Aphid Vectors and Grass Hosts of Barley Yellow Dwarf Virus and Cereal Yellow Dwarf Virus in Alabama and Western Florida

by

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Abstract

Yellow Dwarf (YD) is a major disease problem of wheat in Alabama and is estimated to cause yield loss of 21-42 bushels per acre. The disease is caused by a complex of luteoviruses comprising two species and several strains, including *Barley yellow dwarf virus* (BYDV), strain PAV, and *Cereal yellow dwarf virus* (CYDV), strain RPV. The viruses are exclusively transmitted by aphids. Suction trap data collected between 1996 and 1999 in North Alabama recorded the presence of several species of aphids that are known to be B/CYDV vectors.

Aphids were surveyed in the beginning of planting seasons in several wheat plots throughout Alabama and western Florida for four consecutive years. Collected aphids were identified and bioassayed for their B/CYDV-infectivity. This survey program was designed to identify the aphid (Hemiptera: Aphididae) species that serve as fall vectors of B/CYDV into wheat planting. From 2005 to 2008, bird cherry-oat aphid, *Rhopalosiphum padi* (L.), rice root aphid, *Rhopalosiphum rufiabdominale* (Sasaki), and greenbug, *Schizaphis graminum* (Rondani), were consistently found between October and December. The species of aphids and their timing of appearance in wheat plots were consistent with flight data collected in North Alabama between 1996 and 1999. Both *R. padi* and *R. rufiabdominale* were found to carry and transmit BYDV-PAV and CYDV-RPV. Low overall numbers of collected aphids and low proportion of infective aphid made it difficult to conclusively identify the primary vector of B/CYDV in Alabama.

The source of summer/fall infection of BYDV and CYDV is not known in Alabama. Pasture grasses may provide a means of survival during summer months when wheat is not available in Alabama. Variety plots of three pasture grasses were sampled in summer between 2007 and 2009 for B/CYDV and aphids. Of the three pasture grasses surveyed, bahiagrass was found to consistently harbor BYDV-PAV and CYDV-RPV, while limpograss was found to harbor BYDV-PAV and CYDV-RPV irregularly. This is the first report of BYDV on bahiagrass and limpograss. No aphids were found during this study on limpograss and gamagrass while the aphid *Sipha flava* (Forbes) was found in two out of three years of survey on bahiagrass.

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perspective, “So, you are going to get a degree for crawling around fields and counting insects?”

And as for a dedication, I’m trying still to heed an itinerant preacher’s words long ago, “ …whether you eat or drink or whatever you do (e.g. do research and write a dissertation), do it all for the glory of God.”

Table of Contents

Abstract ii

Acknowledgments iv

List of Tables ix

List of Figures x

List of Abbreviations xi

1 Introduction 1

2 Literature Review 4

2.1 The hosts 5

2.2 The viruses 5

2.3 The vectors 8

2.3.1 Taxonomy and life cycle 8

1.1.1 Aphid movement 10

1.1.2 BYDV and CYDV vectors in Alabama 12

2.4 Virus-host relationships 19

2.4.1 Symptom formation 19

2.4.2 Physiology of yield reduction in infected plants 23

2.4.3 Virus systemic movement inside the plant 24

2.5 Host-vector relationships 25

2.5.1 Aphid reproduction on healthy hosts 25

2.5.2 Aphid dispersal and host finding strategy 26

2.6 Virus-vector relationships 28

2.7 Virus-vector-host relationships 30

2.7.1 Aphid population dynamics and alatae production on infected host 30

2.7.2 Aphid attraction to infected hosts 31

2.7.3 Aphid feeding behavior on infected hosts 32

2.8 Environmental relationships 33

2.8.1 Temperature and rainfall 33

2.8.2 Crop management practices 35

2.8.3 Other field characteristics 38

2.8.4 Natural enemies 39

2.8.5 Plant resistance and tolerance 40

2.8.6 Chemical control and forecasting schemes 46

3 Long-Term Goal, Objectives and Hypothesis 60

4 Species Composition of the Aphid Vectors of Barley Yellow Dwarf Virus and Cereal Yellow Dwarf Virus in Alabama and Western Florida 61

4.1 Introduction 61

4.2 Methodology 62

4.3 Results 65

4.4 Discussion 67

4.5 Conclusion 75

5 Perennial Pasture Grasses as Hosts of Barley Yellow Dwarf Virus and Cereal Yellow Dwarf Virus 81

5.1 Introduction 81

5.2 Methodology 83

5.3 Results 87

5.4 Discussion 88

5.5 Conclusion 92

References 95

Appendix Winged aphids collected on wheat fields in Alabama and western Florida between 2005 and 2009 113

List of Tables

Table 2.1 List of original BYDV strains and their aphid vectors (Hemiptera: Aphididae) ……………………………………………………………..70

Table 4.1 Location, timing and field types used for aphid surveys in Alabama and western Florida between 2005 and 2009 88

Table 4.2 Winged aphids (Hemiptera: Aphididae) collected on wheat fields in Alabama and western Florida between 2005 and 2009 89

Table 5.1 Hosts of barley yellow dwarf viruses reported since D'Arcy (1995) 104

Table 5.2 *Barley yellow dwarf* and *Cereal yellow dwarf viruses* detected using ELISA from pasture grass leaf samples collected in North Florida Research and Education Center, Marianna, FL..105

List of Figures

Figure 4.1 Seasonal abundance of winged aphids collected on wheat fields in Alabama and western Florida between 2005 and 2009 90

Figure 4.2 Seasonal abundance of winged aphids trapped in suction trap in Red Hill, Alabama, between 1996 and 1999 91

List of Abbreviations

BYDV	Barley Yellow Dwarf Virus
B/CYDV	Barley Yellow Dwarf or Cereal Yellow Dwarf
CYDV	Cereal Yellow Dwarf Virus
YD	Yellow Dwarf
ELISA	Enzyme-Linked Immunosorbent Assay
QTL	Quantitative Traits Loci

1 Introduction

In 1951, an epidemic of a disease characterized by "brilliant leaf yellowing dwarfing of the plants" swept through barley, wheat and oat plantings in California (Oswald and Houston 1951). The authors of this original paper called the disease 'Yellow Dwarf' and found that the pathogen was a virus readily transmissible by four prevalent aphid species in California (Oswald and Houston 1952). Extensive research was carried out at Cornell University, where the virus was found to be a virus complex with five strains based on serological properties and aphid vector specificity (Rochow 1969, Rochow and Muller 1971). Evolution of molecular biology techniques has further illumined the relationship of each strain within this virus complex. The causal agents of this disease are now categorized in two virus genera, *Barley yellow dwarf virus* of genus *Luteovirus* and *Cereal yellow dwarf virus* of genus *Polerovirus*, both in the family *Luteoviridae* (van Regenmortel *et al.* 2000). In previous publications, the disease was called Barley Yellow Dwarf (e.g. D'Arcy and Burnett 1995). To accommodate new virus taxonomy the name yellow dwarf (YD), initially designated by Oswald and Houston (1951), is used herein to refer to the disease.

In 1954, J.W. Oswald identified the disease in the Netherlands and suggested that it might also be present in Great Britain. Watson and Mulligan (1957) reported the disease occurred in a wide range of hosts in Great Britain, including wheat, oat, barley, timothy grass and perennial rye grass. Yellow dwarf continues to limit small grain

production in the United States and around the world (Hewings and Eastman 1995). In the last nine years, field surveys from South Dakota, Iowa, Illinois, Indiana and Missouri have reported 7-35% yield loss due to YD (Hesler *et al.* 2005, McKirdy *et al.* 2002, Perry *et al.* 2000, Zwiener *et al.* 2005).

A plant infected with B/CYDV maybe stunted and discolored. Root growth inhibition is common. Yellow dwarf affects yield because it reduces the number of tillers, suppresses heading, causes sterility, reduces the number of kernel per spike and hinders kernel filling (D'Arcy 1995).

In Alabama, a three year study reported that each year more than 15% of sampled winter wheat plants were infected by B/CYDV. The maximum yield loss observed due to this disease was 60%, the equivalent of a loss of 6.2 million dollars assuming a 2006 wheat price and production statistics (Van Riessen 2002).

Three major strategies recommended to manage YD are avoidance of early planting, use of insecticide treated seed and application of foliar insecticide. Efficacy of each strategy varies between localities (Van Riessen 2002, Bowen *et al.* 2002, Flanders *et al.* 2006).

Variation in management efficacy is due to the fact that YD epidemiology is driven by a number of factors that vary between localities. The pathogen is a virus complex of at least two viral genera, *Luteovirus* and *Polerovirus*, with at least six different strains (D'Arcy *et al.* 2000, Mayo 2002). Each strain is transmitted with different efficiency by at least seven species of aphids (Hemiptera: Aphididae) (Power and Gray 1995). The biology and temporal and spatial distribution of each aphid vector is affected by the climate and other properties of its habitat. Thus, there are various possible

combinations of virus strain, vector species and vector biology that characterize YD epidemiology in a specific locality (Irwin and Thresh 1990).

Development of integrated disease management program for YD in Alabama is hindered by the lack of understanding of various components in the local epidemiology, such as primary vector(s) of the disease, summer hosts of both vectors and viruses and the timing of primary infection. The goal of this research project is to investigate different components of YD epidemiology in Alabama. Specifically, the project seeks to identify the aphid vectors that introduce YD viruses to wheat plants in the fall and identify the summer grass hosts of YD viruses and vectors.

2 Literature Review

Yellow dwarf (YD) is a disease of plants of the family *Poaceae*. Losses due to YD are reported in different commodities, including rice, pasture grasses and corn. Nevertheless, globally the disease poses a more serious threat to barley, oat and wheat (Lister and Ranieri 1995).

The most common symptom of YD is stunted growth of the host due to reduced internode elongation. Leaves of infected plants may appear stiff and more erect than the healthy ones. Discoloration is common among older infected leaves (Oswald and Houston 1953). The discolored area enlarges with time from the tip to the base of the infected leaf and may finally cover the whole leaf. Leaves of wheat, rye and triticale usually turn yellow and sometimes red. Serration along the leaf margins was reported among wheat and oats. Root growth inhibition was also reported in plants with YD (Hoffman and Kolb 1997, Kolb *et al.* 1991). YD affects yield by reducing the number of tillers, suppressing heading, causing sterility and reducing number of kernels per spike (D'Arcy 1995).

Symptoms vary with age and physiological condition of the host plant at the time of infection, virus strain and titer, and environmental conditions during disease development. High light intensity and relatively cool temperature (15-18°C) usually favor YD symptom expression. Many uncultivated hosts have been reported to harbor the virus without showing symptoms (D'Arcy 1995).

In this literature review, descriptions about the host plants, the viruses and the vectors will be attempted. Information on characteristics pertaining to vector species present in Alabama is presented together with the indications of the role played by each species in yellow dwarf epidemiology in other states. Since a pathosystem encompasses not only the parts of the system but also interactions between parts, the next portion of this review is dedicated to a survey of literature on the interplay of physical environment, hosts, viruses, and vectors of B/CYDV. In the YD disease system, different combinations of virus strain, aphid species and host species (even varieties) may occur. It will be shown throughout this review that a relationship that holds true for one combination is not necessarily true for another.

2.1 The hosts

Over 100 species in the family *Poaceae* are affected by the disease, including many wild grasses and commonly cultivated commodities such as barley, wheat, oats, sorghum, rye, triticale, corn, and rice (D'Arcy 1995). The wide host range provides many potential hosts for the virus in the absence of cultivated commodities. Wild annuals, perennial grasses, graminaceous weeds, volunteer cereals and even neighboring cultivated grain crops may act as alternative hosts for the virus.

2.2 The viruses

Viruses causing YD were initially grouped in one viral genus, *Luteovirus*, and given one name: *Barley yellow dwarf virus* (BYDV). BYDV was known to possess at least five serologically distinct strains: BYDV-PAV, RPV, MAV, SGV and RMV (Rochow 1961a, Rochow 1961b, Rochow and Muller 1971). The grouping and naming of

these strains were initially based on what was thought to be vector specificity of each strain (Table 2.1), i.e. each strain was reportedly most efficiently transmitted by certain aphid species or combination of species. With new discoveries, it became clear that the vector specificity characteristic of a particular strain was not absolute; in fact, it varied between geographic isolates (e.g. Halbert *et al.* 1992b, Gray *et al.* 1998).

Based on the cytopathology of BYDV infected plants, Gill and Chong (1979) divided BYDV into two groups. BYDV-PAV, BYDV-MAV and BYDV-SGV were placed in a group characterized by single membrane bound vesicles in the plasmadesmata and filaments in the nucleus; BYDV-RPV and BYDV-RMV were placed in a group characterized by double membrane bound vesicles and the absence of filaments in the nucleus. This division is consistent with serological evidence and dsRNA profiles identified in infected tissue (Rochow 1970, Gildow *et al.* 1983).

Recently, the viruses that cause YD were reclassified into distinct but related genera in family *Luteoviridae* based on variations in genomic organization. Each strain is now recognized as a species (van Regenmortel *et al.* 2000). BYDV-PAV and BYDV-MAV are now members of genus *Luteovirus* while *Cereal yellow dwarf virus* strain RPV (CYDV-RPV, previously known as BYDV-RPV) is a member of genus *Polerovirus*. Other BYDV species, namely BYDV-RMV and BYDV-SGV, have not been assigned to a genus (Barker and Smith 1999). In keeping with current scientific taxonomy, the virus previously reported in dated articles as the RPV strain of BYDV will be referred to as CYDV-RPV throughout this literature review.

There have been reports of BYDV isolates from around the world that do not exactly match the vector specificity, serological or genomic profile of the original five

strains. Zhang *et al.* (1983) reported at least two virus isolates from China that caused YD and that had unique vector specificity profiles: BYDV-GPV, the dominant strain found in China, is efficiently transmitted by the aphid *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) and *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae); BYDV-DAV is transmitted by *S. graminum, Metapolophium dirhodum* (Walker) (Hemiptera: Aphididae) and *Sitobion avenae* (Fab.) (Hemiptera: Aphididae). A novel isolate from Alaska was analyzed for its gene sequence and found to be most similar to BYDV-MAV. Yet, the isolate tested negative with serological diagnosis using BYDV-MAV antibodies. It was suggested that the difference in the CP gene region was large enough to cause the negative result, and that consequently the isolate should be treated as a separate strain. The investigators proposed the name BYDV-ORV (oat red-leaf virus) (Robertson and French 2007).

Yellow dwarf in Alabama. In 1975, samples of oat and wheat from Alabama were sent to Cornell University for detection of YD viruses. All infected samples were found to harbor BYDV-PAV (Gildow 1990). Van Riessen (2002) detected BYDV-PAV and CYDV-RPV but no BYDV-MAV from wheat samples collected from Alabama. A recent survey in Alabama showed that in the year 2000, CYDV-RPV was detected in 9.9% of collected samples while BYDV-PAV was detected in 4.3% of samples and 0.4% were infected with both viruses. In 2001, BYDV-PAV was more dominant and detected in 8.2% of collected samples, while CYDV-PAV was detected in 1.9% of samples and both viruses were detected in 2.1% of samples (Bowen *et al.* 2003).

2.3 The vectors

2.3.1 Taxonomy and life cycle

BYDV and CYDV are transmitted exclusively by aphids (Hull 2002). Aphids belong to the superfamily Aphidoidea within the order Hemiptera. Following the classification presented in Blackman and Eastop (2006), super family Aphidoidea is divided into three families: Adelgidae (Conifer Wooly aphids: eg. *Adelges*, *Pineus*), Phylloxeridae (eg. *Phylloxera*, *Moritziella*, *Viteus*) and Aphididae (eg. *Lachnus*, *Myzocallis*, *Aphis*).

Adelgidae and Phylloxeridae possess more primitive features compared to Aphididae, such as the absence of viviparity and siphunculi. Members of Adelgidae live on conifers, whereas the Phylloxeridae utilize only dicotyledons as their hosts. All of Poaceae infesting aphids, some of which act as B/CYDV vectors, belong to the Aphididae.

Species of Aphididae employ various life cycle strategies. Holocyclic species maintain sexual generations in winter time and switch to asexual generations (termed viviparae) during summer. Unlike species of Adelgidae and Phylloxeridae, in which the asexual females lay eggs, the asexual generation of Aphididae reproduces by parthenogenesis, where growth and development of embryos occurs without fertilization by a male. This allows embryos to begin development even before the birth of the mother, and be ready for independent existence outside the mother immediately following birth. Shortening of generation time and overlapping of generations result in dramatic population increases during the parthenogenetic phase of aphids in the family Aphididae (Blackman and Eastop 2000).

Some species of Aphididae seem to have lost the sexual phase of their life cycle. This phenomenon is termed anholocycly. An anholocylic aphid completes its life cycle as viviparae. At least two possible explanations may be offered for the absence of the sexual phase. The start of the sexual phase of most aphids is triggered by a seasonal reduction in day length or a decrease in temperature. In certain environmental settings, such as in a tropical climate or inside a greenhouse, these cues may never materialize resulting in failure to trigger the onset of the asexual phase. The sexual phase may also be lost when slight genetic changes occur within a population of aphids, lowering the response threshold to photoperiod or temperature (Blackman and Eastop 2000). Anholocycly can be facultative. That is, species employing anholocycly in a warmer climate or protected area may be found to be holocylic in a more severe climate (Halbert and Voegtlin 1995). Since the presence of sexual phase is critical in the evolutionary success of every species, Blackman and Eastop (2000) noted that anholocycly may be relatively unimportant in the long term.

Some holocyclic species use different plant species as winter and summer hosts. In these species, the winter host used in the aphid's sexual phase and the plant is termed the primary host. The summer host used in the aphid's asexual phase is termed the secondary host. This host alternating strategy is termed heteroecy. Other aphid species use the same plants throughout the year. This non host-alternating strategy is termed monoecy. All these strategies are represented in B/CYDV vectors (Halbert and Voegtlin 1995).

Typical heteroecious aphids produce eggs on woody hosts during winter. The eggs hatch in spring, giving rise to a form called the stem mother or fundatrix. Usually

one or more wingless (apterous) fundatrigeniae generations are produced asexually on woody hosts. Eventually, winged (alate) forms are developed enabling spring migration to summer hosts, such as grains or grasses. Several viviparous generations are produced on the summer hosts. Normally, the apterous form is present. If the population density in a certain spot becomes too high, it may trigger development of the alate form in the next generation facilitating colonization of new host plants. In autumn, winged male and female aphids develop, termed gynoparae. The gynoparae fly back to the winter hosts. When the host is located, the winged females produce a wingless sexual female form, termed oviparae. The oviparae mate with the winged males before laying overwintering eggs (Halbert and Voegtlin 1995).

Heteroecious true root aphids have a slightly different life cycle. Instead of producing male and female gynoparae, they produce all female autumn migrants termed sexuparae. The sexuparae produce wingless males and oviparae (collectively termed sexuales) on the winter hosts. With few exceptions, the sexuales possess no mouthparts and do not feed. In most root aphids, each oviparae produces a single egg (Halbert and Voegtlin 1995).

1.1.1 Aphid movement

Aphid movement is an important aspect in YD epidemiology (Irwin and Thresh 1990). Plant-to-plant movement of walking apterae results in slow diffusive dispersal. Migratory movement, on the other hand, is not only characterized by the potential long distance it covers, but also by the decrease in the aphid’s responsiveness to host plant stimuli during a part of the movement. Responsiveness to host plant stimuli is again increased by the end of migratory flight, in a phase called targeted flight (Dixon 1998).

Dixon (1998) also postulated that these two classes of aphid movement (e.g. plant-to-plant movement and migratory movement) can be seen as the extremes of a continuum of movement by which aphids track resources in space.

Aphids fly slowly at speeds of approximately 1.6-3.2 km/hour. In the very still conditions close to vegetation, some aphids fly within sight of vegetation, alight at short intervals and do not fly far. This mode of flight is named 'boundary layer migration' (Taylor 1974).

Up to the speed of 5 km/hour, air flow is laminar. Under such a condition, aphids flying upwards out of the boundary layer will enter air masses that are likely to be moving. Aphids fly upwards to a height that balances the tendency to fly upwards in response to the light of the open sky with the tendency to fly downward in response to the light reflected by the earth's surface. The aphid is then borne downwind. Dixon (1998) suggested that after a period of this flight, aphids actively fly downwards in response to increased visual cues from the light reflected by vegetation. Such migration is termed 'stratiform flight'.

Above 5 km/hour, air flow is not laminar, partly because of turbulence produced by surface roughness. Aphids may ride in this turbulence or may be lifted up to a higher air stream. On these upper layers of air stream, an aphid may be rapidly transported long distance. Live aphids have been recovered from air stream as high as 1100 m above sea level (Irwin and Thresh 1988). In this air stream, aphids need to maintain active flight, which consumes energy. The energy storage within the body, associated with percentage of lipid from the whole body weight, is implicated as the factor limiting the distance traveled by aphids during this kind of migration (Thresh and Irwin 1988, Dixon 1998).

Environmental and host related cues affect the course of these movements, as will be discussed later in this review.

1.1.2 BYDV and CYDV vectors in Alabama

There are at least five aphid species that can potentially transmit B/CYDV in Alabama: greenbugs, rice root aphids, bird cherry-oat aphids, English grain aphids, and corn leaf aphids (Flanders *et al.* 2006).

Greenbug, *S. graminum*. The apterous form is bright green with a dark stripe on the spine. The alate form has a brownish-yellow head and prothorax, black thoracic lobe and a yellowish to brownish green abdomen. Generally, it possesses clear siphunculi with dark aphices (Blackman and Eastop 2000).

In Idaho, greenbug is probably holocylic with eggs laid on winter cereals. In areas with harsh winters, greenbug is known to be monoecious holocylic (i.e. having sexual and asexual phase without alternating the host plant), with *Poa pratensis*, Kentucky bluegrass, as the common winter host. Under mild winter situations, anholocylic clones may be found (Blackman and Eastop 2000).

Greenbug lives on various cereals and grasses. Its feeding may cause yellowing and other phytotoxic effects. Several biotypes have been designated based on the feeding reaction of different hosts. However, B/CYDV transmission is not a characteristic clearly defined by biotype. That is, clones of the same biotype may have different transmission efficiency (Halbert and Voegtlin 1995).

During autumn, greenbug is more likely to be found at the base of newly emerging winter wheat plant. In places where spring cereals are cultivated, greenbug

colonizes upper and lower leaves of barley and wheat in summer. The winged forms are produced abundantly upon grain maturation (Halbert and Voegtlin 1995).

In South Carolina, greenbug was among the first aphid species colonizing emergent wheat plants (Chapin *et al.* 2001). Apterous colonies reached peak population levels in December or January and declined in number thereafter. In Idaho, flight activity was found to peak in June and July reflecting emigration flight from maturing grain (Halbert *et al.* 1992a). A survey of BYDV vectors in Virginia showed that greenbug was always present in relative abundance throughout the wheat season (McPherson and Brann 1983). On the other hand, a cereal aphid survey in wheat growing counties of Kentucky conducted in late fall and early spring found very low numbers of *S. graminum* (Johnson and Hershman 1996).

Schizaphis graminum is recognized as a vector of BYDV especially BYDV-SGV (Blackman and Eastop 2000). However, transmission efficiency differs between geographical clones. A population of *S. graminum* from Idaho was found to transmit BYDV-PAV, BYDV-SGV and BYDV–RMV but not CYDV-RPV. BYDV-SGV outbreaks occurred in Idaho, implying potential role of *S. graminum* in YD epidemiology in the area (Halbert *et al.* 1992b, Forster *et al.* 1990). A New York clone of *S. graminum* was able to transmit BYDV-PAV, BYDV-SGV and CYDV-RPV. In contrast, an *S. graminum* clone isolated in South Carolina was not able to vector any of the viruses that cause YD (Gray *et al.* 1998). Thus it was suggested that *S. graminum* may not play a significant role in B/CYDV epidemiology in South Carolina (Gray *et al.* 1998, Chapin *et al.* 2001).

Rice root aphid, *Rhopalosiphum rufiabdominale* (Sasaki) (Hemiptera: Aphididae). The apterous form of this species has hairy antennae and is dark green or olive in color with reddish area at the abdominal posterior end (Blackman and Eastop 2000).

In Japan, rice root aphid was found to be heteroecious holocyclic, switching from *Prunus* (primary host) in winter to roots of many secondary host plants, especially Gramineae, in summer. In other part of the world, *R. rufiabdominale* is mainly anholocyclic on secondary host plants (Blackman and Eastop 2000). Anholocyclic apterae colonize subterranean plant parts, making them difficult to monitor. Due to this and the similarity in appearance to *R. padi*, the importance of rice root aphid may have been overlooked (Halbert and Voegtlin 1995).

Jedlinski (1981) found that *R. rufiabdominale* in Illinois was capable of vectoring BYDV and suggested that subterranean colonies of rice root aphid overwintering on wheat seedlings may explain occasional YD outbreaks amidst an inconspicuous aphid infestation. Together with greenbug, rice root aphid was first to colonize wheat seedlings in South Carolina. Continuous flight activity was recorded from April to December. The population of alatae on wheat typically peaked in December or January and declined markedly for the rest of the wheat growing season (Chapin *et al.* 2001). *Rhopalosiphum rufiabdominale* collected in South Carolina was found to transmit BYDV-PAV, BYDV-SGV and CYDV-RPV efficiently (Gray *et al.* 1998); hence rice root aphid may potentially play a crucial role as a B/CYDV vector. However, there was a lack of correlation between *R. rufiabdominale* abundance and B/CYDV incidence in South

Carolina, leading to a conclusion that *R. rufiabdominale* does not play an important role in YD epidemiology in South Carolina (Chapin *et al.* 2001).

Bird cherry-oat aphid, *Rhopalosiphum padi*. Apterae of this species occur on grasses and cereals (Blackman and Eastop 2000). The apterous form is broadly oval and olive to greenish black in color. The alate form has a pale to dark green abdomen.

Rhopalosiphum padi is holocyclic in many parts of the world with a wide range of cereals and grasses as summer hosts and species of *Prunus* as primary (winter) hosts. In Europe, *R. padi* commonly overwinters on *Prunus padus*, while in the USA *P. virginianae* is the main winter host. Permanent anholocyly is possible under mild winter conditions and was observed in the midwestern US (Halbert and Voegtlin 1995).

Rhopalosiphum padi was the most common BYDV vector found in Kentucky (Johnson and Hershman 1996). In Virginia, *R. padi* was found in relative abundance throughout the wheat season (McPherson and Brann 1983). In South Carolina, *R. padi* alates usually arrived on wheat plants after *S. graminum* and *R. rufiabdominale* alates. Two periods of peak activity were consistently found over nine years of observation. Autumn to early winter alate populations usually peaked in December to early January. The peak of alatae population corresponded with the start of apterae population build up. The apterae population generally stayed below 10/row-meter until early February, then underwent a major increase during February and peaked in late February or March (Chapin *et al.* 2001). In autumn, *R. padi* may be found at or below ground level, feeding on the base of cereals. During spring the aphids colonize leaves, stems and even heads (Halbert and Voegtlin 1995).

Rhopalosiphum padi is implicated as the most important vector of B/CYDV in North America (Hewings and Eastman 1995). *Rhopalosiphum padi* clones from New York, South Carolina and Idaho are known to efficiently transmit BYDV-PAV, CYDV-RPV and to some degree BYDV-RMV (Halbert *et al.* 1992b, Gray *et al.* 1998). In South Carolina, a high degree of correlation was found between the abundance of *R. padi* and both virus incidence and yield loss, confirming the critical role played by *R. padi* in the local YD epidemiology (Chapin *et al.* 2001).

English grain aphid, *Sitobion avenae*. The apterous form is medium sized, broadly spindle shaped, yellowish green to dirty reddish brown in color, with black siphunculi that are a little longer (1.1-1.6 times) than the pale cauda. The dorsal cuticle varies in color, from colorless to dark brown. The alate form is similar in color with more distinct dark markings across the dorsum (Blackman and Eastop 2000).

Sitobion avenae is monoecious holocyclic on many species of Graminae. Anholocyclic overwintering is common in regions with mild winters. It feeds on upper leaves of cereals and on the heads once available. Due to its ability to colonize cereal heads, *S. avenae* may impose a direct effect on cereal yield, either by reducing the number of grains per head or reducing the weight of grains (Halbert and Voegtlin 1995).

Within a season, *S. avenae* was the last aphid species to colonize wheat in South Carolina. In most years, *S. avenae* apterae were first found in January, increasing rapidly during spring and peaking in April or May. There was a consistent spring flight. No autumn flight was recorded in South Carolina (Chapin *et al.* 2001). Along the same line, McPherson and Brann (1983) reported that *S. avenae* was the most abundant BYDV vector found in spring. The survey conducted by Johnson and Hershman (1996) in

Kentucky was done in early spring and *S. avenae* was found to be the second most abundant species of B/CYDV vectors.

Corn leaf aphid, *Rhopalosiphum maidis* (Fitch). The apterous form is rather elongate with short antennae and short, dark siphunculi. The body is yellow green to dark olive green. The alate form has a light green to dark green abdomen without dorsal markings (Blackman and Eastop 2000).

There is a range of karyotypic variation within the species apparently associated with host plant. In the northern hemisphere, the barley colonizing form usually has the 2n=10 karyotype, whereas populations on corn and sorghum usually have the 2n=8 kariotype. Karyotype 2n=8 is also found in certain population colonizing weeds, particularly *Echinocloa crusgalli* and *Panicum avialare.* Another karyotype, 2n=9 is reported from Idaho. The main host for this kariotype is unknown. All three karyotypes can develop small and short-lived colonies on winter wheat in autumn (Blackman and Eastop 2000, Halbert and Voegtlin 1995).

Corn leaf aphid is predominantly anholocyclic on Graminae, although males occur sporadically. Holocylic clones were found in Pakistan with *Prunus cornuta* as the primary host (Blackman and Eastop 2000). The overwintering strategy of *R. maidis* in areas with harsh winter is not known. In the Pacific Northwest of the U.S., *R. maidis* is not found on cereal during winter, yet it was sporadically found among the first species colonizing wheat in spring (Halbert and Voegtlin 1995). One plausible explanation is that the aphids overwinter in areas with warm winters, such as the southern states. Evidence for long distance immigration to the north in spring is available from the midwestern U.S.

Nevertheless, such migration crossing mountainous regions seems unlikely (Halbert and Voegtlin 1995).

In Idaho, the flight activity of corn leaf aphids seems to have no peak period (Halbert *et al.* 1992a). Suction trap data from South Carolina revealed two periods of flight activity: a minor flight in April and May that may represent alate colonization of corn, and a major flight activity in July and August that may reflect the emigration from maturing corn (Chapin *et al.* 2001). Large populations of corn leaf aphids (up to 456.1 ± 212.2 individuals per plant) were observed on maturing corn in South Carolina. Despite the large population buildup on maturing corn, consistently low populations of corn leaf aphids were observed on winter wheat. There is a one or two month long gap between the harvest of corn and emergence of wheat seedlings. The host into which *R. maidis* moved after corn was not known (Chapin *et al.* 2001). In Virginia *R. maidis* made up to 50% of the autumn aphid infestation on winter wheat and persisted on the plant through March (McPherson and Brann 1983). In Idaho, an annual grass *Echinochloa crusgalli,* was available as a bridging host from corn harvest and emerging winter wheat. Nevertheless, *R. maidis* in the area appeared to colonize winter wheat only ephemerally and in low number (Halbert *et al.* 1992a).

BYDV-RMV was first named after the fact that it was transmitted most efficiently by the New York clone of *R. maidis*. Data of repeated transmission tests from 1957 to 1992 showed that this particular clone (maintained at Cornell University) has retained its ability to transmit BYDV-RMV in a highly efficient manner, while being unable to transmit BYDV-MAV, BYDV-PAV, BYDV-SGV and CYDV-RPV (Power and Gray 1995).

A low percentage of corn leaf aphids collected from spring barley in Idaho was found to be viruliferous with BYDV-RMV and BYDV-PAV (Halbert *et al.* 1992b). Populations of Idaho *R. maidis* sampled from the annual weed, *E. crusgalli*, were found to be viruliferous with BYDV. However, no test was conducted to identify the virus strain. It is not easy to infer the role of *R. maidis* in BYDV epidemiology in Idaho. Nevertheless, year long flight activity and the finding of viruliferous aphids on reservoir hosts and emerging wheat may imply that *R. maidis* can transmit the virus from *E. crusgalli* to winter wheat in autumn (Halbert *et al.* 1992b).

As stated before, low populations of corn leaf aphid were observed on winter wheat in South Carolina. Only eight individuals of *R. maidis* were collected during sampling conducted between December to April from 1997 through 1999 in Blackville, South Carolina. Out of this eight, only one was found to be infective with BYDV-PAV. Based on this finding, the role of *R. maidis* in BYDV epidemiology in South Carolina may be insignificant (Chapin *et al.* 2001).

2.4 Virus-host relationships

2.4.1 Symptom formation

Plant age effect on symptom formation. Even in the first papers on YD, the authors recognized the prominent effect of plant age at time of infection on the resulting symptom. Oswald and Houston (1953) stated that "the severity of symptoms is wholly dependent on plant age when infected." Infected wheat seedlings grew to only 1/3 or 1/2 their normal size, the leaves suffered complete chlorosis, and heading was sparse. In wheat plants given time to grow to a stage after tillering before infection, stunting was minimal or not present and the leaves turned bright yellow instead of completely

chlorotic. This trend was uniformly observed between barley, wheat and oat (Oswald and Houston 1953, Watson and Mulligan 1957).

Shoot and root stunting. Stunting of shoots and roots of infected plants may be the result of reduced cell elongation or reduced cell division. Esau (1957a, 1957b) noted reduced meristematic activity of infected plants. Russell and Kimmins (1971) found that the estimate of cell number in the third leaf of infected barley was markedly less than that of the healthy control, signaling the possibility of reduced cell division.

A report by Russell and Kimmins (1971) may be the only paper to date on the effect of BYDV on the host's growth regulators. They found no significant difference in the level of endogenous auxin between infected and healthy plants. On the other hand, significantly less gibberellic acid (GA3) was extracted from diseased plants. Reduction in gibberellic acid may have affected cell division which caused reduced cell counts.

Leaf chlorosis and reddening. The rate of photosynthate translocation in B/CYDV infected plants was reduced relative to healthy plants (Jensen 1968, Jensen and D'Arcy 1995). This causes sugar accumulation in the source leaves. Sugar accumulation in the leaf can lead to a decrease in photosynthesis by repressing the transcription of photosynthesis associated genes (Quirino *et al.* 2000). Reduced photosynthesis was noted as early as four days after infection with BYDV (Jensen and D'Arcy 1995). Down-regulation of photosynthesis associated genes is associated with up-regulation of senescence associated genes which activate chlorophyll breakdown (Biswal and Biswal 1999). This chain of reactions may explain the yellowing of leaves in B/CYDV infected plants. Similarly, the leaf reddening sometimes observed in infected leaves may be attributed to anthocyanin build-up induced by sugar accumulation (Livingston *et al.*

1998). The fact that chlorosis and reddening are more pronounced on older leaves supports this hypothesis because the older leaves act as assimilate sources while the younger ones act as assimilate sinks.

Esau (1957a, 1957b) observed necrotic obliteration of phloem cells in the shoot and root of infected plants. The phloem cells (and to some degree, also xylem cells) underwent necrosis and finally collapsed.

Reduced translocation of assimilate can partially be explained by the necrotic obliteration of infected phloem cells. Burnett's comment that leaf chlorosis is not very pronounced in infected triticale (Burnett 1987) agrees with Esau's observation that phloem degeneration was less extensive in triticale (Esau 1957b).

Viral determinants of symptom formation. Mild and severe isolates of BYDV-PAV differ in their coat protein products (Bencharki *et al.* 1999, Mastari and Lapierre 1999). Mastari and Lapierre (1999) posited that an amino acid change between the coat protein of mild and severe isolates resulted in alteration of the protein local net charge. Change in the protein local net charge may influence disease severity by overcoming host resistance, as shown in the case of Tomato mosaic virus (Weber *et al.* 1993).

In another pathosystem, a viral coat protein has been shown to play a role in the dwarfing of a cereal host. *Rice dwarf virus* (RDV) is a member of the genus *Phytoreovirus* and, as the name implies, causes dwarfing in rice. P2 protein of RDV functions as the viral coat protein and is required for movement but not in virus replication within the leafhopper vector (Culver and Padmanabhan 2007). Zhu *et al.* (2005) showed that P2 protein of RDV interacts with ent-kaurene oxidase, a key component of gibberellic acid biosynthesis of the host. This interaction may have caused

a reduction in the level of GA1, a major form of gibberellic acid in rice, thus inducing dwarfing in infected plants. Reduced gibberellic acid activity was reported from wheat infected by BYDV (Russell and Kimmins 1971). Therefore, a similar mechanism observed in RDV-rice interaction may take place between wheat and BYDV.

As mentioned above, leaf chlorosis and reddening are related to the accumulation of sugar molecules in the leaves of infected plants. Jensen and D'Arcy (1995) noted that sugar accumulation and reduced photosynthesis in the infected plant starts even before vascular bundles are visibly altered. They suggested that reduced production or translocation of regulating substances may be the explanation for this phenomenon. Another possible explanation is that proteins produced by B/CYDV that assist its movement within the plant vascular bundles may affect phloem loading in source leaves.

Herbers *et al.* (1997) used a transgenic line of tobacco that expressed the movement protein of *Potato leafroll virus* (PLRV) to study the effect of the virus movement protein expression on carbohydrate accumulation. PLRV is a member of genus *Polerovirus* with the same movement protein (P4) with CYDV. The study reported that PLRV movement protein expression caused stunting, higher level of sugar and starch and reduced photosynthesis of the transgenic tobacco. In healthy plants, sugar is loaded into phloem for translocation. Theoretically, phloem loading is regulated by two pathways: aploplastic and symplastic. Apoplastic phloem loading does not require plasmodesmata, instead it utilizes the energetized membrane of phloem cells to drive sucrose/H+ symport (where sucrose and H+ ion are transported together actively into phloem tissue). Herbers *et al.* (1997) found that the ATP and ADP levels in the phloem cells of transgenic plants was significantly lower than that in the cells of wild type plant.

A possible explanation is that phosphorylation of expressed movement protein depletes the ATP pool in the phloem cell of the transgenic plant. Symplastic phloem loading requires functional plasmodesmata. PLRV movement protein may interfere with the normal functioning of phloem plasmodesmata. The authors reported distortion of the plasmodesmata in the 7th leaf as well as aberrant structure of young transgenic plant plasmodesmata.

Nass *et al.* (1998) failed to observe any labeling of plasmodesmata in BYDV-infected cells using 17-kDa antiserum, implying that movement protein did not accumulate in the plasmodesmata of infected cells. However, Gill and Chong (1975) noted that plasmodesmata connecting BYDV-infected mature sieve elements with immature sieve element contained densely stained amorphous material and no desmotubules. B/CYDV may affect phloem plasmodesmata in a different way compared to PLRV, but the total effect of disturbed symplastic and apoplastic phloem loading in infected plants may still take place.

2.4.2 Physiology of yield reduction in infected plants

Stoy (1963) studied the photosynthesis, translocation and yield determining factors of healthy wheat and concluded that grain fill was determined by the photosynthesis of the flag leaf, the head and the awns. Jensen and Van Sambeek (1972) reported that flagleaf photosynthesis in BYDV infected red wheat was as much as 60-72% less than in uninfected wheat. This reduction in photosynthesis was proportional to the yield loss. Overall photosynthesis reduction in infected wheat was due to both a reduction in tissue available for photosynthesis and a decline of photosynthetic rate per unit of available tissue (Jensen 1972).

As described above, plant age at the time of infection influences the final symptom severity (Oswald and Houston 1953). Stunting is more pronounced when infection occurs before tillering, consequently early infected cereals suffer greater yield loss. Smith and Sward (1982) reported that yield of wheat inoculated with BYDV before tillering was reduced by 97% compared to uninfected wheat. Yield of wheat inoculated after tillering was reduced by up to 9% relative to uninfected wheat. On barley, Watson and Mulligan (1957) reported a similar trends. Early infected barley was observed to have 40% yield loss, whereas late infected barley yielded 20% less relative to uninfected barley. Other field experiments confirmed that winter cereals infected in autumn were observed to have significantly higher yield loss compared to spring-infected plants (Fitzgerald and Stoner 1967, Herbert *et al.* 1999).

2.4.3 Virus systemic movement inside the plant

Hull (2002) described two types of movement of virus within a plant, short distance transportation through cell to cell movement and long distance transportation through vascular bundles. Since BYDV and CYDV are injected directly into the vascular bundle by their vector, systemic spread in the plant is greatly facilitated.

Jensen (1973) inoculated oat, wheat and barley plants by placing *R. padi* on the second leaf. BYDV was detected in 85% of the third leaves at 48 hours after inoculation regardless of the susceptibility of the wheat variety. Differences between varieties were only clear in the beginning of pathogenesis (i.e. 12 hours after inoculation), when the virus seemed to spread more quickly in more susceptible varieties. There was no information about the virus strain used.

Makkouk *et al.* (1994) reported that, generally, the virus reached the root system and growing point of the host 2-4 days after leaf infection. In barley, the rate of spread of the virus to root and growing tips seemed to be variety dependent. The more susceptible the variety, the quicker the virus reached roots and growing tips. No such difference was detected among wheat varieties.

2.5 Host-vector relationships

2.5.1 Aphid reproduction on healthy hosts

The growth and reproduction of aphids are dependent upon the host plant growth stage. One particular factor often associated with aphid population growth is the level of soluble nitrogen in different plant growth phases. Moreover, it is important to realize that the level of soluble nitrogen in different plant parts fluctuates as the plant ages. Generally, nitrogen level is high in an expanding leaf. Nitrogen level decreases when the leaf stops growing and reaches maturity. Nitrogen level increases again during leaf senescence (Dixon 1998).

Rhopalosiphum padi and *S. graminum* had higher rates of reproduction on the headed stages of wheat rather than on the earlier stages (Kieckhefer and Gellner 1988). Growth stage of barley affected fecundity of *R. maidis* but not of *R. padi*, *S. avenae* or *S. graminum* (Walters and Dixon 1982).

Some aphids showed flexibility of reproductive capability by undergoing embryonic resorption when the quality of the host was poor. In such situation, the largest embryo matures while the smaller ones undergo resorption. Embryonic resorption allows aphids to maintain the immediate reproductive rate at the cost of its lifetime fecundity

(Dixon 1998). *Aphis fabae*, black bean aphid, was shown to ovulate more embryos on the host with good quality, increasing its potential fecundity (Ward *et al.* 1983).

Aphids reared on a host of declining quality may respond by giving birth to more alates, facilitating a higher rate of migration for the next generation. Individual spring migrants of *R. padi* reared on young bird cherry leaves (its primary host) gave birth to fewer alates than when reared on maturing leaves (Dixon and Glen 1971).

Crowding, associated with increasing tactile stimulation within a given time frame, is another stimulus that will induce production of alatae. Considering that crowding may be the result of higher reproduction rate on a host of good food quality, this response may be seen as a mechanism to regulate the population level on a given host (Dixon 1998). *S. avenae* (Watt and Dixon 1981) and *R. padi* (Dixon and Glen 1971) reared in crowded conditions gave birth to more alatae. Moreover, a higher percentage of nymphs of the two species developed into alatae upon experiencing a crowded situation, regardless of the mother's crowding status. It implies that, at least for *S. avenae* and *R. padi*, both mothers and nymphs respond to crowding as a stimulus to produce alatae.

2.5.2 Aphid dispersal and host finding strategy

The presence of alate aphids does not mean necessarily instantaneous dispersal. The take off phase of flight is affected by cues similar to those that trigger alatae production: crowding and declining host quality. For some aphid species, low levels of crowding may delay take off. Alatae may produce offspring before they fly. In general, alatae depart sooner from poor quality hosts than from hosts with good quality. Thus, although capable of flight, alate aphids respond to the current status of their host (Dixon 1998).

At the end of a migratory flight, aphids switch from long distance flying mode to a targeted flying mode. While being less responsive to host-related stimuli in long distance flying mode, in targeted flying mode the aphid responds to a number of cues to assist in host location (Dixon 1998).

Addressing the issue of habitat choice during the end of migratory flight, Favret and Voegtlin (2001) reported that more aphids were found in crop monocultures than in heterogenous natural vegetation (e.g. prairie and woods). The stark contrast between the green plants and brown soil prevalent in monocrop areas might be more attractive to aphids compared to the non-contrasting landscape of prairie or woods. Interestingly, certain aphid species such as *R. padi*, *R. rufiabdominale*, and *S. graminum* chose the crop habitat despite the fact that their more likely hosts (i.e. grass) were to be found in the prairie setting and none of their suitable hosts were to be found in the monoculture crop habitat. Favret and Voegtlin (2001) proposed that these species used the same stimuli present in all row-crop monocultures, regardless of the actual suitability of the crop they perceive. Presumably aphids use visual cues to choose the host habitat during the end of migratory flight.

Aphids are reported to respond to color cues, especially yellow (Dixon 1998). Color is regarded as a good indicator of nutritional status of a host as both nitrogen rich young and senescent leaves tend to be more yellow in color. Moreover, it has been shown that some aphid species, including *R. padi*, are more attracted to water trap baited with plant volatile compared to water trap without the volatile. Thus, olfactory cues also play a role in locating the host (Dixon 1998).

Upon alighting on a host, aphids test the plant surface with the antennae and probe it with the mouth parts. Sensillae on the antennae and the tip of proboscis are used to sense chemical and tactile stimuli. This process can be done without stylet penetration. A decision on the suitability of the host can be made in as short as 60 seconds. This process saves the aphids from incurring the energy cost of stylet penetration to the phloem of the plant, which may take 40 minutes or more depending on the depth of the phloem (Dixon 1998).

Wild grasses or cereal crops? Wild grasses occur together with commercial cereal crops. Although cereal aphids may reproduce successfully on a number of wild grass species, it has been shown that aphids prefer to alight on cereal crops (Kieckhefer 1984). *Schizaphis graminum* reproduced successfully on the seedlings of seven grass species, *S. avenae* on four, *R. padi* on two and *R. maidis* on only one. In six out of eight laboratory tests involving *S. avenae*, *S. graminum*, and *R. padi*, barley was the preferred target for alighting compared to thirteen other warm-season grass species.

2.6 Virus-vector relationships

BYDV and CYDV are transmitted by aphids in a persistent circulative manner (Rochow 1977). Once an aphid acquires the virus, it is potentially infective for life. The viruses do not replicate inside the vector and are not passed transovarially to the offsprings of viruliferous aphid. Aphids that acquire the virus in the nymphal stage retain infectivity in the adult stage (Burgess *et al.* 1999).

Originally, strains of B/CYDV were described on the basis of the most efficient vector species. The term ‘vector specificity’ was thus widely used relating one virus strain to the vector species thought to transmit it most efficiently. Discovery of

geographic virus isolates that did not correspond to the 'vector specificity' profile required a better way of describing a B/CYDV strain (Halbert *et al.* 1992b, Gray *et al.* 1998). For example, the New York isolate of BYDV-RMV (NY-RMV) has been consistently shown to be inefficiently transmitted by New York clones of *R. padi* (Power and Gray 1995), yet *R. padi* collected from South Carolina could transmit NY-RMV quite efficiently (Gray *et al.* 1998).

Power and Gray (1995) suggested describing B/CYDV strains in term of their transmission phenotype, instead of vector specificity. Transmission phenotype of a given strain is defined as the transmission efficiency of the strain by one or more defined aphid biotypes or clones. It is imperative to realize that transmission phenotype of a B/CYDV strain is defined by the experimental system. Geographic collection source of the virus and vector, temperature during which the experiment is conducted, host age and variety susceptibility can affect the transmission phenotype of the examined strain.

Thus, transmission phenotype of a certain virus strain (or, seen from the vector end of the system, transmission efficiency of a given aphid clone) can be affected by numerous factors. Aphids in different developmental stages may have different transmission efficiency. The nymphal stage of *S. graminum*, for example, is more efficient at acquiring and transmitting BYDV-SGV than the adult (Zhou and Rochow 1984).

Length of acquisition and inoculation access period influences aphid transmission efficiency. The minimum access period for both acquisition and inoculation varies with strain-vector combination, but it ranges from 15 to 60 minutes. While infection occurred within this short access period, the transmission efficiency is very low (Gray *et al.* 1991,

Power *et al.* 1991). Increasing the inoculation access period up to 24-48 hours resulted in higher transmission efficiency (Power and Gray 1995, Lowles *et al.* 1996). Due to this long access period needed for optimal transmission efficiency, Irwin and Thresh (1990) defined vectors of BYDV as those who actually colonize the host crop and not the itinerants passing by and probing the crop while in transit.

2.7 Virus-vector-host relationships

Power and Gray (1995) presented a hypothesis on the mutual relationship between virus and vector in the B/CYDV system, whereby the virus improves the suitability of the host for the vector and the vector, in turn, disperses the virus. Irwin and Thresh (1990) concluded that there is indeed a mutualistic interaction between B/CYDV and its vectors. Yet the evidences do not consistently support this hypothesis (see below).

2.7.1 Aphid population dynamics and alatae production on infected host

Virus infected plants may stimulate aphid population and polyphenism (production of winged and unwinged forms). In two studies, *S. avenae* apterae feeding on BYDV-PAV, BYDV-MAV and CYDV-RPV infected wheat had higher fecundity and population growth than apterae feeding on uninfected plants (Quiroz *et al.* 1991, Fereres *et al.* 1989). However, Fiebig *et al.* (2004) reported completely opposite results. In their study, *S.* ave*nae* apterae and alatae on BYDV-MAV and BYDV-PAV infected wheat experienced an increase in development time and a decrease in fecundity, adult weight and intrinsic growth rate compared with those feeding on healthy hosts. A decrease in the host phloem sap quality was cited as a possible explanation of this decrease. Moreover, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), a vector of B/CYDV commonly

found in the western U.S., showed a decrease in fecundity after being reared on wheat infected with BYDV-PAV and CYDV-RPV (Mowry 1990). *Rhopalosiphum padi* longevity decreased when reared on BYDV infected wheat but fecundity increased relative to healthy plants (Araya and Foster 1987).

A simulation study by Kendall *et al.* (1992) showed that the spread of B/CYDV is dependent on the population density of the vector. Hence, in cases where the virus infected plant actually facilitates the growth of the vector population, the potential for disease spread may increase accordingly.

Working on oats, Gildow (1980) showed that *R. padi* reared on BYDV-infected plants produced a higher percentage of alate adults compared to the ones reared on healthy plants. A similar effect was reported for *S. avenae* feeding on BYDV-MAV and -PAV infected wheat (Fiebig *et al.* 2004). This effect was not observed on *S. graminum* feeding on oats (Montllor and Gildow 1986). Apparently aphids feeding on senescing plants also produced a higher percentage of alate adults compared to when they feed on non-senescing hosts. It was postulated that changes in amino acid concentration in diseased or senescing plants may have triggered alate production (Gildow 1980). A shift in alatae production in aphids feeding on BYDV-infected plants could have a significant effect on the epidemiology of the disease, since it promotes disease spread (Irwin and Thresh 1990).

2.7.2 Aphid attraction to infected hosts

Alate aphids were reported to be more likely to land on B/CYDV infected barley and oats than on healthy plants (Ajayi and Dewar 1983). A strong yellowing symptom of infected leaves may serve as the cue for alatae landing (Kring 1972). However, choice

test using detached leaves showed that *S. avenae* and *R. padi* showed no preference over diseased and healthy leaves (Kieckhefer *et al.* 1976). The contradiction of the two reports may be due to the choice of host form (whole plant and detached leaves) or differences in the strains used (no information was given regarding the strains used in either report).

Jimenez-Martinez *et al.* (2004) eliminated visual cues by covering detached leaves of infected and uninfected wheat of two susceptible varieties with dark cloth and tested apterous *R. padi* preference. They found that after thirty minutes of aphid release, consistently for the next 1.5 hours, more aphids were found on the headspace of infected leaves, implying the presence of olfactory cue that attracted apterous aphid to infected host. Power and Gray (1995) reported that apterae of both *S. avenae* and *R. padi* settled on healthy plants rather than infected plants. The authors suggested that apterous aphids settle for healthy than diseased plants due to gustatory cues. Thus, diseased plants may attract the aphids due to the visual and olfactory stimuli. These aphids may later move to healthy plants due to gustatory cues. This pattern will obviously increase the chance of B/CYDV spread from diseased to healthy hosts.

2.7.3 Aphid feeding behavior on infected hosts

Schizaphis graminum initiated committed phloem ingestion faster, interrupted probing less often and ingested longer from phloem of infected oats than from healthy plants (Montllor and Gildow 1986). No information was found regarding the strain of the B/CYDV virus used for the investigation. In another study, Fereres *et al.* (1990) reported that there was no difference between *S. graminum* feeding behavior on healthy wheat and wheat infected with either BYDV-PAV (the virus isolate was collected from Indiana) or CYDV-RPV (New York isolate). No differences were detected on *R. padi* feeding on

diseased and infected oats (Montllor and Gildow 1986). Since longer feeding periods means longer acquisition period, in cases where virus infection affects the aphid feeding behavior in the positive way, the aphid transmission efficiency may increase. However, as already been stated above, studies of virus infection on feeding behavior have not yielded consistent results.

2.8 Environmental relationships

2.8.1 Temperature and rainfall

As in other biological and ecological processes, temperature and rainfall affect many aspects of YD epidemiology. In Alabama, total rainfall for the 12 months preceding wheat harvest was negatively correlated to YD severity, while average temperature for August was positively correlated to disease severity ratings in April (Bowen and Burch 2001).

Temperature has been shown to affect YD epidemiology, either indirectly by affecting the aphid biology or directly by influencing the transmission efficiency of viruliferous aphids. The minimum threshold temperature for aphid development ranges between 4-5°C (Legrand *et al.* 2004). Hutchinson and Bale (1994) reported that exposure to sub-zero temperatures for only six hours reduced longevity, reproductive rate and development rate of a common BYDV vector, bird cherry-oat aphid, *Rhopalosiphum padi*. Bale (1996) maintained that either short exposure to a moderate or high sub-zero temperature or prolonged chilling under low sub-zero temperature may reduce cold tolerance in aphids and lead to death. Short exposure to temperature as low as -5°C is enough to kill a portion of an aphid population, longer exposure to sub-zero temperatures higher than -5°C is expected to have the same deleterious effect (Bale *et al.* 2006).

Studies under controlled condition showed that the proportion of aphids that moved, the mean distance moved by aphids and the subsequent number of infected plants due to the movement of viruliferous aphids increased with temperature (Lowles *et al.* 1999, Smyrnioudis *et al.* 2000). Using four temperatures, 6, 12, 18 and 23°C, Lowles *et al.* (1996) demonstrated that temperature affects BYDV transmission efficiency by British clones of *R. padi* and *S. avenae*. The optimum temperature for BYDV-PAV transmission by the two aphids was between 12 to 18°C. This coincides with the average autumn temperature in Britain.

Mild artificial rain used by Bailey *et al.* (1995) to perturb viruliferous aphids on a host did not facilitate virus spread or result in higher disease incidence compared to the control. Either the intensity of the rain was too low to dislodge the aphid and promote spread or that rain actually disabled the aphid in ways that hindered further virus spread.

Frequency and intensity of rainfall not only influence the YD epidemiology by mechanically dislodging aphids from the plant, but also indirectly by reducing the ambient humidity and affecting host plant water status (Smyrnioudis *et al.* 2000). Working in controlled experiments with apterous *R. padi*, Greek isolates of BYDV-PAV, and wheat plants as hosts, Smyrnioudis *et al.* (2000) reported that drought stress had no effect on mean distance moved between plants by aphids at 5, 10 and 15°C. Drought stress did not cause any differences in the number of infected wheat plants at 5 and 10°C. However, at 15°C more wheat was infected by the virus if it was drought stressed compared to the control. Contradictory results were reported by Bailey *et al.* (1995). In

greenhouse experiments, Bailey *et al.* (1995) reported that drought stress increased the distance traveled by aphids and virus spread on oats, albeit marginally.

Temperature during acquisition and inoculation access period has been shown to influence the transmission efficiency of the vector. Rochow (1969) showed that transmission efficiency of BYDV-RMV by *R. padi* and *S. avenae* increased from 6% and 11%, respectively, at 15°C to 70% and 85% at 30°C. Similarly, Lowles *et al.* (1996) reported that transmission efficiency of BYDV-PAV by *R. padi* and BYDV-MAV by *S. avenae* collected in England increased significantly from around 30% at 6°C to above 50% at 18°C.

2.8.2 Crop management practices

Tillage. In a seven year study on spring cereal in South Dakota, Hesler and Berg (2003) showed greater infestation of *R. padi* in fields without tillage compared to those with preplant tillage. In contrast, in Norway, Andersen (2003), also working on spring cereal, found a higher percentage of *R. padi*-infested, boot stage tillers in spring small grain with tillage than without tillage. Differences in the observation period may be attributed to this discrepancy. Hesler and Berg (2003) assessed their experimental plots regularly between emergence and booting stage, plotted the number of aphids found at each observation date and integrated their observation of *R. padi* abundance by estimating the area under the plot. Andersen (2003) on the other hand, made his observation only at the booting stage.

Planting date. Planting date has been shown to significantly affect the final incidence of BYDV and CYDV. In general, later planting of winter wheat is associated with reduced exposure to aphids, thus facilitating avoidance of virus infection in the

critical early growth stages (Chapin *et al.* 2001, Flanders *et al.* 2006, Hesler *et al.* 2005, Plumb and Johnstone 1995, Van Riessen 2002, Wyatt *et al.* 1988).

Regular assessment of aphid abundance on wheat planted in three different dates (early: 9/15 to 10/12, middle: 10/18 to 11/17 and late: 11/23 to 12/3) in central Georgia showed that aphid abundance was always the lowest in late planted wheat (Flanders *et al.* 2006). In Virginia, wheat planted in November showed significantly reduced YD incidence and increased final yield compared to wheat planted in October. Generally, planting after the first hard freeze is recommended in Kentucky, Virginia, the Carolinas, North Georgia and North Alabama since the freeze will kill most soft-bodied insects, like aphids. In Coastal Plain regions of Georgia and Alabama, though, the first hard freeze usually occurs well after the agronomic planting date recommendation. The agronomic planting date recommendation takes into account that most varieties of winter wheat require four to nine weeks of cold temperature before they produce kernels. In Alabama, three optimal planting dates are recommended for three distinct management regions, North, Central and South Alabama (Mask *et al.* 1997). Van Riessen (2002) reported that planting four weeks later than the recommended optimal date in the Coastal Plain region of Alabama (South Alabama) resulted in a decrease of *R. padi* count but an increase in *S. avenae* count. In both Coastal Plain and Appalachian Plateau regions (North Alabama), wheat planted four weeks after the recommended date showed low BYDV incidence. Nevertheless, the grain yield/ha of the wheat planted four weeks after the recommended date was lower to the wheat planted at recommended date. The author concluded that planting wheat later than the optimal date is not advisable.

A survey conducted in eastern Washington showed that YD incidence is at least 2.5 times higher on wheat planted before 15 September (Wyatt *et al.* 1988).

Royer *et al.* (2005) reported a significant effect of winter wheat planting date on aphid-day accumulation and final yield in Oklahoma. The later the planting date the lower aphid-day accumulation and the higher the final yield.

In South Dakota, Hesler *et al.* (2005) tested three planting dates: early (August 31st – September 9th), middle (September 10th-18th) and late (September 20th– 27th) and documented that generally early and middle planting dates were correlated with higher aphid-day accumulation and YD incidence, whereas the later dates were associated with lower aphid-day accumulation and YD incidence. Interestingly, the differences in aphid-day accumulation between planting dates generally came from the counts of *R. padi* and *R. maidis* individuals. No differences were observed in the aphid-day accumulation of *S. graminum*, *S. avenae* and *R. rufiabdominale* over the three planting dates. Moreover, the yield loss data generally showed no difference between early, middle and late planting dates.

A similar trend was reported from South Carolina where early (early November) and middle (mid-November) planting dates generally did not show differences in aphid-day accumulation, but the late (early December) planting date showed a significantly lower aphid-day accumulation, with all counted species (*S. avenae*, *R. padi*, *S. graminum* and *R. rufiabdominale*) showing the same trend (Chapin *et al.* 2001). In two of three years, YD incidence was significantly lower in the late planting date but showed no differences between early and middle dates. However, no correlation occurred between planting date and yield. In two years, yield did not differ between planting dates, while in

the third year the mid planting showed the highest yield. The optimal planting interval in South Carolina was 15 November to 1 December. Planting too early increased the risk of cold injury in March as April as well as Hessian fly infestation while planting too late resulted in reduced yield potential (Chapin *et al.* 2001).

Plumb and Johnstone (1995) noted that the traditional sowing dates in Europe may have been chosen as a result of 'unconscious influence' such as high BYDV risk in early plantings. In Europe, the traditional planting date was October at a time when few crops were seriously damaged. Nowadays, winter cereal sowing date is set earlier than it was (as early as August) due to increased intensity of cereal production.

2.8.3 Other field characteristics

Foster *et al.* (2004) reported correlations between YD occurrence with other field characteristics such as proximity to the sea, surrounding land use, and field size in Britain. Fields closer to the sea were reported to harbor more *R. padi* and *S. avenae* colonies, and were significantly correlated with higher virus incidence.

Fields with non-cultivated land (such as grassland, moorland, wasteland, running water and shelter belts) surrounding them was associated with higher aphid and BYDV incidence compared to the fields surrounded by cultivated land. An interesting exception was reported with regards to arterial roads and active railways. Fields with these features in the vicinity were associated with high aphid populations, but low virus incidence. Fields with unused railways were associated with a high aphid population and virus incidence.

The lowest occurrences of aphid and YD were reported from the smallest (<1 ha) and the largest (>31 ha) field size. The highest occurrences of both aphid and YD were

reported from fields of 2-7.9 ha in size. Low aphid populations in small fields may be explained by increased predator penetration from the field margin, where they overwinter. On the other side of the spectrum, low aphid number in the largest fields was harder to explain. The authors explained that the largest fields belonged to companies that monitor and manage the fields intensively, reducing probability of aphid population and disease build up.

2.8.4 Natural enemies

The presence of the vectors natural enemies has at least two ways of affecting the epidemiology of a disease. It may decrease the number of vectors, thus reducing the potential for virus spread. On the other hand, it may increase the rate and extend the spread of the virus by increasing vector movement.

Working under laboratory conditions, Smyrnioudis *et al.* (2001) reported that BYDV vectored by *R. padi* was not spread in a more extensive manner in the presence of parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) compared to the control. *Rhopalosiphum padi* showed little reaction (mainly shaking of the abdomen) when attacked by *A. rhopalosiphi*. On the other hand, *R. padi* moved more frequently in the presence of the predator, *Coccinella septempunctata* (Coleoptera: Coccinellidae), which may account for the increased amount of the virus relative to the control.

Bailey *et al.* (1995), working in a laboratory using *R. padi*, BYDV-PAV and larvae of lady beetles, *Coleomegilla maculatus* (Coleoptera: Coccinellidae), demonstrated higher incidence of YD in the presence of predators compared to control. The authors pointed out that aphids disturbed by predators may also release alarm pheromone that alerts other aphids causing them to stop feeding and disperse.

Hence, the benefit of reduced aphid population by predators can by outweighed by increased virus spread due to aphid movement. Nevertheless, Smyrnioudis *et al.* (2001) noted that in field conditions, aphids dislodged from a plant may encounter additional obstacles such as entomopathogenic fungi or ground predators, reducing the aphid survival rate.

2.8.5 Plant resistance and tolerance

Breeding of resistant and tolerant varieties of barley, oat and wheat has always been a part of YD management (Burnett *et al.* 1995). In this review, resistance against YD is defined as by Cooper and Jones (1983) as "reduced viral replication in infected plants," while tolerance is defined as by Burnett *et al.* (1995) as "the development of mild or negligible symptoms in infected plants." Numerous reports of 'field resistance' actually involve tolerance instead of true resistance against YD. Koev *et al.* (1998) noted that tolerance may be a more durable strategy than resistance because there is a much stronger natural selection against resistance in nature.

Sources of natural resistance or tolerance to YD in barley, oats or wheat are available although the physiological or molecular mechanisms are not clear (Burnett *et al.* 1995). A report on a barley breeding program for YD tolerance was published as early as the 1950s. Suneson (1955) worked with barley of variety 'Rojo' which showed intermediate tolerance. The tolerance was conditioned by a single recessive gene named *Yd1*.

In studying the inheritance of what was perceived as 'field resistance' in some varieties of Ethiopian barley to B/CYDV, Rasmussen and Schaller (1959) found that the tolerance was conferred by a semidominant gene, *Yd2*, located on the long arm of barley

chromosome 3H (Collins *et al.* 1996). The gene was later renamed *Ryd2*, but the usage of *Yd2* to refer to this gene is still common among recent publications (e.g. Ovesna *et al.* 2002). In this review, this gene will be referred to as the *Ryd2* gene.

The R*yd2* gene provided a higher level of tolerance than *Yd1*, and has replaced the *Yd1* gene in barley breeding programs (Burnett *et al.* 1995). To date, *Ryd2* is the most widely used natural source of tolerance against B/CYDV in commercial breeding of spring and winter barley (Burnett *et al.* 1995, Ovesna *et al.* 2002, Šíp *et al.* 2004). Basing their scoring system on visual symptoms, Baltenberger *et al.* (1987) reported that barley with the *Ryd2* gene showed more tolerant to BYDV-PAV than CYDV-RPV. There was a reduction of BYDV-PAV titer measured over time with ELISA in barley incorporating the *Ryd2* gene indicating actual suppression of viral replication (Skaria *et al.* 1985, Ranieri *et al.* 1993). No virus titer reduction was observed for the same varieties challenged with CYDV-RPV. These reports indicated that the *Ryd2* gene may actually confer true resistance against BYDV while conditioning the plant for tolerance against CYDV (Burnett *et al.* 1995). In the case of *Ryd2*-BYDV interaction, Ranieri *et al.* (1993) noted that the virus can move out of the point of infection within resistant plants, but the rate of viral replication is suppressed resulting in a decrease in total virus titer.

Ullman *et al.* (1988) compared feeding behavior of *R. padi* on barley lines with and without the *Ryd2* gene. They found that there was no difference in aphid feeding behavior, quantified as the number of sieve element contacts and duration of sieve element ingestion, between the two lines. Thus they concluded that the tolerance or resistance mechanism conferred by the *Ryd2* gene does not involve modification of the vector-host interaction.

Niks *et al.* (2004) reported a novel major barley gene of Ethiopian origin conferring resistance to BYDV. The gene, named *Ryd3*, is mapped to chromosome 6H. Controlled experiments showed that when challenged by a Dutch isolate of BYDV-PAV, plants with *Ryd3* or *Ryd2* had a low percentage of infection. This report is the first in pointing out that *Ryd2* gene not only suppresses BYDV viral replication, in the case of successful infection, but also reduced the chance of successful infection. Moreover, plants with the *Ryd2* gene that were successfully infected by BYDV-PAV showed higher yield components such as number of heads per plant and relative kernel yield per plant than infected susceptible plants.

Apart from tolerance traits with simple Mendelian inheritance ratios, there are other tolerance traits which do not fit such ratios. These are called complex tolerance or resistance traits (Young 1996). Complex traits are usually controlled by multiple loci within the genome (polygenic) and their expressions may be heavily influenced by environment X gene or gene X gene interactions. The genetic loci associated with a particular complex tolerance or resistance trait are collectively called Qualitative Trait Loci (QTL). Several QTL against BYDV in barley have been identified. Toojinda *et al.* (2000) mapped quantitative tolerance to BYDV-PAV and BYDV-MAV associated with chromosome 7H, 4H and 1H. Using a German isolate of BYDV-PAV, Scheurer *et al.* (2001) observed a QTL in the vicinity of the R*yd2* gene and another QTL on chromosome 2H.

Working with tolerant bread wheat lines released by CYMMIT challenged with the Mexican isolate of BYDV-PAV, Singh *et al.* (1993) reported that the tolerance of these lines was due to a partially effective and partially dominant gene named *Bdv1*. *Bdv1*

has remained the sole tolerance gene against BYDV available for wheat breeding (van Ginkel and Henry 2002). Ayala *et al.* (2002) showed that some wheat tolerance to a Mexican isolate of BYDV-PAV is polygenic in nature.

The ability of wheat grasses, *Thynopyrum* spp., wild counterparts of wheat, to suppress virus titer when inoculated with BYDV-PAV (Sharma *et al.* 1995) has been developed as a basis of resistance in wheat breeding (Francki *et al.* 2001, Larkin *et al.* 1995a, Larkin *et al.* 1995b). Intergenic hybridization between grasses from species *T. intermedium* (Host) and *T. elongatum* (Host) followed by backcrossing using wheat as the recurrent parent successfully produced wheat lines with *Thynopyrum* resistant genes to B/CYDV. When wheat lines containing the resistance gene from chromosome 7 of *T. intermedium* were challenged with both BYDV-PAV and CYDV-RPV, the result was complete suppression of CYDV-RPV virus titer in both the shoot and the root and partial suppression of BYDV-PAV titer in the shoot but not in the root (Anderson *et al.* 1998). It has been postulated that the gene within a small region in chromosome 7 of *T. intermedium*, termed *Bdv2*, conferred true resistance to CYDV-RPV but does not hamper viral replication of BYDV-PAV, especially in the root area. Molecular markers have been developed to detect the existence of *Bdv2* successfully introgressed into wheat, facilitating rapid selection of wheat lines with resistance potential against CYDV-RPV (Francki *et al.* 2001). Various quantitative traits loci conferring tolerance to BYDV, *Bdv1* and *Bdv2* have been widely used in commercial breeding programs of bread wheat (Ohm *et al.* 2002, van Ginkel and Henry 2002).

An ELISA-screening study showed reduced viral titer when wheat lines containing the entire chromosomes 1 and 2 of *T. intermedium* were challenged with

BYDV-PAV (Larkin *et al.* 1995b). Francki *et al.* (2001) suggested that these genes provided a possibility of pyramiding *T. intermedium* resistant genes against both BYDV-PAV and CYDV-RPV in wheat.

As reviewed above, there are only a few natural genetic resources of resistance and tolerance against B/CYDV. Efforts to incorporate them into commercial cereals have been difficult due to complex inheritance patterns (Burnett *et al.* 1995). Genetic engineering of commercial cereals has thus become another avenue in developing resistance against YD viruses. McGrath *et al.* (1997) reported transformations of oat with coat protein (CP) genes of BYDV-PAV, BYDV-MAV and CYDV-RPV, and similar transformation of barley with the CP gene of BYDV-PAV. Resistant self-fertile lines were detected by challenging transgenic plants by YD viruses and testing the resultant virus accumulation using ELISA. To test the stability of transgene inheritance, ensuing generations were also tested. Resistant plants were found up to the fourth generation, although less frequently than expected, indicating unpredictability of the inheritance of the resistant phenotype.

Jimenez-Martinez and Bosque-Perez (2004) showed that wheat lines transformed with the CP gene of BYDV-PAV and with a perceived higher resistance did indeed contain lower virus titers than susceptible wheat lines. Moreover, given the same acquisition access period, transmission efficiency of *R. padi* was higher when it fed on the susceptible variety compared to transformed variety. Significantly less volatile concentrations were extracted from the whole head-space of BYDV infected transgenic wheat than from the infected susceptible plants (Jimenez-Martinez *et al.* 2004). Subsequently, in choice tests where apterous *R. padi* were given a choice to aggregate

above YD infected and non-infected transgenic plants, there was no significant difference in the number of aggregating aphids between the two. In a similar test on a susceptible non-transgenic wheat variety, the number of aggregating aphids was significantly higher above infected plants compared to those above uninfected plants. Since volatiles act as one of the cues in attracting aphids, reduced volatile concentration in plants transformed with BYDV-PAV CP may have an impact in the field epidemiology of BYDV.

In another effort to develop resistance towards BYDV-PAV, oat plants were transformed with the 5' half of BYDV-PAV genome which contains open reading frame (ORF) I and II, including RNA-dependent RNA polymerase gene (Koev *et al.* 1998). Screening for resistance to BYDV was conducted on plants from the second and third generation. Transgenic oats challenged with BYDV-PAV showed dramatically reduced symptom severity compared to inoculated non-transgenic plants. Symptom recovery, defined as gradual disappearance of a yellow mosaic symptom back to complete greening of the leaves, was observed later on infected transgenic oats. In some of the recovered transgenic oats, a high virus titer was still detected using ELISA. Thus it is difficult to assess whether the transgene conferred true resistance by limiting viral replication or resulted in a form of tolerance. The mechanism of action by the transgene is also not clear. A few possible mechanisms were suggested: either the resistance was mediated by the 5' end untranslated region, by being complimentary to the viral 3' end genome, interfering with invading viral replication, or the transgenic RNA facilitated degradation of invading viral genome by RNA-mediated gene silencing. Neither of these explanations account for significant virus titer in recovered plants. Alternatively this may be the result of a novel tolerance mechanism mediated by the transgene.

2.8.6 Chemical control and forecasting schemes

Planting varieties with resistance towards B/CYDV that do not have the yield potential of susceptible varieties is not warranted. In the absence of high yielding resistant varieties, usage of chemical control is an option.

Timing of insecticide application to control aphid vectors of B/CYDV can be different from the timing of application to control for losses due to aphid feeding. George and Gair (1979), for example, recommended a control threshold of five *S. avenae* per head prior to flowering for winter wheat in UK. This put the timing of insecticide application towards the end of the wheat growing season. On the other hand, the critical time for the control of B/CYDV infection is at the early growth stage, especially before tillering, as plants infected at this stage tend to produce much less yield compared to those infected at later stages (Cisar *et al.* 1982).

At the early stage of wheat growth, the aphid population is usually low and often difficult to detect without intensive sampling. This difficulty has hampered the use of control threshold based on field aphid counts in YD management. Consequently, insecticide treatment has generally been conducted by prophylactic approach, experience-based or guided by a forecasting scheme (Plumb and Johnstone 1995).

There have been several YD forecasting systems reported from different parts of the world. A variety of forecasting systems have been used. There are forecast schemes that rely on the statistical correlation between aphid population and YD incidence (Plumb *et al.* 1986, Farrel and Stufkens 1992, Kendall and Chinn 1990). On the other end of the spectrum, there are mechanistic simulation models of YD epidemiology, using

correlation of variables derived from controlled experiments or previous literature (Kendall *et al.* 1992, Thackray *et al.* 2009).

A forecasting scheme based on empirical data of alate migration was developed in eastern England (Plumb *et al.* 1986). The scheme utilized information on the number of migrating alate vectors, vector infectivity and crop growth stage. The data on the migrating alate vectors were gathered through the Rothamsted Insect Survey (RIS), a network of aerial traps 12.2 m in height deployed throughout Britain. A similar network of aerial traps was also later deployed in parts of Western Europe. This larger network was named EURAPHID and the cumulative data is available through an online database EXAMINE (http://www.rothamsted.bbsrc.ac.uk/examine/ accessed July 6th 2009). The aphids trapped through RIS traps were identified and summarized weekly, providing regular data on the migration pattern of different aphid species. An additional trapping approach using shorter aerial traps (1.7 m above ground) was conducted at Rothamsted Research Station. In this additional trapping, the aphids were trapped alive. Aphids believed to be B/CYDV vectors were identified under binocular microscopes and tested individually for infectivity by placing them on oat seedlings. After 48-96 hours of feeding period, aphids were recovered from the seedlings and preserved. Originally, the identification of an infective aphid was conducted by observation of YD symptoms on test seedlings which were confirmed with immunospecific electron microscopy (ISEM) or ELISA. Symptoms typically took 10-28 days to develop. This long period needed for symptoms to show became a restrictive factor, since control decisions needed to be taken rapidly. To overcome this, ISEM or ELISA was conducted on test oat seedlings seven

days after the feeding test. Preliminary results suggested that serological tests made this way showed 90% agreement with subsequent symptom development.

The infectivity index was calculated weekly by multiplying the number of each vector aphid species caught in the RIS trap by the proportion of that species found to be infective in the additional trap. The total infectivity index was calculated by summing the indices for all vector species. The cumulative infectivity index was the summation of the weekly indices beginning from the sowing date.

Four year field experiments were set up to find the correlation between YD incidence, yield and infectivity index (Plumb *et al.* 1986). This information was crucial to providing an action threshold value. It was reported that each increase of 50 in the cumulative infectivity index gave a yield decrease of approximately 0.15 t/ha. After factoring the wheat price and control cost, the action threshold value of 50 was used for the Rothamsted area, where these experiments were conducted. The relationship between cumulative infectivity index and yield was reported to apply to much of East Anglia, the principal cereal growing area in England.

Regional variability and even failures of the infectivity index to predict YD incidence have been reported (Foster *et al.* 1993, Lowles *et al.* 1999, McGrath and Bale 1989). In the mild winter of 1988/1989, significant spread of B/CYDV occurred in Britain amidst forecasts of low incidence based on infectivity indices (Harrington *et al.* 1994). This failure has considerably reduced grower confidence in the infectivity index and has resulted in a greater adoption of prophylactic approach to control aphid in winter cereals. The prophylactic approach has been further encouraged by the low cost of pyrethroid insecticides (Oakley and Young 2000).

It has been suggested that although the infectivity index captured the information regarding the primary infection phase of YD epidemiology, it did not take into account the secondary spread of YD during fall. In years or areas with mild winters, exclusion of B/CYDV secondary spread may have translated to failures in predicting final YD incidence and the consequence yield loss (Lowles *et al.* 1999). Furthermore, Plumb *et al.* (1986) noted that although infectivity index measured the introduction of migrant aphid bringing B/CYDV into a newly sown crop, it did not measure the risk of virus infection resulting from within-crop sources such as cereal volunteers and stubble. In Britain, many cereal crops follow previous grass crops that are susceptible to B/CYDV. These two pieces of information: a more complete picture of primary infection and incorporation of the virus secondary spread within the field are common features in the subsequent development of YD forecasting schemes in the UK and elsewhere.

Modifications and improvements have been suggested to refine the infectivity index as a forecasting tool for YD management. Data from northern and southwestern England (McGrath and Bale 1989, Kendall and Chinn 1990) showed that winter barley infection was more closely related to the aerial vector index than to the original infectivity index. The aerial vector index is a refinement of the infectivity index in which the fraction of sexual alate morphs (i.e. gynoparae and males) of *R. padi* was excluded from the index calculation. These morphs were considered relatively unimportant in YD epidemiology because they migrated from the *Rosaceae* hosts which were not susceptible to B/CYDV.

Kendall and Smith (1981) observed that colonization of winter cereals was variable and not always correlated to the alate migrant density. Based on this observation,

another YD risk index based on counts of aphids in the field was suggested. This crop vector index used measurements of aphid abundance and B/CYDV infectivity by directly sampling aphids on the crops. The crop vector index used direct field sampling of aphids, it provided a measure of primary infection both from external sources and from within the fields (e.g. stubble and volunteer cereals). When fitted to YD incidence or yield, crop vector index was a better predictor than aerial vector index or the original infectivity index (Kendall and Chinn 1990).

Kendall *et al.* (1992) developed a YD simulation model that incorporated secondary spread of the virus. The model used the daily maximum and minimum temperature, the original infectivity index, and field aphid density as input variables. The daily maximum and minimum temperatures were used to calculate 'thermal time' for various processes within the model, e.g. crop emergence and growth, virus transmission and acquisition frequency by aphids and the length of the YD latent period in aphids and host plants. Each process had a different base temperature derived from the literature. The infectivity index was used as the variable of infective migrant bringing YD virus into the field. When no infective aphids were detected, a fixed coefficient was used. The aphid density on the crop was used to simulate the secondary spread within the field. Data used to run the model for validation was gathered from southwestern England. The model was validated using data from planting seasons 1980, 1982, 1981 and 1985. A high correlation was found between simulated and observed YD incidence The correlation between simulated YD incidence to observed YD incidence was considerably better than those of the original vector index, aerial vector index or crop vector index.

The mechanistic model proposed by Kendall *et al.* (1992) provided sufficient prediction of final YD incidence and allows for a forecast tailored to fit the situation of individual fields. However, it required a regular measurement of aphid abundance throughout the first months of cereal planting season as one of the inputs. Harrington *et al.* (1994) pointed out that such rigorous scouting is difficult and impractical for farmers to do in winter. To circumvent this problem, a model was developed that simulated wheat colonization rate by aphids, aphid survival rate, breeding rate, aphid movement and YD spread based on temperature and rainfall data.

In the latest UK forecasting system, primary infection is estimated by testing live *R. padi* alate caught using 12.2 m suction traps (Dedryver and Harrington 2004). The tests are done on a regional basis to provide a regionally specific estimation. Number of *R. padi* caught by the traps are translated to the number of aphids landing on the crops based on an established relationship between suction trap captures and the number of aphids found on sticky wire traps. The number of YD infected foci is estimated based on the suction trap captures, proportion of infective aphids and predicted movement of aphids within the crops. A stochastic model of YD secondary spread was developed using the literature-derived relationship between temperature and aphid development, reproduction, mortality and movement. Estimated YD infected foci, when fed into this stochastic model, predicts the secondary spread of the disease. Predicted YD incidence can thus be calculated and a five-level risk index formulated as the output. In a further development of this system, aphid abundance and YD final incidence were surveyed in 623 fields over three years. Multivariate analysis investigated the role of field characteristics in YD epidemiology. The results showed that YD was more likely to occur

on fields that were sown early, closer to the sea, in non-arable areas, east or southwest facing, or distant from arterial roads than on fields without those characteristics (Foster *et al.* 2004). These correlations were used to modify the regional risk index for an individual field based on whether the field has any of above characteristics.

The UK YD forecasting system is delivered via a website (bydv.csl.gov.uk/ accessed July 10th 2009). Users enter crop type, sowing date, seed and other insecticide treatments, location and whether or not arable land is dominant around the field which is used to calculate a tailored risk for the field.

A group in France has developed another YD yield loss forecasting scheme on winter barley based on the proportion of plants infested with *R. padi* (Fabre *et al.* 2003, Fabre *et al.* 2006). Fabre *et al.* (2003) showed that there is a non-linear relationship between the proportion of barley plants infested with *R. padi* and the aphid density in the same field. This variable was used in the forecasting scheme since it was simpler and takes less time to estimate than aphid density. For ten years, the proportion of infested barley plants was recorded regularly for the first two or three months of planting season, plotted over time and used to calculate the area under the curve with a trapezoidal integration method (Fabre *et al.* 2003). Final yield loss from each field was also recorded. Yield loss of 500 kg/ha was set as the economic action threshold based on the cost-benefit calculation of pesticide application. This economic threshold was used to create a binary variable binary logarithmic regression resulted in a highly significant model that acts as a YD risk index.

In a further development of the system (Fabre *et al.* 2006), Bayesian modeling was used to estimate aphid density in the field that existed in the beginning of a season,

based on barley plants infested with *R. padi*. A degree day model was used to estimate subsequent development of the aphid population. Bayesian modeling was used to calculate the probability distribution of growth rate given a known set of degree-day data. The aphid density growth within the plants throughout the season can thus be simulated using inputs of the proportion of plants infested by *R. padi* in the beginning of the season and predicted or historical values of maximum and minimum daily temperature. The area under the curve of simulated aphid density acted as the YD risk. When validated, the simulated YD risk based on observed temperature and 20-year-average temperature showed a significant positive correlation to observed YD risk. In the previous model (Fabre *et al.* 2003), the value of determinate YD risk index had to be compared with a preset decision threshold value. The Bayesian approach allowed for a more informative output by providing an estimate of the probability that the simulated YD risk was larger than the preset decision threshold value.

A YD forecast scheme for winter wheat based on suction trap data was developed in New Zealand (Farrel and Stufkens 1992). The forecast relied on eight years of data correlating aphid numbers caught in a 7.5 m suction trap in June and July and the proportion of crops sown after mid-May (late sowing) with the number of fields in which end-of-season YD incidence exceeded 5% (the tentative economic threshold). A control recommendation was then formulated in August based on trap captures in June and July.

The forecast used the number of aphids caught in suction traps without determining whether the aphids were infective with B/CYDV. Teulon *et al.* (1999) argued that minimal annual variation of the proportion of aphids carrying YD virus to winter cereals makes it unnecessary to determine the amount of infective aphids each

year (Teulon *et al.* 1999). In a later report, Teulon *et al.* (2004) found that the numbers of aphids caught in suction traps were correlated positively with the number of aphids found infesting the crops.

This simple yet functional forecasting system has been made available to the public through a website: www.aphidwatch.com (accessed July 2nd 2009). New data of aphid vectors caught on three to six suction traps in different locations are available weekly. The system uses aphid catch data from June and July to predict YD incidence on late sown wheat (planted after mid-May), and aphid data from April-May to predict YD incidence on early sown wheat (planted before mid May) (Knight and Thackray 2007). The forecast system of Farrel and Stufkens (1992) is used to predict the final YD incidence in both cases. This may have caused slightly less accurate predictions for early-sown wheat since the correlation was built based on late-sown wheat data.

This forecasting scheme underestimated YD incidence in wheat emerging after 1 June in mild winters (Teulon *et al.* 1999). The authors noted that mild winters might have been favorable for aphid survival and YD secondary spread. These components of YD epidemiology were not incorporated to the forecasting scheme.

Another outbreak of YD occurred in New Zealand in 2005 during a mild winter, in which unusually high numbers of alates were caught in the suction traps throughout the winter through spring (Teulon *et al.* 2008). It was postulated that the high alate population throughout the winter permitted continuous secondary spread of the virus. The New Zealand forecast system failed to alert farmers of the subsequent outbreak. Again, the critical need of incorporating secondary spread of B/CYDV into the forecasting system was noted.

In Australia, as in other countries, the key factors driving BYDV epidemics and consequent yield loss are the size and timing of primary infection, the activity of aphid vectors within the crop and the influence of climatic factors on aphid population, movement and viral transmission. In the Mediterranian climate of southwestern Australia, summers are hot and dry. Aphids and viruses persist on perennial grasses in isolated damp locations such as roadside ditches and springs (Hawkes and Jones 2005). In this climate, water is the limiting factor. Rainfall in summer and early autumn is critical in sustaining the perennial grasses that act as the summer hosts for the aphid and virus. Thackray *et al.* (2009), summarizing five years of data, showed a significant linear relationship between total rainfall in March and April (right before the sowing of winter cereals in Australia) and the date aphids were first recorded in wheat. This relationship forms a cornerstone of BYDV PREDICTOR, a mechanistic simulation model used to predict YD incidence and yield loss in southwestern Australia.

BYDV PREDICTOR consists of five sub-models: plant available soil water content; background aphid population density on grass weeds and volunteer cereal and aphid migration to the wheat crop; aphid buildup and movement within wheat crop; infection of wheat with BYDV; and effect of BYDV infection on yield. The model only used data derived from experiments with *R. padi*. A fixed proportion of 5% of immigrating alates are assumed to carry BYDV into wheat crops.

The model requires inputs of daily minimum and maximum temperature, rainfall and pan-evaporation. It is unique among other forecasting systems in not using data of alate migration or aphid crop abundance at all in its calculation. Fixed aphid background population, migration rates and infective aphid proportion replaced the inputs of empiric

data usually derived from suction traps or field scouting. When validated against 10 years field data from five different sites in southwestern Australia, significant positive correlations were found between simulated and observed yield loss (r=0.78).

The result of the southwestern Australia YD forecasting system is provided online at the beginning of the cereal growing season (www.agric.wa.gov.au/PC_92936.html accessed July 10th 2009). It estimates the regional risk of yield loss assuming no insecticide treatment.

The forecasting system functions as a support system in making decisions on insecticide application. The choice of insecticide is critical in YD management. For example, fields sprayed with pirimicarb, deltamethrin and demeton-S-methyl showed dramatic suppression of the *S. avenae* population one week after application. The aphid population rebounded two or three weeks later in fields treated with pirimicarb (McGrath and Bale 1990). Aphid recolonization translated into higher YD incidence on pirimicarb-sprayed fields compared to fields sprayed with deltamethrin and demeton-S-methyl. McKirdy and Jones (1996) reported similar results from western Australia where applications of synthetic pyrethroids, specifically alpha-cypermethrin and beta-cyfluthrin, were more effective in decreasing YD final incidence than pirimicarb and dimethoate, both carbamate insecticides.

No forecasting system is available to guide insecticide treatment in the southeastern states of USA. Insecticide application relies on results of field experiments and field scouting for vector abundance. The optimum timing for insecticide application varies between the northern and southeastern part of this region (Flanders *et al.* 2006). In Virginia, pyrethroid insecticide fall application between the 2-leaf stage and tillering was

shown to produce optimum results in terms of BYDV reduction and final yield. Similarly in central Georgia, where wheat is usually sown in October or early November, fall application of a pyrethroid insecticide around 30 days after planting consistently gave results in terms of yield. However, in the coastal plain region of South Carolina, Georgia and Alabama, wheat is sown late, sometimes as late as December. If *R. padi* is present in economically damaging levels, insecticide application in February is shown to give best results in terms of YD incidence and total yield compared to fall, January or March applications.

Imidacloprid is a systemic neonicotinoid insecticide that is marketed as both a seed treatment and a foliar spray. Alate aphids reared on wheat plants grown from imidacloprid-treated seeds had lower adult longevity and dramatically reduced fecundity (down to zero) compared to aphids reared on untreated plants (Gray *et al.* 1996). The same authors also reported reduced transmission efficiency of BYDV-PAV by *R. padi* and BYDV-MAV by *S. avenae* to imidacloprid-treated 10-day-old oat seedlings compared to untreated controls. Such a reduction was not observed on 24-day-old oat seedlings subjected to the same treatments.

Gray *et al.* (1996) noted that although landing rate of alates did not differ significantly for untreated and imidacloprid-treated wheat plants, the number of apterous aphids in imidacloprid-treated plants remained significantly lower throughout the planting season.

Viruliferous apterous aphids feeding on oat plants grown from imidacloprid-treated seed had interrupted feeding and increased activity. The neurotoxic effect of the insecticide caused rapid incapacitation and shortened feeding period of the aphids (within

four hours) (Knaust and Poehling 1992, Gourmet *et al.* 1994). These effects significantly reduced the spread and final incidence of YD.

In field trials, spring and winter-sown oat and wheat plants grown from imidacloprid-treated seeds had significantly reduced aphid abundance throughout the planting season and final YD incidence compared to untreated control (Flanders *et al.* 2006, Gourmet *et al.* 1996, Gray *et al.* 1996, McKirdy and Jones 1996, Royer *et al.* 2005).

Flanders *et al.* (2006) reported reduced BYDV incidence on plots sown with imidacloprid treated seeds. Inconsistent return was observed in southern parts of Alabama while in the northern parts of the state consistent marginal return was reported.

Using four levels of imidacloprid seed treatment (0, 0.24, 0.48 and 0.96 g/kg seed), Royer *et al.* (2005) reported that, in general, application rate had a negative correlation with aphid abundance and final YD incidence. However, the difference in yield between higher and lower application rates was not always significant. Thus, the higher application rate did not always translate into positive economic return. The wheat planting date also affected the protection level provided by imidacloprid-treated seed to YD and its economic return. Aphid abundance consistently decreased with the increase of application rate in early (11-15 September) and middle planting (22-29 September). In late planted wheat (8-13 October), reduction in aphid abundance was only observed in wheat treated with the highest application rate. Results in this study indicated that the lowest rate of imidacloprid (0.24 g/kg seed) combined with middle planting time (22-29 September) showed the most consistent economic return.

McKirdy and Jones (1996) noted that although imidacloprid deployed as seed treatment provided some control of B/CYDV spread in the first weeks after emergence, an additional foliar spray of pyrethroid insecticide six weeks after emergence was necessary. This report stands in contrast with reports from Europe and US (Knaust and Poehling 1992, Gourmet *et al.* 1996, Gray *et al.* 1996, Royer *et al.* 2005) in which the seed treatment alone was sufficient. High pressure of viruliferous aphid immigrating to wheat fields in western Australia, where these experiments were conducted, for the first twelve weeks after emergence may explain inadequacy of imidacloprid as seed treatment to control YD.

Table 2.1 List of original BYDV strains and their aphid vectors (Hemiptera: Aphididae).

BYDV Strain	Optimum Vector	Citation
PAV	*Sitobion avenae* (Fab.), *Rhopalosiphum padi* (L.)	Rochow 1961a
RPV	*Rhopalosiphum padi* (L.)	Rochow 1961a
MAV	*Sitobion avenae* (Fab.)	Rochow 1961a
SGV	*Schizaphis graminum* (Rondani)	Rochow and Muller 1971
RMV	*Rhopalosiphum maidis* (Fitch)	Rochow 1961b

3 Long-Term Goal, Objectives and Hypothesis

This investigation sought to address the gap of knowledge in yellow dwarf (YD) epidemiology in Alabama, especially by identifying the primary vectors of the virus and potential summer hosts of B/CYDV. This information will be used to design a more accurate and sustainable management strategy for YD in Alabama.

Specific objectives and hypotheses of this work were to:

1. Identify the vector species responsible for B/CYDV primary infection to wheat plants.

   Hypothesis:

   Based on the results of aphid surveys in South Carolina, geographically the closest region to Alabama for which a B/CYDV vector survey has been conducted, *S. graminum, R. padi* and *R. rufiabdominale* are responsible for B/CYDV fall infection to wheat.

2. Identify potential summer hosts of the virus and vectors of B/CYDV.

   Hypothesis:

   Cultivated grasses grown in the summer act as summer hosts for both the viruses and vector of B/CYDV.

4 Species Composition of the Aphid Vectors of Barley Yellow Dwarf Virus and Cereal Yellow Dwarf Virus in Alabama and Western Florida

4.1 Introduction

According to the online database of The National Agricultural Statistics Service (2009), 97,000 ha of wheat were planted in Alabama in 2008, of which 80,000 ha were harvested for grain. The state wheat production in 2008 was valued at $ 85 million.

Barley yellow dwarf (BYD) is a serious impediment to cereal production worldwide (Plumb 1983). As the disease progresses phloem tissues in the root and shoot of infected plants are killed (Esau 1957a, 1957b). Ensuing stunting and discoloration lead to a decrease in yield. In Alabama, BYD can cause yield reductions of up to 60%. The greatest loss due to BYD was reported from the northern part of the state (van Riessen 2002).

The causal pathogens of BYD are two viruses from family *Luteoviridae*, *Barley yellow dwarf virus* (BYDV) and *Cereal yellow dwarf virus* (CYDV). Two strains BYDV have been reported, BYDV-PAV and BYDV-MAV. One strain of CYDV has been reported, CYDV-RPV. Two other viral strains in the virus complex, SGV and RMV, are yet to be classified into any species (Mayo and D'Arcy 1999). A survey in Alabama reported the presence of BYDV-PAV and CYDV-RPV (Bowen *et al.* 2003). The predominance of each virus varied from year to year. Since the reclassification of the viruses introduced a new term 'Cereal Yellow Dwarf' in addition to 'Barley Yellow Dwarf', the diseases will be referred to collectively as Yellow Dwarf (YD) in this paper.

At least 25 species of aphids have been reported to be vectors of YD viruses (Halbert and Voegtlin 1995). In a few reported cases, dominant YD virus strains changed between years at the same location (Rochow 1979), or important aphid vectors varied between adjacent regions (Halbert *et al.* 1992a, Forster *et al.* 1990).

Knowledge of the aphid vectors in a given region is crucial to understanding the regional epidemiology of YD. This warranted a study of potential YD vectors in Alabama. This paper reports two separate studies of YD vectors in the state of Alabama. One study was designed to determine which aphid species are responsible for the transmission of BYDV and CYDV into wheat. The other study utilized suction trap catch data to investigate the seasonal flight activity of potential YD vectors. The overall goal of the studies were to identify the vector species responsible for B/CYDV primary infection to wheat plants. Based on the results of B/CYDV vectors survey in South Carolina (Chapin *et al.* 2001) it is hypothesized that *S. graminum, R. padi* and *R. rufiabdominale* are responsible for B/CYDV fall infection to wheat.

4.2 Methodology

Field collection of live aphids. Surveys of wheat fields were conducted in Alabama and western Florida between October 2005 and January 2009. A pilot study was conducted in October to December 2005 on small grain plots at research stations in Tallassee and Headland, AL to investigate the feasibility of the methodology. Aphids were collected from the wheat plants and assayed for BYDV or CYDV infectivity. In each field, twelve 3 meters long rows were randomly selected and sampled for aphids. When less than thirty individuals were collected from these rows, additional rows were sampled until two hours has passed. The infectivity assay was done in accordance with

the methods of Chapin *et al.* (2001) and Halbert *et al.* (1992a). Live aphids were collected using a paint brush. Each collected aphid was placed directly in a glass tube containing a 7-14 day old 'California Red' oat seedling on a moist substrate. In the pilot study, slanted water agar was used as the substrate. Later, oat seedlings were found to survive on moist cotton or tissue paper for over two weeks. Since the usage of moist cotton or tissue paper considerably reduced preparation time, these substrates were used in subsequent samplings. The tubes containing aphids and oat seedlings were incubated at room temperature to allow for an inoculation access period. After 48-72 hours of inoculation access period, all aphids were removed from the tubes, preserved in 80% ethanol and identified to species according to the identification key described by Blackman and Eastop (2000). The oat seedlings were then treated with lambda-cyhalothrin insecticide to kill all residual aphids, potted and incubated in a protected environment. In the pilot study, the seedlings were incubated for 30-60 days to document symptom formation in infected plants. In subsequent years, an incubation period of two to three weeks was used. By the end of the incubation period, two or three oldest leaves were harvested from each plant and the leaf tissue composites were subjected to triple antibody sandwich enzyme-linked immunosorbent assay (ELISA) using commercial antibodies produced by Agdia Inc. (Elkhart, IN) according to the manufacturer's instruction. Known wheat-based negative and positive controls obtained from Agdia Inc. were added to each plate. A sample was considered positive for the presence of a virus if the ELISA absorbance value was greater than the average plus three standard deviations of the negative controls on a given plate (Sutula *et al.* 1986).

Between 2006 and 2008, the sampling locations included commercial wheat fields in the northern and southern part of Alabama. Beginning in 2007, wheat variety trials at the North Florida Research and Education Center at Marianna in western Florida were sampled (Table 4.1). The North Florida Research and Education Center is located about thirty miles from the Alabama border. Wheat plots at three agricultural research and extension centers in Central and North Alabama became available and were sampled in 2008/2009. The data from western Florida and South Alabama will be referred to as 'south Alabama'. These plots were sampled seven times between October and December 2005.

In the 2006/2007 wheat season, sampling was conducted between November to May. The longer sampling period was conducted to get a more comprehensive picture of aphid species composition throughout the wheat season. In South Alabama, wheat seedlings were just emerging in the first week of December 2006. In wheat season 2007/2008 and 2008/2009, aphid sampling began at wheat emergence and went on until January or February. The sampling period between wheat emergence and January or February afforded a reasonable window to determine aphid species composition during the critical time for fall BYDV infection. In these three years, fields were sampled weekly between the start of sampling period through December and at least twice a month in January through the end of the sampling period. Sometimes, no aphids were collected. Consequently, the weekly data contained a lot of zero values and the data will thus be summarized annually and monthly in this report.

Flight activity of potential virus vectors. One suction trap (Allison and Pike 1988) was operated in Red Hill, AL (latitude 34.26°N, 86.42°W) from late September

1996 through June 1999. The trap collected aphids from a height of 8 m. The trap was located in a valley with mixed agricultural and residential use, surrounded by hills in the Sand Mountain Region of Alabama. Wheat in this valley historically had a high incidence of YD. The trap was placed in a production wheat field in 1996. By 1998, commercial production of wheat in that area had ceased, but a small plot of wheat (approximately 0.5 A) was planted around the base of the trap. Trap catches, preserved in ethylene glycol, were removed weekly and brought into the laboratory for sorting. Trap setup and aphid collection was conducted by Kathy Flanders, Frank Wood and Chuck Howard. Aphids collected from suction traps were identified to species by Susan Halbert from Florida Division of Plant Industry. Remaudière and Remaudière (1997) was used as the standard taxonomic reference, with a few exceptions where newer information was available.

4.3 Results

Field collection: wheat colonizing alates. In four years of field collection, 1142 aphids were collected. However, 31% were lost before they could be identified. As described above the collected aphids were kept in lidded glass tubes. Despite this precaution it was quite frequent to find no aphid in the tube after three days of inoculation feeding period. Aphids may have hidden in the root systems or within the fold of moist cotton or paper towel. But at other times, no aphid could be found within the tube even after complete extraction and search of the oat plant and its media. The aphids may have escaped the tubes sometime during the inoculation feeding period.

Of the total aphids recovered after the assay, 571 were alatae, 206 were apterae and seven were severely damaged so as to make identification impossible. The keys

found in Blackman and Eastop (2000) were limited to alate identification, thus only the alate portion of the collected aphids was identified to species. Of all the alatae, 49% were collected in South Alabama, 43% from Central Alabama and 8% from North Alabama. Eleven species of alate aphids representing nine genera were found on wheat between 2005 and 2009 (Table 4.2).

Field collection: potential YD vectors. Five aphid (Hemiptera: Aphididae) species found on wheat in Alabama and western Florida are known YD vectors: bird cherry-oat aphid, *Rhopalosiphum padi* (L.), rice root aphid, *Rhopalosiphum rufiabdominale* (Sasaki), greenbug, *Schizaphis graminum* (Rondani), corn leaf aphid, *Rhopalosiphum maidis* (Fitch) and English grain aphid, *Sitobion avenae* (Fab.) (Halbert and Voegtlin 1995) (Figure 4.1).

Field Collection: B/CYDV-infective vectors. One thousand and six seedlings used in the bioasssay were tested for infectivity as described. One hundred seedlings wilted and died following the inoculation access period before they could be tested with ELISA. Of all the plants tested, six were found to be infected with B/CYDV. Two of the aphids responsible for infection were *R. rufiabdominale* collected in November 2005 and December 2006, three were *R. padi* collected in November 2005, December 2006 and January 2008, and one infective aphid was lost before identification. All of the infective aphids were collected from South Alabama. Four infective aphids were found to transmit BYDV-PAV, two *R. padi*, one *R. rufiabdominale* and an aphid that escaped before identification. Two infective aphids, a *R. rufiabdominale* collected in November 2005 and a *R. padi* collected in January 2008, were found to transmit CYDV-RPV. All of the infective aphids were alates.

Suction trap: Seasonal flight activity BYDV and CYDV vectors. The five species of B/CYDV vectors found on wheat were also collected from the suction trap in Redhill, AL (Figure 4.2). The highest flight activity of *S. graminum* was detected between October and November with flight activity extending to January. A lower peak of flight activity also occurred in August-September with a low flight activity recorded in April and early May. *Rhopalosiphum rufiabdominale* flight activity was detected between April and December. Trap catches of this species were greatest in April-May and in July and August in Red Hill. Low but consistent numbers of *R. rufiabdominale* were caught from late September until December. A peak of flight activity of *R. padi* was recorded between late November and December with another peak in April and May. Low but consistent flight activity was detected throughout the wheat growing season. *Rhopalosiphum maidis* flight activity was greatest in July and August. Ninety six percent of *R. maidis* were trapped in these two months. Very low flight activity was detected between October and November. *Sitobion avenae* was the least abundant of the five major cereal aphids captured in the suction trap. Only 18 specimens were collected, most of them in May.

4.4 Discussion

The number of aphid species and the number of aphids per species found on wheat were much lower in North Alabama than in South Alabama. Yet, all of the aphid species found on wheat in South Alabama were also found in the suction trap in North Alabama. Fewer numbers of species and individuals caught per species in field sampling in North Alabama may be due to colder temperatures and earlier frosts typical of North compared to South Alabama. Ambient temperature may have limited flight activity and

population buildup of some species in North Alabama which subsequently reduced the chance of a given species to be found in the field at the times of sampling.

Flight activity of cereal aphids are correlated to the ambient temperature. There are lower thresholds of temperature under which the chance of alate to takeoff is very low (Walters and Dixon 1982). These thresholds are variable between aphid species but they are not usually lower than 10°C. Later season wheat planting between 2006 and 2008 meant a shorter time period during which alates could find wheat plants before temperatures got lower than their flight temperature threshold and it may have contributed to the lower numbers of aphid found in these years.

In 2006/2007, a few aphids were successfully collected and identified from North Alabama, whereas in 2007/2008 and 2009/2009 only one alate was found after intensive sampling of three fields. The historical temperature data collected from the nearest weather stations in North Alabama do not show particular differences between these sampling years in terms of the number of days with temperatures lower than 0°C, or average high and average low temperatures. The first frost typically occurred in the first two weeks of November. The only difference between the years was the timing of wheat sowing on the fields selected for sampling. This difference in the timing of wheat sowing was not intentionally manipulated; instead it followed the commercial growers' schedule of the season. In 2006, the commercial fields selected for sampling were sown in October and by November aphids were found in wheat seedlings. In 2007/2008 and 2008/2009, commercial fields selected in North Alabama were sown in early November. The first frost of the year typically occurred the first week of November and by the time the seedlings were germinated in December, it was quite common for the lowest temperature

in a day to be below or near the freezing point. A late sowing date combined with occurrence of low temperatures during November and December may have exposed wheat colonizing aphids to a lethal period of low temperatures. This exposure may induced reduction in the rate of population build up or even decreased the population altogether and resulted in very low aphid catch in 2007/2008 and 2008/2009. Late sowing date was shown to negatively affect colonization and subsequent aphid populations on wheat in Griffin, Georgia (Flanders *et al*. 2006).

Total alates caught in the first sampling year were the highest compared to the other years. A prolonged drought was in place throughout the southeastern United States, including Alabama, beginning in the fall 2005. According to the drought monitor map series published online by the University of Nebraska, Lincoln (2009), parts of Alabama, Georgia and Florida started to get relief from the drought in spring 2009 and by June 2009 only small pockets in these states were still classified as abnormally dry. In a Mediteranean-type environment such as southwestern Australia where the summer is dry and hot, rainfall was closely correlated with the timing of first alate flight into wheat crops (Thackray *et al.* 2009). In this region, wild grasses surviving in ditches were found to be the major summer host of aphids (Hawkes and Jones 2005). Low summer rainfall may have affected the host survival and quality, and thus indirectly affected the aphid population buildup during summer caused a delay of alate flight activity in the subsequent fall (Thackray *et al.* 2009). The drought may have contributed to the low aphid catch in Alabama and western Florida between 2006 and 2009. Bowen and Burch (2001) analyzed YD historical data and found that YD incidence was higher in wheat seasons preceded by a warm summer and less rainfall. It is important to note that this

finding was derived from data in years without drought. Moreover, the basis for higher YD incidence in seasons following summers with less rainfall is unclear.

Aphids were sampled from small sampling plots in the first year while either large commercial fields or large scale variety trials were sampled for the other years. Edge effect, where accumulation of higher aphid counts are found on the edge of a field compared to areas closer to the middle of the field, is a common feature of aphid distribution within a cereal crop (Dean and Luuring 1970) and has been observed with *Sitobion avenae* on winter wheat (Winder *et al.* 1999). In the case of *S. avenae*, the edge effect extended between 30 to 60 m into the field. Consequently aphid sampling within a plot with either length or width dimension smaller than 30 to 60 m may be heavily influenced by the edge effect. This may partially explain the higher number of aphids found in the first year when small plots were monitored.

The five known B/CYDV vectors collected in Alabama and western Florida were identical to survey results in South Carolina (Chapin *et al.* 2001). This species composition of B/CYDV vectors was similar to the results from Virginia (McPherson and Brann 1983) and Kentucky (Johnson and Hershman 1996) with the exception that *R. rufiabdominale* was not found on wheat crops in Virginia and Kentucky.

Sampling results from 2006/2007 (Figure 4.1.b) provided a typical population pattern of B/CYDV vectors found on wheat for the first half of a season. A high number of alates was usually found in the first month of the wheat season. As the temperature dropped in December and January, the number of alates found on wheat decreased. In February, an increasing number of alates was found on wheat in South Alabama. This increase may have been due to the increasing temperatures in February, allowing greater

flight activity from external sources. Alternatively, the increase in aphid population by the end of the winter may have reflected a buildup in the local aphid population that survived the low temperatures of December and January. Temperature is suspected to regulate morphological determination indirectly in *R. padi* (DeBarro 1992, Dixon and Glen 1971). Warmer temperatures allow an increase in population, which causes crowding for both the mothers and nymphs, thus triggering alate formation. The increase in alate aphids found on wheat in South Alabama continued into March and was reduced in April and May. The sharp decrease in April and May may have been due to a heightened rate of predation and parasitization following the increase in aphid population the month before.

Of the five B/CYDV vectors found on wheat, two were consistently found in South Alabama during the fall: *R. padi* and *R. rufiabdominale*. The suction trap data showed high flight activity of *R. rufiabdominale* between August and September while the high flight activity of *R. padi* occurred between November and December (Figure 4.2). In wheat fields in Central and South Alabama, *R. rufiabdominale* was found as soon as sampling began, whether in October or November. The 2005 data showed that *R. rufiabdominale* was found in the field before *R. padi*. This agrees with the results of the wheat aphid colonization survey conducted in the South Carolina coastal plain where *R. rufiabdominale* was found on wheat seedlings before *R. padi* (Chapin *et al.* 2001). The peak month for collecting winged *R. rufiabdominale* on wheat in South Alabama occurred in November as illustrated in the data from 2005, 2007 and 2008. In 2006, wheat seedlings had not germinated until December.

In North Alabama both *R. padi* and *R. rufiabdominale* were found in December 2006 and January 2007. No *R. rufiabdominale* were found after December 2006, although *R. padi* continued to be collected until March 2007. In South Alabama, *R. padi* alates were always present between December and February. *Rhopalosiphum rufiabdominale* is known to colonize root systems of graminaceous plants (Blackman and Eastop 2000). It is possible that after December, *R. rufiabdominale* already colonized the below ground tissues of wheat.

In Alabama, *S. graminum* flight activity peaked once in October and November with lesser flight activity in August-September and between April and early May (Figure 4.2). This contrasted with flight activity on the South Carolina coastal plain, where three distinct periods were observed, a late summer flight in July-September, which tended to have the greatest trap captures, as well as a spring flight in Mar-April and a fall flight period in October-December (Chapin *et al.* 2001). In agreement to the flight pattern observed in Red Hill, AL, *S. graminum* was the only aphid collected on wheat in North Alabama in November 2006 (Figure 4.1b). In Central and South Alabama, *S. graminum* was abundant throughout the sampling period in 2005, especially in late October to December. Only a few individuals of *S. graminum* were found, however, in December 2006 and January 2007. In South Carolina, *S. graminum* was the first aphid to colonize wheat seedlings with a peak field population observed in December or January (Chapin *et al.* 2001). This is in agreement with the results from Alabama (Figure 4.1). The only exception to this was that no *S. graminum* was found in the beginning of 2008/2009 wheat season in South Alabama.

Rhopalosiphum maidis was found on wheat once in February 2007 and once in January 2008. Each collection was in South Alabama (data not shown). Such sporadic collection of *R. maidis* alates was also reported from South Carolina (Chapin *et al.* 2001). These two reports stand in contrast to the report from Virginia where *R. maidis* made up to 50% of autumn aphid infestation on winter wheat and persisted on the plant through March (McPherson and Brann 1983). In South Carolina two peaks of flight activity were consistently found for *R. maidis*: a higher peak in July and August and a lower one between October and November (Chapin *et al.* 2001). In Alabama, in 1995-1998, one peak of flight activity was observed between July and August, while very low flight activity was detected between October and November (Figure 4.2).

Sitobion avenae was found in South Alabama as early as January (2009). Alate *S. avenae* were found continually after January in both South and North Alabama. Very few *S. avenae* were captured in the suction trap in Red Hill, Alabama, with most being captured in May. *Sitobion avenae* is known to colonize winter wheat late in the season. The population typically peaks in spring as reported from South Carolina, Virginia and Kentucky (Chapin *et al.* 2001, McPherson and Brann 1983, Johnson and Hershman 1996).

The transmission assay on collected alates showed that clones of *R. padi* and *R. rufiabdominale* collected in Central and South Alabama transmitted BYDV-PAV and CYDV-RPV and, thus, act as vectors that bring B/CYDV into wheat fields in the fall. The ability of *R. padi* to transmit BYDV-PAV and CYDV-RPV is long established (Rochow 1961a), although there is always a possibility of variability in transmission efficiency between geographic clones (Power and Gray 1995). Clones of *R. padi* from

New York, South Carolina and Idaho were reported to transmit BYDV-PAV efficiently (Halbert *et al.* 1992b, Gray *et al.* 1998). *R. rufiabdominale* clones collected in South Carolina were found to transmit BYDV-PAV, BYDV-SGV and CYDV-RPV efficiently (Gray *et al.* 1998).

Even though *S. graminum*, *R. maidis* and *S. avenae* were collected from wheat in Alabama, no individual of these species was found to be infective with a YD virus. Of these three aphids, *R. maidis* was found very sporadically on wheat while *S. avenae* was only infrequently found before December and, thus, may not play a consistent role as a fall vector of YD viruses. *Schizaphis graminum*, on the other hand, was consistently found in the beginning of the wheat season, especially in South Alabama, albeit at a lower number than *R. rufiabdominale* or *R. padi. Schizaphis graminum* has been reported to transmit BYDV-PAV, BYDV-SGV and BYDV-RMV (Halbert 1992a), but a recent report showed that *S. graminum* clones from South Carolina transmitted YD viruses very poorly (Gray *et al.* 1998).

The low overall percentage of aphids found to be infective with BYDV or CYDV (0.6%) reported in this study was unexpected. In a simulation model used to predict YD incidence in southwestern Australia, a fixed percentage of 5% of incoming alates were assumed to carry the viruses (Thackray *et al.* 2009). In South Carolina, Chapin *et al.* (2001) conducted a similar study on winter wheat spanning a three year period of field collection, tested 2682 aphids and found 85 to be infective, amounting to 3.16% of the total collected aphids. Halbert *et al.* (1992a) collected 9802 aphids from various hosts over four years in southwestern Idaho and recorded an overall 3.19% portion of the collected aphids to be infective with YD viruses. Krebs (1999) described a method to

estimate an optimal sample size given a known estimate of population proportion of interest, an estimate of the confidence interval and a standard error. Assuming 3-5% as the expected percentage of overall aphid populations infective with B/CYDV, a confidence interval of 95% (α=0.05), and an expected standard error of 0.02, the resulting optimal sample size is between 300 and 500. In this study, over 1000 samples were tested for BYDV and CYDV in four years, but the overall proportion of infective aphid was found to be much lower than 3-5%. There is a possibility that aphids at the particular time and places in Alabama were not carrying YD viruses, thus, the very low percentage of infective vectors found in the samples was a true indicator of the state of the infective portion of aphid population. Indeed no visual symptoms of YD were ever recorded in the fields where collections were conducted throughout the years. Another possibility is a problem within the execution of the bioassay protocol. The protocol itself is well established in the study of field vector infectivity (Halbert *et al.* 1992a, Chapin *et al.* 2001). However, in this study, it was quite common to find dead aphids within the assay tubes after three days of inoculation feeding period. Moving aphids from plants to tubes is not without the risk of bruising the insect soft body which may lead to desiccation and death. If this happened frequently, aphids may have died before a sufficiently long period of feeding time to allow for inoculation, rendering the total percentage found in this study lower than the actual proportion of B/CYDV-infective aphids on the field.

4.5 Conclusion

Five known YD vectors were found on wheat in Alabama between October and February 2005-2009: *R. padi*, *R. rufiabdominale*, *R. maidis*, *S. graminum* and *S. avenae*. *Rhopalosiphum padi*, *R. rufiabdominale* and *S. graminum* were observed consistently in

the beginning of winter wheat season with *R. padi* and *R. rufiabdominale* being the major potential vector species in the fall. This is in agreement with suction trap data from 1995-1999 showing high flight activity of *R. padi*, *R. rufiabdominale* and *S. graminum* in the fall and early winter. The vector infectivity data showed that *R. padi* and *R. rufiabdominale* isolated from winter wheat vectored BYDV-PAV and CYDV-RPV. Despite being an efficient vector of BYDV-PAV and CYDV-RPV, *R. rufiabdominale* from South Carolina was not considered an important vector of YD viruses due to the absence of correlation between *R. rufiabdominale* population and YD incidence. On the other hand, surveys of aphid vectors of YD viruses in the United States showed that *R. padi* density is usually correlated with BYDV field incidences (Chapin *et al.* 2001, Clement *et al.* 1986, Halbert and Pike 1985, Halbert *et al.* 1992a). In this study, the frequency of infective vector detection is too low to establish the relative importance of each species as B/CYDV vectors in Alabama, but it is clear that both aphids are capable of introducing BYDV-PAV and CYDV-RPV into Alabama wheat fields. More research is needed to determine oversummering hosts of these two aphid vectors and the viruses causing YD.

Table 4.1 Location, timing and field types used for aphid surveys in Alabama and western Florida between 2005 and 2009.

Season	Sampling duration	Location	Latitude/Longitude	Field Types
2005	October to December	E.V. Smith Research Center at Tallassee, AL	32.32°N, 85.53°W	Small plots
		Wiregrass Research and Extension Center at Headland, AL	31.20°N, 85.20°W	Small plots
2006/2007	November to May	Atmore, AL	31.04°N, 87.33°W	Commercial wheat fields
		Killen, AL	34.52°N, 87.32°W	Commercial wheat fields
		Town Creek, AL	34.40°N, 87.25°W	Commercial wheat fields
2007/2008	September to February	Central Star, AL	34.51°N, 87.27°W	Commercial wheat fields
		Russelville, AL	34.26°N, 87.44°W	Commercial wheat fields
		North Florida Research and Education Center at Marianna, FL	30.46°N, 85.13°W	Small variety trial plots
2008/2009	October to January	Greenbrier, AL	34.39°N, 86.50°W	A commercial wheat field and variety trial plots at research stations
		Tennessee Valley Research and Extension Center at Belle Mina, AL	34.44°N, 86.53°W	Small plots
		Sand Mountain Research and Extension Center at Crossville, AL	34.17°N, 85.56°W	Small plots
		North Florida Research and Education Center at Marianna, FL	30.46°N, 85.13°W	Small variety trial plots
		E.V. Smith Research Center at Tallassee, AL	32.32°N, 85.53°W	Small variety trial plots

Table 4.2 Winged aphids (Hemiptera: Aphididae) collected on wheat fields in Alabama and western Florida between 2005 and 2009.

Species	Number of aphids								
	2005[a]		2006/2007		2007/2008		2008/2009		
	Central	South	North	South[b]	North	South	North	Central	South
Aphis craccivora Koch	0	1	0	0	0	0	0	0	0
Eulachnus rileyi Williams	0	4	0	0	0	0	0	0	0
Histeroneura setariae (Thomas)	11	1	0	0	0	0	0	0	0
Pemphigus sp.	0	0	0	1	0	0	0	0	0
Rhopalosiphum maidis (Fitch) [c]	0	0	0	1	0	0	0	0	1
Rhopalosiphum padi (L.) [c]	18	4	33	31	0	27	0	6	13
Rhopalosiphum rufiabdominale (Sasaki) [c]	117	53	5	17	0	30	0	8	29
Schizaphis graminum (Rondani) [c]	79	17	1	2	0	1	1	0	0
Sipha flava (Forbes)	4	1	1	0	0	10	0	0	2
Sitobion avenae (Fab.) [c]	0	0	1	10	0	2	0	1	1
Tetraneura nigriabdominalis (Sasaki)	1	2	2	16	0	1	0	0	2

a In the first sampling season, no sampling was conducted after December 2005.

b Between 2006 and 2009, data from fields in western Florida and south Alabama were compounded under this category

c Aphid species known to be vectors of B/CYDV (Halbert and Voegtlin 1995)

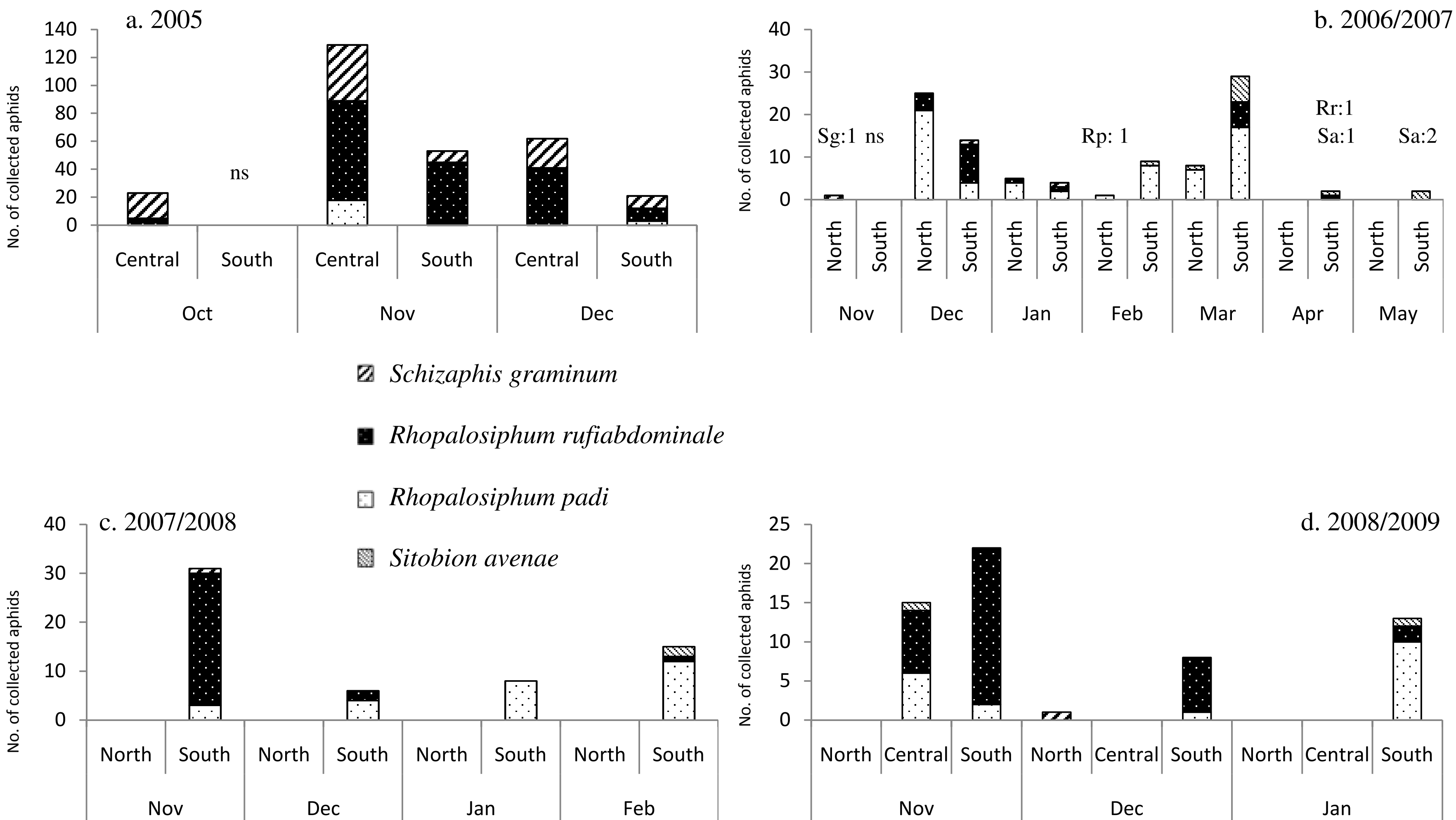

Figure 4.1 Seasonal abundance of winged aphids collected on wheat fields in Alabama and western Florida between 2005 and 2009 (ns: not sampled, Sg: *Schizaphis graminum,* Rr: *Rhopalosiphum rufiabdominale*, Rp: *Rhopalosiphum padi*, Sa: *Sitobion avenae*).

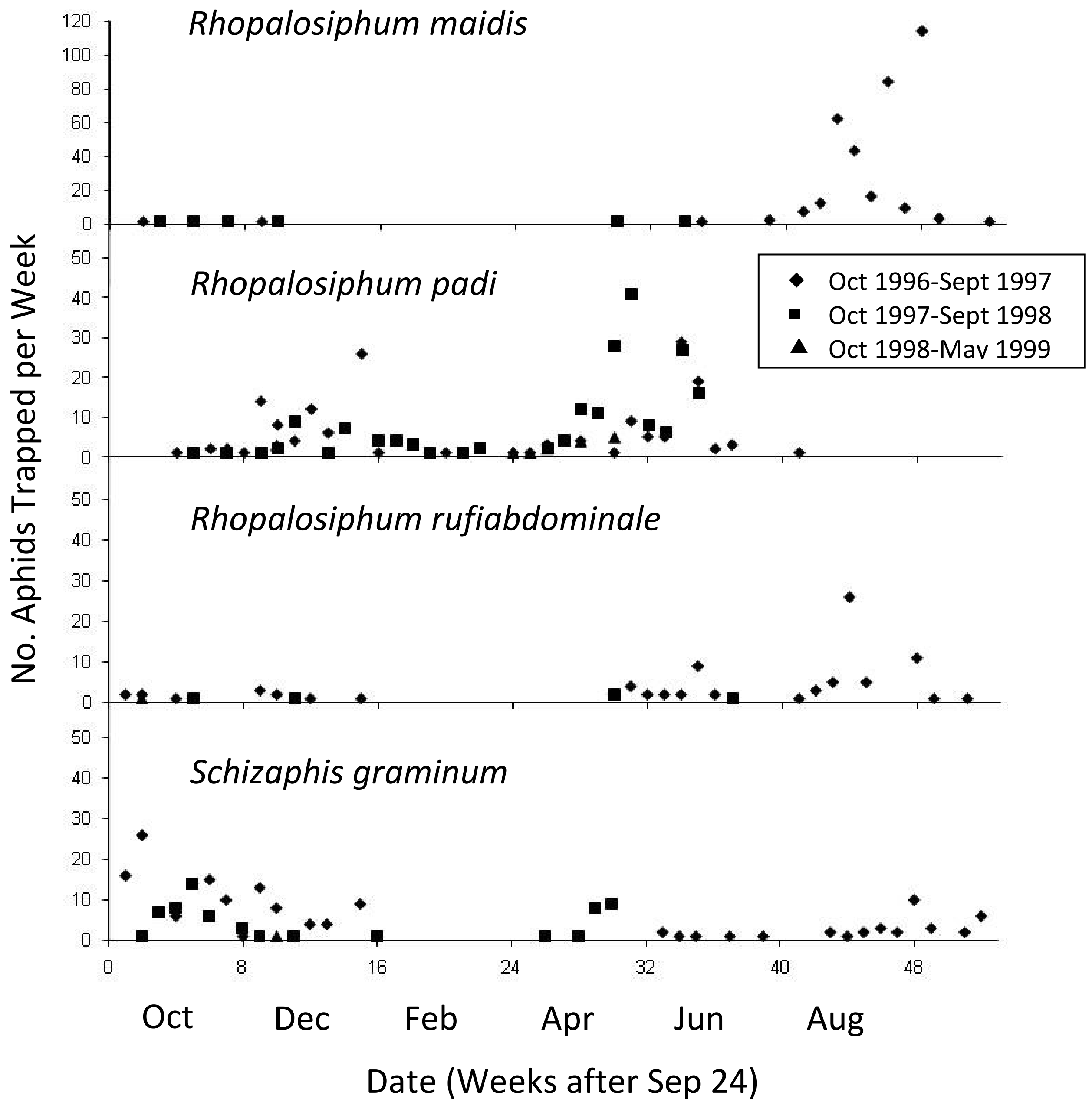

Figure 4.2 Seasonal abundance of winged aphids trapped in suction trap in Red Hill, Alabama, between 1996 and 1999.

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5 Perennial Pasture Grasses as Hosts of Barley Yellow Dwarf Virus and Cereal Yellow Dwarf Virus

5.1 Introduction

In 1951, Oswald and Houston reported the occurrence of a new virus on cereals that was readily transmissible by aphids. Yellowing, sometimes reddening of the infected leaves and plant stunting were typical symptoms of infected plants, resulting in the disease to be called 'yellow dwarf' (Oswald and Houston 1951). The causal agent was identified as *Barley yellow dwarf virus* (BYDV) of family *Luteoviridae* (Shepherd *et al.* 1976). Subsequent research showed that at least five different viral strains existed, each transmitted optimally by one or two aphid species (Hemiptera: Aphididae) (Rochow 1969, Johnson and Rochow 1972). Following a series of cytopathological investigations on oats infected by different BYDV strains, Gill and Chong (1979), proposed that these virus strains be categorized into two sub-groups. The first sub-group consisted of the BYDV strains transmitted optimally by English grain aphid, *Sitobion (Macrosiphum) avenae* (F.), known as BYDV-MAV, by greenbug, *Schizaphis graminum* Rond., known as BYDV-SGV, and by both *S. avenae* and bird cherry-oat aphid, *Rhopalosiphum padi* (L.), known as BYDV-PAV. The second sub-group consisted of the strains transmitted optimally by *R. padi*, known as BYDV-RPV and by corn leaf aphid, *Rhopalosiphum maidis* Fitch, known as BYDV-RMV. In the paper proposing this categorization, Gill and Chong (1979) further suggested that the two groups may belong to two different viruses.

The sequencing of each BYDV strain has led to reorganization of BYD viruses into two different virus species (Mayo and D'Arcy 1999) which corresponds to the distinction proposed by Gill and Chong (1979). The strain BYDV-PAV is classified as BYDV under the genus *Luteovirus* while strain BYDV-RPV is now recognized as a distinct virus, *Cereal yellow dwarf virus* (CYDV) under the genus *Polerovirus* (van Regenmortel *et al.* 2000). In this paper, the disease caused be these viruses will be referred to as yellow dwarf (YD) and the viruses referred to as B/CYDV.

Barley yellow dwarf virus and cereal yellow dwarf virus are known to exclusively infect plants from family Poaceae. The most comprehensive review of the B/CYDV host range was given by D'Arcy (1995), in which it was stated that the viruses can infect "… more than 150 species in 5 of 6 subfamilies of the Poaceae and in 11 of the 25 tribes." In places where the economically important hosts such as wheat, barley or rice are only planted in a specific period in a year, various lawn, weed, pasture and range grasses may act as alternative hosts in which the viruses and vectors survive, facilitating introduction of the viruses to new plantings of commercial hosts (D'Arcy 1995).

Since the publication of the B/CYDV host list by D'Arcy (1995), few new grass species have been reported to host the viruses (Table 5.1). Some of these plants are introduced weeds in the United States, while some are native grasses (USDA online publication, accessed September 2009). The finding of B/CYDV on these grasses illustrates the potential of plants in grasslands as alternative hosts of the viruses causing YD. Three of the newly listed host plants, common reed, weeping lovegrass and blue wildrye, were listed as non-hosts of B/CYDV in older reports. D'Arcy (1995) mentioned that discrepancies between reports may have stemmed from various factors, including

differences in virus strains, inoculum pressure, a change in virus titer in the host over time, and vector feeding behavior.

Malmstorm and Shu (2004) observed bahiagrass with YD-like symptoms in Florida, but were unable to detect B/CYDV using serological tests. Ann Blount of University of Florida and Joseph Anderson of Purdue University isolated BYDV-PAV from bahiagrass grown in Florida using multiplex RT-PCR (Blount personal communication). It was therefore recommended that bahiagrass samples be subjected to additional tests. The goal of the study reported here was confirm the presence of B/CYDV in bahiagrass, and to investigate if any additional summer pasture grasses harbor B/CYDV.

5.2 Methodology

Variety trial plots of pasture grasses at the North Florida Research and Education Center in Marianna, Florida, were surveyed in 2007 and 2008. The plots were surveyed for aphids once a month between June and August. Plant samples were collected once each year between June and August. Three species of pasture grasses were available for sampling, bahiagrass, *Paspalum notatum* Flugge, eastern gamagrass, *Tripsacum dactyloides* (L.), and limpograss, *Hemarthria altissima* (Poir.) Stapf & C.E. Hubbard, none of which were mentioned in D'Arcy's list of B/CYDV hosts (D'Arcy 1995). Eight cultivars of bahiagrass were sampled: Pensacola, Tifton 9, Rapid germination Tifton 9, PICA, AU Sand Mountain, Paraguay 22, Argentine, and Tifton 7. The first five of these cultivars are of diploid type, while the last three are of tetraploid type.

Ten leaves of each grass species that showed typical symptoms of B/CYDV infection, namely yellowing or reddening, were collected per plot. Plots without

symptomatic leaves were not sampled. Collected leaves were tested for virus presence and strain identification using triple antibody sandwich enzyme-linked immunosorbent assay (ELISA) (Agdia Inc., Elkhart, IN) according to the manufacturer's manual. In 2007, the samples were tested for the presence of BYDV-PAV and CYDV-RPV. In 2008, the samples were tested for BYDV-MAV, BYDV-PAV and CYDV-RPV. Known wheat based negative and positive controls obtained from Agdia Inc. were added to each microtiter plate. A sample was considered positive for the presence of virus if the ELISA absorbance value was greater than the three times the average of the healthy controls on a given plate (Sutula *et al.* 1986).

Aphids were sampled alive from the plants and assayed for B/CYDV infectivity. The infectivity assay was done in accordance with the methods of Chapin *et al.* (2001) and Halbert *et al.* (1992a). In the first few trips to the research station, the aphid survey was attempted by searching the plots for aphids. However, no aphid was found using this method. Live aphids were successfully collected by sweeping the plots using a sweep net. Each collected aphid was placed directly in a glass tube containing a 7-14 days old 'California Red' oat seedling on moist cotton or tissue paper. The tubes containing aphids and oat seedlings were incubated under room temperature to allow for an inoculation access period. After 48-72 hours of inoculation access period, all aphids were removed from the tubes, preserved in 80% ethanol and identified to species according to the identification key found in Blackman and Eastop (2000). The oat seedlings were then treated with lambda-cyhalothrin insecticide to kill all residual nymphs, then potted and incubated in a protected environment. By the end of two to three weeks of incubation period, two or three oldest leaves were harvested from each plant and the leaf tissue

composites were subjected to ELISA using commercial antibodies produced by Agdia Inc. (Elkhart, IN) according to the manufacturer's instruction.

Since few aphids were found on bahiagrass, two experiments were conducted to investigate the potential of bahiagrass as a source of B/CYDV infection to commercial small grains. An aphid colony was started from a single *R. padi* collected at Sand Mountain Research and Extension Center, AL that was found to be infective with BYDV-PAV. The aphid was reared on oat seedlings of 'California Red' cultivar in a growth chamber with temperature set on 15°C and 12/12 h light/dark cycle. The relatively low temperature was maintained to induce winged aphid production within the colony. At any given time, six potted oat plants were kept as a food source and virus reservoir for the colony. The plants were changed every four to five weeks by putting six new potted oat seedlings in the growth cabinet. Aphids moved naturally from the older host plants to the new ones. The status of virus infection of the old plants was checked by ELISA before the plants were discarded. After a few cycles of host plant succession in the first colony, an attempt to start a colony of non-viruliferous *R. padi* was conducted. A mature aphid was selected and transferred to a virus free oat leaf blade and kept in a sealed petri dish under room temperature. Every six hours, the petri dish was checked for nymphs. Upon the first sighting, the nymphs were transferred to new oat seedlings and kept in a separate growth chamber with temperature and light regime similar to the first colony. After four weeks, the leaves from this new colony were subjected to ELISA to test for the presence of B/CYDV. As no B/CYDV was found, the colony was maintained by providing new oat seedlings every four to five weeks in the same manner as the maintenance of the viruliferous colony. These two colonies of *R. padi*, one reared on

wheat infected with BYDV-PAV and the other reared on healthy wheat were used in the following experiments.

The first experiment was designed to test whether symptomatic bahiagrass harbored B/CYDV that could be transmitted by *R. padi* to oats. Symptomatic bahiagrass plants were dug from the variety plots and transplanted in pots. Six potted symptomatic bahiagrass plants were kept in a growth room with temperature set on 25°C and 12/12 h light/dark cycle. Ten winged *R. padi* reared on healthy oats were transferred to each potted symptomatic bahiagrass. A pot of healthy oat plants was used as a control, to which ten winged *R. padi* were also transferred. Each potted plant with aphids was kept in a nylon cage and the aphids were given a five day acquisition feeding period. Each aphid was then transferred to a 14-day-old oat seedling of 'California Red' cultivar that would have served as an indicator plant.

The second experiment was conducted to test whether BYDV from oats could be transmitted to healthy bahiagrass using *R. padi* as the vector. Bahiagrass seeds from cultivars Argentine and Pensacola were obtained from Dr. Ann Blount of the University of Florida. Twenty to thirty seeds were sown per pot and the pots were kept in a growth chamber with temperature set on 25°C and 12/12 h dark/light cycle. After six weeks, four pots of each bahiagrass variety that showed vigorous growth were selected. A pot of healthy 14-day-old oat seedlings was used as a control. Ten winged *R. padi* reared on oat plants infected with BYDV-PAV were transferred to each potted plant. Each pot with aphids was kept inside a nylon cage in a growth room with temperature set on 25°C and 12/12 h dark/light cycle. The aphids were given a three day inoculation feeding period. After three days, lambda-cyhalothrin insecticide was sprayed on the plants to kill the

aphids and the plants were kept in the growth room for another three to four weeks. After three or four weeks of incubation period, the leaves from each plant were harvested and subjected to ELISA to detect the presence of BYDV-PAV.

5.3 Results

Field collected leaves of all diploid bahiagrass cultivars and limpograss tested by ELISA were shown to be infected with BYDV-PAV and CYDV-RPV (Table 5.2). BYDV-MAV was detected among diploid bahiagrass samples in 2008. BYDV-PAV was detected but not CYDV-RPV from tetraploid bahiagrass samples. No BYDV or CYDV was detected from gamagrass samples. A total of 27 aphids were collected in 2007 but none were collected in 2008. Aphids were found on bahiagrass, but not found on limpograss and gamagrass. All aphids collected in 2007 on bahiagrass were yellow sugarcane aphid, *Sipha flava* Forbes. None of the aphids that were collected in the field were infective based on the infectivity assay. However, after three days of inoculation feeding period, all of the collected aphids were found dead on the oat seedlings. The cause of this high rate of mortality is not clear. The sweep net collection method may have accidentally injured the aphids.

In the first experiment, no *R. padi* was recovered alive from bahiagrass after the five day feeding period. The aphids on the control (healthy oat plants) were found alive. The experiment was repeated twice with the acquisition feeding period shortened to three days in the second repetition. Even after changing the acquisition feeding period to three days, no *R. padi* was found alive in bahiagrass. Apparently, *R. padi* is unable to survive on bahiagrass.

In the second experiment, as in the first one, no *R. padi* was found alive on bahiagrass seedlings after the five day feeding period. The aphids on the control plants were found alive after the inoculation feeding period. No BYDV-PAV was detected on the bahiagrass leaf samples but virus was detected in the control sample. The experiment was repeated twice with consistent results.

5.4 Discussion

Wheat, oat and barley are among the hosts of B/CYDV that are widely planted and that have high commercial value. Temporal breaks between the seasons of wheat, oat and barley drive the YD viruses and vectors to survive on alternative hosts (Hewings and Eastman 1995). Aphid vectors bring viruses into winter cereals in the fall from these alternative hosts. Irwin and Thresh (1990) pointed out that the inoculum source for fall infection can be local, regional or long distant. Because of the wide host range of B/CYDV, a wide array of plants, both cultivated and wild, may act as the source for infection to small grain. Not all alternative hosts are of equal importance as the sources of inoculum in the infection of small grains (Hewings and Eastman 1995).

In the Pacific Northwest, corn seemed to play a role as the inoculum source of B/CYDV fall infection to winter small grains (Halbert *et al.* 1989). The infectivity of *R. padi* collected on corn in mid-summer was shown to be a good predictor for B/CYDV autumn epidemic on winter wheat in southwestern Idaho. Brown *et al.* (1984) identified irrigated corn as a bridging host during the gap between summer harvest and fall planting of winter grains in eastern Washington. High disease incidence was associated with areas where wheat was planted adjacent to aphid infested corn (Wyatt *et al.* 1988). Even though corn may act as alternative host of both the viruses and vectors of YD, the

importance of corn as the inoculum source for fall infection to cereal seems to vary geographically. In South Carolina, corn was heavily infested by *R. maidis*, which was only rarely found in the wheat plants planted after corn (Chapin *et al.* 2001). In Alabama, wheat planting season for grain harvest is recommended to begin in mid-October in the northern and central parts of the state and early in November for the southern part of the state (Mask *et al.* 1997). Corn is recommended to be planted by early, mid and late March for South, Central and North Alabama (Mask and Mitchell 2009). By the time wheat planting season begins, corn has already been harvested from the field.

In Australia, the UK, Canada, California, Indiana and Virginia, B/CYDV infection and aphid infestation were common among annual and perennial grasses (Clement *et al.* 1986, Kendall *et al.* 1996, Hawkes and Jones 2005, Ilbagi 2006, Malmstrom *et al.* 2005, Masterman *et al.* 1994, Paliwal 1982, Remold 2002) implying a potential of wild grasses to act as B/CYDV inoculum sources for infection to commercial hosts.

Although wild grasses growing near and around cereal fields may harbor the virus, their contribution to B/CYDV epidemics in the adjacent crop is not always clear. Some surveys showed mismatches between strains found in a local population of wild grasses and those found in cereal crop. For example, an investigation in Indiana demonstrated that grasses surrounding studied fields were infected by CYDV-RPV while the wheat fields were dominantly infected by BYDV-PAV (Clement *et al.* 1986). In England, the virus strains found in cereals often differ in their geographic distribution from those of grasses (Plumb 1977). Paliwal (1982) did not verify the strain of the virus, but reported that the grasses collected from Ontario and Quebec mainly harbor YD virus

specifically transmitted by *R. padi*, while the winter wheat was predominantly infected by YD virus specifically transmitted by *S. avenae*.

Masterman *et al.* (1994) reported that aphids collected from weeds in hedge bottoms and field margins during summer in Scotland were infective with the same virus and virus strains found on winter barley the following season. However, no test was conducted on the weeds to confirm the virus presence.

Warm season pasture grasses may also act as alternative hosts of B/CYDV. A three year survey on winter wheat fields in UK showed that fields with surrounding grassy areas, including pastures and moorlands, showed higher numbers of alate and higher mean levels of B/CYDV incidence (Foster *et al.* 2004). Of the 13 plants listed in Table 5.1, weeping lovegrass is utilized as a forage crop (USDA-NRCS 2009). Bahiagrass and limpograss, the two grass species reported here to harbor BYDV, are also utilized as forage crops. Weeping lovegrass, bahiagrass and limpograss are warm-season perennial grasses. Both bahiagrass and limpograss are adapted to the climate in South Alabama, South Georgia and North Florida. Bahiagrass is widely grown as a pasture grass in Alabama, Georgia and Florida. Up to 1,000,000 acres of bahiagrass were planted in Alabama, and 500,000 acres in Georgia (Blount 2004). The large areas of bahiagrass and its capability to serve as B/CYDV host may render the grass important as an inoculum source for fall infection of B/CYDV to winter cereals.

As in the case of corn, the true importance of a particular warm-season pasture grass as a B/CYDV inoculum source for fall infection to commercial cereals is not clear. In Virginia after detecting BYDV-PAV on maturing cultivated winter wheat in April 1999, Sforza *et al.* (2001) reported the presence of BYDV-PAV in tall fescue and

BYDV-MAV on orchardgrass in summer 1999. In the next growing season, a shift in YD virus happened, CYDV-RPV was the predominant YD virus detected in cultivated wheat of the same study site. In January 2000, CYDV-RPV was the only YD virus found on tall fescue and orchardgrass surrounding the field. The observations from Virginia showed that virus movement between wheat and pasture grasses may happen although the direction of the movement may not be reciprocal.

Sipha flava, the only aphid recorded between 2007 and 2008 on bahiagrass, is oval in appearance, yellow colored with long bristle-like hairs on its back (Blackman and Eastop 2000). *Sipha flava* is listed as one of the two major aphid pests of pasture grasses in Florida (Sprenkel 2007). *Schizaphis graminum*, the other aphid pest of pasture grasses, was not found in the two years sampling on the three warm-season grasses reported here. Kindler and Dalrymple (1999) reported that *S. flava* can survive on bahiagrass. These reports supported our observation of *S. flava* on bahiagrass on one of the two sampling years. *Sipha flava* is known to colonize wheat and other grass hosts (Blackman and Eastop 2000). The survey of wheat aphids in Alabama and western Florida showed that *S. flava* is usually found on winter wheat early in the season. A list of B/CYDV vectors compiled by Halbert and Voegtlin (1995) did not include *S. flava*. We found no *S. flava* to be infective with B/CYDV among the individuals collected on bahiagrass. No known vector species was found on bahiagrass or limpograss in the two sampling years, even though B/CYDV was found on both species. The aphid responsible for the introduction of B/CYDV into the two grasses is not known. The two experiments with bahiagrass showed that winged *R. padi* cannot survive on bahiagrass. *Rhopalosiphum padi* is a known aphid vector of B/CYDV and it has been associated with BYDV infection to

wheat (Chapin *et al.* 2001). If bahiagrass played a crucial role in YD epidemiology as a source of B/CYDV, aphid(s) other than *R. padi* must have transmitted the virus from bahiagrass to wheat.

Eastern gamagrass has been reported to host *Sugarcane mosaic virus strain corn dwarf mosaic virus B* (SCMV-MDMV-B) and *Corn dwarf mosaic virus* (MDMV), potyviruses from family *Potyviridae* (Piper *et al.* 1996).

5.5 Conclusion

Two years of testing of bahiagrass, limpograss and gamagrass populations from the North Florida Research and Education Center, Marianna, FL., showed that bahiagrass and limpograss can act as alternative hosts of BYDV-PAV and CYDV-RPV. Additionally, bahiagrass was also shown to harbor BYDV-MAV. Very few aphids were collected and no known B/CYDV vector was found on these grasses in the two years of sampling. *Sipha flava*, an aphid not listed as B/CYDV vector, was the only species collected on bahiagrass. Further research needed to determine the aphid vectors responsible for the introduction of the viruses into bahiagrass and limpograss. More research is needed to determine if bahiagrass and limpograss are serving as sources of B/CYDV for fall infection to wheat in the southeast.

Table 5.1 Hosts of barley yellow dwarf viruses reported since D'Arcy (1995)

Tribe and species	Common name	Reference
Arundineae		
Phragmites communis Trin. [a]	Common reed	Ilbag 2006
Avenae		
Koeleria macrantha (Ledeb) J.A. Schultes	Prairie Junegrass	Malmstorm *et al.* 2005
Cynodonteae		
Chloris truncata R. Br.	Australian fingergrass	Hawkes and Jones 2005
Chloris virgata Sw.	Feather fingergrass	Hawkes and Jones 2005
Eragrostideae		
Eragrostis curvula (Schrad.) Nees[b]	Weeping lovegrass	Hawkes and Jones 2005
Oryzeae		
Erharta calycina Sm.	Perennial veldt grass	Hawkes and Jones 2005
Paniceae		
Pennisetum clandestinum Hochst. ex Chiov.	Kikuyu grass	Hawkes and Jones 2005
Setaria viridis (L.) P. Beauv.[c]	Green bristlegrass	Remold 2002
Setaria pumila (Poir.) Roem & Schult. ssp. pumila	Yellow foxtail	Remold 2002
Urochloa panicoides P. Beauv.	Panic liverseed grass	Hawkes and Jones 2005
Stipeae		
Nassella pulchra (Hitchc.) Barkworth	Purple needlegrass	Malmstorm *et al.* 2005
Triticeae		
Elymus glaucus Buckley [c]	Blue wildrye	Malmstorm *et al.* 2005
Elymus elymoides (Raf.) Swezey	Squirreltail	Malmstorm *et al.* 2005
Elymus multisetus M.E. Jones	Big squirreltail	Malmstorm *et al.* 2005

[a] Reported in Guy *et al.* (1987) as a non-host of BYDV.
[b] Reported in Griesbach *et al.* (1990) as a non-host of BYDV.
[c] Reported in Oswald and Houston (1953) and Bruehl and Toko (1957) as non-hosts of BYDV.

Table 5.2 *Barley yellow dwarf* and *Cereal yellow dwarf viruses* detected using ELISA from pasture grass leaf samples collected in North Florida Research and Education Center, Marianna, FL

Grass species	Number of infected samples / total samples				
	2007		2008		
	BYDV-PAV	CYDV-RPV	BYDV-PAV	CYDV-RPV	BYDV-MAV
Bahiagrass					
Diploid cultivars	20/25	1/25	11/25	4/15	2/15
Tetraploid cultivars	10/15	0/15	2/15	0/15	0/15
Limpograss	10/53	6/53	0/8	0/8	0/8
Gamagrass	0/24	0/24	0/3	0/3	0/3

References

Ajayi, O., and Dewar. A.M. 1983. The effect of barley yellow dwarf virus on field populations of the cereal aphids, *Sitobion avenae* and *Metopolophium dirhodum*. Ann. Appl. Biol. 103:1-11.

AlIison, D. A. and Pike, K. S. 1988. An inexpensive suction trap and its use in an aphid monitoring network. J. Agric. Entomol. 5:103-107.

Andersen, A. 2003. Long-term experiments with reduced tillage in spring cereals: II. Effects on pests and beneficial insects. Crop Prot. 22:147-152.

Anderson, J.M., Bucholtz, D., Greene, A.E., Francki, M.G., Gray, S.M., Sharma, H., Ohm, H.W., and Perry, K.L. 1998. Charaterization of wheatgrass-derived barley yellow dwarf virus resistance in a wheat alien chromosome substitution line. Phytopathology 88:851-855.

Araya, J.E., and Foster, J.E. 1987. Laboratory study on the effects of barley yellow dwarf virus on the life cycle of *Rhopalosiphum padi* (L.). J. Plant Dis. Prot. 94:578-583

Ayala, L., Henry, M., Ginkel, M., Singh, R., Keller, B., and Khairallah, M. 2002. Identification of QTLs for BYDV tolerance in bread wheat. Euphytica 128:249-259.

Bailey S.M., Irwin, M.E., Kampmeier, G.E., Eastman, C.E., and Hewings, A.D. 1995. Physical and biological perturbations: their effect on the movement of apterous *Rhopalosiphum padi* (Homoptera: Aphididae) and localized spread of barley yellow dwarf virus. Environ. Entomol. 24:24-33.

Bale, J. S. 1996. Insect cold hardiness: a matter of life and death. Eur. J. Entomol. 93:369-382.

Bale, J.S., Ponder, K.L., and Pritchard, J. 2006. Coping with stress. Pages 287-311 in: Aphids as Crop Pests. H.F. van Emden and R. Harrington, eds. CAB International, Wellingford.

Baltenberger, D. E., Ohm, H. W., and Foster, J. E. 1987. Reactions of oat, barley, and wheat to infection with barley yellow dwarf virus isolates. Crop Sci. 27:195-198.

Bencharki, B., Mutterer, J., El Yamani, M., Ziegler-Graff, V., Zaoui, D., and Jonard, G. 1999. Severity of infection of Moroccan barley yellow dwarf virus PAV isolates correlates with variability in their coat protein sequences. Ann. Appl. Biol. 134:89-99.

Biswal, B. and Biswal, B. 1999. Senescence-associated genes of leaves. J. Plant Biol. 26:43-50.

Blackman, R.L. and Eastop, V.F. 2000. Aphids on the World's Crops: An Identification and Information Guide, 2nd ed. John Wiley and Sons, Ltd., Chichester.

Blackman, R.L. and Eastop, V.F. 2006. Aphids on the World's Herbaceous Plants and Shrubs. Volume 2. John Wiley and Sons, Ltd., Chichester.

Blount, A.R. 2004. Forage Production in the Southern Coastal Plain Region. Published online.

Bowen, K.L., and Burch, K. 2001. Influence of weather on barley yellow dwarf severity and yields of wheat in Alabama. Phytopathology 91:S10 (abstract).

Bowen, K.L., Flanders, K.L., Mask, P., and Murphy, J.F. 2002. Risk index for barley yellow dwarf virus and cereal yellow dwarf virus of wheat in Alabama. Pages 106-107 in: Barley Yellow Dwarf Disease: Recent Advances and Future Strategies. M. Henry and A. McNab, eds. CIMMYT, Mexico.

Bowen, K.L., Murphy, J.F. Flanders, K.L., Mask, P.L., and Li, R. 2003. Incidence of virus infecting winter wheat in Alabama. Plant Dis. 87:288-293.

Brown, J.K., Wyatt, S.D., and Hazelwood, D. 1984. Irrigated corn as a source of barley yellow dwarf virus and vectors in eastern Washington. Phytopathology 74:46-49.

Burgess, A.J., Harrington, R., and Plumb, R.T. 1999. Barley and cereal yellow dwarf virus epidemiology and control strategies. Pages 211-279 in: The Luteoviridae. H.G. Smith and H. Barker, eds. CAB International, Wellingford.

Burnett, P.A. 1987. Preface. Page ix in: World Perspectives on Barley Yellow Dwarf. P.A. Burnett, ed. CIMMYT, Mexico.

Burnett, P.A., Comeau, A., and Qualset C.O. 1995. Host plant tolerance or resistance for control of barley yellow dwarf. Pages 321-343 in: Barley yellow dwarf: 40 Years of Progress. C.J. D'Arcy and P.A. Burnett, eds. APS Press, St. Paul.

Chapin, J.W., Thomas, J.S., Gray, S.M., Smith, D.M., and Halbert, S.E. 2001. Seasonal abundance of aphids (Homoptera: Aphididae) in wheat and their role as barley yellow dwarf virus vectors in the South Carolina coastal plain. J. Econ. Entomol. 94:410-421.

Cisar, G., Brown, C.M., and Jedlinski, H. 1982. Effect of fall or spring infection and sources of tolerance of barley yellow dwarf of winter wheat. Crop Sci. 22:474-478.

Clement, D.L., Lister, R.M., and Foster, J.E. 1986. ELISA-based studies in the ecology and epidemiology of barley yellow dwarf virus in Indiana. Phytopathology 76:86-92.

Collins, N.C., Paltridge, N.G., Ford, C.M., and Symons, R.H. 1996. The *Yd2* gene for barley yellow dwarf virus resistance maps close to the centromere on the long arm of barley chromosome 3. Theor. Appl. Genet. 92:858-864.

Cooper, J.I., and Jones, A.T. 1983. Responses of plants to viruses: proposals for use of terms. Phytopathology 73:127-128.

Culver, J.N. and Padmanabhan, M.S. 2007. Virus-induced disease: altering host physiology one interaction at a time. Ann. Rev. Phythopathol. 45:221-243.

D'Arcy, C.J. 1995. Symptomatology and host range of barley yellow dwarf. Pages 9-28 in: Barley yellow dwarf: Forty years of progress. C.J. D'Arcy and P.A. Burnett, eds. APS Press, St. Paul.

D'Arcy, C. J., Domier, L. L. and Mayo, M. A. 2000. Family Luteoviridae. Pages 775-784 in: Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses. M.H. van Regenmortel, C.M. Fauquet, D.H.L. Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, J. Maniloff, M.A. Mayo, D.J. McGeoch, C.R. Pringle, and R.B. Wickner, eds. Academic Press, San Diego.

Dean, G. J. and Luuring, B. B. 1970. Distribution of aphids in cereal crops. Ann. Appl. Biol. 66:485-496.

DeBarro, P. J. 1992. The role of temperature, photoperiod, crowding and plant quality on the production of alate viviparous females of the bird cherry-oat aphid, *Rhopalosiphum padi* L. Entomol. Exp. Appl. 65:205-214.

Dedryver C.A. and Harrington R. 2004. BYD epidemiology and forecasting. Pages 155-170 in: Viruses and Virus Diseases of Poaceae. H. Lapierre and P. Signoret, eds. INRA, Paris.

Dixon, A.F.G. 1998. Aphid Ecology, 2nd ed. Chapman and Hall, London.

Dixon A.F.G. and Glen, D.M. 1971. Morph determination in the bird cherry-oat aphid, *Rhopalosiphum padi* L. Ann. Appl. Biol. 68:11-21.

Esau, K. 1957a. Phloem degeneration in Gramineae affected by the barley yellow dwarf virus. Am. J. Bot. 44:245-251.

Esau, K. 1957b. Anatomical effects of barley yellow dwarf virus and maleic hydrazide on certain Poaceae. Hilgardia 27:15-69.

Fabre, F., Dedryver, C.A., Leterrier, J.L., and Plantegenest, M., 2003. Aphid abundance on cereals in autumn predicts yield losses caused by BYDV. Phytopathology 93:1217-1222.

Fabre, F., Pierre, J.S., Dedryver, C.A. and Plantegenest, M. 2006. Barley yellow dwarf disease risk assessment based on Bayesian modeling of aphid population dynamics. Ecol. Model. 193:457-466.

Farrell, J.A. and Stufkens, M.W. 1992. Cereal aphid flights and barley yellow dwarf virus infection of cereals in Canterbury, New Zealand. N.Z. J. Crop Hort. Sci. 20:407-412.

Favret, C. and Voegtlin, D.J. 2001. Migratory aphid (Hemiptera: Aphididae) habitat selection in agricultural and adjacent natural habitats. Environ. Entomol. 30:371-379.

Fereres, A., Lister, R.M., Araya, J.E., and Foster, J.E. 1989. Development and reproduction of the English grain aphid (Homoptera:Aphididae) on wheat cultivars infected with barley yellow dwarf virus. Environ. Entomol. 18:388-393.

Fereres, A., Shukle, R.H., Araya, J.E., and Foster, J.E. 1990. Probing and feeding behavior of *Sitobion avenae* (F.) on three wheat cultivars infected with barley yellow dwarf virus. Environ. Entomol. 18:388-393.

Fiebig, M., Poehling, H.-M., and Borgemeister, C. 2004. Barley yellow dwarf virus, wheat, and *Sitobion avenae*: A case of trilateral interactions. Entomol. Exp. Appl. 110:11-21.

Fitzgerald, P.J., and Stoner, W.N. 1967. Barley yellow dwarf studies in wheat (*Triticum aestivum* L.). I. Yield and quality of hard red winter wheat infected with barley yellow dwarf virus. Crop Sci. 7:337-341.

Flanders, K.L., Herbert, A., Buntin, D., Johnson, D., Bowen, K., Murphy, J.F., Chapin, J., and Hagan, A. 2006. Barley yellow dwarf in small grains in the southeast. Circular ANR-1082. Ala. Coop. Ext. Syst. Auburn, USA.

Forster, R.L., Bishop, G.W., and Sandvol, L.E. 1990. The 1985 barley yellow dwarf epidemic in winter wheat involving barley yellow dwarf virus transmitted by *Schizaphis graminum* and wheat streak mosaic. Pages 266-268 in: World Perspectives on Barley Yellow Dwarf. Burnett, P.A., ed. CIMMYT, Mexico.

Foster, G.N., Blake, S., Tones, S.J., Barker, I., and Harrington, R. 2004. Occurrence of barley yellow dwarf virus in autumn-sown cereal crops in the United Kingdom in relation to field characteristics. Pest Manag. Sci. 60:113-125.

Foster, G.N., Holmes, S.J., and Bone, S.F. 1993. Ten years' experience of infectivity indexing as a method of predicting the risk of barley yellow dwarf virus outbreaks in autumn-sown cereals in the west of Scotland. Pages 97-102 in: Crop Protection in Northern Britain. Williams, G.H., ed. Scottish Crop Research Institute, Dundee, UK.

Francki, M.G., Ohm, H. W., and Anderson, J.M. 2001. Novel germsplasm providing resistance to barley yellow dwarf virus in wheat. Aust. J. Agr. Res. 52:1375-1382.

George, K. S. and Gair, R. 1979. Crop loss assessment on winter wheat attacked by the grain aphid, *Sitobion avenae* (F.), 1974–77. Plant Pathol. 28:143–149.

Gourmet, C., Hewings, A. D., Kolb, F. L., and Smyth, C. A. 1994. Effect of imidacloprid on nonflight movement of *Rhopalosiphum padi* and the subsequent spread of barley yellow dwarf virus. Plant Dis. 78:1098-1101.

Gourmet, C., Kolb, F. L., Smyth, C. A., and Pedersen, W. L. 1996. Use of imidacloprid as a seed-treatment insecticide to control barley yellow dwarf virus (BYDV) in oat and wheat. Plant Dis. 80:136-141.

Gildow, F.E. 1980. Increased production of alatae by aphids reared on oats infected with barley yellow dwarf virus. Ann. Entomol. Soc. Am. 73:343-347.

Gildow, F.E., Ballinger, M.E., and Rochow, W.F. 1983. Identification of double-stranded RNAs associated with barley yellow dwarf virus infection of oats. Phytopathology 73:1570-1572.

Gildow, F.E. 1990. Current status of barley yellow dwarf in the United States: A regional situation report. Pages 266-268 in: World Perspectives on Barley Yellow Dwarf. Burnett, P.A., ed. CIMMYT, Mexico.

Gill, C.C. and Chong, K. 1975. Development of the infection in oat leaves inoculated with barley yellow dwarf virus. Virology 66:440-453.

Gill, C.C. and Chong, K. 1979. Cytopathological evidence for the division of barley yellow dwarf virus isolates into two subgroups. Virology 95:59-69.

Gray, S. M., Bergstrom, G. C., Vaughan, R., Smith, D. M., and Kalb, D. W. 1996. Insecticidal control of cereal aphids and its impact on the epidemiology of the barley yellow dwarf luteoviruses. Crop Prot. 15:687-697.

Gray, S.M., Chapin., J.W., Smith, D.M., Banerjee, N., and Thomas, J.S. 1998. Barley yellow dwarf luteoviruses and their predominant aphid vectors in winter wheat grown in South Carolina. Plant Dis. 82:1328-1333.

Gray, S.M., Power, A.G., Smith, D.M., Seaman, A.J., and Altman, N.S. 1991. Aphid transmission of barley yellow dwarf: virus acquisition access period and virus concentration requirements. Phytopathology 81:539-545.

Guy, P.L. 1988. Pasture ecology of barley yellow dwarf viruses at Sandford, Tasmania. Plant Pathol. 37:546-550.

Harrington, R., Mann, J.A., Plumb, R.T., Smith, A.J., Taylor, M.S., Foster, G.N., Holmes, S.J., Masterman, A.J., Tones, S.J., Knight, J.D., Oakley, J.N., Barker, I., and Walters, K.F.A. 1994. Monitoring and forecasting for BYDV control — the way forward? Asp. Appl. Biol. 37:197-206.

Halbert, S.E., Bishop, G.W., Blackmer, J., Connelly, J., Johnston. R., Sandovol, L., and Pike. K.S. 1989. Barley yellow dwarf infectivity of *Rhopalosipum padi* in corn as an estimate of primary inoculum pressure in irrigated winter wheat. Pages 273-274 in: Barley Yellow Dwarf Virus, the Yellow Plague of Cereals. Burnett, P. A., ed. CIMMYT, Mexico.

Halbert, S.E., Connelly, B.J., Bishop, G.W., and Blackmer, J.L. 1992a. Transmission of barley yellow dwarf virus by field collected aphids (Homoptera: Aphididae) and their relative importance in barley yellow dwarf epidemiology in southwestern Idaho. Ann. Appl. Biol. 121:105-121.

Halbert, S.E., Connelly, B.J., Lister R.M., Klein R.E., and Bishop G.W. 1992b. Vector specificity of barley yellow dwarf virus serotypes and variants in southwestern Idaho. Ann. Appl. Biol. 121:123-132.

Halbert, S. E., and Pike, K. S. 1985. Spread of barley yellow dwarf virus and relative importance of local aphid vectors in central Washington. Ann. Appl. Biol. 107:387-395.

Halbert, S.E., and Voegtlin, D. 1995. Biology and taxonomy of vectors of barley yellow dwarf viruses. Pages 217-258 in: Barley Yellow Dwarf: 40 Years of Progress. D'Arcy, C.J., and P.A. Burnett, ed. APS Press, St. Paul.

Hawkes, J.R., and Jones, R.A.C. 2005. Incidence and distribution of BYDV and CYDV in oversummering grasses in Mediterranean-type environment. Aust. J. Agr. Res. 56:257-270.

Herbert, D.A., Jr., Stromberg, E.L., Chappell, G.F., and Malone, S.M. 1999. Reduction of yield componenets by barley yellow dwarf infection in susceptible winter wheat and winter barley in Virginia. J. Prod. Agr. 12:105-109.

Herbers, K., Tacke, E., Hazirezaei, M., Krause, P, Melzer, Rohde, W., and Sonnewald, U. 1997. Expression of a luteoviral movement protein in transgenic plants leads to carbohydrate accumulation and reduced photosynthetic capacity in source leaves. Plant J. 12:1045-1056.

Hesler, L.S., and Berg, R.K. 2003. Tillage impacts cereal aphid (Homoptera: Aphididae) infestations in spring small grains. J. Econ. Entomol. 96:1792-1797.

Hesler, L.S., Riedell, W.E., and Langham, M.A.C., Osborne, S.L. 2005. Insect infestation, incidence of viral plant diseases, and yield of winter wheat in relation to planting date in the northern Great Plains. J. Econ. Entomol. 98:2020-2027.

Hewings, A.D., and Eastman, C.E. 1995. Epidemiology of Barley Yellow Dwarf in North America. Pages 75-106 in: Barley Yellow Dwarf: 40 Years of Progress. D'Arcy, C.J. and P.A. Burnett, eds. APS Press, St. Paul.

Hoffman, T.K. and Kolb, F.L. 1997. Effects of barley yellow dwarf virus on root and shoot growth of winter wheat seedlings grown in aeroponic culture. Plant Dis. 81:497-500.

Hull, R. 2002. Matthew's Plant Virology 4th ed. Academic Press, San Diego.

Hutchinson, L.A., and Bale, J.S. 1994. Effects of sub-lethal cold stress on the aphid *Rhopalosiphum padi*. J. Appl. Ecol. 31:102-108.

Ilbagi, H. 2006. Common reed (*Phragmites communis*) is a natural host of important cereal viruses in the Trakya region in Turkey. Phytoparasitica 34:441-448.

Irwin, D,. and Thresh, J.M. 1990. Epidemiology of barley yellow dwarf: a study in ecological complexity. Ann. Rev. Phytopathol. 28:393-424.

Irwin, M. E., and J. M. Thresh. 1988. Long-range dispersal of cereal aphids as virus vectors in North America. Phil. Trans. R. Soc. B 321:421-446.

Jedlinski, H. 1981. Rice root aphid, *Rhopalosiphum rufiabdominale*, a vector of barley yellow dwarf virus in Illinois and the disease complex. Plant Dis. 65: 975-978.

Jensen, S.G. 1968. Photosynthesis, respiration and other physiological relationships in barley infected with barley yellow dwarf virus. Phytopathology 58:204-208.

Jensen, S.G. 1972. Metabolism and carbohydrate composition in barley yellow dwarf virus infected wheat. Phytopathology 62:587-592.

Jensen, S.G. 1973. Systemic movement of barley yellow dwarf virus in small grains. Phytopathology 63:854-856.

Jensen, S.G., and D'Arcy, C.J. 1995. Effects of barley yellow dwarf on host plants. Pages 55-74 in: Barley Yellow Dwarf: 40 Years of Progress. D'Arcy, C.J., and P.A. Burnett, eds. APS Press, St. Paul.

Jensen, S.G., and Van Sambeek, J.W. 1972. Differential effects of barley yellow dwarf virus on the physiology tissues of hard red spring wheat. Phytopathology 62:290-293.

Jimenez-Martinez, E.S., and Bosque-Perez, N.A. 2004. Variation in barley yellow dwarf virus transmission efficiency by *Rhopalosiphum padi* (Homoptera: Aphididae) after acquisition from transgenic and nontransformed wheat genotypes. J. Econ. Entomol. 97:1790-1796.

Jimenez-Martinez, E.S., Bosque-Perez, N.A., Berger, P.H., Zementra, R.S., Ding, H., and Eigenborde, S.D. 2004. Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera:Aphididae) to barley yellow dwarf virus-infected transgenic and untransformed wheat. Environ. Entomol. 33:1207-1216.

Johnson, D.W., and Hershman, D.E. 1996. A survey of cereal aphids in Kentucky wheat fields: Common species and distribution. Trans. KY Acad. Sci. 57:15-17.

Johnson, R.A., and Rochow, W.F. 1972. An isolate of barley yellow dwarf virus transmitted specifically by *Schizaphis graminum*. Phytopathology 62:921-925.

Kendall, D.A., Brain, P., and Chinn, N.E. 1992. A simulation model of the epidemiology of barley yellow dwarf virus in winter sown cereals and its application to forecasting. J. Appl. Ecol. 29:414-426.

Kendall, D.A., and Chinn, N.E. 1990. A comparison of vector population indices for forecasting barley yellow dwarf virus in autumn sown cereal crops. Ann. Appl. Biol. 116:87-102.

Kendall, D.A., George, S., and Smith, B.D. 1996. Occurrence of BYDV in some common grasses in southwestern England. Plant Pathol. 45:29-37.

Kendall, D.A., and Smith, B.D. 1981. The significance of aphid monitoring in improving barley yellow dwarf virus control. Pages 399-403 in: Proceedings 1981 British Crop Prot. Conference: Pests and Diseases. Brighton, U.K.

Kieckhefer, R.W. 1984. Cereal aphid (Homoptera: Aphididae) preferences for and reproduction on some warm-season grasses. Environ. Entomol. 13:888-891.

Kieckhefer, R.W., Gellner, J.L. 1988. Influence of plant growth stage on cereal aphid reproduction. Crop Sci. 28:688-690.

Kieckhefer, R.W., Stoner, W.N., and Thysell, J.R. 1976. Preferences of flight-active barley yellow dwarf virus vectors for healthy or diseased leaves. Plant Dis. Rep. 60: 939-941.

Kindler, S.D. and Dalrymple, R.L. 1999. Relative susceptibility of cereals and pasture grasses to the yellow sugarcane aphid (Homoptera:Aphididae). J. Agric. Urban Entomol. 16:113-122.

Knaust, H.J., and Poehling H.-M. 1992. Effect of imidacloprid on cereal aphids and their efficiency as vectors of BYD virus. Pflanzenschutz Nachr. Bayer 45:381-408.

Knight, J.D., and Thackray, D.J., 2007. Decision support systems. Pages 677-688 in: Aphids as Crop Pests. van-Emden, H.F., and R. Harrington, eds. CAB International, Wellingford.

Kolb, F.L., Cooper, N.K., Hewings, A.D., Bauske, E.M., and Teyker, R.H. 1991. Effects of barley yellow dwarf virus on root growth in spring oats. Plant Dis. 75:143-145.

Koev, G., Mohan, B.R., Dinesh-Kumar, S.P., Torbert, K.A., Somers, D.A., and Miller, W.A. 1998. Extreme reduction of disease in oats transformed with the 5' half of the barley yellow dwarf virus-PAV genome. Phytopathology 88:1013-1019.

Krebs, C.J. 1999. Ecological Methodology, 2nd ed. Addison-Wesley, New York.

Kring, J.B. 1972. Flight behavior of aphids. Ann. Rev. Entomol. 17:461-492.

Larkin, P.J., Banks, P.M., and Chen, K. 1995a. Registration of six genetic stocks of wheat with rust and BYDV resistance: Z1, Z2, Z3, Z4, Z5, and Z6 disomic addition lines with *Thinopyrum intermedium* chromosomes. Crop Sci. 35:604.

Larkin, P.J., Banks, P.M., Lagudah, E.S., Appels, R., Xiao, C., Xin, Z.Y., Ohm, H.W., and McIntosh, R.A. 1995b. Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistances. Genome 38:385-394.

Legrand, M.A., Colinet, H., Vernon, P., and Hance, T. 2004. Autumn, winter and spring dynamics of aphid *Sitobion avenae* and parasitoid *Aphidius rhopalosiphi* interactions. Ann. Appl. Biol. 145:139-144.

Livingston III, D.P., Gildow, F.E., and Liu, S.Y. 1998. Barley yellow dwarf virus: Effects on carbohydrate metabolism in oat (*Avena sativa*) during cold hardening. New Phytol. 140:699-707.

Lister, R.M., and Ranieri, R. 1995. Distribution and economic importance of barley yellow dwarf. Pages 29-54 in: Barley Yellow Dwarf: 40 Years of Progress. D'Arcy, C.J. and P.A. Burnett, eds. APS Press, St. Paul.

Lowles, A.J., Tatchell, G.M., Harrington, R., and Clark, S.J. 1996. The effect of temperature and inoculation access period on the transmission of barley yellow dwarf virus by *Rhopalosiphum padi* and *Sitobion avenae*. Ann. Appl. Biol. 128:45-53.

Lowles, A.J., Harrington, R., Mann, J., and Banwo, O.O. 1999. Beyond the infectivity index of barley yellow dwarf virus. Barley Newsletter 43. American Malting Barley Association.

Makkouk, K.M., Comeau, A., and St. Pierre, C.A. 1994. Screening for barley yellow dwarf luteovirus resistance in barley on the basis of virus movement. J. Phytopathol. 141:165-172.

Mastari, J., and Lapierre, H. 1999. Sequences of the 3' halves of the genomes of barley yellow dwarf virus-PA cpA isolates that vary in symptom severity. Eur. J. Plant Pathol. 105:801-811.

Masterman, A.J., Holmes, S.J., and Foster, G.N., 1994. Transmission of barley yellow dwarf virus by cereal aphids collected from different habitats on cereal farms. Plant Pathol. 43:605-13.

Malmstorm, C.M., Hughes, C.C., Newton, L.A., and Stoner, C.J. 2005. Virus infection in remnant native bunchgrasses from invaded California grasslands. New Phytol. 168:217-230.

Malmstorm, C.M., and Shu, R. 2004. Multiplexed RT-PCR for streamlined detection and separation of barley and cereal yellow dwarf viruses. J. Virol. Methods 120:69-78.

Mask, P.L., Flanders, K.L., van Riessen, H.W., and Gazaway, W.S. 1997. Alabama Wheat Production Guide. Circular ANR-992. Ala. Coop. Ext. Serv., Auburn, Alabama.

Mask, P., and Mitchell, C.C. Jr. 2009. Alabama production guide for non-irrigated corn. Ala. Coop. Ext. Serv. Published online.

Mayo, M.A. 2002. ICTV at the Paris ICV: results of the plenary session and the binomial ballot. Arch. Virol. 147:2254-2260.

Mayo, M.A. and C.J. D'Arcy. 1999. Family Luteoviridae: a reclassification of luteoviruses. Pages 15-22 in: The Luteoviridae. Smith, H.G. and H. Barker, eds. CABI Publishing, New York, USA. pp 15-22.

McGrath, P.F., and Bale, J.S. 1989. Cereal aphids and infectivity index for barley yellow dwarf virus in northern England. Ann. App. Biol. 114:429-442.

McGrath, P.F., and Bale, J.S. 1990. The effects of sowing date and choice of insecticide on cereal aphids and barley yellow dwarf virus epidemiology in northern England. Ann. App. Biol. 117:31-43.

McGrath, P.F., Vincent, J.R., Lei, C.H., Pawlowski, W.P., Torbert, K.A., Gu, W., Kaeppler, H.F., Wa, Y., Lemaux, P.G., Rines, H.R., Somers, D.A., Larkins, B.A., and Lister, R.M. 1997. Coat protein mediated resistance to isolates of barley yellow dwarf in oats and barley. Eur. J. Plant Pathol. 103:695-710.

McKirdy, S. J., and Jones, R. A. C. 1996. Use of imidacloprid and newer generation synthetic pyrethroids to control the spread of barley yellow dwarf luteovirus in cereals. Plant Dis. 80:895-901.

McKirdy, S. J., Jones, R. A. C., and Nutter, F. W. 2002. Quantification of yield losses caused by barley yellow dwarf virus in wheat and oats. Plant Dis. 86:769-773.

McPherson R. M., and Brann D. E. 1983. Seasonal abundance and species composition of aphids (Homoptera: Aphididae) in Virginia small grains. J. Econ. Entomol. 76:272–274.

Montllor, C.B., and Gildow, F.E. 1986. Feeding responses of two grain aphids to barley yellow dwarf virus-infected oats. Entomol. Exp. Appl. 42:63-69.

Mowry, T.M. 1990. Russian wheat aphid (Homoptera: Aphididae) survival and fecundity on barley yellow dwarf virus-infected wheat resistant and susceptible to the aphid. Environ. Entomol. 23:326-330.

Nass, P.H., Domier, L.L., Jakstys, B.P., and D'Arcy, C.J. 1998. In situ localization of barley yellow dwarf virus-PAV 17-kDa protein and nucleic acids in oats. Phytopathology 88:1031-1039.

Natl. Agric Stat. Serv. 2009. US and all states crops. Published online by Natl. Agric. Stat. Serv.

National Drought Mitigation Center. 2009. US Drought Monitor. Published online by National Drought Mitigation Center.

Niks, R.E., Habekuß, A., Bekele, B., and Ordon, F. 2004. A novel major gene on chromosome 6H for resistance of barley against the barley yellow dwarf virus. Theor. Appl. Genet. 109:1536-1543.

Oakley, J.N., and Young, J.E.B. 2000. Economics of pest control in cereals in the UK. Pages 663-670 in: The BCPC Conference – Pests and Diseases 2000. BPCP, Brighton, UK.

Ohm, H., Anderson, J., Sharma, H., Ayala-Navarrete, L., and Bucholtz, D. 2002. Spring Oat and Soft Winter Wheat Lines with BYDV Resistance. Pages 58-59 in: Barley Yellow Dwarf Disease: Recent Advances and Future Strategies. Henry, M., and A. McNab, eds. 2002. CIMMYT, Mexico.

Oswald, J.W., and Houston, B.R. 1951. A new virus disease of cereals, transmissible by aphids. Plant Dis. Rep. 35:471-475.

Oswald, J.W., and Houston, B.R. 1952. Barley yellow dwarf, a virus disease of barley, wheat and oats readily transmitted by four species of aphids. Phytopathology 42:15.

Oswald, J.W., and Houston, B.R. 1953. Host range and epiphytology of the cereal yellow dwarf disease. Phytopathology 43:309-313.

Ovesna, J., Šíp, V., Chrpova, J., Vacke, J., Skorpǐk, M., Polakova, K., and Kucera, L. 2002. Exploitation of detected BYDV resistance genes in barley breeding. Pages 78-81 in: Barley Yellow Dwarf Disease: Recent Advances and Future Strategies. Henry, M. and A. McNab, eds. CIMMYT, Mexico.

Paliwal, Y.C. 1982. Role of perennial grasses, winter wheat, and aphid vectors in the disease cycle and epidemiology of barley yellow dwarf virus. Can. J. Plant Pathol. 4:367-374.

Perry, K. L., Kolb, F. L., Sammons, B., Lawson, C., Cisar, G., and Ohm, H. 2000. Yield effects of barley yellow dwarf virus in soft red winter wheat. Phytopathology 90:1043-1048.

Piper, J.K., Handley, M.K. and Kulakow, P.A. 1996. Incidence and severity of viral disease symptoms on eastern gamagrass within monoculture and polycultures. Agricult. Ecosys. Environ. 59:139-147.

Plumb, R.T. 1977. Grass as a reservoir of cereal viruses. Ann. Rev. Phytopathol. 9:361-364.

Plumb, R.T. 1983. Barley yellow dwarf virus – a global problem. Pages 185-198 in: Plant Virus Epidemiology. Plumb, R.T., and J.M. Thresh, eds. Blackwell Scientific Publications, Oxford, UK.

Plumb, R.T., and Johnstone, G.R. 1995. Cultural, chemical and biological method for the control of barley yellow dwarf. Pages 307-319 in: Barley Yellow Dwarf: 40 Years of Progress. D'Arcy, C.J., and P.A. Burnett, eds. APS Press, St. Paul.

Plumb, R.T., Lennon, E.A., and Gutteridge, R.A. 1986. Forecasting barley yellow dwarf virus by monitoring vector populations and infectivity. Pages 387-398 in: Plant Virus Epidemics: Monitoring, Modelling and Predicting Outbreaks. McLean, D.G., Garrett, R.G., and W.G. Ruesink, eds. Academic Press, San Diego, USA.

Power, A.G., and Gray, S.M. 1995. Aphid transmission of barley yellow dwarf viruses: Interaction between viruses, vectors and host plants. Pages 259-292 in: Barley Yellow Dwarf: 40 Years of Progress. D'Arcy, C.J., and P.A. Burnett, eds. APS Press, St. Paul.

Power, A.G., Seaman, A.J., and Gray, S.M. 1991. Aphid transmission of barley yellow dwarf: virus inoculation access periods and epidemiological implications. Phytopathology 81:545-548.

Quirino, B.F., Noh, Y.S., Himelblau, E., and Amasino, R.M. 2000. Molecular aspects of leaf senescence. Trends Plant Sci. 5:278-283.

Quiroz, C., Lister R.M., Araya, J.E., and Foster, J.E. 1991. Effect of symptom variants derived from the NY-MAV insolate of barley yellow dwarf virus on the life cycle of the English grain aphid (Homoptera:Aphididae) and on yield components in wheat and oats. J. Econ. Entomol. 84:1920-1925.

Ranieri, R., Lister, R.M., and Burnett, P.A. 1993. Relationship between barley yellow dwarf virus titer and symptom expression in barley. Crop Sci. 33:968-973.

Rasmusson, D.W., and Schaller, C.W. 1959. The inheritance of resistance in barley to the yellow dwarf virus. Agron. J. 51:661-664.

Remaudière, G., and Remaudière, M. 1997. Catalogue of the World's Aphididae, Homoptera, Aphidoidea. INRA, Paris, France.

Remold, S. 2002. Unapparent virus infection and host fitness in three weedy grass species. J. Ecol. 90:967-977.

Robertson, N.L., and French, R.C. 2007. Genetic analysis of a novel Alaska barley yellow dwarf virus in the family Luteoviridae. Arch. Virol. 152:369-382.

Rochow, W.F. 1961a. Vector specificity of three strains of barley yellow dwarf virus. Phytopathology. 51:578-579.

Rochow, W.F. 1961b. A strain of BYDV transmitted specifically by the corn leaf aphid. Phytopathology 51:809-810.

Rochow, W.F. 1969. Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59:1580-1589.

Rochow, W.F. 1970. Barley yellow dwarf virus. CMI/AAB Descriptions of Plant Viruses no. 32.

Rochow, W.F. 1979. Field variants of barley yellow dwarf virus: detection and fluctuation during twenty years. Phytopathology 69:655-660.

Rochow, W. F., and Muller, I. 1971. A fifth variant of barley yellow dwarf virus in New York. Plant Dis. Rep. 55:874-877.

Rochow, W. F. 1977. Dependent virus transmission from mixed infections. Pages 253-273 in: Aphids as Virus Vectors. Harris, K. F. and K. Maramorosch, eds. Academic Press, New York, USA.

Royer, T.A., Giles, K.L., Nyamanzi, T., Hunger, R.M., Krenzer, E.G., Elliott, N.C., Kindler, S.D., and Payton, M. 2005. Economic evaluation of the effects of planting date and application rate of imidacloprid for management of cereal aphids and barley yellow dwarf in winter wheat. J. Econ. Entomol. 98:95-102.

Russell, S.L. and Kimmins, W.C. 1971. Growth regulators and the effect of barley yellow dwarf virus on barley (*Hordeum vulgare* L.). Ann. of Bot. 35:1037-1043.

Scheurer, K.S., Friedt, W., Huth, W., Waugh, R., and Ordon, F. 2001. QTL analysis of tolerance to a German strain of BYDV-PAV in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 103:1074-1083.

Sforza, P., Moore, S., Stromberg, E., and Herbert, A. 2001. Using a Geographic Information System as a Tool to Improve the Management of Barley Yellow Dwarf in Virginia Wheat. Published online.

Shepherd, R.J., Francki, R.I.B., Hirth, L., Hollings, M., Inouye, T., MacLeod, R., Purcifull, D.E., Sinha, R.C., Tremaine, J.H., Valenta, V., and Wetter, C. 1976. New groups of plant viruses approved by the International Committee on Taxonomy of Viruses, September 1975. Intervirology 6:181-184.

Šíp V., Chrpová J., Vacke J., and Ovesná J. 2004. Possibility of exploiting the Yd2 resistance to BYDV in spring barley breeding. Plant Breeding, 123:24-29.

Singh, R. P., Burnett, P. A., Albarran, M. , and Rajaram. S. 1993. Bdv1: A gene for tolerance to barley yellow dwarf virus in bread wheat. Crop Sci. 33:231-234.

Skaria, M., Lister, R. M., Foster, J. E., and Shaner. G. 1985. Virus content as an index of symptomatic resistance to barley yellow dwarf virus in cereals. Phytopathology 75:212-216.

Sharma, H., Ohm, H., Goulart, L., Lister, R., Appels, R., and Benlhabib, O. 1995. Introgression and characterization of Barley yellow dwarf virus-resistance from *Thinopyrum intermedium* into wheat. Genome 38:406-413.

Smith, P.R., and Sward, R.J. 1982. Crop loss assessment studies on the effects of barley yellow dwarf virus in wheat in Victoria. Aust. J. Agr. Res. 33:179-185.

Smyrnioudis, I.N., Harrington, R., Katis, N., and Clark, S.J. 2000. The effect of drought stress and temperature on spread of barley yellow dwarf virus. Agr. For. Entomol. 2:161-166.

Smyrnioudis, I.N., Harrington, R., Clark, S.J., and Katis, N. 2001. The effect of natural enemies on the spread of barley yellow dwarf virus by *Rhopalosiphum padi* (Hemiptera: Aphididae). Bull. Entomol. Res. 91:301-306.

Sprenkel, R. K. 2007. Insect management in pasture. Published online.

Stoy, V. 1963. The translocation of C14-labeled photosynthetic products from the leaf to the ear in wheat. Physiol. Plantarum 16:851-866.

Sutula, C.L., Gillet, J.M., Morrissey, S.M., and Ramsdell, D.C. 1986. Interpereting ELISA data and establishing the positive-negative threshold. Plant Dis. 70:722-726.

Suneson, C. A. 1955. Breeding for resistance to yellow-dwarf virus in barley. Agron. J. 47:283.

Taylor, L.R. 1974. Insect migration, flight periodicity, and the boundary layer. J. Anim. Ecol. 43:225-238.

Teulon, D.A.J., Stufkens, M.A.W., Nicol, D. and Harcourt, S.J. 1999. Forecasting barley yellow dwarf virus in autumn sown cereals in 1998. Proc. 52nd N.Z. Plant Prot. Conf. Published online.

Teulon, D.A.J., Stufkens, M.A.W., and Fletcher, J.D. 2004. Crop infestation by aphids is related to flight activity detected with 7.5 meter high suction traps. N.Z. J. Plant Prot. 57:227–32.

Teulon, D.A.J., Till, C.F., van Toor, R.F. 2008. Conditions surrounding the outbreak of yellow dwarf virus in autumn/winter-sown cereals in Canterbury during 2005. N.Z. J. Plant Prot. 61:270-276.

Thackray, D. J., Diggle, A. J. and Jones, R. A. C. 2009. BYDV PREDICTOR: a simulation model to predict aphid arrival, epidemics of barley yellow dwarf virus and yield losses in wheat crops in a Mediterranean-type environment. Plant Pathol. 58:186-202.

Toojinda, T., Broers, L.H., Chen, X.M., Hayes, P.M., Kleinhofs, A., Korte, J., Kudrna, D., Leung, H., Line, R.F., Powell, W., Ramsay, L., Vivar, H., and Waugh, R. 2000. Mapping quantitative and qualitative resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). Theor. Appl. Genet. 101:580-589.

Ullman, D.E., Qualset, C.O., and McLean, D.L. 1988. Feeding responses of *Rhopalosiphum padi* (Homoptera: Aphididae) to barley yellow dwarf virus resistant and susceptible barley varieties. Environ. Entomol. 17:988-991

United States Department of Agriculture - National Resource Conservation Service. PLANTS Database. Published online.

van Ginkel, M., and Henry, M. 2002. Breeding for BYDV tolerance/resistance in CIMMYT bread wheats targeted to developing countries. Pages 93-96 in: Barley Yellow Dwarf Disease: Recent Advances and Future Strategies. Henry, M., and A. McNab, eds. 2002. CIMMYT, Mexico.

van Regenmortel, M. H. V., Fauquet. C. M., Bishop, D. H. L., Carstens, E. B., Estes, M. K., Lemon, S. M., Maniloff, J., Mayo, M. A., McGeoch, D. J., Pringle, C. R., and Wickner, R. B. (eds.) 2000. Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego.

van Riessen, H. 2002. Barley yellow dwarf virus and cereal yellow dwarf virus in winter wheat in Alabama. Ph.D. Dissertation. Auburn University.

Walters, K.F.A., and Dixon, A.F.G. 1982. The effect of host quality and crowding on the settling and take-off of cereal aphids. Ann. Appl. Biol. 101:211-218.

Ward, S.A., Dixon, A.F.G., and Wellings, P.W. 1983. The relation between fecundity and reproductive investment in aphids. J. Anim. Ecol. 52:451-461.

Watson, M.A. and Mulligan, T.E. 1957. Cereal yellow dwarf virus in Great Britain. Plant Pathol. 6:12-14.

Watt, A.D. and Dixon, A.E.G. 1981. The role of cereal growth stages and crowding in the induction of alatae in *Sitobion avenae* and its consequences for population growth. Ecol. Entomol. 6:441-447.

Weber, H. Schultze, S., and Pfitzner, A.J.P. 1993. Two amino acid substitutions in the tomato mosaic virus 30-kDa movement protein confer the ability to overcome the Tm-22 resistance gene in tomato. J. of Virol. 67:6432-6438.

Winder, L., Holland J.M., Perry, J.N. 1999. The spatial and temporal distribution of the grain aphid *Sitobion avenae* in winter wheat. Entomol. Exp. Appl. 93:277-290.

Wyatt, S.D., Seybert, L.J., and Mink, G. 1988. Status of the barley yellow dwarf problem of winter wheat in eastern Washington. Plant Dis. 72:110-113

Young, N.D. 1996. QTL mapping and quantitative disease resistance in plants. Ann. Rev. Phytopathology 34:479-501.

Zhang, Q. F. Guan, W. N. Ren, Z. Y. Zhu, X. S. and Tsai, J. H. 1983. Transmission of barley yellow dwarf virus strains from northwestern China by four aphid species. Plant Dis. 67:895-899.

Zhou, G.H. and Rochow, W.F. 1984. Difference among five stages of *Schizaphis graminum* in transmission of a barley yellow dwarf luteovirus. Phytopathology 74: 1450-1453.

Zhu, S., Gao, F., Cao, X., Chen, M., Ye, G. 2005. The rice dwarf virus P2 protein interacts with ent-kaurene oxidases in vivo, leading to reduced biosynthesis of gibberellins and rice dwarf symptoms. Plant Physiol. 139:1935-1945.

Zwiener, C. M., Conley, S. P., Bailey, W. C., and Sweets, L. E. 2005. Influence of aphid species and barley yellow dwarf virus on soft red winter wheat yield. J. Econ. Entomol. 98:2013-2019.

Appendix

Table A1 Winged aphids collected on wheat fields in Alabama and western Florida between 2005 and 2009

Collection Site	Collection Date	Aphid Species *										
		Ac	Er	Hs	Psp	Rm	Rp	Rr	Sg	Sf	Sa	Tn
Tallassee	10/26/05	0	0	2	0	0	0	5	18	1	0	0
Headland	11/03/05	1	0	0	0	0	1	21	7	0	0	0
Tallassee	11/04/05	0	0	2	0	0	1	7	9	1	0	0
Headland	11/10/05	0	0	0	0	0	0	23	1	0	0	0
Tallassee	11/11/05	0	0	0	0	0	8	26	15	1	0	0
Tallassee	11/18/05	0	0	6	0	0	9	38	16	0	0	1
Headland	12/12/05	0	4	1	0	0	3	9	9	1	0	2
Tallassee	12/12/05	0	0	1	0	0	0	41	21	1	0	0
Killen 1	11/25/06	0	0	0	0	0	0	0	1	0	0	0
Killen 2	11/25/06	0	0	0	0	0	0	0	0	0	0	2
Town Creek	11/25/06	0	0	0	0	0	0	0	0	0	0	0
Town Creek	12/03/06	0	0	0	0	0	2	0	0	0	0	0
Killen 1	12/03/06	0	0	0	0	0	1	0	0	0	0	0
Killen 2	12/03/06	0	0	0	0	0	0	0	0	1	0	0

Table A.1 (cont.)

Collection Site	Collection Date	Aphid Species *										
		Ac	Er	Hs	Psp	Rm	Rp	Rr	Sg	Sf	Sa	Tn
Atmore 1	12/08/06	0	0	0	0	0	0	2	0	0	0	0
Atmore 2	12/08/06	0	0	0	0	0	0	0	1	0	0	0
Atmore 3	12/08/06	0	0	0	0	0	2	1	0	0	0	0
Town Creek	12/09/06	0	0	0	0	0	1	0	0	0	0	0
Killen 1	12/09/06	0	0	0	0	0	0	0	0	0	0	0
Killen 2	12/09/06	0	0	0	0	0	0	0	0	0	0	0
Atmore 1	12/14/06	0	0	0	0	0	0	0	0	0	0	0
Atmore 2	12/14/06	0	0	0	0	0	0	1	0	0	0	3
Atmore 3	12/14/06	0	0	0	0	0	0	0	0	0	0	10
Town Creek	12/15/06	0	0	0	0	0	5	1	0	0	0	0
Killen 1	12/15/06	0	0	0	0	0	0	0	0	0	0	0
Killen 2	12/15/06	0	0	0	0	0	0	0	0	0	0	0
Atmore 1	12/19/06	0	0	0	0	0	0	1	0	0	0	1
Atmore 2	12/19/06	0	0	0	0	0	1	3	0	0	0	0
Atmore 3	12/19/06	0	0	0	0	0	1	1	0	0	0	0
Town Creek	12/20/06	0	0	0	0	0	12	3	0	0	0	0
Killen 1	12/20/06	0	0	0	0	0	0	0	0	0	0	0
Killen 2	12/20/06	0	0	0	0	0	0	0	0	0	0	0
Town Creek	01/03/07	0	0	0	0	0	3	1	0	0	0	0
Killen 1	01/03/07	0	0	0	0	0	0	0	0	0	0	0
Killen 2	01/03/07	0	0	0	0	0	1	0	0	0	0	0
Atmore 1	01/11/07	0	0	0	0	0	0	1	1	0	0	0
Atmore 2	01/11/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 3	01/11/07	0	0	0	0	0	1	0	0	0	0	0
Town Creek	01/27/07	0	0	0	0	0	0	0	0	0	0	0

Table A.1 (cont.)

Collection Site	Collection Date	Aphid Species *										
		Ac	Er	Hs	Psp	Rm	Rp	Rr	Sg	Sf	Sa	Tn
Killen 1	01/27/07	0	0	0	0	0	0	0	0	0	0	0
Killen 2	01/27/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 1	01/28/07	0	0	0	0	0	1	0	0	0	0	0
Atmore 2	01/28/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 3	01/28/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 1	02/10/07	0	0	0	0	0	3	0	0	0	0	0
Atmore 2	02/10/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 3	02/10/07	0	0	0	0	0	0	0	0	0	0	0
Town Creek	02/16/07	0	0	0	0	0	1	0	0	0	0	0
Killen 1	02/16/07	0	0	0	0	0	0	0	0	0	0	0
Killen 2	02/16/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 1	02/23/07	0	0	0	0	1	2	0	0	0	1	0
Atmore 2	02/23/07	0	0	0	0	0	2	0	0	0	0	0
Atmore 3	02/23/07	0	0	0	1	0	1	0	0	0	0	0
Town Creek	02/24/07	0	0	0	0	0	0	0	0	0	0	0
Killen 1	02/24/07	0	0	0	0	0	0	0	0	0	0	0
Killen 2	02/24/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 1	03/10/07	0	0	0	0	0	10	2	0	0	0	0
Atmore 2	03/10/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 3	03/10/07	0	0	0	0	0	0	0	0	0	4	0
Town Creek	03/17/07	0	0	0	0	0	6	0	0	0	1	0
Killen 1	03/17/07	0	0	0	0	0	1	0	0	0	0	0
Killen 2	03/17/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 1	03/21/07	0	0	0	0	0	5	4	0	0	1	0
Atmore 2	03/21/07	0	0	0	0	0	0	0	0	0	1	0

Table A.1 (cont.)

Collection Site	Collection Date	Aphid Species *										
		Ac	Er	Hs	Psp	Rm	Rp	Rr	Sg	Sf	Sa	Tn
Atmore 3	03/21/07	0	0	0	0	0	2	0	0	0	0	0
Atmore 1	04/07/07	0	0	0	0	0	0	1	0	0	1	0
Atmore 2	04/07/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 3	04/07/07	0	0	0	0	0	0	0	0	0	0	0
Town Creek	04/15/07	0	0	0	0	0	0	0	0	0	0	0
Killen 1	04/15/07	0	0	0	0	0	0	0	0	0	0	0
Killen 2	04/15/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 1	04/20/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 2	04/20/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 3	04/20/07	0	0	0	0	0	0	0	0	0	0	0
Town Creek	04/22/07	0	0	0	0	0	0	0	0	0	0	0
Killen 1	04/22/07	0	0	0	0	0	0	0	0	0	0	0
Killen 2	04/22/07	0	0	0	0	0	0	0	0	0	0	0
Town Creek	05/01/07	0	0	0	0	0	0	0	0	0	0	0
Killen 1	05/01/07	0	0	0	0	0	0	0	0	0	0	0
Killen 2	05/01/07	0	0	0	0	0	0	0	0	0	0	0
Atmore 1	05/02/07	0	0	0	0	0	0	0	0	0	2	0
Atmore 2	05/02/07	0	0	0	0	0	0	0	0	0	0	2
Atmore 3	05/02/07	0	0	0	0	0	0	0	0	0	0	0
Marianna	11/14/07	0	0	0	0	0	2	20	1	8	0	1
Russelville 1	11/15/07	0	0	0	0	0	0	0	0	0	0	0
Russelville 2	11/15/07	0	0	0	0	0	0	0	0	0	0	0
Central Star	11/15/07	0	0	0	0	0	0	0	0	0	0	0
Marianna	11/20/07	0	0	0	0	0	1	7	0	0	0	0
Russelville 1	11/21/07	0	0	0	0	0	0	0	0	0	0	0

Table A.1 (cont.)

Collection Site	Collection Date	Aphid Species *										
		Ac	Er	Hs	Psp	Rm	Rp	Rr	Sg	Sf	Sa	Tn
Russelville 2	11/21/07	0	0	0	0	0	0	0	0	0	0	0
Central Star	11/21/07	0	0	0	0	0	0	0	0	0	0	0
Marianna	12/11/07	0	0	0	0	0	4	2	0	2	0	0
Russelville 1	12/12/07	0	0	0	0	0	0	0	0	0	0	0
Russelville 2	12/12/07	0	0	0	0	0	0	0	0	0	0	0
Central Star	12/12/07	0	0	0	0	0	0	0	0	0	0	0
Marianna	12/22/07	0	0	0	0	0	0	0	0	0	0	0
Russelville 1	12/23/07	0	0	0	0	0	0	0	0	0	0	0
Russelville 2	12/23/07	0	0	0	0	0	0	0	0	0	0	0
Central Star	12/23/07	0	0	0	0	0	0	0	0	0	0	0
Marianna	01/13/08	0	0	0	0	0	0	0	0	0	0	0
Russelville 1	01/14/08	0	0	0	0	0	0	0	0	0	0	0
Russelville 2	01/14/08	0	0	0	0	0	0	0	0	0	0	0
Central Star	01/14/08	0	0	0	0	0	0	0	0	0	0	0
Marianna	01/25/08	0	0	0	0	0	8	0	0	0	0	0
Marianna	02/09/08	0	0	0	0	0	1	0	0	0	0	0
Marianna	02/14/08	0	0	0	0	0	6	0	0	0	0	0
Marianna	02/29/08	0	0	0	0	0	5	1	0	0	2	0
Marianna	11/07/08	0	0	0	0	0	0	12	0	0	0	0
Tallassee	11/11/08	0	0	0	0	0	6	8	0	0	1	1
Marianna	11/12/08	0	0	0	0	0	2	8	0	2	0	1
Belle Mina	12/04/08	0	0	0	0	0	0	0	0	0	0	0
Greenbrier	12/04/08	0	0	0	0	0	0	0	0	0	0	0
Sand Mountain	12/04/08	0	0	0	0	0	0	0	1	0	0	0
Tallassee	12/04/08	0	0	0	0	0	0	0	0	0	0	0

Table A.1 (cont.)

Collection Site	Collection Date	Aphid Species *										
		Ac	Er	Hs	Psp	Rm	Rp	Rr	Sg	Sf	Sa	Tn
Marianna	12/05/08	0	0	0	0	0	0	5	0	0	0	0
Belle Mina	12/12/08	0	0	0	0	0	0	0	0	0	0	0
Greenbrier	12/12/08	0	0	0	0	0	0	0	0	0	0	0
Sand Mountain	12/12/08	0	0	0	0	0	0	0	0	0	0	0
Marianna	12/13/08	0	0	0	0	0	0	0	0	0	0	0
Belle Mina	12/17/08	0	0	0	0	0	0	0	0	0	0	0
Greenbrier	12/17/08	0	0	0	0	0	0	0	0	0	0	0
Sand Mountain	12/17/08	0	0	0	0	0	0	0	0	0	0	0
Marianna	12/18/08	0	0	0	0	0	0	0	0	0	0	0
Belle Mina	12/26/08	0	0	0	0	0	0	0	0	0	0	0
Greenbrier	12/26/08	0	0	0	0	0	0	0	0	0	0	0
Sand Mountain	12/26/08	0	0	0	0	0	0	0	0	0	0	0
Marianna	12/27/08	0	0	0	0	0	1	2	0	0	0	0
Marianna	01/29/09	0	0	0	0	1	10	2	0	0	1	0

* Ac: *Aphis craccifora,* Er*: Eulachnus rileyi,* Hs: *Histeroneura setariae,* Psp: *Pemphigus sp.,* Rm: *Rhopalosiphum maidis,* Rp: *Rhopalosiphum padi,* Rr: *Rhopalosiphum rufiabdominale*, Sg: *Schizaphis graminum,* Sf: *Sipha flava,* Sa: *Sitobion avenae*, Tn: *Tetraneura nigriabdominalis*