Stolon Development in Four Turf-Type Perennial Ryegrass Cultivars

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ABSTRACT
Perennial ryegrass (*Lolium perenne* L.) is known as a non-creeping, bunch-type species. However, several studies documented the occurrence of a ‘stoloniferous’ or ‘rhizomatous’ habit in this species. This research aimed to study the stolon development in creeping (‘Sienna’ and ‘RPR’) and non-creeping (‘Apple SGL’ and ‘Pizzaz 2’) type cultivars of perennial ryegrass seeded at three rates (10, 20, and 30 g m⁻²). Plots were seeded in September 2015 and from December 2015 to July 2017, two core samples (8 cm diameter and 5 cm depth) were collected from each plot every other month. Stolon length density, weight density, and average diameter were estimated, and the specific weight was calculated. Our results demonstrated the presence of stolons in all the tested cultivars. The cultivar ‘Sienna’ showed the highest stolon production followed by ‘Pizzaz 2’ and ‘Apple SGL’, whereas ‘RPR’ displayed the lowest. The cultivars started to produce stolons in the spring of the first year after establishment, reached the highest level in late summer and maintained production until termination of the study. The lowest seeding rate favored stolon production and their growth in diameter as it produced the highest length density, specific weight, and average diameter.

Core Ideas
- We demonstrate the presence of stolons in all studied cultivars.
- ‘Sienna’ showed more stolons than ‘RPR’ in terms of length and weight density.
- ‘Sienna’ reached higher stolon weight, but not length, density than ‘Pizzaz 2’.
- Stolons were formed in April after the establishment, and rapidly increased in June.
- The lower seeding rate favor production of stolons and their growth in diameter.

PERENNIAL RYEGRASS (*Lolium perenne* L.) is known as a non-creasing, bunch-type species (Beard, 1973).

However, as reported by Wipff and Singh (2015), several studies documented the occurrence of a ‘stoloniferous’ or ‘rhizomatous’ habit in perennial ryegrass with stolons or rhizomes that cannot be considered “true stolons”. These vegetative organs are actually pseudo-stolons or pseudo-rhizomes. As discussed by Wipff and Singh (2015), these structures are actually aerial culms that get trampled or pushed down, and then begin to root at nodes, or crowns that become buried by soil, dung, mulch, or earthworm casts with leaves dying and decomposing leaving only the culm and roots at nodes. Plants of perennial ryegrass with “rhizomatous habit of growth” were found by Mitchell (1956) and Kydd (1966) in pastures. Kydd (1966), studied the effect of stocking rate on perennial ryegrass tillers and observed the formation of elongated stems under high stocking rate. Simons et al. (1974) demonstrated that increasing cutting height and straw mulch encouraged aerial tiller production differently according to genotype. Harris et al. (1979) found crowns below the soil surface connected to what they termed “underground stolons” (i.e., pseudo-rhizomatous) and discussed the definition of the term “stolon” and “rhizome” and “aerial tillers”. Furthermore, they investigated the distribution of 18 morphologically identified ryegrass types grown as spaced plants, and observed that those genotypes had vegetative tillers with elongated internodes on the periphery of the tiller clump spreading to form patches. Similar structures were found by Mitchell (1956) in livestock grazed perennial ryegrass plants. New turf-type cultivars of perennial ryegrass with prostrate growth habits and lateralspreading shoots (creeping-type) have recently been developed (Charbonneau and Brownbridge, 2013). Wipff and Singh (2015), these structures are actually aerial culms that get trampled or pushed down, and then begin to root at nodes, or crowns that become buried by soil, dung, mulch, or earthworm casts with leaves dying and decomposing leaving only the culm and roots at nodes. Plants of perennial ryegrass with “rhizomatous habit of growth” were found by Mitchell (1956) and Kydd (1966) in pastures. Kydd (1966), studied the effect of stocking rate on perennial ryegrass tillers and observed the formation of elongated stems under high stocking rate. Simons et al. (1974) demonstrated that increasing cutting height and straw mulch encouraged aerial tiller production differently according to genotype. Harris et al. (1979) found crowns below the soil surface connected to what they termed “underground stolons” (i.e., pseudo-rhizomatous) and discussed the definition of the term “stolon” and “rhizome” and “aerial tillers”. Furthermore, they investigated the distribution of 18 morphologically identified ryegrass types grown as spaced plants, and observed that those genotypes had vegetative tillers with elongated internodes on the periphery of the tiller clump spreading to form patches. Similar structures were found by Mitchell (1956) in livestock grazed perennial ryegrass plants. New turf-type cultivars of perennial ryegrass with prostrate growth habits and lateralspreading shoots (creeping-type) have recently been developed (Charbonneau and Brownbridge, 2013). Wipff and Singh (2015) filed a patent for “Lolium perenne subsp. stoloniferum with an aggressive determinate-stoloniferous growth habit,” and described this new subspecies as having true stolons.

Perennial ryegrass is widely used as a constituent of winter sports turfgrass in moderate temperature regions and in transition areas (Puhalla et al., 1999), because it performs well during cooler months (Bertrand et al., 2013) when athletic fields are principally used, and because of its excellent wear resistance, quick establishment, and tolerance to close mowing (Goatley et al., 2008; Puhalla et al., 1999). Harris et al. (1979) reported...
that stoloniferous cultivars of perennial ryegrass could have higher competitive ability than the traditional cultivars toward weed control and drought and wear tolerance. Wipff and Singh (2015) suggested that perennial ryegrass varieties with determinate-stolons could have superior turf quality, wear tolerance, and ability to quickly recover from traffic compared to other popular turf varieties. Masin and Macolino (2016) evaluated the competitive ability of creeping-type cultivars of perennial ryegrass to limit annual bluegrass (Poa annua L.) infestation in turfgrass. They found that although annual bluegrass emergence dynamics was the same in all the cultivars tested (‘Sienna’, ‘Apple SGL’, ‘Azimuth’, and ‘Presidio’), the creeping-types (‘Sienna’ and ‘Apple SGL’) reduced seedling emergence.

As mentioned, several studies reported the presence of stolons in perennial ryegrass cultivars, especially in the forage-types, analyzing their morphological structure (e.g., Harris et al., 1979) or their amount in terms of number of stolons per unit surface area (e.g., Matthew et al., 1989). However, to our knowledge, no information is available on stolon production and morphology at establishment and in subsequent years of perennial ryegrass turfs. The objective of this research was to overcome this lack of information by studying stolon development in time of four commercial perennial ryegrass cultivars under three seeding rates. The four cultivars used for this study have been recently introduced to the Italian turf market and included two creeping-types and two traditional types. The non-creeping type cultivars were used as control.

**MATERIALS AND METHODS**

This study was performed in Legnaro (northeastern Italy, 45°20′N, 11°57′E; elevation 8 m) at the Agricultural Experimental Farm of Padova University, Padova, Italy, from September 2015 to July 2017. Legnaro has a humid subtropical climate, with a mean annual temperature of 12.3°C and annual rainfall of 826 mm mostly distributed from April to November. Monthly mean air temperatures and precipitation of the study period are reported in Table 1. The soil at the site was an Oxyaquic Eutrudept, coarse-silty, mixed, mesic (64% silt, 19.7% sand, and 16.3% clay), with a pH of 7.5, 2.4% organic matter, C/N ratio of 9.7, 6.1 mg kg⁻¹ Olsen (P), and 196 mg kg⁻¹ K (buffered BaCl₂ method).

Four cultivars of perennial ryegrass recently introduced to the Italian turf market were seeded on 24 Sept. 2015 at rates of 10, 20, and 30 g m⁻² pure live seed, referred to hereafter as low, medium, and high, respectively. One blend and three cultivars were used: ‘RPR’ (creeping type, composed of ‘Bareuro’ 20%, ‘Barmarga’ 15%, ‘Barclay II’ 30%, ‘Barorlando’ 15%, ‘Barlibro’ 10%, further mentioned as cultivar), ‘Sienna’ (creeping-type, in Europe ‘New Orleans’), ‘Apple SGL’ (commercialized as traditional type), and ‘Pizzaz 2’ (commercialized as traditional type). Traditional cultivars (i.e., non-creeping type cultivars) were included as controls. The experimental design was a split-plot with three replications having cultivars as the whole plot (6 × 2 m) and seeding rate as subplots (2 × 2 m), for a total of 36 subplots. Irrigation was applied daily during establishment to prevent drought stress and weekly in the summer (June–August) as 100% ET (ET Gauge meter, Spectrum Technologies, Inc.) using a overhead sprinkler system. Before seeding, the soil was fertilized with a starter fertilizer (8 N–10.4 P–20 K) at a rate of 50 kg ha⁻¹ of N. A fertilizer (15 N–3.9 P–12.5 K), containing 5% of slow-release N (isobutylidenediurea) was then applied in March, May, September, and December at a rate of 50 kg ha⁻¹ of N, for a total annual N application of 200 kg ha⁻¹. During growing seasons plots were mowed once a week with a walk-behind rotary mower set at a height of 30 mm and clippings were removed. A postemergence herbicide (Bayer Dicotex, 2,4- D, Dicamba, MCPA, and MCP-P) was applied in the spring of each year to control dicotyledons, while annual and perennial grasses were periodically removed manually.

From December 2015 to July 2017, two core samples (8 cm diameter × 5 cm depth) were collected from each plot every other month. Stolons were cleaned and analyzed following the method proposed by Pornaro et al. (2018). The stolons measured consisted of all aboveground elongated horizontal stems whether they were stolons or pseudo-stolons, including crowns and nodes, according to the definition proposed by Wipff and Singh (2015).

The method provides image analysis through the use of WinRHIZO technology (Regent Instrument Inc., Quebec, QC, Canada) for measuring stolon length and average diameter. Stolon dry weight was then measured after drying the stolons in an oven at 105°C for 36 h. Stolon length density (cm dm⁻²) and weight density (g dm⁻²) were then calculated based on the turfgrass area sampled (50.2 cm²) derived from the stolon length

**Table 1. Monthly mean air temperatures and monthly precipitations during 2015, 2016, and 2017, and long-term averages (1963–2007) at the Agricultural Experimental Farm of Padova University in Legnaro, northeastern Italy.**

<table>
<thead>
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<tbody>
<tr>
<td>Jan.</td>
<td>4.1</td>
<td>3.1</td>
<td>0.9</td>
<td>3.5</td>
<td>16</td>
<td>31</td>
<td>14</td>
<td>52</td>
</tr>
<tr>
<td>Feb.</td>
<td>5.7</td>
<td>7.6</td>
<td>6.3</td>
<td>4.8</td>
<td>62</td>
<td>145</td>
<td>7</td>
<td>48</td>
</tr>
<tr>
<td>Mar.</td>
<td>9.5</td>
<td>9.7</td>
<td>11.1</td>
<td>8.7</td>
<td>70</td>
<td>43</td>
<td>12</td>
<td>57</td>
</tr>
<tr>
<td>Apr.</td>
<td>13.4</td>
<td>14.0</td>
<td>13.7</td>
<td>12.6</td>
<td>47</td>
<td>21</td>
<td>56</td>
<td>70</td>
</tr>
<tr>
<td>May</td>
<td>18.6</td>
<td>17.0</td>
<td>18.2</td>
<td>18.1</td>
<td>82</td>
<td>192</td>
<td>40</td>
<td>73</td>
</tr>
<tr>
<td>June</td>
<td>22.4</td>
<td>19.4</td>
<td>23.7</td>
<td>21.3</td>
<td>62</td>
<td>134</td>
<td>45</td>
<td>86</td>
</tr>
<tr>
<td>July</td>
<td>26.8</td>
<td>24.8</td>
<td>24.6</td>
<td>23.2</td>
<td>15</td>
<td>58</td>
<td>45</td>
<td>73</td>
</tr>
<tr>
<td>Aug.</td>
<td>24.3</td>
<td>23.0</td>
<td>25.3</td>
<td>23.3</td>
<td>45</td>
<td>51</td>
<td>8</td>
<td>75</td>
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<tr>
<td>Sept.</td>
<td>19.5</td>
<td>20.7</td>
<td>17.8</td>
<td>18.8</td>
<td>36</td>
<td>67</td>
<td>145</td>
<td>73</td>
</tr>
<tr>
<td>Oct.</td>
<td>14.0</td>
<td>13.3</td>
<td>13.7</td>
<td>14.2</td>
<td>87</td>
<td>119</td>
<td>7</td>
<td>78</td>
</tr>
<tr>
<td>Nov.</td>
<td>8.1</td>
<td>9.2</td>
<td>8.1</td>
<td>8.7</td>
<td>12</td>
<td>135</td>
<td>94</td>
<td>78</td>
</tr>
<tr>
<td>Dec.</td>
<td>3.3</td>
<td>3.0</td>
<td>2.8</td>
<td>4.4</td>
<td>0</td>
<td>0</td>
<td>44</td>
<td>63</td>
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measured with WinRHIZO software and stolon dry weight. Furthermore, stolon specific weight (mg dm⁻¹) was calculated as the ratio of weight density to length density.

Analysis of variance was performed using linear mixed effect models to test cultivar, seeding rate, and sampling date effect on the measured parameter (stolon length density, weight density, specific weight, and average diameter). Data of the first and second sampling dates (December and February) were not statistically analyzed as none of the samples had stolons. The parameters were transformed when necessary to achieve normality and homoscedasticity of the residuals. The least significant difference test with the Bonferroni correction was used at the 0.05 probability level to identify significant differences among means. The statistical analyses were performed using R version 3.4.0 (R Development Core Team, 2015) and additional packages nlme for fitting mixed models, and multcomp for post hoc comparisons.

<table>
<thead>
<tr>
<th>Date (Da)</th>
<th>Cultivar (cv)</th>
<th>Seeding rate (SR)</th>
<th>Da × cv</th>
<th>Da × SR</th>
<th>cv × SR</th>
<th>Da × cv × SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>***</td>
<td>***</td>
<td>*</td>
<td>ns†</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Not significant at the 0.05 probability level.

**RESULTS AND DISCUSSION**

The analysis of variance revealed significant main effects of date, cultivar, and seeding rate for stolon length density, whereas their interactions were not significant (Table 2). For stolon weight density the interactions between date and seeding rate and between date and cultivar were significant. The interaction between date and cultivar and the seeding rate effect were significant for stolon specific weight and average diameter.

Stolon length density quickly increased from April to June 2016 when it reached its maximum value and then decreased during the following August (Fig. 1a). From August 2016 until the end of the study period, no differences were observed among sampling dates. The cultivars with the highest values of stolon length density were ‘Sienna’ and ‘Pizzaz 2’ followed by ‘Apple SGL,’ and ‘RPR’ exhibited the lowest value (Fig. 1b). It is interesting to note that all cultivars produced stolons even if commercialized as non-creeping type. Furthermore, lower value of stolon length density was found for ‘RPR’, a creeping-type cultivar, than ‘Apple SGL’ and ‘Pizzaz 2’, traditional cultivars. When data were averaged among cultivars and sampling dates, low seeding rate resulted in higher value of stolon length density than high seeding rate (Table 3).

In line with stolon length density, stolon weight density showed a strong increase from April to June 2016 for all three seeding rates (Fig. 2). However, in June 2016, the low seeding rate reached higher values than the other two rates, followed by a marked decrease from June to October. In August 2016, differences were found only between the low and the high rate, whereas there were no differences among seeding rates on the remaining dates. Plots seeded at lower rates were probably characterized by a lower shoot density at the beginning of the first growing season. This lower density may have favored a rapid stolon production from April to June when ideal growing condition occurred (Table 1). These results indicate that intra-specific competition could play a significant role in stolon production. Beard (1973) gave evidence to the prevention of solon...
production due to intense intraspecific competition. Specific to the interaction between cultivar and sampling date for stolon weight density, ‘Sienna’ had an increase in stolon weight density from June to August 2016, whereas ‘Pizzaz 2’ and ‘Apple SGL’ exhibited a decrease (Fig. 3a). Moreover, different from all the other cultivars, ‘Sienna’ had a decrease in stolon weight at the last sampling date (July 2017). Among cultivars tested ‘Sienna’ maintained high stolon density throughout the study period. In particular, ‘Sienna’ showed the highest stolon length density in August 2016, October 2016, and April 2017, and significant differences between the two creeping types ‘Sienna’ and ‘RPR’ were observed from June 2016 to July 2017 (Fig. 3a). The stolon specific weight increased for all cultivars from April to August 2016 and maintained constant values until the end of the study period (Fig. 3b). The cultivar ‘Sienna’ displayed a rapid increase in stolon specific weight from June to August 2016. No differences among cultivars were observed within each date, with the exception of July 2017 when ‘RPR’ reached higher values than ‘Sienna.’ Furthermore, the stolon specific weight of the plots at low seeding rate was higher than those at medium and high seeding rate (Table 3).

Differences in average diameter among cultivars within each date were observed only in April 2016 (Fig. 3c). Other stoloniferous turf species could vary greatly in stolon diameter (Giolo et al., 2013; Pompeiano et al., 2012; Rimi, 2012) and diversity among cultivars can be the result of long-term breeding efforts to produce high quality cultivars characterized by fine texture while breeding programs for creeping perennial ryegrasses are relatively recent. Except for ‘Pizzaz 2’ the stolon diameter increased from April to June 2016. The cultivars ‘Sienna,’ ‘Pizzaz 2,’ and ‘Apple SGL’ displayed a decrease in values from June to August 2016, subsequently stolon diameter did not vary for any of the cultivars. Seeding rate affected stolon diameter, indeed stolons in plots at low seeding rate had a larger diameter than those in plots at medium and high seeding rate (Table 3).

Our results demonstrate the presence of stolons in all the tested cultivars including ‘Apple SGL’ and ‘Pizzaz 2,’ which were not promoted in the turf-market as creeping-type. The cultivar with the highest stolon production was ‘Sienna,’ whereas the cultivar with the lowest production was ‘RPR’ and not a non-creeping type (‘Pizzaz 2’ or ‘Apple SGL’) as we expected. It was interesting to note that ‘Sienna’ maintained higher stolon density than ‘Pizzaz 2’ for several sampling dates (Fig. 3a), but no significant differences in stolon length density were observed between these two cultivars (Fig. 1b). In this study, we demonstrated that stolon production started in the spring following establishment.

Table 3. Effect of seeding rate [10 (low), 20 (medium), and 30 g m⁻² (high)] on stolon length density, specific weight, and average diameter of four perennial ryegrass cultivars.

<table>
<thead>
<tr>
<th></th>
<th>Length density (cm dm⁻²)</th>
<th>Specific weight (mg dm⁻¹)</th>
<th>Average diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>250 a†</td>
<td>4.84 a</td>
<td>0.66 a</td>
</tr>
<tr>
<td>Medium</td>
<td>225 ab</td>
<td>4.00 b</td>
<td>0.60 b</td>
</tr>
<tr>
<td>High</td>
<td>211 b</td>
<td>3.61 b</td>
<td>0.57 b</td>
</tr>
</tbody>
</table>

† Values with the same letter are not significantly different (LSD test at the 0.05 probability level).
The first stolons were observed in April 2016, and the most productive period was from April to June. In this period, we found the major differences among cultivars and, starting from August 2016, stolon production, in terms of both length density (Fig. 1a) and weight density (Fig. 3a), seemed to stabilize and no further relevant variations were noted over time. This result could have been caused by the intraspecific competition effect that might have prevented further solon production (Beard, 1973). It seems that on this date, the turfgrass reached full maturity and stability. It was also interesting to note that in June 2016 the low seeding rate showed a peak of weight density not observed for the other two seeding rates (Fig. 2). The lower competition among plants under low seeding rate favored not only the production of stolons, but also their growth in diameter (Table 3).

To date, no information is available on stolon length density of turf-type perennial ryegrass, nor on stolon weight density. The main studies on stolon quantity and morphology were conducted on forage-type perennial ryegrass subjected to different grazing pressures (e.g., Harris et al., 1979; Matthew et al., 1989), so a comparison with our results is inconsistent. Masin and Macolino (2016) studied the capacity of creeping-type perennial ryegrass, including ‘Sienna,’ to reduce weed infestation in turfgrass. They suggested that differences in morphological features among cultivars affect the spring germination–emergence process and persistence of annual bluegrass (Poa annua L.). The high stolon production of ‘Sienna’ observed in this study supports what was reported by Masin and Macolino (2016) who noticed the high ability of this cultivar to compete with annual bluegrass. In a study on the competitive interaction among annual bluegrass, Kentucky bluegrass (Poa pratensis L.), and perennial ryegrass, Brede and Duich (1986) observed that, on the basis of root and shoot growth parameters, competitive ability of perennial ryegrass is generally affected by belowground rather than aboveground traits. However, Busey (2003) suggested that plant morphology is not the only trait playing an important role for turfgrasses to outcompete weeds. Brede (1992) found greater shoot density in ‘Mustang’ compared with ‘Kentucky-31’ tall fescue while no difference was found in their susceptibility to invasion by bermudagrass [Cynodon dactylon (L.) Pers.], Stolon weight density is a parameter commonly investigated in warm season turfgrasses. Rimi et al. (2013), in a study analyzing the influence of different levels of nitrogen fertilization on bermudagrass and seashore paspalum (Paspalum vaginatum Sw.), found stolon weight density to be in general higher than 10 g dm⁻². Schiavon et al. (2016), analyzing seasonal changes in carbohydrate and protein content of seeded bermudagrasses, found stolon weight density ranging from 4 and 8 g dm⁻². In this case study, we found that stolon weight density of some perennial ryegrasses, is much lower than that of some seeded bermudagrasses.

**CONCLUSION**

The results demonstrated that both creeping and non-creeping type perennial ryegrass cultivars involved in this study were characterized by a notable stolon production. Among studied cultivars, ‘Sienna,’ a creeping-type cultivar, was the one with more stolons, followed by the two non-creeping type cultivars ‘Pizzazz 2’ and ‘Apple SGL’ that had a greater stolon production than ‘RPR,’ the other creeping-type. Stolons were produced in the spring of the first year after establishment and reached their maximum level in approximately 3 mo (April–June). After a decline in late summer, stolon density was maintained throughout the entire follow-up period (12 mo). The results of this study also demonstrated that stolons were more abundant and thicker in plots seeded at 10 than 20 and 30 g m⁻², suggesting that seeding rate can be used to control stolon production.

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**REFERENCES**


