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Article

Seed Yield and Lodging Assessment in Red Fescue (*Festuca Rubra* L.) Sprayed with Trinexapac-Ethyl

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Abstract: Red fescue (*Festuca rubra*) is used in seed mixtures for lawns and pastures. It is prone to lodge at flowering, and plant growth regulators (PGRs) are used to prevent lodging, ensuring sufficient pollination. Seed yield and lodging were studied over three years in a red fescue field established with four seeding rates (2, 4, 6 and 8 kg ha⁻¹) and sprayed each year with three doses of the PGR trinexapac-ethyl (250 g L⁻¹) (0, 0.3, 0.6 and 1.2 L ha⁻¹). Half of each plot was sprayed with the PGR and the other half was left unsprayed as control. The degree of lodging was assessed by analysing drone images in the second year of the experiment and using a 10-point scale for scoring lodging at the ground. Generally, application of PGR increased the seed yield but the effect varied between years. There was no interaction between the PGR dosage and seeding rate. We found a positive correlation between the blue intensity of the images and lodging. PGR dosage significantly affected lodging evaluated by visual ranking and the blue intensity of the images, while the seeding rates did not affect lodging. Lodging affected seed yield negatively.

Keywords: aerial images; consecutive harvest; image analysis; plant growth regulators; PGR; lodging; seed production

1. Introduction

Red fescue (*Festuca rubra* L.) is the second-largest grass seed crop in Denmark, and most of the produced seeds are exported [1]. Consecutive harvests of red fescue fields are common, and 50% of the total 21,000 ha are harvested for two succeeding years and 4% for three succeeding years [2]. Red fescue is harvested consecutively, as the seed yield increases from the first to the second production year [1]. If the seed yield is similar or even lower in an aging red fescue crop, this is most probably caused by low production of inflorescence in the aging stand [3]. Seed certification standards have recently been altered in Denmark. Today, there is no upper limit on the number of years in which seeds can be harvested on the same crop as long as the certification criteria are met [2]. Research has so far solely focused on seed yield by manipulating, for example, plant density to estimate the optimum number of consecutive harvests [4]. In grass seed production, the most labour-intensive and costly field operation is the establishment and farming during the first seed production year. Therefore, efforts should be taken to continue seed harvest on the same crop in several consecutive years to spread the cost of establishment over more than one production year [5].

Rapid growth response to high nitrogen (N) fertility rates makes grasses more vulnerable to lodging. Seed yield N responses are commonly polynomial, with an optimum followed by a decline at higher N rates [6,7]. Lodging happens when fertile tillers have insufficient stem strength to support their weight, and it can impede pollination, seed development and, consequently, seed yield [8,9]. Trinexapac-ethyl is a plant growth regulator (PGR) which has to be applied when the first node is

visible (late April or May) [10,11]. Moddus M (250 g L⁻¹ trinexapac-ethyl, Syngenta Nordic A/S) has an off-label registration in some of the grasses in Denmark, including *F. rubra*. Generally, application of Moddus M in Denmark increases seed yield less than reported from other countries [12]. In grass species, this sterol biosynthesis inhibitor reduces the level of endogenous gibberellins, resulting in internode compression [13,14]. It has given excellent control of stem elongation, resulting in reduced lodging, and has significantly increased seed yields in cold-season grasses [15–20].

Unmanned aerial vehicle (UAV) imagery is a tool for the assessment of several crop growth characteristics [21,22]. Multispectral, hyperspectral and true colour (red, green and blue (RGB)) are all suitable for quantifying the different crop growth characteristics, and each type of camera has its advantages and disadvantages [23]. Compared with the multispectral or hyperspectral sensors, RGB sensors have limitations in spectral resolution, but they have lower costs and higher spatial resolution, and it is possible to calculate vegetation indices and estimate plant height from the same set of photographs [24,25]. Processing of these images using structure from motion (SfM)-based software [26] enables the generation of highly detailed orthophotos that deliver centimetre resolution [27,28].

Moreover, UAVs can work on-demand with high flexibility at critical moments, depending on the agronomic goals involved [29]. UAV technology has been adapted and utilised by diverse groups in agricultural investigations [30].

This study aimed to (1) assess yearly seed production in three succeeding harvests of red fescue sprayed with different doses of PGR, (2) use aerial images to determine the level of lodging and (3) evaluate the relationship between seed yield and lodging of plots, which had different dosages of trinexapac-ethyl and seeding rates.

2. Materials and Methods

2.1. Experimental Design

A red fescue (*F. rubra*) trial was established in 2015 at the research station of the University of Copenhagen in Taastrup (55°40' 8.16" N; 12°18' 18.82" E), Denmark in a field with clayey soil. Red fescue (cv. Maxima) was undersown in spring barley (cv. Odyssey) on 15 April 2015. The experiment consisted of four seeding rates (2, 4, 6 and 8 kg ha⁻¹) and four Moddus M (250 g L⁻¹ trinexapac-ethyl, Syngenta Nordic A/S) doses (0, 0.3, 0.6 and 1.2 L ha⁻¹) applied on 12 May 2016, 15 May 2017 and 9 May 2018, when the flag leaf was fully unrolled and the ligule just visible (BBCH = 39). Each plot was 12 × 1.5 m, of which half was sprayed with the PGR and the other half was left unsprayed as a control. Treatments were replicated four times for doses that were >0. There were controls (dose = 0) for all treatments, as half of the plots were kept unsprayed. The grass seed plots were combine harvested in three consecutive years (8 July 2016, 17 July 2017 and 2 July 2018), and the grass straw was removed. The grass seeds were air-dried, and after cleaning, seed samples were weighed and the weight was converted to seed yield ha⁻¹. The barley was harvested on 2 September 2015, and afterwards, the red fescue field was trimmed to 5 cm on 3 September. In 2016, the area was trimmed on 27 July, 30 August, 19 September and 12 October, and in 2017 on 3 October. Chemicals, including the plant nutrition, herbicides and fungicides applied during the experiment, are presented in Table 1.

Table 1. Chemicals applied during the experiment.

Applied Chemicals		Date of Application	Application Rate
Plant Nutrition			
NPK 21-3-9 + Mg S		24 April 2015	476 kg ha ⁻¹
Manganese nitrate		12 June 2015	1.5 l ha ⁻¹
NPK 21-3-10 + Mg S		21 September 2015	344 kg ha ⁻¹
NPK 21-3-9 + Mg S		21 March 2016	312.8 kg ha ⁻¹
NPK 21-3-10 + Mg S B		17 October 2016	371.4 kg ha ⁻¹
NPK 22-3-10 + S		9 March 2017	326 kg ha ⁻¹
NPK 23-3-7 + Mg S B Cu		12 October 2017	347 kg ha ⁻¹
NS 27-4		4 April 2018	273 kg ha ⁻¹
Herbicides	Active Ingredient		
Trimmer 50 SG	500 g kg ⁻¹ tribenuron-methyl160 g L ⁻¹ ioxynil + 240 g L ⁻¹ bromoxynil	20 May 2015	10 L ha ⁻¹
Briotril 400 EC			0.25 L ha ⁻¹
Agropol (additive)			0.15 L ha ⁻¹
Metaxon			1 L ha ⁻¹
Tomahawk 180 EC	750 g L ⁻¹ MCPA180 g L ⁻¹ fluroxypyr	12 June 2015	0.4 L ha ⁻¹
Agropol (additive)			0.15 L ha ⁻¹
Hussar OD	300 g L ⁻¹ mefenpyr-diethyl + 100 g	8 September 2015	0.02 L ha ⁻¹
Renol (additive)	L ⁻¹ iodosulfuron-methyl-Na		0.5 L ha ⁻¹
Focus Ultra	100 g L ⁻¹ cycloxydim	25 September 2015	1.5 L ha ⁻¹
Dash (additive)			0.5 L ha ⁻¹
Hussar OD	300 g L ⁻¹ mefenpyr-diethyl + 100 g L ⁻¹	04 May 2016	0.05 L ha ⁻¹
Legacy 500 SC	iodosulfuron-methyl-Na		0.05 L ha ⁻¹
Renol (additive)	500 g L ⁻¹ diflufenican		0.5 L ha ⁻¹
Fungicides			
Proline EC 250	250 g L ⁻¹ prothioconazole	26 June 2015	0.2 L ha ⁻¹
Comet	250 g L ⁻¹ pyraclostrobin		0.15 L ha ⁻¹
Flexity	300 g L ⁻¹ metrafenon		0.125 L ha ⁻¹

In 2017, lodging was assessed in sprayed subplots by visual ranking done by two persons on the ground using a scale from 1 to 10. One indicated no lodging and 10 indicated that the whole stand was lying down on the ground (Figure 1). Plots were ranked on 27 June and 17 July and photographed with a UAV on 27 June. Lodging was also assessed by UAV imagery. The UAV was a Phantom 4 (DJI, Shenzhen, China) quadrotor equipped with a built-in GNSS and an integrated RGB camera. The camera was a Sony 12.4 megapixel with a 1/2.3" CMOS sensor (4000 × 3000 pixels) and a 20 mm focal length (35 mm equivalent). The flight altitude was 40 m and the ground sampling distance (GSD) was 0.017 m pixel⁻¹. An orthomosaic was generated in Pix4DMapper (<https://www.pix4d.com/>) and plots were cropped from the orthomosaics with in-house software. A variety of indices were calculated for each plot to quantify the colour differences recognised in the areal orthomosaic (Figure 1). The blue intensity (0–255) correlated slightly better with the visual rankings of lodging than the average intensity of all colours expressing the brightness of the plots. Therefore, the blue intensity was used as an indicator of lodging.



Figure 1. An aerial image of the experimental plots and their corresponding scores of lodging on 27 June 2017. A score of 1 indicates no lodging and of 10 indicates that the whole stand was lying on the ground. Rows (1a) and (2a) show unsprayed subplots and rows (1b) and (2b) show sprayed subplots.

2.2. Weather Data

Weather data was provided from the research weather station in the area (55°67' N, 12°30' E) (Figure 2).

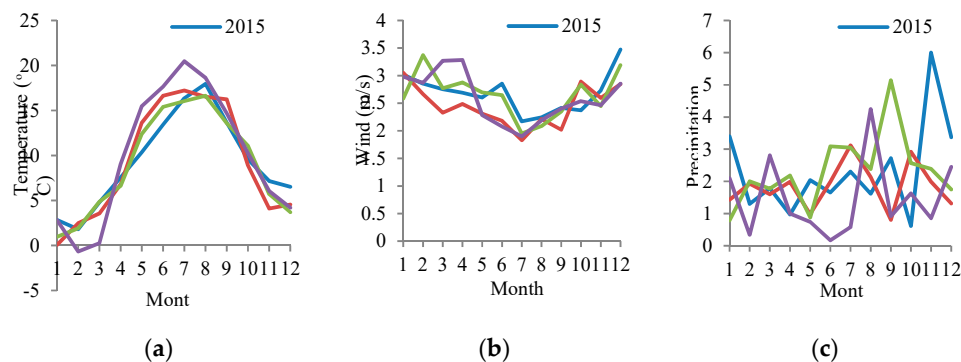


Figure 2. Weather data during the experiment: (a) temperature, (b) wind and (c) precipitation. Measurement heights: 2, 2 and 1.5 m above ground level for temperature, wind and precipitation, respectively.

2.3. Statistical Analyses

First, the seed yield difference between the sprayed and unsprayed plots was calculated to assess the average PGR effect. A *t*-test was used to test the difference between the sprayed and unsprayed subplots.

Afterwards, the seed yields in PGR sprayed plots were subjected to a two-way analysis of variance (ANOVA) with PGR dosage and seeding rate as factors, followed by Fisher's least significant difference (LSD) test. Further, a test for means separation was done.

The Pearson correlation coefficient test was used to test the correlations between (1) the visual rankings of lodging and the blue intensity of images and (2) the seed yield and the blue intensities for all plots.

Statistical analysis was done using SAS® version 9.4 software (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA).

3. Results

PGR increased the average seed yield ($p < 0.0001$) (Table 2). The increase in seed yield due to PGR was significantly higher in 2017 compared with 2016 and 2018 ($p = 0.0003$) (Table 2), but the PGR doses did not affect the seed yield increase differently in any of the years.

Table 2. Seed yield difference (kg ha^{-1}) and standard deviation between the sprayed and unsprayed subplots over 3 years of the experiment.

Year	Differences between Seed Yields (kg ha^{-1})
2016	67.6 (66.02)
2017	317.7 (51.8)
2018	45.0 (28.5)

In all plots, the seed yield in 2017 was significantly higher than in 2016 and 2018 ($p < 0.0001$) (Table 3). The dosages of the PGR did not affect the seed yield differently, nor did seeding rates affect the seed yields differently, and there were no interactions between PGR treatments and seeding rates in any of the years.

Table 3. Seed yield (kg ha^{-1}) and standard deviation in sprayed and unsprayed subplots over 3 years of the experiment.

Year	Seed Yield (kg ha^{-1})	
	Sprayed Subplots	Unsprayed Subplots
2016	1729 (42.8)	1680 (37.7)
2017	2176 (39.5)	1858 (24.5)
2018	1640 (25.1)	1573 (21.5)

PGR doses significantly affected lodging evaluated by visual ranking and UAV imagery ($p \leq 0.001$), while the seeding rates were without any impacts. The visual ranking was unaffected by ranking date. A regression analysis of the visual ranking as a response of PGR dosages showed that the correlation coefficient was -0.63 for 27 June and -0.67 for 17 July. There was no interaction between PGR doses and seeding rates ($p = 0.14$). Adding 0.6 and 1.2 L ha^{-1} of PGR resulted in the same low ranking of lodging (≈ 2), while 0.3 L ha^{-1} of PGR resulted in severe lodging (≈ 7). There was a high correlation between lodging and the blue intensity of images ($p < 0.001$; coefficient correlation: 0.92 for the ranking done on 27 June and 0.91 for the ranking done on 17 July) (Figure 3). The blue intensity revealed that lodging was more pronounced in the unsprayed plots than in plots sprayed with 0.3 L ha^{-1} of PGR. There was a negative correlation between seed yield and lodging indicated by the blue intensity ($p \leq 0.01$; correlation coefficient: -0.39) (Figure 4).

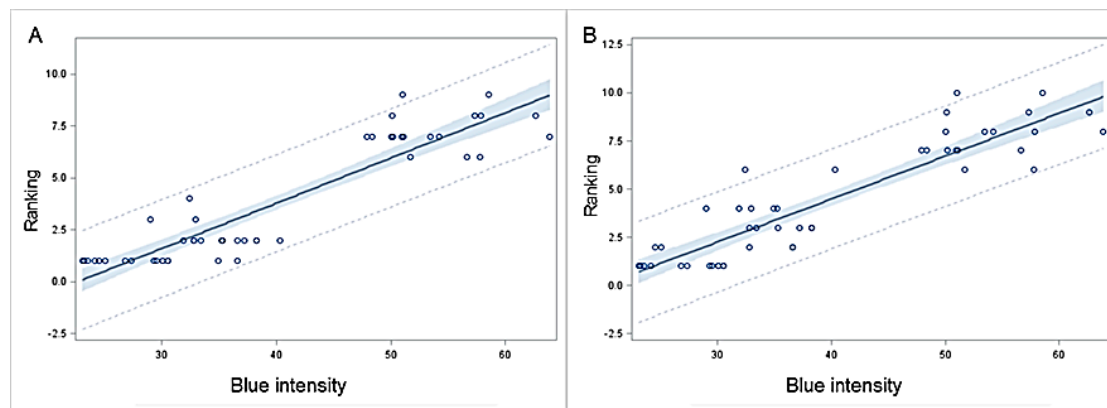


Figure 3. The relationships between lodging ranked from the ground (y -axis) and the blue plot intensity (0–255) (x -axis) for plant growth regulator (PGR)-treated plots. The shaded area around the

regression line corresponds to the 95% confidence limits for the line, and the dotted lines correspond to the 95% prediction limits of single observations. (A) Ranked on 27 June and (B) ranked on 17 July.

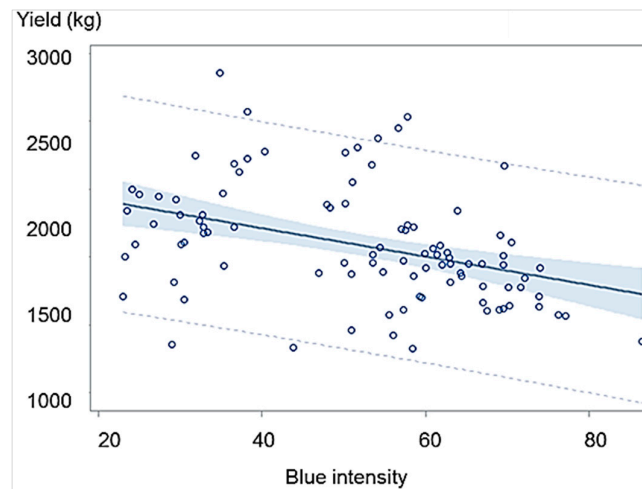


Figure 4. Relationship between blue intensity indicating lodging and the seed yield (kg ha^{-1}) for all plots in the experiment. See further explanations in the legend of Figure 3.

4. Discussion

Red fescue is a persistent grass with a root system that can grow on soils with low levels of plant nutrients. It tolerates extreme abiotic stresses such as winter cold and summer drought [31]. Two consecutive harvests of red fescue in Denmark are common. It is feasible to have a consecutive harvest of the same crop of red fescue for more years than what is standard Danish practice [5]. In an aging red fescue crop, lower production of inflorescence resulting in lower seed yield is likely [3], though different red fescue varieties have shown different responses [5]. The seed yields varied over the three years. In both PGR-sprayed and unsprayed subplots, the most substantial seed yield was obtained in 2017, followed by 2016 and 2018. Very different weather conditions characterised the growing seasons. In 2017, the summer in Denmark was cold, wet and cloudy and had no days with temperatures above 30°C . In contrast, the weather in 2018 was unusually dry, warm and sunny and provided many days with temperatures above 30°C . It was the warmest summer since 1874 (Figure 2) [32]. Deleuran et al. [5] did not find any significant seed yield differences from the first to the fourth year of red fescue cultivar stands in Denmark [5].

We did not observe any effect of seeding rate on the seed yield. Deleuran and Boelt [1] also reported that sowing rates ($2, 4$ and 6 kg ha^{-1}) did not affect the seed yield in the first harvest year of red fescue, but they observed a reduction of the seed yield at the highest seeding rate in the second year [1]. The natural growth habit of red fescue involves a steady proliferation of tillers, which eventually become too dense to form seed heads [4]. Both the juvenility of the tillers and the crop density in the first year affect the number of inflorescences. When the stand ages, the crop density mainly affects the number of inflorescences [3]. The competition between reproductive and vegetative tillers is possibly the reason for the limited seed yield at high sowing rates. Often, densely sown seed crops have a great proportion of vegetative tillers [3]. We did not observe any significant effect of PGR dosages on seed yield. However, the degree of lodging affected the seed yield negatively. Mathiassen et al. [12] reported significant seed yield responses at early and late application of Moddus M in red fescue cv. Maxima [12]. Boelt and Gislum [33] reported that the degree of lodging could be controlled by trinexapac-ethyl (Moddus M). Both seed weight and seed yield increased with the declining degree of lodging before the harvest [33]. The high temperature increased the efficacy of Moddus M at early growth stages (BBCH 31–33 and 49–51) but was of minor importance at the late application (BBCH 53–57) [12]. Mathiassen et al. [12] found that air humidity had a low impact on the efficacy of trinexapac-ethyl (Moddus M) [12]. Haldrup [34] observed that

the application of an extra 30 kg N ha⁻¹ with a mixture of 0.4 L Moddus M and 1.25 L CCC 750 (750 g L⁻¹ chlormequat chloride; DLA Agro amba) resulted in a seed yield response of 217 kg ha⁻¹, while 30 kg N ha⁻¹ alone only resulted in a seed yield response of 58 kg ha⁻¹ [34]. He found a seed yield increase of 73 kg ha⁻¹ when growth regulations were applied without any extra N [34].

Seeding rates did not affect lodging. Red fescue produces rhizomes, making it able to cover the ground after some time even at low initial crop densities. The ability to invade the whole area quickly may explain why lodging and seed yield in the second year of the crop was unaffected by seeding rate.

UAVs equipped with high-resolution consumer digital cameras can capture the visible part of the reflected electromagnetic energy in RGB bands via imagery with centimetre resolution (e.g., [24]). Based on visual inspections of images, lodging and brightness were positively correlated, and the blue intensity correlated slightly better with the ground truth of lodging than the overall brightness. Drone imagery was fast and objective. Estimation of lodging, however, requires calibration, which means that a suitable vegetation index (or colour intensity) has to be calibrated against ground truth. A drone can easily cover one hectare per minute. The correlation between lodging (estimated from drone images) and seed yield was not large ($R^2 = -0.39$). Other factors than lodging affected the seed yield, such as climate conditions, as shown by Mathiassen et al. [12]. Drone imagery is considered a successful tool to estimate lodging and may be a useful tool for site-specific application of PGRs. UAV imagery may be more reliable than ground imagery, as it is objective. Different UAV teams with different equipment will mostly be able to reproduce results in terms of comparisons of experimental plots based on vegetation indices derived from uncalibrated RGB cameras [35].

5. Conclusion

Application of PGR increased the seed yield, but the effect varied between years. Adding 0.6 and 1.2 L ha⁻¹ of PGR resulted in the same low ranking of lodging (≈ 2), while 0.3 L ha⁻¹ of PGR resulted in severe lodging (≈ 7). We found a positive correlation between the blue intensity of the images and lodging. PGR dosage significantly affected lodging evaluated by visual ranking and the blue intensity of the images. The seeding rates did not affect lodging or yield, and there was no interaction between the PGR dosages and seeding rate. Lodging affected seed yield negatively. Consequently, we recommend farmers to use between 0.6 and 1.2 L ha⁻¹ of Moddus M. As a low seeding rate results in the same yield as a high seeding rate, farmers could save seeds (and money) by using only 2 kg of seeds per ha⁻¹. Drone imaging appears to be a useful tool for ranking lodging in red fescue fields.

Author Contributions: C.A. was responsible for funding acquisition and the design of the experiment. Z.B. and C.A. ranked the lodging, while J.R. was responsible for capturing and analysing drone images and J.C.W. processed the images. Z.B. and J.R. did the statistical analyses. Z.B. wrote the first draft of the article and all authors reviewed, edited and accepted the final manuscript.

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