

First Report of *Cucumber green mottle mosaic virus* Infecting Greenhouse Cucumber in Canada

K.-S. Ling and R. Li, USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC ; and W. Zhang, Greenhouse Branch, Crop Research and Extension Division, Alberta Agriculture and Rural Development, Brooks, Alberta, Canada

In early 2013, greenhouse cucumber growers in Alberta, Canada, observed virus-like disease symptoms on mini-cucumber (*Cucumis sativus*) crops (e.g., 'Picowell'). Two types of symptoms were commonly observed, green mottle mosaic and necrotic spots. In the early infection, young leaves of infected cucumber plants displayed light green mottle and blisters. The infected plants were stunted in growth, with darker green blisters and green mottle mosaic symptoms on mature leaves. Disease incidence varied from one greenhouse to another. In some severe cases, diseased plants were widely distributed inside the greenhouse, resulting in 10 to 15% yield losses based on grower's estimation. Nine symptomatic samples were collected and subjected to total RNA isolation using the TRIzol reagent (Invitrogen, Carlsbad, CA). Laboratory analyses were conducted using real-time RT-PCR systems for *Cucumber green mottle mosaic virus* (CGMMV) (1), *Melon necrotic spot virus* (MNSV, Ling, unpublished), and *Squash mosaic virus* (SqMV) (3). All nine samples were positive for CGMMV and seven of them were in mixed infections with MNSV. Two samples were selected for validation for the presence of CGMMV using conventional RT-PCR (2) with a new primer set (CGMMVMP F1: 5'-ATGTCTCTAAGTAAGGTGTC-3' and CGMMV3'UTR R1: 5'-TGGGCCCTACCCGGGG-3') and two previous online published primer sets, one for CGMMV MP (5' TAAGTTTGCTAGGTGTGATC-3', GenBank Accession No. AJ250104 and 5' ACATAGATGTCTCTAAGTAAG-3', AJ250105), and another for CGMMV CP (5' ACCCTCGAAACTAAGCTTTC-3', AJ243351 and 5' GAAGAGTCCAGTTCTGTTTC-3', AJ243352). The expected sizes of RT-PCR products were obtained and sequenced directly. Sequences from these three products overlapped and generated a 1,282-bp contig (KF683202). BLASTn analysis to the NCBI database showed 99% sequence identity to CGMMV isolates identified in Asia, including China (GQ277655, KC852074), India (DQ767631), Korea (AF417243), Myanmar (AB510355), and Taiwan (HQ692886), but only 92% sequence identity to other CGMMV isolates identified in Europe, including Spain (GQ411361) and Russia (GQ495274), and 95% to CGMMV isolate from Israel (KF155231). The strong sequence identity to the CGMMV Asian isolates suggests that the Canadian CGMMV isolate identified in Alberta was likely of Asian origin. In two bioassay experiments using one sample prepared in 0.01 M phosphate buffer, the similar green mottle mosaic symptoms were observed on systemic leaves in the mechanically inoculated plants and the presence of CGMMV, but not MNSV, was confirmed through real-time RT-PCR on four different cucurbits, including three *Cucumis sativus* cultivars (six plants in 'Marketer,' five plants in 'Poinsett 76,' six plants in 'Straight 8'), seven plants of *C. melo* 'Athena,' six plants of *C. metulifer* (PI201681), and two plants of *Citrullus lanatus* 'Charleston Gray.' To our knowledge, CGMMV has only been reported in Asia, Europe, and the Middle East, and this is the first report of CGMMV in the American continents. CGMMV is highly contagious and is seed borne on cucurbits. With the increasing trend in growing grafted watermelon and other cucurbits in the United States and elsewhere, it is even more important now that a vigilant seed health test program for CGMMV should be implemented.

References: (1) H. Chen et al. J. Virol. Methods 149:326, 2008. (2) K.-S. Ling et al. Plant Dis. 92:1683, 2008. (3) K.-S. Ling et al. J. Phytopathol. 159:649, 2011.

