Analysis of winter wheat and leafhoppers for wheat dwarf virus

- Factors causing outbreaks of wheat dwarf disease

Lina Karm Togo

Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Plant Biology
Agriculture Programme – Soil and Plant Sciences
Master thesis/Department of Plant Biology, SLU, 200
Uppsala 2024

Analysis of winter wheat and leafhoppers for wheat dwarf virus

- Factors causing outbreaks of wheat dwarf disease

Analys av höstvete och dvärgstritar för vetedvärgvirus

- *Faktorer som orsakar utbrott av vetedvärgsjuka*

Lina Karm Togo

Supervisor:	**Anders Kvarnheden, SLU, Department of Plant Biology**
Assistant supervisor:	Elham Yazdkhasti, SLU, Department of Plant Biology
Examiner:	Anders Hafrén, SLU, Department of Plant Biology

Credits:	30 hp
Level:	Second cycle, A2E
Course title:	**Master thesis in Biology, A2E**
Course code:	**EX0898**
Programme/education:	**Agriculture Programme Soil and Plant Sciences**
Course coordinating dept:	**Department of Aquatic Sciences and Assessment**
Place of publication:	**Uppsala**
Year of publication:	**2023**

Title of series:	Examensarbete/Institutionen för växtbiologi, SLU: part number 200
Part number:	
Keywords:	Sweden, *Psammotettix alienus*, DAS-ELISA, cloning, sequencing, BLASTn, BLASTx, MEGA, SDT

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Plant Biology

Abstract

Wheat dwarf disease is caused by wheat dwarf virus (WDV) and is transmitted by the leafhopper *Psammotettix alienus.* The disease has been reported in many European countries, as well as in West Asia, China, and one country in North Africa. There are five strains (ABCDE) of WDV, with isolates of strains A, B and C infecting barley, and isolates of strains D and E infecting wheat. Although mixed infections can occur, in Sweden only the WDV-E strain has been found so far and the hypothesis is that it is still the same strain found in Sweden as the first sequenced WDV isolate from 1969.

During the summer and autumn of 2022, symptomatic winter wheat and leafhopper samples were collected and distributed by the Swedish Board of Agriculture to investigate the presence of WDV from different locations. Winter wheat leaves from three counties (Södermanland, Västra Götaland and Östergötland) and six fields all tested positive for WDV with DAS-ELISA. Leafhopper samples from 28 locations distributed in the counties Uppsala, Stockholm, Södermanland, Örebro and Östergötland were tested for WDV through PCR with primer pair 1877-1896 and 328-309, which gives a PCR product of 1.2 kb. Three leafhopper samples, two from Uppsala (Haga, Säby) and one from Östergötland (Skarpenberga), tested positive and were further characterised with the help of cloning and sequence determination. Four clones from Haga and three clones from Säby were obtained and the sequenced isolates were analysed with the programmes BLASTx, BLASTn, MEGA11 and SDT.

The sequences of the isolates from Haga and Säby showed 97.5% identity to those of other previously sequenced WDV-E isolates including the isolate from 1969. In a phylogenetic analysis, three sequences grouped together with subtype B also of the WDV-E strain. This subtype B has been found before in co-infections with the common genotype of WDV (Ramsell et al. 2008).

In conclusion, the WDV isolates in Sweden have not changed much over time. It is still the same strain, with 97.5% nucleotide identity between isolates. Instead, main factors for outbreaks are probably volunteer plants, symptomless reservoirs, and dry climate with temperatures above 15 °C, which will increase the activity of the leafhopper.

Keywords: WDV, strain, wheat-infecting

Table of contents

Popular science summary

One important plant pathogen is wheat dwarf virus (WDV), which causes yellowing and dwarfed plants with no or poor fed grains in wheat and barley. Infected plants are usually seen as patches of dwarfed and yellowing plants in the field. The virus is spread by a leafhopper called *Psammotettix alienus*, which can feed on infected plants and then transmit the virus to susceptible plants. WDV can persist in many grasses with no symptoms such as ryegrass, common wild oat, common windgrass and meadowgrass. Infected volunteer plants of barley or wheat that are self-set from previous year´s crop may also be a source of inoculum. There are five strains of WDV with isolates of strains A, B, and C infecting barley and D, E infecting wheat. In Sweden, only one of the wheat-infecting strains, E, has been reported. Over the past century, incidences of WDV have been reported from many parts of Europe as well as China, west Asia and northern Africa, causing significant yield losses. To reduce losses, studying the vector, the virus, hosts, environment and monitoring of disease outbreaks and presence of vector/virus are necessary.

In Sweden, wheat dwarf disease has been reported almost every year with large outbreaks in 2009, 2010 and 2017. In this master thesis, symptomatic winter wheat and leafhoppers collected from yellow water traps were sent in by the Swedish Board of Agriculture to test for the presence of WDV. The results showed presence of WDV in all symptomatic winter wheat and three leafhopper samples out of 28 locations. All samples of winter wheat and leafhoppers were from the central parts of Sweden, which is an area commonly affected by wheat dwarf disease.

WDV isolates from positive leafhopper samples were sequenced to identify strain and possible changes in the genome. The virus isolates were almost identical to other isolates sequenced 20 years ago and had a 97.5 % pairwise identity to the first sequenced WDV isolate from 1969. This contributes to earlier reports of low genetic diversity in Swedish isolates in comparison to the region of the Fertile Crescent where higher genetic diversity of cereals and grasses co-exists with a greater variability of WDV.

List of tables

List of figures

Introduction

The general division of cereal virus diseases is into those caused by soil-borne viruses transmitted by the plasmodiophorid *Polymyxa graminis*, and those transmitted by insects, such as aphids and leafhoppers, or by mites (Ordon et al., 2009; Jin et al., 2023). Wheat dwarf disease is one of the virus diseases transmitted by leafhoppers. It is a widely distributed disease reported from several parts of the world among them are Northern Africa, Europe, West Asia and China (Serfling et al., 2017; Rojas et al., 2018). It is caused by wheat dwarf virus (WDV), which belongs to the family of *Geminiviridae* and genus *Mastrevirus*, and of which there are five known strains. WDV infects and causes disease in winter wheat and barley (Muhire et al., 2013; Rojas et al., 2018), but there are also reports of severe infections by WDV in durum wheat, triticale, and rye (Lapierre & Hariri, 2008; Pfrieme et al., 2023). Because plant viruses cannot infect an intact plant the transmission of WDV is dependent on the vector *Psammotettix alienus* or *P. provincialis* (Vacke, 1961; Palmer & Rybicki, 1998; Ekzayez et al., 2011).

The vector is a member of the family Cicadellidae (leafhoppers), which has over 20 000 described species distributed all over the world. Leafhoppers are plant-sucking insects and different species have been found to transmit different viruses in a persistent circulative manner (Hull, 2014). The leafhopper *P. alienus* is the most studied vector for WDV and the persistent circulative manner of transmission through the vector means that the virus circulates through the anterior of the body, back through the salivary glands and into the plant. The virus does not multiply within the vector (Wang et al., 2014). In detail, the virus can be transmitted within minutes from one plant through the vector and to a new plant. The virus can also accumulate inside the leafhopper midgut, hemicoel and salivary glands and stay there for the entire life of the insect (Wang et al., 2014).

Components in the epidemics of wheat dwarf disease are populations of leafhoppers and reservoirs of volunteer plants of cereal crops, like wheat, barley, rye, oat or triticale, which may serve as inoculum of the virus. Symptomless wild grasses, like *Avena fatua, Aspera spica-venti* and *Poa pratensis,* have been testing positive for WDV, some with low incidence but nonetheless they have the possibility to serve

as reservoirs (Ramsell et al., 2008). This is the case also for *Lolium* spp., which are cultivated grasses (Vacke, 1972; Yazdkhasti et al., 2021).

An earlier analysis of ryegrass and leafhopper samples gathered from different counties in Sweden has shown that the number of leafhoppers and incidence of wheat dwarf disease correlate positively. The temperature during autumn was also found to influence the population size of the leafhoppers, where higher temperatures increased the activity of the leafhoppers. Significant damage with periodic re-appearance of wheat dwarf disease in the central parts of Sweden could be linked with changes in agricultural practises and presence of alternative host plants (Yazdkasti, 2022).

1.1 Epidemiology in Sweden

In Sweden, the first reports of what is believed to be infections of WDV on wheat are from the beginning of the 20th century and at that time it was called “slidsjuka” (Jordbruksverket, 2021). Slidsjuka translates into sheath disease which was used to describe the disease symptom of partially stuck ears in the leaf sheaths (Pfrieme et al., 2023). A report by Nilsson-Ehle (1918) discusses the possible cause and symptoms when the disease caused great damage in the years 1902, 1912, 1915 and 1918. The most affected areas were in the mid-part of Sweden mentioning Östergötland as the worst affected county, but the symptoms were also extensive in the counties of Uppsala and Södermanland. The disease was noted in the county of Skåne as well, which is in the south of Sweden, but only to a lesser extent. Later transmission experiments in 1969 proved that *P. alienus* was the vector that transmitted the virus causing wheat dwarf disease in Sweden, also discussing the similarities from the previous reports of the so-called slidsjukan (Lindsten et al., 1970).

After some severe outbreaks during the 1940s, the disease frequency decreased until 1996 when some affected fields again were noticed. The following year, hundreds of fields were affected in the counties of Uppsala, Södermanland and Västra Götaland (Sigvald, 2006). After 1997, wheat dwarf disease has been reported almost annually with severe outbreaks in 2009, 2010 and 2017. So far, only the wheat-infecting strain called WDV-E has been reported, indicating that the genetic diversity of the Swedish WDV population is low (Yazdkhasti, 2022).

1.2 Cicadellidae (leafhoppers)

In Sweden, there are almost 300 different species of leafhoppers and several of them can be pests on different crops (Douwes, 1998). For a very long time, *P. alienus* was considered the only vector for WDV, but one study from Syria found that *P. provincialis* could transmit WDV to barley crops as well (Ekzayez et al., 2011). Other studies have shown that *P. alienus* can transmit other diseases as well such as wheat blue dwarf disease caused by phytoplasma (Wilson & Turner, 2021; Zhao et al., 2010). Determining *Psammotettix* species can be very difficult because it requires the characteristics of the male genitalia, and many studies do not classify the leafhopper species when analysing virus. Therefore, little is known of how many different *Psammotettix spp.* that are vectors and for which diseases (Abt et al., 2018).

Figure 1. Adult leafhopper "Psammotettix alienus" photo by Pavel Šinkyřík is licensed under CC BY-NC 4.0.

Psammotettix alienus is a small brownish insect that was first described by Dahlbom in 1850 (Wilson & Turner, 2021) (Figure 1). The males can measure up to 3.8-4.1 mm and females 4.1-4.3 mm. All Cicadellidae insects have mate recognition and localisation via vibrational signals. The general pattern of leafhopper mating signalling starts with the male attention call and the stationary female responding signal and recordings can tell differences between *Psammotetix* species. After the female has laid her eggs, it is possible that she will mate again (Derlink et al., 2018).

1.3 Symptoms and disease cycle

The characteristics of wheat dwarf disease for winter wheat and winter barley are dwarfing, yellowing of leaf tips and reduced spikes on short rachis. The disease cycle in Sweden can start with infections early in autumn that lead to very weakened or dying plants. A secondary spread of the virus is carried out by the nymphs, which hatch in spring and jumps from infected crops to new plants causing even greater damage (Lindsten & Lindsten, 1999). Symptoms in the field (Figure 2) are often seen as patches of damaged crops in field edges or where the crop has emerged earlier (Vacke, 1972; Jordbruksverket, 2021). Young plants are more susceptible and after the stem extension has started the plant becomes resistant (Lindblad & Sigvald, 2004).

Figure 2. Symptoms in a field affected by WDV infection with a border between two fields of different cultivation practices. The right field had been ploughed and the left had no-till. Picture taken in Västra Götaland county by Lars Johansson (Plant protection agency in Skara, 2022).

In warmer regions, the leafhopper can have up to four generations per year compared to colder regions, as in Sweden, where two generations are more common. Elevated risk of transmission is during autumn when adult leafhoppers lay their eggs in newly sown fields of winter cereals and the second infection in spring when the nymphs start feeding (Figure 3). The virus does not follow the egg, but the nymph can transmit the virus from infected hosts to crops within the field

(Abt et al., 2015). Leafhoppers are more active during dry weather and temperatures above 15°C, and this can cause a greater spread of the disease (Lindblad & Arenö, 2002; Jordbruksverket, 2021).

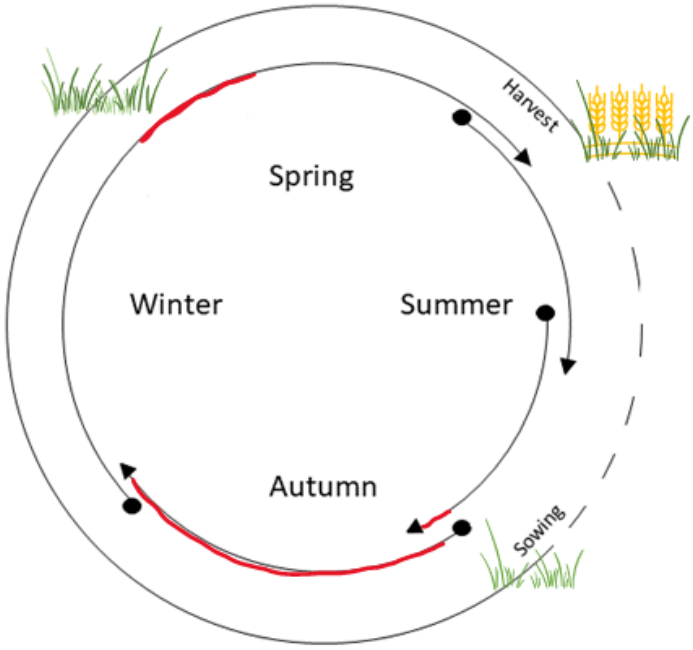

Figure 3. Disease cycle. The inner circle demonstrates four generations of leafhoppers and the outer circle winter cereals. The red label marks periods for risk of transmission of WDV from infected plants to cereal crops via leafhoppers.

1.4 Control measures

This geminivirus group of plant viruses causes great losses in agricultural food, feed, and fibre production globally and is often managed by pesticide use (Rojas et al., 2018). To apply integrated pest management (IPM) against wheat dwarf disease, there are several practises that can be combined. At the moment there are no resistant cultivars, therefore, in temperate regions, it is possible with late sowing in autumn to avoid temperatures above 15°C when leafhoppers are more active. If no crop rotation is applied, ploughing between cultivation periods to avoid volunteer plants and host weeds (figure 2), such as *Apera spica-venti* or *Avena fatua*, can reduce virus reservoirs (Ramsell et al., 2008; Jordbruksverket, 2021).

Other host plants that are possible sources of outbreaks are *P. pratensis* and *P. annua* (Lindsten & Lindsten, 1999), as well as *L. perenne* (Yazdkhasti et al., 2021). It should be kept in mind that cultivated grasses, as such, in large scale or in field edges, can maintain the virus inoculum between years.

The use of pesticides should always be a last resort in IPM considering that the long-term effects for the environment are difficult to measure including the risk of resistant pests. Applying insecticides when certain risk factors have been met can reduce the spread in the field and it is most effective for reducing the secondary infection in spring. These risk factors are measured by a combination of monitoring of leafhoppers and the presence of the virus in leafhoppers, as well as previous disease outbreaks in the area (Rojas et al., 2018) and agricultural practices, including early sowing and reduced tilling.

Climate change has been predicted to increase the population sizes of leafhoppers in autumns, which in turn could increase the incidence of wheat dwarf disease. This is because a warmer climate would prolong their reproduction period and the populations would grow (Roos et al., 2011; Abt et al., 2015). For future aspects some alternatives could be used to avoid the use of insecticides. One alternative is finding resistance genes against the virus and creating more resilient crops. In a report from Buerstmayr and Buerstmayr (2023), two significant QTLs were identified contributing to resistance against WDV in wheat, which could be a valuable resource when breeding for resistance. The other is finding a way to disrupt the leafhoppers' transmission of WDV to the crop. Some studies of the vibrational signalling of insects suggest that disruption of mating patterns could reduce disease indices (Derlink et al., 2018; Fattoruso et al., 2021; Berardo et al., 2022). Making accurate species classifications of *Psammotettix* in fields can help to determine if insecticide use is necessary, but better knowledge of the transmission of WDV through other *Psammotettix* species is also needed. A study from France showed that *P. alienus* was the most common species in cereal fields and that it sometimes occurred together with *P. confinis* (Abt et al., 2018). Looking at a compilation from the Swedish website artportalen.se, the most reported species is *P. confinis* followed by *P. nodosus*, which are not known to transmit WDV. Many of the reported *Psammotettix* species are found in agricultural fields or grasslands, but there are two species whose habitats are in the seashore and mountains (Table 1).

Table 1. Reported findings of Psammotettix leafhoppers in Sweden between 2000 and 2022

Scientific name	Habitat*	Number of reported leafhoppers
Psammotettix		18
P. albomarginatus	A	12
P. alienus	A	57
P. cephalotes	A, F	17
P. confinis	A, U	606
P. dubius	A, S	28
P. excisus	A	3
P. frigidus	M	3
P. nodosus	A, W	491
P. poecilus	A, U	11
P. putoni	S	1
P. sabulicola	A, S	2
P. lapponicus	A, M	-
P. pallidinervis	A	-

** F=Forest, A= Agricultural landscape, U=Urban environment, W=Wetlands, S=Seashore, M=mountain. All Psammotettix species are registered as viable and reproducing 2020. Source: artportalen.se*

1.5 WDV genome

WDV belongs to the family of *Geminiviridae* and the genus *Mastrevirus.* Viruses in this family have a genome of circular single-stranded (ss)DNA encapsidated in a geminate formed capsid. Mastreviruses have small monopartite genomes with around 2700 nucleotides. Most of them infect monocotyledonous plants in the family Poaceae but there are those infecting dicots as well (Rojas et al., 2018). The WDV genome has around 2750 nucleotides, but the genome size differs between WDV strains give or take one or two nucleotides (Kvarnheden et al., 2002; Abt et al., 2015). The synthesis of ssDNA starts in a loop structure in the long intergenic region (LIR) (Figure 4). This loop structure is conserved in all geminiviruses. To initiate replication, the virus depends on the host DNA polymerase, and the coding region functions in two directions (Fiallo-Olivé et al., 2021). The genomes of mastreviruses, including WDV, encode four proteins: the coat protein (CP), movement protein (MP) and two different replication proteins (Rep and RepA) (Figure 4). The different replication proteins are produced because of an intron in the *Rep* gene. Sequences for regulation of replication and transcription are in LIR and the short intergenic region (SIR). Synthesis of dsDNA starts with the help of a primer in SIR, while synthesis of ssDNA starts in LIR (Kvarnheden et al., 2002).

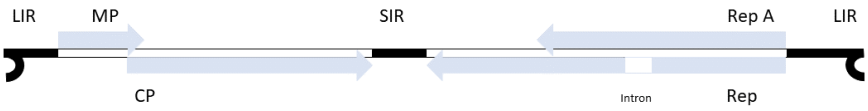

Figure 4. Genomic organization of WDV. LIR: long intergenic region; MP: movement protein gene; CP: coat protein gene; SIR: small-intergenic region; Rep: replication-associated protein gene including intron and RepA: replication-associated protein A gene.

WDV are categorised into five different strains (ABCDE), where A, B and C are barley-infecting strains and D, and E are wheat-infecting strains. Because the demarcation criteria for the genus *Mastrevirus* are set to 78% nucleotide identity for species and 94% for strains, the barley and wheat strains are considered as the same species (Muhire et al., 2013).

1.6 Aim of project

During the summer of 2022, symptomatic winter wheat samples were collected by the Swedish Board of Agriculture. The symptoms and history of the fields indicated that it was an infection of WDV, and the samples were sent to the Swedish University of Agricultural Science (SLU) for testing. Knowing what disease exists in the field helps the farmer in planning future agricultural practises like crop rotations, soil preparation and pesticide use. The aim was to test these wheat samples, report the results and discuss possible factors that could have caused the outbreak.

The Swedish Board of Agriculture also has cooperation with farmers scattered around in Sweden. During spring and autumn 2022, yellow water traps were placed in fields or field edges to collect insects. A total of 28 locations contributed and the leafhoppers found were sorted and sent to SLU for testing of WDV. The aim was to test the leafhoppers, report the results and sequence virus isolates. Sequencing the virus is of interest because it can help indicate changes in the genome that might lead to epidemics or altered host specificity. It might also be possible to see the relationship between isolates from different locations.

There was also a comparison of the results for 2021 (Appendix 1), as well as the wide survey (2002-2020) of wheat dwarf disease incidence in Sweden (Yazdkhasti, 2022). The comparison discussed the questions:

- In which locations were the WDV-positive leafhoppers from 2021 compared with the analysed winter wheat and leafhopper samples from 2022?
- Which factors could be behind these results?

The expected result of this thesis is that it is still the same strain of WDV occurring in the fields in Sweden as the first sequenced WDV isolate from 1969 as described in McDowell et al. (1985). The isolate from 1969 is from a Swedish wheat plant collected in the field in 1969 (Lindsten et al., 1970) and was later supplied by Klas Lindsten to the Imperial College of Science and Technology in the UK for sequencing.

Materials and methods

Plant material consisted of symptomatic dried leaves of winter wheat, collected in 2022 from six fields located in the counties of Västra Götaland, Södermanland and Östergötland (Figure 5). From one of the locations, wheat samples had been collected a two different time points (June and July). The other plants were collected in July. Since there where several plants collected from each location, they were divided into five replicates for each location (Table 2). These samples were analysed with the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

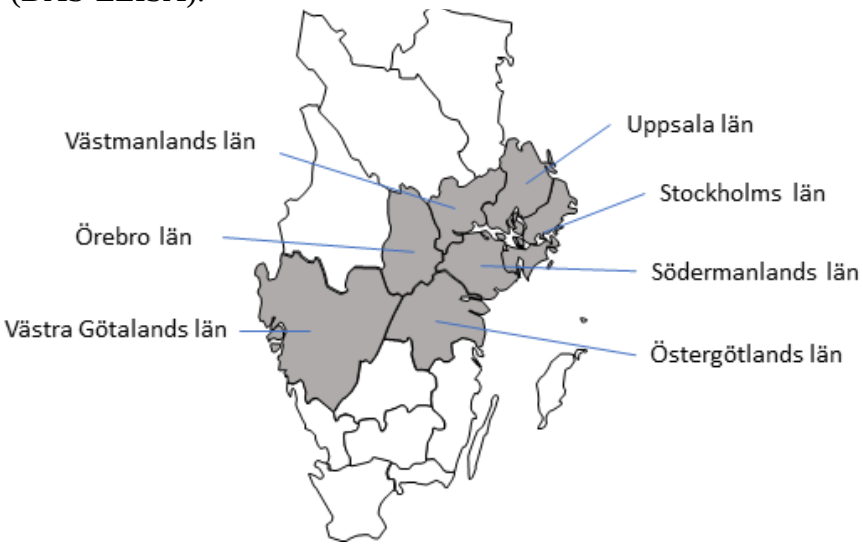

Figure 5. Counties with high incidence of wheat dwarf disease in Sweden (Yazdkhasti, 2022). For the present study, wheat samples were collected from the counties of Västra Götaland, Södermanland and Östergötland.

Table 2. Location for collected wheat samples

Location	County
Fyrö Gård (Valla)	Södermanland
Prästgården (Horn, Skövde)	Västra Götaland
Axtorp (Horn, Skövde)	Västra Götaland
Västra Husby	Östergötland
Evertsholm, Söderköping	Östergötland
Skarpenberga, Å (2022-06-13)	Östergötland
Skarpenberga, Å (2022-07-13)	Östergötland

Leafhopper samples stored in 70% ethanol were from different locations at different dates, collected in yellow water traps in or next to fields of winter oilseed rape,

winter wheat or oat (Tables 3 and 4). All samples were collected and distributed by the Swedish Board of Agriculture in 2022 from the counties of Örebro, Uppsala, Stockholm, Södermanland and Östergötland (Figure 5).

Table 3. Leafhopper samples from Plant protection agency in Linköping

County	Location	Leafhoppers*	Field type
Örebro	Valla	1	Oilseed rape
Östergötland	Boberg	13	Oilseed rape
Östergötland	Yxstad	6	Oilseed rape
Östergötland	Säby	19	Oilseed rape
Östergötland	Renstad	3	Oilseed rape
Östergötland	Hagelstad	19	Oilseed rape
Östergötland	Förråd	8	Oilseed rape
Östergötland	Hyttringe	6	Oilseed rape
Östergötland	Skarpenberga	6	Oilseed rape
Östergötland	Borringe	8	Wheat
Östergötland	Skälboö	22	Wheat
Östergötland	Gårdeby	40	Wheat
Södermanland	Fyrö gård	12	Wheat
Östergötland	Östervarv	13	Oilseed rape
Östergötland	Helleberga	17	Oilseed rape
Östergötland	Gammelkil	1	Oilseed rape
Östergötland	Skälsund	2	Oilseed rape

**Number of leafhoppers per sample, a maximum of 10 leafhoppers were used for each PCR test*

Table 4. Leafhopper samples from Plant protection agency in Uppsala

County	Location	Date	Leafhoppers*	Field type
Uppsala	Nyborg	16/05	1	Wheat
		23/05	1	Wheat
Stockholm	Frölunda	16/05	3	Wheat
		23/05	1	Wheat
Uppsala	Haga, Enköping	13/05	1	Wheat
		20/05	1	Wheat
Stockholm	Markim	16/05	2	Oat
		30/05	2	Oat
Uppsala	Britehov	05/09	5	Wheat
Uppsala	Fyrisvall, Storvreta	30/08	19	Oilseed rape
		27/09	14	Wheat
		13/10	36	Wheat
Uppsala	Brunnby	23/09	5	Wheat
Uppsala	Säby, Ultuna egendom	12/09	4	Wheat
		13/10	4	Wheat
Uppsala	BCA lantbruk	15/08	2	Oilseed rape
		02/09	1	Oilseed rape
Uppsala	Sätuna, Björklinge	30/08	7	Oilseed rape
		27/09	6	Wheat
		13/10	14	Wheat
Uppsala	Lilla Vallskog	30/08	29	Oilseed rape
		05/09	10	Oilseed rape
		13/10	4	Oilseed rape

** Number of leafhoppers per sample, a maximum of 10 leafhoppers were used for each PCR test*

2.1 DAS-ELISA of winter wheat

To measure the virus titre in the wheat samples, DAS-ELISA was performed. This method uses specific antibodies that react with WDV antigens in the sample. These

form a complex that in the last enzymatic step will give a yellow colour, which can be measured in a spectrophotometer (Loewe, 2013).

Wheat samples were homogenized in approximately 2 ml of sample buffer and stored at -20°C. One positive and one negative control were made from winter wheat samples, which had tested positive and negative, respectively, in a previous analysis. The ELISA was carried out according to Yazdkhasti et al. (2021) and the protocol provided with the WDV antiserum (Loewe Biochemica). The absorbance was measured at 405 nm in a spectrophotometer after 2 hours of dark reaction at room temperature. Samples were considered positive if the absorbance value was twice as high as for the negative controls. Additional negative controls with buffer were used as back-up if negative controls failed.

2.2 DNA extraction and PCR detection of WDV in leafhoppers

A total of 35 PCR tests were carried out for the leafhopper samples (Table 3 and 4). For PCR tests of samples from the Plant protection agency in Uppsala (Table 4), leafhoppers from the same location, but different dates, were merged into a single DNA extraction. A maximum of 10 individuals were used for each DNA extraction and subsequent PCR.

2.2.1 DNA extraction from leafhoppers

To remove ethanol, the leafhoppers were washed with water three times and allowed to dry on a paper towel. In the first round of DNA extractions, leafhoppers from Fyrisvall 13/10 and Lilla Vallskog 30/08 were put in 2 ml Eppendorf tubes containing a glass bead, frozen in liquid nitrogen and homogenized using a Tissue Lyser (Qiagen) for 2 minutes. For a better homogenisation of the following samples, a metal bead was used instead and the leafhoppers were grinded for 3 minutes. For the DNA extraction, Sigma´s GenElute Mammalian Genomic DNA Miniprep kit was used with step 2b to 8 as in the manufacturer's protocol. In step 9, the first round of elution was done with 50 µl of Elution Solution, followed by incubation for 5 minutes and the second round of elution was then done with 100 µl of Elution Solution.

Five samples (Skarpenberga E62, Östervarv E3, Helleberga E2, Gammelkil E46 and Skälsund E63) were processed with Sigma-Aldrich GenElute -E single spin tissue DNA kit. The leafhoppers in the sample from Helleberga was split into two samples. Concentration and purity of the DNA extracts were analysed using a

NanoDrop 1000 (Thermo Fisher Scientific) and the DNA was stored at -20°C or 4°C for later analysis.

2.2.2 PCR and gel electrophoresis

For PCR, Thermo scientific™ DreamTaq Green PCR Master Mix (2x) was used according to the manufacturer's protocol. The total volume for the reaction was set to 25 µl, with 1.5 µl of DNA extract and 0.5 µM of each primer. The positive control was taken from a previous DNA extract of leafhoppers, for which WDV had been confirmed, and for the negative control, nuclease free water was added. The primer pair 1877-1896 (5'-CTTAC GGAGT AGAGA TGTTC-3') and 328-309 (5'-AACAG AGTGT AAGCA AGCCA-3') designed by Kvarnheden et al., (2002) was used. The expected amplification product is a fragment of 1201 bp that corresponds to nucleotide positions 1877-2750 and 1-328 of WDV-E (original isolate called WDV-F, F for France), which is the entire LIR plus the 5'ends of *Rep* and *MP* of the WDV genome. The PCR conditions were as in Kvarnheden et al. (2002) with minor adjustments: initial incubation for 2 min at 94°C, followed by 35 cycles with 30 s at 94°C, 1 min at 55°C and 2 min at 72°C, and final extension for 10 min at 72°C.

Gel electrophoresis was run with 0.8% or 1% agarose, TAE buffer and Midori Green for 30 to 50 min at 80 V. The GeneRuler 1 kb DNA ladder from Thermo scientific was used as a size marker.

2.3 Cloning of amplicons for WDV-positive leafhoppers

For cloning of amplicons for WDV from leafhoppers, a new PCR was run with the same settings as described above, but in a larger volume (50 µl). The results of the PCR were visualized by gel electrophoresis, where 7 µl of the PCR solution was used. For purification, 40 µl of the remaining PCR solution was used together with the GeneJET™ PCR Purification Kit (Thermo scientific). The purification of the amplicons was carried out according to the manufacturer's recommendations and the concentration of the purified amplicons was estimated using a Nanodrop.

For ligation of the purified PCR products, CloneJET PCR Cloning kit (Thermo scientific) was used following the kit protocol and the instructions for sticky-end cloning. The molar ratio between vector (pJET1.2/blunt) and insert was set to 1:3, and the incubation was prolonged to 1 h at room temperature.

For the transformation, 5 µl of the ligated DNA products were transformed into competent cells of *Escherichia coli* DH5α (Invitrogen) according to manufacturer's

protocol. As a transformation control, pUC19 plasmid was used. From each transformation, cells were spread on two plates of LB-agar containing 100 µg/ml ampicillin with 100 µl transformation solution on one plate and a second plate with resuspended cell pellets, produced by centrifugation at 2500 rpm for 3 min. The plates were incubated over night for about 18 hours at 37°C.

Plasmid DNA was purified using the Thermo scientific GeneJET plasmid miniprep kit following the kit protocol. One colony was used to inoculate 4 ml LB medium containing 100 µg/ml ampicillin. The bacterial culture was incubated overnight at 37°C and with shaking at 180 rpm. The next day, 1.5-3 ml bacterial culture was harvested by centrifugation, and used for plasmid purification with the steps for protocol A (Plasmid DNA purification using centrifuges), where plasmid DNA was eluted in a volume of 50 µl. The bacterial culture was also used for making single colony streaks on LB ampicillin plate. After overnight incubation at 37°C, the plate was stored at 4°C. Single colony streaks were used as back-ups if repeated plasmid purifications were needed.

To increase the DNA concentration in a repeated plasmid purification, 3 ml of bacterial culture was harvested in two rounds of centrifugation, and the incubation period for the Lysis solution was prolonged to 10 min and the elution volume was reduced to 2x 15 µl. The concentration and purity of the plasmid DNA were determined using a NanoDrop.

For plasmid digestion, FastDigest *Bgl*II (Thermo scientific) and a protocol called Fast digestion of DNA were used. The *Bgl*II restriction enzyme recognises a palindromic site (AGATCT) and cuts between the adenine and guanine on both DNA strands leaving a sticky end with 3’ protrusive termini (Sambrook et al., 2001). This releases the ligated insert from the pJET1.2/blunt cloning vector making it possible to verify insert size by gel electrophoresis (Petterson, 2020). The total volume of the reaction mixture was 20 µl and the volume of purified plasmid used for the digest was adjusted depending on DNA concentration, where a volume of 5 µl or 10 µl were used. The digested plasmid DNA was analysed by electrophoresis in an 1% agarose gel to see which clones that had the expected insert size.

2.4 Sequencing of the WDV from leafhoppers

Sequencing of the virus genome was conducted to confirm if the isolate belongs to the common wheat-infecting strain (WDV-E) previously found in Sweden. Clones with the highest DNA concentration and having an insert size of 1.2 kb were sent

for Sanger sequencing at Macrogen, both forward and reverse reads were ordered. The sequencing results, the chromatograms, were checked visually for errors and the primer sequences were removed. Programmes used for analysis in GenBank were BLASTn and BLASTx (Basic Local Alignment Search Tool). To find accessions with highest nucleotide identity to the sequenced clones, BLASTn search was used (Altschul et al., 1990). BLASTx uses translation of a nucleotide sequence to search a protein database and identify proteins with highest amino acid identity.

For this study, the programme MEGA 11 (Molecular Evolutionary Genetics Analysis) version 11.03.13 was used to construct a phylogenetic tree (Kumar et al., 2018). A total of 17 nucleotide sequences were used, including the seven sequences from this study and those of five different WDV strains used in Yazdkhasti et al. (2021): FN806787 (WDV-A), FJ620684 (WDV-B), JQ647455 (WDV-C), JN791096 (WDV-D), MN453813 (WDV-E). In addition, four accessions with the highest identity from the BLASTn search were included (AM491482, AM491483, AM491475, AM491477) as well as the WDV isolate from 1969 (X02869; McDowell et al., 1985). The sequences of all accessions were adjusted to match the region of LIR, partial *Rep* and *MP* genes (1162 nt) and were aligned using the Clustal W algorithm. The alignment was used to compute the distances for neighbour-joining analyses with the Kimura two-parameter method. To test the robustness of the internal branches, bootstrap analysis was carried out with 500 replicates.

The pairwise nucleotide identity between the sequences was calculated in SDT (Sequence Demarcation Tool) to create a colour-coded pairwise identity matrix that visually aids the classifications of the sequences (Muhire et al., 2014).

Results

3.1 DAS-ELISA of winter wheat samples

The symptomatic winter wheat samples were tested for WDV infection using DAS-ELISA. From six locations, five samples were analysed for each location and the locations represented the counties Södermanland, Västra Götaland and Östergötland. For the location Skarpenberga, two sets of samples were collected at different time points.

All locations had at least one positive out of five samples (Table 5). The negative control is not taken in consideration, because it was unusually high compared to the buffer and absorbance values twice the buffer indicate a more reliable limit for a sample to be positive. Samples from the location (Skarpenberga) that had been collected at different time points are considered as positive for both time points. The spectrophotometric measurements revealed a high titre of WDV in many samples, and this tells us that the suspected winter wheat samples indeed were infected by WDV (Table 5).

Table 5. Absorbance values from DAS-ELISA test for WDV infection of wheat samples

Location/Sample	1	2	3	4	5
Fyrö Gård (Valla)	0.099[a]	**0.443**[b]	0.140	0.090	0.098
Prästgården (Horn, Skövde)	**0.270**	**0.889**	**0.728**	**0.750**	**0.823**
Axtorp (Horn, Skövde)	**0.840**	**0.675**	**0.581**	**0.677**	**0.838**
Västra Husby	0.093	**0.904**	0.103	**0.641**	0.136
Evertsholm, Söderköping	0.217	**0.620**	**0.746**	**0.878**	**0.892**
Skarpenberga, Å (220613)	**0.899**	**0.995**	**0.938**	**0.851**	**0.888**
Skarpenberga, Å (220713)	0.098	0.115	0.099	**0.661**	0.111
Positive control	0.928				
Negative control	0.365				
Buffer	0.117				

[a]*Values from 2 h of incubation with substrate.* [b]*Values 2x 0.117 (Buffer) are considered as positive for WDV and marked in bold.*

3.2 DNA extraction from leafhoppers and PCR detection of WDV

3.2.1 DNA extraction from leafhoppers

A total of 36 DNA extractions were made from the leafhoppers gathered from 28 locations. The DNA extracts of the leafhopper samples had varying DNA concentration, which ranged from 3.3 ng/µl to 706.9 ng/µl. From the column for DNA purification, the first eluate had generally higher DNA concentration compared to the second eluate (Tables 6 and 7), except for leafhoppers from Nyborg, Frölunda and Lilla Vallskog (30/08) in Uppsala County (Table 6). The low DNA concentrations for samples Lilla Vallskog (30/08) and Fyrisvall (13/10) could be due to the grinding with glass beads and the leafhoppers may then not have been sufficiently homogenised (Table 6).

Table 6. DNA concentrations in DNA extracts from leafhoppers

Location	Date	Leafhoppers in sample*	1st elution (ng/µl)	2nd elution (ng/µl)
Uppsala (spring)				
Nyborg, Upplands Bro	16/05 23/05	(merged) 2	9.7	26.6
Frölunda, Kungsängen	16/05 23/05	(merged) 4	19.2	47.0
Haga, Enköping	13/05 20/05	(merged) 2	95.7	16.8
Markim	16/05 30/05	(merged) 4	86.7	17.9
Uppsala (autumn)				
Britehov	05/09	5	143.2	32.9
Fyrisvall, Storvreta	30/08	10	118.0	35.8
	27/09	10	751.1	81.7
	13/10	10	148.8	28.5
Brunnby	23/09	5	337.8	37.2
Säby, Ultuna egendom	12/09	4	16.0	5.5
	13/10	4	303.2	83.0
BCA lantbruk, Hagby	15/08 02/08	(merged) 3	227.3	27.5
Sätuna, Björklinge	30/08	7	71.4	24.4
	27/09	6	235.2	34.5
	13/10	10	275.3	56.0
Lilla Vallskog	30/08	10	8.7	21.6
	05/09	10	549.7	71.0
	13/10	4	223.0	29.8

**Leafhoppers collected by Plant protection agency in Uppsala.*

Five samples (Skarpenberga E62, Östervarv E3, Helleberga E2, Gammelkil E46 and Skälsund E63) were processed with GenElute-E single spin tissue DNA kit from Sigma-Aldrich (Table 7), and no second elution was made with this kit. These DNA extracts had a DNA concentration ranging from 3.3 ng/µl to 134.4 ng/µl. Compared to Sigma's GenElute Mammalian Genomic DNA Miniprep kit, a lower amount of DNA was obtained. However, the low DNA concentrations should not have affected further downstream analysis. Generally, DNA extracts from samples with more leafhoppers had a higher DNA concentration and for PCR testing, the DNA concentration should ideally be above 100 ng/µl (Tables 6 and 7). One thing to take into account is that the extracted DNA is a combination of DNA from leafhoppers, virus, and other possible organisms.

Table 7. DNA concentrations in DNA extracts from leafhoppers

Location **Linköping**	Sample	Leafhoppers in sample*	1st elution (ng/µl)	2nd elution (ng/µl)
Valla, Örebro	T151	1	162.4	16.7
Borringe	E15	8	513.3	77.3
Skälboö	E67	10	673.1	93.4
Gårdeby (Västra Husby)	E71	10	583.6	214.7
Fyrö gård	D95	10	552.9	130.2
Boberg	E4	10	469.4	101.6
Yxstad	E21	6	123.0	27.6
Säby	E61	10	706.9	150.3
Renstad	E22	3	20.0	5.0
Hagelstad	E41	10	256.2	100.6
Förråd	E42	8	357.7	102.9
Hyttringe	E1	6	166.3	11.3
Skarpenberga	E62	6	94.5	
Östervarv	E3	10	134.4	
₁Helleberga	E2	8	69.7	
₂Helleberga	E2	9	97.0	
Gammelkil	E46	1	3.3	
Skälsund	E63	2	12.4	

** Leafhoppers collected by Plant protection agency in Linköping.*

3.2.2 PCR detection of WDV in leafhoppers

All 36 DNA extracts from leafhoppers were used for analysis of WDV using PCR. Gel electrophoresis of PCR tests showed a PCR-band (1.2 kb) of the expected size for leafhoppers from five out of 28 locations. The locations that clearly tested positive for WDV were Haga and Säby from Uppsala County (Figure 6). There were false positive results for Renstad and Valla (Figure 7). A positive result for Skarpenberga from Östergötland County (Figure 8).

Both positive and negative controls indicated a successful PCR, where the positive control gave a clear band after gel electrophoresis for the expected size of 1.2 kb (Figures 6, 7 and 8).

For Uppsala County there were a total of 18 samples from eleven locations, and 16 samples tested negative for WDV. Two samples from the locations Haga and Säby (date 12/9) tested positive for WDV, with Haga having a weak band and Säby a clearer band with the expected size of 1.2 kb (Figure 6). Even though the sample Säby date 13/10 gave a negative result, the location Säby is still considered positive for WDV because the leafhoppers that were from 13/10, did not carry WDV it does not mean that WDV disappeared from the area. WDV were still present in symptomless reservoirs of other host plants.

For Örebro County, only leafhoppers from one location were tested, Valla, which gave a weak PCR band barely visible in gel electrophoresis with the size of 1.2 kb (Figure 7). There was also one sample from Fyrö Gård in Södermanland County that tested negative (result not shown). For the County of Östergötland, there were a total of 16 samples, and leafhoppers from two locations, Renstad (Figure 7) and Skarpenberga (Figure 8) gave WDV-positive results. A second PCR and gel was run for samples Fyrö Gård and Renstad that came out negative (result not shown).

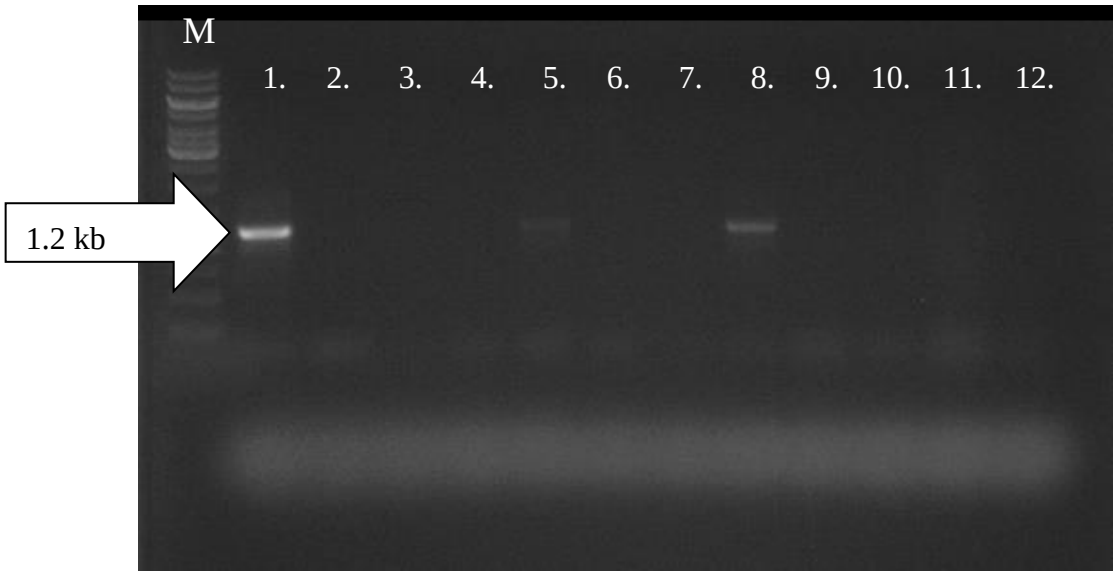

Figure 6. Gel electrophoresis after PCR to detect WDV in leafhoppers. Samples from Uppsala agency. M = size marker and the arrow marks the 1.2 kb PCR-band. Order of samples in lanes: 1. Positive control, 2. Negative control, 3. Nyborg, 4. Frölunda, 5. Haga (weak band), 6. Markim, 7. Fyrisvall 30/8, 8. Säby 12/9 (band), 9. BCA lantbruk Hagby, 10. Sätuna 30/8 11. Lilla Vallskog 5/9 and 12. Lilla Vallskog 13/10.

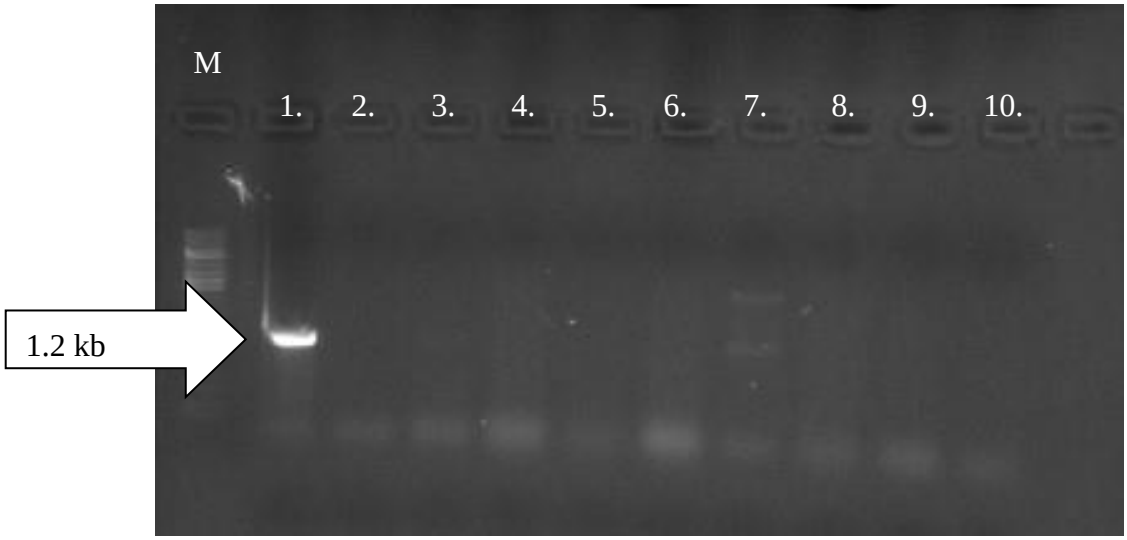

Figure 7. Gel electrophoresis after PCR to detect WDV in leafhoppers. Samples from Linköping agency. M = size marker and the arrow marks the 1.2 kb PCR-band. Order of samples in lanes: 1. Positive control, 2. Negative control, 3. Valla (weak band), 4. Boberg, 5. Yxstad, 6. Säby, 7. Renstad (two bands), 8. Hagelstad, 9. Förråd, 10. Hyttringe.

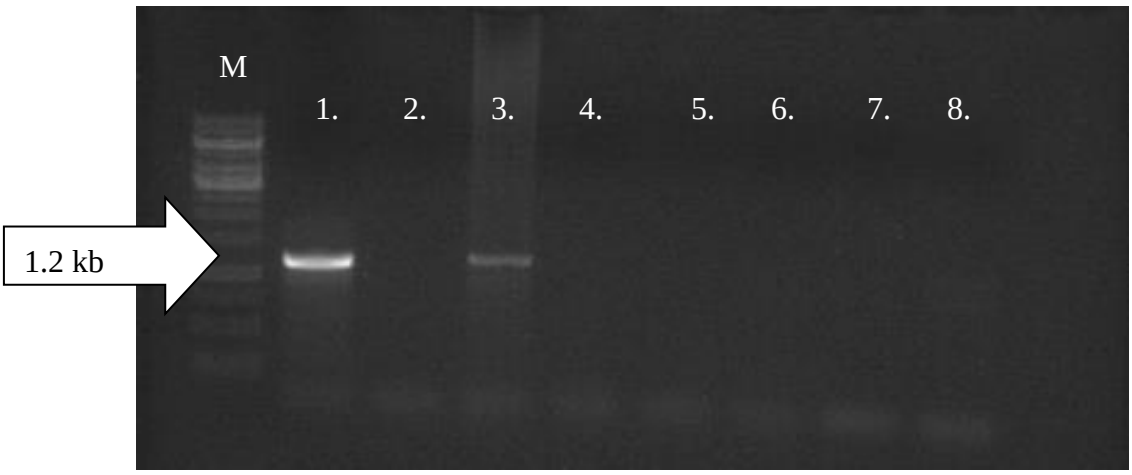

Figure 8. Gel electrophoresis after PCR to detect WDV in leafhoppers. Samples from Linköping agency. M = size marker and the arrow marks the 1.2 kb PCR-band. Order of samples in lanes: 1. Positive control, 2. Negative control, 3. Skarpenberga (band), 4. Östervarv, 5. ₁Helleberga, 6. ₂Helleberga, 7. Gammelkil, 8. Skälsund.

3.3 Cloning of amplicons

For the cloning, the PCR was repeated in a larger volume for previously positive samples. Gel electrophoresis then showed positive results for Säby, Haga (Uppsala County) and Skarpenberga (Östergötland County). Because the second PCR for the leafhopper samples Valla and Renstad (counties of Örebro and Östergötland, respectively) was negative, no cloning was conducted for those samples. The

purified PCR products of 1.2 kb for Säby had a DNA concentration of 19.8 ng/µl, Haga 17.0 ng/µl and Skarpenberga 21.8 ng/µl.

After cloning to identify clones with the expected insert, the plasmids were digested with *Bgl*II. In total 30 clones were digested, and gel electrophoresis showed that 14 clones had an insert with the expected size of 1.2 kb (Figures 9 and 10).

For the sample from Haga, eleven clones were obtained: H1-H5 (Figure 9) and H1a-H6a (Figure 10). Five clones (H1, H2a, H3a, H4a, and H5a) had inserts of the expected size (Figures 9 and 10). For the sample from Säby, eleven clones were obtained: S1-S5 (Figure 9) and S1a-S6a (Figure 10). These gave seven clones (S1, S2, S4, S5, S1a, S2a, S4a, S2 and S4) with inserts of the expected size (Figures 9 and 10). For the sample from Skarpenberga, six clones were obtained (E1-E6), none of which had inserts of the expected size (Figure 10). A larger band could be seen at approximately 3 kb for isolated plasmids. This corresponded to the pJet1.2 vector, which has a size of 2.9 kb (Figures 9 and 10).

After cloning, a total of six clones for Säby (S2, S4, S5, S1a, S2a and S4a) and four clones for Haga (H1, H2a, H3a and H5a) with the expected insert size of 1.2 kb and the highest DNA concentration were chosen for sequence determination.

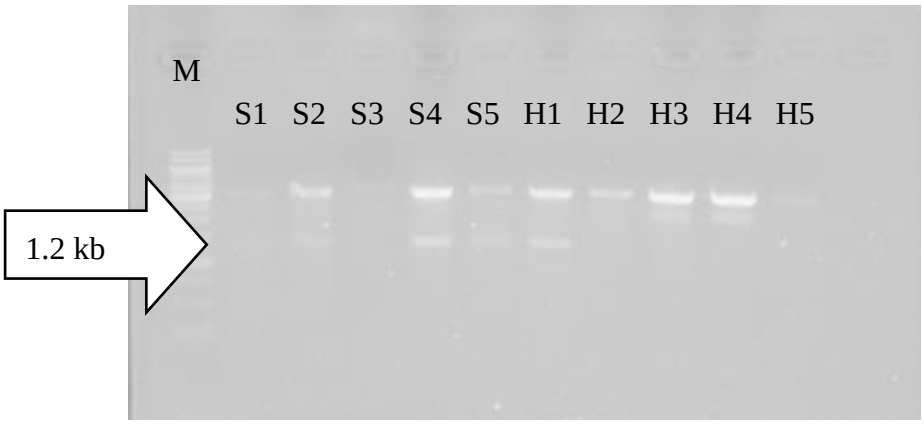

Figure 9. Gel electrophoresis of BglII-digested plasmids for cloning of PCR-fragments of WDV from leafhoppers. M = size marker and the arrow marks the 1.2 kb insert.

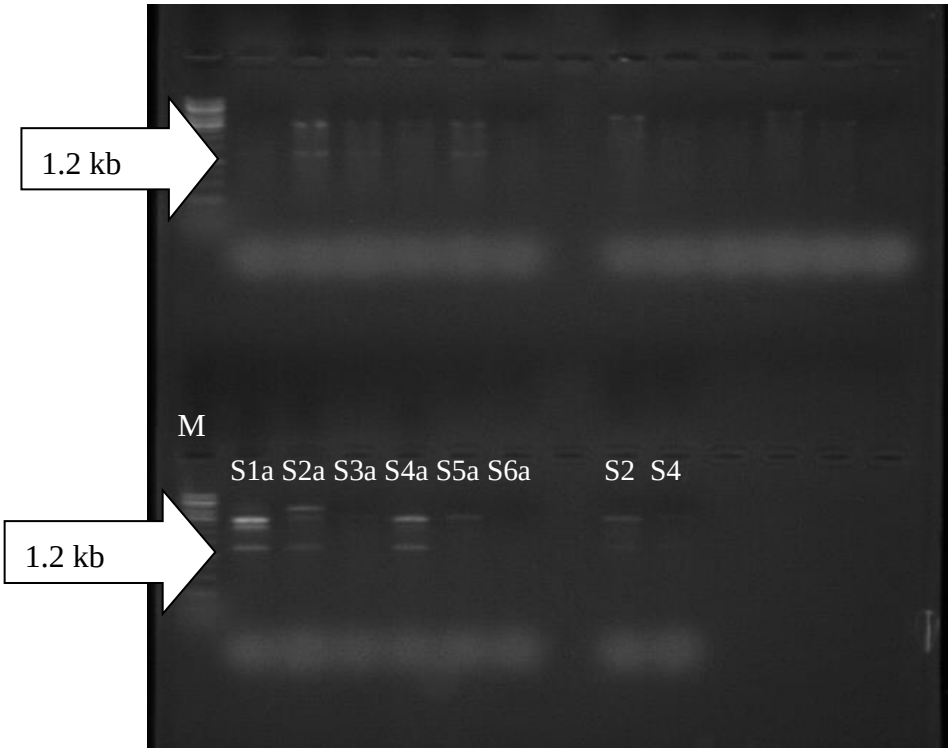

Figure 10. Gel electrophoresis of BglII-digested plasmids for cloning of PCR-fragments of WDV from leafhoppers. M = size marker and the arrow marks the 1.2 kb insert.

3.4 Sequence analyses

The determined sequences for clones H1, H2a, H3a and H5a (from Haga) as well as S1a, S2a and S4a (from Säby) had clear sequencing signals in the chromatograms and were further analysed. The sequencing of clones S2, S4 and S5 did not produce clear signals and these clones were excluded.

3.4.1 BLAST searches

A sequence search of GenBank was performed using BLASTn and the two accessions with the highest identity for each clone were noted. These accessions with highest identities were all for isolates of WDV-E from Sweden. The identity ranged from 99.74% to 99.31% (Table 8). The clones H3a, S1a, S2a and S4a showed highest identity with accession number AM491482 (isolate Öjebro). The clones H1, H2a and H5a showed highest identity with accession number AM491475 (isolate Hacksta), which is mentioned in Ramsell et al. (2008) as a subtype B that was always found in mixed infections together with the main WDV genotype. This states a mixed infection to what was found for leafhopper sample Haga with clones H1, H2a, H3a and H5a. Isolate Öjebro (AM491482) was isolated from a common meadow-grass (*Poa pratensis*) in 2001 from the county of

Östergötland and isolate Hacksta (AM491475) was isolated from a wheat plant in 2001 from the county of Uppsala. The sequence of H3a shared highest identity to accession number AM491477 (Haga), which was for a leafhopper sample collected in 2002 from the same location as in this study. The sequences of WDV isolates from leafhopper collected in Haga are almost identical although 20 years separate them.

Table 8. GenBank accession with highest nucleotide identity to the clones of this study

Clone	Accession	Identity (%)	Location	County
H1*	**AM491482**	**99.48**	**Öjebro**	**Östergötland**
	AM491475	**99.40**	**Hacksta**	**Uppsala**
H2a	**AM491482**	**99.65**	**Öjebro**	**Östergötland**
	AM491475	**99.65**	**Hacksta**	**Uppsala**
H3a	AM491477	99.57	Haga	Uppsala
	AM491483	99.31	Öjebro	Östergötland
H5a	**AM491482**	**99.48**	**Öjebro**	**Östergötland**
	AM491475	**99.40**	**Hacksta**	**Uppsala**
S1a	AM491477	99.74	Haga	Uppsala
	AM491483	99.48	Öjebro	Östergötland
S2a	AM491477	99.74	Haga	Uppsala
	AM491483	99.48	Öjebro	Östergötland
S4a	AM491477	99.66	Haga	Uppsala
	AM491483	99.40	Öjebro	Östergötland

**Subtype B marked in bold.*

A search using translated nucleotide sequences of the clones (H1, H2a, H3a, H5a, S1a, S2a and S4a) with BLASTx showed some amino acid changes in RepA compared to other WDV RepA sequences in the protein database. Most of these amino acid changes are not expected to make any differences to the structure or function of the protein (Table 9). However, the proline substitution in RepA of clone H1 could influence the protein function, but further studies are needed to investigate the effect.

Table 9. Identification of amino acid changes in RepA of new WDV isolates from leafhoppers

Clone	Protein Accession	Nucleotide Accession	Amino acid changes	Comment
H1	CAM33192.1	Hacksta AM491475	Q95P and S147P	Proline could influence protein functions
H2a	CAM33192.1	Hacksta AM491475	No changes	
H3a	CAM33196.1	Haga AM491477	No changes	
H5a	CAM33192.1	Hacksta AM491475	N193D	Conservative substitution
S1a	CAM33196.1	Haga AM491477	I107L	Conservative substitution
S2a	CAM33196.1	Haga AM491477	I107L	Conservative substitution
S4a	CAM33196.1	Haga AM491477	I107L	Conservative substitution

3.4.2 Phylogenetic analysis

For the phylogenetic analysis, the sequences of the clones were analysed together with those of the four isolates (AM491477, AM491483, AM491482 and AM491475) showing highest sequence identity (Table 8), as well as the first sequenced WDV isolate from 1969 (McDowell et al., 1985) and five isolates of different WDV-strains (ABCDE) used in Yazdkhasti et al. (2021). The four sequences from isolates AM491477, AM491483, AM491482 and AM491475 were used to give a broader view of the relationship between virus isolates in the WDV-E strain.

One group in the phylogenetic analysis consisted of WDV-E, where the clones of this thesis were included, and the bootstrap value for this clade was 100%. The clones H5a, H2a and H1 formed a cluster together with AM491482 and AM491475, with a bootstrap value of 100% and is marked with Subtype B. Another group was formed with the clones H3a, S4a, S2a and S1a together with AM491477 and AM491483 with a bootstrap value of 90%. The Swedish isolate from 1969 (X02869) was in between these groups and a Swedish ryegrass isolate MN453813 was also separated from these groups. Accessions for different WDV strains (A, B, C and D) from Europe and Iran, mentioned in Yazdkhasti et al. (2021), were used as a demonstration to highlight that the Swedish isolates belong to the WDV-E strain (Figure 11).

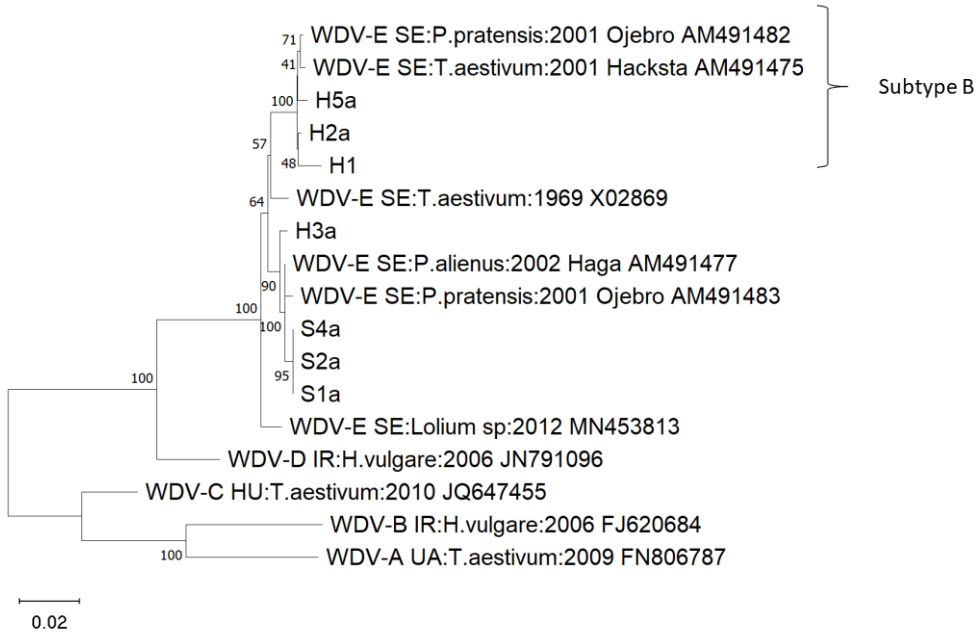

Figure 11. Neighbour-joining analysis based on the LIR, 5´ ends of Rep/RepA and MP (total of 1162 nt). Accession numbers for published isolates can be found in GenBank. The subtype B is marked with a bracket. Horizontal lines are proportional to the number of nucleotide differences between branch nodes. Numbers denote bootstrap values of 500 replicates.

3.4.3 Pairwise sequence identities

The sequence demarcation tool (SDT) was used to calculate the pairwise nucleotide identity between the 17 isolates used in the phylogenetic analysis (Figure 11). All WDV-E isolates shared an identity of 97.5% or higher. Sequences of subtype B (H1, H2a and H5a) shared 98.5 to 100% identity and the subtype B isolates shared 97% identity with the main type isolates S1a, S2a and S4a. Isolates of the other WDV strains shared lower identity with those of the WDV-E strain, for WDV-D it was 94%, WDV-C 88% and WDV-B/WDV-A 84% (Figure 12).

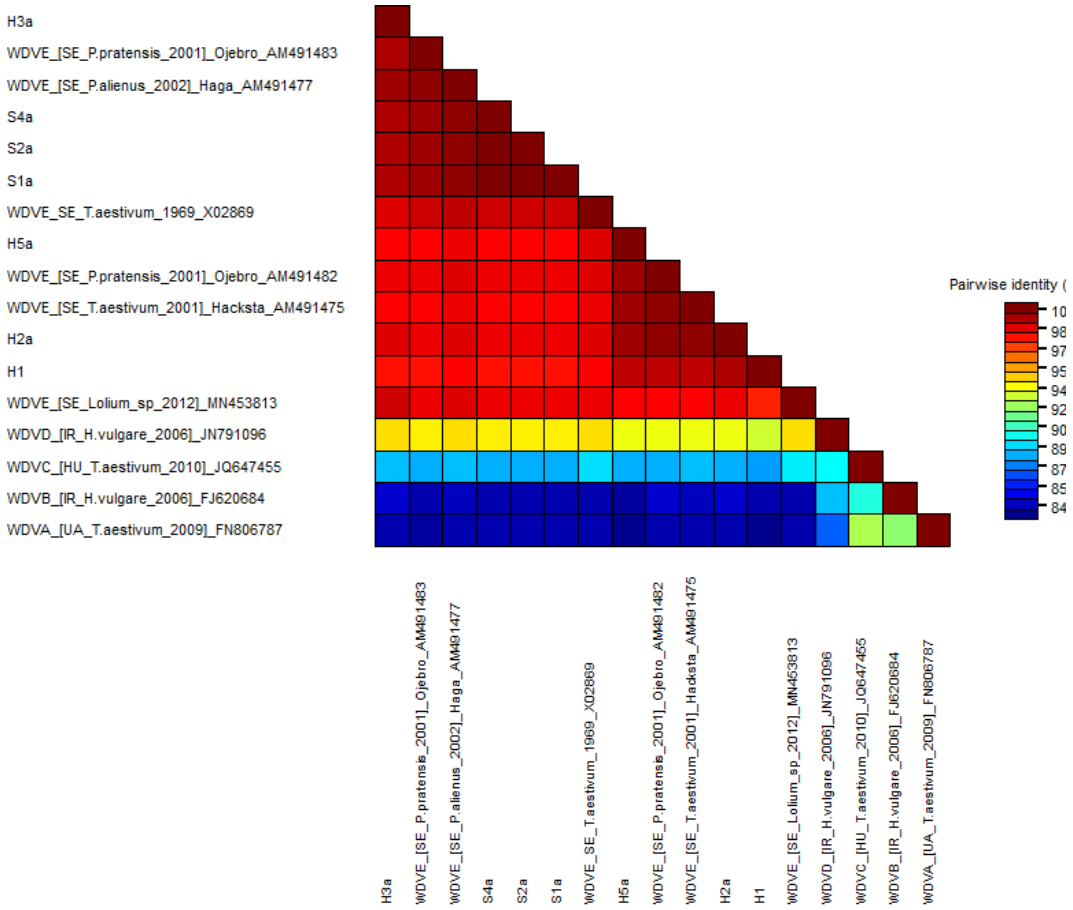

Figure 12. Pairwise nucleotide identity (%) based on LIR as well as partial Rep and MP genes (1162 nt) between clones H1, H2a, H3a, H5a, S1a, S2a, and S4a as well as other WDV isolates.

Discussion

Summarizing the results for the symptomatic winter wheat samples, all locations Fyrö Gård (Södermanland County), Prästgården, Axtorp (Västra Götaland County), Västra Husby, Evertsholm and Skarpenberga (Östergötland County) had at least one out of five samples with positive values for WDV. The results confirmed that WDV was present in all the sampled fields.

Looking at the leafhopper samples where three of these locations (Västra Husby, Fyrö Gård and Skarpenberga) were represented for the wheat samples, only the leafhopper sample from Skarpenberga (Östergötland County) gave a positive result for WDV. However, this sample did not give any clones for sequencing (Table 6).

In the testing of leafhoppers collected in 2021 (appendix 1), seven samples out of 35 had positive results for WDV. The leafhoppers testing positive were from Högsta (Uppsala County), Yxstad, Skälboö, Hagelstad, Förråd (Östergötland County), Säbylund (Örebro County) and Lunda Prästgård (Södermanland County). None of these specific locations from 2021 had reports of wheat dwarf disease the following year in winter wheat nor did any leafhoppers of these location test positive for WDV in this study. However, comparing the WDV-positive leafhoppers from this study (Haga, Säby and Skarpenberga) to the counties tested in 2021, WDV were present in the same counties Uppsala and Östergötland. There was also WDV-positive winter wheat from two of the same counties Södermanland and Östergötland compared to the testing in 2021.

In the PhD thesis of Yazdkhasti (2022), there is a compilation of observations of wheat dwarf disease incidence in Sweden. The survey spans from 2002 till 2020 and the years 2002, 2003, 2009, 2010 and 2017 are mentioned as years with high disease incidence. The severity was then estimated to three in a scale of five for the counties of Uppsala, Stockholm and Västmanland. For these counties, there was a strong correlation between high vector populations, higher temperature during autumn and incidence of wheat dwarf disease the following summer. Where WDV is present in the environment, these factors can largely explain the occurrence of high disease incidence. The other counties Södermanland, Östergötland and Västra Götaland did not have these correlations and the severity of disease ranges from zero to two. To add to this compilation, this study's observation, and analyses of plants with wheat dwarf disease in fields from the counties of Södermanland, Västra Götaland and Östergötland would give a disease severity of two out of five: "plants with disease common in some fields, few field severely affected". There were no reports of wheat dwarf disease in the counties of Uppsala, Stockholm or Västmanland in 2022, nor were there reports of wheat dwarf disease for 2021. However, this does not exclude that there could have been disease-affected wheat plants in additional places. Symptomless reservoirs or minor outbreaks of wheat dwarf diseases are not discovered or reported. These factors can explain why WDV keeps re-occurring in susceptible winter wheat.

The sequenced WDV clones for Haga and Säby showed a close relationship to other Swedish WDV-E isolates and shared more than 99% nucleotide identity with them, which is similar to results of analyses from Yazdkhasti et al. (2021) and Ramsell et al. (2008), where WDV-E isolates had 98 - 99% identity.

Through a BLASTx search, one conservative amino acid change in RepA was identified for translated sequences of clones H1, H5a, S1a, S2a and S4a. However, this change is not expected to result in any structural or functional changes to the viral protein. Clone H1 also had two proline substitutions in RepA, which could have influenced protein function. Clones H2a and H3a had no changes in RepA. However, one interesting finding was that clone H3a, which originated from the same location as accession number AM491477 (Haga), was almost identical in sequence although 20 years separate them. The report from Ramsell et al. (2008) further describes the low genetic diversity in Swedish WDV isolates analysed at that time. The search of the sequences in BLASTx for amino acid changes mainly showed results for *RepA* because it is longer than the sequenced part encoding the MP. For further studies, the MP could be analysed for changes and infectious clones could be used to study the effects of amino acid differences in RepA.

Another finding was that of WDV-E subtype B, which has been found before. Two variants of WDV-E isolates (AM491482 and AM491483) were then both found in a single plant of *P. pratensis* in Öjebro, the latter sequence mentioned belonged to subtype B (Ramsell et al., 2008). AM491482 had high sequence identity to clones H1, H2a and H5a, while AM491483 (subtype B) had high identity to clones H3a, S1a, S2a and S4a. These connections were also demonstrated in the phylogenetic analysis. Clones H1, H2a, H5a and H3a are all from the same leafhopper sample, indicating that there were two variants of WDV-E in the leafhoppers from Haga. This tells us that the two variants of WDV-E were present in the feeding habitat of the leafhopper population in Haga.

The SDT analysis demonstrated the high sequence identities among WDV-E isolates in comparison to isolates of other WDV strains. All WDV-E isolates, including the clones isolated in this report, shared more than 97.5% identity and they were all Swedish isolates. One should mention that only part of the genome was sequenced and analysed. There could be changes in other parts of the genome that can give rise to changes in virulence factors. The LIR has very conserved sections, but does not encode proteins, and mutations here would not affect the amino acid sequences of proteins, which are virulence factors. Mutations in LIR could, however, affect gene expression and replication.

There are many theories of virus evolution, and among them are Darwin´s theory of evolution by natural selection, the punctuated equilibrium, and the symbiosis model (Pagan, 2018). Selection pressure acts upon the evolution of viruses (Monjane et al., 2020) and an important source for genetic diversity in virus populations comes from recombination events (Pagan, 2018). Why WDV has slow evolution in Sweden could partly be explained by the lack of genetic diversity in the WDV population. No other WDV strains have been found so far, and therefore, evolution through recombination would not occur. Host plants are not as diverse in Sweden as, for example, the diversity of cereals and grasses in the Fertile Crescent (Parizipour et al., 2017; Balla et al., 2022). Although there are host reservoirs of symptomless grasses in Sweden, the problem arises when the virus is transmitted to cultivated wheat that lacks resistance or tolerance. Even though many forces, like selection pressure, punctuated equilibrium, and the symbiosis model, act upon the evolution of WDV-E in Sweden, looking at the sequences from 1969 or from 2002, there have not been any known changes in the genome that have led to more virulent strains. This indicates that the virus population might have reached a peak of fitness, where further changes through mutations impair its adaptation.

Even so, the dynamics of leafhopper populations in Sweden could also reduce the transmission events. The climate is colder as well as the vegetative period shorter compared to warmer regions, only producing two or three generations per year of the leafhoppers. Compared to aphids, leafhoppers do not spread long distances, which reduces the possibilities for long-distance virus transmission. This also reduces the possibility for the virus population to come in contact with other WDV-strains.

The leafhoppers were not classified into species in this study, but *P. alienus* is said to be a very common species found in or near fields (Abt et al., 2018). At the Swedish website Artportalen.se for amateurs to report species, the most reported *Psammotettix* species (Table 1) are *P. confinis* and *P. nodosus* with 606 and 491 reports, respectively, during the years 2000-2022. These volunteer reports cannot be subjected to any scientific analysis, but this shows that an inventory of the different species could be of good use. This includes for example using simultaneous easy methods for classification of leafhoppers when testing for WDV.

Identification of factors related to feeding, habitat preferences, predators and competition between *Psammotettix* species, as well as mating disruptive patterns, might help in reducing populations and virus transmission by *P. alienus* (Derlink et al., 2018).

4.1 Conclusions

To answer the hypothesis if it is still the same strain of WDV occurring in the fields of Sweden as the first sequenced WDV isolate from 1969 and described in McDowell et al. (1985), the answer is, yes. All WDV-E isolates showed over 97.5% nucleotide identity to each other.

The presence of WDV in symptomless reservoirs makes it difficult to predict disease outbreaks over time and space. This could be one of the reasons why it has kept re-occurring since the early 20th century. Several reports (Lindsten & Lindsten, 1999; Mehner et al., 2003; Lindblad & Sigvald, 2004; Manurung et al., 2004) suggest that volunteer plants play a crucial role in the epidemics of wheat dwarf disease. Considering the slow evolution of WDV and lack of other strains in Sweden, breeding and use of resistant wheat cultivars could be a good method of decreasing crop losses.

This knowledge further supports that the combination and the quantity of hosts, vectors + virus, and suitable environment, will define the magnitude of disease outbreaks.

References

Abt, I. & Jacquot, E. (2015). Wheat dwarf. In: Tennant, P & Fermin, G. *Virus diseases of tropical and subtropical crops.* UK: CAB International, 27–41. https://doi.org/10.1079/9781780644264.0027

Abt, I., Derlink, M., Mabon, R., Virant-Doberlet, M. & Jacquot, E. (2018). *Integrating multiple criteria for the characterization of Psammotettix populations in European cereal fields*. Bulletin of entomological research, 108 (2), 185–202. https://doi.org/10.1017/S0007485317000669

Altschul, S.F., Gish, W., Miller, W., Meyers, E.W. & Lipman, D.J. (1990). *Basic local alignment search tool*. Journal of molecular biology, 215 (3), 403–410. https://doi.org/10.1006/jmbi.1990.9999

Balla, M.Y., Gorafi, Y.S.A., Kamal, N.M., Abdalla, M.G.A., Tahir, I.S.A. & Tsujimoto, H. (2022). *Exploiting wild emmer wheat diversity to improve wheat A and B genomes in breeding for heat stress adaptation*. Frontiers in plant science, 13, 895742. https://doi.org/10.3389/fpls.2022.895742

Berardo A., Fattoruso V., Mazzoni, V. & Pugno, N. M. (2022) *Coupling computational vibrational models and experimental biotremology to develop a green pest control strategy against the greenhouse whitefly* ***Trialeurodes vaporariorum.*** Journal of the royal society interface, 19 (195), 20220311. https://doi.org/10.1098/rsif.2022.0311

Buerstmayr, M. & Buerstmayr, H. (2023). *Two major quantitative trait loci control Wheat dwarf virus resistance in four related winter wheat populations*. Theoretical and applied genetics, 136 (5), 103. https://doi.org/10.1007/s00122-023-04349-3

Derlink, M., Abt, I., Mabon, R., Julian, C., Virant-Doberlet, M. & Jacquot, E. (2018). *Mating behavior of Psammotettix alienus (Hemiptera: Cicadellidae): Behavior of the leafhopper P. alienus.* Insect science, 25 (1), 148–160. https://doi.org/10.1111/1744-7917.12379

Douwes, P. (1998). *Insekter: en fälthandbok*. 2 rev. uppl. Stockholm: Interpublishing.

Ekzayez, A., Kumari, S. & Ismail, I. (2011). *First report of Wheat dwarf virus and its vector (Psammotettix provincialis) affecting wheat and barley crops in Syria*. Plant disease, 95 (1), 76. https://doi.org/10.1094/PDIS-09-10-0628

Fattoruso, V., Anfora, G. & Mazzoni, V. (2021). *Vibrational communication and mating behavior of the greenhouse whitefly Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae).* Scientific reports, 11 (1), 6543. https://doi.org/10.1038/s41598-021-85904-0

Fiallo-Olivé, E., Lett, J.-M., Martin, D.P., Roumagnac, P., Varsani, A., Zerbini, F.M. & Navas-Castillo, J. (2021). *ICTV Virus taxonomy profile: Geminiviridae 2021.* Journal of general virology, 102:001696. https://doi.org/10.1099/jgv.0.001696

Hull, R. (2014). Chapter 12 - *Plant to plant movement.* Plant virology. Fifth edition. Elsevier Inc, 669–751. https://doi.org/10.1016/B978-0-12-384871-0.00012-1

Jin, J., Shen, T., Shu, L., Huang, Y., Deng, Y., Li, B., Jin, Z., Li, X. & Wu, J. (2023). Recent achievements in antiviral agent development for plant protection. *Journal of agricultural and food chemistry*, 71 (3), 1291–1309. https://doi.org/10.1021/acs.jafc.2c07315

Jordbruksverket. (2021) *Skadegörare i jordbruksgrödor. Vetedvärgvirus.* Växtskyddsinfo. [Faktablad] Växtskyddsinfo (jordbruksverket.se)[2022-11-23]

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). *MEGA X: Molecular evolutionary genetics analysis across computing platforms.* Molecular biology and evolution, 35 (6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Kvarnheden, A., Lindblad, M., Lindsten, K. & Valkonen, J.P.T. (2002). *Genetic diversity of Wheat dwarf virus*. Archives of virology, 147 (1), 205–216. https://doi.org/10.1007/s705-002-8313-x

Lapierre, H.D. & Hariri, D. (2008). *Cereal viruses: wheat and barley*. Encyclopedia of Virology. 490–497. https://doi.org/10.1016/B978-012374410-4.00701-9

Lindblad, M. & Arenö, P. (2002). *Temporal and spatial population dynamics of Psammotettix alienus, a vector of Wheat dwarf virus*. International journal of pest management, 48 (3), 233–238. https://doi.org/10.1080/09670870110118731

Lindsten, K. & Lindsten, B. (1999). *Wheat dwarf: an old disease with new outbreaks in Sweden*. Journal of plant diseases and protection, 106 (3), 325–332

Lindblad, M. & Sigvald, R. (2004). *Temporal spread of wheat dwarf virus and mature plant resistance in winter wheat*. Crop protection, 23 (3), 229–234. https://doi.org/10.1016/j.cropro.2003.08.011

Lindsten, K., Vacke, J. & Gerhardsson, B. (1970). *A preliminary report on three cereal virus diseases new to Sweden spread by Macrosteles and Psammotettix leafhoppers.* National Swedish Institute for Plant Protection Contributions 14, 285-97

Liu, Y., Du, Z., Wang, H., Zhang, S., Cao, M. & Wang, X. (2018). *Identification and characterization of wheat yellow striate virus, a novel leafhopper-transmitted nucleorhabdovirus infecting wheat*. Frontiers in microbiology, 9, 468. https://doi.org/10.3389/fmicb.2018.00468

Loewe (2012). Catalog/Downloads/Manuals, ELISA. URL: LOEWE (loewe-info.com) [2022-12-16]

MacDowell, S.W., Macdonald, H., Hamilton, W.D.O., Coutts, R.H.A. & Buck, K.W. (1985). *The nucleotide sequence of cloned Wheat dwarf virus DNA.* The EMBO journal, 4 (9), 2173–2180. https://doi.org/10.1002/j.1460-2075.1985.tb03912.x

Manurung B, Witsack W, Mehner S, Gruntzig M, Fuchs E, 2004. *The epidemiology of Wheat dwarf virus in relation to occurrence of the leafhopper Psammotettix alienus in Middle-Germany*. Virus research 100, 109–13.

Mehner, S., Manurung, B., Gruntzig, M., Habekuss, A., Witsack. W. & Fuchs, E., (2003). *Investigations into the ecology of the Wheat dwarf virus (WDV) in Saxony-Anhalt, Germany*. Journal of plant diseases and protection, 110, 313–323.

Monjane, A.L., Dellicour, S., Hartnady, P., Oyeniran, K.A., Owor, B.E., Bezuidenhout, M., Linderme, D., Syed, R.A., Donaldson, L., Murray, S., Rybicki, E.P., Kvarnheden, A., Yazdkhasti, E., Lefeuvre, P., Froissart, R., Roumagnac, P., Shepherd, D.N., Harkins, G.W., Suchard, M.A., Lemey, P., Varsani, A. & Martin, D.P. (2020). *Symptom evolution following the emergence of maize streak virus*. eLife, 9, e51984. https://doi.org/10.7554/eLife.51984

Muhire, B.M., Martin, D.P., Brown, J.K., Navas-Castillo, J., Moriones, E., Zerbini, F.M., Riviera-Bustamente, R., Malathi, V.G., Briddon, R.W., and Varsani, A. (2013) *A genomwide paire-identity-based proposal for the classification of viruses in the genus Mastrevirus (family Geminiviridae).* Archives of virology, 158, 1411-1424. http://doi.org/10.1007/s00705-012-1601-7

Muhire, B.M., Varsani, A. & Martin, D.P. (2014). *SDT: a virus classification tool based on pairwise sequence alignment and identity calculation*. PLoS one, 9 (9), e108277. https://doi.org/10.1371/journal.pone.0108277

Nault, L.R. & Ammar, E.D. (1989). *Leafhopper and planthopper transmission of plant viruses.* Annual review of entomology, 34 (1), 503–529. https://doi.org/10.1146/annurev.en.34.010189.002443

Nilsson-Ehle, H. (1918). Årets svåra vetesjukdom. *Landtmannen*, 1, 564-566

Ordon, F., Habekuss, A., Kastirr, U., Rabenstein, F. & Kühne, T. (2009). *Virus resistance in cereals: Sources of resistance, genetics and breeding.* Journal of phytopathology, 157 (9), 535–545. https://doi.org/10.1111/j.1439-0434.2009.01540.x

Pagan, I. (2018). *The diversity, evolution and epidemiology of plant viruses: A phylogenetic view*. Infection, genetics and evolution, 65, 187–199. https://doi.org/10.1016/j.meegid.2018.07.033

Palmer, K.E & Rybicki, E.P. (1998). *The molecular biology of mastreviruses*. Advances in virus research, 50, 183-234. https://doi.org/10.1016/S0065-3527(08)60809-X

Parizipour, M.H.G., Schubert, J., Behjatnia, S.A.A., Afsharifar, A., Habekuß, A. & Wu, B. (2017). *Phylogenetic analysis of Wheat dwarf virus isolates from Iran*. Virus genes, 53 (2), 266–274. https://doi.org/10.1007/s11262-016-1412-0

Pettersson, J. (2020). *Detection of poleroviruses causing virus yellows in sugar beet.* MSc thesis. Dept. of Plant Biology, Swedish University of Agricultural Sciences.

Pfrieme, A., Will, T., Pillen, K. & Stahl, A. (2023) *The past, present and future of wheat dwarf virus management- A review*. Plants, 12, 3633. https://doi.org/10.3390/plants12203633

Ramsell, J.N., Lemmetty, A., Jonasson, J., Andersson, A., Sigvald, R. & Kvarnheden, A. (2008). *Sequence analyses of Wheat dwarf virus isolates from different hosts reveal low genetic diversity within the wheat strain*. Plant pathology, 57 (5), 834–841. https://doi.org/10.1111/j.1365-3059.2008.01862.x

Rojas, M.R., Macedo, M.A., Maliano, M.R., Soto-Aguilar, M., Souza, J.O., Briddon, R.W., Kenyon, L., Rivera Bustamante, R.F., Zerbini, F.M., Adkins, S., Legg,

J.P., Kvarnheden, A., Wintermantel, W.M., Sudarshana, M.R., Peterschmitt, M., Lapidot, M., Martin, D.P., Moriones, E., Inoue-Nagata, A.K. & Gilbertson, R.L. (2018). *World management of geminiviruses.* Annual review of phytopathology, 56 (1), 637–677. https://doi.org/10.1146/annurev-phyto-080615-100327

Sambrook, J & Russell, D.W. (2001). *Molecular cloning: a laboratory manual. Vol*. 1. 3. ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Serfling, A., Kopahnke, D., Habekuss, A., Novakazi, F., & Ordon, F. (2017). Wheat diseases: an overview. In: Langridge, P (ed.) *Achieving sustainable cultivation of wheat*. Cambridge, UK: Burleigh Dodds Science Publishing, 319–326.

Sigvald, R. (2006). *Vetedvärgsjuka*. Faktablad om växtskydd, jordbruk. Uppsala. SLU. 83J. faktablad_om_vaxtskydd_83j.pdf (slu.se) [2023-03-15]

Vacke, J. (1961). *Wheat dwarf virus disease.* Biologia plantarum (Praha), 3, 228–233.

Vacke, J. (1972). *Host plants range and symptoms of wheat dwarf virus.* Vedecke Prace Vyzkumnych Ustavu Rostlinne Vyroby v Praze-Ruzyni, 17, 151-162.

Wang, Y., Mao, Q., Liu, W., Mar, T., Wei, T., Liu, Y. & Wang, X. (2014). *Localization and distribution of Wheat dwarf virus in its vector leafhopper, Psammotettix alienus*. Phytopathology, 104 (8), 897–904. https://doi.org/10.1094/PHYTO-09-13-0251-R

Wilson M. R. & Turner J. A. (2021). *Insect vectors of plant disease*. Amgueddfa Cymru - National Museum Wales. http://insectvectors.science/. [2023-08-28].

Yazdkhasti, E. (2022). *Epidemiology of Wheat dwarf virus.* PhD thesis. Dept. of Plant Biology, Swedish University of Agricultural Sciences.

Yazdkhasti, E., Hopkins, R.J. & Kvarnheden, A. (2021). *Reservoirs of plant virus disease: Occurrence of Wheat dwarf virus and barley/cereal yellow dwarf viruses in Sweden*. Plant pathology, 70 (7), 1552–1561. https://doi.org/10.1111/ppa.13414

Zhao, L., Dai, W., Zhang, C. & Zhang, Y. (2010). *Morphological characterization of the mouthparts of the vector leafhopper Psammotettix striatus (L.) (Hemiptera: Cicadellidae).* Micron, 41 (7), 754–759. https://doi.org/10.1016/j.micron.2010.06.001

Acknowledgements

For professional guidance and support throughout this project I thank my supervisor Prof. Anders Kvarnheden. You have been very helpful bringing this project forward and I´m grateful for all the time you spent in educating and instructing me during this thesis. I would also like to thank Dr. Elham Yazdkhasti who helped me get started with the lab work and everyone in the department of Plant Biology who helped me in the lab when I had any questions.

Special thanks to my opponent, Tua Lilja for making this experience a good one. As well as many thanks to associate Prof. Anders Hafrén for coming forward as my examinator and Jordbruksverket for supplying the winter wheat, leafhopper samples and pictures.

My personal acknowledgements and gratitude for all the help and moral support from my family during this process. Lastly, final thanks to all personal working in the department of Plant biology for welcoming me, as well as staff working in the building who kept me company at lunch breaks.

Appendix 1

Leafhopper testing of WDV 2021 (Wärdig & Kvarnheden, unpublished).

Växtskydds-central	cw prov#	Gröda	Lokal	Fältnr PoV	Län	stritar /prov	Extraktion	DNA konc ng/µl	PCR	WDV pos/neg	Kommentar
Linköping	1	höstraps	Helleberga (6480322, 519423)	1	E	7	2022-03-02	24,8	V	neg	
Linköping	2	höstraps	Hyttringe - Karlstad (6486285, 512381)	2	E	3	2022-03-03	40,8	V	neg	
Linköping	3	höstraps	Östervarv Mellangården - Lårstad (6481488, 502318)	3	E	3	2022-03-03	44,3	V	neg	band med mindre storlek
Linköping	4	höstraps	Yxstad (6471341, 485920)	21	E	2	2022-03-03	17,4	V	pos	
Linköping	5	höstraps	Renstad gård - Karleby (6466106, 495495)	22	E	1	2022-03-03	29,5	V	neg	
Linköping	6	höstraps	Forsa (6468894, 513456)	4	E	1	2022-03-03	40	V	neg	
Linköping	7	höstraps	Förråd (6484258, 545731)	41	E	3	2022-03-03	9,4	V	pos	
Linköping	8	höstraps	Hagelstad - Skärkind (6480783, 558015)	42	E	6	2022-03-03	18,2	V	pos	
Linköping	9a	höstraps	Säby (6496637, 583826)	61	E	~10	2022-02-24	37,4	V	neg	
Linköping	9b	höstraps	Säby (6496637, 583826)	61	E	~10	2022-02-24	36,3	V	neg	~30 st totalt
Linköping	9c	höstraps	Säby (6496637, 583826)	61	E	~10	2022-03-02	38	V	neg	
Linköping	10	höstraps	Skarpenberga (6484473, 586165)	62	E	5	2022-03-03	96,8	V	neg	
Linköping	11	höstraps	Varningsrutor i Örebro - Säbylund	62	T	3	2022-03-03	38,4	V	pos	
Linköping	12	höstraps	Lunda Prästgård (6512593, 600810 - gårdens koordinater)	92	T	3	2022-03-03	39,3	V	pos	
Linköping	13	höstraps	Förråd (6483120, 546935)	(41)	E	8	2022-03-02	30,3	V	neg	
Linköping	14	höstraps	Skälbo0 (6472891, 582377)	(61)	E	8	2022-03-02	48,2	V	pos	
Uppsala	15	höstvete	Nyborg, Upplands Bro		AB	1	2022-03-03	3,5	V	neg	
Uppsala	16	höstvete	Frölunda, Kungsängen		AB	1	2022-03-03	3,8	V	neg	
Uppsala	17	höstvete	Österby, Sigtuna		AB	2	2022-03-03	10,1	V	neg	
Uppsala	18	höstvete	Fyrisvall, Storvreta		C	4 (3+1)	2022-03-03	49,1	V	neg	6e och 13e sept
Uppsala	19	höstvete	Fyrisvall, Storvreta		C	9	2022-03-02	121,4	V	neg	
Uppsala	20	höstvete	Fyrisvall, Storvreta		C	3	2022-03-03	31,2	V	neg	
Uppsala	21	höstvete	Husby, Örsundsbro		C	4 (1+3)	2022-03-03	17	V	neg	6e och 20e sept
Uppsala	22	höstvete	Hacksta, Enköpings Näs		C	2	2022-03-03	12,6	V	neg	
Uppsala	23	höstvete	Hacksta, Enköpings Näs		C	2 (1+1)	2022-03-03	63,1	V	neg	20e och 27e sept
Uppsala	24	höstvete	Lövsta, Uppsala		C	1	2022-03-03	13,8	V	neg	
Uppsala	25	höstvete	Högsta		C	5	2022-02-24	16,3	V	pos	
Uppsala	26a	höstvete	Högsta		C	~6	2022-02-24	35,1	V	neg	14 st totalt
Uppsala	26b	höstvete	Högsta		C	~7	2022-03-02	36,2	V	neg	
Uppsala	27	höstvete	Högsta		C	7	2022-03-02	62,4	V	pos	
Uppsala	28	höstvete	Högsta		C	5	2022-03-03	20,4	V	neg	
Uppsala	29	höstvete	Lilla Vallskog		C	5	2022-02-24	9,8	V	neg	
Uppsala	30	höstvete	Lilla Vallskog		C	2	2022-02-24	4	V	neg	
Uppsala	31	höstvete	Lilla Vallskog		C	6	2022-03-02	65,9	V	neg	
Uppsala	32	höstvete	Lilla Vallskog		C	5	2022-03-03	19,3	V	neg	
Uppsala		höstvete	Haga, Enköping		C	0					Ingen strit

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. You will find a link to SLU's publishing agreement here:

- https://libanswers.slu.se/en/faq/228318.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

Nr. 200
Uppsala, 2024

Department of Plant Biology
SLU
Box 7080
75007 Uppsala, Sweden