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Impact Factor: 3.02

ISSN: 0191-2917

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plant disease

Editor-in-Chief: Alison E. Robertson
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May 2016, Volume 100, Number 5

Page 1030

<http://dx.doi.org/10.1094/PDIS-09-15-1017-PDN>

DISEASE NOTES

First Report of *Cocksfoot mottle virus* Infecting *Dactylis glomerata* in Oregon and the United States

S. C. Alderman, **R. C. Martin**, and **B. S. Gilmore**, USDA-ARS FSCRU, Corvallis, OR 97331; **R. R. Martin**, USDA-ARS HCRU, Corvallis, OR 97330; **G. D. Hoffman**, Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331; **C. S. Sullivan**, Linn County Extension, Tangent, OR 97389; and **N. P. Anderson**, Yamhill County Extension, McMinnville, OR 97128.[Citation](#)[Open Access.](#)

ABSTRACT

Cocksfoot mottle virus (CfMV) is a mechanically and beetle-transmitted, non-seed-transmitted sobemovirus associated with orchardgrass (*Dactylis glomerata* L.) stand decline in Europe, Japan, New Zealand (Mahy and Van Regenmortel 2010), and Canada (Bittman et al. 2006). Additional hosts, reported from New Zealand (Delmiglio et al. 2010), include *Festuca novae-zelandiae*, *Lolium* spp., *Poa anceps*, *Poa cita*, *Chionochloa rubra*, and *Dichelachne crinita*. To determine if the virus is present in the United States, surveys for CfMV were conducted in 2014 and 2015 in orchardgrass seed production fields in the Willamette Valley in Oregon, where most of the orchardgrass seed produced in the United States is grown. During June, in each of 2014 and 2015, 18 orchardgrass fields were selected arbitrarily. Stand age of the seed production fields ranged from 2 to 24 years. Two of the fields were sampled in both years. Four samples (each containing 4 leaves, one from each of four plants) were collected along each of four transects in an M-shape pattern from each field. All plants sampled appeared healthy. In 2014, samples were placed in separate plastic bags and in 2015 samples were placed in deep well plates (VWR, International LLC, Radnor, PA). In each year, samples were transported over ice and stored at 5°C until processed. Samples were homogenized and tested for CfMV using DAS-ELISA, with antibodies derived from an isolate of CfMV from British Columbia, Canada. In 2014 and 2015, CfMV was detected in 61% and 72% of the fields, respectively. Symptoms were not present at the time of sampling. In 2015, eight ELISA positive samples were further examined with RT-PCR. RNA was extracted with the Direct-zol RNA MiniPrep kit (Zymo

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Article History

Print: 8 Apr 2016

Ahead of Print: 3 Mar 2016

First Look: 19 Dec 2015

Accepted: 14 Dec 2015

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you via a subscription
from the Oregon State
Univ

Research, Irvine, CA) following the manufacturer's instructions and used for cDNA synthesis using the SuperScriptIII First-Strand Synthesis SuperMix kit (Life Technologies, ThermoFisher, Waltham, MA). PrimeSTAR HS DNA Polymerase (Takara Clontech, Kyoto, Japan) was used following the manufacturer's instructions with CfMV specific primers CfCP-F1 (GATGGAGCCAGTCTCTCG) and CfCP-R2 (ATCCGTCAATCTTCAAGC) (Delmiglio 2008) for PCR with the following program: 98°C for 2 min; 40 cycles of 98°C for 15 s, 55°C for 5 s, and 72°C for 45 s; followed by 72°C for 7 min. The predominant band at 750 bp was reamplified and sequenced using CfCP-F1 and CfCP-R1 primers. A BLAST search indicated 94.4 to 95.4% identity with CfMV isolates found in native and exotic grasses of New Zealand (Delmiglio 2008). Sequences were submitted to GenBank (Accession No. KT984653 to KT984660). To our knowledge, this is the first report of CfMV in orchardgrass seed production fields in Oregon and in the United States. Results of this study are significant given the severity of CfMV in Canada and elsewhere, and this could account for the winter die-out occurring in orchardgrass seed-production fields in Oregon. Additional studies will be needed to determine to what extent CfMV is associated with stand decline in Oregon and what vectors may be present.



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Section: ▼

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