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Studies on grass viruses in Austria

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Abstract

Hitherto only limited information was available on viruses infecting *Poaceae* in Austria. Besides the *Barley yellow dwarf* complex only the occurrence of *Wheat dwarf virus* has been reported. In the present study some new viruses were detected: the soil-borne bymoviruses *Barley yellow mosaic* virus and *Barley mild mosaic* virus were detected on winter barley; *Brome streak mosaic virus* was isolated from *Hordeum murinum*; *Cocksfoot streak mosaic virus* and *Ryegrass mottle virus* were isolated from *Dactylis glomerata*, the latter being the first report for Central Europe. Other, yet unidentified viruses were present in wild grass species along the field edge. Further studies are necessary to identify and characterise these viruses. It can be concluded that the extent of virus infections in grasses is higher than expected and that the influence of virus infections in nature has been significantly underestimated.

Keywords

Arrhenatherum elatius, cocksfoot streak mosaic virus, *Dactylis glomerata*, ryegrass mottle virus, tritimovirus

Introduction

While several reports on virus diseases of grasses have been published during the past decade (LAPIERRE and SIGNORET 2004, PLUMB 2006), only limited information is available on viruses infecting *Poaceae* in Austria. In addition to viruses of the *Barley yellow dwarf* complex (BYDV-PAV, BYDV-MAV, CYDV-RPV) only the occurrence of *Wheat dwarf virus* (WDV) has been reported (OBERFORSTER 2003, HUSS and PLANK 2008). A range of wild and cultivated grass species can serve as hosts, however, the epidemiology and incidence of these viruses in annual and perennial grasses is largely unknown.

Wheat dwarf virus

The differentiation of the wheat infecting WDV strain from the barley strain (*Barley dwarf virus*, BDV) is possible by direct sequencing using rolling circle amplification (SCHUBERT et al. 2007) or with a BYD specific antibody (RABENSTEIN et al. 2005). By means of a BDY specific monoclonal antibody we identified BDY in winter barley var. 'Hannelore' originating from a field in Antiesenhofen (Upper Austria). It is not known whether the WDV and BDY incidence and strain composition in wild grasses in Austria differs from that in Germany. In a survey conducted last year in Germany we only found BDV in winter- or spring barley and at one point in loose silky bent grass (*Apera spica-venti* L. Beauv.), but never in wheat, spelt or triticale which were infected only by the WDV strain. At one point WDV was also detected in *Poa pratensis* L. and in one sample of spring barley var. 'Grace'.

New viruses

The production of winter barley in Austria is now also threatened by the recently discovered soil-borne bymoviruses *Barley yellow mosaic* virus and *Barley mild mosaic* virus which are currently spreading (HUSS and RABENSTEIN 2012). On the other hand, the cultivation of barley and other cereals in Austria is obviously not endangered by a new mite-transmitted virus recently isolated from wall barley (*Hordeum murinum* L.) plants. The virus from *H. murinum* belongs to the genus *Tritimovirus* (RABENSTEIN et al. 2004) and was identified as an isolate of *Brome streak mosaic* virus (BrSkMV) which was easily transmissible by mechanical inoculation to barley, oats and some winter wheat species (RABENSTEIN et al. 2011). Although the mite species *Aceria tosichella* (Keifer [Amrine] is an expanding pest worldwide (NAVIA et al. 2013) and a population of this mite species transmitted BrSkMV under controlled conditions (STEPHAN et al. 2008), apparently factors such

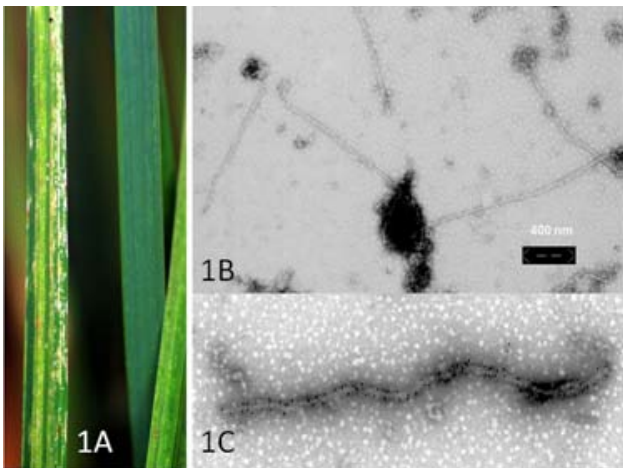

Figure 1: **Symptoms in form of chlorotic and necrotic streaks along the leaf blade on cocksfoot plant sample StP1 (A), containing flexible elongated virus particles about 800 nm in length (B), which were decorated with gold labelled antibodies to *Cocksfoot streak mosaic virus* (C)**

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as adjustment and specialization of the vector population on host plants and low transmission efficiency seem to prevent an effective distribution in central Europe.

Two further new viruses were isolated from cocksfoot plants (*Dactylis glomerata* L.) collected near the experimental station in Lambach/Stadl-Paura (Upper Austria). The first sample (StP1) showed chlorotic and necrotic streaks along the leaf blade (*Figure 1A*) and contained flexible elongated virus particles about 800 nm in length (*Figure 1B*). The virus was detected by ELISA and immunogold labeling with an antiserum to a recently identified tritimovirus detected in *D. glomerata* plants in Germany (*Figure 1C*).

The virus with the preliminary designation *Cocksfoot streak mosaic virus* (CfSMV) has a genome organization characteristic for potyviruses with a single stranded RNA consisting of 9383 nucleotides coding for a polyprotein of 3035 amino acids. The polyprotein of this new virus from cocksfoot shows the highest sequence identity to *Wheat streak mosaic virus*, the type member of the genus *Tritimovirus*. A vector for the CfSMV is unknown but, like other tritimoviruses (BrSkMV and WSMV), it is possibly transmitted by mites (RABENSTEIN et al. 2010).

The other cocksfoot sample (StP2) was representative of plants with symptoms in form of chlorotic streaks or spots on their leaves (*Figure 2A*). Infected plants often died completely in the field or showed heavily bleached leaves after transfer to the greenhouse. Similar symptoms appeared after mechanical inoculation of healthy cocksfoot or barley plants with infected leaf sap. Infected plants contained isometric virus particles with a diameter of about 30 nm (*Figure 2B*).

Plants infected with isolate StP2 reacted in ELISA with antisera to strains of *Ryegrass mottle virus* (RgMoV) isolated in 1997 from ryegrass or brome grass in Germany (RABENSTEIN et al. 1998a). In addition, the serological reactivity was confirmed by IEM decoration assays showing a clumping of virus particles with RgMoV antibodies (*Figure 2C*). RgMoV was first isolated from ryegrasses

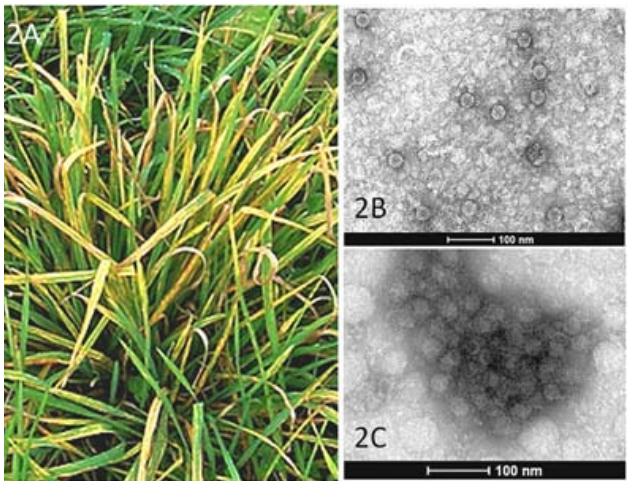

Figure 2: **Chlorotic streaks and spots on cocksfoot plant sample StP2 (A), containing isometrical virus particles with a diameter of about 30 nm (B). Decorated and clumped particles after treatment with antiserum to *Ryegrass mottle virus* (C)**

Figure 3: **Necrotic and dead plants in a cultivated area of Italian ryegrass cv. 'Meroa RvP'**

and cocksfoot in Japan by TORIYAMA et al. (1983). In Germany the virus was first detected in ryegrass breeding clones and in *Bromus* species (RABENSTEIN et al. 1998b). The occurrence of RgMoV in *D. glomerata* plants is new for Central Europe. There are no reports on yield losses caused by RgMoV infections in fodder grasses in the literature. Unexpectedly, however, the virus was found with high incidence in a cultivated area (17 ha) of Italian ryegrass (*Lolium multiflorum* Lam.) cv. 'Meroa RvP' last year at one location in Saxony-Anhalt in Germany (*Figure 3*). A more detailed analysis revealed that more than 60% of the plants in the field grown for dairy cattle feeding were infected by RgMoV causing a considerable reduction in yield and forage quality.

Interestingly, an investigation of several wild grass species along the field edge showed that other, yet unidentified viruses were present. For example, in mixed infected tall oat-grass (*Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl) both flexible, filamentous and isometric viruses were observed by electron microscopy. Similarly, hitherto unknown viruses could be isolated from a tall oat-grass sample collected last year in Austria. The viruses were transmitted by mechanical inoculation to several test plants like winter wheat cv. 'Alcedo', *Lamarckia aurea* L., *Lagurus ovatus* L., and *A. elatius*. With the exception of wheat all inoculated plants contained both forms of virus. Further studies are necessary to identify and characterise these viruses.

Conclusions

From our studies it can be concluded that probably the extent of virus infections in grasses is much higher than expected and that the influence of virus infections in nature has been significantly underestimated. Virus symptoms on wild grasses can differ from those seen in field crops (e.g. obvious foliar discoloration, mosaic, streaks, mottling etc.) and thus may have been overlooked. Further studies are required to test this hypothesis. The situation may be turn out to be more complex since recent findings showed that plant viruses may alter insect behaviour in order to enhance their spread to new hosts (INGWELL et al. 2012).

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