Variation Among Orchardgrass (*Dactylis glomerata*) Germplasm for Choke Prevalence Caused by *Epichloë typhina*

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Abstract

Orchardgrass, or cocksfoot (*Dactylis glomerata* L.), is a cool-season forage grass susceptible to the choke disease caused by *Epichloë typhina*. Choke has been reported in orchardgrass seed production fields across the temperate regions of the world, but fungicides have not been efficacious in reducing choke incidence or prevalence. To assess the potential for genetic resistance or tolerance of orchardgrass to choke, we evaluated the variation in orchardgrass cultivars and accessions for choke prevalence and characterized infected plants for endophyte secondary metabolite and mating type gene presence. Significant variation was detected across years and locations. Choke

prevalence did not always increase with the age of the stand, nor did choke prevalence correlate with flowering time or swathing time of the entries. Both mating types of *E. typhina* were detected in approximately equal proportions, and no evidence for loline, ergot alkaloid, or indole-diterpene biosynthesis was found. Plants with multiple infected tillers often showed more than one mating type present in the plant, indicating multiple infection events rather than a single infection event that spread to multiple tillers. Both accessions and cultivars with significant choke, and no choke, were detected, which constitute sources of germplasm for further testing and breeding.

Orchardgrass, or cocksfoot (*Dactylis glomerata* L.), is one of the top four cool-season forage grasses worldwide (Stewart and Ellison 2010) but is susceptible to the choke disease. Choke is caused by *Epichloë typhina*, an inconspicuous systemic fungal endophyte in vegetative tissues that initiates its own sexual cycle during host flowering (Craven et al. 2001). Upon initiation of the sexual cycle, the *E. typhina* surrounds the host's immature inflorescence before it can emerge from the surrounding leaf sheaths and prevents (chokes) its further development and seed set. Choke has been reported in orchardgrass seed production and other fields in the United States, Europe, and Asia (Cagaš and Macháč 2012; Large 1954; Li et al. 2009; Pfender and Alderman 1999). In Oregon's Willamette Valley, incidence of choke was first reported in 1996 (Pfender and Alderman 1999), with subsequent seed yield losses growing from 9% to over 30% (Pfender and Alderman 2006).

Multiple reports have studied the occurrence, spread, and possible fungal infection routes of choke on orchardgrass, as well as vectors that might aid in fertilization of the sexual fungus. What has emerged is a complicated and unclear pattern. Once choke is detected within a field, it appears to pervade in a quadratic response (Pfender and Alderman 2006). The scale and spread of choke are not completely consistent with small-scale plant wounding (Alderman 2013; Leyronas and Raynal 2008), such that wounding caused by swathing during orchardgrass seed harvest and while ascospores are abundant has remained a likely cause for fungal spread (Western and Cavett 1959).

Throughout the vegetative plant growth, the endophyte remains inconspicuous, growing slowly between the intercellular spaces of the plant host and maintaining growth primarily near the crown of the plant (Christensen et al. 2008; Kirby 1961). The intercellular growth of the endophyte changes abruptly upon an unknown signal

or signals from the plant at flowering, whereupon the endophyte is able to ramify through the host, developing stromata that engulf (choke) the developing seed heads. *E. typhina* in orchardgrass is considered aggressive, in that each endophyte-infected tiller will choke, and those tillers without choke are endophyte-free (Schardl et al. 2009). This aggressive nature is in contrast to the related *E. festucae*, in which asymptomatic tillers of host grasses (e.g., *Festuca rubra* L.) can be infected. Because the *E. typhina* endophyte is only conspicuous when orchardgrass begins to flower, fungicide treatments have had little or no effect (Pfender and Alderman 2003). Additionally, *E. typhina* is heterothallic and requires fertilization with the opposite mating type to develop and release ascospores that can lead to horizontal transmission. Although several insects have been proposed as travel vectors for fungal fertilization, none have been shown to account for the majority of fertilization of the fungus (Hoffman and Rao 2014; Rao et al. 2012; Rao and Baumann 2004). Thus, many questions still exist surrounding infection, growth, and fertilization, and no efficacious treatment or control has yet been found.

Breeding of disease-tolerant or -resistant cultivars is a lengthy process, but when less expensive or effective management options are lacking this approach holds merit. In the case of choke and orchardgrass, mechanisms of possible tolerance or resistance would be difficult to define but may include gene products that prevent or dilute the plant signal that induces the fungal sexual stage, phenology mechanisms to avoid times when fungal spores are prevalent, or plant structure or secondary metabolites that limit fungal entry or survival in mature tissues. Although the explanatory causes of choke would be difficult to define, resistance or tolerance can be detected in replicated field trials by a lack or paucity of choke in replicated entries over time. The objective of our research was to determine if there is variation for the percentage of infected plants among orchardgrass collections and varieties, to characterize *E. typhina* and the mating type frequency among these collections and varieties, and to identify germplasm sources that are less symptomatic of choke for further evaluation and plant breeding.

Materials and Methods

Forty-eight orchardgrass varieties and wildland collections (hereafter called entries) were planted in two sites: one near Albany, OR, at 44°37'32.86" N/122°55'57.63" W, and one near Corvallis, OR, at 44°30'53.30" N/123°13'18.75" W. The entries represented the wide range of flowering times present in orchardgrass and germplasm from Mediterranean as well as Continental origins (Table 1). Each

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location was planted with three replications of 16 plant plots, in a randomized complete block design. Seedlings of each entry were germinated in Logan, UT, and transplanted at the two Willamette Valley, OR, locations in the summer of 2012. Plots were maintained thereafter with fertilizer, pesticide for rust diseases, and herbicide as needed and directed in the Oregon State University orchardgrass fertilizer guide (Doerge et al. 2000) and the Pacific Northwest weed and pest management handbooks (<https://pnwhandbooks.org/>). Choke prevalence data were collected from 2013 through 2016. Plant mortality was recorded prior to choke scoring each year. Temperature and precipitation data were extracted from the Oregon State University PRISM database (prism.oregonstate.edu) for Linn County, OR, where both research plots reside. Spring temperature data comprised average monthly maximum and minimum temperatures from March through May of each year, and spring precipitation was the sum of precipitation across those three months.

For each year of data collection, heading and swathing dates were determined at the Albany site (Table 1). Heading dates of individual plants were recorded as the day from January first when three panicles emerged from the boot and were recorded as the date when 50% of the plants in a plot had headed. Swathing occurs after seed has ripened and comprises cutting and laying down the tillers prior to combine harvesting. Entry plots were swathed at both locations on the same day but were based on maturity of seed at the Albany location and when seed moisture was at 42 to 46%, such that swathing dates were also recorded as the number of days from January 1. Plant material was discarded 1 week after swathing. Choke prevalence scoring occurred at anthesis and was recorded as the number of plants in each 16-plant plot that had stromata covering one or more panicles (Fig. 1). All plots were scored twice, by different people, to ensure accuracy. To account for plant mortality within plots, the number of plants with infected tillers was divided by the total number of plants

Table 1. Orchardgrass cultivars and accessions evaluated for choke prevalence^a

Entry	Source/information	Growth type	Heading date	Swathing date	Mortality (Albany)	Mortality (Corvallis)
AC Killarney	Agri-Food Canada	Continental	126.1	193.0	1.8	24.4
Baraula	Barenbrug	Continental	127.2	188.7	2.5	15.9
Barexcel	Barenbrug	Continental	112.5	182.0	1.1	19.1
Baridana	Barenbrug	Continental	118.3	183.7	1.5	13.0
Barlegro	Barenbrug	Continental	121.1	183.3	5.1	22.4
Barlemas	Barenbrug	Continental	107.9	178.0	0.9	32.6
Benchmark Plus	FFR Cooperative	Continental	114.9	180.3	0.4	14.7
Crown Royale	Grassland Oregon	Continental	113.7	182.0	0.7	9.9
Dg12R01	Barenbrug	Continental	129.2	195.3	3.1	11.1
DglGRL	Barenbrug	Continental	108.1	177.3	2.6	14.5
Gr. Tekapo	Grasslandz Tech. Lmtd.	Unknown	114.0	182.0	15.0	21.6
Harunemidori	NARCH, Japan	Continental	114.8	179.3	1.7	5.1
Icon	Forage first	Continental	110.1	178.7	1.6	9.3
Intensiv	Barenbrug	Continental	128.5	184.0	0	16.1
NFOG-101	Noble Res. Inst.	Continental	107.0	180.7	35.6	25.8
PI 189142	Netherlands	Continental	116.1	186.7	0	17.4
PI 189388	New Zealand	Continental	114.7	182.0	2.9	18.2
PI 217416	Denmark	Continental	112.6	181.0	1.4	26.4
PI 223250	Afghanistan	Continental	107.2	179.3	3.9	20.9
PI 231484	Algeria	Continental	116.1	186.7	0.7	4.9
PI 235471	Switzerland	Continental	117.5	183.7	3.1	18.9
PI 237602	Portugal	Continental	119.7	195.3	33.6	53.7
PI 237610	Iran	Continental	116.3	184.7	0	3.2
PI 242800	Turkey	Unknown	107.8	178.7	25.8	55.2
PI 250928	Iran	Continental	116.5	181.7	1.5	21.4
PI 253966	Iraq	Continental	105.8	179.7	8.2	18.6
PI 292592	Israel	Unknown	105.6	182.3	46.4	48.8
PI 308542	Colombia	Unknown	104.4	174.0	4.3	16.3
PI 311374	Spain	Mediterranean	111.8	183.3	46.0	47.9
PI 311402	Spain	Mediterranean	120.3	186.0	2.0	20.1
PI 368879	Portugal	Mediterranean	113.3	183.0	29.7	48.3
PI 371948	Bulgaria	Continental	108.4	180.3	2.8	11.2
PI 499409	China	Continental	136.9	198.0	4.2	53.3
PI 516543	Morocco	Mediterranean	112.6	185.7	44.2	55.7
PI 527484	France	Continental	112.0	181.0	17.2	28.6
PI 538922	Russia	Continental	127.7	198.0	0.2	29.0
PI 577042	Yugoslavia	Continental	117.6	181.3	3.3	16.9
PI 578584	Morocco	Mediterranean	127.2	188.7	45.2	63.6
PI 578597	Israel	Unknown	108.7	178.0	16.3	36.4
PI 578603	Cyprus	Mediterranean	112.4	183.0	40.0	68.5
PI 578610	Algeria	Mediterranean	117.7	189.3	54.9	73.5
PI 598862	Morocco	Mediterranean	112.6	184.0	47.5	51.8
PI 632495	Tunisia	Mediterranean	113.2	191.3	48.0	76.1
PI 634258	Albania	Unknown	111.4	184.7	6.2	14.4
PI 636581	China	Continental	128.0	195.3	2.4	21.4
Potomac	Check Cultivar	Continental	115.2	179.3	0.8	4.6
Syn9914	Barenbrug	Continental	120.2	183.0	0.3	13.0
Toyomidori	NARCH, Japan	Continental	139.9	198.0	7.5	13.8
LSD (0.05)			2.9	3.2	6.8	13.0

^a LSD = least significant difference.

(maximum 16) in each plot, and choke prevalence data were recorded as the percent of plants exhibiting choke per entry.

To characterize the *E. typhina* strains and mating types, orchardgrass tissue containing *E. typhina* stromata was collected individually from infected plants immediately below the stroma present on a reproductive tiller. During each collection, reproductive tillers without choke were also collected as controls. In 2014, 129 infected plants were randomly sampled from both locations; in 2015, all infected plants were sampled from both locations; and in 2016, all infected plants were sampled from the Albany location. Also, in 2016, eight plants with at least 10 choked tillers were evaluated by polymerase chain reaction (PCR) to determine if endophyte variation existed within a plant. DNA was extracted from the samples using the Qiagen MagAttract Kit (Qiagen, Valencia, CA). PCR was performed with eight *Epichloë*-specific primer sets to evaluate the fungal diversity based on the following genes: *tefA* encoding the translation elongation factor-1- α , *perA* encoding peramine synthetase and using primer sets to different T2 and R protein domains, *lolC* encoding the first step of loline production, *dmaW* encoding dimethylallyl tryptophan synthase in the first step in ergot alkaloid production, *idtG* encoding the first step of indole diterpene production, and *mtAC* and *mtBA* mating type genes. PCR conditions and primer sequences of these genes were previously published in Charlton et al. (2014).

Heading date, plant mortality, swathing date, and choke prevalence were analyzed as nearest-neighbor mixed models using the ASReml-R package in R (Butler et al. 2009; R Core Team 2013). For each trait, years, locations, orchardgrass populations, and their interactions were modeled as fixed effects. Blocks within locations were modeled as random effects. The analysis also modeled the covariance associated with repeated measures of the plots across years and the spatial variation within each location based on the locations

of plots in the row and column field structure (Smith and Casler 2004). For the fixed effects the model estimated best linear unbiased estimates. Rank and Pearson correlations were estimated using the CORR procedure of SAS (SAS Institute 2011). Discriminant analysis was used to determine if groups of entries categorized by growth type, heading date, or swathing date would be able to be discriminated by choke prevalence. First the STEPDISC procedure in SAS was used to determine if choke prevalence at either or both of the locations was an appropriate variable. Then the DISCRIM procedure of SAS with pooled variance was used to test the significance of the discriminatory variables.

Results

The effect of year, year by location, and year by entry were each significant at $P < 0.001$. Although no choke data were collected during the 2012 establishment year, years 2013 to 2016 had overall choke levels significantly greater than zero, except 2013 at the Albany location (Fig. 2). Years 2014 and 2016 had relatively higher choke prevalence, whereas 2013 and 2015 showed less choke (Fig. 2). Concerning the year by entry effect, Spearman rank correlations of choke prevalence in entries were highly significant (Table 2), such that further analyses for choke prevalence were combined across years. Concerning the year by location effect, the rank correlations between year and location ranged from $r = 0.40$ to 0.50 ($P < 0.01$), significant yet moderate to the extent that both magnitude and rank changes occurred between the locations. Therefore, further analyses for choke prevalence were conducted separately by location.

Because climate in the spring season might affect the *E. typhina* infection rate, temperature and precipitation data were collected for each year across the months of March to May (Fig. 3). Although the average minimum and average maximum temperatures did not

Fig. 1. A, Replicated field plots of orchardgrass near Albany, OR, at the time of choke data collection. B, Stroma (white) surrounding the immature inflorescences on an *Epichloë typhina*-infected plant. C, Fertilized stroma.

vary appreciably, the spring precipitation varied (Fig. 3) and reflected the choke prevalence (Figs. 2 and 3).

At both locations, PI 223250 exhibited no choke over the course of the experiment, whereas four entries showed choke prevalence significantly greater than zero (Fig. 4). At the Albany location, four entries exhibited no choke over the course of the experiment, whereas 17 entries, including 9 of the 13 cultivars, had a prevalence significantly greater than zero (Fig. 4). At the Corvallis location, 14 entries had no choke, whereas 7 had choke prevalence significantly greater than zero (Fig. 4). The 'Baraula' cultivar had a particularly high percentage of choke, exhibiting choke even in year 2 (data not shown) and having 40 and 57% of plants infected across the 4 years at the two locations. The significance detected in spatial analysis did not reveal any specific patterns of choke spread but was rather clustered approximate to Baraula entries (darker blocks, Supplementary Fig. S1). Entries with little or no choke prevalence across the 4 years mainly included wildland collections: PI 223250, PI 292592, PI 578603, PI 578584, and PI 371948. Of the cultivars tested, 'AC Killarney', 'Barlegro', 'Grasslands Tekapo', and 'Harunemidori' had the lowest choke prevalence.

To estimate the relationship of choke prevalence to heading date or swathing date, correlations were computed. Heading dates were highly correlated across years, whereas swathing dates were not (Table 2). Heading dates ranged from 104 (April 15) to 140 (May 21) days from January 1 but were only moderately correlated with choke prevalence ($r = 0.29$ at Albany and $r = 0.20$ at Corvallis, both $P < 0.05$). When the late-heading cultivar Baraula was removed from analysis, the correlations between heading date and choke prevalence at the two locations were not significant. Swathing dates ranged from 174 (June 23) to 198 (July 18) days from January 1, were correlated with heading dates at $r = 0.75$ ($P < 0.001$), but were not correlated with choke prevalence at either location. Based on discriminant analyses, choke prevalence was not significant in its ability to discriminate heading or swathing dates at either location.

Fig. 2. The overall percent of *Epichloë typhina*-infected orchardgrass plants at two locations in Oregon, from 2013 to 2016.

Table 2. Spearman rank correlations across years for the percent of *Epichloë typhina*-infected plants, plot heading date (Albany location only), and plot swathing date (Albany location only)^a

Year	Parameter	2013	2014	2015
2014	Choke prevalence	0.71**	...	...
	Heading date	0.90**	...	...
	Swathing date	-0.43**	...	...
2015	Choke prevalence	0.55**	0.84**	...
	Heading date	0.84**	0.92**	...
	Swathing date	0.69**	-0.17	...
2016	Choke prevalence	0.43**	0.65**	0.74**
	Heading date	NA	NA	NA
	Swathing date	NA	NA	NA

^a NA = not applicable.

To determine if summer dormant/winter active Mediterranean orchardgrass ecotypes varied in choke prevalence compared with summer active/winter dormant Continental orchardgrass ecotypes, discriminant analysis was further conducted. The cultivars and accessions were grouped into Continental (33 entries), Mediterranean (nine entries), and unknown (six entries) based on country of origin (Table 1). Stepwise discriminant analysis identified choke prevalence from both Corvallis and Albany locations for inclusion into the model to discriminate ecotype, with a Wilk's lambda value of $F_{4,84} = 2.36$ ($P = 0.06$). Using choke prevalence as an indicator, the unknown category was separated with 100% accuracy, the Mediterranean ecotypes were grouped into either Mediterranean or unknown categories, but the Continental ecotypes were placed into all three categories and thus poorly discriminated from the other two ecotypes (Table 3).

In total, 560 individual plants were tested by PCR to evaluate the endophyte within the study fields. The endophyte was only detected in the choked tillers, whereas tillers with developing inflorescences were endophyte-free. All infected samples across both locations were consistent and tested positive for the *tefA*, *perA*-T2, and mating type markers (Supplementary Fig. S2). The endophyte-infected orchardgrass samples showed no evidence of the potential to produce lolines, ergot alkaloids, or indole-diterpenes, because the genes encoding early pathway steps were not detected. For mating types, either *mtAC* or *mtBA* amplified per individual tiller sample, and approximately equal frequencies of mating types were detected at the two locations (Table 4). In 2016, plants with more than 10 individual choked tillers were selected to determine if a plant could have multiple infections, which would be evidenced by the presence of more than one mating type associated with a single plant. Of the eight individual plants tested, four had mixed infections in which both *mtAC* and *mtBA* were present. To validate this result, we identified 98 individual plants from 16 plant lines that were tested in both 2015 and 2016. Of these infected plants, 23% of the samples showed both mating types (Table 5).

Discussion

Although choke prevalence varied by year, it did not necessarily increase each year. Previous studies have seen similar results (Cagaš and Macháč 2012; Leyronas and Raynal 2008) and have found that moisture and cool temperatures were necessary for ascospores germination (Alderman 2013). Because of such, we extracted the spring seasonal temperature and precipitation data prior to each orchardgrass flowering season to test for possible correlations of choke prevalence and seasonal weather. Although the numbers were insufficient to conduct statistical tests, a trend in similarity between spring precipitation and choke prevalence was seen. Because years with higher spring precipitation apparently corresponded to higher choke prevalence, further investigation of an ability of spring precipitation to predict choke prevalence may be a valuable course of future study.

Fig. 3. Mean minimum and maximum temperatures, and sum of precipitation, for the months of March to May in Linn County, OR, for 4 years.

The Corvallis location had a higher percentage of choke than the Albany location in 2013, a smaller rise of choke incidence in 2014, and a lower prevalence of choke than the Albany location in 2016 (Fig. 2). Interestingly, the two locations were separated by approximately 25 km, and the Corvallis location had a history of choke incidence in adjacent fields, yet the Corvallis location exhibited a lower overall choke prevalence after 2013. One reason for this difference may have been poorer stand health at the Corvallis location. The average mortality per plot at the Corvallis location was 28%, significantly higher than the 13% mortality of the Albany location ($P = 0.02$). The higher mortality corresponded to higher weed pressure and open space among plants in the Corvallis location. Previously, another assessment of choke also found that poorer stand

health coincided with less choke prevalence (Western and Cavett 1959).

It is possible that late flowering orchardgrass entries may avoid optimal fungal growth conditions in spring or that orchardgrass entries with either early or late swathing dates may avoid times of high ascospore flow (Western and Cavett 1959). However, choke prevalence was not correlated with either heading or swathing dates, nor was it able to discriminate among early or late heading or swathing entries. For example, AC Killarney and Baraula are both late-heading cultivars of orchardgrass, with the former showing the lowest choke prevalence among cultivars, whereas the latter showed the highest prevalence (Fig. 4). In a similar vein, early heading orchardgrass entries were found with both low and high choke prevalence.

Fig. 4. Percent of *Epichloë typhina*-infected orchardgrass plants within entries at two locations in western Oregon, U.S.A. Error bars represent least significant difference values at $P = 0.05$, and the dashed lines correspond to the threshold after which entries had significantly more choke than zero.

One reason for the lack of association of swathing dates in particular might be that *E. typhina* ascospores are released over an extended period of time (Western and Cavett 1959), which likely encompassed the entire range of swathing dates. It would therefore be unlikely that seed producers could avoid swathing during high spore counts at seed harvest. From a plant-breeding perspective, however, these data do suggest that choke-tolerant orchardgrass varieties could be developed across heading or swathing dates.

In this study, the climate in western Oregon did not favor summer dormant/winter active Mediterranean ecotypes of orchardgrass, but several such entries were included and persisted through the experiment. In contrast to heading and swathing dates, there was partial evidence for an ability of choke prevalence to discriminate Mediterranean and unknown ecotypes of orchardgrass from Continental ecotypes, but not vice versa (Table 3). There was a preponderance of Mediterranean and unknown entries with low choke prevalence, whereas Continental ecotypes were evenly spread across the range of prevalence (Table 1, Fig. 4), which suggests that choke-

tolerant cultivars can be developed from both Continental and Mediterranean ecotypes of orchardgrass.

Characterization of endophytes with PCR markers used to identify the potential of a strain to produce known alkaloids has provided insight into collections of native and forage grasses (Charlton et al. 2014; Shymanovich et al. 2017; Young et al. 2014). Consistent with previously analyzed *E. typhina* from orchardgrass (Schardl et al. 2012), the endophyte-infected orchardgrass from Oregon did not exhibit PCR products from genes involved in loline, ergot alkaloid, or indole-diterpene biosynthesis. Although one of the markers that correlates with peramine biosynthesis was present, all strains lacked the region that encodes the *perA* reductase domain and, therefore, would likely not be able to produce peramine (Berry et al. 2015). These results confirm earlier findings and show that orchardgrass infected with choke-causing *E. typhina* lacked the known endophyte-synthesized alkaloid products.

E. typhina is a heterothallic species that requires the opposite mating type to complete the sexual cycle, which results in the release of ascospores for horizontal transmission (Leuchtmann and Schardl 1998). Evaluation of the *E. typhina* strains across the two locations showed that both mating types were present in approximately equal abundance, consistent with the requirement of stromata fertilization. Interestingly, when multiple choked tillers were tested from a single plant, both mating types were sometimes found. Although it is still unclear when and how an orchardgrass plant is infected, our data indicate that a mature plant with multiple crown meristems could have different areas of the crown infected by different ascospores, possibly at the same or different times. This situation would usually be difficult to detect in a field setting, in which it can be hard to delineate the crown of a single plant in a sward, but in this study each plant was established from a single seed as spaced plants. Further evaluation of the samples with markers such as simple sequence repeats would be needed to better estimate endophyte variation.

The PI 578603 and PI 578584 Mediterranean ecotype accessions and the Continental ecotype accessions PI 223250, PI 538922, and PI 371948 showed little or no choke prevalence at both locations. Of the cultivars included in this study, AC Killarney, Grasslands Tekapo, and Barlegro showed the least overall choke prevalence, whereas Baraula, Barlemas, and Intensiv had the highest overall choke prevalence. The high level of choke found in Baraula in particular presents an optimal cultivar to further examine choke infection and growth in orchardgrass. Although careful consideration of other agronomic factors will be necessary to include these potentially choke-tolerant accessions into orchardgrass breeding schemes, these

Table 3. Discriminant analysis for the ability of choke prevalence on orchardgrass at the Albany and Corvallis locations to distinguish among Continental, Mediterranean, and unknown orchardgrass ecotypes

From	To			Total
	Continental	Mediterranean	Unknown	
Continental	10	13	10	33
Mediterranean	0	3	4	7
Unknown	0	0	6	6
Total	10	16	20	46

Table 4. Mating type frequency of *Epichloë typhina*-infected orchardgrass at two field locations^a

Year	Location	N	MTA	MTB
2014	Albany	81	32 (40%)	49 (60%)
	Corvallis	48	20 (42%)	28 (58%)
2015	Albany	165	84 (51%)	81 (49%)
	Corvallis	41	20 (49%)	21 (51%)
2016	Albany	225	123 (55%)	102 (45%)

^a Mating type is based on presence of *mtAC* for mating type A (MTA) and *mtBA* for mating type B (MTB).

Table 5. Evaluation of individual orchardgrass plants tested in both 2015 and 2016 for mixed infection based on the presence of both mating types

Plant line	Number of choked plants			MTA ^b	MTB ^b	Mix (MTA and MTB) ^b
	2015	2016	Both ^a			
Baraula	28	43	22	5	8	9
Barexcel	18	18	13	6	5	2
syn9914	7	16	7	5	2	0
Potomac	13	13	7	5	2	0
Dg12R01	9	12	6	3	2	1
Baridana	11	10	6	3	0	3
PI 237610	7	7	6	1	3	2
Crown Royale	7	9	5	3	0	2
Intensiv	6	10	5	4	0	1
PI 217416	6	16	4	1	2	1
Icon	6	7	4	1	3	0
PI 577042	4	7	4	2	1	1
Toyomidori	6	5	4	1	3	0
Barlemas	4	6	3	1	1	1
PI 527484	2	6	1	1	0	0
PI 189388	1	9	1	1	0	0
Total	...	...	98	43 (44%)	32 (33%)	23 (23%)

^a The number of infected plants found in both 2015 and 2016.

^b Mating type is based on presence of *mtAC* for mating type A (MTA) and *mtBA* for mating type B (MTB); mix indicates both *mtAC* and *mtBA* were present in either 2015 or 2016 sample. Note: a single tiller only contains one mating type.

accessions and cultivars present promising orchardgrass breeding material.

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