CHOKE STROMA EXPRESSION IN ORCHARDGRASS WITH DIFFERENT NITROGEN APPLICATION RATES AND TIMINGS

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Introduction

Choke disease in orchardgrass seed production fields in the Willamette Valley is caused by a fungus, *Epichloë typhina*, that mostly grows unobserved within the plant. The fungus, native to Europe, was inadvertently introduced into cultivated orchardgrass fields in western Oregon in the late 1990s (Alderman et al. 1997). In the presence of an abundance of host plants in close proximity, the fungus spread rapidly. By 2000, approximately 90% of orchardgrass seed production fields in Oregon were host to the fungus (Pfender and Alderman 2006). It appears that seed yield loss is proportional to the percentage of flowering tillers choked (Large 1954, Pfender and Alderman 2006).

During vegetative growth of the orchardgrass the fungus develops internally. However, when the plant switches to the flowering phase, the fungus proliferates externally forming stromata. This occurs at the tip of the tiller where the leaves are folded together. Stromata are about 1.5 to 2.5 inches in length and are greenish-white when immature, white after fertilization, and burnt-orange when the mature ascospores (infective spores) are ready to be released. The fungal hyphae of the stroma 'cement' together the folded leaves of the upper tiller and prevent the inflorescence from growing upwards. As a result, no seeds are produced on the affected tillers; hence the expression of the pathogen is called choke disease (Sampson and Western 1954).

There does not appear to be anyway to eliminate the choke pathogen from orchardgrass once the plant is infected. Efforts at control have focused on reducing the spread of newly infected plants by reducing the number of infective ascospores released each spring. Eleven different fungicides were tested to determine whether they could reduce the amount of stromata surface producing ascospores. While several fungicides did inhibit fertilization to some degree, the decline was not believed to be great enough to have an impact on ascospore numbers (Alderman et al. 2008). A similar approach explored the use of the fungus *Dicyma pulvinata*, which grows on the fungal stromata of choke. This fungus was very effective in greenhouse studies in reducing the percent of perithecia (ascospore producing structures) on sprayed stromata, but was much less effective in field trials (Alderman *et al*. 2009). Another possible mechanism of limiting the spread of choke is to use an insecticide to kill the fly *Botanophilla labata*, whose feeding and egg laying behaviors lead to the cross fertilization of the stromata from plants infected with the two alternate mating type strains (Bultman *et al*. 1998). An initial insecticide trial conducted in Oregon was inconclusive (Alderman *et al*. 2008). It is now thought that because of the multiple ways fertilization of stromata can occur (Rao *et al*. 2012), interrupting only one mechanism of fertilization is not adequate to limit the production of ascospores.

Ongoing orchardgrass variety trials show some promise in identifying choke resistant orchardgrass lines, but breeding this material from its Mediterranean (dry habitat) adapted sources into varieties suitable for the orchardgrass growing regions in the US, will be a long term project.

Thus, for now, orchardgrass seed farmers in the Willamette Valley will continue to live with the problem of choke. This means taking out fields after 5-6 years, rather than the decades that were once common. In the current study, we examined whether manipulating late winter fertilization practices could reduce the severity of choke expression, and perhaps extend the productive life of orchardgrass fields by a few years. Not all tillers of an infected plant are choked each year, and there is considerable variation between years in the percentage of tillers choked. Our hypothesis was that early and quick tiller growth would allow the flowering tillers to outgrow the development of the fungus and the production of stromata. We manipulated the nitrogen (N) environment of the orchardgrass by varying the amount of late winter applied N, and also compared single versus split applications. We were particularly interested in seeing if the split application would lead to a reduction in the percentage of tillers with stromata.

Methods

The study was initiated in the fall of 2009 using six year old orchard grass plots that developed stromata three years earlier. During the previous three years the plots had been surveyed for the presence of choke disease and the choke fly. The orchardgrass plots received minimal amounts of nitrogen fertilizer during previous years.

At the onset of this study, plots received 40 lbs of N/acre in the form of urea each fall when the plants began to grow with the commencement of fall rains. Soil test phosphorus and potassium levels were moderate to high because of previous fertilizer applications.

Year 2010. The randomized complete block design had 5 late winter N application entries comprised of varying application rates and timings (single versus split). Split applications were made at early (early February) and typical (late February- early March) timings. In 2010 the early date was Feb. 4, and the typical date was Feb. 25. There were three single application entries at the typical date: 80; 120; 160 lbs N/acre. There were two split application entries: 120 lbs- with 40 lbs applied early plus 80 lbs typical; and 160 lbs- with 60 lbs early plus 100 lbs typical. The sixth entry was 120 lbs typical plus a gibberellic acid formulation (ProGibb 40%). The ProGibb rate was 20 gm AI/acre, sprayed on April 20. The sulfur coated urea fertilizer (40-0-0-5.6) was applied with a drop spreader.

Years 2011 and 2012. The experiment was conducted for a second and third year; N entries were laid on the same entry plots from the previous year. The exception was that an additional high N rate (80 lbs early and 120 lbs typical) replaced the ProGibb plots from the previous year since the ProGibb treatment had no effect during the first year of field trials or in a separate potted plant study. In 2011 the early application date was February 7, and the typical was March 4. In 2012 the early application date was February 2, and the typical was March 4.

In all years, we assessed the trial for the effectiveness of the N treatment in reducing choke severity in early June. In each plot two 5-adjacent plant subsamples were selected, and the number of tillers with stromata, the number of flowered tillers, and total tillers, were counted for each 5-plant subsample. The three variables were analyzed using Proc GLM (SAS 9.92), with N application entry as the main effect, and the single versus split applications compared using a contrast statement.

Results

Over the course of the three years of the N rate and timing trial there were small but significant differences in the entries for some of the variables. For proper interpretation of the results the following aspects of the methods are important to consider. 1) The trial was initiated in plots that had for several years been receiving less fertilizer than typical production practices. Therefore, soil and mineralizable N were low compared to typical production fields at the beginning of the study. 2) Individual fertilizer entries were applied to the same plots each year. Therefore, there was likely a buildup of soil and mineralizable N in the higher N entries over the course of the study. The exception was the 2010 ProGibb (growth regulator) entry which we changed to the highest N entry (80 lbs N early, 120 lbs N typical) in 2011 and 2012.

In 2010 there were no differences in N rates or between single and split applications (Table 1). This may have been a result of the previously low N applications, which may also have been the cause of the low total number of tillers per plant recorded that year.

In 2011 there were a smaller proportion of choked tillers in the entries with higher N (Table 2). The contrast for single versus split applications was also significant; with split applications having a higher proportion of choked tillers. There was a trend in higher numbers of flowering tillers in the higher N treatments but this was not significant (Table 2). There was no difference in the number of total tillers.

In 2012 there were again a smaller proportion of choked tillers in the high N entries (Table 3). In this year the split versus single application contrast was not significant. The very high rainfall after the early February application could have washed some of the early applied N out of the system. The patterns in the number of flowering tillers and total tillers in 2012 were the same as in 2011. There was a trend in a higher number of flowering tillers in the high N entries (P=0.0855), no difference in total number of tillers, and no significant single versus split application contrast (Table 3).

Discussion

The results do not support the hypothesis, that an early application of nitrogen may give the orchardgrass plant a tiller growth advantage over the process of tiller fungal infection. One possible reason for this is that the early N may be washed from the system before it can be taken up by the plants, resulting in lower total N uptake in these entries. Virtually all the orchardgrass plants in the trial plots had tillers that were choked. Commercial production fields are typically replanted to another crop once the incidence of choked tillers reaches 10 – 20%. Our findings may have been different if trial was conducted in a less infected field.

Higher total N rates did result in a lower proportion of choked tillers, but this did not translate into significantly greater number of flowering (yield producing) tillers. However, there was a consistent trend in this direction in the last two years. The differences between the lowest and highest values were rather large, 63% in 2011, and 120% in 2012; but high variances limited the significance of the statistical tests.

Yield samples were not taken from the plots so we do not know exactly how the N rate and timing entries affected seed yield. In 2010 on-farm field trials, only one of the three fields responded to a supplemental early application of N (40 lbs/A) with an increase in seed yield (Mellbye [OSU], Boren and Cacha [Crop Protection Services], unpublished data).

These data suggest there is no strong benefit to an early application of N to reduce the severity of choke. Any reduction in choke severity, and increased seed yield, would need to offset the additional cost of the fertilizer application. Higher rates of total N fertilizer appear to confer some advantage in reducing choke expression, but the highest rates were not significantly better than the medium rates typically used in production fields.

References

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Table 1. Incidence of choke, and number of tillers per plant at the Hyslop Experiment Station nitrogen-choke incidence trial in 2010.

Fertilizer Treatment [1,2]	Proportion of stroma/plant	No. of flowering tillers/plant	Total no. of tillers/plant
P-value =	0.9247	0.8358	0.3060
160	0.47 ± 0.05	7.8 ± 1.62	14.3 ± 2.4
120 + PG	0.49 ± 0.05	7.5 ± 1.28	14.2 ± 1.6
40 + 80	0.49 ± 0.02	6.4 ± 0.67	13.6 ± 1.5
60 + 100	0.50 ± 0.03	8.8 ± 1.44	18.3 ± 2.3
120	0.52 ± 0.09	8.0 ± 1.96	16.0 ± 2.5
80	0.56 ± 0.08	7.1 ± 0.82	17.0 ± 1.2
Split vs. Single Application			
P-value =	0.9929	0.8147	0.6233

[1] Rank of treatments in table based on proportion of tillers with stroma.
[2] Rate in pounds of N per acre, PG = ProGibb

Table 2. Incidence of choke, and number of tillers per plant at the Hyslop Experiment Station nitrogen-choke incidence trial in 2011.

Fertilizer Treatment [1,2]	Proportion of stroma/plant	No. of flowering tillers/plant	Total no. of tillers/plant
P-value =	0.0495	0.1357	0.9845
40+80	0.71 ± 0.02 a	11.0 ± 0.7 a	41.2 ± 2.9 a
80	0.70 ± 0.06 a	13.8 ± 3.5 a	41.0 ± 1.0 a
60+100	0.62 ± 0.05ab	16.4 ± 2.9 a	43.0 ± 1.9 a
120	0.61 ± 0.07ab	14.8 ± 2.5 a	42.1 ± 3.3 a
160	0.54 ± 0.08 b	17.3 ± 2.9 a	38.9 ± 2.9 a
80+120	0.54 ± 0.07 b	18.0 ± 2.5 a	37.7 ± 0.9 a
Split vs. Single Application			
P-value =	0.0485	0.2440	0.8653

Mean values in the same column followed by different letters differ significantly (LSD $P<0.05$).
[1] Rank of treatments in table based on proportion of tillers with stroma.
[2] Rate in pounds of N per acre

Table 3. Incidence of choke, and number of tillers per plant at the Hyslop Experiment Station nitrogen-choke incidence trial in 2012.

Fertilizer Treatment [1,2]	Proportion of stroma/plant	No. of flowering tillers/plant	Total no. of tillers/plant
P-value =	0.0078	0.0855	0.2439
80	0.78 ± 0.02 a	4.3 ± 0.7	20.1 ± 1.5
40+80	0.71 ± 0.06 ab	5.8 ± 0.9	19.7 ± 1.2
120	0.69 ± 0.05 abc	5.0 ± 2.1	18.4 ± 3.3
160	0.63 ± 0.03 bcd	7.5 ± 0.5	23.7 ± 2.7
80+120	0.60 ± 0.03 cd	9.5 ± 1.5	24.5 ± 0.7
60+100	0.59 ± 0.03 d	8.1 ± 0.6	21.1 ± 2.1
Split vs. Single Application			
P-value =	0.6811	0.5895	0.7429

Mean values (mean and SE) in the same column followed by different letters differ significantly (LSD:$P<0.05$). Without block 3.

[1] Rank of treatments in table based on proportion of tillers with stroma.

[2] Rate in pounds of N per acre