New Disease Reports

First report of *Pea necrotic yellow dwarf virus* in The Netherlands

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Pea necrotic yellow dwarf virus (PNYDV) is a nanovirus that was first detected in pea crops (*Pisum sativum*) in Saxony-Anhalt, Germany in 2009 (Grigoras *et al*., 2010). In 2016, PNYDV was detected countrywide in both Germany and Austria not only on pea but also on faba bean (*Vicia faba*), vetch (*V. sativa*) and lentil (*Lens culinaris*) causing severe yield losses (Gaafar *et al*., 2016).

During a routine survey of twelve green pea crops in the Province of Flevoland (The Netherlands), plants with virus-like symptoms were noticed (Fig. 1). Symptomatic plant material was pooled from each field and analysed by ELISA for typical pea viruses: *Alfalfa mosaic virus*, *Cucumber mosaic virus*, luteo-/poleroviruses, *Pea enation mosaic virus* (PEMV), potyviruses, and *Red clover vein mosaic virus*-like carlaviruses, and nanoviruses. PEMV was detected in all fields while luteo-/poleroviruses were found in one field. Two samples each from different pea fields reacted positively using a broad nanovirus monoclonal antibody mixture (Gaafar *et al*., 2016). The lack of reaction with a monoclonal antibody mixture designed to detect only *Faba bean necrotic stunt virus* and *Faba bean necrotic yellows* virus suggested infection with *Pea necrotic yellow dwarf virus* (PNYDV). This was confirmed by PCR using PNYDV-specific primers targeting the eight PNYDV components producing bands of approximately 1 kb (Table 1). All PCR products were cloned using the NEB PCR cloning kit (New England Biolabs, Germany) and at least four clones for each component were sequenced in both directions. The sequences of the eight components of the two Dutch isolates (NL HZ16-186 and NL HZ16-189) had between 96.7 and 99.9% identity with the equivalent PNYDV components of an isolate from Germany and between 96.7 and 99.8% with an Austrian isolate (Table 1). The sequences of the Dutch PNYDV isolates have been deposited in GenBank (KY593279- KY593294).

To our knowledge, this is the first report of PNYDV in The Netherlands. This indicates that nanoviruses are far more spread throughout Europe than previously thought (Grigoras *et al*., 2014). As PNYDV is aphid-transmitted in a circulative, non-propagative manner, it is expected that more nanovirus diseases will occur in the future as changes in climatic conditions (especially milder winters in Central Europe) favour aphid survival thus facilitating the spread of these viruses (Ziebell, 2017).

Figure 1

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Table 1: List of the primers used for *Pea necrotic yellow dwarf virus* identification and pairwise comparisons between the sequences of the Dutch isolates (NL HZ16-186 and NL HZ16-189) and isolates from Austria (GenBank Accession No. KC979043- KC979050) and Germany (GU553134 and JN133279-JN133285).

PNYDV component	Primer name	Primer sequence	Pairwise comparisons	References	NL HZ16-186	NL HZ16-189
					Identity (%)	
DNA-C	priPeaCdir	GCCGGAAGCTTGCCGGACTGACGGAG		KC979045	99.2	99.0
	priPeaCrev	AGCTTCCGGCAAGACGCAGTAATTG		JN133280	99.5	99.1
DNA-M	priPeaMdir	TACCTGAACGTCCTGTATCTT		KC979046	98.7	98.3
	priPeaMrev	TCAGGTACTGAATTACTTGCC		JN133281	98.3	97.4
DNA-N	priPeaNdir	GAAGAACCCAGGAAGGTGTTGC		KC979047	99.4	98.9
	priPeaNrev	GGTTCTTCCAATTTACCTTTCATGG		JN133282	99.9	99.2
DNA-R	priPeaRdir	GGAATACCAAGGTGAGTTACGG		KC979043	99.8	99.7
	priPeaRrev	TATTCCCTGAGAGTCCCGGAC		GU553134	99.8	99.5
DNA-S	priPeaSdir	AACCTCCGGATATCACCAGAT		KC979044	99.3	98.7
	priPeaSrev	CCGGAGGTTTTATTTCAAAACCAAC		JN133279	99.3	99.8
DNA-U1	priPeaU1dir	TGGTGAAGAAATTGCAGGTGAT		KC979048	98.0	98.7
	priPeaU1rev	TTCACCAGTTTCTCGTCAGAAC		JN133283	98.3	98.8
DNA-U2	priPeaU2dir	GATCAAGAACAAGGTTAGTTTATG		KC979049	98.2	96.7
	priPeaU2rev	TCTTGATCGGAGACGAACTGGA		JN133284	98.2	96.7
DNA-U4	priPeaU4dir	ATCAAGTCTGAAGATGATACG		KC979050	99.1	99.3
	priPeaU4rev	GACTTGATTTCAACATCTCTTTCAC		JN133285	99.8	99.7

Figure 2

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