INCIDENCE OF VIRUSES IN FESCUE (*FESTUCA* SPP.) SEED PRODUCTION FIELDS IN THE WILLAMETTE VALLEY, 2016

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Introduction

The Willamette Valley is the primary production area for grasses grown for seed in the United States. In 2015, Oregon grass seed growers produced 212.8 million pounds of fescue seed, valued at $163.5 million, on 152,000 acres (Anderson, 2015).

Fescue plants are known to be vulnerable to two viruses, Barley yellow dwarf virus (BYDV-MAV and BYDV-PAV) and Cereal yellow dwarf virus (CYDV-RPV). Both are now endemic to wheat and barley fields throughout the Willamette Valley. Although these viruses can be economically devastating in oats, barley, and wheat, CYDV-RPV (genus *Polerovirus*), BYDV-MAV, and BYDV-PAV (both of genus *Luteovirus*) can persist for years in many perennial grass hosts. Symptoms include yellowing or reddening of leaf tips, a reduction in root mass, and subsequent stunting of plant growth. However, in some cases, infected plants express no obvious visible symptoms (Watson and Mulligan, 1960; Catherall, 1966; Miller et al., 2002).

CYDV-RPV, BYDV-MAV, and BYDV-PAV are exclusively aphid transmitted, and many grasses and cereals are hosts for these viruses (D'Arcy and Domier, 2000). To test for the presence of virus in collected plant samples, an enzyme-linked immunosorbent assay (ELISA) was used. The ELISA method is a sensitive and reliable serological laboratory test, which provides a more accurate determination of the presence of viruses in Willamette Valley fescue fields than relying on visual symptoms alone.

Methods and Materials

Fescue sampling

To determine the occurrence of these viruses, leaf samples were collected from 16 fescue seed production fields located throughout the Willamette Valley. Fescue fields sampled were predominantly forage- and turf-type tall fescue (*Festuca arundinacea* L.). One field each of Chewings fescue (*Festuca rubra* L. subsp. *commutata*) and strong creeping red fescue (*Festuca rubra* L. subsp. *rubra*) was sampled. Both of these are fine fescue types. The age of fescue seed production fields surveyed ranged from 2 to 20 years.

The fescue fields were sampled in April and May of 2016. Fifteen samples were collected per field, with seven of the samples collected along two transects of a V pattern and the other eight samples collected based on visual symptoms indicative of a possible virus infection. Suspect samples were collected from the periphery of bare and sparsely populated areas in the field or areas where plants displayed yellow or reddish leaf tips.

ELISA testing

Samples were collected and placed into 48 deep well plates (VWR International LLC, Radnor, PA). The plates were placed on ice after collection and then stored at 4°C until processing. Samples were homogenized in phosphate-buffered saline solution that contained 2% PVP-44, 0.1% nonfat skim milk powder, and 0.05% Tween-20 using a Meku leaf juice press (Meku Erich Pollähne GmbH, Leopoldshoehe, Germany). Each sample was tested for the presence of three viruses (BYDV-MAV, BYDV-PAV, and CYDV-RPV) by ELISA (enzyme-linked immunosorbent assay), resulting in 45 separate readings per field. The ELISA kits (Agdia, Elkhart, IN) were used according to the manufacturer's instructions. After the substrate was added, the sample plates were stored at room temperature overnight and then read with an ELx808 plate reader (BioTek, Winooski, VT) at an absorbance of λ 405 nm.

Each sample plate had two blank wells containing grinding buffer in lieu of samples. The values of these wells were averaged to obtain the Blank Value of that plate. Samples were considered positive if their absorbance reading was greater than three times the Blank Absorbance Value. Evaluations for each virus consisted of two parameters: the percentage of virus-positive fields (100 x [number of fields positive/total number of fields tested]) and the incidence of disease, which was based on the number of positive samples in each field. Disease incidence ratings were as follows: one or two positives = mild; three to five positives = moderate; and six or more positives = severe.

Results and Discussion

In 2016, virus surveys revealed that all 16 fescue seed production fields tested negative for BYDV-MAV. However, 94% of the fields tested positive for

Table 1. Results of 2016 fescue seed production virus surveys.

Virus	---------- Number of fields with indicated levels of infection[1] ----------				% of infected fields
	0 samples infected	Mild infection	Moderate infection	Severe infection	
BYDV-MAV	16	0	0	0	0%
BYDV-PAV	1	3	1	11	94%
CYDV-RPV	2	1	3	10	88%

[1]Disease incidence: mild infection = one or two positive samples; moderate infection = three to five positive samples; severe infection > five positive samples

BYDV-PAV, 88% tested positive for CYDV-RPV, and many fields had a high incidence level of both viruses (Table 1). Interestingly, all of the fescue fields surveyed tested positive for either BYDV-PAV, CYDV-RPV, or both viral pathogens. In four of the surveyed fields, more than 66% of individual samples tested positive for either BYDV-PAV, CYDV-RPV, or both (Table 2). The strong red creeping fescue field had only one positive sample, and one tall forage fescue field had only two positive samples.

We do not have enough information to explain the wide range of results, but we feel that they need to be investigated further. Previous reports suggest fescue plants are asymptomatic when infected with BYDV and CYDV (Watson and Mulligan, 1960). The data collected from the field survey were examined to determine whether selecting suspected symptomatic plants resulted in a higher frequency of virus-infected plants compared to random plant samples representative of individual fields (Table 3). There were more ELISA-positive samples in the suspected disease samples than in the random samples in the majority of the fields surveyed (12 of 16). In three fields, the random samples had more positive results. Only one field resulted in the same number of virus-infected samples regardless of sampling procedure. The *p* values of the two-tailed t-test for BYDV-PAV and CYDV-RPV were both 0.01, which means we collected more infected samples when selecting plants that appeared symptomatic than when we randomly collected samples from a chosen area. This would indicate that the tall fescue plants were displaying symptoms or that the observed stand thinning was related to virus infection and not to random chance.

Table 2. Number of samples testing positive for BYDV-PAV and CYDV-RPV.[1]

Field number	Fescue type[2]	Samples with BYDV-PAV	Samples with CYDV
1	TTF	7	4
2	TTF	8	11
3	TTF	8	7
4	TTF	8	9
5	TTF	10	13
6	TTF	14	7
7	TTF	5	7
8	FTF	2	3
9	FTF	6	7
10	FTF	14	6
11	FTF	10	5
12	FTF	6	2
13	FTF	15	6
14	FTF	2	0
15	Chewings	0	7
16	Strong red	1	0

[1]Total number of samples tested per field = 15.
[2]Fescue type: TTF = turf tall fescue; FTF = forage tall fescue

Future Research

In October 2016, we planted a virus trial consisting of 9 grass species representing 101 different varieties (Table 4). The purpose of this trial is to observe BYDV and CYDV incidence/severity levels in different varieties and to observe stand persistence over 3 years. The trial includes 22 varieties of fine fescue, 25 varieties of orchardgrass, 28 varieties of perennial ryegrass, 25 varieties of tall fescue, and 1 variety of festulolium. A tall fescue plant infected with BYDV and/or CYDV will be planted into each plot in April 2017, and *Rhopalosiphum padi* (the aphid vector of these viruses) will be introduced into the field twice a month during the summer. Rates of infection will be assessed by the presence of visual symptoms in late spring and summer and by yearly ELISA testing.

Table 3. Comparison of virus-positive samples—symptomatic samples (8) versus random samples (7) collected from 16 fields.

	---------- Positive for BYDV-PAV ----------		--------- Positive for CYDV-RPV --------	
Field number	Symptomatic samples	Representative field samples	Symptomatic samples	Representative field samples
1	4	3	2	2
2	6	2	8	3
3	6	2	6	1
4	6	2	6	3
5	7	3	8	5
6	8	6	6	1
7	5	0	7	0
8	1	1	3	0
9	3	3	3	4
10	7	7	2	4
11	5	5	2	3
12	4	2	2	0
13	8	7	4	2
14	1	1	0	0
15	0	0	6	1
16	1	0	0	0
Sample Σ	72	44	65	29
p value, t-test		0.01		0.01

Table 4. Types of grass in virus trial planted in fall 2016.

	---- Number of entries ----	
Type of grass	Turf type	Forage type
Chewings fescue	6	—
Hard fescue	6	—
Sheep fescue	1	—
Slender creeping red fescue	2	—
Strong creeping red fescue	7	—
Festulolium	—	1
Orchardgrass	—	25
Perennial ryegrass	25	3
Tall fescue	17	8

Conclusion

Survey results indicate that BYDV-PAV and CYDV-RPV exist in fescue fields throughout the Willamette Valley. We have planted a test plot with the hope of identifying varieties that are resistant or tolerant to virus infection or resistant to aphid feeding, because finding resistance to these viruses or their vectors will remain the best management solution. Fields will continue to be monitored in the coming years, with emphasis on examining field infection in different varieties to identify tolerant and resistant cultivars, as well as varieties that possess aphid-aversion qualities that could reduce virus spread. The information gleaned from this project will be used to inform growers of the pervasiveness of these viruses so that they can better manage the risk to production.

Growers must decide whether spraying for aphids is an economically feasible option to help control these viruses. If it is, an aphid flight monitoring system could be beneficial to help growers with timely insecticide application information for preventing the spread of the yellow dwarf viruses.

References

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