EFFECT OF THE BIRD CHERRY-OAT APHID

(RHOPALOSIPHUM PADI) ON WHEAT AND

CONTROL OF THE APHID/BARLEY

YELLOW DWARF COMPLEX

WITH GAUCHO

(IMIDACLOPRID)

INSECTICIDE

By

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NOMENCLATURE

BCO .... bird cherry-oat

BYD .... barley yellow dwarf

BYDVs .... barley yellow dwarf viruses

DI .... disease incidence

DS .... disease severity

ELISA .... enzyme linked immunosorbent assay

FD .... feeding duration

GB .... greenbugs

GC .... growth chamber *or* Gaucho concentration

HRWW .... hard red winter wheat

NA .... number of aphids

NYBYD-ES .... New York barley yellow dwarf experimental system

PCR .... polymerase chain reaction

PTS .... percent treated seed

TKW .... thousand-kernel weight

TRT .... treatment

Chapter I

Introduction

Oklahoma, which usually ranks second or third in the United States for hard red winter wheat production, harvested 4.3 million acres of wheat in 1999. This important field crop provides not only grain that is milled into flour for baking bread, but in Oklahoma wheat also provides forage for cattle during the fall and winter. Because wheat is used for this dual purpose, grain production is often sacrificed in efforts to improve forage for grazing by planting wheat as early as late August. However, planting during this period usually results in lower grain yields than planting in October, which is generally considered optimum for grain production. Two major reasons for the reduction of grain yield as a result of planting early include increases in insect activity and plant disease development.

Aphids, especially greenbugs (GB) (*Schizaphis graminum* L.) and bird cherry-oat (BCO) aphids [*Rhopalosiphum padi* (L.)], are pests of wheat that occur every year in Oklahoma. GB directly damage wheat by feeding and inducing a phytotoxic response, but the infestation level at which economic damage in Oklahoma occurs is uncertain. BCO aphids are not thought to damage wheat as severely as GB (Burnett and Gill, 1976); however, the threshold at which BCO aphids cause economic damage is also uncertain.

Although aphids alone may damage wheat, they may also transmit disease-causing viruses during feeding (Gray and Power, 1995). The most common viruses transmitted by aphids in Oklahoma are the barley yellow dwarf viruses (BYDVs), which cause the disease barley yellow dwarf (BYD). BYDVs are members of the taxonomic group Luteovirus, which are characterized by viruses composed of isometric particles 22-25 nm in diameter that cause yellowing symptoms on their hosts, are restricted to phloem tissues, and are transmitted solely by aphids (Martin and D'Arcy, 1995).

Barley yellow dwarf (BYD) is considered to be the most serious disease on cereal crops worldwide. First described in 1951 (Oswald and Houston, 1952), BYD is restricted to the family Poaceae, which includes cereals (wheat, oats, barley, maize and rice), perennial grassy weeds and range grasses. A major symptom of BYD is stunted growth that results from reduced internode elongation. Other symptoms include older leaves turning yellow, red, or purple along the margins and tips 7-20 days after infection. BYD can also dramatically reduce root systems and cause sterility in flowers (D'Arcy, 1995). Because BYD symptoms can be confused with other diseases or with nutrient deficiencies, identification is commonly made with the enzyme-linked immunosorbant assay (ELISA) (French, 1995; Lister and Rochow, 1979). In years when there is little aphid activity in Oklahoma, little BYD is observed. However, in years such as 1996-97, GB and BCO aphid levels were high, and significant damage from the aphid/BYDV complex occurred (Hunger *et al*, 1997).

The pathosystem of BYD is extremely complex. Over 20 aphid species are capable of transmitting one or more of five BYDVs with varying degrees of efficiency (Gray and Power, 1995). The host range of BYDV is wide; many cereals, grasses, and perennial grassy weeds are susceptible to infection.

Furthermore, environmental conditions may dramatically influence the severity of a disease epidemic. These components by themselves, and the interactions between each, entail a complex pathosystem. Thus, although BYD has been extensively studied since it was first described, further work is necessary to better understand BYD epidemiology in order to provide improved disease management recommendations and forecasting models.

The purposes of my research were to 1) determine the effects of BCO aphids on seedling root and shoot length and yield of hard red winter wheat, and 2) investigate the ability to control BYD by planting mixtures of insecticide-treated and untreated seed. In chapter III, damage to hard red winter wheat caused by varying levels of aviruliferous BCO aphids is reported. Chapters IV and V present results from studies that investigate the planting of mixtures of insecticide-treated and untreated wheat seed to control aphids and BYD. This strategy is based on the principle that less disease occurs on crops that have varying degrees of susceptibility to a pathogen (i.e., mixed cultivars or multilines) than crops that have plants that are all susceptible (susceptible monocrops) (Wolfe and Barrett, 1980).

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Chapter II

Literature Review

History and Economic Importance of Barley Yellow Dwarf

Symptoms of barley yellow dwarf (BYD) were likely observed long before the discovery that a virus caused this disease, which now is known to be caused by the barley yellow dwarf viruses (BYDVs) (Plumb, 1992). Oswald & Houston (1952) first reported that a virus caused BYD in 1951 when yellowing was observed in barley growing in California. Subsequent observations of yellowing and stunting of wheat and reddening of oats followed. An attempt to isolate fungi from diseased plant roots was unsuccessful. Also, greenbug populations in the field were low and could not provide the explanation. Finally, they suspected that a virus caused the symptoms. Virus transmission tests using aphids as vectors confirmed the presence of an aphid-transmitted virus. In 1959, Allen and Houston (Lister and Ranieri, 1995) demonstrated that BYD extended far beyond California by diagnosing BYD in fields from Arizona, Oregon, Washington, Illinois, Minnesota, Wisconsin, Maryland, and Arkansas via transmission tests. By 1963, Canada, Mexico, Western Europe, Australia, New Zealand, Jordan, Egypt, India, Pakistan, and Japan were added to the growing list of geographic areas reported to have BYD. Today, the disease is thought to occur throughout the world where cereal crops are grown (Plumb, 1992).

BYD is considered to be the most serious disease of cereal crops worldwide. Although difficult to actually determine, the lost value in wheat due to

BYD is estimated at $387 million when BYD damage is 5% (Lister and Ranieri, 1995). Yield losses of 17% have been attributed to BYD for wheat growing in the field, and yield losses of 50% have been reported for wheat seedlings artificially infected with BYDVs in the U.S. and other parts of the world (Lister and Ranieri, 1995). Frequently, yield components are reduced by BYD. Components most significantly reduced when wheat is infected with BYDVs are the number of kernels per spike and kernel weight. Hoffman (Hoffman and Kolb, 1998) reported 11-30% reduction in number of kernels per spike and 3-19% reduction in kernel weight. The number of spikes per unit area, however, was rarely reduced. This study suggests that infections by BYD results in smaller spikes as opposed to fewer spikes.

Yield losses from BYD are dependent on disease incidence and severity levels, which in turn depend on many factors. Vector movement and reproduction as well as virus replication in host plants are highly dependant on temperatures in the field (De Barro, 1992). Young seedlings are more susceptible to damage caused by BYD than older plants (McKirdy and Jones, 1997). The viruses and vectors present in the field determine the efficiency of virus transmission (Gray *et al*, 1991) and degree of virulence (Ranieri *et al*, 1993). Furthermore, the presence of aphid parasites may significantly reduce vector populations (Jones, 1972). Finally, variation in tolerance to BYD can be found among different cultivars (Ranieri et al, 1993). Because many factors influence the incidence and severity of BYD, yield losses are difficult to both predict and determine.

Host Range and Virus Properties

BYDVs are members of the taxonomic group Luteoviruses. Luteoviruses are characterized by isometric particles 22-25 nm in diameter that cause yellowing of their hosts, are transmitted only by aphids in a persistent manner, and are associated with phloem tissues in roots and shoots of infected plants (Hewings, 1995).

One hundred-fifty species in the family Poaceae serve as hosts of BYDVs including important agronomic cereal crops such as wheat, rye, oats, barley, rice and maize are included in their host range. Perennial grassy weeds and range grasses may be important alternate hosts that harbor BYDVs during seasons when cereal crops are fallowed (Martin and D'Arcy, 1995).

Virus Nomenclature and Taxonomy

In 1969, Rochow (1969) characterized five BYDVs based on the transmission efficiency of four aphid species: the bird cherry-oat (BCO) aphid (*Rhopalosiphum padi*), the corn leaf aphid (*R. maidis*), the English grain aphid [*Sitobion avenae* (formerly *Macrosiphum avenae*)], and the greenbug (GB) (*Schizaphis graminum*). The aphid species that demonstrated transmission most efficiently identified each virus, resulting in the virus nomenclature BYDV-rpv, -rmv, -mav, and -sgv respectively. A fifth virus, BYDV-pav, was transmitted with similar efficiencies by both the BCO aphid and the English grain aphid. Rochow (1969) designated this system of classification for BYDVs as the New York

Barley Yellow Dwarf Experimental System (NYBYD-ES), and this system became widely used through the mid-1990s (Martin and D'Arcy, 1995).

However, since the time BYDVs were biologically characterized by Rochow in the NYBYD-ES, biotechnology has provided improved research tools that have been employed to further characterize BYDVs. Studies examining ultrastuctural, serological, and DNA hybridization characters of BYDVs have given clues about relationships among BYDVs and other Luteoviruses that could not be described using transmission studies alone. Observations from studies using these tools suggest that the BYDVs can be divided into two groups: (1) BYDV-pav, -mav, and –sgv, and (2) BYDV–rmv and –rpv (Gill and Chong, 1979; Martin and D'Arcy, 1995; Miller and Rasochova, 1997). Both BYDV-rmv and –rpv are found in plant cells bound by a double membrane along the endoplasmic reticulum. In contrast, BYDV-pav, -mav, and –sgv are single membrane bound and found near the plasmadesmata. Enzyme-linked immunosorbent assays (ELISA) have been used to quantitatively determine close serological relationships within the two groups. Finally, DNA hybridization and sequence analysis suggest BYDV-pav and –mav are more closely related than the other BYDVs. Although advances in cytopathology, molecular biology, and serology have helped further describe the BYDVs, the NYBYD-ES remains an important resource. The biological characterization of these viruses allows a description of transmission efficiencies of four aphids that are considered important vectors in the United States and throughout the world because the transmission efficiencies determine virus spread and subsequent yield losses.

Vectors of BYDVs

BYDVs depend solely on aphids to be transmitted (Irwin and Thresh, 1990). Of at least 20 aphid species that transmit BYDVs (Voegtlin and Halbert, 1995), two species commonly infest wheat in Oklahoma, namely the BCO aphid and GB. In the United States, both species rank among the most important aphid vectors of BYDVs (Gray *et al*, 1998; Halbert *et al*, 1992; Smith *et al*, 1968; Smith and Richards, 1963). Thus, the spread of BYD in wheat in Oklahoma is thought to be highly dependent on infestations by these aphids. Other sporadic aphid pests that infest Oklahoma wheat include the corn leaf aphid (*Rhopalosiphum maidis*), the English grain aphid (*Sitobion avenae*), the rice root aphid (*Rhopalosiphum rufiabdominalis*) and the Russian wheat aphid (*Diuraphis noxia*).

Because of their important roles as vectors in the epidemiology of BYD, the life histories of BCO aphids and GB should be considered in some detail (Voegtlin and Halbert, 1995). Infestations by both aphids can occur on winter wheat seedlings in fall and early winter, when they colonize leaves and sheaths of young seedlings. BCO aphids have a tendency to migrate to areas of increased moisture, and hence are frequently found near the crown and often at the soil line or below. GB, in contrast, tend to inhabit both upper and lower leaves. Both species are parthenogenic (having the ability to reproduce asexually). During severe winters, populations of BCO aphids and GB on wheat may greatly decline, but can rapidly increase in the spring when warm conditions favor reproduction. BCO aphids are reported to overwinter on members of the genus *Prunus* where sexual reproduction occurs. GB, however, do not require an alternate host for sexual reproduction. Both aphid species can colonize wheat until plant maturity in late spring and early summer prior to harvest, when aphids migrate to other hosts such as sorghum, range grasses and weeds. These hosts may then provide the sources of aphids for infestations that occur in the fall on winter wheat seedlings.

Unlike BCO aphids, GB induce a phytotoxic response while feeding that is indicated by pin-head sized brown spots. These phytotoxins directly damage plants and result in yield reductions (Voegtlin and Halbert, 1995). Damage caused by BCO aphids is less obvious, and typically no signs of feeding are evident on leaves previously colonized.

Epidemiology and The Virus/Vector Complex

Aphids must acquire particles of BYDVs during feeding if they are to spread the virus. BYDVs are not passed directly from adult aphids to young nymphs (Irwin and Thresh, 1990). According to Gray *et al* (1991), aphid acquisition of viruses BYDV-pav and –rpv by BCO aphids can occur within 15 mins. However, 1-3 hrs of feeding time is required for 50% of those aphids to transmit the viruses. Virus acquisition by GB is reported to be twice as long as for BCO aphids, and transmission by GB requires feeding periods of 4-6 hours and 10-12 hrs for BYDV-mav or –pav, respectively. During aphid acquisition periods, particles from host phloem tissue enter the aphid stylet, progress through the food canal and proceed to the mid- and hindgut. Luteovirus-specific receptors on the hindgut membrane are thought to facilitate transport of particles into the hemocoel. Particles are further transported to the salivary glands. Once virus is acquired, aphids transmit BYDVs to plants by regurgitating saliva (and virus) into the phloem of susceptible plants while feeding. Transmission can occur in distant fields (primary spread) or nearby plants in the same field (secondary spread) (Irwin and Thresh, 1990). Inoculation of BYDVs on winter wheat as a result of primary and secondary spread can occur throughout the growing season and during dormant periods in winter. During inoculation, particles move from the hemocoel into the accessory salivary gland, where virus-specific receptors may be found. These virus-specific receptors may determine the efficiency of virus transmission (Gray and Power, 1995).

Although our knowledge of BYDVs has expanded since the nomenclature was first established by the NYBYD-ES, this system remains a valuable component to the understanding of virus transmission. The four aphid species have demonstrated consistent transmission efficiencies among the virus/aphid combinations. It is important to note, however, that these efficiencies are not universally applicable to other aphids of the same species (different biotypes) nor the viruses they transmit from other parts of the world. The NYBYD-ES is based on the use of aphid progeny from original NYBYD-ES colonies. One example of this variation in transmission was demonstrated when a California clone of GB transmitted BYDV-pav inefficiently (<10% infection rate). This transmission rate is much lower than reported by the NYBYD-ES (37%) (Gray and Power, 1995).

Diagnostic Methods

A major symptom of BYD is stunted growth that results from reduced internode elongation. Other symptoms include older leaves turning yellow, red, or purple along the margins and tips 7-20 days after infection. Because BYD symptoms can be confused with other diseases or with nutrient deficiencies, diagnosing BYD based on the symptoms alone is risky. Thus, detecting BYDVs in plants suspected of infection provides a more reliable diagnosis.

Early detection of BYDVs was based on transmission assays similar to those used in the NYBYD-ES. Prior to the mid 1970s, serological and DNA hybridization methods were not available. One significant disadvantage of the use of transmission assays is that test results (symptom expression or lack there of) take weeks to obtain. However, this method has proved useful for detecting uncharacterized isolates of BYDV.

In the late 1970s, ELISA was applied to the BYDV pathosystem, and has proved useful for the detection and diagnosis of BYDVs. ELISA is based on enzyme-linked antibodies that bind to virus particles. Presence of virus is determined when a substrate is added that reacts with an enzyme. Although other serological assays were developed prior to ELISA, their use in diagnosis was handicapped. Special equipment was needed and assays were not developed for multiple sample testing. ELISA made it possible to rapidly detect BYDVs in multiple samples simultaneously and hence, ELISA remains the most common and preferred method of detection (Clement *et al*, 1986; D'Arcy *et al*, 1992; Irwin and Thresh, 1990). Like transmission assays, ELISA requires that all

BYDVs be independently tested as antibodies have not been developed to detect all viruses in one test.

By 1991, a protocol was published for the detection of BYDVs with the polymerase chain reaction (PCR), a nucleic acid assay (Robertson *et al*, 1991). Following RNA extraction from phloem tissue and construction of cDNA, sequences specific to the BYDVs are amplified with DNA primers. PCR products are subjected to gel electrophoresis for broad detection of all BYDVs. For detection of specific BYDVs, PCR products can be digested after amplification, and specific profiles for each virus result in the electrophoresis. To date, the PCR process used to detect BYDVs is employed only in some commercial testing laboratories. This is mainly due to the high cost of equipment required for PCR, namely a thermocycler and gel apparatus. However, PCR will most likely become more popular for testing because of its high sensitivity and usefulness in detecting many other plant pathogens (French, 1995).

Control

Current strategies to control BYD on winter wheat include (1) planting tolerant varieties, (2) applying insecticides to eliminate aphid vectors, and (3) planting late in the fall. Although there are no wheat varieties highly resistant or immune to BYD, some varieties such as 2137, 2163, and Custer exhibit low levels of resistance or tolerance.

BYD control can also be achieved via insecticides (Araya and Foster, 1987; Gray *et al*, 1996; Irwin and Thresh, 1990). Reductions in vector populations result in reductions in BYD transmission, which in turn translates to lower disease incidence. Seed-applied systemic insecticides may provide effective control because of their ability to prevent aphid populations from becoming established. Insecticides applied after the establishment of aphid colonies may not be as effective against primary spread of BYD. The seed-applied insecticide Gaucho is very effective when applied at 3 oz./cwt. and is recommended by the company (Gustafson, Inc.) at 2 or 3 oz./cwt for control of BYD (Hunger *et al*, 1997).

Infections of BYDVs that occur in the fall result in greater yield losses than spring infections (Fitzgerald and Stoner, 1967; Hammon *et al*, 1996; Irwin and Thresh, 1990; Mann *et al*, 1997; McGrath *et al*, 1987). Planting late avoids or reduces levels of fall aphid infestations (Hammon,Pearsonet al, 1996; McKirdy and Jones, 1997). Furthermore, plants infected with BYDVs in the fall are more susceptible to winter kill (Irwin and Thresh, 1990). Early planting periods correspond with warmer temperatures that favor aphid activity. By planting late in the fall, aphid infestations and the consequent spread of BYD may be avoided. However, this practice is rarely employed where cattle grazing is practiced due to lower forage potentials associated with planting late.

Monocultivar planting systems have become common in today's agriculture. However, evidence suggests that less disease occurs with genetically diverse crops than in crops with little genetic diversity; better disease control has been demonstrated in multiline cultivar plantings as compared to monoline cultivar plantings, in mixed cultivars as compared to single cultivars, and in mixed crops as compared to mono-crops (Andow, 1991; Browning and Frey, 1969; Power, 1991; Wolfe, 1985; Wolfe and Barrett, 1980). Explanations for this observation vary depending on the disease in question; however, all hypotheses focus on the transmission of the pathogen. Lower transmission rates occur in crops that are more genetically diverse than in those crops with less diversity. In systems that involve insect vectors of plant viruses, Power (1990, 1991) proposed two hypotheses: (1) aphids tend to emigrate after having moved on an undesirable host or non-host plant, sometimes resulting in long distance movement and thus leaving the crop (Power, 1990), and (2) aphids in multi-line crops feed on more plants in a given time than those feeding on mono-line crops. Feeding on more plants means less time on plants, which may interfere with the acquisition and transmission of the virus (Power, 1991).

BYD control measures are consistent with disease control measures for other field crops and other diseases by manipulating the components of disease. Cultivar selection allows for planting of tolerant cultivars, insecticides reduce aphid vector populations, and planting late in the fall usually results in an environment unfavorable for high aphid infestation levels and disease pressure. Thus, these control measures are employed in pursuit of limiting losses from aphids and BYD.

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CHAPTER III

RESPONSE OF WINTER WHEAT TO BIRD CHERRY-OAT APHID INFESTATIONS

Abstract

The bird cherry-oat (BCO) aphid [*Rhopalosiphum padi*] is an important vector of barley yellow dwarf virus on cereal crops. However, the effects of aviruliferous BCO aphids on winter wheat are unclear, and hence were examined in this study. Caged wheat seedlings were grown hydroponically at 16.5 degrees C with a 16:8 photoperiod. Ten-day-old seedlings were infested with 0, 10, 20 or 30 aviruliferous BCO aphids for 2, 4, 6, 8 or 10 days. Nymphs were removed daily to maintain original infestation levels. At 20 days after planting, length of roots and shoots was quantified using Rootedge software. Seedlings were transplanted into clay pots, vernalized, and grown to maturity in a greenhouse. For each treatment, number of heads, number of seeds, and grain weight were recorded. Results indicated that low population levels of aviruliferous BCO aphids adversely affected root and shoot length of seedling wheat, and increasing aphid density decreased number of heads, number of seeds and grain weight.

In Oklahoma, hard red winter wheat is the most economically important field crop, and is planted in the fall and harvested the following May to June. Insects and diseases frequently occur on wheat in Oklahoma, and often cause yield reductions. The most common insects that occur on Oklahoma wheat are the greenbug (GB) and the bird cherry-oat (BCO) aphid. Population levels of these aphids can explode when temperatures are warm during the growing season, and injury caused by these aphids is more severe when seedlings are infested during the fall compared with older plants infested in the spring (Kieckhefer and Gellner, 1982; Pike and Schaffner, 1985). The mechanism for injury caused by GB appears to be different than that caused by BCO aphids. GB induces a phytotoxic response when feeding, resulting in brown and yellow spots. Injury caused by BCO aphids, however, is not as obvious (Riedell and Kieckhefer, 1995), and no visual evidence is observed on leaves where BCO aphids have fed.

Aphids can also indirectly injure wheat by transmitting disease-causing viruses during feeding. The most common viruses transmitted by GB and BCO aphids in Oklahoma are the barley yellow dwarf viruses (BYDVs), which cause the disease, barley yellow dwarf (BYD). Symptoms of BYD on winter wheat include yellowing and stunting (D'Arcy, 1995). BYD occurs every year on winter wheat in Oklahoma, but yield losses are greatest when infection occurs in the fall. Consistent with reports in Australia (McKirdy and Jones, 1997), spring infections in Oklahoma often result in minimal or no yield losses.

Determining the independent effects of both aphids and viruses is essential to understanding interactions that lead to yield losses caused by the aphid/BYD complex (Irwin and Thresh, 1990). Several studies have reported damage caused by aviruliferous aphids (Kieckhefer and Gellner, 1992;

Kieckhefer *et al*, 1995; Pike and Schaffner, 1985; Riedell and Kieckhefer, 1995; Riedell *et al*, 1999). For example, Riedell and Kieckhefer (1995) infested BCO aphids, GB, and Russian wheat aphids on planted spring wheat planted in pots at the 2-leaf stage. Aphids were allowed feeding durations of 2, 4, 6 and 12 days, but aphid days (number of aphids per plant X number of days feeding) remained constant for each treatment. For the 2-day treatment, no damage in root length or root dry weight occurred for any of the aphids. However, damage was significant for the 4- and 12-day treatments but not the 6-day treatment.

Another example of the effects of aviruliferous aphids on wheat was reported by Pike and Schaffner (1985), who examined the effects of low-level fall infestations of BCO aphids, GB, and mixed infestations of BCO aphids and GB on winter wheat in the field. Single plants were infested with 2-4 aviruliferous aphids and then covered with an insect cage. Unlike the experiments conducted by Riedell and Kieckhefer (1995), aphid population levels were not held constant, but rather, aphids were allowed to reproduce inside the caged environment. BCO aphid populations increased at a rate greater than GB populations resulting in higher cumulative aphid days for BCO aphids. In addition, BCO aphids were hardier during cold temperatures during the winter. Plant heights, root and foliage weights, test weights and grain yields were significantly reduced by infestations of BCO aphids at the 2-leaf stage. Little damage occurred from infestations at the 4-leaf stage and no damage occurred from infestations at the 2-tiller stage.

In another study, Kieckhefer and Gellner (1992) infested 10 and 15 BCO aphids per plant and 2, 4, 6, 8, 10, and 15 GB per plant for 30 days on winter wheat planted in pots at the 2-leaf stage. Their findings suggested that yield losses caused by BCO aphids occur at 10 aphids per plant, or 300 aphid days. Yield losses associated with greenbug population levels, however, were significant when 15 GB were infested for 30 days, or 450 aphid days.

These studies suggest BCO aphids can cause significant damage to wheat when infestations occur on young seedlings, and that greater numbers of aphids and longer feeding durations are associated with increased damage. However, determining damage caused by BYDVs alone is difficult. Studies reporting the effects of BYDVs on wheat may be misleading due to assumptions that the vectors used cause minimal or no damage. For example, Riedell *et al* (1999) investigated the effects of BCO aphids, BYDV, and the BYDV/aphid complex on winter wheat. The resulting yield reductions for each treatment were 21%, 46%, and 58% respectively. The BYDV treatment was achieved by allowing 25 viruliferous BCO aphids to feed for two days. For aphid only treatments, 25-30 aviruliferous BCO aphids had feeding durations of ten days. The same feeding duration was used for the aphid/BYDV treatment. However, the effect of 25 aphids feeding for two days used for the BYDV treatment was not considered and may have confounded the results in this study.

The BCO aphid appears to significantly contribute to damage of wheat as a result of feeding in addition to being a vector of BYDVs. In Oklahoma, BCO aphids are found at low infestation levels in the fall, and little is known about the role of these levels in the BYDV/aphid complex. Hence, the purpose of this study was to determine the effects of low-level aviruliferous BCO aphid infestation levels and short feeding durations on seedling roots and shoots and yield of winter wheat.

METHODS AND MATERIALS

Aphid colonies

For this study, a BCO aphid colony was obtained from the USDA-ARS (Stillwater, OK), and was maintained on 'Karl 92' wheat seedlings grown at 16.5C with a 16:8 photoperiod in 10 in. plastic pots caged with clear plastic tubes.

Growth chamber (GC) variability

A preliminary experiment was conducted to test variability of wheat seedling growth in the growth chamber (GC) used in the experiments. Twelve positions were designated in a 3 row x 4-column matrix (Fig. 3.01). Twenty days after planting, seedling roots and shoots were scanned, and root and shoot lengths were measured with Rootedge software (Kaspar and Ewing, 1997). Results were analyzed using the proc mixed procedure (SAS Institute, 1998). The analysis indicated a row by column interaction for both shoot and root length. Because variability in root and shoot length was observed, a Latin square design

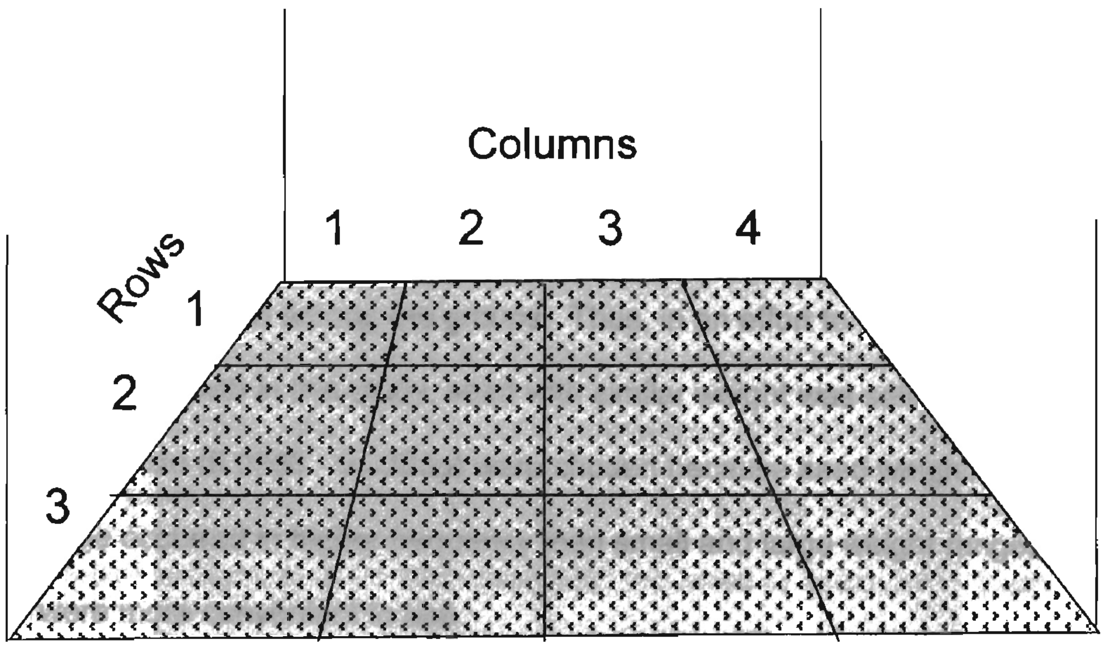

Fig. 3.01 Spatial arrangement of caged pots in an experiment designed to determine effects on wheat seedling growth as a result of variability in the growth chamber. There were 12 cages (4 columns X 3 rows). Each caged pot contained 3 seedlings.

was used in this study in an effort to negate the effect of treatment location in the GC.

Effect of seed size on growth uniformity

A preliminary study was conducted to determine the effects of seed size on growth uniformity. One hundred Karl 92 wheat seeds were weighed and divided into 3 groups: small (0.0351 – 0.0367 g/seed); medium (0.0368 - .0384 g/seed); and large seed (0.0385 – 0.0399 g/seed). After 10 days, visual observations of seedlings grown hydroponically from these seeds revealed differences in growth among the groups. Thus, in an effort to further minimize variability in growth in this study, seed with similar weight was selected. Seeds of medium size (0.0368 - 0.0383g) were chosen because seed weighing within this range occurred in higher frequency than small and large weight ranges, and thus were more convenient to use.

Experimental plants

Sixty-four seeds of the hard red winter wheat 'Karl 92' were planted in clear hydroponic plastic pouches (Mega-International, Minneapolis, MN), one seed per pouch. Each pouch was modified by punching 2 holes at the base to allow water uptake, and then wrapped with aluminum foil to prevent light from reaching the roots. Four aluminum stands (Mega-International, Minneapolis, MN) were used to support the pouches (16 pouches per stand). Each stand was placed in a 4 in. x 4 in. plastic tub. A solution (1g/L) of Peters 20-10-20 fertilizer

+ micronutrients was prepared, and 1.5 L was dispensed in each tub. Tubs were covered with 24 in. x 6 in. x 6 in. aluminum cages and placed in the GC (Conviron PG W 36) at 16.5C with a 16:8 photoperiod. Light intensity measured 192.1μ $Es^{-1}m^{-2}$ at 6 in. above the GC floor. Hydroponic solution levels were maintained daily for each tub. Ten days post planting, 48 seedlings were visually selected for treatments based on uniform foliage growth.

Application of treatments

Ten-day-old seedlings were infested with 10, 20, or 30 aviruliferous BCO aphids per seedling for 2, 4, 6, 8, or 10 days and returned to the growth chamber. There were 16 treatments; one for each combination of BCO aphids and duration of feeding (=15 treatments) plus a control with zero aphids was included. Alphabetical assignments for each treatment are listed in Table 3.01. Each seedling represented one subsample. Each treatment had three subsamples, which were present in one cage. Four replications of the experiment were conducted through time in the same GC. Due to variability in root and shoot length in the GC described previously, each treatment was subjected to a different row and column position for each replication. Thus, all treatments were subjected to all rows and columns. Fig. 3.02 illustrates the positions of treatments in the GC for all four replications.

Table 3.02 illustrates how treatments I and N, L and P, A and B, and H and J have equal aphid days [number of aphids X feeding duration (days)] and can be compared. For example, treatment I consisted of 10 aphids feeding for 4

Table 3.01 Treatments (A-P) as defined by the number of aphids infested per seedling and their feeding duration (days).

Treatment	Number of BCO Aphids	Feeding Duration (days)	*Aphid Days
A	20	4	80
B	10	8	80
C	30	10	300
D	10	2	20
E	20	8	160
F	30	6	180
G	30	8	240
H	20	6	120
I	10	4	40
J	30	4	120
K	0	0	0
L	10	6	60
M	20	10	200
N	20	2	40
O	10	10	100
P	30	2	60

* Aphid days is a product of the number of aphids and their feeding duration (days). Some treatments have equal aphid days, but differ by the equation components.

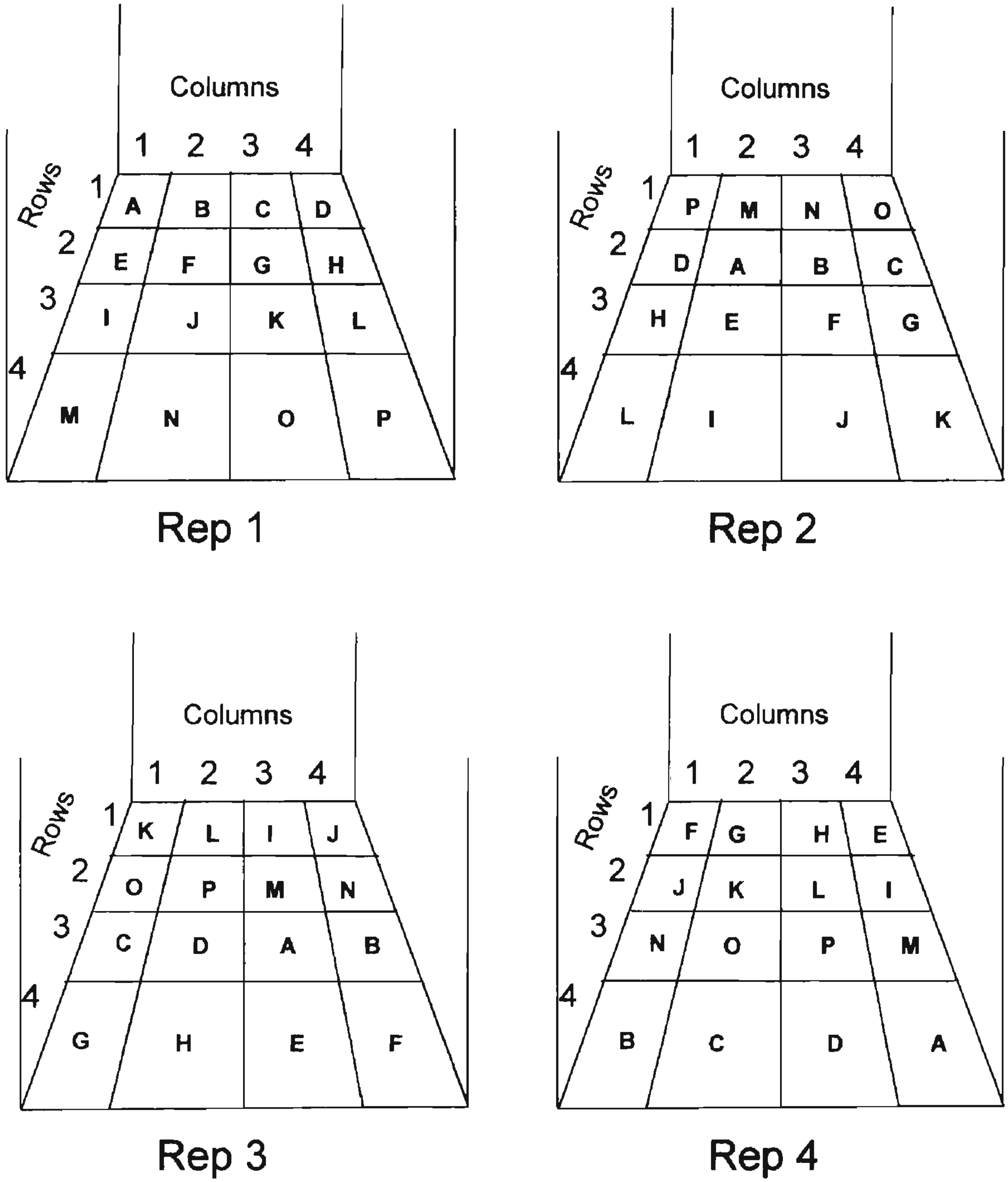

Fig. 3.02 Spatial arrangement of treatments (A-P) in the growth chamber for each replication. This Latin square design ensures that each treatment is not subjected to the same row and column for all replications.

Table 3.02 Treatments I and N, L and P, A and B, and H and J have equal aphid day values and can be compared, while the numbers of aphids and feeding durations differ.

Treatment	Number of BCO Aphids	Feeding Duration (days)	*Aphid Days
A	20	4	80
B	10	8	80
C	30	10	300
D	10	2	20
E	20	8	160
F	30	6	180
G	30	8	240
H	20	6	120
I	10	4	40
J	30	4	120
K	0	0	0
L	10	6	60
M	20	10	200
N	20	2	40
O	10	10	100
P	30	2	60

* Aphid days is a product of the number of aphids and their feeding duration (days). Some treatments have equal aphid days, but differ by the equation components.

days, and treatment N consisted of 20 aphids feeding for 2 days. Both treatments received 40 aphid days, yet the number of aphids and feeding durations for treatments I and N differ. Such comparisons may help determine the limitations of the aphid-day term.

Nymphs were removed daily to maintain original infestation levels. At the end of a feeding duration for each treatment, aphids were killed using malathion insecticide. At 20 days after planting, roots and shoots from seedlings were scanned using a Hewlett Packard 5100c desktop scanner, and Rootedge software was used to quantify root and shoot lengths (total additive linear lengths of roots and shoots) (Kaspar and Ewing, 1997). Immediately after procuring these measurements, seedlings were transplanted into 6 in. clay pots with Scotts Metro-Mix 702 growing medium and vernalized at 10C for 8 weeks in a walk-in cold chamber. After vernalization plants were grown to maturity in a greenhouse. For each treatment, number of heads, number of seeds, and grain weight were recorded. Both seedling and post-harvest data were analyzed using the proc mix and proc reg procedures (SAS Institute, 1998).

RESULTS

Growing seedlings hydroponically in pouches greatly enhanced the ability to observe effects of BCO aphids on the roots of wheat seedlings (Fig. 3.03). Results (probability values) from the statistical analysis of all data are presented

Treatment "K" Control 0 aphids infested	Treatment "C" 30 aphids for 10 feeding days

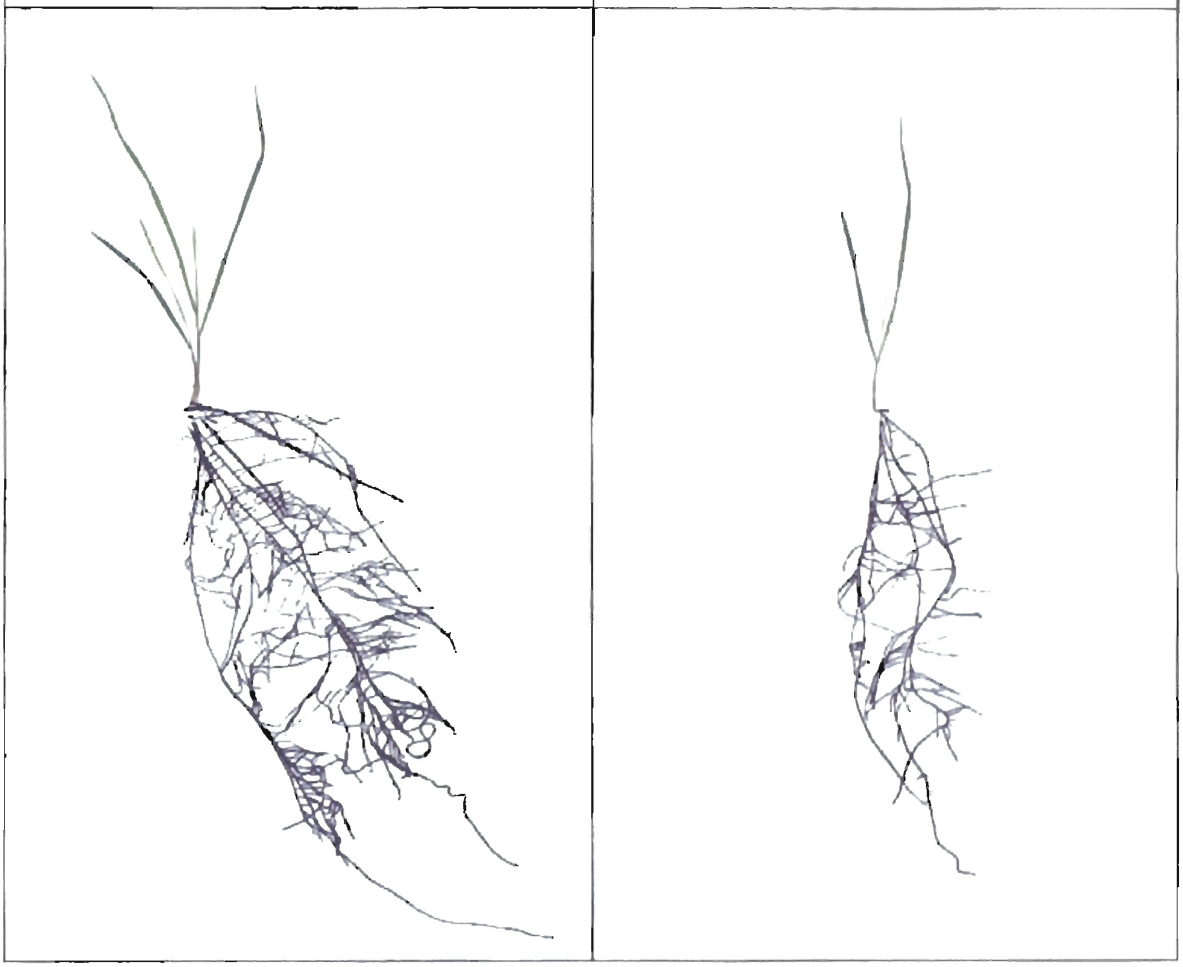

Fig. 3.03 Visual comparison of the control, treatment "K" (0 aphids infested) and treatment "C" (30 aphids infested for 10 days) on wheat seedlings.

in Table 3.03. The effects of aphid incidence and feeding duration on root and shoot length, number of heads, number of seeds and grain weight were assessed using contrasts in analysis of variance (ANOVA) with Proc Mixed (SAS, Cary, NC). Seven contrasts were created, one for the main effect of aphid incidence (2 df), one for the main effect of feeding duration (4 df), one to access aphid incidence by feeding duration interaction (8 df), and 4 to compare equivalent aphid incidence-feeding duration combination (aphid days). The 4 contrasts for aphid days were to compare treatments that received 80 aphid days, 120 aphid days, 40 aphid days, and 60 aphid days.

Regression using Proc Reg (SAS, Cary, NC) was performed to assess the numeric relationship that root and shoot length had to aphid incidence and feeding duration. This was done in addition to the ANOVA models because both aphid incidence and feeding duration are numeric variables and regression better illuminates these relationships. Interaction of aphid incidence and feeding duration were fit and if not significant, later removed to maintain a simpler model. Dummy variables were created to compare slopes as associated with the different levels of the factors in question.

This analysis indicated a significant interaction between the number of aphids and feeding duration on both root and shoot length of seedling wheat, but no effect by feeding duration on root or shoot length, and only an effect on root length by number of aphids. Because of this interaction, the effect of feeding duration at each aphid infestation level was examined, and revealed no significant effect on feeding duration at 10 aphids, but there was a significant

Table 3.03 Probability values from regression analysis examining the effect of number of aphids and feeding duration on root and shoot length, and on number of heads, number of seeds, and grain weight. Values are interpreted as significant (S, P≤.05) or not significant (NS, P>.05).

S = Not Significant NS = Not Significant	**Root Length (seedling)**	**Soot Length (seedling)**	**Number of Heads**	**Number of Seeds**	**Grain Weight**
Number of Aphids (NA)	S P=.0064	NS P=.7313	S P=.0239	S P=.0217	S P=.0199
Feeding Duration (FD) (days)	NS P=.3891	NS P=.2556	S P=.0027	NS P=.1269	NS P=.0501
NA X FD	S P=.0001	S P=.0011	NS P=.3563	NS P=.8376	NS P=.9269
Feeding Duration (10 aphids)	NS P=.2524	NS P=.3169			
Feeding Duration (20 aphids)	S P=.0017	S P=.0069			
Feeding Duration (30 aphids)	S P<.0001	S P=.0002			
Number of Aphids (2 days)	S P=.0167	NS P=.2937			
Number of Aphids (4 days)	S P<.0001	S P=.0030			
Number of Aphids (6 days)	S P<.0001	S P=.0251			
Number of Aphids (8 days)	S P=.0004	S P=.0038			
Number of Aphids (10 days)	S P<.0001	S P<.0001			

effect of feeding duration on root and shoot length at 20 and 30 aphids (Table 3.03). The regression lines for these data are presented in Fig. 3.04 (root length) and 3.05 (shoot length). The control (0 aphids infested) was included in each regression, and is indicated by similar y-intercepts among regression lines.

The analysis also indicated that number of aphids significantly affected root length at all feeding durations (2, 4, 6, 8, and 10 days), and also significantly affected shoot length at feeding durations of 4, 6, 8, and 10 days (Table 3.03). The regression lines for these data are presented in Fig. 3.06 (root length) and 3.07 (shoot length).

The interaction of number of aphids and feeding duration was not significant for number of heads, number of seeds, and grain weight (Table 3.03), so the effects of number of aphids and feeding duration could be examined directly. This analysis indicated that number of aphids affected number of heads, seed number, and grain weight (Table 3.03, Fig. 3.08, 3.09, and 3.10). However, feeding duration only affected the number of heads (Table 3.03 and Fig. 3.11). Regression lines presented in Figs. 3.8, 3.9, 3.10, and Fig. 3.11 represent effects for all feeding durations (pooled data).

Treatments I and N, L and P, A and B, and H and J had equal number of aphid days (Table 3.02). Thus, root and shoot lengths from such treatments were compared. No significant differences in root and shoot lengths were identified in the analysis. Hence, the aphid day term accurately reflected the combined effects of number of aphids and feeding duration on root and shoot lengths.

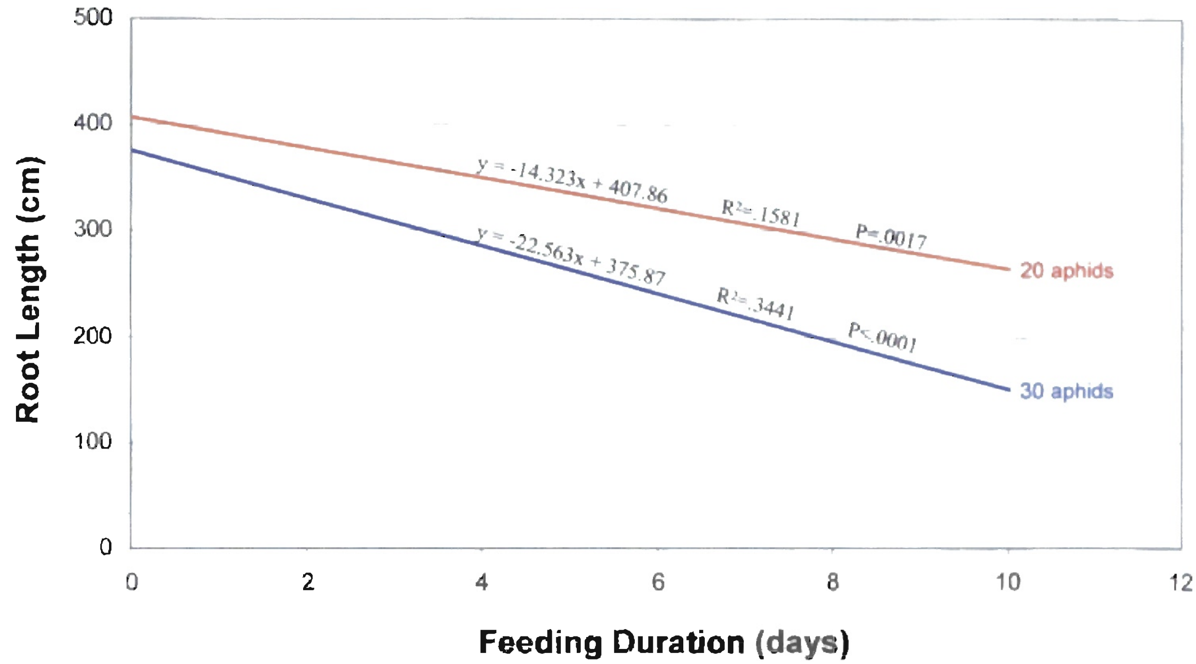

Fig. 3.04 Regression of number of aphids (20 or 30) on root length for 0, 2, 4, 6, 8, and 10 days feeding.

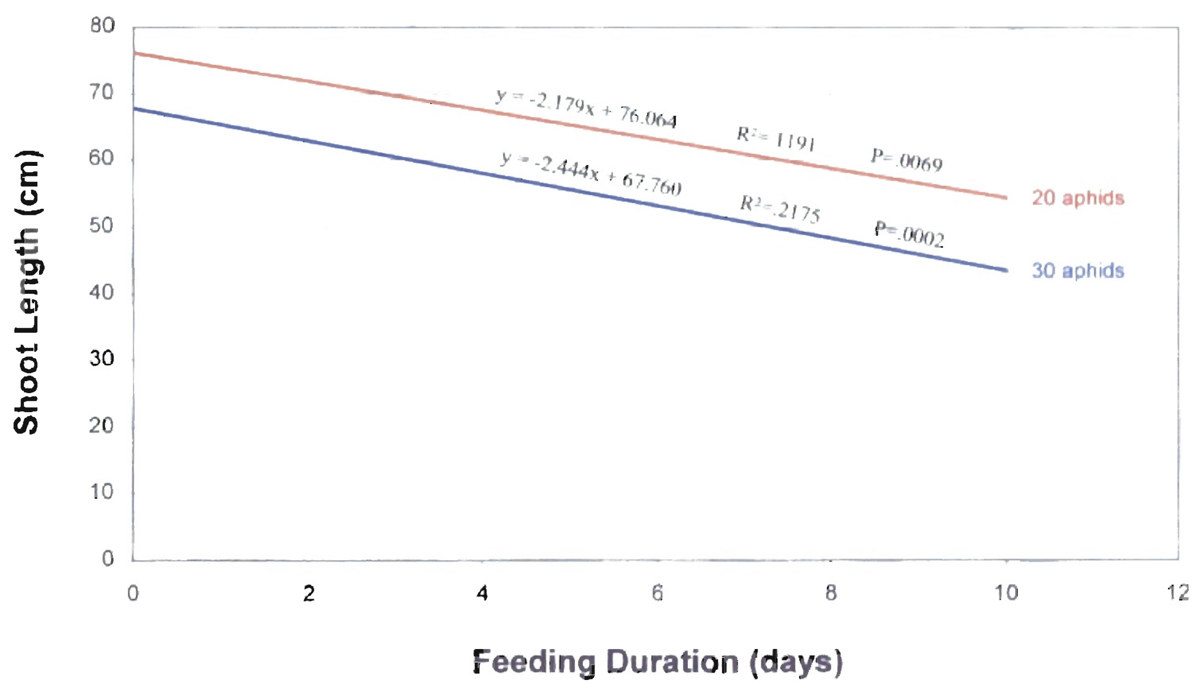

Fig. 3.05 Regression of number of aphids (20 or 30) on shoot length for 0, 2, 4, 6, 8, and 10 days feeding.

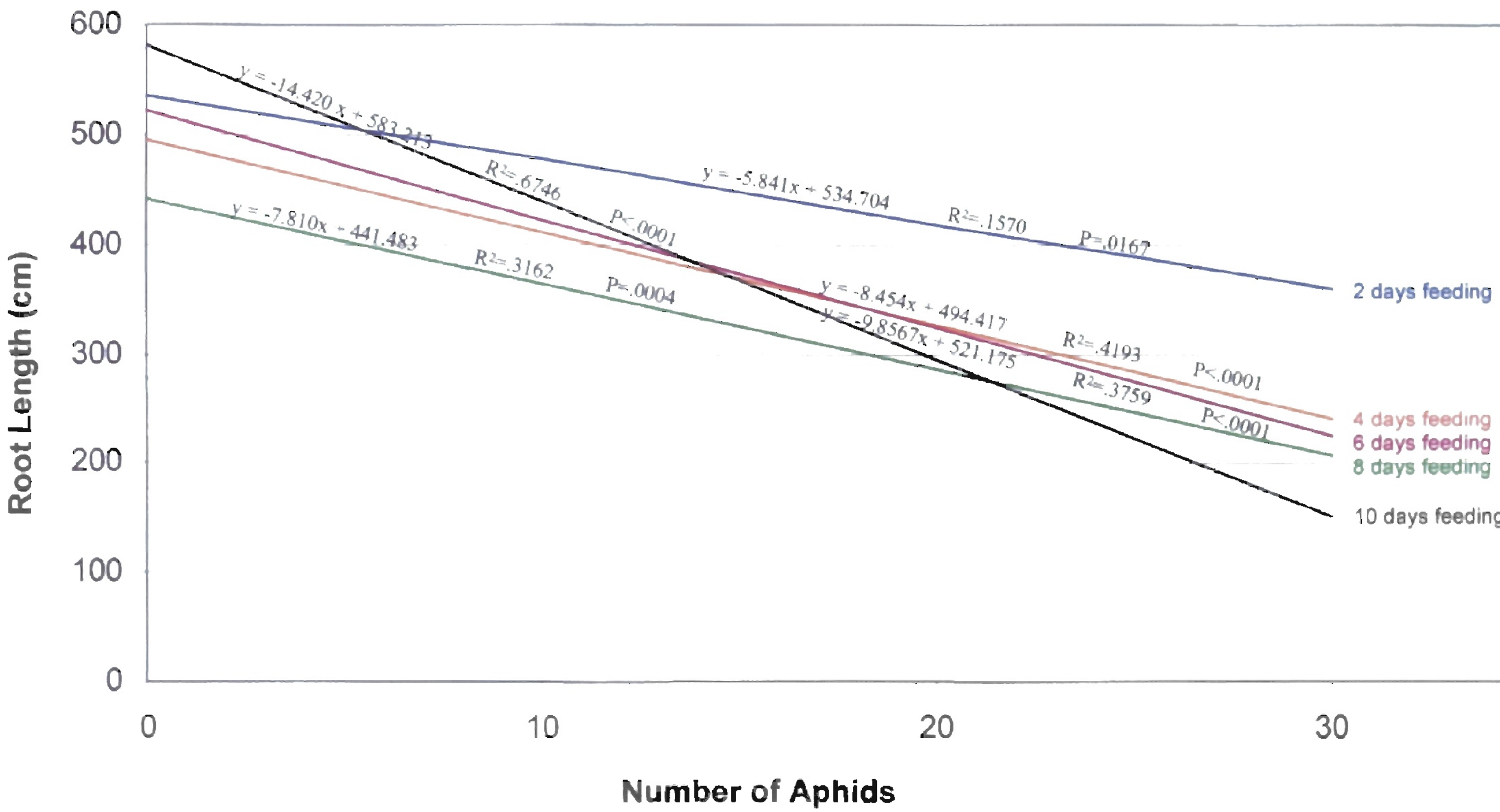

Fig. 3.06 Regression of number of aphids on root length after 2, 4, 6, 8, and 10 days feeding.

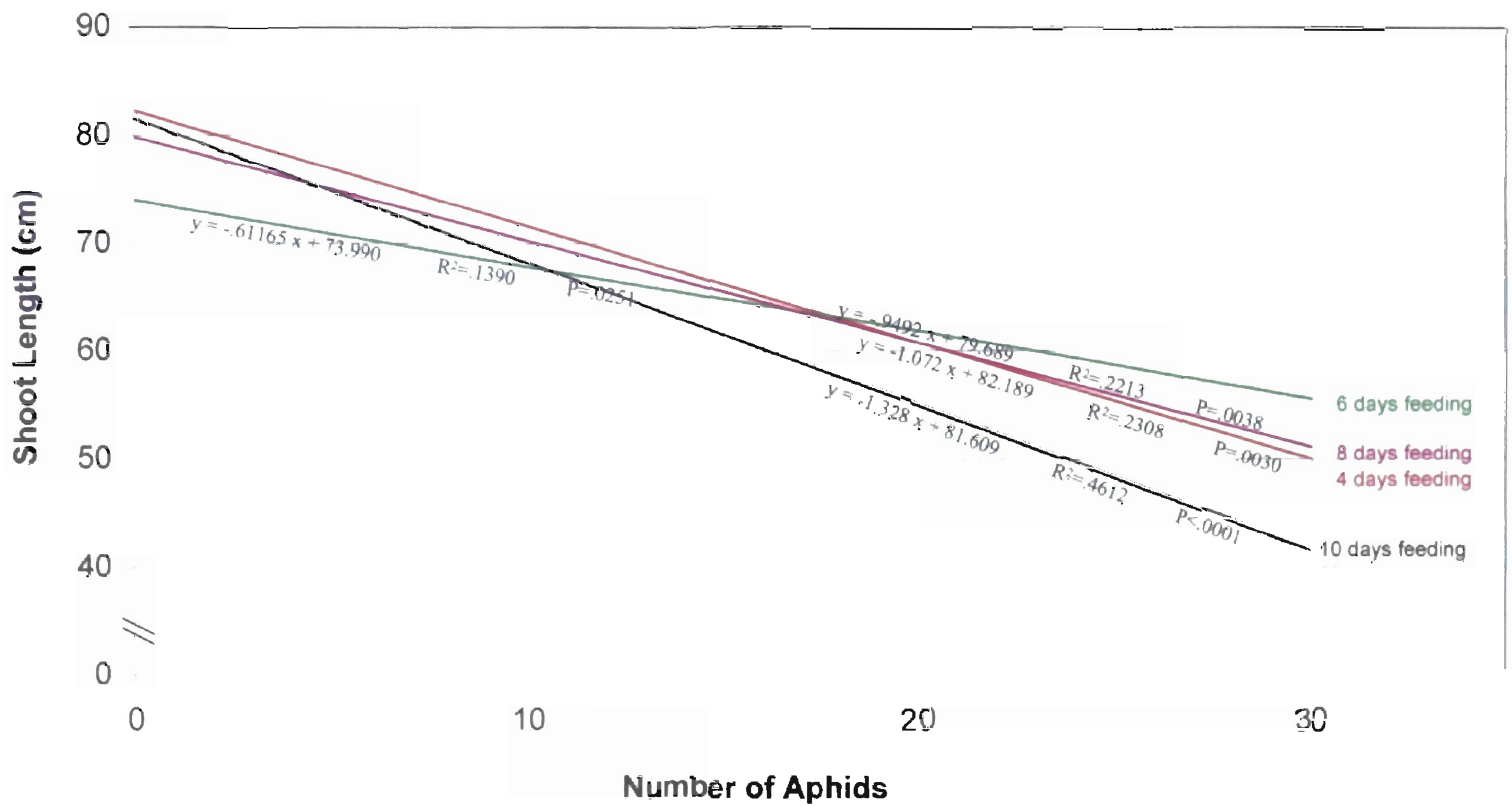

Fig. 3.07 Regression of number of aphids on shoot length for 4, 6, 8, and 10 days feeding.

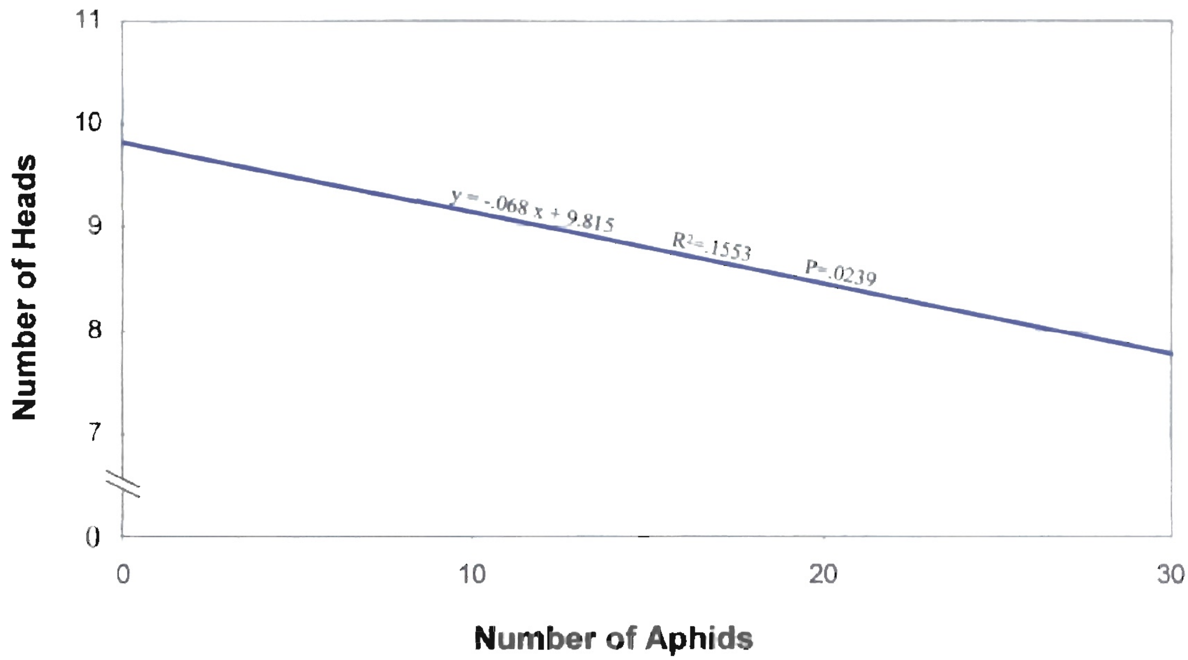

Fig. 3.08 Regression of significant effects of number of aphids on number of heads.

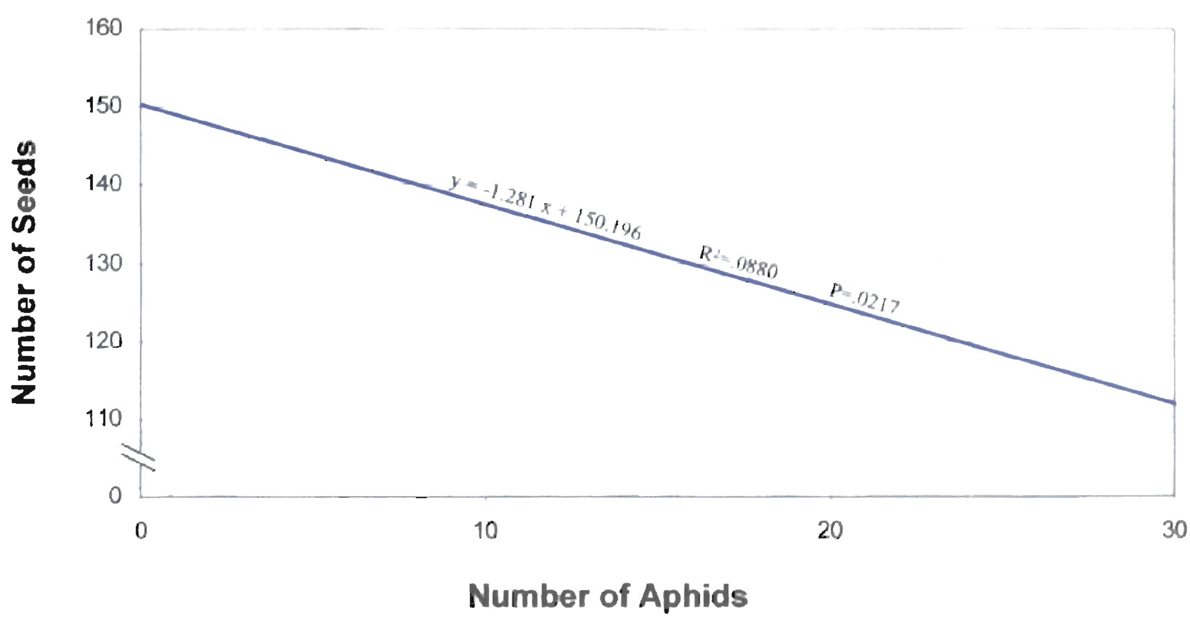

Fig. 3.09 Regression of significant effects of number of aphids on number of seeds.

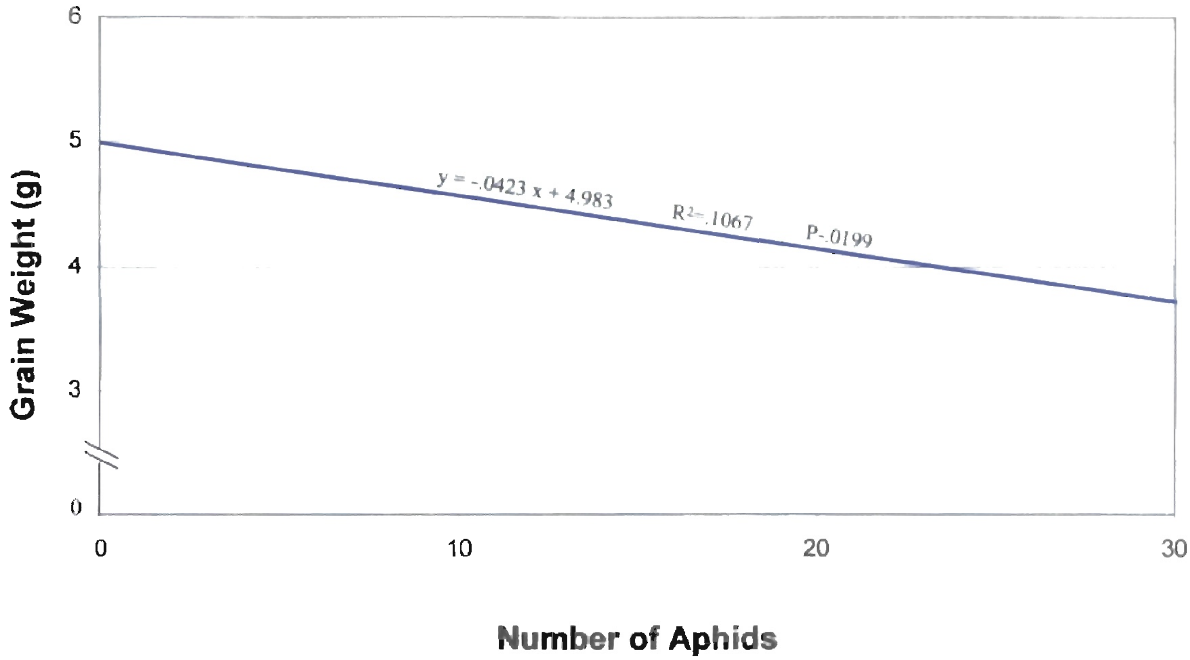

Fig. 3.10 Regression of number of aphids on grain weight.

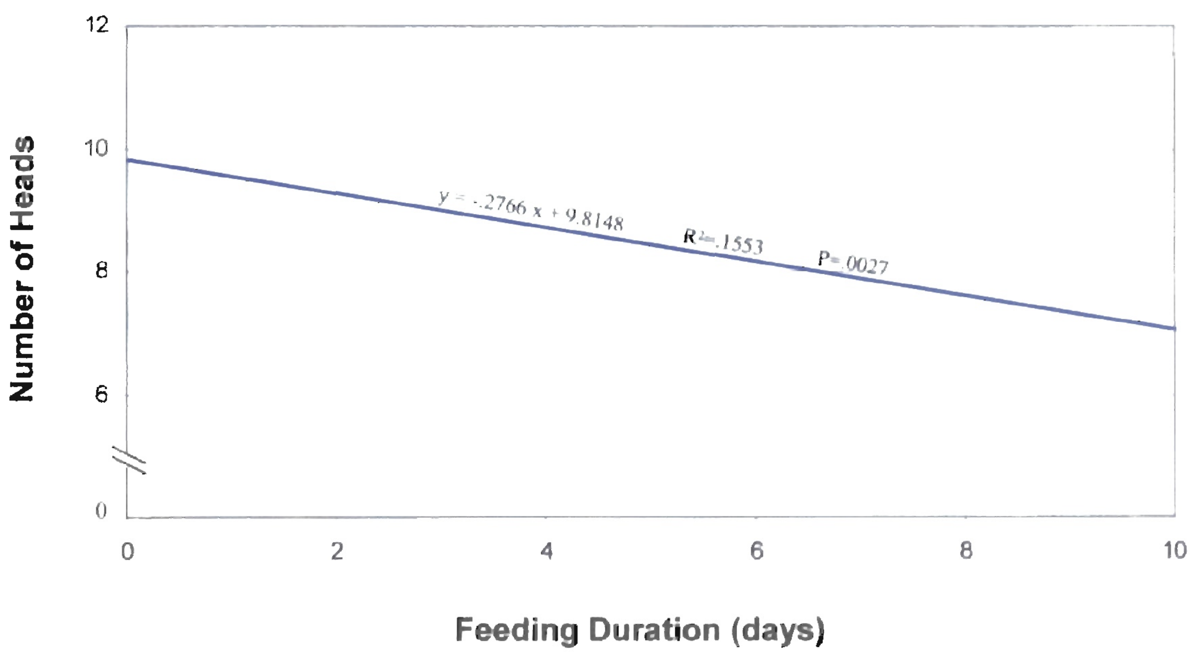

Fig. 3.11 Regression of feeding duration on number of heads.

DISCUSSION

The system of growing seedlings hydroponically in transparent pouches used in these experiments provided an excellent mechanism by which to observe the effects of BCO aphids on root and shoots of seedling wheat. This system also allowed for easy maintenance of aphid infestation levels, and most importantly, facilitated the non-destructive application of Rootedge software (Kaspar and Ewing, 1997) to quantify seedling root and shoot length as affected by aphids. Additionally, this system allowed transplanting seedlings into soil following evaluation of root and seedling growth; however, not all seedlings survived, and it seemed that seedlings weakened by aphid infestation were the most susceptible to death following transplanting.

Results from this study suggested that low infestation levels of BCO aphids damaged wheat. The effects of number of aphids and feeding duration were greater on seedling roots and shoots than on yield and yield components. This finding suggests winter wheat may recover from early damage caused by such infestations, which is consistent with previous studies (Kieckhefer and Gellner, 1982; Kieckhefer *et al*, 1995; Pike and Schaffner, 1985; Riedell and Kieckhefer, 1995; Riedell *et al*, 1999).

However, the significant effect of feeding duration on number of heads and insignificant effect on number of seed and grain weight may be indicators that yield is compensated by possible increases in number of seed per head or heavier grain. In contrast, Kieckhefer and Gellner (1982) found reductions in tillers caused by BCO aphids.

Riedell *et al* (1999) suggested that additional environmental stress factors that occur in the field such as freeze damage may result in increased injury caused by aphids and BYD. Stresses such as this do not occur in a controlled environment. In Oklahoma, BCO aphid infestation levels of 20 or 30 aphids per plant are unlikely to occur on 10-day-old seedlings. However, stress factors in addition to longer feeding durations (>10 days) may result in significant yield reductions caused by BCO aphid infestation levels as few as 10 aphids per plant.

BCO aphids, as virus vectors, and their injurious effects on HRWW as phloem feeders, are key components to the BYD complex where BCO aphids occur. Other components, such as the BYDVs involved in disease, timing of aphid infestations and virus infections, environmental conditions, and their synergistic effects as they work together in a complex equation, further challenge our ability in the understanding of BYD. Hence, the findings reported in this study indicate that low-level aviruliferous BCO aphid infestations injure wheat seedlings, which should be recognized in future studies identifying the affects of the BYDV complex when BCO aphids are used.

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Chapter IV

Control of Aphids and Barley Yellow Dwarf by Planting Mixtures of Gaucho Insecticide-Treated and Untreated Seed

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Abstract

In this study, a potential method of controlling aphids and barley yellow dwarf (BYD) on hard red winter wheat (HRWW) was investigated using mixtures of Gaucho-insecticide treated and untreated wheat seed. The use of such mixtures, if effective, could result in lower costs associated with the insecticide used. Two field plots were planted near Stillwater, Oklahoma on 19-Sep and 20-Oct-97 with seed of the HRWW variety Karl 92 treated with Gaucho (480F) insecticide. Nine treatments were defined by the Gaucho concentration (GC) (1, 2, and 3 oz. cwt.) and percentage of treated seed (PTS) (33, 67, and 100). Parameters measured and analyzed were aphid incidence, BYD incidence and severity, tiller height, fertile head density, grain weight and thousand-kernel weight (TKW). Increases in aphid incidence, and BYD incidence and severity, and decreases in tiller height, fertile head density, grain weight and TKW were observed in the 19-Sep-97 planted plots compared to the 20-Oct-97 planted plots. No effects of treatment or planting date X treatment interaction on any parameter were observed. However, decreases in PTS resulted in linear increases in aphid incidence and BYD severity, and linear decreases in tiller height, fertile head density, grain weight and TKW. Effect of PTS on BYD incidence was insignificant. Also, no effect of GC on any parameter, and no GC

X PTS interaction was observed. Findings in this study suggest that mixing Gaucho-treated and untreated seed results in higher aphid population levels and greater BYD incidence and severity.

Hard red winter wheat (HRWW), which is the most economically important field crop in Oklahoma, is planted in the fall, remains dormant during the winter months, and then is harvested the following summer. Insects and diseases occur every year on HRWW and often result in yield reductions. Perhaps the most destructive insects that occur on HRWW grown in Oklahoma are greenbugs (GB) (*Schizaphis gramineum*) and the bird cherry-oat (BCO) aphid (*Rhopalosiphum padi*). Populations of these aphids can explode when temperatures are warm during the growing season, and damage caused by aphids is more severe when aphid infestations occur on seedling wheat during the fall than when older plants are infested in the spring (Kieckhefer and Gellner, 1982; Pike and Schaffner, 1985).

Aphids can also injure wheat by transmitting disease-causing viruses during feeding. The most common such viruses transmitted by greenbugs and BCO aphids in Oklahoma are the barley yellow dwarf viruses (BYDVs), which cause the disease barley yellow dwarf (BYD). Symptoms of BYD on winter wheat include yellowing and/or purpling of the foliage, and stunting (D'Arcy, 1995). BYD occurs every year on HRWW wheat in Oklahoma. Yield losses caused by BYD depend on several factors. Infections that occur in the fall (seedling stages) cause greater losses than infections that occur in the spring (Hammon *et al*, 1996; McGrath *et al*, 1987; McKirdy and Jones, 1997). Numbers of viruliferous aphids, which can vary from season to season and field to field, determine the amount of potential virus inoculum present (Pike and Schaffner, 1985; Tetrault *et al*, 1963). Temperatures in the fall and spring are favorable for both aphids and virus, whereas temperatures during the winter and early summer are often too extreme to favor aphid and disease outbreaks (De Barro, 1992; Michels and Behle, 1989).

Several strategies are employed for the control of BYD. These strategies are based on the manipulation of components of the plant disease triangle: the host, the pathogen, and the environment (Agrios, 1996) (Fig. 4.01). This three-component triangle, however, may not apply to pathosystems that have a fourth component - an insect that vectors the pathogen. Because aphids are the sole vectors of BYDVs, a pyramid provides a more accurate depiction of BYD disease components (Fig. 4.02) and will be used in this discussion.

Current strategies to control BYD on HRWW with reference to the manipulation of the BYD disease pyramid components include (1) planting tolerant varieties (manipulation of the host), (2) planting late in the fall (manipulation of the environment), and (3) applying insecticides to eliminate aphid vectors (manipulation of the vector or host and vector).

(1) Although there are no wheat varieties highly resistant or immune to BYD, some varieties such as 2137, 2163, and Custer have low levels of resistance or tolerance. Thus, planting such varieties may help reduce losses from the aphid/BYDV complex.

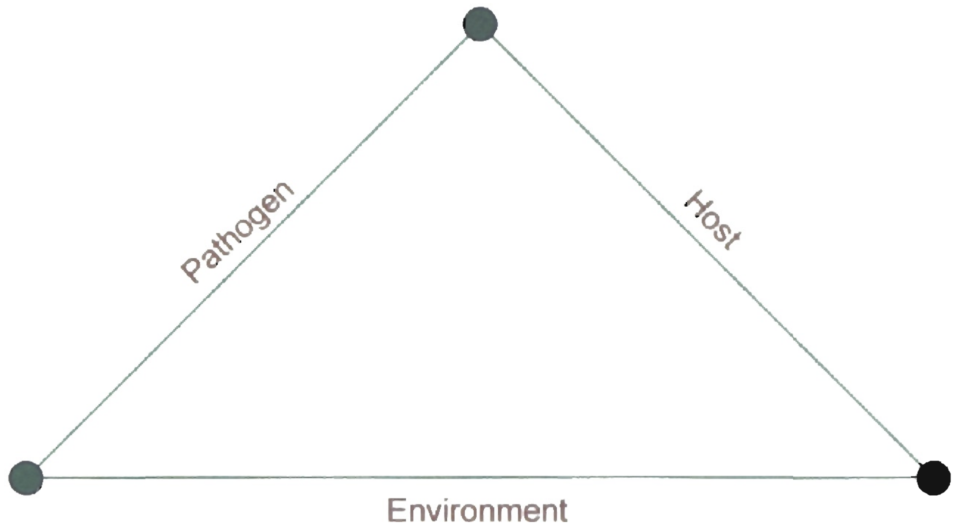

Fig. 4.01 The plant disease triangle and it's components: the host, the pathogen, and the environment. The presence of each component determines the amount of disease severity. Disease severity is high when a *virulent pathogen* is on a susceptible *host* plant in an *environment* that favors disease development.

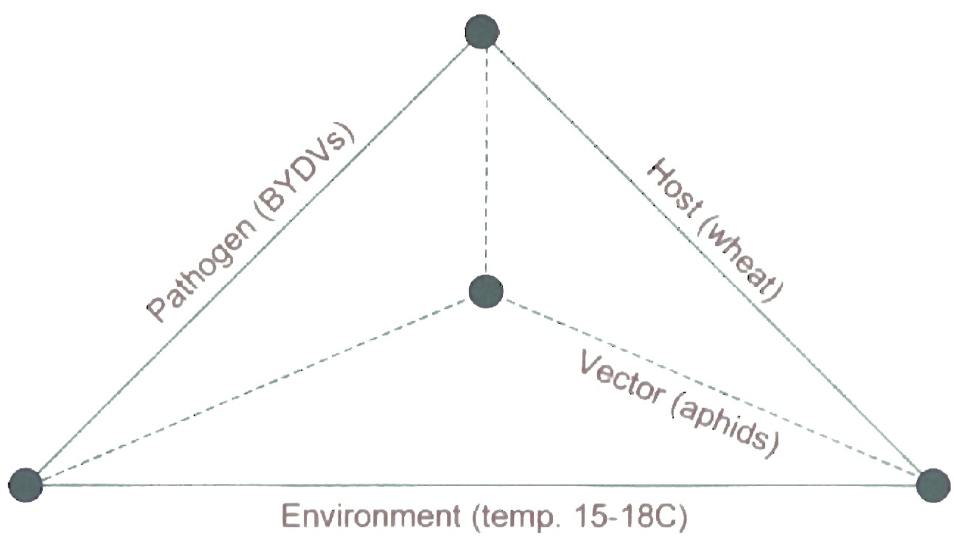

Fig. 4.02 The plant disease pyramid and it's components including the host, the pathogen, then environment and the vector of the pathogen. For barley yellow dwarf on wheat, disease is high when barley yellow dwarf viruses (BYDVs) [the pathogen(s)] are present in or nearby fields planted with susceptible varieties (the host), in addition to the presence and abundance of aphids (the vectors) that efficiently transmit the BYDVs. Temperatures ranging from 15-18C and high light intensity constitute an environment favorable for both aphids and BYDVs.

(2) Planting late in the fall tends to reduce losses from aphids and BYD (Hammon *et al*, 1996; McGrath *et al*, 1987; McKirdy and Jones, 1997). Because aphid populations are less likely to establish on late-planted wheat, lower disease incidence can be expected. More vegetation and hence protection is available for the support of aphid populations in early-planted wheat (mid-September) than in late-planted wheat (October and November). Oklahoma wheat also provides forage for cattle during the fall and winter. Farmers commonly plant wheat as early as late August in an attempt to gain such forage.

(3) BYD control can also be achieved via insecticides (Araya and Foster, 1987; Gray *et al*, 1996; Irwin and Thresh, 1990). Reductions in aphid populations result in reduced transmission of BYDVs, which in turn should translate into lower disease incidence and severity. Seed-applied systemic insecticides may provide optimal control because of their ability to prevent aphid populations from becoming established. This strategy not only involves manipulation of the vector component of the disease pyramid, but also involves manipulation of the host plant; i.e., aphids are not directly targeted with insecticides, but are rather targeted through the wheat on which they feed. Insecticides applied after the establishment of aphid colonies (vector manipulation only) may not be as effective against primary spread of BYD because primary infections may occur prior to insecticide applications. The seed-applied insecticide Gaucho (Imidacloprid, Bayer Ag, Germany) is recommended for control of aphids at rates from 1-3 oz per cwt of seed, and for reduction of BYD at rates $\geq$2.0 oz. per cwt. Treatment at these higher rates has

demonstrated effective control of aphids and BYD (Hunger *et al*, 1997). However, the cost of treating wheat seed at these rates (~ $7.00/bushel) may exceed the economic return in a weak grain market.

Imidacloprid, a nicotinoid, appears to stimulate movement followed by paralysis and incapacitation. Gourmet *et al* (1994) observed lower BCO aphid reproduction and higher fecundity with Gaucho treated oats compared to untreated oats. The initial spread of BYD increased apparently as a result of the stimulated movement associated with Gaucho. However, BYD incidence was reduced compared to the untreated oats most likely due to the rapid neurotoxic action of Gaucho.

In this study, a potential method of controlling BYD on winter wheat was investigated using mixtures of Gaucho-insecticide treated and untreated wheat seed. The use of such mixtures, if effective, could result in lower costs associated with the insecticide used. The hypothesis of this study is that mixtures of insecticide-treated and untreated seed would provide control comparable to a homogeneous seed treatment. The basis of this hypothesis relates to the theory that genetic diversity within crops contributes to insect and disease reduction. Less disease occurs within genetically diverse crops than within crops with little genetic diversity. This concept has been demonstrated using multiline cultivars as compared to monoline cultivars, using mixed cultivars as compared to single cultivars, and using mixed crops as compared to mono-crops (Andow, 1991; Browning and Frey, 1969; Power, 1991; Wolfe, 1985; Wolfe and Barrett, 1980). In all of these cases, lower disease incidence and severity occurred in crops with the greater diversity. Explanations for this observation vary depending on the disease in question; however, all hypotheses focus on the transmission of the pathogen. Lower transmission rates occur in crops that are more genetically diverse than in those crops with less diversity. In systems that involve fungal and bacterial pathogens, inoculum is typically spread by wind and/or rain to other plants. Pathogens that land on non-host or resistant plants are trapped, and unable to propagate for secondary transmission. Thus, an overall reduction in disease occurs.

However, the mechanism behind disease control in genetically diverse plantings that have systems that involve insect vectors of pathogens is more complex and less documented. Unlike abiotic vectors (i.e., wind and rain), insects may choose their host when migrating from plant to plant. Aphids tend to emigrate after having moved on an undesirable host or non-host plant, sometimes resulting in long distance movement and thus leaving the crop (Power, 1990). Furthermore, aphids in multi-line crops feed on more plants in a given time than those feeding on mono-line crops. Feeding on more plants means less time on plants, which may interfere with the acquisition and transmission of viruses (Power, 1991). These theories of mechanisms for such reduction in transmission may work in wheat fields that have mixtures of plants that are treated and untreated with insecticide. Viruliferous aphids that migrate to Gaucho-treated plants may transmit BYDVs to those plants, but quickly die as a result of insecticide. Thus, secondary spread of BYD would be limited.

Hence, experiments were designed and conducted to determine if treating less seed at a higher Gaucho concentration provides less, equal, or better control of aphids and BYD than treating more seed at lower Gaucho concentration, while the total amount of Gaucho applied remains equal.

METHODS AND MATERIALS

Field Plots. Two field plots were planted near Stillwater, Oklahoma on 19-Sep and 20-Oct-97 with seed of the HRWW variety Karl 92 treated with Gaucho (480F) insecticide (Gustafson, Inc., Plano, TX) using a seed treater (Hege, unknown model) as indicated in Table 4.01. Treatments resulted in nine Gaucho-treated seed mixtures plus an untreated control (0 oz. Gaucho). Plots were arranged in a randomized-block design with 5 replications (Fig. 4.03a and 4.03b). Each plot consisted of three, 5-ft. rows opened with a five-row small grains drill (H & N Equip, model 547) with 8.5 in. spacing. Oats were planted in the two outside rows in an effort to attract aphids and to increase the incidence and severity of BYD. Wheat seed was hand planted in the center three rows about 1/2-3/4 in. deep at a rate of (close to) 1.50 bushels per acre. On 24-Nov-97, Glean herbicide (E.I. du Pont de Nemours and Co., Inc., Wilmington, DE) was applied at both plots at 1/3 oz. per acre in 20 gal. of water for weed control, and on 28-Apr-98, Tilt (Novartis, Greensboro, NC) was applied at 4 oz. per acre in 20 gal. of water to both plots to limit powdery mildew, rust, and other foliar fungal diseases.

Table 4.01 Treatments (1-10) as defined by the concentration of Gaucho (1, 2, and 3 oz. cwt.) and percentage of seed treated and untreated.

Treatment Number	Gaucho Rate (oz/cwt)	% Seed*	
		Treated	Untreated
1	0	0	100
2	1	33	67
3	1	67	33
4	1	100	0
5	2	33	67
6	2	67	33
7	2	100	0
8	3	33	67
9	3	67	33
10	3	100	0

* Represents mixtures of Gaucho treated and untreated seed.

Fig. 4.03a Diagram of early-planted (19-Sep-97) field plots. The randomized block design consisted of 10 treatments (north to south) and 5 replications (east to west).

5	4	1	10	5
6	5	5	4	1
8	8	9	1	10
9	6	4	8	3
3	1	2	2	7
4	10	3	3	6
7	2	8	5	9
10	3	7	7	8
1	7	10	6	2
2	9	6	9	4
Rep 1	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**

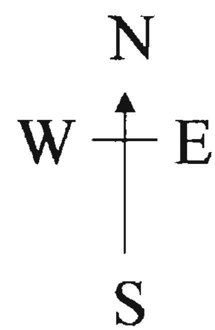

Fig. 4.03b Diagram of late-planted (20-Oct-97) field plots. The randomized block design consisted of 10 treatments (south to north) and 5 replications (west to east).

9	9	6	6	2
1	2	10	9	10
5	1	7	7	3
4	7	8	5	6
7	10	3	3	1
10	4	1	2	8
2	3	4	8	7
3	6	9	1	5
8	8	5	4	9
6	5	2	10	4
Rep 5	**Rep 4**	**Rep 3**	**Rep 2**	**Rep 1**

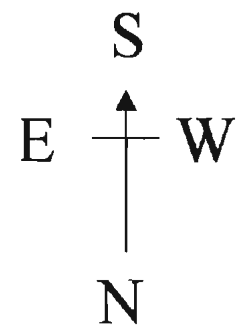

Table 4.02 illustrates how treatments 3 and 5, 4 and 8, and 7 and 9 have equal amounts of Gaucho, but differ in the amount of seed treated with Gaucho at the designated rate. For example, treatment 4 consisted of 33% Gaucho-treated seed at a 3 oz. rate, and treatment 8 consisted of 100% Gaucho treated seed at a 1 oz. rate. Both treatments received a 3 oz. rate. However, the use of mixtures of Gaucho-treated and untreated seed alters the distribution of Gaucho: While a percentage of seed remains untreated, there is a corresponding increase in concentration of treated seed. As mentioned above, the total amount of Gaucho for each treatment remains constant.

Aphid incidence and BYDV presence. For both planting dates, aphid incidence was determined by counting the number of BCO aphids and GB in a randomly selected linear ft. of row in each plot on 20-Nov-97, 26-Nov-97, 11-Dec-97, 19-Dec-97, 02-Jan-98, 19-Jan-98, 03-Feb-98, 20-Feb-98, 06-Mar-98, 24-Mar-98 and 10-Apr-98. Presence of BYDVs was determined by testing samples with double antibody sandwich (DAS) Enzyme Linked Immunosorbent Assay (ELISA) kits (Agdia Incorporated, Elkhart, Indiana) using polyclonal antibodies. Negative controls were prepared from healthy Karl 92 seedling leaves. Samples for ELISA were collected from each treatment on 29-May-98 and 03-Jun-98 Each sample consisted of five leaves exhibiting strong BYD symptoms, and was assayed for BYDV-rpv, -rmv, and –pav, because prior testing indicated that these three BYDVs were the most common in Oklahoma. Sample assays for each virus were conducted twice. Optical absorbance was

Table 4.02 Treatments 3 and 5, 4 and 8, and 7 and 9 have equal amounts of Gaucho and can be compared, while the combinations of percent treated and untreated seed differ.

Treatment Number	Gaucho Rate (oz/cwt)	% Seed*	
		Treated	Untreated
1	0	0	100
2	1	33	67
3	1	67	33
4	1	100	0
5	2	33	67
6	2	67	33
7	2	100	0
8	3	33	67
9	3	67	33
10	3	100	0

* Represents mixtures of Gaucho treated and untreated seed.

determined with a microplate reader (BIO-TEK Industries, Atlanta, Model EL-307). BYD disease incidence and severity were rated on 24-Apr and 08-May. Disease incidence was determined by examining ten flag leaves for BYD symptoms that were randomly selected from the middle row of each treatment. Disease severity was determined by visual ratings using the following scale: 1=no symptoms; 2=trace to slight discoloration of leaves, no stunting; 3=moderate discoloration, slight stunting; 4=extensive discoloration, moderate stunting; and 5=severe discoloration, severe stunting. On 20-May, tiller height and fertile head density were quantified. Tiller height was determined by selecting ten tillers randomly for each treatment and measuring the height of these tillers from the ground to start of the head. Fertile head density was measured by counting the number of fertile heads in a randomly selected linear ft. of row in each plot. The middle row of each treatment was harvest by hand, and total grain weight and thousand-kernel weight were quantified.

RESULTS

Mild temperatures and sufficient moisture during the fall and winter of 1997-98 provided ideal conditions for growth of plants in both the early- and late-planted plots. Plants in the early-planted plots, which were never mowed to simulate grazing, were significantly damaged by a freeze that occurred on 21-Mar-98. The freeze reduced plant fertility in the early-planted plots, which confounded results for grain yield, thousand-kernel weight, and fertile head density. Plants in the late-planted plots were not affected by the freeze because these plants were not as physiologically mature as plants in the early planted plots when the freeze occurred.

Probability values generated from this analysis for all research parameters measured are presented in Table 4.03. All data in this study was analyzed using the general linear models procedure of SAS (SAS Institute, 1998). Aphid response was transformed using the natural log transformation. Measurements were taken at eleven different time points, so repeated measures analysis was necessary. PROC GLM (SAS Institute, Cary, NC) was used to assess the effects of treatment, time, and the treatment by time interaction. Further models were used that utilized the numeric nature of treatment in order to gain inferences of the relationships of aphid incidence, BYD incidence and severity, tiller height, number of heads, and grain weight to percent treated seed and Gaucho rate. This relationship described further with the use of regression techniques.

Aphids

All plots relied on natural infestation by aphids, and results were measured and are presented as "aphid incidence." In determining aphid incidence, all types of aphids present were counted; however, the majority of the aphids present were BCO aphids. Other than this aphid, GB were occasionally observed in the plots. Aphid incidence of the BCO aphid alone was used in the analysis because

Table 4.03 Probability values from general linear models procedure examining the effect of planting date, treatment, time, percent treated seed, and Gaucho concentration on bird cherry-oat (BCO) aphid incidence, barley yellow dwarf (BYD) incidence and severity, tiller height, fertile head density, grain weight, and thousand kernel weight (TKW).

S = Not Significant NS = Not Significant	BCO Aphid Incidence	BYD Incidence	BYD Severity	Tiller Height	Fertile Head Density	Grain Weight	TKW
Planting Date (PD)	S P<.0001	S P<.0001	S P=.0003	S P=.0324	S P=.0004	S P<.0001	S P<.0001
Treatment (TRT)	NS P=.3122	NS P=.3152	NS P=.3251	NS P=.1382	NS P=.2659	NS P=.2376	NS P=.2609
PD X TRT	NS P=.5215	NS P=.1179	NS P=.1179	NS P=.9262	NS P=.1275	NS P=.4020	NS P=.4090
Time	S P<.0001						
PD X Time	NS P=1.000						
Date		S P<.0001	S P<.0001				
TRT X Date		NS P=.7256	NS P=.9264				
Percent Treated Seed (PTS)	S P=.0215	NS P=.0825	S P=.0468	S P=.0017	S P=.0139	S P=.0248	S P=.0367
Gaucho Conc. (GC)	NS P=.3620	NS P=.4681	NS P=.4599	NS P=.5896	NS P=.6760	NS P=.9969	NS P=.5541
PTS X GC Interaction	NS P=.4585	NS P=.2303	NS P=.2193	NS P=.8199	NS P=.3418	NS P=.2453	NS P=.8596

low GB infestation levels were observed (usually <10 greenbugs/ft of row) and did not contribute to the analysis.

Fig. 4.04 illustrates increases and decreases in BCO aphid population levels throughout the season at both planting dates. BCO aphids were first observed on plants in the early-planted (19-Sep-97) plots on 26-Nov-97 at an incidence of <10 aphids/ft of row. BCO aphid incidence remained at this level until late February, at which time BCO aphids increased to nearly 10,000/ft. of row by early April. In contrast, BCO aphids were first observed on plants in the late-planted (20-Oct-97) plots on 06-Mar-98, and remained at <10 BCO aphids/ft of row through March and April. By 21-Apr-98, BCO aphid populations in both plots were drastically reduced, and no BCO aphids were observed in either plot through the remainder of the season.

BCO aphid incidence was significantly higher in the early-planted (19-Sep-97) plots than the late-planted (20-Oct-97) plots (Table 4.03), but there was no effect of 'treatment' (TRT) or interaction of 'planting date' (PD) X TRT on BCO aphid incidence. BCO aphid population levels significantly increased through time ('Time') (Table 4.03, Fig. 4.05). Data in Fig. 4.05 was generated by combining BCO aphid incidence of both PDs (19-Sep-97 and 20-Oct-97), which was done because no PD X Time interaction was observed.

The effects of 'Percent Treated Seed' (PTS) and 'Gaucho Concentration' (GC) were also analyzed. Increasing PTS corresponded to a significant linear decrease in BCO aphid incidence (Table 4.03, Fig. 4.06). However, no significant effect of GC and no interaction of PTS X GC on BCO on aphid

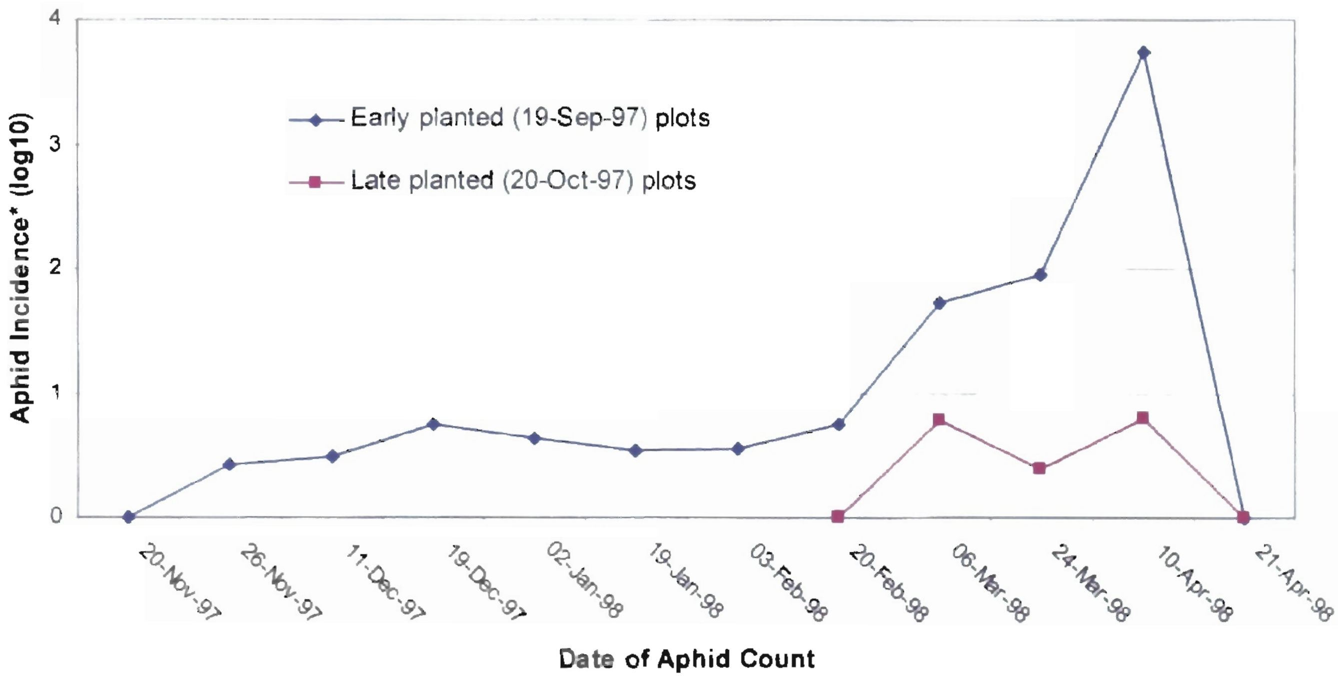

Fig. 4.04 Incidence of bird cherry-oat (BCO) aphids observed in early- and late-planted wheat from 20-Nov-97 to 10-Apr-98.

* Aphid incidence was determined by the number of BCO aphids per linear ft. of row, and has been transformed and graphed as log values.

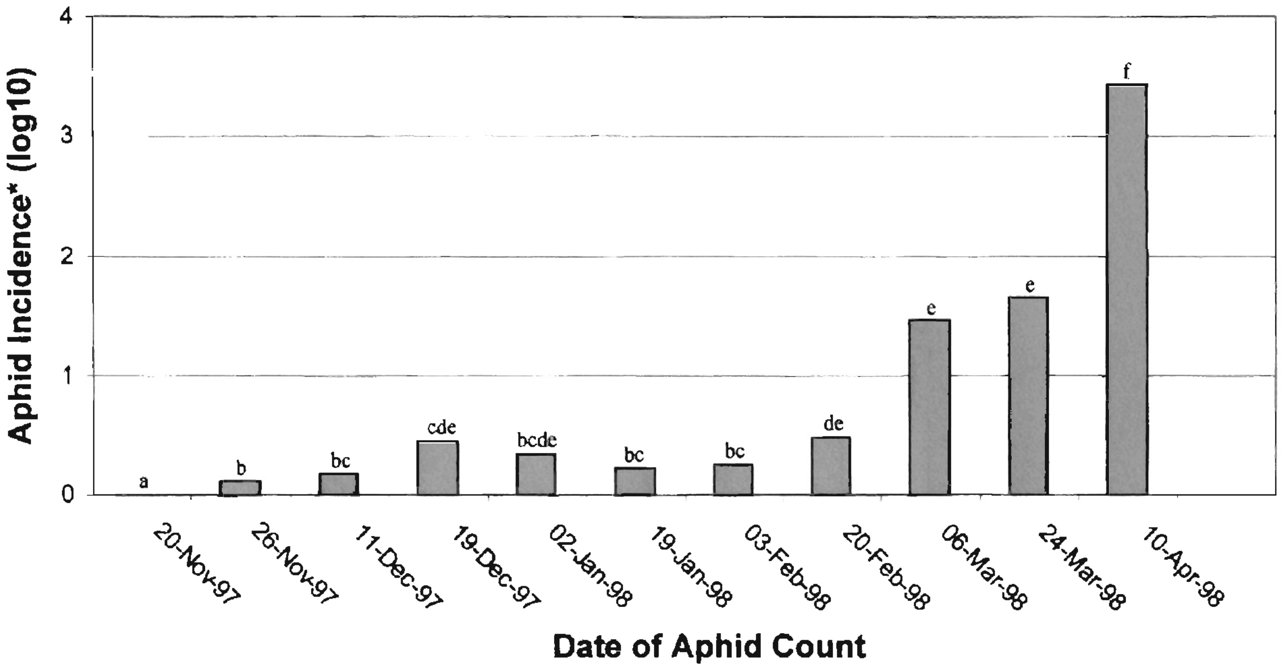

Fig. 4.05 Incidence of bird cherry-oat (BCO) aphids observed from 20-Nov-97 to 10-Apr-98. Each bar represents combined incidence of early-planted (19-Sep-97) and late-planted (20-Oct-97) field plots. Mean aphid incidence with the same letter are not significantly different.

* Aphid incidence was determined by the number of BCO aphids per linear ft. of row.

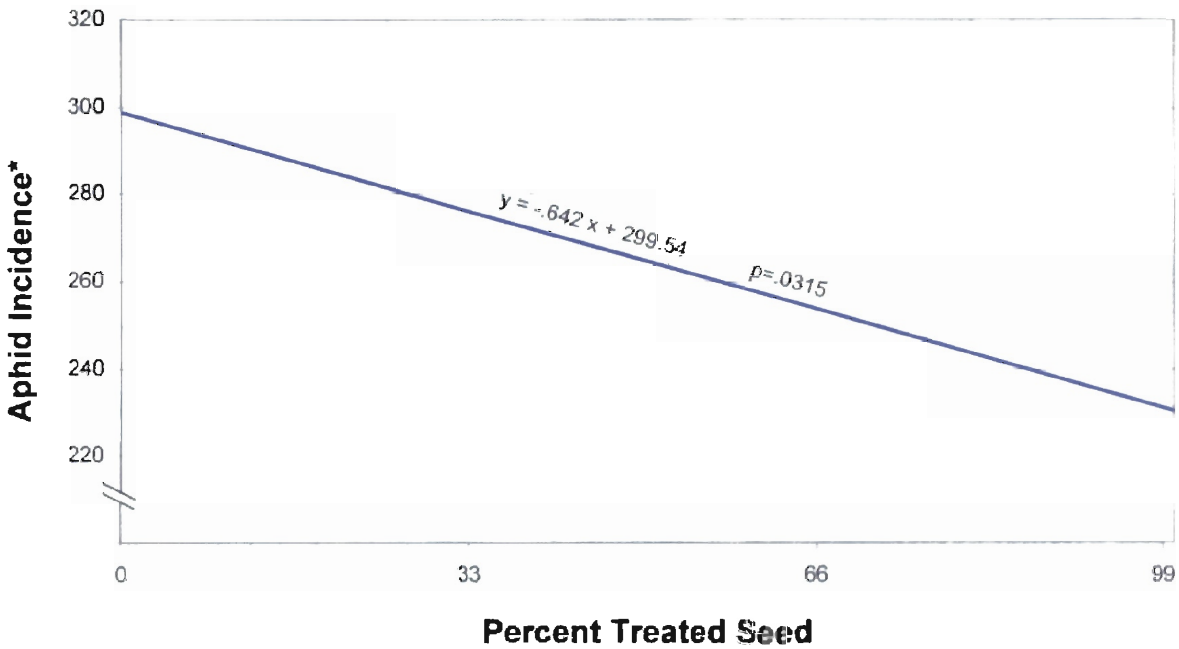

Fig. 4.06 The percentage of seed treated with Gaucho (0, 33, 67, 100) had a significant linear effect on bird cherry-oat (BCO) aphid incidence.

* Aphid incidence was determined by the number of BCO aphids per linear ft. of row.

incidence were identified. Aphid incidence was significantly higher with mixtures with 33 PTS compared to 100 PTS. All other comparisons were insignificant.

<u>Barley Yellow Dwarf</u>

Foliar samples collected from plants in both the early- (19-Sep-97) and late-planted (20-Oct-97) plots were tested with ELISA to ascertain the presence/absence of BYDV-pav, -rmv, and –rpv. Testing was conducted only for these three BYDVs because the test for BYDV-sgv did not function properly, and past testing in the Plant Disease Diagnostic Laboratory at Oklahoma State University indicated that these three BYDVs were the most common in Oklahoma. Results from this testing are presented in Appendix A, and indicate that BYDV-pav was the most common BYDVs found in the samples from both the early- (19-Sep-97) and late-planted (20-Oct-97) plots.

Although aphids were observed on plants in the early-planted (19-Sep-97) plots in late November, symptoms of BYD were not observed until mid-April. In contrast, aphids were first observed on plants in the late-planted (20-Oct-97) plots in late February and BYD symptoms were first observed in early-May. BYD disease incidence (DI), was significantly higher in the early-planted (19-Sep-97) plots than in the late-planted (20-Oct-97) plots (Table 4.03, Fig. 4.07). Data in Fig. 4.07 was generated by combining DI measurements taken at two dates ('Date') (24-Apr-98 and 08-May-98), which was done because no TRT X Date interaction was observed. Also, DI increased significantly when determined on 08-May-98 as compared to 24-Apr-98 (Fig. 4.08). Data in Fig. 4.08 was

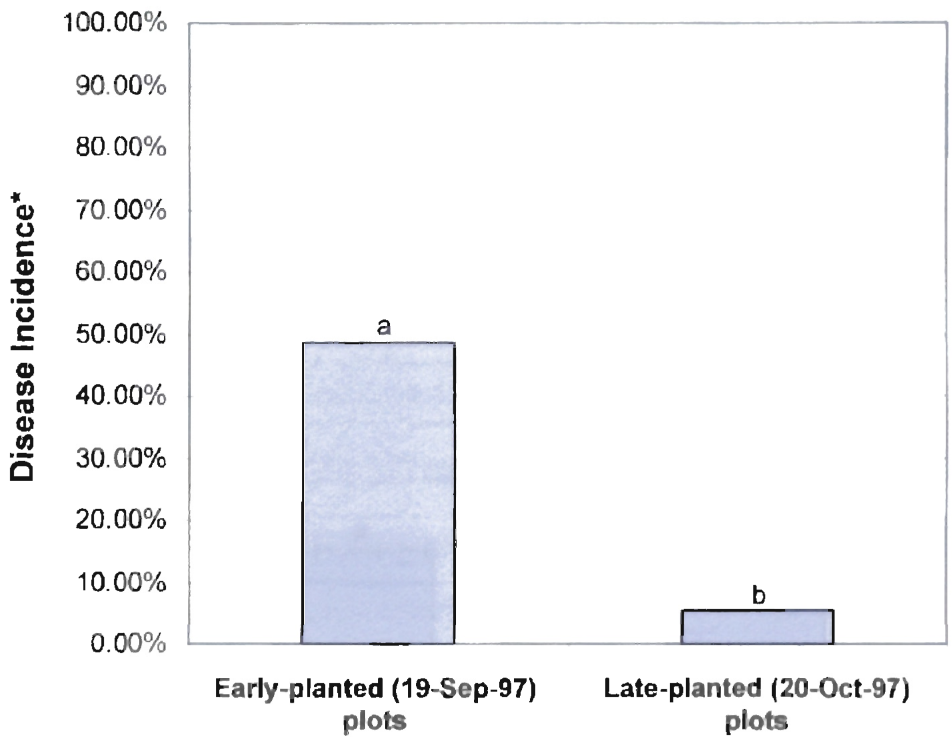

Fig. 4.07 Barley yellow dwarf disease incidence (DI) in early- and late-planted wheat. Data was generated by combining DI measurements taken at two dates (24-Apr-98 and 08-May-98), which was done because no treatment X date of measurement interaction was observed.

* Disease incidence was determined by the percentage of flag leaves showing BYD symptoms (P=.0001).

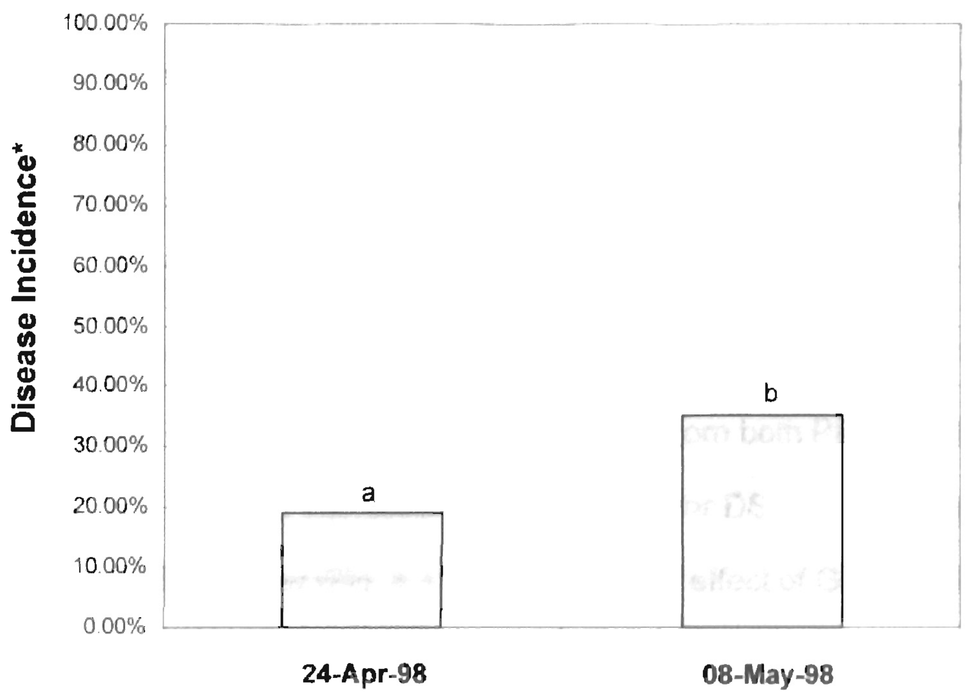

Fig. 4.08 Barley yellow dwarf disease incidence (DI) on 08-May-98 and on 24-Apr-98. Data was generated by combining DI measurements from early- and late-planting dates, which was done because no treatment X planting date interaction was observed for DI.

* Disease incidence was determined by the percentage of flag leaves showing BYD symptoms (P=.0001).

generated by combining DI measurements from both PDs, which was done because no TRT X PD interaction was observed for DI. The effects of PTS and GC on DI were also analyzed. However, the effects of PTS and GC on DI were not significant, and no PTS X GC interaction occurred.

Similarly, BYD disease severity (DS) was significantly higher in the early-planted (19-Sep-97) plots than in the late-planted (20-Oct-97) plots (Table 4.03, Fig. 4.09). Data in Fig. 4.09 was generated by combining DS measurements taken at two dates (24-Apr-98 and 08-May-98), which was done because no TRT X Date interaction was observed. Also, DS increased significantly when determined on 08-May-98 as compared to 24-Apr-98 (Fig. 4.10). Data in Fig. 4.10 was generated by combining DS measurements from both PDs, which was done because no TRT X PD interaction was observed for DS. A significant linear effect of PTS on DS occurred (Fig. 4.11). However, no effect of GC on DS was found and no PTS X GC interaction was observed for DS.

Tiller Height

Tiller height, determined by selecting ten tillers randomly for each treatment and measuring the height of these tillers from the ground to start of the head, was measured prior to harvest on 20-May-98. Tiller height was significantly lower in the early-planted (19-Sep-97) plots than the late-planted (20-Oct-97) plots (Table 4.03, Fig. 4.12). No PD X TRT interaction was observed. Effects of PTS and GC on tiller height were also analyzed. Increases in PTS resulted in a linear increase in tiller height (Table 4.03, Fig. 4.13). No

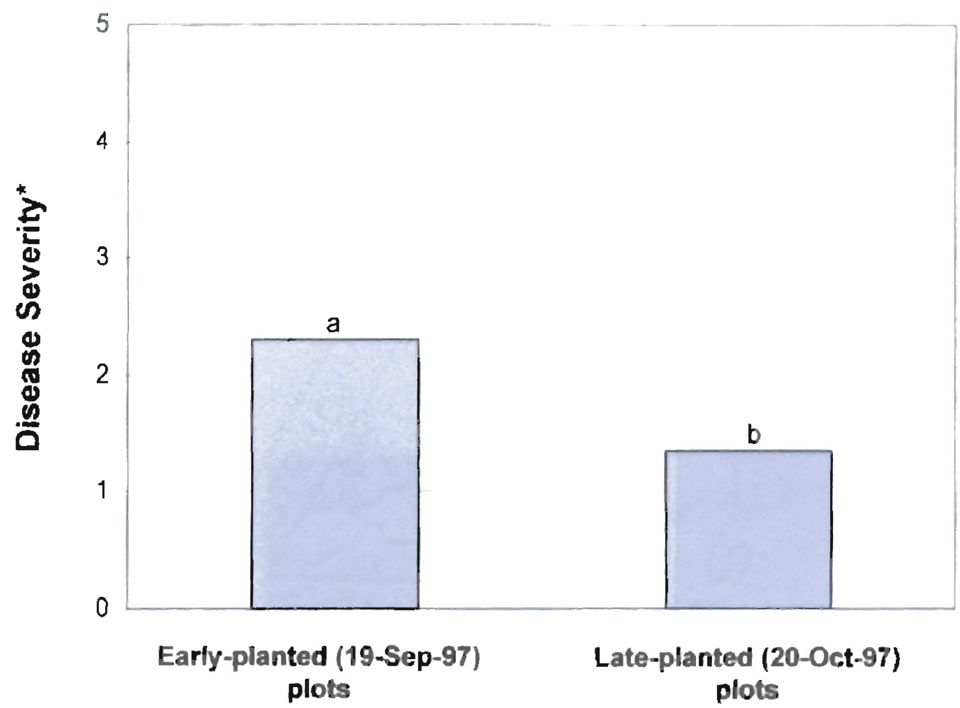

Fig. 4.09 Barley yellow dwarf disease severity (DS) in early- and late-planted wheat. Data was generated by combining DS measurements taken at two dates (24-Apr-98 and 08-May-98), which was done because no treatment X date of measurement interaction was observed.

* Disease severity was determined by visual ratings using the following scale: 1=no symptoms; 2=trace to slight discoloration of leaves, no stunting; 3=moderate discoloration, slight stunting; 4=extensive discoloration, moderate stunting; and 5=severe discoloration, severe stunting (P=.0003).

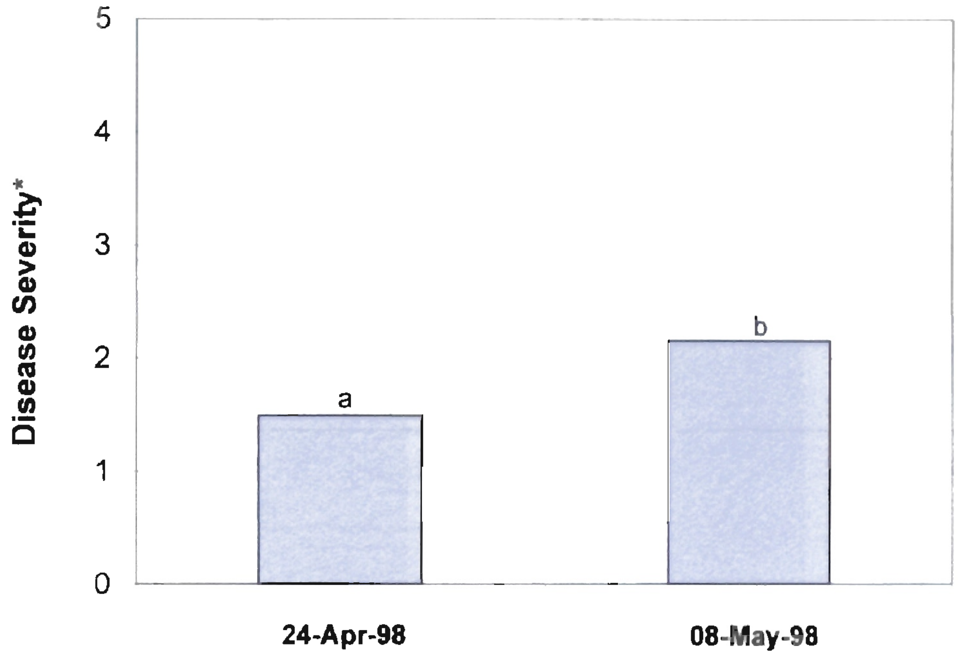

Fig. 4.10 Barley yellow dwarf disease severity (DS) on 08-May-98 and on 24-Apr-98. Data was generated by combining DS measurements from early- and late-planting dates, which was done because no treatment X planting date interaction was observed for DS.

* Disease severity was determined by visual ratings using the following scale: 1=no symptoms; 2=trace to slight discoloration of leaves, no stunting; 3=moderate discoloration, slight stunting; 4=extensive discoloration, moderate stunting; and 5=severe discoloration, severe stunting (P=.0003).

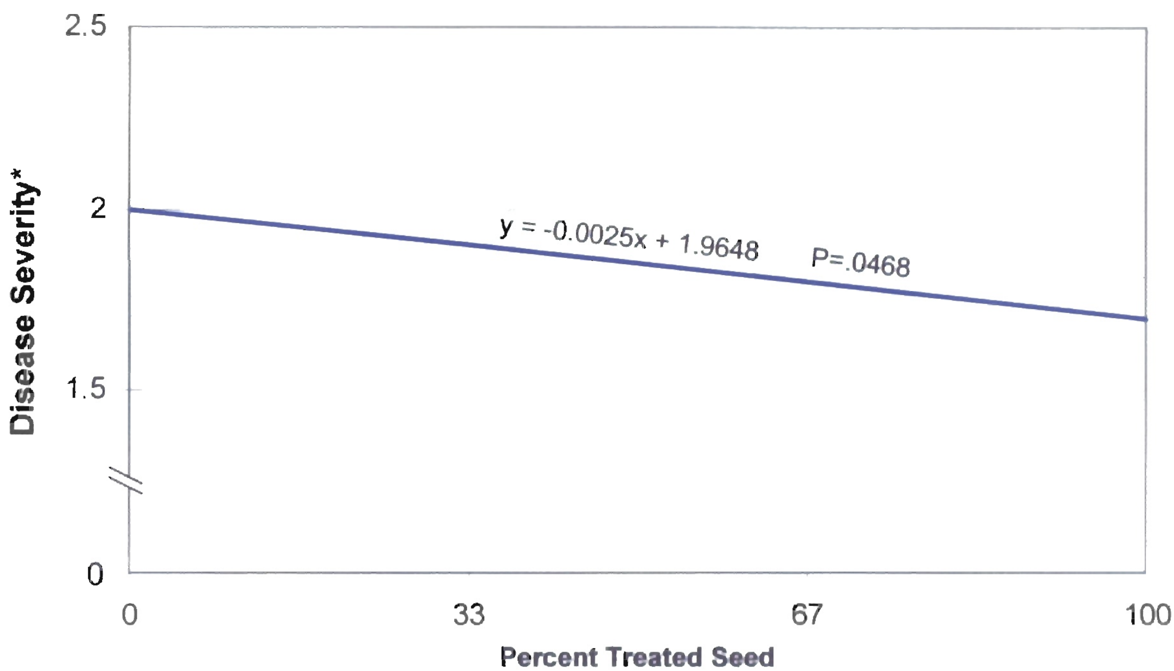

Fig. 4.11 The percentage of treated seed with Gaucho (0, 33, 67, and 100) had a significant linear effect on barley yellow dwarf disease severity.

* Disease severity was determined by visual ratings using the following scale: 1=no symptoms; 2=trace to slight discoloration of leaves, no stunting; 3=moderate discoloration, slight stunting; 4=extensive discoloration, moderate stunting; and 5=severe discoloration, severe stunting (P=.0468).

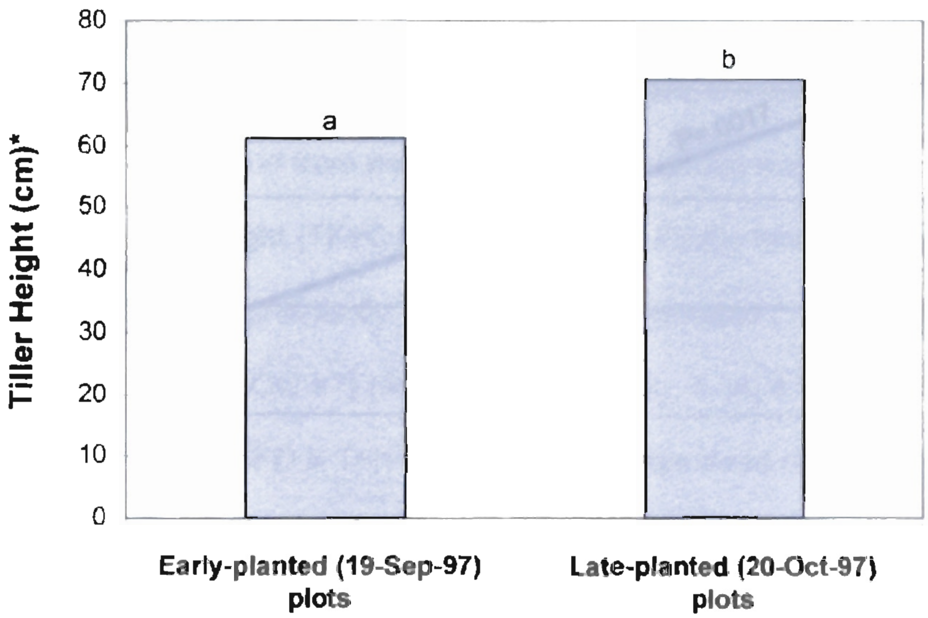

Fig. 4.12 Tiller heights in the early-planted (19-Sep-97) plots were significantly lower than tiller heights in the later-planted (20-Oct-97) plots.

* Tiller height was determined by selecting ten tillers randomly for each treatment and measuring the height of these tillers from the ground to start of the head.

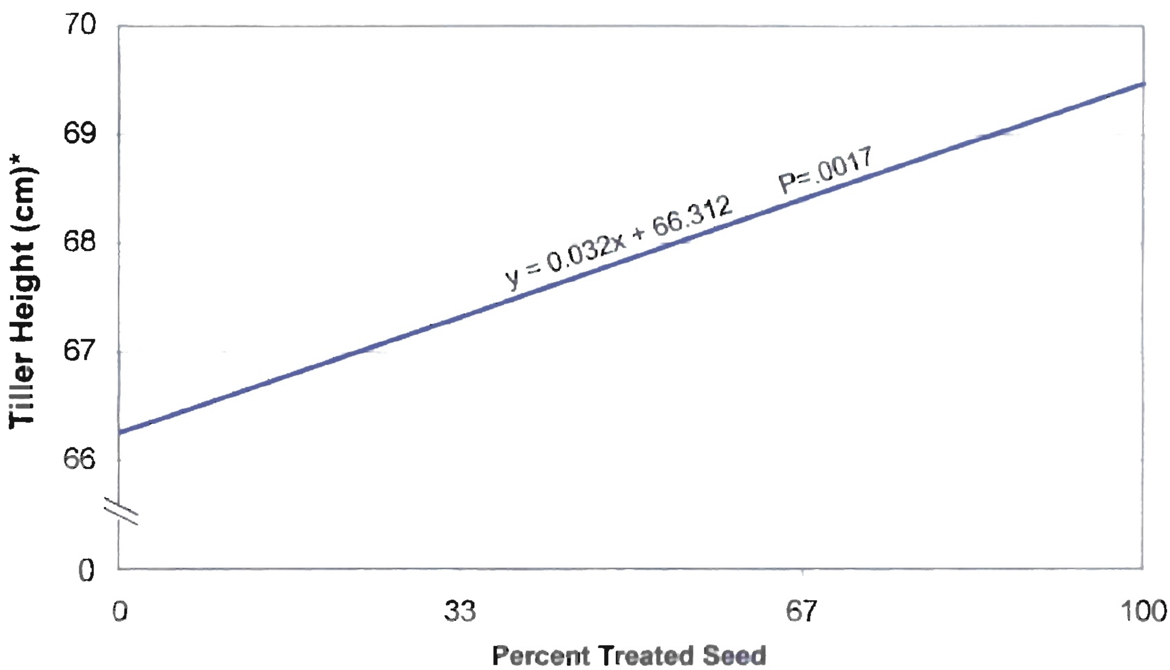

Fig. 4.13 The percentage of treated seed with Gaucho (0, 33, 67, and 100) had a significant linear effect on tiller height. Data was generated by combining tiller height measurements from early- and late-planting dates, which was done because no treatment X planting date interaction was observed for tiller height.

* Tiller height was determined by selecting ten tillers randomly for each treatment and measuring the height of these tillers from the ground to start of the head.

PTS X GC interaction occurred. Both the control (0% seed treated) and mixtures with 33 PTS had significantly lower tiller heights compared to 100 PTS. All other comparisons were insignificant.

Yield and its Components

On 20-May-98, fertile head density (determined by the mean number of fertile heads/ft. of row for each plot) was determined and the middle row of each plot was harvested by hand from both planting dates. Grain was later weighed and thousand-kernel weight (TKW) was determined. Fertile head density, grain weight, and TKW were significantly lower in the early-planted (19-Sep-97) plots than the late-planted (20-Oct-97) plots (Table 4.03, Fig. 4.14, 4.15, and 4.16). No effect of TRT, and no PD X TRT interaction on fertile head density, grain weight, and TKW were observed. Effects of PTS and GC on fertile head density, grain weight, and TKW were also analyzed. Increasing PTS resulted in significant linear increases for each of these parameters (Fig. 4.17, 4.18, and 4.19). No effects of GC on these parameters were observed. PTS X GC interactions for these parameters were insignificant. Fertile head density in mixtures with 33 PTS was significantly lower compared to fertile head density in 100 PTS. All other comparisons were insignificant.

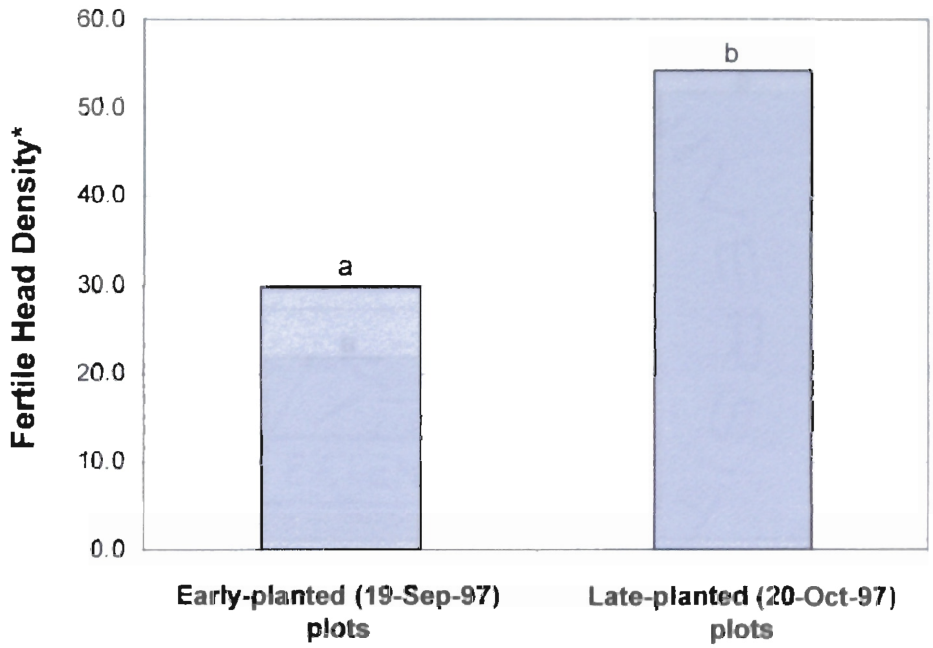

Fig. 4.14 Fertile head density in the early-planted plots were significantly lower than the fertile head density in the later-planted plots.

* Fertile head density was measured by counting the number of fertile heads in a randomly selected linear ft. of row.

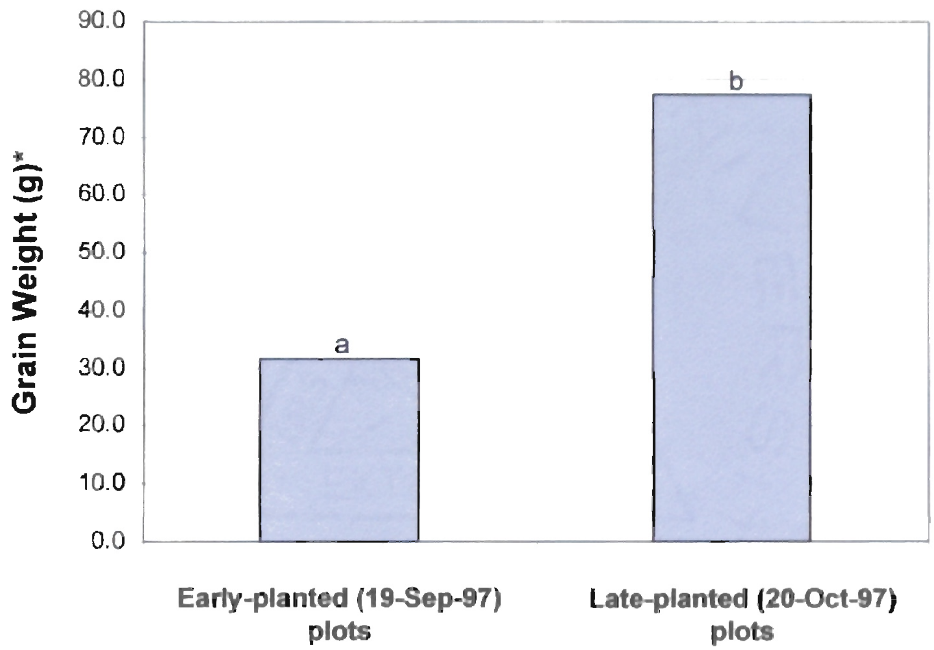

Fig. 4.15 Grain weight in the early-planted plots was significantly less than the grain weight in the later-planted plots.

* Grain weight was determined by weighing the seed harvested from the middle row of each treatment. Each bar represents the mean grain weight of all treatments for the respective planting date.

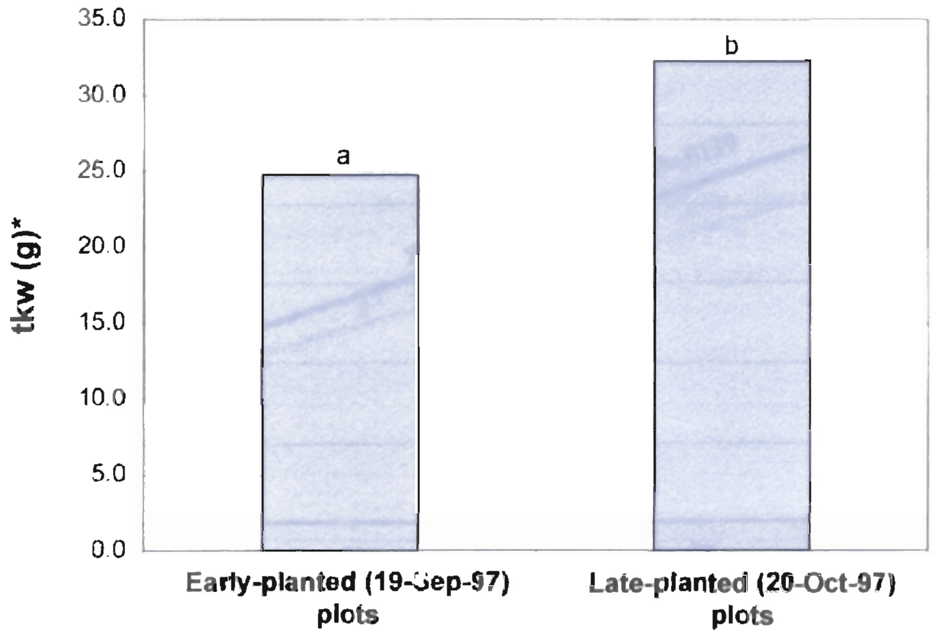

Fig. 4.16 The thousand kernel weight (tkw) of grain harvested in the early-planted plots was significantly less than the tkw of grain harvested in the later-planted plots.

* Tkw was determined from the grain harvested from the middle row of each treatment (P=.0001).

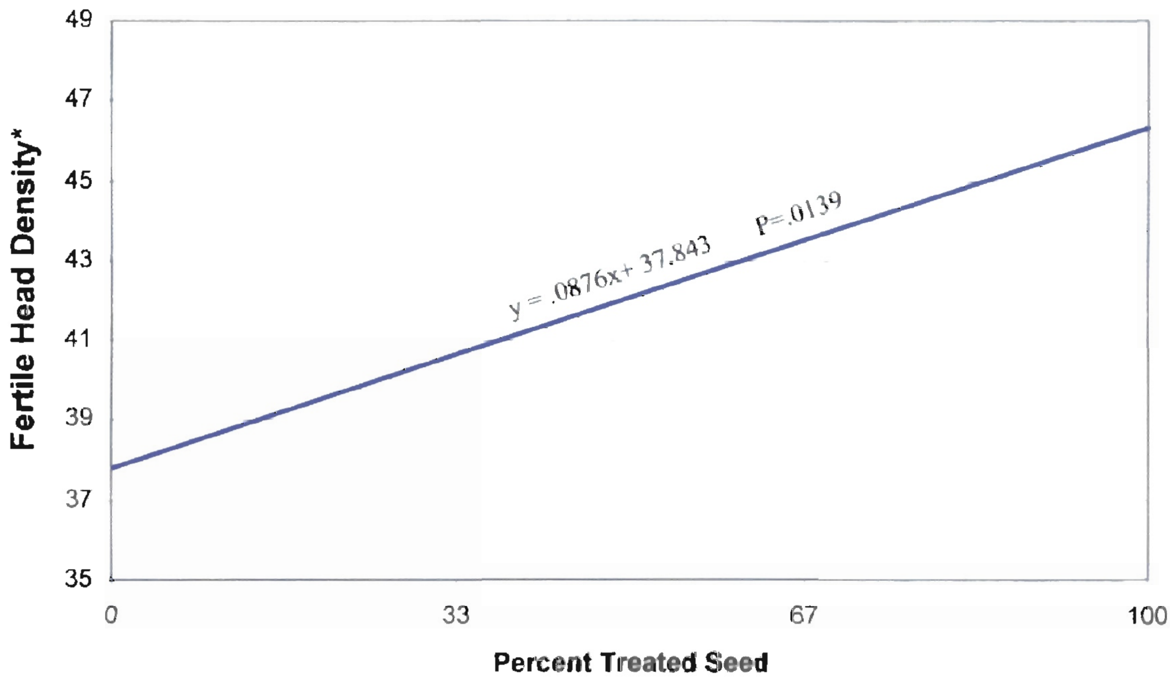

Fig. 4.17 The percentage of seed treated (0, 33, 67, and 100) had a significant linear effect on fertile head density. Data was generated by combining fertile head density measurements from early- and late-planting dates, which was done because no treatment X planting date interaction was observed for fertile head density.

* Fertile head density was measured by counting the number of fertile heads in a randomly selected linear ft. of row.

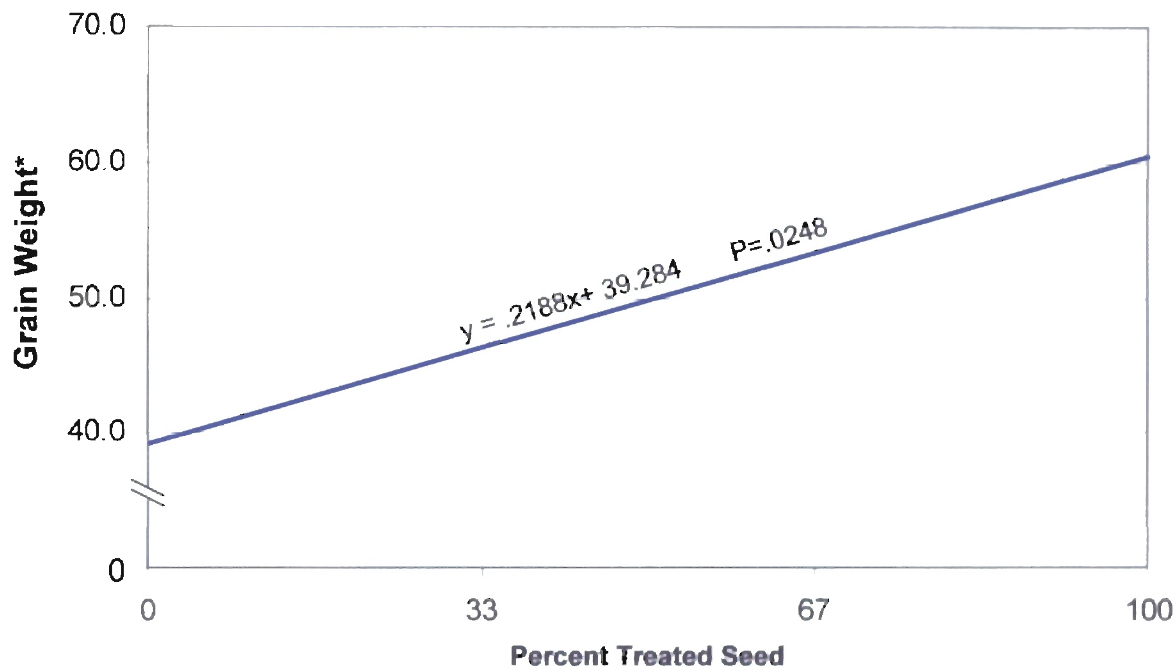

Fig. 4.18 The percentage of treated seed with Gaucho (0, 33, 67, and 100) had a significant linear effect on the grain weight. Data was generated by combining grain weight measurements from early- and late-planting dates, which was done because no treatment X planting date interaction was observed for grain weight.

* Grain weight was determined by weighing the grain harvested from the middle row of each treatment.

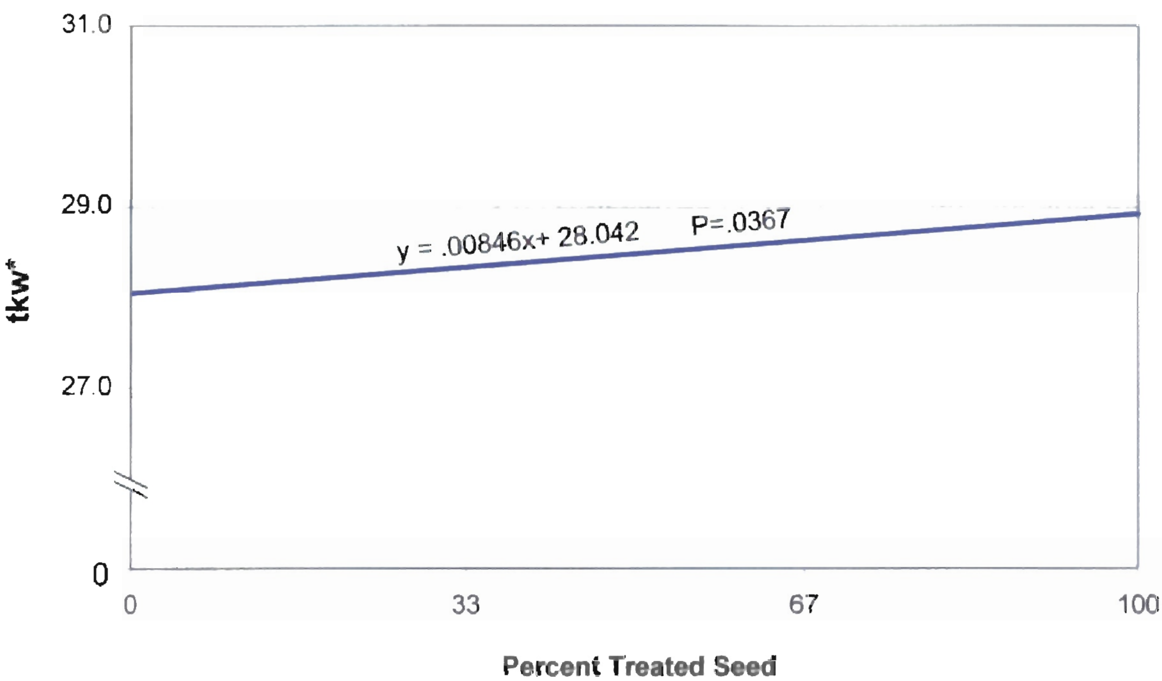

Fig. 4.19 The percentage of treated seed with Gaucho (0, 33, 67, and 100) had a significant linear effect on the thousand kernel weight (tkw). Data was generated by combining tkw measurements from early- and late-planting dates, which was done because no treatment X planting date interaction was observed for tkw.

* Tkw was determined from the grain harvested from the middle row of each treatment.

DISCUSSION

The effect of planting date on aphid and BYD control in this study agrees with the work of others (Hammon *et al*, 1996; McGrath *et al*, 1987; McKirdy and Jones, 1997). It is assumed that a freeze, which occurred in April, caused more damage to the early-planted (19-Sep-97) plots than the later-planted (20-Oct-97) plots. In addition, the late-planted (20-Oct-97) plots appeared to escape fall aphid infestations. Both aphids and BYD occurred in these plots, but not until the spring of '98. This resulted in less damage compared to the severe aphid and BYD damage that occurred in the early-planted plots.

The concentration of Gaucho had no effect on aphids or BYD control, which contradicts other studies that show significant decreases in aphid populations and BYD control correlated with an increase in Gaucho concentration (Gourmet *et al*, 1994; Gourmet *et al*, 1996; Hunger *et al*, 1997; McKirdy and Jones, 1996). Because the control (untreated seed) was included in the analysis, another interpretation of the insignificance of Gaucho rate would be that there was no effect of Gaucho.

The primary objective of this study was to examine the effects of using mixtures of Gaucho insecticide-treated and untreated seed to control aphids and BYD. Results from experiments investigating this objective demonstrated that such mixtures cause increases in aphid incidence and BYD incidence and severity; i.e., the lower the proportions of Gaucho-treated seed, the higher the aphid population levels and greater BYD incidence and severity. An explanation of this phenomenon can only be speculative based on this study alone. As previously mentioned, Power (1991) suggested that aphids tend to emigrate after having moved on an undesirable host or non-host plant, which results in more aphids feeding on more plants in a given time than those feeding on genetically homogeneous crops. It was proposed that the consequence of more aphids feeding on more plants would result in lower feeding periods, which in turn would result in lower transmission rates of BYDVs. However, Gourmet *et al* (1994) reported feeding periods of BCO aphids on Gaucho-treated oats to average 5 hrs before emigration to other plants. Feeding periods of this extent are far beyond the acquisition and transmission periods reported by Rochow (1963). Thus, it is possible that Gaucho-treated plants may become infected with BYDVs, especially at a low Gaucho concentration. Furthermore, aphids exposed to mixtures of Gaucho-treated and untreated-plants could migrate to untreated hosts after primary infestations on Gaucho-treated plants. As aphid densities increase on untreated plants, migration to plants treated with Gaucho would be expected, thereby increasing the BYD disease pressure. In summary, plants with Gaucho may not serve as an aphid "trap" as anticipated in a mixed system. Instead, it may result in an increase in movement from Gaucho treated plants to untreated plants, and consequently, favor spread of BYDV.

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Appendix A Presence of BYDVs (pav, rpv, and rmv) as determined by ELISA in early- and late-planted plots (see pages 53-59 for description of sampling methods and field plots).

PD = Planting Date
R1 = Absorbence value of rep 1 from ELISA.
R2 = Absorbence value of rep 2 from ELISA.
X = Mean of R1 and R2
PT = Positive Threshold
VP = Virus Presence
+ = Virus present
- = Virus abscent

PD	TRT	% Treated	Gaucho Conc.	Rep.	PAV					RMV					RPV				
					R1	R2	X	PT	VP	R1	R2	X	PT	VP	R1	R2	X	PT	VP
19-Sep	1	0	0	1	2.000	2.000	2.000	0.0124	+	0.020	0.022	0.021	0.055	-	0.01	0.01	0.01	0.02	-
19-Sep	1	0	0	2	2.000	5.000	3.500	0.0124	+	0.057	0.053	0.055	0.055	+	0.01	0.02	0.01	0.02	-
19-Sep	1	0	0	3	2.000	2.000	2.000	0.0124	+	0.013	0.032	0.023	0.055	-	0.01	0.01	0.01	0.02	-
19-Sep	1	0	0	4	2.000	2.000	2.000	0.0124	+	0.038	0.037	0.038	0.055	-	2.00	1.36	1.68	0.02	+
19-Sep	1	0	0	5	0.325	0.355	0.340	0.0138	+	0.175	0.186	0.181	0.332	-	0.00	0.01	0.00	0.05	-
19-Sep	2	33	1	1	2.000	2.000	2.000	0.0124	+	0.015	0.023	0.019	0.055	-	0.02	0.02	0.02	0.02	-
19-Sep	2	33	1	2	2.000	2.000	2.000	0.0124	+	0.042	0.030	0.036	0.055	-	0.02	0.02	0.02	0.02	-
19-Sep	2	33	1	3	2.000	2.000	2.000	0.0124	+	0.032	0.058	0.045	0.055	-	0.01	0.01	0.01	0.02	-
19-Sep	2	33	1	4	2.000	2.000	2.000	0.0124	+	0.022	0.034	0.028	0.055	-	0.67	0.66	0.67	0.02	+
19-Sep	2	33	1	5	2.000	2.000	2.000	0.0124	+	0.018	0.059	0.039	0.055	-	0.01	0.02	0.02	0.02	-
19-Sep	3	67	1	1	2.000	2.000	2.000	0.0124	+	0.026	0.031	0.029	0.055	-	0.00	0.01	0.01	0.02	-
19-Sep	3	67	1	2	1.578	1.616	1.597	0.0124	+	0.039	0.032	0.036	0.055	-	1.25	1.29	1.27	0.02	+
19-Sep	3	67	1	3	2.000	2.000	2.000	0.0124	+	0.001	0.027	0.014	0.055	-	0.02	0.02	0.02	0.02	-
19-Sep	3	67	1	4	2.000	2.000	2.000	0.0124	+	0.022	0.020	0.021	0.055	-	0.45	0.41	0.43	0.02	+
19-Sep	3	67	1	5	2.000	2.000	2.000	0.0138	+	0.163	0.150	0.157	0.332	-	0.03	0.03	0.03	0.05	-
19-Sep	4	100	1	1	2.000	2.000	2.000	0.0124	+	0.025	0.016	0.021	0.055	-	0.27	0.29	0.28	0.02	+
19-Sep	4	100	1	2	0.951	0.537	0.744	0.0124	+	0.023	0.016	0.020	0.055	-	0.01	0.02	0.01	0.02	-
19-Sep	4	100	1	3	2.000	2.000	2.000	0.0124	+	0.025	0.038	0.032	0.055	-	0.02	0.04	0.03	0.02	+
19-Sep	4	100	1	4	1.996	2.000	1.998	0.0124	+	0.032	0.034	0.033	0.055	-	0.00	0.00	0.00	0.02	-
19-Sep	4	100	1	5	2.000	2.000	2.000	0.0124	+	0.023	0.041	0.032	0.055	-	2.00	2.00	2.00	0.02	+

Appendix A (cont)

		%	Gaucho		PAV					RMV					RPV				
PD	TRT	Treated	Conc.	Rep.	R1	R2	X	PT	VP	R1	R2	X	PT	VP	R1	R2	X	PT	VP
19-Sep	5	33	2	1	2.000	2.000	2.00	0.01	+	0.037	0.050	0.04	0.05	-	0.013	0.020	0.02	0.02	-
19-Sep	5	33	2	2	1.439	2.000	1.72	0.01	+	0.031	0.029	0.03	0.05	-	0.068	0.067	0.07	0.02	+
19-Sep	5	33	2	3	1.255	1.232	1.24	0.01	+	0.039	0.029	0.03	0.05	-	0.018	0.011	0.01	0.02	-
19-Sep	5	33	2	4	1.487	1.466	1.48	0.01	+	0.043	0.024	0.03	0.05	-	0.024	0.019	0.02	0.02	+
19-Sep	5	33	2	5	2.000	2.000	2.00	0.01	+	0.157	0.140	0.15	0.33	-	0.007	0.003	0.01	0.05	-
19-Sep	6	67	2	1	1.788	1.776	1.78	0.01	+	0.048	0.044	0.05	0.05	-	0.030	0.032	0.03	0.02	+
19-Sep	6	67	2	2	0.712	0.741	0.73	0.01	+	0.028	0.020	0.02	0.05	-	1.080	1.042	1.06	0.02	+
19-Sep	6	67	2	3	2.000	2.000	2.00	0.01	+	0.017	0.020	0.02	0.05	-	0.761	0.805	0.78	0.02	+
19-Sep	6	67	2	4	2.000	2.000	2.00	0.01	+	0.012	0.022	0.02	0.05	-	0.901	0.988	0.94	0.02	+
19-Sep	6	67	2	5	2.000	2.000	2.00	0.01	+	0.011	0.020	0.02	0.05	-	1.121	1.559	1.34	0.02	+
19-Sep	7	100	2	1	2.000	2.000	2.00	0.01	+	0.024	0.030	0.03	0.05	-	0.016	0.013	0.01	0.02	-
19-Sep	7	100	2	2	2.000	2.000	2.00	0.01	+	0.008	0.021	0.01	0.05	-	0.023	0.023	0.02	0.02	+
19-Sep	7	100	2	3	2.000	1.939	1.97	0.01	+	0.021	0.026	0.02	0.05	-	0.007	0.007	0.01	0.02	-
19-Sep	7	100	2	4	2.000	2.000	2.00	0.01	+	0.033	0.032	0.03	0.05	-	0.007	0.016	0.01	0.02	-
19-Sep	7	100	2	5	2.000	2.000	2.00	0.01	+	0.169	0.153	0.16	0.33	-	0.011	0.052	0.03	0.05	-
19-Sep	8	33	3	1	1.678	1.778	1.73	0.01	+	0.052	0.030	0.04	0.05	-	1.147	1.274	1.21	0.02	+
19-Sep	8	33	3	2	1.636	1.699	1.67	0.01	+	0.015	0.015	0.02	0.05	-	0.020	0.019	0.02	0.02	-
19-Sep	8	33	3	3	1.852	1.908	1.88	0.01	+	0.009	0.016	0.01	0.05	-	0.011	0.016	0.01	0.02	-
19-Sep	8	33	3	4	1.966	1.898	1.93	0.01	+	0.036	0.033	0.03	0.05	-	0.026	0.033	0.03	0.02	+
19-Sep	8	33	3	5	2.000	2.000	2.00	0.01	+	0.007	0.016	0.01	0.05	-	0.014	0.011	0.01	0.02	-
19-Sep	9	67	3	1	2.000	2.000	2.00	0.01	+	0.026	0.036	0.03	0.05	-	0.617	0.615	0.62	0.02	+
19-Sep	9	67	3	2	2.000	2.000	2.00	0.01	+	0.019	0.024	0.02	0.05	-	0.015	0.015	0.02	0.02	-
19-Sep	9	67	3	3	1.611	1.580	1.60	0.01	+	0.020	0.014	0.02	0.05	-	0.898	0.866	0.88	0.02	+
19-Sep	9	67	3	4	1.972	1.934	1.95	0.01	+	0.018	0.029	0.02	0.05	-	1.338	1.370	1.35	0.02	+
19-Sep	9	67	3	5	2.000	2.000	2.00	0.01	+	0.025	0.026	0.03	0.05	-	0.009	0.006	0.01	0.02	-
19-Sep	10	100	3	1	1.462	1.420	1.44	0.01	+	0.011	0.013	0.01	0.05	-	0.009	0.025	0.02	0.02	-
19-Sep	10	100	3	2	2.000	2.000	2.00	0.01	+	0.018	0.019	0.02	0.05	-	0.031	0.034	0.03	0.02	+
19-Sep	10	100	3	3	2.000	1.996	2.00	0.01	+	0.015	0.024	0.02	0.05	-	1.859	1.902	1.88	0.02	+
19-Sep	10	100	3	4	1.636	1.579	1.61	0.01	+	0.051	0.044	0.05	0.05	-	0.017	0.014	0.02	0.02	-

Appendix A (cont)

PD	TRT	% Treated	Gaucho Conc.	Rep.	PAV					RMV					RPV				
					R1	R2	X	PT	VP	R1	R2	X	PT	VP	R1	R2	X	PT	VP
19-Sep	10	100	3	5	2.000	2.000	2.00	0.01	+	0.164	0.152	0.16	0.33	-	0.010	0.021	0.02	0.05	-
20-Oct	1	0	0	1	2.000	2.000	2.000	0.054	+	0.032	0.065	0.0485	0.0769	-	0.003	0.004	0.004	0.03	-
20-Oct	1	0	0	2	1.270	1.288	1.279	0.089	+	0.059	0.081	0.07	0.1883	-	0.033	0.041	0.037	0.21	-
20-Oct	1	0	0	3	0.046	0.053	0.050	0.089	-	0.135	0.04	0.0875	0.1883	-	0.005	0.016	0.011	0.21	-
20-Oct	1	0	0	4	1.789	1.831	1.810	0.089	+	0.069	0.077	0.073	0.1883	-	0.005	0.012	0.009	0.21	-
20-Oct	1	0	0	5	1.400	1.407	1.404	0.089	+	0.054	0.104	0.079	0.1883	-	0.01	0.014	0.012	0.21	-
20-Oct	2	33	1	1	0.399	1.429	0.914	0.054	+	0.052	0.095	0.0735	0.0769	-	0.004	0.002	0.003	0.03	-
20-Oct	2	33	1	2	0.033	0.010	0.022	0.089	-	0.008	0.035	0.0215	0.1883	-	0.005	0.009	0.007	0.21	-
20-Oct	2	33	1	3	2.000	2.000	2.000	0.089	+	0.106	0.126	0.116	0.1883	-	0.007	0.006	0.007	0.21	-
20-Oct	2	33	1	4	2.000	2.000	2.000	0.089	+	0.084	0.167	0.1255	0.1883	-	0.017	0.018	0.018	0.21	-
20-Oct	2	33	1	5	0.078	0.043	0.061	0.089	-	0.111	0.09	0.1005	0.1883	-	0.019	0.018	0.019	0.21	-
20-Oct	3	67	1	1	2.000	2.000	2.000	0.054	+	0.031	0.033	0.032	0.0769	-	0.002	0.004	0.003	0.03	-
20-Oct	3	67	1	2	1.645	1.355	1.500	0.089	+	0.073	0.049	0.061	0.1883	-	0	0.01	0.005	0.21	-
20-Oct	3	67	1	3	1.156	1.072	1.114	0.089	+	0.116	0.031	0.0735	0.1883	-	0.817	0.802	0.81	0.21	+
20-Oct	3	67	1	4	1.729	1.798	1.764	0.089	+	0.064	0.156	0.11	0.1883	-	0.03	0.011	0.021	0.21	-
20-Oct	3	67	1	5	0.079	0.052	0.066	0.089	-	0.081	0.094	0.0875	0.1883	-	0.024	0.027	0.026	0.21	-
20-Oct	4	100	1	1	0.000	0.007	0.004	0.089	-	0.021	0.106	0.0635	0.1883	-	0.002	0.005	0.004	0.21	-
20-Oct	4	100	1	2	1.938	1.930	1.934	0.089	+	0.056	0.134	0.095	0.1883	-	0.005	0.002	0.004	0.21	-
20-Oct	4	100	1	3	2.000	2.000	2.000	0.089	+	0.107	0.069	0.088	0.1883	-	0.025	0.008	0.017	0.21	-
20-Oct	4	100	1	4	0.038	0.000	0.019	0.089	-	0.179	0.14	0.1595	0.1883	-	0.012	0.01	0.011	0.21	-
20-Oct	4	100	1	5	2.000	0.743	1.372	0.089	+	0.041	0.132	0.0865	0.1883	-	0.017	0.01	0.014	0.21	-
20-Oct	5	33	2	1	1.937	1.947	1.942	0.089	+	0.044	0.177	0.1105	0.1883	-	0.001	0.002	0.002	0.21	-
20-Oct	5	33	2	2	1.512	1.178	1.345	0.089	+	0.01	0.027	0.0185	0.1883	-	0.006	0.003	0.005	0.21	-
20-Oct	5	33	2	3	1.242	1.298	1.270	0.089	+	0.057	0.02	0.0385	0.1883	-	0.02	0.017	0.019	0.21	-
20-Oct	5	33	2	4	0.076	0.071	0.074	0.089	-	0.084	0.185	0.1345	0.1883	-	0.009	0.01	0.01	0.21	-
20-Oct	5	33	2	5	2.000	2.000	2.000	0.089	+	0.078	0.072	0.075	0.1883	-	0.021	0.007	0.014	0.21	-
20-Oct	6	67	2	1	2.000	2.000	2.000	0.054	+	0.049	0.046	0.0475	0.0769	-	0	0	0	0.03	-
20-Oct	6	67	2	2	1.724	1.698	1.711	0.089	+	0.105	0.099	0.102	0.1883	-	0.004	0.009	0.007	0.21	-
20-Oct	6	67	2	3	2.000	2.000	2.000	0.089	+	0.023	0.037	0.03	0.1883	-	0.034	0.009	0.022	0.21	-

Appendix A (cont)

PD	TRT	% Treated	Gaucho Conc.	Rep.	PAV					RMV					RPV				
					R1	R2	X	PT	VP	R1	R2	X	PT	VP	R1	R2	X	PT	VP
20-Oct	6	67	2	4	2.000	1.999	2.000	0.089	+	0.091	0.166	0.1285	0.1883	-	0.02	0.046	0.033	0.21	-
20-Oct	6	67	2	5	0.075	0.063	0.069	0.089	-	0.065	0.061	0.063	0.1883	-	0.017	0.022	0.02	0.21	-
20-Oct	7	100	2	1	1.990	1.996	1.993	0.089	+	0.017	0.131	0.074	0.1883	-	0.181	0.179	0.18	0.21	-
20-Oct	7	100	2	2	1.255	0.876	1.066	0.089	+	0	0.056	0.028	0.1883	-	0.011	0.002	0.007	0.21	-
20-Oct	7	100	2	3	2.000	2.000	2.000	0.089	+	0.06	0.04	0.05	0.1883	-	0.005	0.002	0.004	0.21	-
20-Oct	7	100	2	4	0.049	0.058	0.054	0.089	-	0.046	0.107	0.0765	0.1883	-	0.006	0.01	0.008	0.21	-
20-Oct	7	100	2	5	0.819	0.817	0.818	0.089	+	0.004	0.048	0.026	0.1883	-	0.017	0.011	0.014	0.21	-
20-Oct	8	33	3	1	1.972	1.995	1.984	0.089	+	0.061	0.103	0.082	0.1883	-	0	0.001	5E-04	0.03	-
20-Oct	8	33	3	2	1.979	1.719	1.849	0.089	+	0.061	0.035	0.048	0.1883	-	0.004	0.004	0.004	0.21	-
20-Oct	8	33	3	3	1.831	1.837	1.834	0.089	+	0.105	0.06	0.0825	0.1883	-	0.017	0.013	0.015	0.21	-
20-Oct	8	33	3	4	1.337	1.285	1.311	0.089	+	0.057	0.112	0.0845	0.1883	-	0.024	0.024	0.024	0.21	-
20-Oct	8	33	3	5	1.330	1.799	1.565	0.089	+	0.103	0.089	0.096	0.1883	-	0.166	0.178	0.172	0.21	-
20-Oct	9	67	3	1	0.384	0.445	0.415	0.089	+	0.024	0.108	0.066	0.1883	-	0.006	0.003	0.005	0.21	-
20-Oct	9	67	3	2	0.025	0.030	0.028	0.089	-	0.027	0.055	0.041	0.1883	-	0.003	0.005	0.004	0.21	-
20-Oct	9	67	3	3	0.966	0.828	0.897	0.089	+	0.052	0.029	0.0405	0.1883	-	0.011	0.009	0.01	0.21	-
20-Oct	9	67	3	4	0.907	0.896	0.902	0.089	+	0.061	0.1	0.0805	0.1883	-	0.023	0.013	0.018	0.21	-
20-Oct	9	67	3	5	1.904	1.771	1.838	0.089	+	0.092	0.102	0.097	0.1883	-	0.119	0.115	0.117	0.21	-
20-Oct	10	100	3	1	1.692	1.711	1.702	0.054	+	0.054	0.026	0.04	0.0769	-	0.003	0.006	0.005	0.03	-
20-Oct	10	100	3	2	1.988	1.995	1.992	0.089	+	0.067	0.073	0.07	0.1883	-	0.011	0.007	0.009	0.21	-
20-Oct	10	100	3	3	2.000	2.000	2.000	0.089	+	0.048	0.026	0.037	0.1883	-	0.003	0.004	0.004	0.21	-
20-Oct	10	100	3	4	1.992	2.000	1.996	0.089	+	0.083	0.115	0.099	0.1883	-	0.006	0.029	0.018	0.21	-
20-Oct	10	100	3	5	0.092	0.084	0.088	0.089	-	0.181	0.186	0.1835	0.1883	-	0.021	0.016	0.019	0.21	-

Chapter V

Control of Aphids and Barley Yellow Dwarf by Planting Rows of Gaucho Insecticide-Treated Seed Among Rows Planted with Untreated Seed

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Abstract

In this study, a potential method of controlling aphids and barley yellow dwarf (BYD) on hard red winter wheat (HRWW) was investigated using rows with Gaucho-insecticide treated seed planted among rows with untreated seed. The use of such mixtures, if effective, could result in lower costs associated with the insecticide used. Field plots were planted near Stillwater, Oklahoma on 28-Sep-98 with seed of the HRWW variety Karl 92 treated with Gaucho (480F) insecticide. Six treatments were defined by the Gaucho concentration (GC) (1, 2, and 3 oz. cwt.) and proportion of rows planted with Gaucho-treated seed. Parameters measured and analyzed were greenbug (GB) and bird cherry-oat (BCO) aphid incidence, BYD incidence, fertile head density, grain weight, test weight, and thousand-kernel weight (TKW). Increases in GB and BCO aphid incidence in time were significant, and treatment X time interaction was insignificant. No effects of treatment on any parameter were observed. Thus, results of this study were not conclusive.

Hard red winter wheat (HRWW), which is the most economically important field crop in Oklahoma, is planted in the fall, remains dormant during the winter months, and then is harvested the following summer. Insects and diseases occur every year on HRWW and often result in yield reductions. Perhaps the most destructive insects that occur on HRWW grown in Oklahoma are greenbugs (GB) (*Schizaphis gramineum*) and the bird cherry-oat (BCO) aphid (*Rhopalosiphum padi*). Populations of these aphids can explode when temperatures are warm during the growing season, and damage caused by aphids is more severe when aphid infestations occur on seedling wheat during the fall than when older plants are infested in the spring (Kieckhefer and Gellner, 1982; Pike and Schaffner, 1985).

Aphids can also injure wheat by transmitting disease-causing viruses during feeding. The most common such viruses transmitted by greenbugs and BCO aphids in Oklahoma are the barley yellow dwarf viruses (BYDVs), which cause the disease barley yellow dwarf (BYD). Symptoms of BYD on winter wheat include yellowing and/or purpling of the foliage, and stunting (D'Arcy, 1995). BYD occurs every year on HRWW wheat in Oklahoma. Yield losses caused by BYD depend on several factors. Infections that occur in the fall (seedling stages) cause greater losses than infections that occur in the spring (Hammon *et al*, 1996; McGrath *et al*, 1987; McKirdy and Jones, 1997). Numbers of viruliferous aphids, which can vary from season to season and field to field, determine the amount of potential virus inoculum present (Pike and Schaffner, 1985; Tetrault *et al*, 1963). Temperatures in the fall and spring are favorable for both aphids and virus, whereas temperatures during the winter and early summer are often too extreme to favor aphid and disease outbreaks (De Barro, 1992; Michels and Behle, 1989).

Several strategies are employed for the control of BYD. These strategies are based on the manipulation of components of the plant disease triangle: the host, the pathogen, and the environment (Agrios, 1996) (Fig. 5.01). This three-component triangle, however, may not apply to pathosystems that have a fourth component - an insect that vectors the pathogen. Because aphids are the sole vectors of BYDVs, a pyramid provides a more accurate depiction of BYD disease components (Fig. 5.02) and will be used in this discussion.

Current strategies to control BYD on HRWW with reference to the manipulation of the BYD disease pyramid components include (1) planting tolerant varieties (manipulation of the host), (2) planting late in the fall (manipulation of the environment), and (3) applying insecticides to eliminate aphid vectors (manipulation of the vector or host and vector).

(1) Although there are no wheat varieties highly resistant or immune to BYD, some varieties such as 2137, 2163, and Custer have low levels of resistance or tolerance. Thus, planting such varieties may help reduce losses from the aphid/BYDV complex.

(2) Planting late in the fall tends to reduce losses from aphids and BYD (Hammon *et al*, 1996; McGrath *et al*, 1987; McKirdy and Jones, 1997). Because aphid populations are less likely to establish on late-planted wheat, lower disease incidence can be expected. More vegetation and hence protection is available for the support of aphid populations in early-planted wheat (mid-September) than

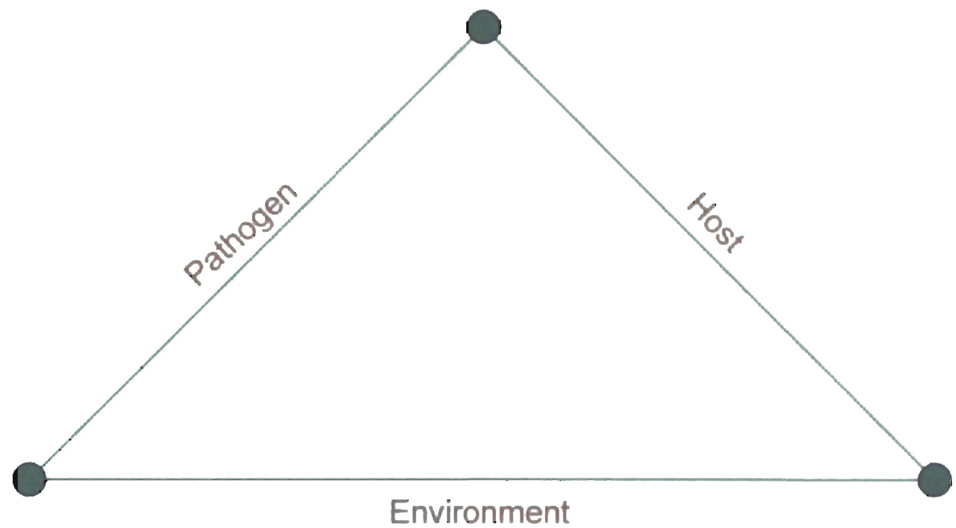


Fig. 5.01 The plant disease triangle and it's components: the host, the pathogen, and the environment. The presence of each component determines the amount of disease severity. Disease severity is high when a *virulent pathogen* is on a susceptible *host* plant in an *environment* that favors disease development.

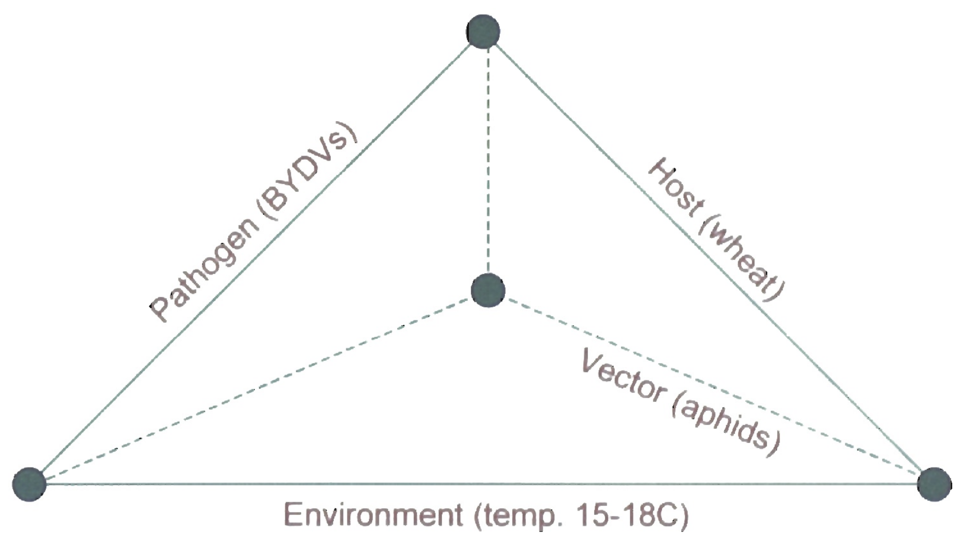


Fig. 5.02 The plant disease pyramid and it's components including the host, the pathogen, the environment and the vector of the pathogen. For barley yellow dwarf on wheat, disease is high when barley yellow dwarf viruses (BYDVs) [the pathogen(s)] are present in or nearby fields planted with susceptible varieties (the host), in addition to the presence and abundance of aphids (the vectors) that efficiently transmit the BYDVs. Temperatures ranging from 15-18C and high light intensity constitute an environment favorable for both aphids and BYDVs.

in late-planted wheat (October and November). Oklahoma wheat also provides forage for cattle during the fall and winter. Farmers commonly plant wheat as early as late August in an attempt to gain such forage.

(3) BYD control can also be achieved via insecticides (Araya and Foster, 1987; Gray *et al*, 1996; Irwin and Thresh, 1990). Reductions in aphid populations result in reduced transmission of BYDVs, which in turn should translate into lower disease incidence and severity. Seed-applied systemic insecticides may provide optimal control because of their ability to prevent aphid populations from becoming established. This strategy not only involves manipulation of the vector component of the disease pyramid, but also involves manipulation of the host plant; i.e., aphids are not directly targeted with insecticides, but are rather targeted through the wheat on which they feed. Insecticides applied after the establishment of aphid colonies (vector manipulation only) may not be as effective against primary spread of BYD because primary infections may occur prior to insecticide applications. The seed-applied insecticide Gaucho (Imidacloprid, Bayer Ag, Germany) is recommended for control of aphids at rates from 1-3 oz per cwt of seed, and for reduction of BYD at rates $\geq$2.0 oz. per cwt. Treatment at these higher rates has demonstrated effective control of aphids and BYD (Hunger *et al*, 1997). However, the cost of treating wheat seed at these rates (~ $7.00/bushel) may exceed the economic return in a weak grain market.

Imidacloprid, a nicotinoid, appears to stimulate movement followed by paralysis and incapacitation. Gourmet *et al* (1994) observed lower BCO aphid reproduction and higher fecundity with Gaucho treated oats compared to untreated oats. The initial spread of BYD increased apparently as a result of the stimulated movement associated with Gaucho. However, BYD incidence was reduced compared to the untreated oats most likely due to the rapid neurotoxic action of Gaucho.

In this study, a potential method of controlling BYD on winter wheat was investigated using combinations of rows planted with Gaucho-insecticide treated seed and rows planted with untreated seed. The use of such mixtures, if effective, could result in lower costs associated with the insecticide used. The hypothesis of this study is that mixtures of insecticide-treated and untreated seed would provide control comparable to a homogeneous seed treatment. The basis of this hypothesis relates to the theory that genetic diversity within crops contributes to insect and disease reduction. Less disease occurs within genetically diverse crops than within crops with little genetic diversity. This concept has been demonstrated using multiline cultivars as compared to monoline cultivars, using mixed cultivars as compared to single cultivars, and using mixed crops as compared to mono-crops (Andow, 1991; Browning and Frey, 1969; Power, 1991; Wolfe, 1985; Wolfe and Barrett, 1980). In all of these cases, lower disease incidence and severity occurred in crops with the greater diversity. Explanations for this observation vary depending on the disease in question; however, all hypotheses focus on the transmission of the pathogen. Lower transmission rates occur in crops that are more genetically diverse than in those crops with less diversity. In systems that involve fungal and bacterial pathogens, inoculum is typically spread by wind and/or rain to other plants. Pathogens that land on non-host or resistant plants are trapped, and unable to propagate for secondary transmission. Thus, an overall reduction in disease occurs.

However, the mechanism behind disease control in genetically diverse plantings that have systems that involve insect vectors of pathogens is more complex and less documented. Unlike abiotic vectors (i.e., wind and rain), insects may choose their host when migrating from plant to plant. Aphids tend to emigrate after having moved on an undesirable host or non-host plant, sometimes resulting in long distance movement and thus leaving the crop (Power, 1990). Furthermore, aphids in multi-line crops feed on more plants in a given time than those feeding on mono-line crops. Feeding on more plants means less time on plants, which may interfere with the acquisition and transmission of viruses (Power, 1991). These theories of mechanisms for such reduction in transmission may work in wheat fields that have mixtures of plants that are treated and untreated with insecticide. Viruliferous aphids that migrate to Gaucho-treated plants may transmit BYDVs to those plants, but quickly die as a result of insecticide. Thus, secondary spread of BYD would be limited.

Hence, experiments were designed and conducted to determine if treating less seed at a higher Gaucho concentration provides less, equal, or better control of aphids and BYD than treating more seed at lower Gaucho concentration, while the total amount of Gaucho applied remains equal.

METHODS AND MATERIALS

Fields Plots. Field plots for were planted near Stillwater, Oklahoma on Sep-21-98 with seed of the HRWW variety Karl 92 treated with Gaucho (480F) insecticide (Gustafson, Inc., Plano, TX) using a seed treater (Hege, unknown model) as indicated in Table 5.01. Treatments resulted in six combinations of 12 rows planted with Gaucho-treated and untreated seed plus an untreated control (0 oz. Gaucho). Plots were arranged in a randomized block design with 5 replications (Fig. 5.03). Each plot consisted of twelve, 10-ft. rows prepared with a Hege seven row small grains drill (H & N Equip, model 500) with 7 in. spacing. Wheat seed was hand planted about 1/2-3/4 in. deep at a seedling rate of close to 1.50 bushels per acre. Oats were planted between the plots in an effort to attract aphids and increase BYD pressure. On 16-Nov-98, Glean Herbicide (E.I. du Pont de Nemours and Co., Inc., Wilmington, DE) was applied to plots for weed control, and on 12-Apr-99, Tilt (Novartis, Greensboro, NC) was applied to plots in an effort to reduce powdery mildew, rust, and other foliar fungal diseases.

Artificial aphid infestations. On 03-Nov-98, BCO aphids obtained from the USDA-ARS (Keith Mircus, Stillwater, OK) were raised on Karl 92 wheat seedlings infected with BYDV-pav and –rpv in a growth chamber (GC) [Conviron PG W 36] at 16.5C with a 16:8 photoperiod. Light intensity measured 192.1μ Es-1m-2 at 6 in. above the GC floor. On 17-Nov-98, aphids were collected from the GC and allocated into 35 parts, one part for each field plot. Each plot was infested with

Table 5.01 Treatments (1-7) defined by Gaucho rate (1, 2, and 3 oz. cwt.) and percentage of rows that were treated and untreated with Gaucho.

Trt.	Gaucho Rate*	Percentage of Rows* Treated	Percentage of Rows* Untreated
1	0	0	100
2	1	100	0
3	2	100	0
4	2	50	50
5	3	100	0
6	3	67	33
7	3	33	67

* Represents mixtures of Gaucho treated and untreated seed.

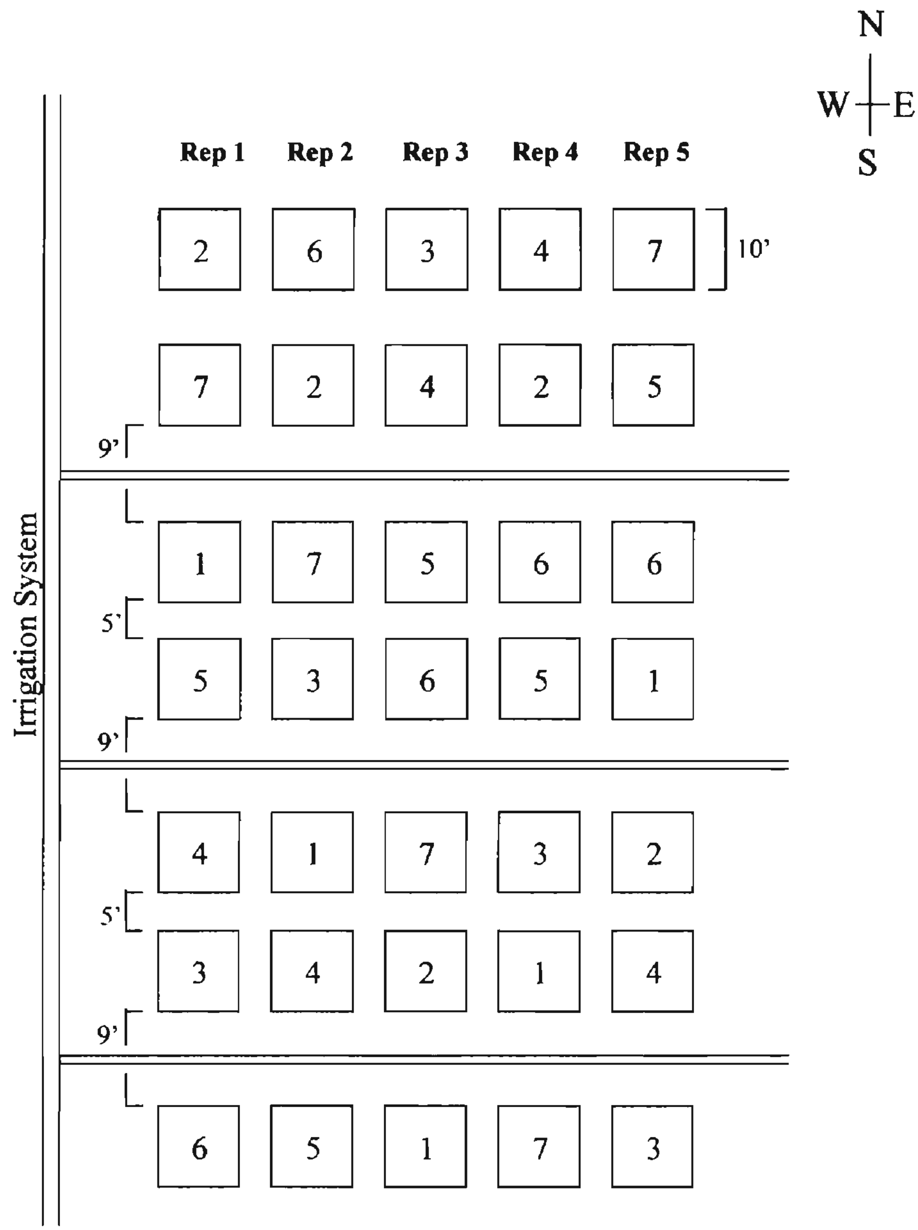


Fig. 5.03 Diagram of barley yellow dwarf field plots including dimensions of plots and distances between plots. The randomized block design consisted of 7 treatments (north to south) and 5 replications (west to east).

0.05 g of BCO aphids. Infestations were performed by placing one petri dish containing the allotted BCO aphids at ground level in the center of each plot.

Aphid incidence and BYDV presence. Aphid incidence was determined by counting the number of BCO aphids and GB in a randomly selected linear ft. of row within each of the middle four rows on 28-Feb-99, 14-Mar-99, 06-Apr-99, and 30-Apr-99. BYD disease incidence was quantified on 23-Apr-99 by randomly selecting 10 flag leaves from a randomly selected row of each treatment. On 28-Jun-99, fertile head density was quantified by counting the number of fertile heads in a randomly selected linear ft. of row within each of the middle four rows. The middle four rows of each treatment were harvested by machine (Hege, model 125). Total grain weight, test weight, and thousand-kernel weight were quantified.

RESULTS AND DISCUSSION

High temperatures and drought during the summer and fall of '98 required field plots to be irrigated throughout the fall growing season to improve plant growth. Plots were mowed to simulate grazing in mid-November and again in late-February.

Two factors confounding the results were observed and included, (1) an infestation by the fall armyworm, and (2) incidence of take-all disease. In early

October, fall armyworm infestations caused severe damage throughout the plots. Stands affected by the fall armyworm were reduced and stunted throughout the remainder of the growing season. In addition to the fall armyworm infestation, patches of take-all decline disease were observed in the spring of '99 when plants began to head. *Gaeumannomyces graminis*, the fungus that causes take-all, survives on debris in the soil during the off-season and invades the roots of wheat during the growing season. Plants infected with the disease become stunted, quickly die, and do not yield grain. Unfortunately, management of take-all decline in the plots could not be achieved because this disease cannot be controlled in a single year. Practices such as crop rotation and deep tilling are recommended for control and multiple growing seasons are required.

Probability values generated from this analysis for all research parameters measured are presented in Table 5.02. All data in this study was analyzed using the general linear models procedure of SAS (SAS Institute, 1998). Aphid incidence was taken at five different time points, so repeated measures analysis was necessary. PROC GLM (SAS Institute, Cary, NC) was used to assess the effects of treatment, time, and the treatment by time interaction. Further models were used that utilized the numeric nature of treatment in order to gain inferences of aphid incidence, BYD incidence, fertile head density, and grain weight, test weight and thousand kernel weight (tkw). This relationship described further with the use of regression techniques.

Table 5.02 Probability values from the mixed procedure examining the effect of treatment and time on bird cherry-oat (BCO) aphid and greenbug incidence, BYD incidence, fertile head density, grain weight, test weight and thousand kernel weight (TKW).

S = Significant NS = Not Significant	BCO Aphid Incidence	Greenbug Incidence	BYD Incidence	Fertile Head Density	Grain Weight	Test Weight	TKW
Treatment (Trt.)	NS P=.3949	NS P=.5762	NS P=.8399	NS P=.0962	NS P=.9232	NS P=.5124	NS P=.3640
Time	S P<.0001	S P<.0001					
Trt. X Time Interaction	NS P=.7276	NS P=.7985					

Aphids

In determining aphid incidence, BCO aphids and GB were counted seperately; however, the majority of the aphids present were BCO aphids. Artificial infestations of viruliferous BCO aphids were conducted 17-Nov-98. A freeze occurred on 20-Nov-98, and field observations indicated BCO aphids from the artificial infestations did not become established. No aphids were identified in plots until natural infestations of BCO aphids were observed on 14-Mar-99 (<10 aphids/linear ft. of row) (Fig. 5.04). GB, however, were not observed until 14-Mar-99 (<1 aphid/linear ft. of row). No significant effect of treatment ('TRT') on BCO aphid or GB incidence was identified (Table 5.02). However, significant increases in both BCO aphids and GB incidence was observed through 'Time' (Table 5.02, Fig. 5.04), with no TRT X Time interaction (Table 5.02). Observed BCO aphid incidence peaked in early-April '99 (~45 aphids/linear ft. of row), steadily declined to ~40 aphids/linear ft. of row by the end of April '99, and vanished by mid-May '99. In contrast, observed GB incidence peaked in early-May '99, but only to about 10 aphids/linear ft. of row. Like BCO aphids, GB vanished by mid-May '99 (Fig. 5.05). No BCO aphids or GB were identified through the remainder of the season.

Barley Yellow Dwarf

Artificial infestations of viruliferous BCO aphids were conducted on 17-Nov-98 in an effort to facilitate fall infestations of viruliferous BCO aphids. The establishment of such infestations would most likely have resulted in higher BYD

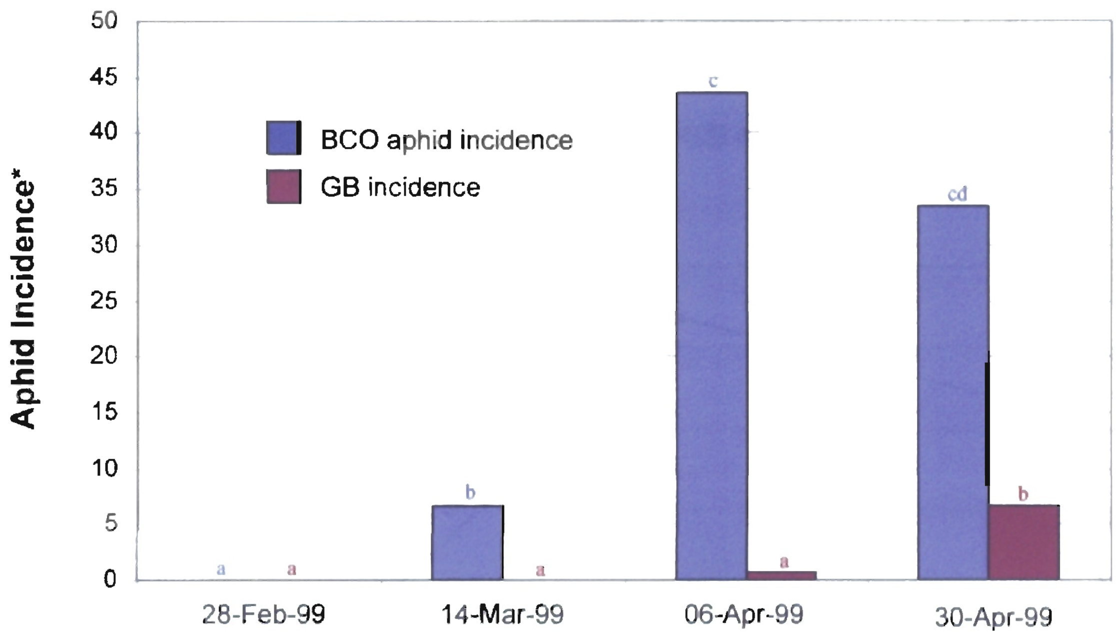
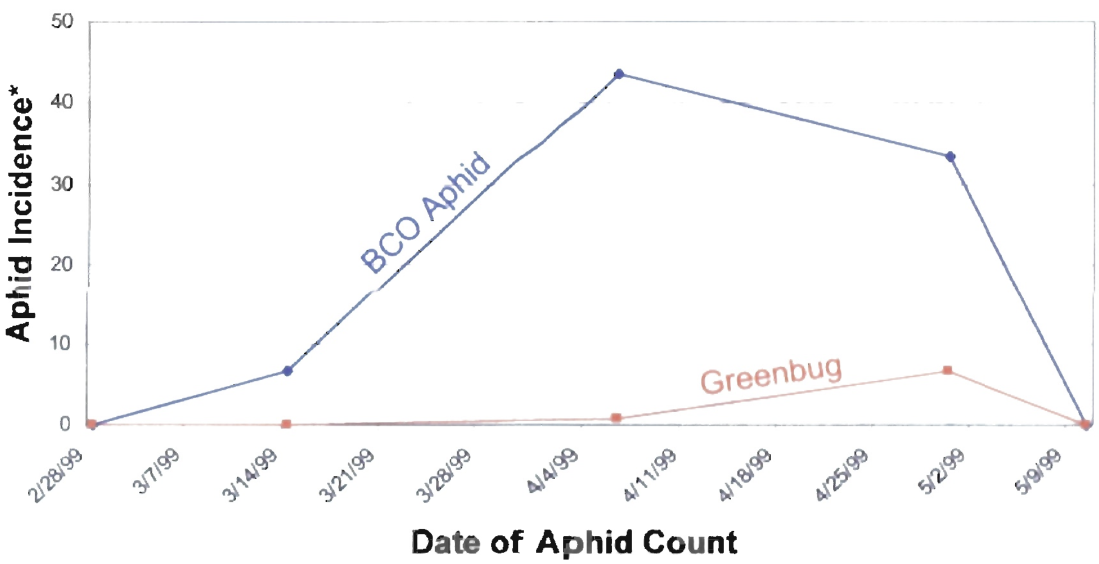


Fig. 5.04 Incidence of greenbugs (GB) and bird cherry-oat (BCO) aphids observed from 28-Feb-99 to 30-Apr-99. Mean aphid incidence with the same letter are not significantly different.

* Aphid incidence was determined by the number of GB and BCO aphids per linear ft. of row.

Fig. 5.05 Incidence of BCO aphids and GB observed in wheat from 28-Feb-99 to 25-Apr-99.



* Aphid incidence was determined by the number of bird cherry-oat (BCO) aphids and greenbugs (GB) per linear ft. of row.

incidence and severity as opposed to the natural infestations that occurred in the spring of '99. Although the artificial BCO aphid infestations appeared to be unsuccessful in terms of establishing BCO aphid colonies, field observations and subsequent testing of plants in mid-April '99 confirmed the presence of BYDV-pav and -rpv. However, the occurrence of BYD was very limited as was indicated by slight stunting in the centers of some plots where artificial infestations were conducted. Thus, the presence of BYD was suspected to be a result of the artificial viruliferous BCO aphid infestations, although this could not be confirmed. BYD severity visually was not significant among TRT, and thus BYD severity readings were not performed. BYD disease incidence, however, was measured and the statistical analysis of the effect of TRT on BYD disease incidence was not significant.

Yield and its Components

Wet weather during late-June and early-July '99 caused a delay in harvesting the field plots. By 28-Jun-99, weather and field conditions allowed measurement of fertile head density and the plots were harvested. Grain weight, test weight and TKW were later determined. No effects of TRT on fertile head density, test weight, or TKW were significant (Table 5.02).

The primary objective in this study was to determine the value of planting rows with Gaucho-treated wheat seed among rows planted with untreated wheat seed on the incidence of aphids and BYD. The analysis of data did not indicate any effect of planting rows with Gaucho-treated seed among rows untreated on any of the parameters measured. The fall armyworms and take-all undoubtedly weakened the analysis. Furthermore, the absence of aphid incidence in the fall (other than the two-three day period when viruliferous BCO aphids were present from artificial infestations conducted in November) minimized potential treatment effects, as Gaucho-insecticide is most active 60 days after planting. Also, no effects of treatments were expected on the low aphid incidence levels observed from Mar-99 through Apr-99. Thus, the effect of planting rows with Gaucho-treated seed among rows planted with untreated seeds for controlling aphids and BYD were not determined.

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VITA

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Thesis: EFFECT OF THE BIRD CHERRY-OAT APHID *(RHOPALOSIPHUM PADI)* ON WHEAT AND CONTROL OF THE APHID/BARLEY YELLOW DWARF COMPLEX WITH GAUCHO (IMIDACLOPRID) INSECTICIDE

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