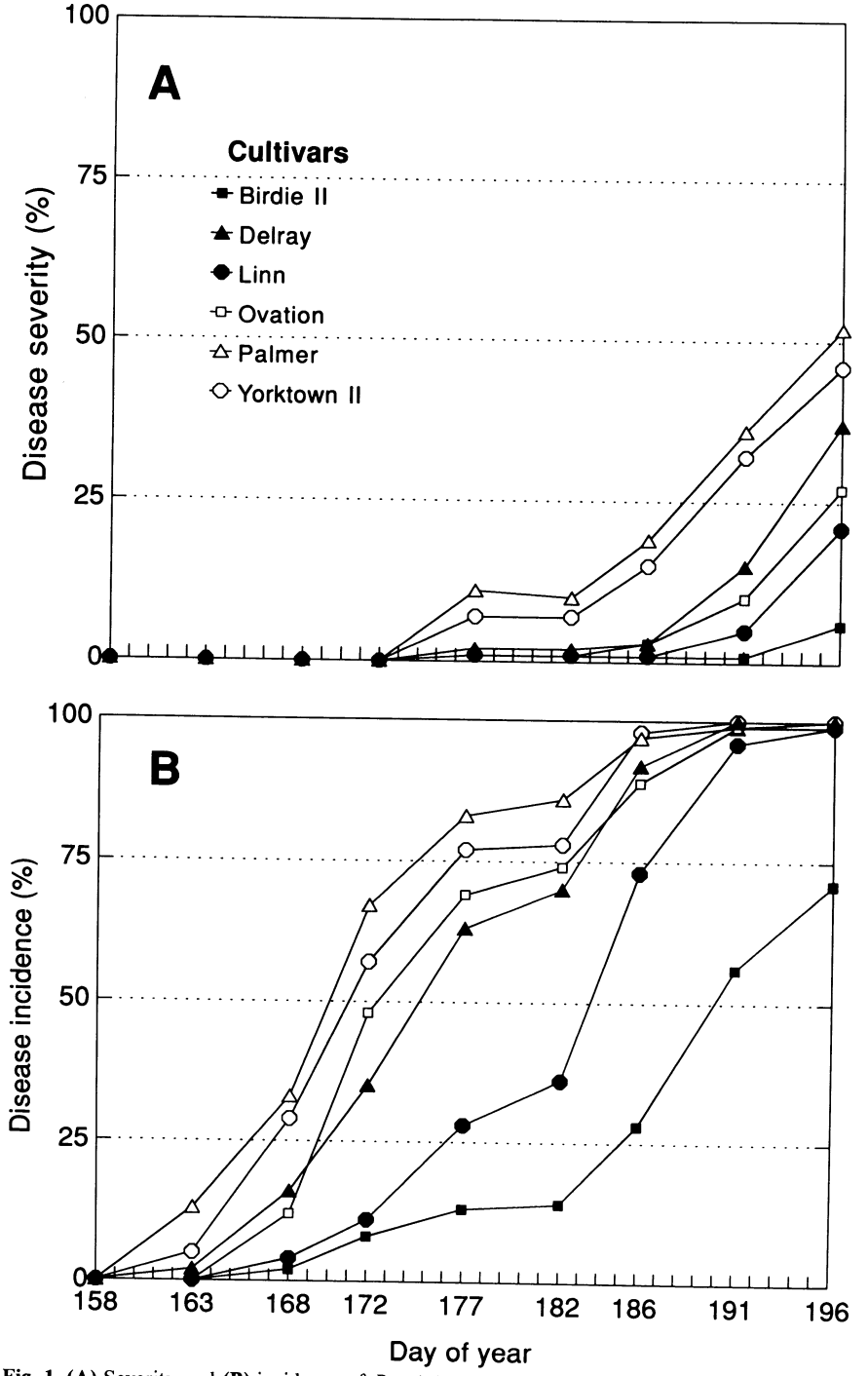

Fig. 1. (A) Severity and **(B)** incidence of *Puccinia graminis* subsp. *graminicola* in six perennial ryegrass cultivars between 7 June (calendar year day 158) and 15 July (calendar year day 196) 1991.

were infected by stem rust. When stem rust development between CY day 158 and 196 was plotted (Fig. 1), the incidence of stem rust infection and average percentage of severity of individual plants indicated that stem rust developed more slowly in Birdie II and Linn than in the other four cultivars.

Comparison of inoculation methods for stem rust development. Individual plant reactions to stem rust at 8 or 14 wk and in the field were combined into two disease reactions for each inoculation time: R-- or S-- at 8 wk, -R- or -S- for 14 wk, and --R or --S for the field, where - is where the disease reaction was disregarded (Table 2). Disease was severe when plants were inoculated at 8 wk of age—no plants of Palmer and Yorktown II and only 19 plants of Birdie II were resistant to stem rust (R--); 81–100% of plants in the six cultivars were rated susceptible (S--). Of the plants inoculated at 14 wk of age, the number that showed reactions resistant to stem rust (-R-) ranged from four in Palmer to 83 in Birdie II; reactions susceptible to stem rust (-S-) ranged from 91 in Palmer to 17 in Birdie II. In the field, the number of plants resistant to stem rust (--R) ranged from 12 in Palmer to 81 in Birdie II; the number of plants susceptible to stem rust (--S) ranged from 83 in Palmer to 19 in Birdie II. When disease reactions were combined for 14-wk-old and field plants (-RR), the number of plants resistant to stem rust ranged from two in Palmer to 71 in Birdie II.

DISCUSSION

Birdie II was the cultivar the most resistant to stem rust, and Palmer and Yorktown II were the least resistant cultivars. In the three experiments, stem rust resistance in Linn was ranked greater than Ovation, and Ovation was ranked greater than Delray.

Controlled inoculations of seedlings have been used successfully to evaluate disease resistance in open-pollinated species and are acceptable as long as stem rust reactions are similar between young plants and adults. When the reactions are dissimilar, judgment is needed in young plant screening. Our data show that when 8-wk-old plants were inoculated, resistance was present in some plants but was lacking or not expressed in others. At 8 wk, 19 plants of Birdie II, 17 plants of Linn, and no plants of Palmer or Yorktown II showed resistant reactions. When plants of these cultivars were inoculated at 14 wk old, 83 plants of Birdie II, 45 plants of Linn, four plants of Palmer, and six plants of Yorktown II were classified as resistant. If stem rust screening had been done only on 8-wk-old plants, some plants resistant at 14 wk old would have been eliminated from the population. If screening for stem rust resistance had been done only in the field, some of the plants rated resistant may have escaped disease. This likely happened with Palmer, Yorktown II, and Delray where 12, 16, and 32 plants, respectively, were rated resistant in the field but had zero to one plant resistant at 8 wk old and four to 16 plants resistant at 14 wk old.

An effective selection procedure to identify plants resistant to stem rust would be to use a controlled inoculation, followed by a field evaluation. Our data show it is preferable to use controlled inoculations with 14-wk-old plants rather than 8-wk-old plants. When 8-wk-old plants were inoculated, fewer than 20% of them were resistant to stem rust. This indicates selection pressure for resistance was too severe in plants of this age, perhaps because genes for resistance are not expressed in 8-wk-old plants or because of an influence of temperature or inoculum density on the disease response (25). In these experiments, generally, a more comparable number of 14-wk-old plants and plants in the field were resistant to stem rust. When 14-wk-old plants were used in combination with field evaluations (-RR reaction), two plants in Palmer, three in Yorktown II, and 71 in Birdie II were rated resistant to stem rust. This double-screen procedure eliminated escapes and provided an opportunity to incorporate a controlled-inoculation procedure into a screening and selection process for plant breeding. It also provided a controlled-inoculation procedure so the host-pathogen-environment interaction could be evaluated with results closely applicable to field situations. Our results support those of Rose-Fricker et al (27), who reported young plant reactions to stem rust were not always the same as adult plant reactions.

Data show a definite delay in stem rust incidence (Fig. 1A) and severity (Fig. 1B) in two of the six cultivars. Birdie II and Linn, in addition to having more stem rust resistance, were also slower to rust. Slow rusting describes a reduced rate of epidemic development (21) and is a valuable trait in cereal crops (35). Its importance in perennial ryegrass seed production is unknown because of the extensive use of fungicides. Postinfection components influencing slow rusting include the length of latent period, frequency of infection, size of uredinia, duration of sporulation, and quantity of urediniospores produced. The only component studied here was size of uredinia; one or more of the other components also may influence the rate of stem rust development in the field. Care should be taken when selecting for resistance in controlled conditions not to eliminate plants with slow rusting characteristics.

Table 2. Stem rust reaction (susceptible [S] or resistant [R]) of 8- and 14-wk-old plants of perennial ryegrass inoculated with *Puccinia graminis* subsp. *graminicola* in controlled conditions and stem rust reaction in the field

Stem rust reaction	Cultivar (no. of plants)					
	Birdie II	Linn	Ovation	Delray	Yorktown II	Palmer
R--[a]	19	17	6	1	0	0
S--	81	75	94	99	100	95
-R-[b]	83	37	37	16	6	4
-S-	17	55	63	84	94	91
--R[c]	81	45	32	32	16	12
--S	19	47	68	68	84	83
-RR[d]	71	22	15	8	3	2

[a]Stem rust reaction rated as 8-wk-old plants; disease reaction as 14-wk-old plants and reaction in the field disregarded (-). Total plants rated for Birdie II = 100; Linn = 92; Ovation = 100; Delray = 100; Yorktown II = 100; and Palmer = 95.

[b]Stem rust reaction rated as 14-wk-old plants; disease reaction as 8-wk-old plants and reaction in the field disregarded (-).

[c]Stem rust reaction rated in the field; disease reaction as 8- and 14-wk-old plants disregarded (-).

[d]Stem rust reaction rated as 14-wk-old plants and as adult plants in the field; disease reaction as 8-wk-old plants disregarded (-).

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In the article "Site Relationships of *Armillaria* Species in New York" by J. T. Blodgett and J. J. Worrall, footnote y for Table 2 (page 172) erroneously includes *Fagus grandifolia, Pinus resinosa*, and *P. sylvestris* and should read: [y]All other species that show a significance level greater than 0.05 alone, including: *Acer pensylvanicum, A. rubrum, A. saccharinum, Alnus rugosa, Betula lenta, B. papyrifera, Carpinus caroliniana, Carya cordiformis, C. ovata, Castanea dentata, Fraxinus americana, F. nigra, F. pennsylvanica, Juglans cinerea, J. nigra, Lirioidendron tulipifera, Nyssa sylvatica, Ostrya virginiana, Picea abies, Pinus strobus, Populus deltoides, P. grandidentata, P. tremuloides, Prunus serotina, Robinia pseudoacacia, Sassafras albidum, Tilia americana, Tsuga canadensis, Ulmus americana, Amelanchier* spp., *Cornus* spp., *Crataegus* spp., *Prunus* spp., *Rhododendron* spp., and *Ulmus* spp.

Footnote y for Table 3 (page 172) erroneously includes *Fraxinus americana* and should read: [y]All other species that show a significance level greater than 0.05 alone, including: *Acer pensylvanicum, A. rubrum, A. saccharinum, Alnus rugosa, Betula lenta, B. papyrifera, Carpinus caroliniana, Carya cordiformis, C. ovata, Castanea dentata, Fagus grandifolia, Fraxinus nigra, F. pennsylvanica, Juglans cinerea, J. nigra, Liriodendron tulipifera, Nyssa sylvatica, Ostrya virginiana, Picea abies, Pinus resinosa, P. strobus, P. sylvestris, Populus deltoides, P. grandidentata, P. tremuloides, Prunus serotina, Robinia pseudoacacia, Sassafras albidum, Tilia americana, Tsuga canadensis, Ulmus americana, Amelanchier* spp., *Cornus* spp., *Crataegus* spp., *Prunus* spp., *Rhododendron* spp., and *Ulmus* spp.