Barley Yellow Dwarf Epidemiology

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Abstract

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Barley yellow dwarf (BYD) disease is induced by viruses that are vectored by aphids. The viruses infect grasses and cause severe damage on oats, barley and wheat worldwide. This thesis focuses on the following aspects of the BYD disease epidemiology: occurrence and genetic variability of viruses causing BYD disease, aphid population parameters and host plant damage caused by virus infection.

The virus species *Barley yellow dwarf virus*-PAV (BYDV-PAV), *Barley yellow dwarf virus*-MAV (BYDV-MAV) and *Cereal yellow dwarf virus*-RPV (CYDV-RPV) were found in Latvia. The three year incidence of BYDV/CYDV ranged from 9 to 15% in symptomatic leaf samples of spring cereals and from 2 to 19% in random samples of pasture grasses.

Sequence analyses of partial coat protein encoding region revealed close genetic relationships among all isolates of BYDV-MAV. The isolates from Sweden and Latvia are the first published BYDV-MAV sequences from Europe. Swedish and Latvian isolates of BYDV-PAV were found in two host-specific groups. A distinct variant of BYDV-PAV was discovered in Latvia and proposed to belong to a new species.

Monitoring of aphid flight activity over eight years indicated cereal aphid *Rhopalosiphum padi* (L.) predomination over *Sitobion avenae* (Fab.) and *Metopolophium dirhodum* (Walk.). Positive linear correlations were observed between suction trap catches and population size of *R.padi* in a field, and between spring and summer migrations of *R.padi*, but not for their summer/autumn and autumn/next spring migrations. Aphid density in a field was well predicted from proportion of tillers infested and the Nachman model.

A specific BYDV-PAV isolate inoculated to oats at four different growth stages decreased the grain biomass and plant height, especially when inoculated in early growth stages, but increased the number of tillers and panicles per plant. The infection did not affect 1000-kernel weight and grain volume weight.

In conclusion, the results of this thesis add new knowledge and contribute to understanding the parts of the complex system of BYD disease.

Keywords: Aphids, BYDV/CYDV, viruses, occurrence of the virus, coat protein encoding region, yield loss and time of infection

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Appendix

Papers I-IV

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Bisnieks, M., Kvarnheden, A., Sigvald, R. & Valkonen, J.P.T. 2004. Molecular diversity of the coat protein-encoding region of Barley yellow dwarf virus-PAV and Barley yellow dwarf virus-MAV from Latvia and Sweden. *Archives of Virology* 149, 843-853.

II. Bisnieks, M., Kvarnheden, A., Turka, I. & Sigvald, R. 2006. Occurrence of barley yellow dwarf virus and cereal yellow dwarf virus in pasture grasses and spring cereals in Latvia. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* 56, 171-178.

III. Bisnieks, M., Wiktelius, S., Lindblad, M., Turka, I. & Sigvald, R. Sampling and forecast of aphid species populations in spring cereals in Latvia. (Manuscript).

IV. Bisnieks, M., Persson, T., Eckersten, H. & Sigvald, R. 2005. The effects on yield and components of yield in oats infected with BYDV-PAV at different growth stages. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 112, 521-528.

Introduction

The scientific investigation of plant diseases now known to be caused by viruses did begin in late nineteenth century, whereas the earliest known written record describing what was almost certainly a virus disease is a poem in Japanese from 752 AD (Hull, 2002). Plant viruses, like fungi and bacteria, are infectious agents that can cause considerable losses in agriculture. Viruses were recognized as plant pathogens in 1898, during early studies on the etiology of tobacco mosaic disease in the Netherlands (Bos, 2000). Since that time, virus research has become more specialized and wide-ranging as a part of plant pathology, a multidisciplinary field that deals with all levels of biological organizations from molecules to ecosystems. Plant pathologists do epidemiological research in one form or another, concerned with elucidating the general principles underlying development of epidemics or direct problem-solving, for example with disease forecasting or crop loss assessment. Some researchers have put the main emphasis on etiology and virus characterization, whereas others primarily have been concerned about resistance breeding, vector ecology, or mechanisms of transmission. In this thesis I combined four papers describing four distant issues, which are essential parts of plant disease epidemiology: virus genetic diversity, surveys of virus occurrence, vector population dynamics and host plant yield damage.

As pointed out by Zadoks (2001), plant disease epidemiology gained its status as a separate discipline in 1963 after the first international meeting of plant disease epidemiologists in Pau, France and the appearance of a book by Vanderplank, *Plant Diseases: Epidemiology and Control* in 1963. Plant disease epidemiology is a discipline describing the dynamics of disease in time and space and it is concerned simultaneously with populations of pathogens and host plants within an environmental context (Milgroom & Peever, 2003). In the plant disease system for Barley yellow dwarf (BYD) an additional component exist - the vector, aphids that transmit the causal agents of the disease.

This work has been addressed to increase knowledge about BYD, which is caused by a virus (Oswald & Houston, 1951). A number of virus species can induce the disease of BYD (see below). This work deals with virus species classified as Barley yellow dwarf virus (BYDV) and Cereal yellow dwarf virus (CYDV). The BYD has been recognized as one of the most damaging diseases of cereal crops worldwide (D'Arcy, 1995). All cereals and majority of other grasses are susceptible to BYD (Huth, 2000).

In BYD epidemiology, the so called disease triangle has become commonly applied. This pyramid adds interactions with the environment to those between viruses, vectors and host plant. Although the picture of disease triangle is oversimplified, it may serve a useful purpose in drawing attention to some of the issues when solving questions in management of crop protection (Harrington, 2002).

Both epidemiology and population genetics are integral parts of population biology and they share many concepts. The main focus of population genetics is to

understand the evolutionary processes shaping and maintaining genetic variation within and among populations. With the advent of molecular biology and the ability to compare regions of genomic DNA representing conserved sequences, the development of laboratory tests increased at an amazing rate for all groups of plant pathogens. During the last decade phylogenetic analysis became a powerful tool to investigate specific global epidemiological issues and to identify potentially new genotypes of different viruses. The ability to differentiate virus species is also important for assessing the biological impact (e.g. yield) of any virus disease management strategy.

Components of Barley yellow dwarf disease system

Vector ecology and virus-vector interaction

Viruses often need insects as vectors for their transmission from plant to plant. Thus, the epidemiology of the virus largely depends on the dispersal pattern of the vector population (Fiebig *et al*., 2004). Plumb (1983) defined BYD as the disease with specific symptoms and effects that are caused by different persistently aphid-transmitted viruses. In aphids, next to whiteflies the most important insect vectors of plant viruses (Carter & Harrington, 1991), dispersal can be induced by various factors, among others crowding (Watt & Dixon, 1981; Maudsley *et al*., 1996) and the decreasing nutritional conditions of the host plants (Walters & Dixon, 1982). An extensive and effective way of monitoring the aphid migratory flights was started from Rothamsted (UK) by 12 m high suction traps in 1963. Since then a network of suction traps has been established across Europe, including Latvia.

Aphids posses phenotypic plasticity, the ability of a genotype to develop different phenotypes in different environments (Halkett *et al.*, 2004). In temperate climates, many species of aphids are holocyclic, i.e., they have several parthenogenetic generations during spring and summer and one sexual, egg producing generation in fall.

For *R. padi* sexual reproduction is associated with host-alternation from various herbaceous plants to bird cherry, *Prunus padus*. The host-alternating *R.padi* has different reproductive strategies. In response to autumn daylength and temperature the holocyclic parthenogenetic females give birth to males and a special type of parthenogenetic females, called gynoparae (Dixon & Glen, 1971). Males and gynoparae fly to bird cherry, where the latter give birth to sexual females, oviparae, which after maturation mate with males (Leather *et al.*, 1989). In autumn, the different morphs, holocyclic (gynoparae and males) and anholocyclic alate exules (virginoparae), are produced, but the proportions of the two groups are largely affected by geographical region, the presence of primary hosts and climate (Rispe *et al.*, 1998). Anholocyclic morphs predominate under conditions of mild winters, whereas holocyclic ones survive in regions with cold winters

(Wiktelius *et al*., 1990; Hulle *et al.*, 1998). In northern countries *R.padi* predominates as holocyclic, where the sexual generation produces overwintering eggs using *Prunus padus* as a primary host and this aphid is the principal vector of BYDV/CYDV in these regions (Lindsten, 1977; Wiktelius, 1987b; Kurppa *et al.*, 1989). The sexual generation of aphids breaks the link with virus transmission, as the primary host is not susceptible to BYDV/CYDV.

In spring, the first migrants of the *R.padi* on primary host *P.padus* need at least 14^{o}C in order to fly to grasses or cereals (Kurppa, 1991) and to feed on infected host before they can act as vectors of BYDV/CYDV. A part of initial colonizers that bring the virus in cereals create the primary foci of infection within the crop. Later subsequent aphid generations determine the spread of secondary spread of the virus. The relative importance of primary and secondary spread in cereals differs in different regions (Plumb, 1995).

Different BYD-causing viruses are transmitted more efficiently by different aphid species, a fact that was originally used by Rochow (1969) to distinguish the virus. Although, currently, it is more common to identify field isolates by serological or molecular techniques (Lister & Rochow, 1979; Robertson *et al.*, 1991), the aphid transmission phenotype is the most important diagnostic characteristic in terms of the epidemiology of the disease and designing control strategies that target the vector (Lucio-Zavaleta *et al.*, 2001).

Each luteovirus (see below) is transmitted only by a limited number of aphid species, showing high vector-specificity: *Barley yellow dwarf virus*-MAV (BYDV-MAV) is transmitted by the grain aphid *Sitobion avenae; Barley yellow dwarf virus*-PAV (BYDV-PAV) is transmitted by *S. avenae* and the bird-cherry aphid *Rhopalosiphum padi; Cereal yellow dwarf virus*-RPV (CYDV-RPV) is transmitted by *R. padi.* Aphid transmission specificity of viruses shows high stability over many years (Rochow, 1969). Virus-vector specificity appears to depend on the specificity of receptor sites on the membrane surface in the aphid's accessory salivary gland and the viral capsid protein (Gildow & Rochow, 1980; Gildow & Gray, 1993). However, with respect to the relative transmission efficiencies of BYDV or CYDV species, Lucio-Zavaleta *et al.* (2001) indicated that it is not appropriate to assume that it is consistent across all or even most clonal populations of any aphid species. Whereas, Guo *et al.* (1997) proved that transmission efficiency of vector aphids correlate with virus titre retained in the aphids.

Recent findings have indicated that there is an essential interaction for virion stability between a luteovirus and the extracellular protein Buchnera GroEL produced by endosymbiotic bacteria in the aphid hemolymph (Filichkin *et al.*, 1997; Young & Filichkin, 1999). Virions, when ingested with phloem sap from infected plants, are transported through the gut into the hemocoel by receptor-mediated endocytosis-exocytosis (Gildow, 1993; Gildow & Gray, 1993). The hemolymph acts as a reservoir in which acquired virus particles are retained in an infective form for the aphid's lifespan, without replication. The high degree of vector specificity of luteoviruses among aphid species implies an intimate

relationship between aphid components, the Buchnera GroEL and receptor molecules, and surface domains of the viral capsid. Van den Heuvel *et al.* (1997) suggested that the Buchnera GroEL and read trough domain (RTD) interaction protects the virus from rapid degradation in the aphid.

Among insects, the aphids have evolved to be the most successful exploiters of higher plants as a food source. In many parts of the world *R.padi* is considered as the primary risk factor for BYDV/CYDV incidence and aphid related yield loss on cereals (Chapin *et al.*, 2001).

Taxonomy and diversity of viruses causing Barley yellow dwarf disease

Barley yellow dwarf virus is a member of genus *Luteovirus* and the type member of the *Luteoviridae* family (formerly luteovirus group) (D'Arcy, 2000). The old genus *Luteovirus* was separated into two genera – genus *Luteovirus* containing species of former subgroup I, BYDV-PAV and BYDV-MAV, and genus *Polerovirus* containing species of former subgroup II, *Potato leafroll virus* (PLRV), BYDV-RPV and other non-BYD-causing viruses. PLRV was chosen as the type species along with former BYDV-RPV renamed as *Cereal yellow dwarf virus*-RPV (CYDV-RPV). In addition, there are unclassified and tentative members within the family *Luteoviridae*, BYDV-SGV and BYDV-RMV, which share similar biology with respect to aphid transmission, but differ genetically. Currently, a more extensive strategy is employed taking into account also genome composition. Taxonomy for luteoviruses was greatly affected after emerging knowledge from sequenced isolates of BYDV.

For many virus types, the serological relatedness of a virus was sufficient to assume the identity or novelty of the isolate being tested. However, among luteoviruses this has proved not to be a definitive character. Classification of luteoviruses was based on serological relatedness, physicochemical properties of the virus particles and biological properties, such as vector relations and tissue localization (Mayo & D'Arcy, 1999). Luteoviruses are single-stranded, positive sense RNA viruses that are restricted to the phloem in plants and transmitted exclusively by aphids in a persistent circulative manner. The phloem limitation of luteoviruses is not due to their inability to replicate in cells other than phloem cells, but rather due to failure of their movement in other cells than phloem (Gill & Chong, 1975, Young *et al.*, 1991). Distinct BYD-causing virus species of the luteovirus group initially shared the same virus name and were differentiated by a suffix according to species of aphids that most efficiently transmitted that strain as identified in experiment by Rochow (1969).

There are two specific features of luteoviruses that set them apart from other viruses. One is the coat protein readthrough domain that is essential for aphid transmission (van den Heuvel *et al.*, 1994). The second feature is the ability to interact with other viruses in such a way that luteovirus coat protein is covering the non-luteoviral RNA (Harrison, 1999). The coat protein (CP) of luteoviruses is

the most conserved viral gene and therefore can be used to compare and differentiate specific virus species. Comparisons among the amino acid sequences of luteovirus CP show that the CP is 60% or more identical among viruses in genus *Luteovirus* or in genus *Polerovirus*, less than 50% identical between pairs of viruses in either genus (Mayo & D'Arcy, 1999). In this study, the partial nucleotide sequence of the CP of BYDV-PAV and BYDV-MAV species from Latvia and Sweden were described and compared to that of other luteoviruses.

Host Plants

BYD-causing viruses are transmitted to over 100 species of cultivated and wild grasses, within the family *Poaceae* (Irwin & Tresh, 1990). The various effects of viruses on plants are not equally harmful. The type and severity of host reaction to virus infection depend greatly on the crop genotypes, virus strains, age of plant at the time of infection and are influenced by environmental conditions. Host reactions to virus diseases are therefore extremely variable, as are the resulting losses (Bos, 1982).

Infection by BYD-causing viruses causes destructive effects on yield and quality of cereal crops. The most severe effects have been reported on oats, where reddening of the leaves and blasting of the florets are easily observed. Other symptoms are stunted growth and late heading (Yount *et al*., 1985). Symptoms on barley and wheat consist primarily of chlorosis and stunting and usually are less pronounced than in oats. Plant physiological processes are interfered by the virus that multiplies specifically within the phloem of the host plant. The infected phloem cells are destroyed and translocation of assimilates produced by leaves is reduced. This results in carbohydrate accumulation, which in turn increases dry weight, inhibits photosynthesis and reduces chlorophyll content that subsequently cause discoloration and thickening of leaves (Jensen, 1968). The severity of BYD effect on plants is determined also by the time of infection (Smith, 1967), the virus species involved (Rochow, 1969; Baltenberger *et al.*, 1987), and the cultivar genotype (Jedlinski, 1972).

BYDV has been found in various parts all over Scandinavia (Lindsten, 1977). In the majority of grasses obvious symptoms are not recognized (Catherall, 1966; Lindsten & Gerhardson, 1969). Nevertheless, both wild and perennial grasses play an important role in the epidemiology of BYD, providing a large and permanent virus source that spreads annually to cereal crops (Plumb, 1977; Kurppa *et al.*, 1989; Guy, 1991). However, some studies have revealed that the predominant strains of BYDV in grasses often differ from those causing the epidemics in the nearby cereal crops (Fargette *et al*., 1982; Paliwal, 1982; Henry & Dedryver, 1991; Moriones *et al.*, 1991).

Maize is known to be an important BYDV reservoir and a secondary host of *R.padi* in southern regions and areas of continental climate (Brown *et al.*, 1984; Clement *et al.*, 1986; Halbert *et al.*, 1992). A diversity of pasture grasses also serve as *R.padi* secondary hosts and reservoirs of the BYDV, but generally these

grasses are not considered a significant source of infection due to their very low aphid populations (Henry & Dedryver, 1991). Maize is not a common crop grown in Latvia and thus different grasses may play a more important role than in France as a virus source. However, not only local conditions prescribe the appearance of *R.padi* population in grasslands and cereals, but also the long-distance migration from very distant regions (Kurppa, 1989; Wiktelius, 1984).

There is an interest in the ability to forecast how crop losses will vary in relation to at which stage of crop development that plants become infected. When healthy plants of a susceptible variety are exposed to virus inoculum, the virus may or may not become established in the plants and replicate. If infection does occur, and the virus multiplies and becomes systemic, the host plant can act as a source of inoculum from which further spread may occur. Thus, the knowledge about the time of infection and the abundance of viruliferous vectors is important when considering crop safety. The time of infection has an epidemiological aspect not only for virus spread from plant to plant, but also for the effect on individual plant development and level of yield.

Economic importance of virus disease

Detailed studies on plant viruses have been conducted mainly due to the impact that the disease they cause have on crop productivity worldwide. Virus diseases are frequently less conspicuous than those caused by other plant pathogens and last for much longer. The effects of virus infection can be various such as yield reduction, crop failure, increased sensitivity to frost ad drought, increased sensitivity to attack by other pathogens and pests, defects of visual attraction, reduced storing quality, etc. In spite of difficulties to gather data of crop losses due to virus diseases on global basis, there have been various collections of them from comparative trials and estimates (e.g. Hull & Davies, 1992; Waterworth & Hadidi, 1998).

BYD-causing viruses occur world-wide, infect a wide range of *Gramineae* species, and cause great yield losses in some years. Luteoviruses are one of the most ecologically successful and economically important taxa of plant viruses. Globally the problems due to BYD are the most serious in wheat, oats and barley. In most situations, oats and barley suffer most severe. Costly BYD epidemics have been reported in many parts of the world and reviewed, e.g., by Conti *et al.*, (1990), Burnett (1984, 1990), Comeau and Makkouk (1992). The incidence of BYD in any given year is hardly predictable. It depends on host and pathogen dynamics, environmental conditions that favour disease development, aphid population dynamics. Altogether it makes difficulties to justify every application of control measures.

As with other plant virus diseases, control measures for BYD are as follows: prevention of virus spread and use of virus resistant cultivars. In spite of the economic importance of BYD, few natural resistance genes have been identified in any of the major crops. Durable genetic resistance or tolerance to BYDV is the best and most cost-effective option for bringing this unpredictable disease under

control. Luteovirus-resistant gene, Y2, found in Ethiopian barley line has given an early success of resistance to a range of BYDV by restricting virus concentrations. A well characterized resistance gene, Yd_2, exist in barley, but it does not protect against all strains of BYDV (Skaria *et al.*, 1985). No natural resistance genes have been identified in oat or wheat. Toward overcoming this limitation, e.g., Koev *et al.* (1998) engineered virus-derived transgenic resistance in oat and supposed that the transgene acted by restricting virus accumulation. Although reduction of virus multiplication is desirable for longer term control of the disease and to diminish its spread in the environment, some resistant materials have proven sensitive to virus infection in the field (Ayala *et al*., 2001b). Due to the fact that viruses continually mutate it is unlikely that breeding for resistance or the development of transgenic plants can give a permanent solution.

Among other control measures are, e.g., host plant resistance to aphids (Basedow, 1981; Kuo, 1986), biological control with the manipulations of predator, e.g *Cocinellid*, and parasite , e.g. *Aphelinid* and *Aphidiid*, populations (Zuniga, 1990), cultural practice such as manipulation of sowing time in relation to aphid phenology.

Overall aims of the study

This project aimed to deliver a better understanding of, and to contribute to a disease management strategy for "Barley yellow dwarf". The long-term aim of this work is to develop forecast methods that will enable prediction of the risk for disease spread and justify chemical applications or the consideration of other methods. A better understanding of the epidemiology of BYD will help farmers to avoid BYDV/CYDV infection or minimize its influence.

Identification of the viruses and monitoring their vectors responsible for the disease spread were key aspects of this work. The work was accomplished using ELISA and molecular DNA techniques, suction trap catches, field counts and virus transmission procedures.

Specific objectives of the individual studies were:

1. To develop and refine a molecular technique for detection of the CP-encoding region of BYDV-PAV and BYDV-MAV from leaf samples (I).

2. To reveal the sequence variability of BYDV-PAV and BYDV-MAV by analyzing the CP-encoding region of six isolates from Latvia and four isolates from Sweden (I).

3. To identify the virus species of BYDV/CYDV and assess their occurrence in spring cereals and pasture grasses (II).

4. Determine the status of grass species in pastures as BYDV/CYDV reservoirs (II).

5. To monitor cereal aphid flight activity and their abundance in spring cereal fields (III).

6. To evaluate the relationships between abundance of aphids in suction trap catches and field surveys, between the seasonal migrations of *R. padi*, and between proportions of plants infested versus average aphid density on tillers (III).

7. To investigate and to quantify the yield loss potential in oats (cv. Stork) infected with PAV-Sto isolate of BYDV at four different growth stages: 11, 13, 31 and 39 (IV). To evaluate the impact of the isolate on the grain weight, biomass, number of tillers and panicles (IV).

Materials and Methods

Study I Molecular diversity of the coat protein-encoding region of PAV and MAV isolates of BYDV

Leaf samples of spring barley, spring oats and pasture grasses were collected from Latvia and Sweden in 2000-2001, and tested with triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA) for BYDV-PAV and BYDV-MAV. Six samples positive for BYDV-PAV and four samples positive for BYDV-MAV were further analysed by sequencing the central part (502 bp) of the CP gene. The CP gene of BYDV-PAV isolates was amplified by immunocapture reverse transcription polymerase chain reaction (PCR) with primers designed by Robertson *et al*., (1991) (Table 1). New primers were designed and the PCR cycle was slightly changed for isolates of BYDV-MAV (Table 1).

Table 1 A list of PCR primers used in this study

Origin	Primer	Sequence
Robertson *et al.* (1991)	Lu1	5‘ CCA GTG GTT RTG GTC 3’
	Lu4	5‘ GTC GTA CCT ATT TGG 3’
Bisnieks *et al.* (I)	M3	5’ ATG AAT TCA GTA GGC CGT AG 3’
	M4	5’ CGG ATC AGG TTT GGG CTC TG 3’

PCR products were purified and cloned. Two or three clones of each virus isolate were sequenced in both directions. The nucleotide sequences of 502 bp of the cloned PCR fragments were aligned with those of 29 other virus isolates of diverse geographic origin from the family *Luteoviridae* that were available in GenBank. Multiple sequence alignments were made using Clustal W (Thompson *et al.*, 1994). Phylogenetic analyses were carried out using Phylogenetic Analysis Using Parsimony (PAUP) (Swofford, 2002).

Study II Occurrence of BYDV/CYDV in spring cereals and pasture grasses

During the study period, 2000-2002, a total of 2589 leaf samples (367 spring oats, 743 spring barley, 1479 predominant grass species) were collected from 44 fields of spring oats, 84 fields of spring barley, and 26 pastures at two sites of Latvia. Two to ten leaf samples of spring cereals with BYD symptoms were collected at each crop during the growth stages 31 (first node detectable) and 61 (beginning of anthesis) according to Zadoks *et al.* (1974). Three to five dominant pasture grasses were selected for sampling at each pasture (Table 2). Ten to 60 leaf samples were collected of each selected species (*Phleum pratense*, *Lolium perenne*, *Poa spp.*, *Dactylis glomerata*, *Festuca elatior* and *Bromus inermis*) at five places with regular intervals on a diagonal transect across the pasture.

Table 2 Parameters included in the study of BYDV/CYDV occurrence in Latvia

Virus tested	Pasture grass species tested	Crop species tested	Area tested	Years
BYDV-PAV BYDV-MAV CYDV-RPV	*Phleum pratense*, *Lolium perenne*, *Dactylis glomerata*, *Festuca elatior,* *Bromus inermis,* *Poa spp.*	Barley Oats	Cesis Saldus	2000 2001 2002

Leaf samples were put into plastic bags and kept on ice during transport to the laboratory of the Department of Plant Biology and Protection, Latvia University of Agriculture in Jelgava, where they were stored at – 20°C until analysis (I). Each leaf collected from cereals and grasses was tested for BYDV-PAV, BYDV-MAV and CYDV-RPV. Absorbance at 405 nm was measured by an ELISA Reader. The absorbance of the sample was considered positive when A_{405} nm reading was at least twice the mean of the healthy control.

The probability of a leaf sample being infected with BYDV-PAV, BYDV-MAV and CYDV-RPV or in any mixture of them was estimated as a function of year, region and crop using logistic regression (McCullagh & Nelder, 1989) with the SAS Procedure GENMOD (SAS Institute, 1996).

Study III Sampling and forecast methods of cereal aphids in spring cereals in Latvia

All data recorded in this study were obtained from two sites of Latvia: Saldus (56°40N, 22°30E) and Cēsis (57°18N, 25°15E). Eight-year data of *R. padi*, *S. avenae* and *Metopolophium dirhodum* flight activity were obtained from suction trap catches collected by specialists of Latvian State Plant Protection Service.

From 2 to 15 fields were surveyed within 30 km from each suction trap during 1998-2003. At each field, 80-100 tillers were examined.

Three different relationships were investigated:

1. Relationships between migrations of *R. padi* were estimated with regression analysis.
2. Spring migration of *R. padi* was related to the maximum aphid density in a field.
3. The log of the mean aphid density was related to a double logarithmic transformation of the reciprocal of the proportion of non-infested tillers (*Nachman*, 1981).

Study IV The impact of BYDV-PAV infection time on oat yield

In this study, oats (cv. Stork) were sown near Uppsala in 2002 and inoculated with PAV-Sto isolate of BYDV. The experiment was performed in randomised complete block design with four blocks (as replication) and five treatments. Oats were isolated after germination with net cages of $1m^3$. Oats were artificially inoculated by transfer of viruliferous bird cherry-oat aphids (*R. padi*) at one of four different growth stages: 11 (first leaf extended), 13 (third leaf extended), 31 (first node detectable) and 39 (flag leaf just visible) according to Zadoks *et al.* (1974). Around 200 aphids were placed along each of the three middle rows and allowed to feed on plants for six days and then killed by spraying insecticide. Oats were hand-harvested and dried in a cold air. The measurements recorded were number of tillers and panicles, plant height, biomass, weight of threshed and cleaned grains, grain volume weight and 1000-grain weight. Treatment means were compared using the least significant difference test at 5% level.

Results and Discussion

Coat protein diversity of BYDV-PAV and BYDV-MAV isolates (I)

Partial nucleotide sequences of coat protein (CP) genes of ten BYDV-PAV and BYDV-MAV isolates from Latvia and Sweden were determined in this study and compared with 29 isolates from the family *Luteoviridae* that were available in GenBank. In a phylogenetic analysis, all isolates of PAV, PAS and MAV, including those from Latvia and Sweden, formed one group (genus *Luteovirus*) whereas isolates of CYDV-RPV, *Barley yellow dwarf virus-GPV* (BYDV-GPV) and PLRV formed another group (genus *Polerovirus*).

Four well-supported groups were observed within the genus *Luteovirus*: PAV, PAS, MAV and one group with a distinct isolate from Latvia (PAV-Sal1) and PAV-CN. The diversity within MAV was low, considering that the analysis included isolates from distant parts of the world (Europe, U.S.A. and China). In contrast, the variability among PAV isolates was greater and showed two major clusters, with PAV isolates from Latvia and Sweden placed to both clusters.

Based on analysis of CP sequences, geographical origin of isolates did not show an influence on genetic diversity. Instead, isolates of BYDV-PAV were separated into two major clusters according to the host species from which they were isolated. PAV isolates from oat and barley were placed into group I, while PAV isolates from grasses and barley were placed in the group II. A larger number of virus isolates from different hosts needs to be sequenced to verify the suggested grouping according to host species.

There was one isolate of the most common serotype PAV that was distinctly different from other PAV isolates. The isolate PAV-Sal1 from Latvia showed the highest nucleotide identity (86%) with a divergent PAV isolate from China (PAV-CN) and 77% identity with MAV isolates. A difference at amino acid level exceeding 10% for any viral gene product can be used as a criterion to distinguish species within the family *Luteoviridae*. PAV-Sal1 shows no more than 86% amino acid identity to any other previously characterised virus isolate, which indicates that it belongs to a new species. The name, oat yellowing virus (BYDV-OYV), was proposed. This study shows that there is no geographic grouping of BYDV-PAV and BYDV-MAV isolates, despite the worldwide occurrence. In addition, it suggests that there may be many unknown species to be discovered.

Occurrence of BYDV in pasture grasses and spring cereals (II)

The incidence of BYDV/CYDV in grasses has often been shown to vary during the year and differ between geographic regions (Dempster & Holmes, 1995). The three common BYD-causing viruses, BYDV-PAV, BYDV-MAV and CYDV-RPV, occur in pasture grasses and cereals within two explored regions in Latvia and probably exist also in the other regions not inspected in this study. The results confirmed that all three species, BYDV-PAV, BYDV-MAV and CYDV-RPV, were present in these samples of spring cereals and pasture grasses collected in Latvia.

Pasture grasses

In both regions, the overall incidence of BYDV/CYDV in pasture grasses during 2000 to 2002 ranged from 2 to 19% and was highest in 2000. This level of incidence is relatively low compared to results from other studies: e.g., 90 % in England (Doodson, 1966) and 93 % in Scotland (Dempster & Holmes, 1995). Our results are consistent with those obtained from surveys in leys (mainly *L. perenne*) of northern France and southern England (5-11 %) (Henry *et al*., 1993), in ryegrass pastures of Australia (0-17 %) (Coutts & Jones, 2002), and in Germany (<10 %) (Huth, 2000).

BYDV/CYDV infection was detected in six common pasture grass species tested, but only PAV and MAV in *Poa* species. *Poa* spp. are known to be natural hosts of BYDV/CYDV and are frequently infected in the UK (Masterman *et al.*, 1994; Kendall *et al.*, 1996). Failure to detect CYDV-RPV in *Poa* spp. could be due to the small number of plants tested (n=45), low virus concentration, or virus resistance, which has not been investigated for Latvian *Poa* cultivars. Among

selected grass species, the estimated probability to find any of the three viruses was highest for *F. elatior*, followed by *D. glomerata*, and *L. perenne*.

With regards to particular grass species, our results are in agreement with those reported in Sweden (Lindsten & Gerhardson, 1969), France (Henry & Dedryver, 1991), USA (Fargette *et al*., 1982) and Australia (Henry *et al*., 1992), in that a high virus incidence was found in samples of *Festuca* spp. and also, but to a lesser extent in samples of *Lolium* spp. In addition, we found a high virus incidence in *D. glomerata*. Both *F. elatior* and *D. glomerata* were the most common grass species among and within pastures. Previous studies have demonstrated no relationship between BYDV incidence and change in proportion of *L. perenne* in mixed species perennial pastures (Coutts & Jones, 2002). Although, Malmstrom *et al.*, (2005) suggest that BYDV may contribute to changes in community composition in the grasslands as a result of differences in host tolerance to infection or population characteristics, such as stand structure and seed bank size.

When comparing among serotypes, all grass species collected, except *B. inermis*, were infected predominantly with MAV in year 2000. In 2001, BYDV-PAV dominated in grass samples, except for *L. perenne*, in which all three viruses were common. Isolates of CYDV-RPV were rather rare: merely found in *L. perenne* and *D. glomerata* among six grass species tested. In 2002, only 10 samples out of 450 were positive for any of the three viruses.

Comparing the occurrence of serotypes between the regions, pasture grasses infected with serotypes of PAV and RPV were more common in Cēsis than in Saldus ($P<0.001$ and $P<0.05$, respectively), while there was no difference for MAV. The incidence of the PAV and RPV differed ($P<0.01$) in Cēsis, but did not in Saldus.

The aspects that could explain the variation of virus occurrence between years and regions are well known, i.e. vector species, abundance of vectors, composition of grass species, growth conditions for grasses etc. Thus, theses factors should be analysed in details and related to particular virus occurrence in order to find any relationship that would help to explain or define specific occurrence of virus.

Cereals

A low proportion, on average 13%, of BYD-symptomatic cereal samples reacted positively in TAS-ELISA. Most of the infected samples contained only a single serotype of BYDV/CYDV. The proportion of leaf samples infected with each of the three serotypes varied among years. Both PAV and MAV were more prevalent than RPV ($P<0.001$) in 2002, whereas in 2001, no significant differences were found between samples infected with a specific serotype. This demonstrates that there is no predominance of a single serotype in cereals of Latvia.

Occurrence of virus infection was found in 20 out of 35 fields in 2001 and in 43 out of 93 fields in 2002. Within fields the overall infection of BYDV/CYDV over the two years was more common in barley than in oats ($P<0.01$). The more

frequent infections of BYDV/CYDV in barley than in oats observed in this study could be the result of a biased collection of symptomatic plants. The golden yellow leaves of barley were easier to recognize than the reddish oat leaves, which could be easily confused with nutrition deficiency. The other factors that can cause symptoms similar to those caused by viruses of BYDV/CYDV are, for example, aster yellows mycoplasma and moisture or temperature stress.

This is the first report of selected BYD-causing viruses in spring cereals and pasture grasses in Latvia and in the Baltic states. Since different approaches were used to collect leaf samples from cereals and pasture grasses, our results are not comparable between these groups of plants. Furthermore, the distribution of serotypes in cereals may be somewhat biased due to the sampling of only symptomatic leaves. Nevertheless, this study has shown that BYDV/CYDV are common in cereals and perennial grasses in Latvia and that BYD is a potential treat to cereal production in this country, particularly when conditions favour the occurrence of epidemics.

Aphid vector sampling and forecast methods (III)

Many aspects of vector biology affect their ability to transmit BYD-causing viruses. Of most epidemiological importance is the time and size of migratory flights (Plumb, 1983).

Abundance and dynamics of the cereal aphids

The abundance of the three species of cereal aphids in Latvia varied substantially among years and sites. *R. padi* was the most abundant in all years followed by *S. avenae* and *M. dirhodum*. The same order of species abundance has been reported in Finland (Rautapää, 1976) and Sweden (Wiktelius *et al*., 1985), but different in UK, where *S. avenae* predominated (Basky & Harrington, 2000).

Over the eight years, the difference of *R. padi* abundance was 14 and 20 fold, in the regions of Saldus and Cēsis, respectively. However, the years of extremes did not match in both regions. In five years out of eight, the catch of *R. padi* in suction trap was greater in Cēsis than in Saldus. In 1996, the greatest, seven fold difference of total *R. padi* abundance was recorded in Cēsis over Saldus, mainly due to the autumn migrants. Reasons for great variability of aphid populations between sites consistently over many years could be explained by habitat diversity, particularly grassland fragmentation between the regions (Braschler *et al.*, 2003). Variation in abundance of *S. avenae* and *M. dirhodum* between regions of the same year was less pronounced than for *R. padi*, although the counts could be too small for a reasonable comparison.

Patterns of R. padi migrations

All three distinct *R. padi* flights that occur seasonally (spring, summer, and autumn) were detected, but their relative size varied a lot among the years. Large numbers of *R. padi* were observed in both regions during summer migration in 1999 and 2002, with a following 28 to 88 fold decrease in the magnitude of

population size in autumn. The greatest flight activity in autumn over the study period occurred at Cesis in 1996, 8 orders of magnitude larger than at Saldus.

The mean weekly abundance of *R. padi* over eight years indicates that a small spring peak converges with the large summer flight activity, while in autumn an intensive flight is extended over longer time than in spring and summer (Fig. 1). An overlap of spring and summer migrations seems to occur particularly in week 24 (mid-June). Total weekly catches over eight years were relatively smaller in autumn than in summer and that is opposite to other eight-year results from Hungary and UK reported by Basky and Harrington (2000).

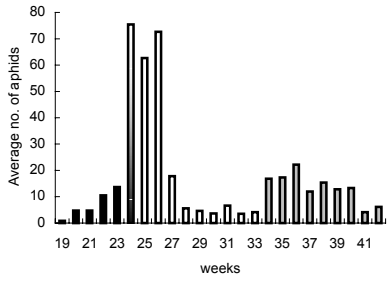

Fig. 1 Mean abundance of *R.padi* in suction trap catches in Latvia between 1996-2003. Bars filled in different colors represent flight activity in spring (black), summer (white) and autumn (gray).

Wiktelius (1990) reported an inverse linear relationship between summer and autumn migrations of *R.padi* in Sweden and explained the possible causality by a delayed natural predation. Natural predators are capable to follow large aphid numbers from cereals to grasslands (Wiktelius, 1987a). However, Ekbom *et al.*, (1992) suggest that high predation rates during the exponential growth period appear to have little effect on aphid population growth. This contrasts to our findings, where no relationship was found between these two migrations from the eight-year data. The lack of correlation in our data could be explained by the fact that the magnitude of autumn migration, which is formed mainly in grasslands (Wiktelius, 1987a), depends on the nutritional quality of the grasses (A'Brook, 1981; Breton & Addicott, 1992). Since precipitation, that greatly varies from year to year, affects the growth and quality of grasses, the size of summer migration may not be the sole and the direct determinant for the numbers of autumn migrants.

There was a positive linear relationship between abundance of *R. padi* in spring and summer migrations for both regions, which is consistent with previous results by Wiktelius (1990) and is well discussed incorporating predation into the aphid population growth model by Ekbom *et al.* (1992). However, relationships between spring migration and autumn migration in the previous year were different between regions. A positive correlation was found between aphid migration in

spring and the autumn in previous year in Cesis. However, a negative insignificant relationship of the two parameters was obtained in the other region, Saldus.

Relationship between spring migration and maximum density of R. padi in the field

A positive correlation between the maximum density of *R. padi* on tillers and spring migration was significant for data over the three years (2001-2003) from Latvia. The slope of the line is relatively steeper and has a lower intercept than the one reported for Sweden (Wiktelius, 1987b): From a given equation in our work, for example, if 1, 10 or 50 aphids are captured in a suction trap sample it would indicate the maximum density of 0.07, 1.1 or 12 aphids on average per tiller of spring barley, respectively.

Relationship between population density and spatial distribution

Distribution of species in space has long been of interest for theoretical and practical use. Distribution data have been widely used to address many important ecological questions - evolutionary dynamics of species, species-habitat association, effects of climate change, patterns of species diversity and conservation. Analytical and empirical approaches have been applied to solve the above questions as well as how the distribution of a species may be defined and measured.

A model that is used widely to describe the relationship between population density and spatial distribution, particularly in agricultural entomology, is that developed by Nachman (1981). Ekbom (1987) suggests this model as a simple method for field counts of *R.padi*. The method is based on incidence counts of aphids rather than actual number counts and thus provides basic information for designing efficient labour-saving sampling procedures for population estimation and decision making in pest management.

We tried to fit the model to the data from Latvia. Nachman's model gave a good fit ($a = 0.216$ and $b = 0.744$; SE = 0.021; $r^2 = 0.97$; n = 32) to the relationship between the proportion of tillers without aphids and mean density of *R.padi*. Using the parameter estimates ($a = 0.637$ and $b = 1.242$, SEM = 0.043, $r^2 = 0.96$; n = 32), the proportion of tillers with or without aphids can be estimated from mean density with equation:

$$\ln(m) = A + B \ln[-\ln(P_0)],$$

where (P_0) – proportion of sample units without aphids; (m) – mean density of aphids per sampling unit. For example, samples with mean densities of 1 and 5 aphids per shoot correspond to ≈27 and ≈70% infested tillers, respectively. This model proves to be suitable for application in Latvia and can be used for ones who wish to develop the decision-making plans once the economic threshold of *R.padi* is known.

Virus effect on yield (IV)

Crop damage depends on many factors (cereal species and variety, virus species and isolate, and environmental conditions), but most of all, on the abundance of

viruliferous aphids and the time of infection. Time of infection relative to plant phenology has economic implications by affecting individual plant damage as reported here and by restricting the time available for an epidemic. Oats infected at four different growth stages were compared for grain weight and biomass, number of tillers and panicles, plant height and thousand-kernel weight.

Grain weight and biomass

In our study, grain yield loss, compared to uninfected plants, gradually decreased from 71 % to 9 % with advanced growth stage at inoculation. Early-infected (GS11 and GS13) plants produced significantly less grain weight and biomass than late infected (GS31 and GS39) and uninfected plants. Biomass did not differ between late infected and uninfected plants, whereas grain weight did, except for the latest inoculation (GS39). The biomass of plants infected at GS39 was, on average, 12% greater than that of uninfected plants.

Tillers

Increased tillering has been considered to be a form of resistance to the virus (Burnett & Gill 1976). Goulart *et al*., (1989) suggested that the escape from infection or reinforced growth activity is more likely to occur at the stem elongation stage than at the three-leaf stage. In our study, enhanced production of tillers was observed in plants infected at GS13 or later compared to plants infected at GS11 and uninfected plants. Similarly, we observed more panicles of infected plants compared to healthy plants. This has not been observed in earlier experiments (Baltenberger *et al*., 1987; Goulart *et al*., 1989).

Plant height

Plant height varied a lot between treatments. Inoculations with PAV-Sto isolate had a severe impact on growth of Stork oats, reducing the plant height of early-infected plants by up to 29%, compared to uninfected plants. Reduction in plant height due to BYD in oats infected before GS13 has been shown in earlier reports (Endo & Brown, 1957; Goulart *et al*., 1989) or at day 20 after sowing (Comeau 1987), although for some cultivars contradictory results have been found (Baltenberger *et al*., 1987).

1000-kernel weight

The infection with PAV-Sto isolate had no effect on 1000-kernel weight (TKW). Earlier reports of experiments with other isolates have indicated similar results (Baltenberger *et al*., 1987; Gildow & Frank, 1988; Gourmet *et al*., 1996; McKirdy *et al*., 2002). In contrast, Goulart *et al.* (1989) found a slight increase in TKW for plants infected at two- or three-leaf stage compared to uninfected plants, but no such effect for plants infected at later stages of growth. However, in barley (Edwards *et al*., 2001) and wheat (Baltenberger *et al*., 1987) a clear decrease in TKW has been observed in BYDV infected plants. The difference in BYDV-effect on grain weight in different cereals might be ascribed to a difference in patterns of spikelet formation between oats on one hand (Landes & Porter, 1990) and wheat and barley on the other (Waddington *et al*., 1983).

Conclusions

- Close genetic relationships were found for BYDV-MAV and BYDV-PAV isolates regardless of their geographic origins.

- Results of sequence analysis of the coat protein gene revealed the existence of a new, distinct isolate of BYDV (PAV-Sal1) from Latvia, which was proposed to be named BYDV-OYV (OYV for oat yellowing virus).

- The results indicate that BYD-causing viruses, namely BYDV-PAV, BYDV-MAV and CYDV-RPV, are common in pasture grasses and spring cereals; however, their occurrence tends to be occasional and varies between years, regions and host plants.

- Oats infected at such early growth stages as GS11 and GS13 was severely damaged in terms of grain weight. The decreased grain weight was only 64 and 78 % compared to uninfected oats.

- From eight-year data of aphid monitoring it is clear that *R. padi* is the most predominant species among cereal aphids in Latvia.

- Data from monitoring of spring migrants can be used to predict aphid population in the field.

- The use of Nachman model is applicable in Latvia to estimate aphid density in the field by sampling the number of infested tillers, which is faster and labour cost effective compared to direct aphid counting.

Future perspectives

The five species of BYD-causing viruses first described by Rochow based on serological relationships have withstood the time. Now identification and comparison of viruses have become precise and unique with implementation of molecular methods, since when many different variants of BYDV/CYDV were found. Our finding of a new tentative virus species of BYDV creates a larger interest to find other diverse BYDV/CYDV-related viruses and to improve the understanding of genetic variation among BYD-causing viruses. With regular improvements, wider accessibility and increasing speed to run laboratory tests of sequencing, it is not a difficult task.

The survey of the occurrence of BYDV/CYDV in Latvia during 2000 to 2002 is far too limited to draw reliable conclusions about the complex factors determining BYD epidemiology. There is a need for more detailed study to find good relationships between the occurrence of BYDV/CYDV and the most important

factors that regulate it. The next step would be to test the influence of various aspects on virus occurrence and incidence, e.g., distribution of true virus sources, and the role of distance to permanent virus sources.

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