



## First outbreak of *Chrysanthemum stem necrosis virus* (CSNV) on potted *Chrysanthemum* in Belgium

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In Belgium, potted *Chrysanthemum x morifolium* cultivation covers about 400 ha, of which 375 ha is in open fields and the remaining portion in glasshouses. This represents nearly half of total European production. Among the most important pests and diseases with a significant impact on chrysanthemum production are the western flower thrips (*Frankliniella occidentalis*) and spotted wilt (*Tomato spotted wilt virus* TSWV), for which the thrips acts as a vector. So far, *Chrysanthemum stem necrosis virus* (CSNV), a distinct member of the genus *Tospovirus* (Bezerra *et al.*, 1999), has only been described in a limited host range and with a restricted geographical distribution. However, even with limited data available on losses caused by CSNV in its major areas of distribution i.e. Brazil and Japan, the phytosanitary risk to chrysanthemum and tomato crops, in areas where its vector *F. occidentalis* is known to occur, is considered high. Moreover, while symptoms of CSNV and TSWV are comparable, CSNV-induced disease often develops faster and is more destructive (Bezerra *et al.*, 1999). In October 2012, plants suspected to be virus-infected in showing wilting and having numerous necrotic lesions surrounded by yellow areas, were received from a seasonal chrysanthemum grower, growing under glass in western Belgium. Initially TSWV infection was suspected but the necrotic zones quickly extended to stems (Fig. 1), ultimately killing most of the crop in the case of cvs. Ludo and Jorca Pink (Fig. 2). Losses for the cv. Mirage Yellow (Fig. 3) also grew to more than 50%. Cultivar Miral White and an assorted cultivar mix were also infected, although symptoms remained less severe. Cuttings from all of these cultivars originated from Brazil.

After an initial screen for TSWV with TSWV reagent set (Art. No. 190165, BIOREBA, Switzerland), followed by a CSNV test (AS-0529; DSMZ, Germany), both by DAS-ELISA, further detection and identification of the virus by PCR was based on the nucleocapsid (*N*) gene sequence. Total RNA was extracted from leaf and stem tissue using a Spectrum Total Plant RNA kit (Sigma-Aldrich, Belgium). The specific CSNV-N5/N3 PCR primers developed by Takeshita *et al.* (2011) were used to amplify the *N* gene ORF (approx. 950 bp) and these fragments were cloned (Clonejet PCR cloning kit, Thermo Scientific, Belgium) and sequenced. CSNV infection was confirmed on all sampled plants with symptoms from all five infected cultivars, and no variability was noticed in

their respective *N* gene sequences. The consensus 937 bp sequence of the *N* gene is available from GenBank (Accession No. KC525102).

Symptomless plants did not test positive for CSNV nor any other tospoviruses. Infected plant material from the cv. Ludo was mechanically inoculated on a series of indicator plants and resulted in symptoms corresponding to those described by Bezerra *et al.* (1999). RT-PCR on symptomatic indicator plants again confirmed the presence of CSNV. This is the first report of a CSNV outbreak in Europe in 10 years. Previous outbreaks in the Netherlands (Verhoeven *et al.*, 2003), Slovenia (Ravnikar *et al.*, 2003) and UK (Mumford *et al.*, 2003) were all declared eradicated (EPPO, 2005). The infected production facility is now under eradication measures and the virus will remain subject to official control in 2013.

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Figure 1



Figure 2



Figure 3

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