New insights into virus yellows distribution in Europe and effects of beet yellows virus, beet mild yellowing virus, and beet chlorosis virus on sugar beet yield following field inoculation

Roxana Hossain1 | Wulf Menzel2 | Celin Lachmann1 | Mark Varrelmann1

1Department of Phytopathology, Institute of Sugar Beet Research, Göttingen, Germany
2Plant Virus Department, Leibniz-Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Correspondence
Roxana Hossain, Department of Phytopathology, Institute of Sugar Beet Research, Holtenser Landstraße 77, D-37079 Göttingen, Germany.
Email: hossain@ifz-goettingen.de

Funding information
Bundesministerium für Ernährung und Landwirtschaft, Grant/Award Number: 2814901615

Abstract
Beet yellows virus (BYV), beet mild yellowing virus (BMYV), beet chlorosis virus (BChV), and beet mosaic virus (BtMV) cause virus yellows (VY) disease in sugar beet. The main virus vector is the aphid *Myzus persicae*. Due to efficient vector control by neonicotinoid seed treatment over the last decades, there is no current knowledge regarding virus species distribution. Therefore, Europe-wide virus monitoring was carried out from 2017 to 2019, where neonicotinoids were banned in 2019. The monitoring showed that closterovirus BYV is currently widely spread in northern Europe. The poleroviruses BMYV and BChV were most frequently detected in the northern and western regions. The potyvirus BtMV was only sporadically detected. To study virus infestation and influence on yield, viruses were transmitted to sugar beet plants using viruliferous *M. persicae* in quadruplicate field plots with 10% inoculation density simulating natural infection. A plant-to-plant virus spread was observed within 4 weeks. A nearly complete infection of all plants was observed in all treatments at harvest. In accordance with these findings, a significant yield reduction was caused by BMYV and BChV (~23% and ~24%) and only a moderate reduction in yield was observed for BYV (~10%). This study showed that inoculation at low densities mimics natural infection, and quick spreading induced representative yield effects. Within the background of a post-neonicotinoid era, this provides the basis to screen sugar beet genotypes for the selection of virus tolerance/resistance and to test the effectiveness of insecticides for the control of *M. persicae* with a manageable workload.

KEYWORDS
*Myzus persicae*, sugar beet, virus yellows disease

1 INTRODUCTION
Sugar beet (*Beta vulgaris*), a root crop that accumulates sucrose and provides about one quarter of the world’s sugar, is mainly grown in Europe, Asia, and parts of North America (Biancardi et al., 2010). In the EU sugar beet is grown on $1.6 \times 10^6$ ha arable land with a white sugar yield (WSY) of 17.6 Mt in 2018/19, the major producers being France, Germany, and Poland (Wirtschaftliche
HOSSAIN et al.

Vereinigung Zucker/ Verein der Zuckerindustrie, 2020). With the intensification of sugar beet cultivation, some pests and diseases became a major threat for sugar production, restricting the agronomic yield potential of the crop (Bennett et al., 1956). One of the most economically important viral diseases is virus yellows (VY), caused by a complex of different aphid-transmissible virus species. In Europe, beet yellows virus (BYV), beet mild yellowing virus (BMYV), and beet chlorosis virus (BChV) are most abundant, while beet mosaic virus (BtMV) is only rarely observed. The spread of beet western yellows virus (BWYV) is so far restricted to the USA and Asia and not relevant for the European sugar beet cultivation (Stevens et al., 2005; Xiang et al., 2008). Myzus persicae, the green peach aphid, is the main vector for all aphid-transmitted virus species in sugar beet fields (Limburg et al., 1997; Schliephake et al., 2000; Kozlowska-Makulksa et al., 2009).

Beet yellows virus belongs to the genus Closterovirus in the family Closteroviridae. Infections with BYV lead to yellowish discoloration of the older leaves and subsequently reddish necrosis may occur (de Koeijer and van der Werf, 1999). BYV can be transmitted by more than 20 different aphid species in a semipersistent mode of transmission. In addition to M. persicae, Aphis fabae can contribute to virus transmission (Limburg et al., 1997). BYV virions move via the phloem, but can also colonize mesophyll and epidermal cells and have been detected in plasmodesmata that connect the different cell types of the phloem (Dolja, 2003; Dolja & Koonin, 2013).

Beet mild yellowing virus, Beet western yellows virus, and Beet chlorosis virus belong to the genus Polerovirus in the family Luteoviridae. In Beta species, the viruses induce yellow to orange leaf discoloration, which may cause premature foliage death (Lewellen et al., 1999). Poleroviruses are persistently transmitted by their aphid vectors (Gray & Gildow, 2003). Studies have shown that BMYV and BChV are efficiently transmitted by M. persicae (100%) and Macrosiphum euphorbiae (83%-98%) (Kozlowska-Makulksa et al., 2009). Unlike closteroviruses, poleroviruses are strictly limited to the cell types of the host's phloem, that is, parenchyma, sieve elements, and companion cells (Boissinot et al., 2017).

Beet mosaic virus belongs to the genus Potyvirus in the family Potyviridae. Symptoms of BtMV infection initially appear as yellowish speckles before the typical mosaic-like structures appear. In addition, the leaves are often malformed (Dunning & Byford, 1982). BtMV is transmitted via a nonpersistent mechanism (Gallet et al., 2018). The main vectors for the transmission of BtMV in the field are M. persicae and A. fabae (Dusi & Peters, 1999). However, the virus can also be transmitted by many other species such as Myzus ascalonicus (Semal, 1956), M. euphorbiae, Acrithosiphon pisum, Metopolophium dirhodum, and Rhopalosiphum padi (Dusi & Peters, 1999).

Studies to investigate the occurrence and spread of VY species are rare. The most prominent study was published in 2005 and was based on a sampling of around 260 sugar beet leaves with symptoms in 10 countries on three continents (Stevens et al., 2005). BMYV was most frequently found in northern and western regions of Europe, BChV was also found in southern areas of Europe and Chile, and BYV was predominantly detected in southern Europe, Turkey, the USA, and Chile. Field studies to investigate the influence of infection with VY species on sugar beet yield have so far been carried out exclusively with an inoculation density of 100%, meaning that each plant was individually inoculated with at least 10 wingless M. persicae individuals (Smith & Hallsworth, 1990; Stevens et al., 2004). Infection with BYV led to yield losses of up to 47% (Smith & Hallsworth, 1990). Infection with BMYV reduced yield up to 29% (Smith & Hallsworth, 1990) and was shown to result in an 18%-27% reduction in sugar yield. More varying losses in sugar yield of 8%-24% were observed for BChV (Stevens et al., 2004). The field studies showed that BMYV is more damaging to root and sugar yields when inoculated early in the growing season (May and June) compared to BChV, but when plants become infected later (July), BChV has a greater impact on yield (Stevens et al., 2004). BYV infection later than July has only minor effects on sugar beet growth (Smith & Hallsworth, 1990). Yield loss induced by BtMV infection was not observed to be higher than 10% (Dunning & Byford, 1982). However, the studies conducted so far do not resemble natural infestation in the field. The disease starts from individually infected plants in the field, leading to so-called infection patches, and can spread to the entire sugar beet field if supportive conditions are present in the further course of the vegetation period.

As the yellowing viruses were satisfactorily controlled by combating the vector with insecticides from the neonicotinoid class since the early 1990s, VY disease lost its economic importance and working groups across Europe gave up their interest in monitoring the occurrence and distribution of the virus species involved. However, pesticides are coming into focus, concerning costs, safety, environmental impact, and the development of resistance in the target organisms (Luterbacher et al., 2004). Also of particular importance is that seed pelleting with neonicotinoids has been banned in sugar beet cultivation since 2019 in most of the sugar beet-producing countries in Europe. As a result, virus-carrying aphids can colonize the crop earlier in the vegetation period leading to high potential yield losses. Because M. persicae has already developed resistance to the remaining classes of insecticidal active substances, resistance breeding will offer the only practical solution for disease control in the future (Luterbacher et al., 2004).

It is not known how the occurrence of the yellowing viruses has changed after almost 30 years of effective vector control. Hence, the aim of this study was to gain an up-to-date overview of occurrence and distribution of the VY species, concentrating on a number of European countries through countrywide virus monitoring. In addition, by applying an inoculation method with 10% inoculation density, the natural infection course of the disease in a field was simulated in order to determine the yield effects under viral infection. Effective genetic resistance to VY species is currently not available in sugar beet, reflecting the success of former pesticide usage for control and plant breeder’s priorities, for example, higher yield and quality (Luterbacher et al., 2004). The field inoculation experiments carried out in this study provide the basis for establishing screening tests for sugar beet genotypes in order to identify and select resistance/tolerance and to ensure...
yield security even under aphid infestation by growing resistant/tolerant varieties.

2 | MATERIALS AND METHODS

2.1 | Europe-wide virus monitoring: leaf sampling and virus detection

Leaf samples displaying virus-like symptoms were selectively collected from sugar beet fields in 10 European countries (Belgium, Denmark, France, Germany, Hungary [only in 2017], Italy, Netherlands, Sweden, Spain, and UK) over a 3-year period (2017–2019). Thirty leaves per field were taken for sampling; occasionally also individual samples were analysed. The detection of the yellowing virus was carried out by standard DAS- or TAS-ELISA (double/triple antibody sandwich enzyme-linked immunosorbent assay) according to the instructions of the antiserum manufacturer. The detection of BYV and BtMV was performed using specific ELISA tests (DSMZ [German Collection of Microorganisms and Cell Cultures], Braunschweig, Germany), whereas the detection of poleroviruses (LOEWE) was not discriminatory due to the cross-reaction of the antibodies used, but covered all relevant species.

2.2 | Discrimination of poleroviruses by reverse transcription PCR and sequencing

RNA was extracted from fresh or frozen leaf tissue using the RNeasy Plant Mini kit (QIAGEN), following the manufacturer’s instructions. Based on alignments of sequences available in GenBank of the different sugar beet-infecting poleroviruses (BWYV, BChV, BMYV), two highly conserved regions were identified as suitable for the selection of the generic primers 1374BB-s3 (5′-CAGCCAGTGGTTGTGGTC-3′) and 1378BB-as4 (5′-GCTATCGATGAAGAACCATTGCCTT-3′). These primers span a part of the ORF3-encoded coat protein, and amplify a 482 bp fragment (nucleotide positions 3,609–4,090 for GenBank accession no. NC_004756), possibly slightly deviating for individual isolates. Amplified products were sequenced directly following purification using the NucleoSpin PCR Clean-up kit (Macherey-Nagel) following the manufacturer's instructions. The species assignment of the obtained sequences was carried out based on comparison to GenBank reference sequences for BMYV, BChV, and BWYV.

2.3 | Determining impact of virus infection on sugar beet yield parameters under field conditions

In 2019, the test sites were located in Sieboldshausen and Wollbrechthausen near Goettingen, Germany. Seeds of the susceptible cultivar Vasco (Sesvanderhave) were sown in 3-row field plots of 8 m length with 100 plants each in four replicates, by the end of March. The replicates for the two treatments, inoculated and non-inoculated, were in the same block, but were separated from each other by non-inoculated border rows. As inoculation variants, BMYV, BChV, and BYV were chosen. To investigate possible synergistic effects of yellowing virus species in plants, a coinoculation of BChV and BYV was also included in the experiment. For inoculation purposes, seeds were treated only by fungicides (thiram and hymexazole). For control plots and border rows, seeds were additionally treated with neonicotinoid (thiamethoxam) to prevent unintentional virus spread. For the production of viruliferous M. persicae for the inoculation experiments, healthy aphids were placed on virus-infected mother plants that were previously produced in sufficient quantities in the greenhouse for virus acquisition for at least 48 hr. Plants were inoculated at the end of May. A total of 10% of the plants per plot were inoculated with 10 viruliferous wingless aphids. For coinoculation, aphids individually viruliferous for BChV or BYV were placed in a 1:1 ratio on the plants to be inoculated. Monitoring of virus symptom development was performed once per month from July to October (four times). Visual assessment was carried out in front of the plots. At the first observation time point, percentage of plants showing yellowing symptoms was estimated. The plants displaying virus symptoms were then counted, and the numbers compared (data not shown). The counting and estimation yielded nearly the same values, so that at further observation time points only an estimation of the number of plants displaying symptoms was carried out and considered sufficient. In addition to the visual observation of symptoms, 10 randomly selected leaves were taken from each inoculated and noninoculated plot at the time of the first scoring and analysed by DAS-ELISA to confirm either virus infection or absence of virus in noninoculated control plots. At the later scoring time points, no further leaf sampling was performed.

2.4 | Yield and quality analysis

Sugar beets from inoculated and non-inoculated plots were harvested by a sugar beet harvester by the end of October. Beets were washed and freed from leaf residues. Yield was determined by weighing the total plot for each repetition. Beet brei was prepared and analysed according to routine methods of the sugar industry (ICUMSA [International Commission for Uniform Methods of Sugar Analysis], 2007).

2.5 | Statistical analysis

For the field trial with virus inoculation, a completely randomized block design was not practicable because the potential risk for cross-contamination of the different virus species was too high. In order to avoid an unintentional mixing of the virus species, experiments were carried out at two different locations near Goettingen with a randomized design without blocks for each virus species. Because
the effects of the location are difficult to estimate and yield data
for the genotype used were missing, statistical differences between
the different virus species in terms of yield reduction could not be
calculated. The statistical analysis was limited to two samples, which
were tested by a t test to determine the significant differences be-
tween virus-inoculated and corresponding non-inoculated plots in a
fourfold repetition. Significant differences were determined using
SigmaPlot and indicated by *p ≤ .05, **p ≤ .01, and ***p ≤ .001.

3 | RESULTS

3.1 | Europe-wide monitoring to detect VY species in sugar beet

All species of the VY complex could be detected. BYV or the poleroviruses
were predominantly found in all years, whereas BtMV was detected rela-
tively rarely. The virus species were identified in 8 out of 10 European coun-
tries, with no virus detection at all in Hungary and Sweden; however, the
results for Hungary were based on low overall sample numbers. Because
the number of samples per country was not consistent and varied from
one year to the next, the results are expressed as percentage of samples
that tested positive (Table 1).

In 2017, a total of 3,091 samples were collected. In 683 samples,
yellowing viruses could be detected, which represents a per-
centage of 27.9%. Within the infected samples, 18.9% tested positive for
BYV, and 6.8% and 2.2% for the poleroviruses and BtMV, re-
spectively. The country with the highest percentage of BYV-infected
samples was Spain (63.3%), followed by the UK (56.8%) and France
(30.9%). Lower percentages were found in Germany (16.6%), the
Netherlands (7.3%), and Denmark (3.2%). In Belgium, Italy, Hungary,
and Sweden, BYV was not detected at all. Countries with the high-
est percentage of polerovirus-infected samples were France and the
UK (25.6% and 13.5%, respectively), followed by Germany (4.6%)
and Italy (3.3%). In the remaining countries there was no evidence for
polerovirus occurrence. BtMV was detected in the UK (24.3%),
France (5.5%), Germany (1.3%), and the Netherlands (0.8%).

In 2018, a total of 1,611 samples were collected, of which 203
samples were infected by yellowing viruses, which represents a per-
centage of 12.6%. Within the infected samples, 5.6% reacted pos-
tive for BYV, 7.1% for the poleroviruses, and 0.8% for BtMV. BYV
was detected in three countries, in France (30.3%), the UK (4.8%),
and Germany (2.5%). Poleroviruses were detected in Germany
(8.3%) and the UK (3.6%). BtMV was found in Spain (13.3%), the UK
(7.5%), and Germany (0.4%).

In 2019, in which the use of neonicotinoid seed treatment was pro-
hibited in most of the European monitoring countries (except Belgium
and Hungary), a total of 1,334 samples were collected. Yellowing virus
species were detected in 480 samples, which represents a per-
centage of 28%. Among the infected samples, 9.7% were positive for BYV. 25.7%
for the poleroviruses, and 0.5% for BtMV. The country with the high-
est percentage of BYV infection was Spain (91.2%), followed by the UK
(35.5%). Lower percentages were observed for Italy, Germany, Belgium,
France, and the Netherlands, ranging from 1% to 7.4%. The countries
with highest percentages of polerovirus infections were France (67.3%),
the Netherlands (40.8%), Belgium (26.7%), and the UK (25.8%), followed
by Germany (18.9%), Spain (5.9%), and Italy (3.3%). No poleroviruses were
detected in the remaining countries. BtMV was detected only in Spain
(2.9%) and Germany (0.8%).

3.2 | Polerovirus discrimination and verification of
virus infection

As the serological method applied did not distinguish between the
different sugar beet-infecting polerovirus species, the assignment to

| TABLE 1 | Detection of sugar beet infecting viruses (beet yellows virus [BYV], poleroviruses, and beet mosaic virus [BtMV]) in monitoring in 10 European countries in 2017-2019 |
| Country | No. of samples | BYV (%) | Poleroviruses (%) | BtMV (%) |
| | 2017 | 2018 | 2019 | 2017 | 2018 | 2019 | 2017 | 2018 | 2019 |
| Germany | 2,290 | 961 | 610 | 16.6 | 2.5 | 7.4 | 4.6 | 8.3 | 18.9 | 1.3 | 0.4 | 0.8 |
| UK | 74 | 67 | 31 | 56.8 | 4.8 | 35.5 | 13.5 | 3.6 | 25.8 | 24.3 | 7.5 | 0 |
| Netherlands | 123 | 52 | 76 | 7.3 | 0 | 1.3 | 0 | 0 | 40.8 | 0.8 | 0 | 0 |
| France | 363 | 208 | 269 | 30.9 | 30.3 | 3.0 | 25.6 | 0 | 67.3 | 5.5 | 0 | 0 |
| Belgium | 30 | 30 | 30 | 0 | 0 | 3.3 | 0 | 0 | 26.7 | 0 | 0 | 0 |
| Italy | 60 | 60 | 30 | 0 | 0 | 6.7 | 3.3 | 0 | 3.3 | 0 | 0 | 0 |
| Denmark | 31 | 82 | 100 | 3.2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sweden | 30 | 121 | 120 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Spain | 60 | 30 | 68 | 63.3 | 0 | 91.2 | 0 | 0 | 5.9 | 0 | 13.3 | 2.9 |
| Hungary | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 3,091 | 1,611 | 1,334 | 18.9 | 5.6 | 9.7 | 6.8 | 7.1 | 25.7 | 2.2 | 0.8 | 0.5 |

Note: Samples (30 samples per field and occasionally individual samples) were analysed by DAS/TAS-ELISA.
In 2017–2019, a total of 186 samples from different countries were subsequently selected to determine the exact species.

In Germany, BMYV was predominantly found, in 20 of 28 samples, and BChV in 8 samples. In France, BChV was detected in one sample. In the UK, BChV was found in six of nine samples, and BMYV in the remaining samples.

In 2018, samples from the same countries were chosen, 23 samples from Germany, 15 from France, and one from the UK. In the samples from Germany, 22 were infected with BMYV and one with BChV. In France, BChV was predominantly found (17 of 18 samples). BMYV was detected in one sample. In the UK, BChV was found in six of nine samples, and BMYV in the remaining samples.

In 2019, in addition to the three aforementioned countries (Germany 45, France 28, and UK 5 samples), samples from Spain (4 samples), the Netherlands (8 samples), and Belgium (2 samples) were analysed. In Germany, there was a relatively balanced ratio of 20 samples infected with BMYV and 25 with BChV. In France, 11 samples were infected with BMYV and 17 with BChV. In the UK, two samples were found to be infected with BMYV and three with BChV. In Spain, three of the four analysed samples were shown to contain BMYV and one BChV. In the Netherlands, five of eight samples were infected by BMYV and three by BChV. Both samples analysed from Belgium were infected with BMYV. BWYV was not identified in any of the samples tested during the 3 years.

Table 2: Identification of the polerovirus species Beet mild yellowing virus (BMYV), Beet chlorosis virus (BChV), and Beet western yellows virus (BWYV) by reverse transcription PCR in selected ELISA-positive samples in 2017–2019

| Year | Country | No. of samples | BMYV | BChV | BWYV |
|------|---------|----------------|------|------|------|
| 2017 | Germany | 28             | 20   | 8    | 0    |
|      | France  | 18             | 1    | 17   | 0    |
|      | UK      | 9              | 3    | 6    | 0    |
| 2018 | Germany | 23             | 22   | 1    | 0    |
|      | France  | 15             | 1    | 14   | 0    |
|      | UK      | 1              | 0    | 1    | 0    |
| 2019 | Germany | 45             | 20   | 25   | 0    |
|      | France  | 28             | 11   | 17   | 0    |
|      | UK      | 5              | 2    | 3    | 0    |
|      | Spain   | 4              | 3    | 1    | 0    |
|      | Netherlands | 8       | 5    | 3    | 0    |
|      | Belgium | 2              | 2    | 0    | 0    |

In contrast, the combination of BYV and BtMV, and even triple infections with poleroviruses, were more common (data not shown).

3.3 Symptom development after M. persicae inoculation and virus transmission in the field

First symptoms for BMYV, BChV, BYV, and the coinoculation of BChV and BYV, occurred around 3 weeks after M. persicae inoculation (Figure 1). Symptoms first appeared on plants inoculated with M. persicae and then spread to neighbouring plants and the entire plot. Typical BYV symptoms were yellowing of the leaf margins as well as necrotic spots. BMYV and BChV in single infection showed typical yellowing, which initially occurred at the leaf margins, but later spread over the entire leaf surface. The necrotic spot expression that was observed for BYV became more pronounced in coinfection with BChV. In the later course of the vegetation period, the yellowing for all virus species became more intense, and leaves developed necrosis and died off. In addition, there was a massive regrowth of heart leaves. Results of the estimated infection rates for all scoring time points in the plots are displayed in Figure 2.

Verification of virus infection in selected samples from Germany, France, the UK, and Spain by RT-PCR and sequencing detected all possible combinations of mixed virus infection. The mixed infection of a polerovirus and BtMV was found only once in Germany in 2017.

FIGURE 1 Virus symptoms caused by the different virus species in field-grown sugar beets following inoculation. (a) Beet yellows virus, (b) beet chlorosis virus, (c) beet mild yellowing virus, and (d) beet chlorosis virus and beet yellows virus coinoculation [Colour figure can be viewed at wileyonlinelibrary.com]
A 100% infection rate in the plots was reached at the third scoring time point at the beginning of September.

Infection with BChV resulted in 81% of plants already displaying symptoms at the first scoring time point. A total of 70% of the randomly collected leaves were ELISA-positive (data not shown). By the end of July, 94% of the plants within the plots showed symptoms, and 100% infection rate was reached at the third scoring date at the beginning of September.

In BYV-infected plots, 55% of the plants showed yellowing at the first scoring time point. ELISA testing of 10 randomly selected leaves resulted in 50% virus-positive plants. The infection rate increased continuously to 90% until the beginning of September, but showed no change at the last date in October, which means that an infection rate of 100% was not reached here until harvest.

In BYV-infected plots, 55% of the plants showed yellowing at the first scoring time point. ELISA testing of 10 randomly selected leaves resulted in 50% virus-positive plants. The infection rate increased continuously to 90% until the beginning of September, but showed no change at the last date in October, which means that an infection rate of 100% was not reached here until harvest.

In coinoculated BChV/BYV plots, 91% of the plants already showed yellowing symptoms at the first scoring time point. Randomly selected leaves were 62.5% virus-positive for poleroviruses and 35% virus-positive for BYV (data not shown). Within the coinoculation plots, infection rates of 100% were already reached by the end of July (second scoring). Due to the quick spread of M. persicae distributing the virus within the plots, a significant occurrence of yellowing was also observed in the control plots. However, infection rates were below 15% and occurred late in corresponding BChV and BYV/BChV non-inoculated plots, and were negligible in corresponding BYV and BMYV non-inoculated plots. Virus detection via ELISA was not performed.

### 3.4 Effect of virus infection on root yield and white sugar yield

The root yield data (t/ha) are displayed in Table 3. The mean root yield in non-inoculated plots was 105 t/ha. This value is slightly above the average yield achieved in the same year on nearby sites, for

| Virus species | Root yield (t/ha) Inoculated | Non-inoculated | WSY (t/ha) Inoculated | Non-inoculated |
|---------------|-----------------------------|----------------|-----------------------|----------------|
| BMYV          | 78.75 ± 3.50                | 101.83 ± 1.75  | 12.04 ± 0.60          | 17.00 ± 0.14   |
| BChV          | 78.37 ± 1.32                | 102.79 ± 0.46  | 12.03 ± 0.20          | 17.06 ± 0.15   |
| BYV           | 100.41 ± 3.78               | 111.11 ± 3.20  | 16.50 ± 0.60          | 18.60 ± 0.53   |
| BChV/BYV      | 64.77 ± 7.60                | 102.08 ± 2.49  | 9.71 ± 1.20           | 17.05 ± 0.45   |

Note: All values are mean values ± SD.
example, in federal variety trials (89.45 t/ha) in Sieboldshausen and Wollbrechtshausen, Goettingen. Plots infected with BMYV and BChV achieved nearly the same average root yield of approximately 78 t/ha. This was a significant root yield reduction of 23% (p ≤ .0001) for BMYV and 24% (p ≤ .0001) for BChV compared to corresponding non-inoculated control plots. In plots infected with BYV, approximately 100 t beet/ha were harvested. Compared to the noninfected control, this was a significant reduction of 11% (p ≤ .005). The lowest yield was obtained in plots coinoculated with BChV and BYV; on average 65 t beet/ha was harvested. This led to the highest significant reduction of 37% (p ≤ .0001) as compared to all other treatments (Figure 3).

The mean WSY (t/ha) of inoculated and non-inoculated plots are summarized in Table 3. A reduced root weight after infection with the different VY species resulted in a corresponding reduction in sugar content, and accordingly WSY. Our data show that after virus infection, potassium was significantly reduced, while sodium as well as amino-N contents were significantly increased, leading to a deterioration of beet processability and hence reduction of the WSY (data not shown).

Infection with BMYV and BChV led to equal values in WSY (12 t/ha). This was a reduction of 29% (p ≤ .0001) compared to control plots. Sugar beets inoculated with BYV showed a WSY of 16.5 t/ha, which was a reduction of 11% (p ≤ .0023). The reduction in WSY was highest in the coinoculation treatment with BChV/BYV (9.7 t/ha) compared to the average of non-inoculated controls (17.5 t/ha), which represented a reduction of 43% (p ≤ .0001; Figure 3).

4 DISCUSSION

The Europe-wide monitoring of the virus species involved in VY from 2017 to 2019 provides an up-to-date overview of the occurrence and spread of BYV, the poleroviruses BMYV and BChV, and BtMV in 10 countries (Belgium, Denmark, France, Germany, Hungary, Italy, Netherlands, Sweden, Spain, and UK). Apart from BWYV, all known species of the VY complex could be identified. Viruses were detected in samples from eight of the 10 countries involved. In Hungary and Sweden, none of the virus species could be detected, although the occurrence of the yellowing viruses has been proven in these countries. It is possible that the virus contents were below the limit of detectability by ELISA. However, the total rate of actual virus-infected samples was in general rather low, considering that leaves

![FIGURE 3](image-url) Effect on sugar beet root yield and white sugar yield (WSY) after inoculation with beet mild yellowing virus (BMYV), beet chlorosis virus (BChV), beet yellows virus (BYV), and beet chlorosis virus/beet yellows virus coinoculation, respectively, in relation to the respective non-inoculated control plot. Bars indicate the standard deviation from four biological replicates. Significances were determined by t test, **p ≤ .01; ***p ≤ .001
with virus-like symptoms were selected for sampling. The symptom of leaf yellowing due to a virus infection can be quite an unspecific symptom, and can easily be confused, especially if the sampler is inexperienced. Thus, yellowing of leaves can be caused not only by viral infections, but also a variety of other biotic or abiotic factors, for example, soil compaction and low microbial activity in the soil can cause nutrient (magnesium or boron deficiency) and water stress. Insects like the capsid bugs from the Miridae family can also cause yellowing due to their sucking activity on the leaves (Draycott, 2008). Some fungal diseases caused by Fusarium or Verticillium species can also cause chlorotic tissue (Hanson & Jacobsen, 2009; Strausbaugh et al., 2016). Finally, herbicides can have toxic properties that can lead to leaf yellowing (Draycott, 2008).

The detection of poleroviruses showed a trend towards an increased occurrence in 2019. Whereas only 6.8% and 7.1% of the samples tested positive for poleroviruses in 2017 and 2018, respectively, this proportion increased to 25.7% in 2019. It is striking that countries in which no poleroviruses could be detected in 2017 and 2018 showed a considerable increase in 2019. This is particularly true for the Netherlands and Belgium, but also for Spain, where beet-infecting poleroviruses have been detected rather rarely in the past (Stevens et al., 2005). A clear upward trend could also be observed in France and Germany, with France showing overall the highest values for poleroviruses in 2017 and 2019. However, as Belgium was authorized to continue to use seed pelleting containing neonicotinoids in 2019, it must be assumed that the changes in the occurrence of the virus species also largely depend on natural and disease specific variations that have been observed over decades for yellowing viruses (Hauer et al., 2017). It has been reported to depend, for example, on the availability of the primary winter host Prunus spp. for M. persicae, the climate, the antagonist potential, the time of aphid attack during the vegetation period, and also the efficacy of control measures against virus vectors (Bass et al., 2014; Hauer et al., 2017). The virus monitoring carried out in this study confirms the previously observed year-to-year fluctuations of virus occurrence. In Germany, for example, BMYV clearly dominated BChV in 2017 and 2018, while in 2019 BMYV and BChV were both detected with similar frequency. In France, on the other hand, BChV dominated in the monitoring years 2017 and 2018, whereas in 2019 BMYV was also identified more frequently. Due to the relatively small number of samples analysed for the other countries, a meaningful evaluation is not possible.

The occurrence of BYV did not follow a clear trend, but reflects the natural fluctuations that were reported from previous studies (Stevens et al., 2005). According to this virus monitoring, BYV seems most widely distributed in Spain. However, contrary to previous observations, southern Europe is no longer the main area of distribution. BYV has also been detected in central and northern European countries. This means that virus resistance/tolerance traits need to be identified against several virus species, and in regions with occurrence of multiple species, combinations of traits might be required to control VY. The country with the most frequent detection of BYV besides Spain was the UK. Looking at the countries in detail, the incidence of BYV was declining for the Netherlands, France, and Denmark. While no BYV was detected in Belgium and Italy in 2017 and 2018, it was detected in 2019. Whether a lack of seed pelleting with neonicotinoids was already having an influence, and whether an increased spread can be expected in the coming years, cannot be concluded at this stage. The occurrence and spread of BtMV can generally be classified as very low, which confirms its currently assumed economic insignificance for European sugar beet cultivation.

In our study we could show that in some countries mixed infections of BYV, BtMV, and the poleroviruses are important factors to be considered. Our analysis showed that all possible combinations of virus species could be detected in the same sample. To what extent the viruses in mixed infections are transmitted simultaneously by a single aphid or in independent steps to sugar beets is still unknown. The occurrence of the different yellowing viruses should be further monitored in the coming years, in particular in the light of a longer lasting ban of the neonicotinoids.

The field trial was designed to provide up-to-date information on the symptom development and potential yield reduction as well as quality effects under nearly natural conditions. In order to ensure this, a method with 10% inoculation density was established. Considering that in nature only about 0.8-1.8% of the M. persicae adults are actually viruliferous (Stevens et al., 1995), the application method of 10% inoculation density simulates a strong natural infection pressure, in contrast to the formerly used 100% inoculation density; however, it is still closer to the natural conditions in a field. The study aimed to generate measurable yield effects even at lower inoculation densities than previously applied, primarily to reduce the amount of work required. In the case of transmission of M. persicae, one must also expect that some insects may be decimated by injuries, antagonists, or even wind and heavy rain.

The virus species of the VY complex exhibited different dynamics within the plots and expressed symptoms at different levels. In our field trial, virus symptoms of BChV spread faster within the plots than those of BMYV. The typical BChV-induced yellowing was particularly pronounced in contrast to BMYV infection, where only mild yellowing was observed on less than half of the plants at the first scoring time point. It was shown that BYV spread more slowly and did not reach a 100% infection rate like the individual poleroviruses. However, symptom severity was intensified as soon as BYV was inoculated together with the polerovirus BChV, so that infection rates of 100% were already reached at the second scoring time point at the end of July (about 8 weeks after infection). Similar observations have already been reported by Wintermantel (2005), who investigated synergistic effects in greenhouse coinoculation experiments between BYV, BtMV, and the polerovirus BWYV, which has a similar significance in the USA as BMYV in Europe. It was shown that symptom expression was increased and synergistic effects on stunting and plant biomass production occurred during coinfection (Wintermantel, 2005).

The ELISA test performed at the first symptom scoring primarily confirmed the authenticity of the infection and the accuracy of the symptom evaluation. The values from the visual assessment and the ELISA test do not necessarily need to be identical, as it is to be expected that symptomless plants may also harbour viruses.
A certain percentage of plants with symptoms could also be detected in the control plots. This indicated incomplete control of virus spread, because no insecticide spray application was made during the course of the vegetation period. Furthermore, the natural influx of viruliferous aphids cannot be excluded. However, the symptoms appeared so late that only a very low effect on root yield was measured.

Each virus species in the VY complex differs in its effect on yield (Stevens et al., 2004; Wintermantel, 2005). Single infection of BMYV and BChV led to approximately identical yield losses of about 20%–25%. This is consistent with studies in which a much higher inoculation density was chosen. Previous studies described BChV as less damaging and yield losses more variable (8%–24%) than those caused by BMYV. However, higher impact on yield caused by BChV compared to BMYV was observed when plants were inoculated later in the growing season (Stevens et al., 2004). For BMYV, Stevens and associates showed that infection leads to 18%–27% losses in sugar yield. Our studies again reflect the fact that an efficient virus spread is already guaranteed at a lower inoculation density, leading to identical results to previous studies that used densities of 100%, and thus both material and working time can be saved in conducting such experiments.

Surprisingly, the yield losses in BYV-infected plots were comparatively low, which differs from previous studies. BYV was always described as the most economically relevant virus, which generates yield losses of up to 50% (Smith & Hallsworth, 1990). Clover et al. (1999) described the infection with BYV as markedly decreasing sugar yield, which is related to the reduction of storage root growth. Another study on leaf growth and leaf area index (LAI) showed that BYV caused a higher reduction in leaf light interception than BMYV, resulting from a lower LAI and a higher proportion of yellowed leaf area after BYV infection (de Koeijer & van der Werf, 1999). The mechanism by which BYV reduces sugar beet growth and root yield is unknown (Clover et al., 1999). Nevertheless, these data are to be considered as preliminary results, as only one sugar beet variety, one location, and one year has been investigated so far. If our findings are confirmed, the relevance and order of importance of the different virus species may need to be reconsidered.

As already expected following the observations of strong symptoms and the early achievement of infection rates of 100%, the highest yield loss of about 40% occurred in the plots with the coinoculation of BChV and BYV. Due to the fact that in a single infection with BYV the yield decreases only moderately, we assume that poleroviruses might have greater relevance for reduction of yield and quality parameters. Furthermore, considering the preliminary data on coinoculation of BYV with BChV, showing that BYV causes serious yield losses and quality reduction, this may be due to synergism with other yellowing viruses. Therefore, it will be important to repeat such coinoculation experiments with different virus combinations to study the synergism effects in more detail.

With the ban on neonicotinoid seed treatment since 2019, sugar beet cultivation will face new challenges. Sugar beet seedlings are no longer protected, especially in the critical emergence phase. Aphids can colonize the plants earlier, partly because the changed climatic conditions mean that more adults, possibly viruliferous individuals, can survive the winter. This not only affects the time of first colonization, but consequently also the yield that can be achieved from infected fields at the end of the vegetation period. The effect of the remaining classes of insecticidal active substances may be severely limited in regions where M. persicae populations with resistance properties occur, so that control via crop protection must be assessed as very critical. In order to keep yields stable even after M. persicae infestation, it is essential to breed virus-tolerant/resistant varieties. The challenge of breeding will be that VY is not just one pest, but a number of viruses from different virus families are involved, and therefore solutions for overall control must be found via conserved resistance mechanisms. For this purpose, it is important to continue virus monitoring, as it is not yet possible to estimate how the occurrence of the virus species will change in the growing regions without the use of neonicotinoids. The breeding process should also be supported by the determination of aphid flight times and by the identification of viruliferous M. persicae, and also resistance properties against insecticides for spray application, in order to be able to achieve the goal of bringing tolerant/resistant varieties onto the market.

Artificial field inoculation on which this work is based on provides the prerequisite for supporting breeders in the selection of suitable breeding material. Field inoculation with an inoculation density of 10% is a good option in terms of time and work intensity for screening sugar beet genotypes under natural growing and inoculation conditions. This will enable farmers to ensure a secure harvest in the near future by growing virus-tolerant or virus-resistant varieties.

ACKNOWLEDGEMENTS

We would like to thank all cooperating European sugar beet breeders of the “Gemeinschaft zur Förderung von Pflanzeninnovation e. V.” (GFPi) that are MariboHilleshoeg, KWS SAAT SE & Co. KGaA, SESVANDERHAVE and Strube Research GmbH & Co. KG for supplying field samples. The project was funded by the Federal Ministry of Food and Agriculture (BMEL) on the basis of a resolution of the German Federal Parliament. The project “Estimation of the risk of infestation by sugar beet yellowing viruses – Foresighted development of control strategies taking into account the neonicotinoid and insecticide resistance problem of the insect vector” (funding code 2814901615) is funded by the Federal Agency for Agriculture and Food (Bundesanstalt für Landwirtschaft und Ernährung, BLE) as part of the programme to promote innovation.

CONFLICT OF INTEREST

There is no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ORCID

Roxana Hossain https://orcid.org/0000-0003-3536-0957
REFERENCES

Bass, C., Puinean, A.M., Zimmer, C.T., Denholm, I., Field, L.M., Foster, S.P. et al. (2014) The evolution of insecticide resistance in the peach potato aphid, Myzus persicae. Insect Biochemistry and Molecular Biology, 51, 41–51.

Bennett, C.W., Price, C. & McFarlane, J.S. (1956) Effects of virus yellows on sugar beet with a consideration of some of the factors involved in changes produced by the disease. American Society of Sugar Beet Technologists Journal, 9, 479–494.

Biancardi, E., McGrath, J.M., Panelia, L.W., Lewellen, R.T. and Stevanato, Pj. (2010) Sugar beet. In: Bradshaw, J.E. (Ed.) Root and Tuber Crops, New York: Springer, pp. 173–219.

Boissinot, S., Pichon, E., Sorin, C., Piccini, C., Scheidecker, D., Ziegler-Graff, V. et al. (2017) Systemic propagation of a fluorescent infectious clone of a polerovirus following inoculation by agrobacteria and aphids. Viruses, 9, 166.

Clover, G.R.G., Azam-Ali, S.N., Jaggard, K.W. & Smith, H.G. (1999) The reaction of sugar beet breeding lines and hybrids to beet chlorosis luteovirus. Journal of Sugar Beet Research, 36, 76.

Dolja, V.V. & Koonin, E.V. (2013) The closterovirus-derived gene expression and RNA interference vectors as tools for research and plant biotechnology. Frontiers in Microbiology, 4, 83.

Draycott, A.P. (2008) Sugar Beet. New Jersey: John Wiley & Sons.

Dunning, A. & Byford, W. (1982) Pests, Diseases and Disorders of the Sugar Beet. Deleplanque: Brooms Barn Experimental Station.

Dusi, A.N. & Peters, D. (1999) The effects of beet yellows virus on the growth and physiology of sugar beet (Beta vulgaris). Plant Pathology, 48, 129–138.

Dolja, V.V. (2003) Beet yellows virus: the importance of being different. Molecular Plant Pathology, 4, 91–98.

Dolja, V.V. & Koonin, E.V. (2013) The closterovirus-derived gene expression and RNA interference vectors as tools for research and plant biotechnology. Frontiers in Microbiology, 4, 83.

Gray, S. & Gildow, F.E. (2003) Luteovirus-aphid interactions. Annual Review of Phytopathology, 41, 539–566.

Hanson, L.E. & Jacobsen, B.J. (2009) Fusarium yellows. In: Harveson, R.M., Hanson, L.E. and Hein, G.L. (Eds.) Compendium of Beet Diseases and Pests. St Paul, MN: APS Press, pp. 28–30.

Hauer, M., Hansen, A.L., Manderyck, B., Olsson, Å., Raaijmakers, E., Stevens, P. & Frese, L. (2004) Sources of resistance to diseases of sugar beet in related Beta germplasm: I. Foliar diseases. Euphytica, 139, 105–121.

Lewellen, R.T., Wisler, G.C., Liu, H.Y., Kaffka, S.R., Sears, J.L. & Duffus, J.E. (1999) Reaction of sugar beet breeding lines and hybrids to beet chlorosis luteovirus. Journal of Sugar Beet Research, 36, 76.

Limburg, D.D., Mauk, P.A. & Godfrey, L.D. (1997) Characteristics of beet yellows closterovirus transmission to sugar beets by Aphid fabae. Phytopathology, 87, 766–771.

Luterbacher, M.C., Asher, M.J.C., DeAmbrogio, E., Biancardi, E., Stevenato, P. & Frese, L. (2004) Sources of resistance to diseases of sugar beet in related Beta germplasm: I. Foliar diseases. Euphytica, 139, 105–121.

Schliephake, E., Graichen, K. & Rabenstein, F. (2000) Investigations on the vector transmission of the Beet mild yellowing virus (BMVY) and the Turnip yellow virus (TuYV). Journal of Plant Diseases and Protection, 81–87.

Semal, J. (1956) Transmission of Beet mosaic virus from Stellaria media and Capsella bursa-pastoris by Myzus ascalonicus Doncaster. Nature, 178, 501–502.

Smith, H.G. & Hallsworth, P.B. (1990) The effects of yellowing viruses on yield of sugar beet in field trials, 1985 and 1987. Annals of Applied Biology, 116, 503–511.

Stevens, M., Smith, H.G. & Hallsworth, P.B. (1995) Detection of the luteoviruses, beet mild yellowing virus and beet western yellow virus, in aphids caught in sugar beet and oilseed rape crops, 1990–1993. Annals of Applied Biology, 127, 309–320.

Stevens, M., Hallsworth, P.B. & Smith, H.G. (2004) The effects of Beet mild yellowing virus and Beet chlorosis virus on the yield of UK field-grown sugar beet in 1997, 1999 and 2000. Annals of Applied Biology, 144, 113–119.

Stevens, M., Patron, N.J., Dolby, C.A., Weekes, R., Hallsworth, P.B., Lemaire, O. et al. (2005) Distribution and properties of geographically distinct isolates of sugar beet yellowing viruses. Plant Pathology, 54, 100–107.

Staats, C.A., Eujayl, I.A. & Martin, F.N. (2016) Pathogenicity, vegetative compatibility and genetic diversity of Verticillium dahliae isolates from sugar beet. Canadian Journal of Plant Pathology, 38, 492–505.

Wintermantel, W.M. (2005) Co-infection of Beet mosaic virus with beet yellowing viruses leads to increased symptom expression on sugar beet. Plant Disease, 89, 325–331.

Wirtschaftliche Vereinigung Zucker/Verein der Zuckerindustrie (2020) Sugar production in the European Union 2018/19. Available at: http://www.zuckerverbvaende.de/zuckerkmarkt/zahlen-und-fakten/eu-zuckerkmarkt/zuckererzeugung.html [Accessed 13 March 2020]

Xiang, H.Y., Shang, X.Q., Han, C.G., Li, D.W. & Yu, J.L. (2008) First identification of Beet western yellows virus on sugar beet and lettuce in China. Plant Pathology, 57, 390.

How to cite this article: Hossain R, Menzel W, Lachmann C, Varrelmann M. New insights into virus yellows distribution in Europe and effects of beet yellows virus, beet mild yellowing virus, and beet chlorosis virus on sugar beet yield following field inoculation. Plant Pathol. 2021;70:584–593. https://doi.org/10.1111/ppa.13306