THE EFFECT OF GREENBUG DAMAGE ON CARBOHYDRATE LEVELS IN WINTER WHEAT SEEDLINGS

By

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THE EFFECT OF GREENBUG DAMAGE ON CARBOHYDRATE LEVELS IN WINTER WHEAT SEEDLINGS

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PREFACE

My stay in Stillwater has been the best of times and the worst of times. I leave with academic knowledge, but more importantly with fond rememberences of kindness and helpfulness from the people of Oklahoma State University, the United States Department of Agriculture, the town of Stillwater, and the state of Oklahoma.

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Finally, I would like to dedicate this body of work to myself because it was I who lived the monastic life in Stillwater, shed the tears, and spent all the sleepless nights studying and extracting carbohydrates in the laboratory.

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CHAPTER I

INTRODUCTION

Aphids have fascinated and frustrated man for a long time. The aphid is characterized by polymorphism, an intricate life style that is closely inter-related with host plants, and the ability to reproduce both asexually and sexually. The modes of aphid feeding and reproduction have led to a close and specific association with certain plants through evolutionary history and affected aphid size and population structure. In turn, these consequences have influenced aphid life history patterns and dispersal distribution (Dixon 1985).

Aphids belong to the insect superfamily Aphidoidea, within the order Homoptera, the plant-sucking bugs. Although the oldest known aphid fossil, Triassoaphid cubitus, is from the Triassic, it is thought that aphids originated from the now extinct Archesyntinidae in the Carboniferous era, or early Permian, 280 million years ago. A conspicuous evolution of aphids was later associated with the appearance of flowering plants, the angiosperms, and the host plants of most present-day aphids are angiosperms, although some aphids live on gymnosperms and a few species attack ferns and mosses (Dixon 1973).

There are about 4,000 described species of aphids. Compared with 10,000 grasshoppers, 12,000 geometrid moths, and 60,000 weevils, the aphids are a contained group. Aphids, however, are serious pests of agricultural crops and forest trees. Several generations are born each year and their reproductive potential is great. Their complex life cycles and polymorphism enable them to exploit host plants and respond to most contingencies of their environment. The greatest number of aphid species occurs in temperate regions, and one plant species in four is infested (Dixon 1985).

Salient Features of Aphids

Mouthparts: their structure and mode of operation

After alighting on a plant, aphids walk over the surface testing it with the antennae and by probing it with their mouthparts. While walking over and probing the surface of a plant, an aphid obtains information about physical properties and chemistry of both the surface and internal chemistry of the plant. This initial investigation of the surface involves little or no stylet penetration, but often enables an aphid to sense the suitability of a plant within sixty seconds. This is the time it takes an aphid to penetrate the epidermis and exude saliva. Once an aphid settles, it probes deep into the plant (Dixon 1985).

Aphids feed by inserting their slender mouthparts, the stylets, into plant cells. The stylet bundle consists of an

outer pair of mandibular stylets and an inner pair of maxillary stylets (Figure 1). The maxillary stylets are held together by interlocking grooves and ridges, and in the channels formed between them run the food and salivary canals. In the resting position the stylet bundle lies in a groove along the anterior surface of the aphid's proboscis. The terminal segment of the proboscis grips the stylet bundle (Figure 2) (Dixon 1973). When penetrating the tissues of a plant the stylets go between the cells, and only rarely pass through a cell, until they reach the sieve tubes of the host plant (Dixon 1973).

While probing plant tissues aphids secrete saliva that forms a sheath around the stylets (Figure 3). The protein sheath is mainly material which is secreted by the salivary glands (Figure 4). The salivary sheath gives rigidity to the flexible stylets and enables aphids to control the direction of the probe by restricting the bending, except at the apex of the stylet (Pollard 1973).

Ingestion and Digestion of Plant Sap

Most aphids obtain food from sieve tubes in phloem tissue of plants. Phloem sap is under 10 to 30 atmospheres of pressure (Dixon 1985) and aphids probably rely on turgor pressure to force sap up the extremely fine food canal in the stylets. However, turgor pressure does not account for aphid feeding success on artificial diets which are not under

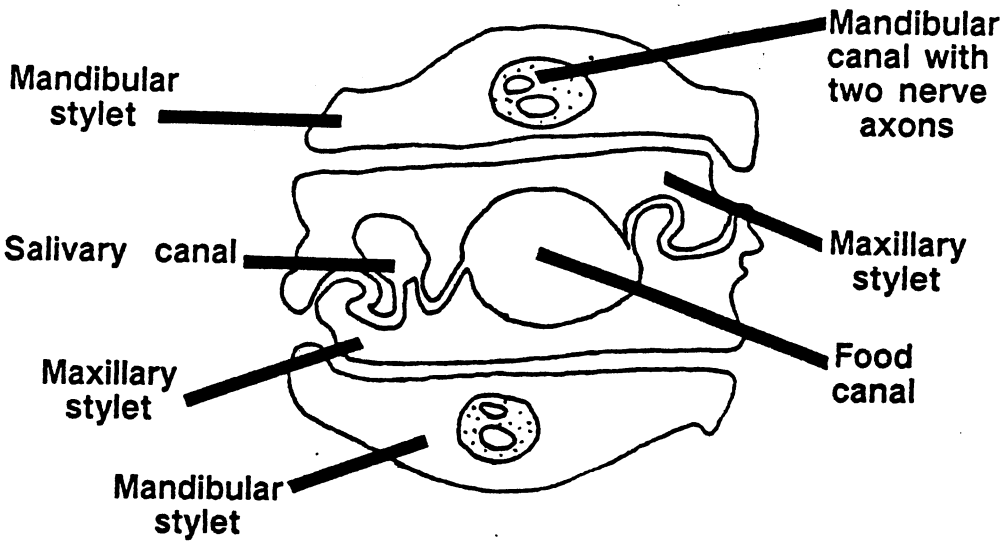


Figure 1. Diagram of a transverse section through the stylet bundle of an aphid (Dixon 1973).

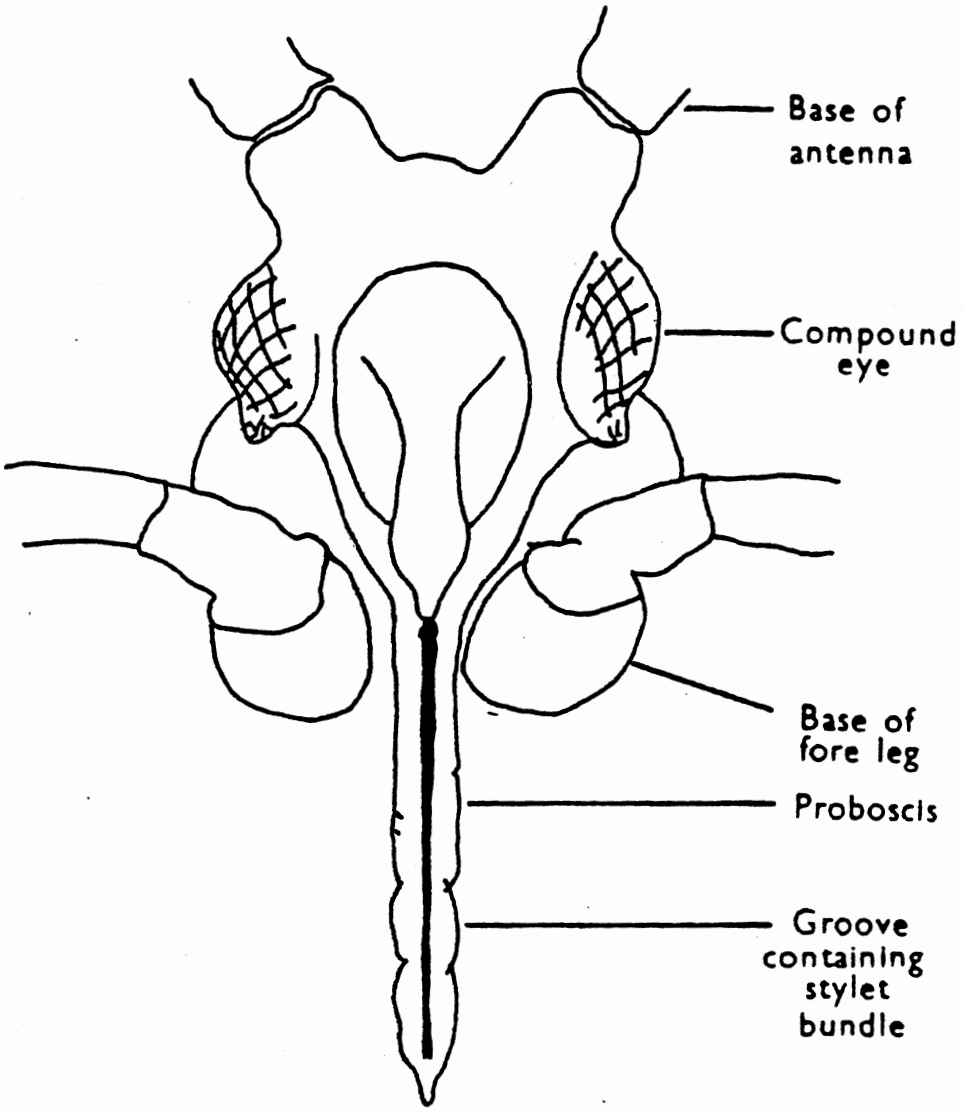


Figure 2. Anterior view of the head and proboscis of an aphid to show the groove in the proboscis in which lies the stylet bundle (Dixon 1973).

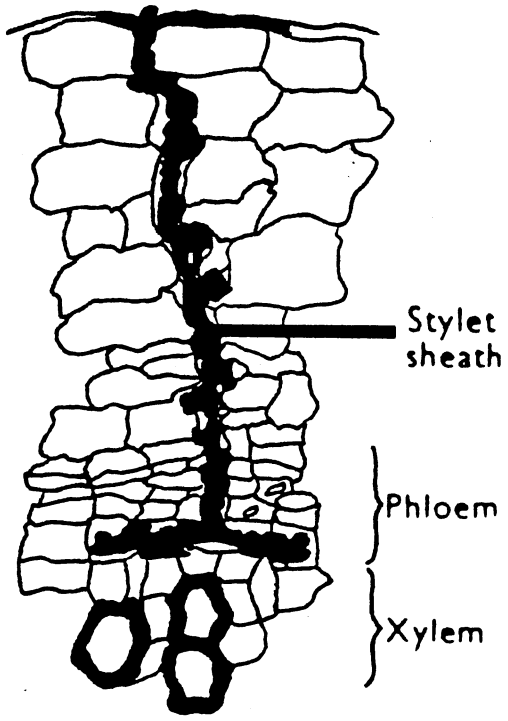


Figure 3. Stylet sheath laid down in the tissues of a plant by an aphid feeding on the phloem sieve tubes (Dixon 1973).

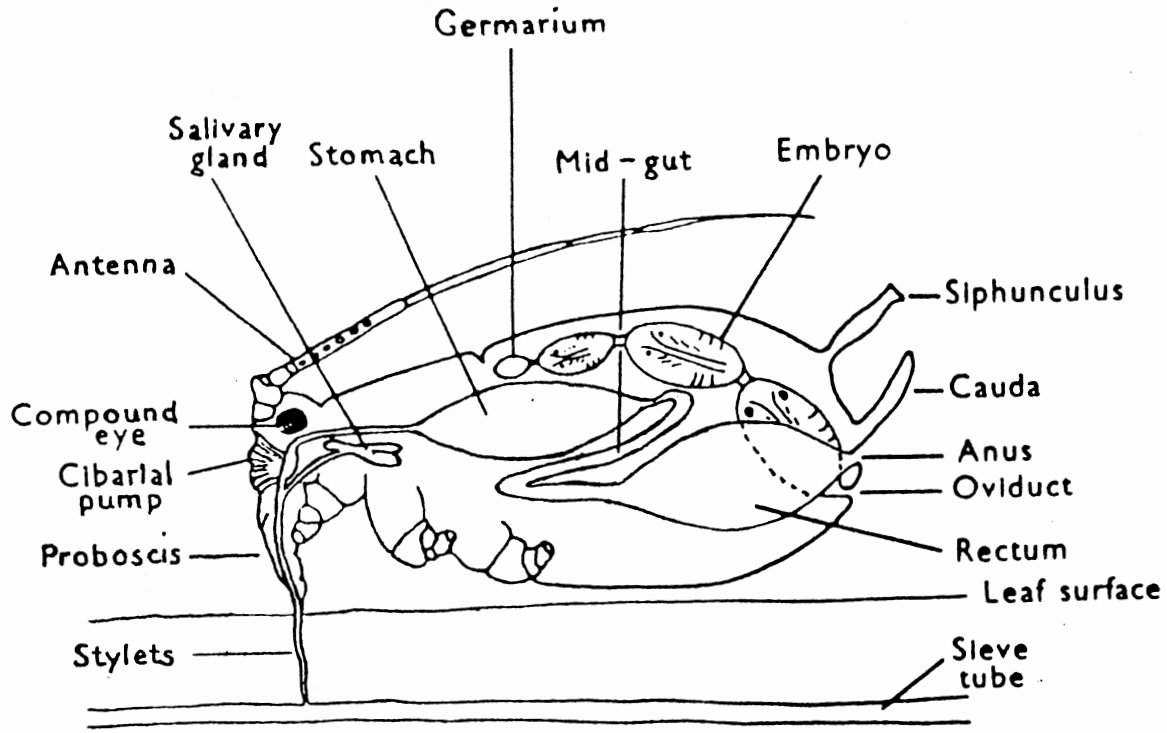


Figure 4. Diagram of an aphid feeding on a plant to illustrate the internal anatomy relative to the external features of the aphid (Dixon 1973).

pressure. In this case, aphids probably use the cibarial pump to control the flow of food (Figure 4).

Phloem sap is rich in sugars but poor in amino acids, which are essential for growth. Aphids ingest a large amount of sap in order to acquire sufficient amino acids. The residual solution of digested food, mainly sugars, is stored in the rectum before ejection to the exterior in the form of a droplet of honeydew.

Nitrogen Metabolism and Excretion

The excretory system in most insects consists of Malpighian tubules which regulate the balance of water and ions in body fluids, and remove nitrogenous wastes. However, aphids lack Malpighian tubules and excrete nitrogenous wastes in the form of ammonia through the gut to obviate toxic effects. Symbiotic organisms within certain cells of the body are thought to detoxify part of the ammonia produced by the aphid and also to fix atmospheric nitrogen. The cells containing these organisms are called mycetomes and are extremely well developed in young aphids (Dixon 1973).

Gonads and Embryogenesis

All aphids appear to show diploid parthenogenesis. There is no reduction division and development starts from a cell containing a full complement of chromosomes including 2X chromosomes. Males are produced by the loss of an X

chromosome at the first division. Males produce haploid sperm which contain an X chromosome only. Fertilization of haploid eggs of sexual females results in a diploid cell with two X chromosomes, therefore, all aphids hatching from fertilized eggs are females (Dixon 1973).

At birth, nymphs are structurally similar to adults. Those destined to become winged adults possess wing buds. At each moult there is an increase in size and a gradual change towards adult form. There is no dramatic transition in form between the last juvenile stage and the adult.

Polymorphism

An aphid life cycle is made up of a sequence of morphs that differ in both behavior and structure. In a few species there can be as many as eight morphs that differ in their external morphology. Polymorphism is characteristic of aphids and reaches its greatest development in host-alternating species (Dixon 1985). In many aphids, parthenogenetic females show alary dimorphism. That is, they can be either alate (winged) or apterous (wingless).

During periods of stress, such as mid-summer and mid-winter, a few species can produce either aestivating or hibernating morphs. In autumn most aphids produce sexual forms: wingless females and usually winged males, although apterous males are known and a few species have both apterous and alate males. After mating, sexual females lay eggs,

enabling many species to survive the winter and hatch the following spring (Dixon 1985).

In addition to the sexual morphs in highly polymorphic species of aphids, there are also alate and apterous parthenogenetic morphs. Crowding, quality of the host plant, temperature, and photoperiod have been implicated in the development of alate forms (Dixon 1985).

Energy for Flight

Energy for flight is obtained by respiration of fats and glycogen. Glycogen is used first and then, after the first hour of flight, fat becomes the principal fuel. In the conversion of fats, water is released which enables the aphid to maintain its water content even after prolonged flight in dry air.

After reproduction has begun (in some species), indirect flight muscles begin to degenerate. This prevents further flight and it has been suggested that the break-down products from muscles are used in the reproductive process (Dixon 1985). Breakdown of flight muscles is rapid in some aphids, but in other species it can take fourteen to twenty-one days.

Escape Responses and Defense Mechanisms

Aphids are seldom distributed evenly over host plants, but are usually found clustered together on leaves and stems. Such clustering may result from the aggregation of

parthenogenetically born young, the aggregation habits of some species following dispersal flights, or disruption from clusters. Aphid species differ in their propensity to aggregate following mechanical or chemical disruption. This propensity appears to be correlated roughly with the degree of myrmecophily (ant attendance). Tight clusterings of myrmecophilous aphids minimize the "tending territory" of attendant ants, thus facilitating honeydew collection and the protection of aphids from predators (Nault and Phelan 1984).

The general impression is that aphids are helpless, sedentary, and thin-skinned creatures which are entirely defenseless. If a predator seizes an appendage of an aphid the latter may attempt to kick the predator away, or pull the appendage free. If the predator is small relative to the aphid, then the predator can often be kicked off the plant. In some species of aphids, if pulling fails, the aphid sheds the leg held by the predator.

For most aphid species, defense is centered around paired, tubular cornicles. When attacked by a predator or parasitoid an aphid secretes sticky droplets of triglycerides from its cornicles (Figure 5). The release of droplets is reflexive and under nervous control. If daubed on the predator's head, the cornicle secretions can gum the predator's mouthparts and irritate its sensory organs, resulting in release of the aphid prey.

The principal alarm pheromone utilized by several aphid

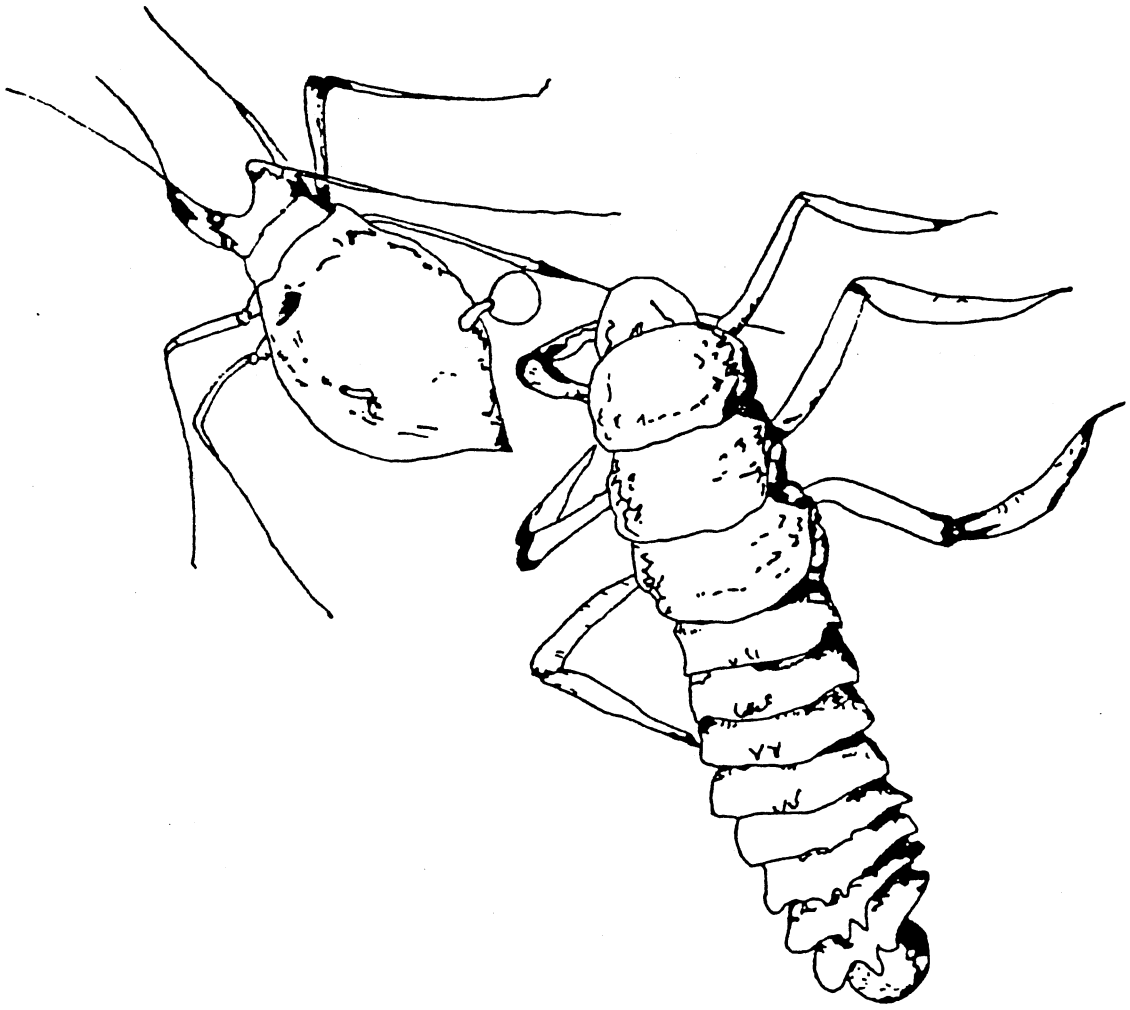


Figure 5. An aphid attempting to escape from a larva of a ladybird beetle which has seized a hind leg of the aphid. The aphid attempts to pull its leg free, at the same time daubing a droplet of siphuncular secretion on to the head of the predator (Dixon 1973).

species is (E)-beta-farnesene (EBF). Aphids also utilize the cyclic sesquiterpene, (-)-germacrene A, as a pheromone. These highly volatile compounds result in rapid tranmission of alarm messages while high lability provides rapid decomposition of the molecule so that the alarm message does not persist (Nault and Phelan 1984).

Aphids respond to alarm pheromones in a variety of ways. The most marked response is by falling or jumping from the host. Some will walk away from the predator or drop off the host plant. Certain aphids will either back up, wiggle, or jerk, but not remove mouthparts from feeding sites.

Greenbugs

Economic Importance

The greenbug, Schizaphis graminum (Rondani), was described in Italy and the first reported infestation of this insect in the United States was in Virginia in 1882 (Daniels 1977 and Starks and Burton 1977). Since then, at least nineteen outbreaks have occurred. The aphid is an economic pest of cereals in the Central, Northwestern, and Southeastern United States. The greenbug costs an estimated $300 million each year in yield loss and control expenditures on wheat, barley, oats, rye, grain sorghum and other hosts in North and South America, Asia, and Africa. Economic damage from greenbugs occurs every year, but periodic outbreaks, about every seven years, cause large-scale crop losses over

widespread areas. An outbreak in 1976 was estimated by the Oklahoma Agricultural Extension Service to cost Oklahoma wheat producers $80 million (Burton et al. 1985).

Biotypes

A biotype is an individual or a population within a species which is distinguishable by criteria other than morphology. Five distinct biotypes of greenbug have been identified: A, B, C, D, and E. Biotypes A, B, C, and E designations were based on plant reaction to plant resistance. Biotype D was designated based on an insecticide response. Dickinson Selection 28-A (DS 28 A, CI 13833) wheat was resistant to biotype A, but not to the other biotypes. Biotype B was found in 1958 and predominated in the Southern Great Plains. The resistance of DS 28-A was overcome by biotype B (Tyler et al. 1985). Biotype C was recognized in 1968 when there was a severe outbreak of greenbugs on grain sorghum. Prior to this time greenbugs were not known to attack sorghum. Biotype D is an organophosphate insecticide-resistant strain (Peters et al. 1975). It showed resistance to disulfoton. The latest major greenbug biotype, designated E, was discovered in 1980 when Amigo wheat and advanced breeding lines having Amigo-derived resistance showed suseptible reactions to greenbugs collected near Bushland, Texas (Porter et al. 1982). Biotypes C and E comprise the majority of current field populations with biotype E

predominating in most areas. The reason for the development of biotypes of the greenbug in the United States is partly because the pest reproduces rapidly and by parthenogenesis. Also, plant resistance in crops has been obtained by simple, single gene action (Starks et al. 1983). There is no record of how long biotype A had been in existence or how many previous biotypes had existed, and it is unknown how long the present biotype E will endure before another change occurs (Daniels 1981).

A wide array of factors contribute to the ability of greenbugs to rapidly reach pest proportions. They have alate (winged) and apterous (wingless) adult forms. All the apterous types are females. Outbreaks in the spring are thought to be caused by winged females moving into northern areas on wind currents from the south. The pest has a high parthenogenetic reproductive rate and most of these give birth to living young (viviparism). There is sexual reproduction in the life history of greenbugs but its importance is unknown because the development of a sexual form and eggs have not been observed in the field, only in the greenhouse. Greenbugs which are pale green when they first emerge and have a dark-green stripe down the back when fully grown, are typified by sexually maturing in a short time (about one week), producing several offspring (50 to 100 young), and by having several generations during the growing season. In Indiana, there may be twenty generations a year.

In Texas and Oklahoma even more generations are possible (Starks and Burton 1977). Under optimal conditions (favorable weather, absence of parasites and predators, space, and unlimited food source), in a lifetime a single aphid could give rise to one-half million offspring.

Methods of Control

Before the advent of organophosphates in 1949, farmers were almost helpless in effecting satisfactory control of greenbugs. Early practices included burning straw and dragging chains through the field. Also, sprays of kerosene and solutions of whale oil soap were applied (Wood 1971). Organophosphorus chemicals afforded reliable control at relatively small dosages and reasonable cost for about twenty years, but in 1974 tolerance to the insecticide disulfoton became pronounced (Peters et al. 1975, Starks and Burton 1977). The use of insecticides can also extract an ecological price. Insects which were once incidental pests may became primary pests through resistance to insecticides. The insecticides can kill predators and parasites which might keep the newly developing primary pests below economically significant thresholds of loss.

Natural enemies, weather, and climate are other factors that help suppress aphid build-up. Greenbug populations are reduced by several insect enemies, including predaceous lady beetles, lacewings, damsel bugs, syrphid fly larvae, several

species of spiders, and small parasitic wasps. The braconid wasp, Lysiphlebus testaceipes (Cresson), is the major primary parasite of this aphid on the High Plains of Texas (Chedester 1979). It lays its eggs within the body of the greenbug. After hatching, the young feed, develop, and emerge as adults through holes they cut in the host's body wall. A survey of parasitoids of the greenbug in 1968 and 1969 (Jackson et al. 1970) and in 1972 (Archer et al. 1973) indicated that L. testaceipes was the most abundant primary parasitoid collected from the aphids in Oklahoma. In the Texas Panhandle, in 1977, there was a low greenbug population even during the winter months, mainly a result of heavy parasitization by L. testaceipes. In the fall of 1976, most greenbugs in the Texas Panhandle area were parasitized (Daniels and Chedester 1980). This wasp reproduces much more slowly than greenbugs when the temperature is below 18°C. Consequently, during long periods of cool weather, greenbugs can increase without much interference from the wasps (Starks and Burton 1977).

Late in the growing season, lady beetles are frequently found in fields heavily infested with greenbugs. Lady beetles are energetic feeders on aphids, but they often enter a field after economic damage has taken place. Efforts to introduce lady beetles into fields earlier, before crops are damaged, have not been successful (Starks and Burton 1977).

Greenbugs can reproduce and develop at temperatures from

4 to 38°C, but reproduce most rapidly between 21 and 24°C. Effects of winter temperature on greenbugs were studied in twelve counties of the Texas Panhandle for twenty-six years (Daniels and Chedester 1980). From these studies it was concluded that the average temperature must be below -7°C for a consecutive period of at least a week to kill more than 95% of an unprotected (without snow cover) greenbug population.

Damage to Host

Wheat is an annual grass that grows from sea level to altitudes over 3,000 m. It is best adapted to well-drained, clay-loam soils and to temperate, arid, or semi-arid environments. Botanically, wheat belongs to the genus Triticum L. in the grass family Gramineae. The genus is divided into several species based on morphology and in part on chromosome number. Common wheat, T. aestivum, and club wheat, T. compactum, have twenty-one chromosome pairs (three genomes), whereas durum wheat, T. durum, has fourteen chromosome pairs (two genomes). These three wheats account for approximately 90% of the cultivated crop (Wiese 1977).

Winter wheat is a preferred host of the greenbug and can be attacked both in fall and spring on the Southern Great Plains. Greenbugs feed intracellularly on aerial portions of wheat plants by sucking sap. In the process of feeding, they apparently inject a toxin-like substance that causes highly

visible damage ranging from chlorotic spots to severe necrosis. Heavy unrestricted infestations can result in dead leaves and eventual plant mortality (Burton 1986). Entire wheat fields may be decimated by offspring from swarms of migrating greenbugs.

Research has focused primarily on effects of greenbug infestations on above ground portions of wheat. However, a few studies have shown that greenbug feeding causes a reduction in plant root systems. Ortman and Painter (1960) and Daniels (1965) showed that roots of infested plants had fewer root hairs and adventitious roots. The roots were also shorter than those of uninfested plants. Pike and Shaffner (1985) showed that injury to wheat was highest where aphid infestations started at the two-leaf stage, intermediate to none at the four-leaf stage, and none at the two-tiller stage. This damage reduced root and shoot weights and grain yields. Burton (1986) showed that greenbug feeding does interfere with root and shoot growth and continues to interfere, probably throughout the life of the plant, perhaps indicating why greenbug seedling damage influences yield.

Other studies have associated phytophagous insect damage to the reduction of plant cold hardiness and have implicated the importance of carbohydrates and other root reserves to cold hardiness of plants (Levitt 1981, Olien and Smith 1981, Olien and Lester 1985). Cold hardiness of alfalfa, Medicago sativa L., was decreased by the pea aphid, Acyrthosiphon

pisum (Harris) (Harper and Freyman 1979, 1983). Wellso et al. (1985) observed a 20% decrease in fructans in seedlings damaged by the bird-cherry oat aphid, Rhopalosiphum padi L. Wellso et al. (1986) also demonstrated that Hessian fly, Mayetiola destructor (Say), larval feeding reduces fructans, glucose, and fructose in wheat crowns and, in turn, reduces cold hardiness of plants grown under controlled conditions.

Nonstructural Carbohydrates

Nonstructural carbohydrates are those that can be accumulated and then readily mobilized for metabolism or translocation to other plant parts (Smith 1981). Nonstructural carbohydrates, including the reducing sugars, glucose and fructose; a nonreducing sugar, sucrose; fructans; and starches, are the major reserve constituents. They are a readily metabolized source of energy needed for growth and respiration of the plant (White 1973). Reserves are essential for survival and for producing plant tissues when carbohydrate utilization exceeds photosynthetic activity (Smith 1981).

Type, distribution in the plant, and relative proportions of individual carbohydrate reserve components vary among and within grass species and under various climatic conditions during the growing season (White 1973). The primary storage carbohydrates in grass species of temperate origin are sucrose and fructans, whereas starch and

sucrose are the reserves of those grasses of subtropical or tropical origin (Weinmann and Reinhold 1946, Smith 1968, and Ojima and Isawa 1968). Smith (1981) found that native and introduced grass species in the Hordeae tribe (barley) accumulated only short-chained fructans; species in the Aveneae (wheat) accumulated fructans, predominantly of long-chain length; and species of the Festuceae (fescue) represented both types of accumulators. In addition, these grasses also accumulated small amounts of starch (1 to 6% dry weight).

An estimate of the concentration of total nonstructural carbohydrates (TNC) is often desired rather than an estimate of each fraction. A number of methods have been used to extract and measure TNC from plant tissue. The method of extraction used can greatly influence values obtained due to differences in the type of carbohydrate stored (Smith 1964). These extraction procedures include the Heinze and Murneek (1940) alcohol extraction; Weinmann's (1949) procedure using takadiastase, followed by acid hydrolysis; the Lindahl et al. (1949) modification of the Weinmann technique; and the widely used Smith method (1969; revised 1981).

The most common methods for TNC analysis have been those using takadiastase enzyme or acid solutions (Smith 1973). When using the takadiastase method, the enzyme hydrolyzes disaccharides and starches to monomers. Fructans extracted in the water are not hydrolyzed by the diastatic enzyme and

must be hydrolyzed in the filtrate with acid. Acidic solutions are used to hydrolyze the sugars and nonstructural polysaccharides to monomers. However, a strong acid solution may hydrolyze some structural carbohydrates (cellulose and hemicellulose) while a weak acid solution may not hydrolyze all of the starch. Fructans are readily hydrolyzed to monomers by weak acid solutions, but high acid concentrations can destroy fructose.

Hot and cold water have also been utilized in the removal of nonstructural carbohydrates. Free sugars are highly water soluble, but nonstructural polysaccharides (fructans and starches) are differentially soluble in water. Since fructan accumulators contain small concentrations of starches, cold or hot water will not remove all of the nonstructural carbohydrates. Thus, the term "cold or hot water-soluble carbohydrates" is not synonymous with that of "total nonstructural carbohydrates" (Smith 1973).

Agronomic research led to the understanding of diurnal variation of nonstructural carbohydrates in plants (Miller 1924, Krotkov 1943, Holt and Hilst 1969, Smith 1973). Concentrations of total sugars and water-soluble carbohydrates in temperate grass herbage appear to increase during the morning hours until sometime in the afternoon and then decrease until daylight the next day. Most of the diurnal variation in sugars appears to be due to changes in sucrose concentration (Smith 1973). One of the earliest and

most definitive studies of diurnal variation in carbohydrate content of crops was published by Miller (1924). In his experiments, total sugar content, defined as sucrose plus reducing sugars, of the leaves of both corn and sorghum began to increase at 4:00 to 6:00 A.M., reached a maximum between 12:00 noon and 5:00 P.M., then decreased gradually until 4:00 to 6:00 A.M. the following morning. Most of the diurnal variation in total sugars was accounted for by change in sucrose content, the level of reducing sugars being fairly constant. Krotkov (1942) observed a two- to four-fold increase in the hot alcohol extracted sugars (expressed as per cent of fresh weight) of seedling wheat, T. vulgare, leaves during the period 9:00 A.M. to 4:00 P.M.

Holt and Hilst (1969) studied the daily variation in carbohydrate content in alfalfa, M. sativa, bromegrass, Bromus inermis, tall fescue, Festuca arundinacea, and Kentucky bluegrass, Poa pratensis. Water soluble carbohydrate percentages in alfalfa followed a curvilinear diurnal trend from a low at 6:00 A.M. to a maximum level at 12:00 noon and a slight decrease by 6:00 P.M. Nonstructural polysaccharide content (exclusive of fructans) followed a nonlinear daily trend with the most rapid increase occurring in the afternoon.

The grasses underwent linear increases in water soluble carbohydrate percentages from 6:00 A.M. to 6:00 P.M. The nonstructural polysaccharide content of the grasses also

followed an increasing linear trend in the daytime.

Fructans

Occurrence. Fructans are widely distributed in the plant kingdom. They are present not only in monocotyledons and dicotyledons but also in blue-green algae, Tolypothrix tenuis (Tsusue and Fujita 1964).

The first fructans known were those from certain members of the Compositae. In 1804, Rose extracted them from rhizomes of elecampane, Inula helenium, and the fructans chemically related to those from Inula were later termed inulins. Then in 1864, Sachs located inulin in tubers of dahlias, Dahlia variabilis, the Jerusalem artichoke, Helianthus tuberosus, and I. helenium. By the end of the nineteenth century, the number of plants known to contain fructans had increased considerably and fructans were reported in rhizomes of grasses and in cereals such as barley, rye, and wheat (Pontis and del Campillo 1985). Much of the present knowledge of fructans comes from Edelman and Jefford's (1968) work with inulins in the Jerusalem artichoke.

Chemistry. The general properties of fructans can be summarized as follows: they are low molecular weight polymers of D-fructofuranose, they are stored in the cell vacuoles in a dissolved or colloidal state and they replace starch as reserve material. Fructans extracted are usually

laevo-rotatory from -19 to -55 degrees and they are not colored by iodine (Meier and Reid 1982). They are quite susceptible to acid hydrolysis and while having varying solubilities in cold water, are very soluble in hot water.

Inulins. As far as is known, the fructans of the dicotyledons are all inulins. However, it should be remembered that virtually all the fructans which have been studied in detail are from the Compositae (Pontis and del Campillo 1985). Inulins consist of a chain of D-fructofuranosyl residues linked by β-2->1-glycosidic linkages and they usually carry a D-glucosyl residue at the end of the chain linked α-1->2, as in sucrose. Values of 20 to 40 fructose residues have been reported as the average degree of polymerization (DP) in Compositae, calculated either on the basis of methylation studies or from the glucose content, assuming the presense of one glucose residue per molecule. The specific optical rotations of purified inulins from the Compositae are generally around -40 degrees (Meier and Reid 1982).

Phleins. Large variations in solubility and specific rotation suggest that the chemical structure of fructans of monocots may differ widely. Structural analysis and molecular weight determinations with modern methods have revealed that fructans of the monocotyledons are of the phlein type or of the branched type, and, in a few cases,

also of the inulin type (Pontis and del Campillo 1985). Phleins are D-fructofuranose polymers of β-2->6 glycosidic linkages which contain one D-glucose residue per molecule linked α-1->2 as in sucrose. They predominate in the leaf, stem, and root tissue of many monocotyledonous plants, particularly in Gramineae. They have a DP of 10 to 260 fructose residues and have been isolated mainly from temperate herbage grasses. The longest-chain fructans are found in stem base tissue where they vary from a DP of approximately twenty-six, as found in bromegrass, B. inermis, to approximately two hundred sixty, as found in timothy, Phleum pratense (Smith 1973). The short-chained phleins (10 to 26 DP) can be extracted with greater than 65% ethanol, while the long-chained ones (90 to 260 DP) are extracted with less than 20% ethanol (Meier and Reid 1982).

Branched. The branched type is a mixed linkage type with a phlein or inulin backbone. The fructans contain both 2->6- and 2->1-β-D-fructofuranosidic linkages. Each have one or more short branches usually consisting of a single fructose residue. They contain one glucose residue per molecule linked as in sucrose.

Biochemistry and Physiology of Formation, Mobilization and Seasonal Changes of Fructans

Dicotyledonous Fructans

Formation of Dicot Fructans. Knowledge of the biosynthesis and degradation of fructans comes mainly from the work of Edelman and Jefford (1968) carried out with inulin type polymers present in Jerusalem artichoke tubers, H. tuberosus. On the other hand, the knowledge of the enzymes involved in the synthesis and degradation of phlein type fructans is scant, and metabolic studies of phlein fructans has only recently begun. There is little information about fructan metabolism or enzymology in algae (Pontis and del Campillo 1985).

In Jerusalem artichoke tubers, Edelman and Jefford (1968) found three enzymes associated with fructan metabolism:

1. Sucrose-sucrose transfructosylase (SST), which forms 1F-fructosylsucrose (GF2), the initial homologue of the 2->1-fructan series, from sucrose according to:

Sucrose + Sucrose ------> Fructosylsucrose + D-glucose

This enzyme is not an invertase and it is unable to promote polymerization above the trisaccharide level.

SST seems to occupy a key role in the initiation of fructan synthesis. Thus, Scott (1968) found a definite correlation between the occurrence of SST and the new

synthesis of fructans in Jerusalem artichoke tubers and onion bulbs, Allium species. Pontis et al. (1972) ascribed to sucrose a regulatory role in the induction of SST activity. The production of 1F-fructosylsucrose in explants of Jerusalem artichoke tubers was clearly dependent on the sucrose level. Similar results have been reported for lettuce leaf discs, Lactuca sativa L., incubated in a sucrose medium (Chandorkar and Collins 1974). Research of Pollock (1982), Housley and Pollock (1985), and Hogan and Hendrix (1986) on wheat, T. aestivum, support the findings of Pontis. SST can rapidly produce sufficient trisaccharide even at low temperatures when phloem transport and growth are restricted but photosynthesis is still functioning (Wagner et al. 1983).

2. β-2->1-Fructan: β-2->1-fructan-1-fructo-furanosyltransferase (FFT), is responsible for chain elongation. This enzyme transfers single terminal -D-fructofuranosyl residues to the same position of another molecule according to:

Sucrose-Fru(n) (donor) + Sucrose-Fru(m) (acceptor)------>
Sucrose-Fru(n-1) + Sucrose-Fru(m+1)

where (n) may be any number from one (trisaccharide) to approximately thirty-five and (m) for the acceptor molecule is any number from zero (sucrose) to approximately thirty-five (Pontis and del Campillo 1985). Sucrose can be an acceptor but not a donor molecule for this enzyme. 1F-fructosylsucrose and its higher homologues can act both as donors and acceptors.

It has been found that sucrose, which can act as an acceptor of fructosyl residues, competes with the trisaccharide so successfully that continued transfer to form material of a higher DP is first partially and then totally prevented. Thus, the following exchange reaction is dominant in the presence of equal concentrations of sucrose and fructosylsucrose:

Fructosylsucrose + (14C)Sucrose ------>
Sucrose + (14C)Fructosylsucrose

Similarly, polymers of high DP also show great affinity, with respect to FFT, as acceptors. Therefore, in a mixture of fructosylsucrose and such primers, higher molecular weight polymers are formed, instead of oligosaccharides of a low DP that would arise in the absence of such primers by self transfer (Pontis and del Campillo 1985).

In order to avoid interference of sucrose in the reaction catalyzed by FFT, Edelman and Jefford (1968) have speculated that the reaction catalyzed by SST may take place in the cytoplasm, whereas FFT might be localized at the tonoplast. Thus, the trisaccharide formed in the cytoplasm may be converted to tetrasaccharide by FFT at the tonoplast and then released into the vacuole, while the sucrose also produced in the reaction may be released back into the cytoplasm. Polymers in the vacuole may undergo further transformations by action of the membrane-bound FFT, whereby fructans of higher DP are again released into the vacuole and terminally resulting sucrose is released into the cytoplasm.

However, the hypothesis does not take into account the pH optima determined for the two enzymes involved, SST and FFT. All SST preparations so far isolated exhibit an optimum pH about 5.5, whereas FFT exhibits an optimum pH in the neutral or alkaline region. Pontis and del Campillo (1985) think it rather unlikely that SST would be localized in the cytoplasm. They also find it difficult to visualize the localization of FFT at the tonoplast, as in order to interact with fructan molecules impinging on it. The FFT enzyme should be in the inner face of the tonoplast in contact with the acidic vacuolar contents. Wagner et al. (1983) experimented with the protoplasts from fructan-enriched barley leaves. It was found that all the fructans (greater than trisaccharide), as well as the SST, activity were found to be associated exclusively with the vacuoles.

3. β-2->1-Fructan 1-fructanohydrolase. Two enzymes with very similar properties have been described. Their activity is expressed by the equation:

Sucrose F(n) + H_2O ------>Sucrose F(n-1) + F

These enzymes break only the β-2->1- linkage between a terminal fructosyl group and its adjacent fructose residue. Rate of reaction depends on the DP and nature of the group at the nonreactive end of the molecule. The enzymes showed no transfructosylase activity (Edelman and Jefford 1968).

Mobilization of Dicot Fructans. In Jerusalem artichokes a partial depolymerization of inulin occurs during cold

storage in winter, whereas complete depolymerization to sucrose and fructose occurs during sprouting. According to Edelman and Jefford (1968) the two hydrolases are, together with FFT, responsible for partial depolymerization of fructans in winter, whereas the two hydrolases are solely responsible for degradation of fructans during sprouting in spring. The two hydrolases are enzymes which hydrolyze single fructose residues off the chain end. Both enzymes are inhibited by sucrose against which they are inactive (Meier and Reid 1982).

Seasonal Changes of Dicot Fructans. In Jerusalem artichoke tubers seasonal changes of fructans and other carbohydrates were studied by Bacon and Loxley (1952), who measured variations in optical rotation of water extracts made at different times of the year. They found that extracts from late summer and autumn had the most negative optical rotation (about -22), indicating the presence of large amounts of high molecular weight inulin, whereas extracts from winter and spring (January to April) had the most positive optical rotation (approximately +8), indicating the presence of large amounts of fructan oligosaccharides of low molecular weight. This has been explained by Edelman and Jefford (1968), who pointed out that changes in average chain length can occur during tuber dormancy without a net change in carbohydrate content. This implies that fructose residues released by action of fructan hydrolases must be converted to

glucose, from which more sucrose may be produced, thus allowing FFT to catalyze a redistribution of fructose residues and increase the amount of fructans of smaller molecular size at the expense of larger ones. This mechanism would permit a rapid change in size and number of molecules, and hence a rapid change in osmotic pressure. Frost resistance should be improved by such a process. Rutherford and Flood (1971) showed seasonal changes in hydrolase and invertase activities in Jerusalem artichoke tubers. Changes in hydrolase activity accord with conversion of inulin to oligosaccharides, particularly during cold storage.

Monocotyledonous Fructans

Formation of Monocot Fructans. Biosynthesis of fructans in monocotyledons seems to proceed, as in dicotyledons, via a transferase system in which the first acceptor is sucrose and the fructose donor is sucrose also (Meier and Reid 1982). In dicots fructose transfer seems always to be onto the primary hydroxyl at C-1 of the fructose residue in sucrose or an oligo- or polysaccharide, whereas, in monocots the transfer takes place onto any one of the three primary hydroxyl groups in sucrose, or onto any of the two primary hydroxyls in the fructose residues of oligo- or polysaccharides. Consequently, fructans from monocots show great variation in structure.

Rocher (1967) studied synthesis of phlein-type fructans

in leaves of ryegrass, Lolium italicum, and showed that they started with the formation of kestose with sucrose as acceptor and donor of fructose residues. It is unclear whether sucrose and/or kestose is/are the fructose-donor(s) for the prolongation of the kestose molecule.

Bhatia and Nandra (1979) studied the fructan-forming enzymes extracted from the American aloe, Agave americana. They purified a fructosyltransferase which could only use sucrose as a fructosyl donor and which could synthesize inulin-type oligosaccharides up to a DP of approximately eleven.

Pollock et al. (1983) have shown that sucrose is the major precursor of fructan in ryegrass, L. tenulentum.

Hogan and Hendrix (1986) studied the labeling of fructans in winter wheat stems, T. aestivum, to test the hypothesis that the fructose moiety from the translocated sucrose is used preferentially in the biosynthesis of fructans. Results indicated: (a) a large percentage of labeled sucrose was translocated and unloaded in an unaltered state; and (b) sucrose contributed its fructose moiety to fructan synthesis in stems.

Mobilization of Monocot Fructans. There is little information regarding the depolymerization of fructans present in monocotyledons. Evidence that fructan degradation proceeds in a way similar to that outlined for the Compositae has been given by Smith (1976) and by Mino and Maeda (1974).

Smith (1976) showed the presence of a β-fructofuranosidase with exo-action in the culm base tissue of tall fescue, F. arundinacea. This enzyme degraded phlein starting at the fructose end and only fructose residues were released until a molecule of sucrose remained. The enzyme was specific for cleaving 2->6-linkages and did not attack inulin. In addition to this enzyme the same tissue also contained an invertase. Evidence of Mino and Maeda (1974) indicates that, in haplocorms of timothy, P. pratense, a phlein hydrolase exists that does not act on inulin.

Seasonal Changes of Monocot Fructans. Fructan accumulation is not restricted to specific organs (Waite and Boyd 1953, MacKenzie and Wylam 1957, Smith 1967) but the amount that occurs in a given tissue will depend on the stage of development (Smith 1967, 1973). Smith (1967) reports that in stem bases of both timothy, P. pratense, and bromegrass, B. inermis, total water-soluble carbohydrate and fructans decreased in spring (from the beginning of April to the middle of May) and increased again in summer (until the end of July). According to Waite and Boyd (1953) fructan content in stems of orchard grass, Dactylis glomerata, meadow fescue, F. pratensis, and timothy, P. pratense, showed two peaks: the first in late spring (May) and the second in summer (July/August). The first peak occurred during the change from vegetative to reproductive development. The second peak occurred during seed development. The percentage of fructans

in stems then decreased as carbohydrate was translocated for starch storage in seeds. Three phases of fructan metabolism could be distinguished during the annual growth cycle of four British forage grasses; timothy, P. pratense, meadow fescue F. pratensis, and two ryegrass selections, L. perenne (Pollock and Jones 1979). The first, where growth was most rapid (April and May), had little or no detectable fructans, and low levels of low molecular weight sugars. This was followed by nonstructural carbohydrate accumulation during later stages of reproductive growth and during autumn and winter growth of vegetative tillers. In the latter instance, growth was slower and fructan levels rose to a peak in December; fructan breakdown later began around the time of leaf elongation.

In wheat, Colin and Belval (1923) found increasing quantities of fructans in different plant parts until flowering. According to Escalada and Moss (1976), the fructan content in wheat kernels reaches a maximum before the phase of rapid starch synthesis. Barnell (1938) noted also that the fructans in the heads are obviously transitory reserves, which disappear during maturation of the seeds when large amounts of starch are formed. Preece (1957) reported a fructan content of 1% in whole mature wheat grains.

Carbohydrate Accumulation

The accumulation of carbohydrate reserves in plant

tissue is a dynamic system of energy balance. Accumulation of reserves indicates an excess of fixation over utilization whether caused by an increase in photosynthesis, a decline in the requirement for fixed carbon, or a perturbation in transport restricting distribution (Pollock et al. 1983). Accumulation of fructans is greatly influenced by growth rate, plant development stage, and the environment (White 1973). Higher concentrations usually are found at mature rather than at young growth stages, at low rather than high soil nitrogen levels, and at cool rather than warm temperatures (Smith 1973).

Numerous studies, mainly reported in the agricultural literature, suggest that whenever the supply of assimilate by photosynthesis exceeds the immediate demand for growth or grain filling, the fixed carbon may be diverted into fructan synthesis (Wagner et al. 1983). The large fructan reserves thus formed may be remobilized such as during growth in early spring, during regrowth after mowing, and during grain filling. Thus, fructans play a key role as buffer between supply by photosynthesis and utilization for growth (i.e. crop yield) (Wagner et al. 1983).

The carbohydrate reserves of orchard grass, D. glomerata, and bermuda grass, Cynodon dactylon, grown in growth chambers decreased when growth and respiration demands were greater than the photosynthetic rate and increased when growth and respiration demands were less than the

photosynthetic rate (Blaser et al. 1966). Pollock (1979) found that fructan accumulation was maximal in periods when growth was restricted. McCaig and Clarke (1982) reported that carbohydrates accumulated in the stem of winter wheat, T. aestivum, to a maximum at anthesis and then declined toward maturity. Their results support the concept that the accumulation of stem carbohydrates results from a phase difference between the ability of the plant to produce carbohydrate and the requirement for it. Therefore, the stem may act as a temporary storage organ to partially correct an imbalance. A readily available stem carbohydrate reserve could contribute to grain filling or energy requiring processes within the plant.

Winter Hardiness

The survival of winter wheat seedlings in harsh winter conditions requires a remarkable hardiness. The plants must endure cycles of freezing and thawing, frost heaving, slow desiccation, and compaction under accumulating ice and snow. Midwinter thaws reduce hardiness even when they are short.

Whether a field of seedlings survives the winter to become a harvestable crop depends largely on the young plant's ability to withstand deep cold. Wheat seedlings of hardy varieties must acquire a tolerance for such cold prior to the first killing frost each fall. As days grow shorter and nights colder, plants adapt for freezing. This

acclimation process, known as hardening, is critical to winter survival. Considerable time is required for the adaptive changes that impart cold hardiness. Six to eight weeks have been reported to be required for maximum development of hardiness in winter wheat (Pomeroy and Fowler 1973).

As the environment of an established plant becomes colder, alterations in metabolism and membranes are required to establish forms that will maintain function and semipermeability at low temperatures (Olien and Smith 1981). This adaptation permits continued metabolic activity and is a prerequisite for hardening. Results of research by Olien and Lester (1985) indicate that part of the biochemistry of hardening is induced by light freezing. Winter cereals readily adapt to chilling and then at these cool temperatures proceed to harden by developing systems that protect them from freeze injuries.

The correlation between temperature and the level of fructans, as well as their DP, has been studied in Gramineae by Smith (1968). He showed that in timothy, P. pratense, temperature influenced levels of fructans accumulated but had little effect on levels of total sugars and starch. Moreover, not only did fructans accumulate but modifications of fructan chain length also occurred.

The studies of Eagles (1967) with two climatic races of cocksfoot (also known as orchard grass), D. glomerata, showed

that the relationship between photosynthesis, respiration, translocation, and conversion of simple sugars to structural compounds, and the accumulation of fructans, was most marked at low temperatures. Eagles (1967) studied the variations of fructan content in Mediterranean (Portuguese) and North European (Norwegian) populations of cocksfoot at four different temperatures. He reached the conclusion that the ecological significance of the accumulation of fructans in the Norwegian population at low temperatures ($5^{o}C$) may be associated with the high degree of cold resistance of this population and the greater requirement for a substrate for respiration during the longer winter. The surplus for this respiratory requirement may be remobilized in spring and used for rapid production of leaf area in the short growing season of the Norwegian sub-arctic region.

Pollock and Ruggles (1976) also established that orchard grass, D. glomerata, accumulated fructans more rapidly at $5^{o}C$ than at 15 to $20^{o}C$. Wagner et al. (1983) found that a temperature regime of cool nights ($5^{o}C$) induced the conspicuous accumulation of nonstructural carbohydrates in leaf blades of wheat, T. aestivum, and barley, Hordeum vulgare, seedlings.

Fructans are found in the vacuoles of the mesophyll cells of winter wheat, T. aestivum (Wagner et al. 1983). Storage of fructans in vacuoles is one way of overcoming the osmotic constraints of accumulation of low molecular weight

sugars.

The deposition of carbon in the form of fructans offers several advantages over deposition as starch in nondehydrated plant tissue (Wagner et al. 1983). By filling vacuoles with highly water-soluble fructans, use is made of the largest compartment of plant cells. In contrast, accumulation of starch in chloroplasts may eventually impair photosynthesis. The mean molecular size of a fructan can be changed easily using only a simple transferase system and thus fructans are ideally suited for osmoregulation during temperature or water stress. Alteration of the osmotic potential may also be used to protect cells against freezing by lowering the freezing point. Even high molecular weight fructans which do not substantially increase the osmotic potential may act as efficient antifreeze agents. Fructans may therefore play other roles besides that of a temporary carbohydrate reserve (Wagner et al. 1983).

CHAPTER II

MATERIALS AND METHODS

Total Nonstructural Carbohydrate (TNC) Experiment

Objectives

The objectives of this experiment were as follows:

1. Investigate the effect of aerial greenbug feeding on the total nonstructural carbohydrate levels in roots and shoots of winter wheat seedlings.
2. Determine if TNC levels were effected over time after cessation of greenbug feeding, thus indicating whether or not root and shoot biomass reduction caused by greenbug feeding is a result of carbohydrate partitioning.

Test Plants (TNC)

Studies were conducted under greenhouse conditions using a randomized block design during the months of February, March, and April 1986. The following factors were considered when controlling variables within the experiment: light, temperature (21 +/- 5^{o}C), air flow, spacing of cone-tainers, watering schedule, fertilizing schedule, plant

depth, harvest time, greenbug age, greenbug numbers on test plants, and quality seeds. A commercial winter wheat cultivar, TAM-W-101, was first germinated then planted, one seed per container, in sterile fritted clay (Absorb-n-Dry) (Burton 1986) in Supercell Cone-Tainers (3.8 x 26.1 cm, 164 cc) supported by cell tray racks (Ray Leach "Cone-Tainer" Nursery, 1500 N. Maple Street, Canby, OR 97013). Plants were watered daily and fertilized twice per week with Peter's Peat-Lite Special (analysis 15-16-17), a water soluble fertilizer (Peter's Fertilizer Products, P.O. Box 238, Folgelsville, PA 18051).

Test Insect and Infestation Procedures (TNC)

Greenbugs, biotype E, from laboratory colonies were used to infest plants at the two-leaf stage. Using a fine, moistened camel-hair brush, adult greenbugs were placed on each plant. The infested and noninfested plants, including border rows, were covered with plastic cages fitted with cloth-covered ventilation holes (Starks and Burton 1977). The aphids were allowed to feed freely but their numbers were restricted to the initial number per plant. After feeding seven days visible damage had occurred and the greenbugs were removed from the plants. The plants were maintained for five weeks post feeding for weekly sampling of plant tissue for TNC extraction.

Root and Shoot Preparation (TNC)

In each herbage collection, shoots were sampled in situ by clipping at the root/shoot junction. The fritted clay was thoroughly washed from the roots. Fresh root and shoot tissue was microwaved from 50 to 60 seconds (depending upon the age of the plant) then oven dried at 65°C for twenty-four hours (Trent and Christiansen 1986). The dried tissue was weighed then ground in a cyclone mill (Udy Inc., Ft. Collins, CO 80524) to pass through a 1.0 mm screen. Ground samples were weighed to determine per cent recovery from the cyclone mill. Dried tissue was stored under cool, dry conditions.

Extraction Procedure (TNC)

The technique employed was a modification of Smith's (1981) TNC extraction procedures. It synchronizes the sucrose and fructan hydrolysis with starch gelatinization (Trent and Christiansen 1986). Solutions of D-glucose and D-fructose were included with samples to construct standard curves. Approximately 50.0 mg of ground shoot and root herbage were weighed and samples placed in 25 ml Erlenmeyer flasks. Ten ml of 2.5 mM sulfuric acid were added to each flask. Flask stoppers were fitted with a syringe needle to reduce pressure while minimizing evaporation during immersion in boiling water. After one hour in the water bath (gelatinizes the starch), the solutions were cooled prior to adding 9 ml of 0.2 M acetate buffer. This solution was

agitated to mix acid and buffer and 1 ml of 0.5% amyloglucosidase [glucoamylase; 1,4-α-D-Glucan glucohydrolase; 11,800 units/g (from Rhizopus)] was added (hydrolyzes any starch to monomers). Final volume was 20 ml. Solutions were checked to insure a pH of 4.4. The flasks were then agitated in a shaker bath (50°C) to mix enzyme and sample solutions. The final mixture was centrifuged for fifteen minutes and decanted into 4 ml beaker cups for refrigerated storage.

Reagents for the acetate buffer included:

1. Two volumes 0.2 N acetic acid: dilute 12.0 ml glacial acetic acid C. P. reagent (99.5% pure) with distilled water and bring to 1 liter volume.

2. Three volumes 0.2 N sodium acetate: dissolve 16.4 g anhydrous sodium acetate with distilled water and bring to 1 liter volume.

3. Check final pH and adjust to 4.9 with sodium hydroxide or hydrochloric acid. Add a small amount of thymol. Store in colored bottles.

Analysis Procedure (TNC)

Analysis of TNC in samples was based on the colorimetric reaction of reducing sugars with p-hydroxybenzoic acid hydrazide (PAHBAH), using a Lachat Quickchem System IV, Model 170 Flow-injection Analyzer (Lachat Chemicals Inc., Mequon, WI 53092) (Trent and Christiansen 1986). Absorbance was measured by a colorimeter (410 nm) interfaced to a programmer/printer. Glucose standards were used to calibrate the colorimeter. The automated method was expeditious and

showed high repeatability and accuracy.

Fructan Experiment-Test I (F1)

Objectives (F1)

The objectives of this experiment were as follows:

1. Investigate effect of greenbug feeding on fructose levels in leaves, stems, and roots of winter wheat seedlings.
2. Determine if greenbug feeding changes either the amount or DP of fructans in leaves, stems, and roots of winter wheat seedlings.

Test Plants (F1)

The study was conducted in a growth chamber using a completely randomized design during the months of June and July, 1986. Daytime temperature was 21^{o}C while the nighttime temperature was lowered to 8^{o}C to induce production of fructans. The photoperiod was 16:8 hours (day:night). The winter wheat cultivar, TAM-W-101, was planted, one seed per container, in sterile fritted clay in Supercell Cone-Tainers. Plants were watered as needed and fertilized every three days.

Test Insect and Infestation Procedure (F1)

Ten greenbugs, biotype E, from laboratory colonies were used to infest plants at the two-leaf stage. Infested and

noninfested plants, including border rows, were covered with plastic cages fitted with cloth-covered ventilation holes. Aphids were allowed to feed and reproduce freely. Greenbugs were removed from plants after feeding fourteen days. At this time plants appeared visibly damaged. Plants were maintained for one week post feeding.

Leaf, Stem, and Root Preparation (F1)

For the analysis, the shoot was clipped from the root system prior to its removal from the cone-tainer. The shoot was divided into fractions and designated leaf and stem. Fritted clay was thoroughly washed from the roots. Fresh weights were taken prior to extraction of fructans.

Extraction Procedure (F1)

The technique employed is patterned after Pollock's (1979) extraction procedure. Three extractions were performed. The first preparation of tissue was made when greenbugs had been feeding three days; the second at the time of aphid removal (they had been feeding fourteen days); and the third extraction was one week post greenbug removal. At each extraction plant parts were cut into 0.5 cm sections and dropped into boiling 80% ethanol for three minutes. After two extractions in distilled water at 60^{o}C for five minutes and one further extraction in boiling 80% ethanol for three minutes, the extracts were pooled. It should be noted that

at the first extraction, due to small plant size, only 5 ml of each solvent was used for a total extract volume of 20 ml. The second and third extraction required 20 ml of each solvent for a total extract volume of 80 ml. Alcohol was removed using an Evapo-Mix^{R} apparatus. Samples were shell frozen in acetone and dry ice and then lyophilized to complete dryness. The first extraction was rehydrated in 1 ml distilled water while the second and third were diluted in 2 ml distilled water. After dilution samples were cleansed in 0.60 g Amberlite MB-3^{R} which is a mixture of Amberlite IR-120^{R} cation exchange resin (hydrogen form) and Amberlite IRA-410^{R} anion exchange resin (hydroxide form). Samples were drawn off and centrifuged for fifteen minutes at 10,000g. The supernatant was decanted and prepared for the cysteine-carbazole color test and thin-layer chromatography.

Analysis Procedure (F1)

Cysteine-Carbazole Color Test (F1). A modified procedure of the cysteine-carbazole reaction was utilized for determination of fructose (Nakamura 1968). By incubating the components of the color reaction at 40^{o}C for one hour, fructose is determined with good sensitivity and specificity. Fructose, when occurring in an amount as small as 0.4 µg and in the presence of 250-fold excess of glucose, can be determined with an error of about 10%.

Procedure: In small test tubes 2.5 ml cold 75%

sulfuric acid were added. To these tubes were added, slowly and without mixing, 50 μl of sample solution (4 μl were added for second and third extraction), 370 μl distilled water, 85 μl 2.4% cysteine hydrochloride and 85 μl 0.12% carbazole in 95% ethanol (TABLE I). The tubes were shaken and then incubated for one hour in a 40°C water bath. After incubation color was measured by a spectrophotometer (560 mu) against a reagent blank.

Thin-Layer Chromatography (Fl). Fischer Silica Gel G Redi-Plates^{R} (Fischer Scientific Company, 711 Forbes Ave., Pittsburgh, PA 15219) with a calcium sulfate inorganic binder were used. The inorganic binder was chosen over an organic binder to insure faster separation and better resolution.

For each extraction TLC plates were activated in a 70°C oven for thirty minutes. They were allowed to cool at room temperature for twenty minutes prior to spotting 80 μg of each sample on the plates. Fructose, sucrose, and stachyose standards were also applied.

The solvent system was a 2:1:1 solution of 1-butanol, glacial acetic acid, and distilled water. Plates were developed three times. They were dried between each development. Once developed, the plates were dried and sprayed with a phosphoric acid-urea spray which consisted of 11.5 ml 85% phosphoric acid, 88.5 ml water-saturated butanol, 3 g urea, and 5 ml absolute ethonol. Plates were heated in a 65°C oven for eighty-five minutes to insure full color

development. Color development did not begin for twenty-five minutes.

Fructan Experiment-Test II (F2)

Objectives (F2)

Objectives of the second fructan experiment were essentially the same as the first experiment but a resistant variety of wheat, Century, was included along with the susceptible TAM-W-101.

Test Plants (F2)

This study was conducted under greenhouse conditions using a randomized block design during October and November, 1986. A temperature regime of 21 +/- 5^{o}C daytime and 15 +/- 5^{o}C nighttime was used. An artificial light source controlled the photoperiod at 16:8 (day:night). Two commercial winter wheat cultivars, TAM-W-101, and Century, were planted, one seed per container, in sterile fritted clay in Supercell Cone-Tainers. Plants were watered daily and fertilized every three days. Century is the result of a three-way cross between the lines Payne, TAM-W-101, and Amigo. It is characterized by resistance to greenbug biotype C, the resistance derived from the germplasm, Amigo.

Test Insect and Infestation Procedure (F2)

Ten biotype C greenbugs from laboratory colonies were

used to infest plants at the two-leaf stage. All plants were covered with plastic cages fitted with cloth-covered ventilation holes. Aphids were placed on designated plants and allowed to feed and reproduce freely. Greenbugs were removed after feeding ten days.

Leaf, Stem, and Root Preparation (F2)

Plant parts were prepared as in the first fructan experiment with the exception of shoots being thoroughly washed prior to fructan extraction to remove aphid honeydew.

Extraction Procedure (F2)

Instead of three extractions, two were performed in this experiment: the first when aphids had been feeding for three days, and the second one week post greenbug removal (aphids had been feeding for ten days).

As in the former experiment, due to the lack of plant material at the first extraction time, only 5 ml of each solvent were used. For the second extraction 20 ml each were required.

There are three procedural differences between this test and the first fructan experiment. The first difference involves the amount of extract dried. In the first experiment total leaf, stem, and root samples were dried. In this test only 5 ml of each sample were dried. The second divergence involves the drying method. Instead of drawing

off the alcohol with the Evapo-Mix^{R}, shell freezing samples, and lyophilizing to complete dryness, samples in this test were placed in an automatic liquid evaporator which drew off both ethanol and water simultaneously. This method was considerably easier and more time efficient. The third difference involved rehydration of samples prior to the cysteine-carbazole color reaction. In this experiment, both extraction samples were rehydrated with 1 ml distilled water was used from the first extraction material, while 2 ml were utilized for the second and third extraction.

Analysis Procedure (F2)

Cysteine-Carbazole Color Test (F2). The Nakamura (1968) modified procedure was employed for this experiment. Identical reagents were used and the same spectrophotometer was engaged. Equal amounts and concentrations of reagents were utilized (TABLE I). Equivalent incubation conditions were used.

Thin-Layer Chromatography (F2). Exact chromatographic conditions stipulated previously for the first fructan experiment were utilized in this experiment.

CHAPTER III

RESULTS AND DISCUSSION

To my knowledge, the following work is the first attempt to confirm effects of greenbug, Schizaphis graminum (Rondani), feeding on total nonstructural carbohydrates and fructans in winter wheat, Triticum aestivum.

Total Nonstructural Carbohydrate (TNC) Experiment

Root and Shoot Dry Weight Analysis (TNC)

Greenbug damage to small wheat plants or seedlings has been implicated as an important cause of yield reductions (Burton et al. 1984). Although greenbugs feed on the aerial portions of wheat plants, Ortman and Painter (1960) found that a high infestation of greenbugs caused a maximum 55% reduction of wheat plant root systems. Daniels (1965) reported that greenbugs caused more damage to roots of wheat plants than to aerial portions and roots of infested plants had fewer root hairs and adventitious roots than the noninfested plants. In the Pike and Shaffner (1985) experiments, injury to wheat was highest where aphid infestations started at the two-leaf stage.

In this study, analysis of TAM-W-101 dry weights

consistently showed that greenbug biotype E feeding caused a significant ($P<0.05$) reduction of growth in both roots and shoots (TABLE II).

Reduction in biomass was caused when sixteen greenbugs were initially placed on designated plants and maintained at that level by population adjustment every other day. After seven days of feeding, and as visual damage appeared severe, greenbugs were removed. As a result of greenbug feeding, extensive chlorosis and necrotic spots appeared on leaves and stems of treated plants. Over the following five weeks the symptoms of damage began to disappear and plants appeared to recover. However, when the growth of the roots and shoots were measured, differences between damaged and undamaged plants were significant ($P<0.05$) from the time of greenbug removal (zero days) through the fourth week post greenbug removal (twenty-eight days). However, at thirty-five days significant treatment differences in root and shoot dry weight no longer occurred between infested and noninfested plants (TABLE II and III). Plants were approximately eight weeks old at this time and considerable binding of roots had occurred in cone-tainers, probably reducing growth rates of larger plants, and perhaps allowing smaller plants to continue growing to a similar weight.

The explanation for the reduction of growth caused by greenbug damage is unknown. Maxwell and Painter (1962) noted a relationship between auxins in honeydew and auxins in host

plants. They suggested that aphids were effective in removing auxins from plant sap, concentrating them in honeydew, causing auxin depletion, resulting in stunted plant growth. Today it is generally thought that greenbugs inject a toxin during feeding, although the toxin has not been located or identified at this time (Burton 1986). Ultrastructural studies of greenbug feeding damage by Al-Mousawi et al. (1983) indicated existence of a toxin-like substance and identified two types of feeding damage in TAM-W-101. The first type of damage involved vascular bundles and was observed as early as one hour post infestation. The second type damaged mesophyll parachyma surrounding damaged vascular tissues where macroscopic chlorotic halos developed by four to six days post infestation (Al-Mousawi et al. 1983). Greenbug damage to wheat, particularly to seedlings, was demonstrated in the field to be irreversible (Burton et al. 1985). Even though plants appeared to recover from a visual standpoint, early damage, followed by immediate greenbug removal, ultimately reduced yield several months later at harvest. Results from this study generally confirm these results.

Root and Shoot TNC Analysis

Nonstructural carbohydrates constitute energy reserves of plants. Sucrose and starch are the predominant reserve constituents of tropical-origin grasses, while sucrose and

fructans are stored by temperate-origin grasses. Seeds generally contain starch (White 1973).

TNC concentration is an estimate of carbohydrate energy readily available to the plant. Estimating the status of carbohydrate reserves indicates periods of storage and utilization and potential of plants to regrow following cutting or grazing (Smith 1972). Many pasture and range management practices are based upon knowledge of how various environmental factors and herbage removal treatments affect carbohydrate reserves (White 1973). A knowledge of the kinds and trends of energy providing carbohydrates in stem bases and/or roots is important to cutting and grazing management studies (Smith 1972). This understanding helps managers to maintain high yields of desirable species and to control the undesirable ones (White 1973). Therefore, estimating the concentration of TNC usually is desired in management studies rather than estimating each individual fraction of nonstructural carbohydrate (Smith 1981).

Greenbugs extract an enormous amount of sap from their host plants and must, in some way, influence TNC. Some of this sap is eventually excreted as honeydew. Auclair (1963) reported a sugar concentration of 10 to 15% in sap from stylets of aphids feeding on herbaceous plants. A depletion of carbohydrate from the plant would mean a lower energy reserve to initiate and continue growth or overwinter. The balance between photosynthesis and respiration would be

disrupted. In this TNC study, greenbug feeding had a direct effect over time on dry weight of roots and shoots of winter wheat, possibly from plant toxicity. Therefore, it was postulated that greenbug damage, either by carbohydrate extraction or plant toxicity, would influence TNC concentration of wheat seedlings. However, in this experiment, instead of increasing or decreasing the concentration, greenbug feeding caused no significant ($P>0.05$) difference in TNC concentration in roots and shoots (TABLE IV and V).

Fructan Experiments: Test I and Test II

These studies were conducted to determine the effect of greenbug damage on fresh weight of leaves, stems, and roots of winter wheat seedlings; whether greenbug feeding decreased fructose concentration in the individual plant parts; and to ascertain changes in the amount and DP of fructans in the particular plant fraction.

In both tests greenbugs feeding under controlled conditions on winter wheat seedlings, were compared to uninfested (control) seedlings. Results of fresh weight determinations and statistical analysis for leaves, stems, and roots for the first and second test are presented in TABLE VI and VII, respectively. TABLE VI compares fresh weights over three extraction times of greenbug susceptible wheat cultivar, TAM-W-101, while TABLE VII contrasts fresh

weights of greenbug susceptible TAM-W-101 and greenbug resistant Century over two extraction times.

It is necessary to note that test I and test II were conducted in a plant growth chamber and under greenhouse conditions, respectively.

Fresh Weight Analysis: Test I and Test II

For test I and test II, ten greenbugs each of biotype E and C were used to infest wheat plants. The greenbugs were allowed to feed and reproduce freely. During test I, when greenbugs had fed for three days on the susceptible cultivar, TAM-W-101, there was a significant ($P<0.05$) increase in leaf and root weight (TABLE VI). Stem and root weights were slightly greater, but not significant ($P>0.05$).

During test II after three days of greenbug feeding on TAM-W-101, only the stem fresh weight showed a significant difference ($P<0.05$) between treated and untreated wheat plants. After three days infested leaves and roots were unaffected by aphid feeding (TABLE VII).

Greenbug removal in test I occurred when aphids had been feeding fourteen days. In the TNC experiment greenbugs were removed after feeding seven days. The cooler growth chamber temperatures slowed the reproductive rate, therefore, additional time was required to achieve the level of damage deemed necessary to cause a reduction in growth. Plants in test I sustained severe damage and appeared incapable of

recovery from injuries. Leaves and stems had sustained extensive chlorosis and necrotic damage.

After feeding fourteen days on wheat seedlings, leaf, stem, and root fresh weights in test I had reversed from a greater weight in treated plants to a greater weight in control plants. Instead of the significant difference ($P<0.05$) which was present in damaged leaf weights and root weights after three days of feeding there was now no significant ($P>0.05$) difference in the damaged leaf weights and root weights. Stems exhibited no significant ($P>0.05$) difference at three days of feeding, but after fourteen days of feeding, a significant ($P<0.05$) difference had manifested itself between treated and untreated plants (TABLE VI).

Afflicted plants in test I continued to grow (without greenbugs) in spite of the visually devastating effects of feeding. However, as the plants grew, there was a continuation of differences between damaged and undamaged plants.

One week post greenbug removal there were significant differences ($P<0.05$) between the infested and noninfested plants. This coincides with growth patterns of roots and shoots found by Burton (1986). He found that the magnitude of difference increased in roots by four-fold from zero to fourteen days post greenbug removal. This pattern was evident for shoot growth also, as indicated by shoot weights

and the unchanging root-shoot ratio. The results of this study show the same growth patterns for roots and shoots in a damaged and undamaged situation.

In test II greenbugs were removed from TAM-W-101 and Century plants ten days after infestation. Aphid numbers attained in test I were not observed in test II. Since the greenbugs had entered a reproductive phase during the test, eggs were laid instead of giving birth to live young, thus reducing the number of feeding aphids. TAM-W-101, susceptible to biotype C greenbug, exhibited more chlorosis on leaves and stems than did the resistant cultivar, Century. However, the intensity of damage on TAM-W-101 was less severe in this test than the observed damage in test I due to fewer aphids. Even so, the reduction in fresh weight of TAM-W-101 due to greenbug damage, manifested itself one week post aphid removal. At this time, leaf, stem, and root fresh weights exhibited a significant difference ($P<0.05$) between infested and noninfested plants.

In test II, greenbug resistant characteristics of Century were evident in all plant parts at three days post infestation and one week post greenbug removal. Increased wet weights did occur for uninfested plants at both times. However, wet weight differences were not significant ($P>0.05$) between infested and noninfested plants (TABLE VII).

Visible damage was evident on the resistant Century plants, but chlorotic injury due to greenbug feeding was

notably less, and even nonexistent on some plants. A higher greenbug mortality rate was apparent on Century plants when compared to the greenbug susceptible TAM-W-101.

Some plant resistance to aphids is related to physical features, however, most resistance appears to be due to physiological and biochemical characteristics of the cultivars (Al-Mousawi et al. 1983). In a ten day study Al-Mousawi et al. (1983) found that biotype C greenbug resistant TAM-W-101 x Amigo did not develop macroscopic symptoms. At ten days post infestation, most cell organelles were normal in vascular tissues as well as in the mesophyll, with the exception of a few scattered necrotic mesophyll cells.

Fructose Levels: Test I and Test II

A cysteine-carbazole reaction was used for determination of fructose concentration. By incubating components for the color reaction at 40^{o}C for one hour, fructose is determined with good sensitivity and specificity. With this method, fructose, in small quantities such as 0.4 μg, in the presence of a 250-fold excess of glucose, can be determined with an error of about 10%.

Three days after the initial infestation with greenbug biotype E, damaged TAM-W-101 wheat plants in test I exhibited higher fresh weight values than undamaged plants. Therefore, higher fructose values detected in damaged plants is not surprising (TABLE VIII). A significant difference ($P<0.05$)

in the sugar level was observed in leaves and roots between the damaged and undamaged plants. However, increased fructose concentration in the stem area was not significant ($P>0.05$).

For test I greenbugs were allowed to feed for fourteen days. At the time of removal the severity of damage was visually acute with pervading chlorosis and necrotic areas on leaves and stems. A greater concentration of fructose was detected in undamaged plants, but statistical analysis failed to produce significant differences ($P>0.05$) between infested and noninfested plants. Due to the intensity of the greenbug damage this is amazing.

This same trend of increased carbohydrate levels in each plant fraction but a lack of significant difference was also observed one week post greenbug removal.

In test II, greenbug biotype C susceptible TAM-W-101 displayed greater fresh weights for controls for all plant parts at three days post infestation and one week post greenbug removal (TABLE VII). A higher fructose level in all noninfested plant fractions was presumed. This did occur at three days post infestation (TABLE IX). However, by one week post infestation, there was a greater concentration of fructose in the greenbug damaged leaves, stems, and roots. When greenbugs had fed for three days, only the leaf fructose level proved significant ($P<0.05$) (TABLE IX). One week after greenbugs were eliminated the stems and roots exhibited

significant differences ($P<0.05$), while the leaf carbohydrate level was nonsignificant (TABLE IX).

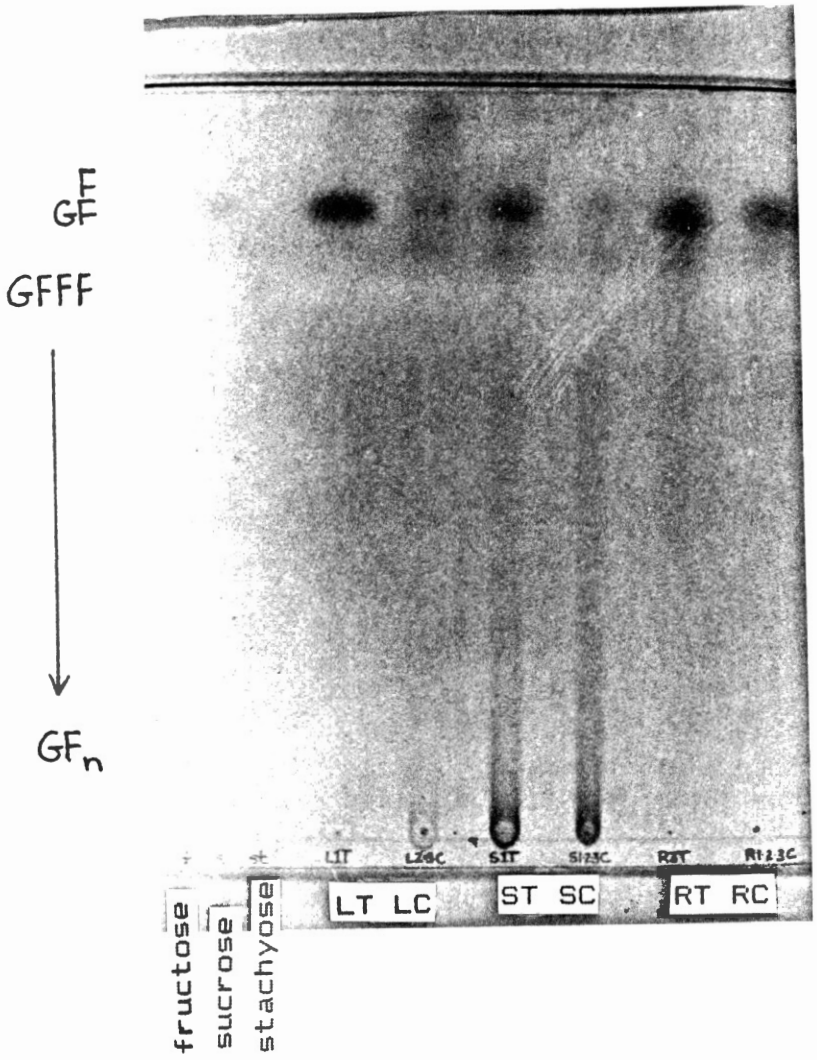
Aphids had no effect on fresh weights of greenbug biotype C resistant Century wheat plants, either at three days post infestation or one week post removal (TABLE VII). When the greenbugs had been feeding three days there was a higher concentration of fructose in plant parts without aphids. One week post greenbug removal, opposite effects were observed and higher levels occurred in leaves and stems. However, noninfested roots continued to have a greater quantity of the sugar. In spite of these trends significant levels of all plant parts at both times were nonsignificant ($P>0.05$) (TABLE IX).

Thin-Layer Chromatography

Thin-layer chromatography (TLC) experiments were conducted to examine effects of greenbug damage on the amount of fructan present in leaves, stems, and roots of winter wheat seedlings. Also investigated was the effect of greenbug feeding on DP of fructans in plant parts.

Test I: Extraction I (TLC). TAM-W-101 wheat seedlings were seventeen days old at the time of extraction I (Test I-TLC) and greenbugs had been for feeding three days. The TLC plate (Figure 6) indicates that sucrose is the main sugar in both insect damaged and undamaged leaves, while fructans are absent. Sucrose is found in a much greater concentration in

Figure 6. Test I, Extraction 1. Accumulation of fructans in leaf, stem, and root tissue of TAM-W-101 wheat seedlings.

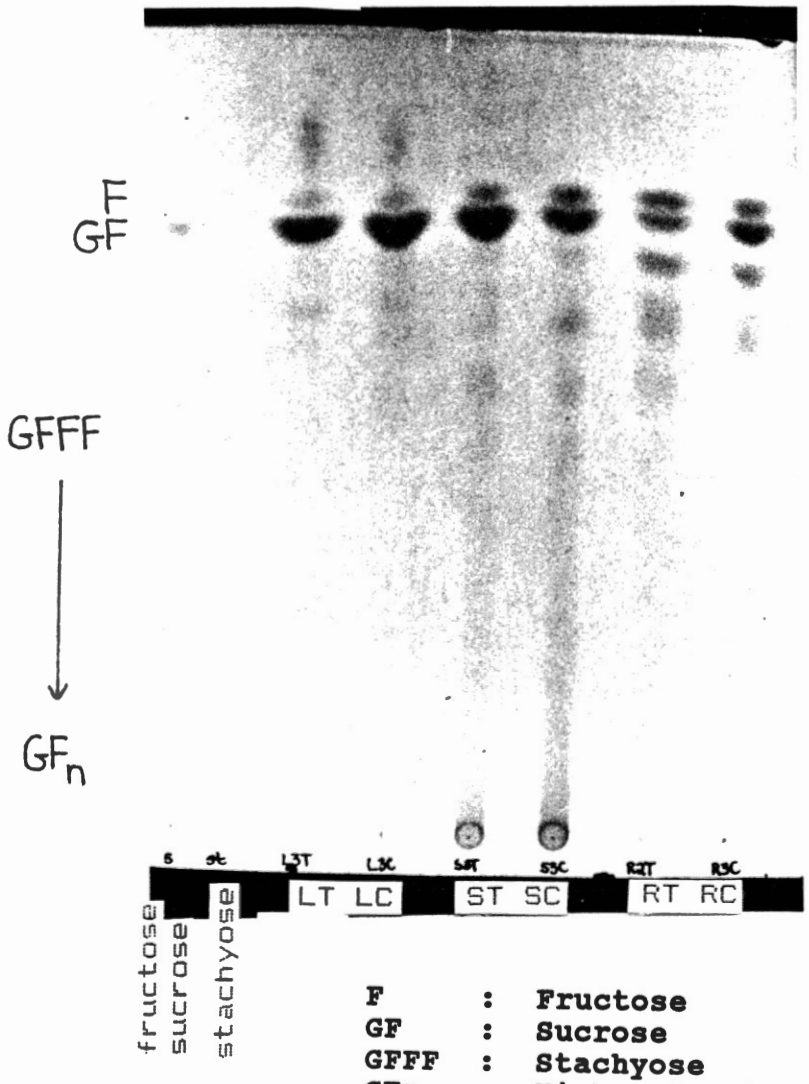


F	:	Fructose	LT:	Leaf Treated	
GF	:	Sucrose	LC:	Leaf Untreated	(Control)
GFFF	:	Stachyose	ST:	Stem Treated	
GFn	:	Higher Fructans	SC:	Stem Untreated	(Control)
			RT:	Root Treated	
			RC:	Root Untreated	(Control)

damaged leaves than in undamaged ones. Carbohydrates in the roots appear to occur in equal amounts between infested and noninfested plants. Fructans in the roots are in minute quantities at this stage and sucrose is the principal sugar. Damaged stems accumulated more sucrose than undamaged stems, but fructan concentration appeared equal. The majority of fructans in this extraction are located in the stem area. This coincides with findings of other investigators (Smith 1973, Hogan and Hendrix 1986). Nonstructural carbohydrates may be stored temporarily in all plant parts, however, major storage areas of these reserves are usually the lower regions of the stem (White 1973). Pollock's (1979) investigation of four forage grasses indicated that lower internodes accumulated the largest proportion of fructans, although there were differences between varieties. McCaig and Clarke (1982) found that both short-chained carbohydrates and oligosaccharides were more prevalent in stems than in green leaves of two cultivars of wheat. Evidently, greenbug damage had no effect on DP of fructans in the plant parts at this stage of growth or damage.

Test I: Extraction II (TLC). Sucrose was the primary carbohydrate found in all plant parts when plants were seventeen days old [Extraction I-(Test I-TLC)]. However, at thirty days [Extraction II-(Test I-TLC)], the synthesis of fructans had begun, particularly in the stem area, and to a lesser degree in the roots (Figure 7). Leaves showed a DP of

Figure 7. Test I, Extraction 2. Accumulation of fructans in leaf, stem, and root tissue of TAM-W-101 wheat seedlings.



F : Fructose
GF : Sucrose
GFFF : Stachyose
GFn : Higher Fructans

LT: Leaf Treated
LC: Leaf Untreated (Control)
ST: Stem Treated
SC: Stem Untreated (Control)
RT: Root Treated
RC: Root Untreated (Control)

approximately three. Housley and Daughtry (1987) found that sucrose, the main carbon source arriving in seeds of winter wheat, Triticum aestivum, is utilized to support metabolic activities. Sucrose is also necessary for fructan synthesis (Hogan and Hendrix 1986).

In leaves of ryegrass, Lolium temulentum, increasing sucrose concentration results in fructan synthesis (Housley and Pollock 1985). Kinetic studies have shown that sucrose is the major precursor of fructan in ryegrass, L. temulentum (Pollock 1982). Wagner et al. (1983) studied induction of fructans in leaf blades of wheat, T. aestivum and barley, Hordeum vulgare. It was found that initially, as this study has shown, sucrose was the main species formed. Later, after fourteen days, sucrose levels remained constant and rapid synthesis of fructans began (Figure 8). Particularly evident in wheat, the trisaccharide (kestose or isokestose) appeared first and then, sequentially, higher polymers of fructose. In spite of the severity of damage caused by greenbug feeding at this extraction time (greenbugs had been feeding fourteen days) there does not appear to be appreciable differences in fructan level between infested and noninfested plants and DP appears unchanged (Figure 7).

Test I: Extraction III (TLC). Extraction III (Test I-TLC) was done when plants were thirty-seven days old, and seven days after greenbug removal. At this time, the stem area contained the bulk of larger fructans, with a DP greater

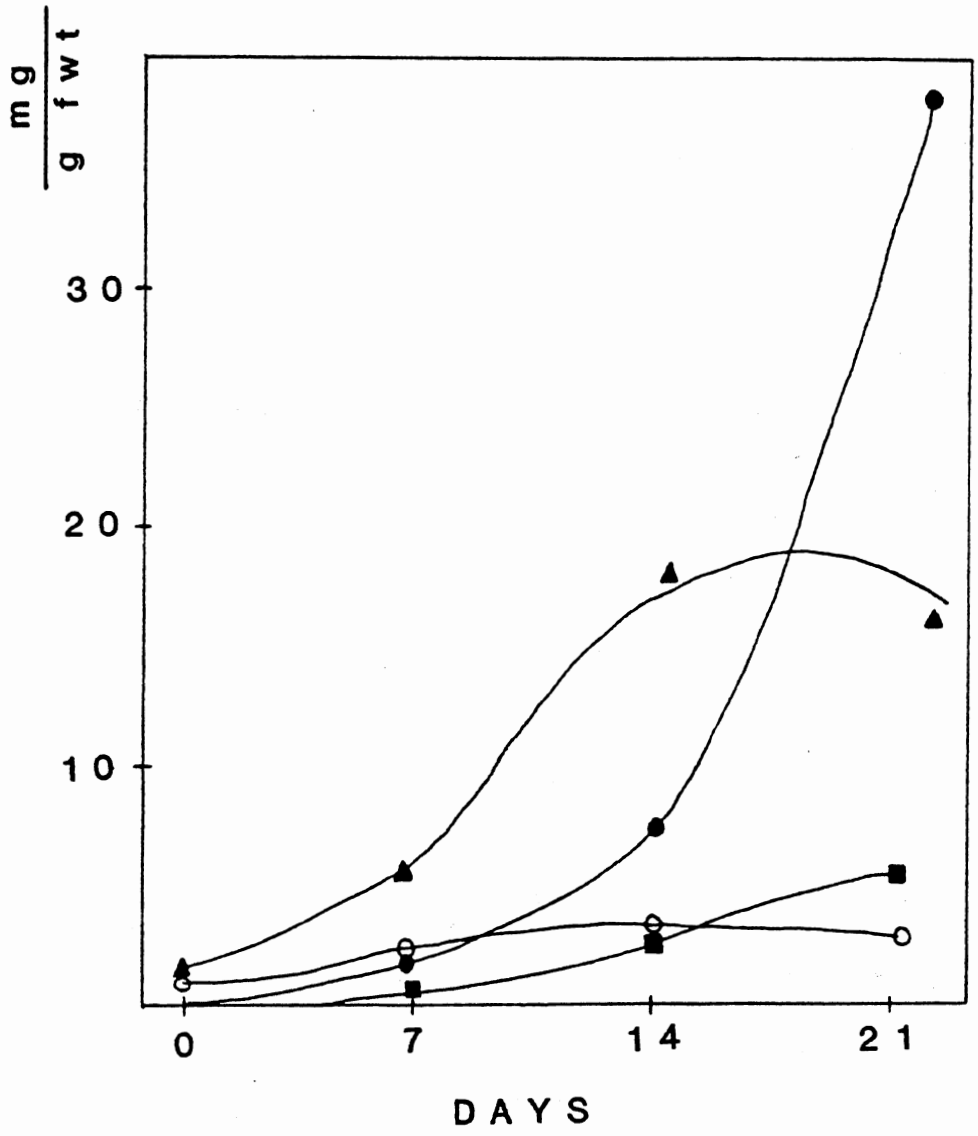


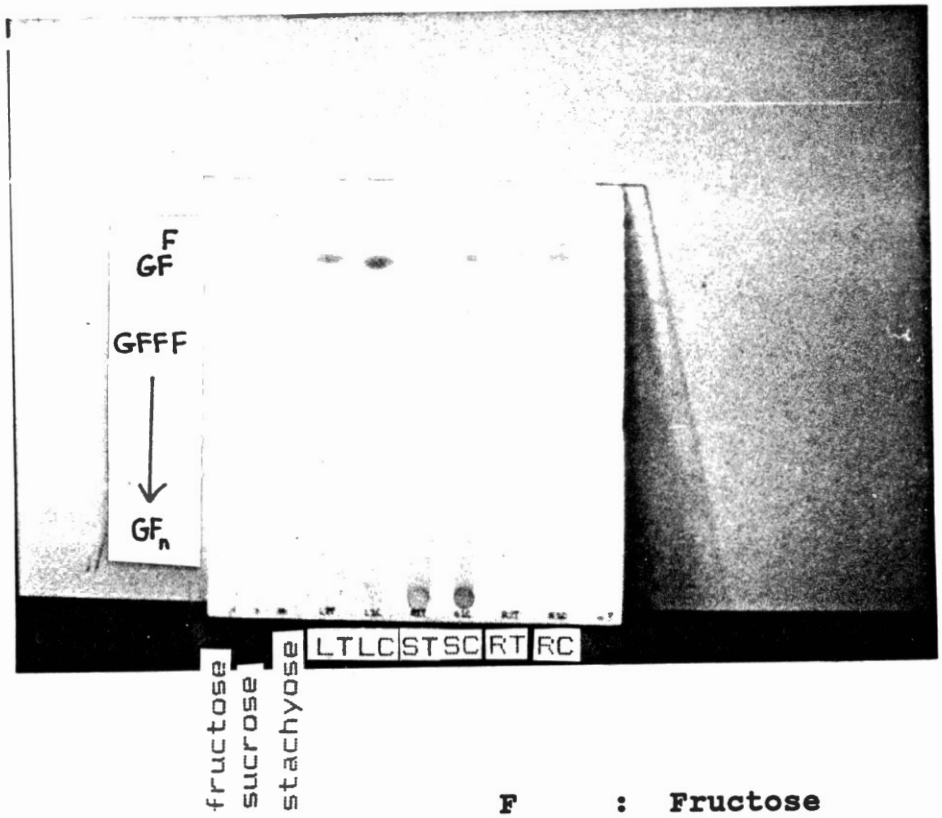
Figure 8. Cold stress induced accumulation of fructans in seconday leaf blades of *H. vulgare* seedlings (Wagner et al. 1983).

Fructose ○ Trisaccharide ■
Sucrose ▲ Fructans DP>3 ●

than eight (Figure 9). Root concentration is comprised of fructans with a DP of five. Sucrose is the predominant sugar in leaves, while fructans are lacking. It is unclear why fructans are not present in leaves at this time. Their synthesis was begun when the plants were thirty days old [Extraction II-(Test I-TLC)]. Now at thirty-seven days, leaf fructans are absent. FFT is the enzyme which promotes polymerization above the trisaccahride level. Sucrose can be an acceptor but not a donor for this enzyme. It has been found that sucrose can compete with the trisaccharide formed by the SST enzyme for fructose moieties so successfully that continued transfer of sucrose to form material of a higher degree of polymerization is prevented (Pontis and del Campillo 1985). In this study greenbug feeding damage caused no dissimilarities in the amount or DP of carbohydrates between treated and untreated plants (Figure 9).

Test II: Extraction I (TLC). In this study sucrose also appears to be the predominant carbohydrate in damaged and undamaged leaves, stems, and roots of greenbug susceptible TAM-W-101 (Figure 10). At this time plants were sixteen days old and greenbugs had been feeding for three days. Higher molecular weight fructans are absent from leaves and roots in TAM-W-101. However, a faint band appears below sucrose in treated and untreated leaves. Possibly, this is kestose or isokestose, a trisaccharide which is a

Figure 9. Test I, Extraction 3. Accumulation of fructans in leaf, stem, and root tissue of TAM-W-101 wheat seedlings.



F : Fructose
GF : Sucrose
GFFF : Stachyose
GFn : Higher Fructans

LT: Leaf Treated
LC: Leaf Untreated (Control)
ST: Stem Treated
SC: Stem Untreated (Control)
RT: Root Treated
RC: Root Untreated (Control)

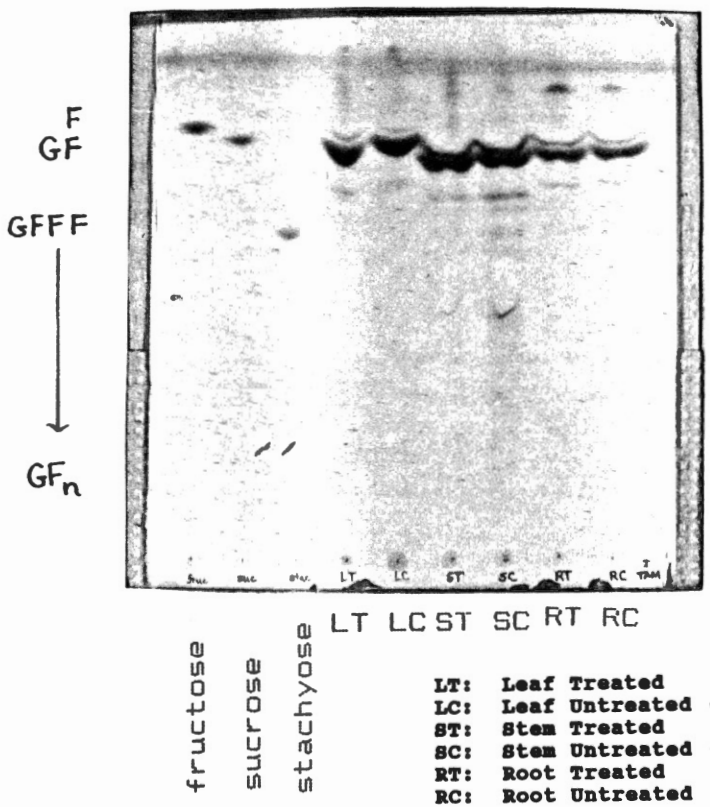
proposed precursor of phlein-type fructans (Pollock 1982). The trisaccharide is indistinct in infested and noninfested roots. Initiation of fructan production was begun at this time in the stem area, even though individual bands are rather obscure. At this time the damaged stem visibly contains a greater quantity of fructans, but greenbug feeding appears to be having no effect on DP.

A vague, but noticeable trisaccharide band is formed underneath sucrose in the treated leaf portion on the Century TLC plate (Figure 11). This band is absent in the untreated leaf. It is interesting that no fructan production has occurred in greenbug damaged or undamaged stems at this time, while the generation of fructose polymers has reached a DP of five in noninfested roots. Lower and higher molecular weight fructans are absent in aphid damaged roots.

Test II: Extraction II (TLC). Plants were thirty days old at extraction II (Test II-TLC), one week post greenbug removal (the greenbugs fed for ten days). In both cultivars sucrose remains the primary sugar species in leaves, while active synthesis of fructans is underway in both stems and roots. The result of greenbug feeding on stems and roots of TAM-W-101 (Figure 12) is negative in reference to a change in the concentration or DP of the fructans. However, in the undamaged leaf five faint bands appear, whereas, in the damaged leaf bands are absent.

No appreciable differences were apparent from the TLC

Figure 10. Test II, Extraction 1. Accumulation of fructans in leaf, stem, and root tissue of TAM-W-101 wheat seedlings.



LT: Leaf Treated
LC: Leaf Untreated (Control)
ST: Stem Treated
SC: Stem Untreated (Control)
RT: Root Treated
RC: Root Untreated (Control)

F : Fructose
GF : Sucrose
GFFF : Stachyose
GFn : Higher Fructans

Figure 11. Test II, Extraction 1. Accumulation of fructans in leaf, stem, and root tissue of Century wheat seedlings.

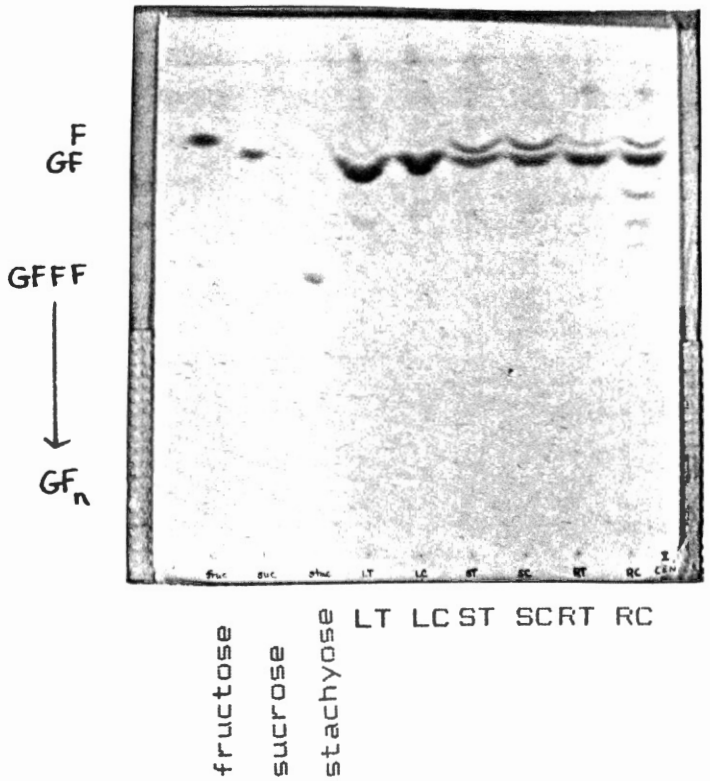
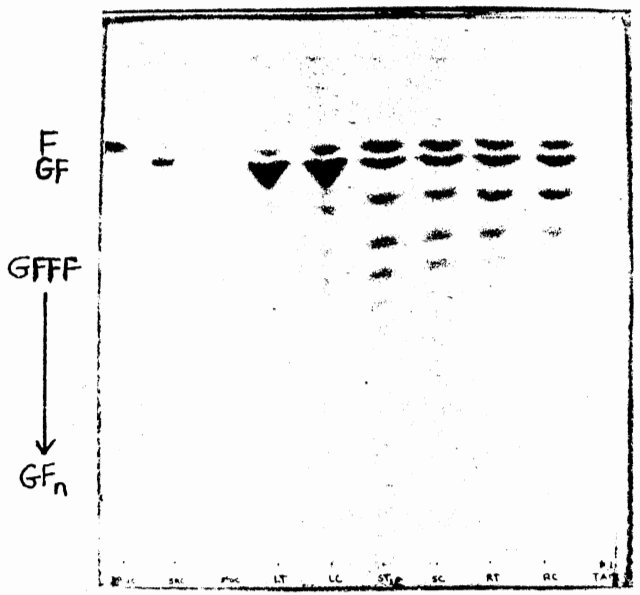


plate between insect damaged and undamaged resistant Century plant parts (Figure 13).

Greenbug biotype C and E susceptible wheat, TAM-W-101, exhibited significant ($P<0.05$) differences in root and shoot dry weights in the TNC experiment. However, the infestation did not generate a significant ($P<0.05$) depletion in the TNC concentration in the initial experiment in spite of root and shoot dry weight differences. This trend was also observed in leaf, stem, and root fresh weights in both fructan experiments.

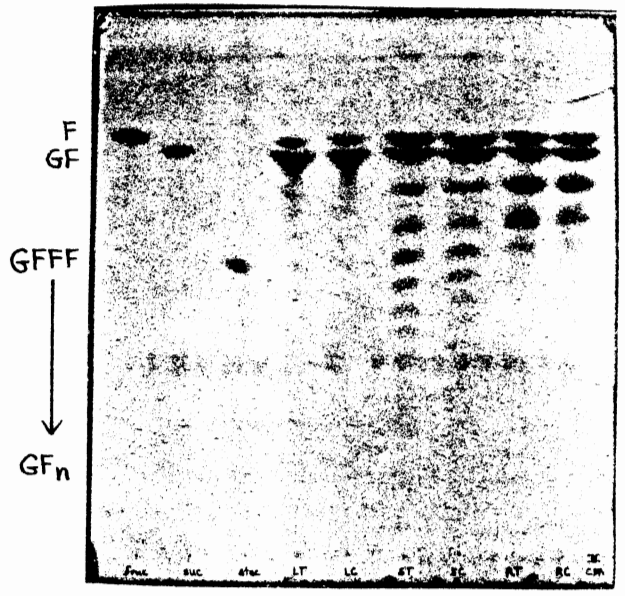
Greenbug biotype C resistant characteristics of Century were evident in the second fructan experiment. No significant ($P>0.05$) differences were evident in fresh weight, fructose concentraction, or TLC plates between infested and noninfested wheat seedlings.

Figure 12. Test II, Extraction 2. Accumulation of fructans in leaf, stem, and root tissue of TAM-W-101 wheat seedlings.



LT: Leaf Treated
LC: Leaf Untreated (Control)
ST: Stem Treated
SC: Stem Untreated (Control)
RT: Root Treated
RC: Root Untreated (Control)

Figure 13. Test II, Extraction 2. Accumulation of fructans in leaf, stem, and root tissue of Century wheat seedlings.



F : Fructose
GF : Sucrose
GFFF : Stachyose
GFn : Higher Fructans

CHAPTER IV

SUMMARY AND CONCLUSIONS

In the TNC and both fructan experiments greenbug feeding damage did not result in an appreciable reduction in carbohydrate concentration. Also, no visibly notable change occurred in the DP in either fructan experiment. This lack of difference between insect damaged and undamaged wheat seedlings is intriguing and perplexing, especially in light of the extensive chlorosis and necrosis that plants incurred and the amount of nutritive reserves which are removed during the feeding process. These uneffected carbohydrate concentrations may be explained by Refai et al. (1955) and Harper and Freyman (1979, 1983). Refai et al. (1955) discovered that larval Hessian fly feeding caused twice as much total sugar to accumulate in wheat leaves ten days after infestation compared with control plants. The authors suggested that plant phosphorylases which are involved in the splitting of β-1->4 glycosidic bonds were inactivated or inhibited, thus causing foliar sugars to increase.

Harper and Freyman (1979, 1983) discussed the loss of winter hardiness in seedling alfalfa and alfalfa grown in the field for one, two, and three years, due to feeding by the pea aphid. In the seedling study (1979) the aphid

infestation significantly reduced cold hardiness, crown weight, and height and weight of top growth. Moisture and carbohydrate concentration were not affected. The lack of a significant decrease in carbohydrate concentration was unexpected. They speculated that lack of a significant reduction in sugar concentration was due to a marked reduction in crown weight, and consequently, the amount of carbohydrate per plant would also be reduced. Their 1983 study on one, two, and three year old alfalfa plants produced similar results. The pea aphid again reduced cold hardiness of alfalfa without a measurable parallel reduction in carbohydrate reserves. Perhaps lack of decline in carbohydrate levels resulted from collapsed leaf cells following feeding and interrupted translocation of reserves.

Phytophagous insect damage has been associated with a reduction in plant cold hardiness by removing nutritive reserves. Wellso et al. (1985) noted that feeding by bird-cherry oat aphids reduced the amount of fructans in crown tissue by about 20% when compared with extracts from noninfested winter wheat seedlings. Studies by Wellso et al. (1986) indicate that Hessian fly larval feeding reduces fructans, glucose, and fructose in wheat crowns and in turn reduces cold hardiness of plants grown under controlled conditions. Insect feeding caused a 20% fructan depletion when compared to controls.

Auclair (1963) reported that sucrose, which is the major

transportable form of carbohydrate in plants, was the main constituent found in stylets of aphids feeding on herbaceous plants. If the aphids preferentially removed sucrose, one would expect a reduction of fructans since induction of the oligosaccharide is dependent upon sucrose concentration. On the other hand, an interruption of translocation by greenbug feeding should produce a surplus of sucrose in the plant fractions. Sucrose plays a regulatory role in the induction of SST enzymatic activity to produce the initial trisaccharide from which fructans are synthesized. SST can rapidly produce sufficient trisaccharide even at low temperatures when phloem transport is restricted but photosynthesis is still functioning (Wagner et al. 1983).

If greenbugs are promoting cellular collapse in leaf, stem, and root tissue, and sucrose translocation is interrupted, then possibly the excess sucrose which can act as an acceptor in the presence of FFT, is competing with the trisaccharide formed by SST so continued transfer to form fructans of higher DP is prevented.

Greenbugs feed on leaf blades and stems of winter wheat seedlings. They are phloem feeders and remove nutritive reserves. Effects of aphid feeding are shown in Figures 6 to 12. These figures show that greenbug feeding is not reducing fructan concentration nor changing the DP of fructose polymers.

I do not understand this lack of depletion. Further

studies should be conducted to explore the relationship of insect feeding and the biochemical aspects of yield and biomass loss in plants.

REFERENCES CITED

Al-Mousawi, A. H., P. E. Richardson, and R. L. Burton. 1983. Ultrastructural studies of greenbug (Hemiptera: Aphididae) feeding damage to susceptible and resistant wheat cultivars. Ann. Entomol. Soc. Am. 76: 964-971.

Archer, T. L., R. H. Cate, R. D. Eikenbary, and K. J. Starks. 1974. Parasitoids collected from greenbugs and corn leaf aphids in Oklahoma in 1972. Ann. Entomol. Soc. Am. 67: 11-14.

Auclair, J. L. 1963. Aphid feeding and nutrition. In Smith, R. F. and Mittler, T. E. (Eds.), Ann. Rev. Ent. 8: 439-490. Annual Reviews, Inc., Palo Alto, California.

Bacon, J. S. D., and R. Loxley. 1952. Seasonal changes in the carbohydrates of the Jerusalem artichoke tuber. Biochem. J. 51: 208-213.

Barnell, H. R. 1938. Distribution of carbohydrates between component parts of the wheat plant at various times during the season. New Phytol. 37: 85-112.

Bhatia, I. S., and K. S. Nandra. 1979. Studies on fructyosyl transferase from Agave americana. Phytochemistry. 18: 923-927.

Blaser, R. E., R. H. Brown, and H. T. Bryant. 1966. The relationship between carbohydrate accumulation and growth of grasses under different microclimates. Int. Grassland Congress., Proc. 10th, 147-150. Helsinki, Finland.

Burton, R. L, D. D. Simon, K. J. Starks, and R. D. Morrison. 1985. Seasonal damage by greenbugs (Homoptera: Aphididae) to a resistant and susceptible variety of wheat. J. Econ. Entomol. 78: 395-401.

Burton, R. L. 1986. Effect of greenbug (Homoptera: Aphididae) damage on root and shoot biomass of wheat seedlings. J. Econ. Entomol. 79: 633-636.

Chandorkar, K. R. and F. W. Collins. 1974. Enzymological aspects of de novo synthesis of fructooligosaccharides in leaf disks of certain Asteraceae. IV. The activity of sucrose-sucrose 1-fructosyltransferase. Can. J. Bot. 52: 1369-1377.

Chedester, L. D. 1979. Development of a braconid wasp, Lysiphlebus testaceipes (Cresson). Texas A&M Agr. Expt. Stn. Prog. Rept. PR-3546. College Station, Texas.

Colin, H. and H. Belval. 1923. Les hydrocarbones solubles du grain de ble au cours du developpement. Comptes Rendus. 177: 343-346.

Daniels, N. E. 1965. Effect of greenbug feeding on the roots of winter wheat. Texas A&M. Expt. Stn. Prog. Rept. PR-2330. College Station, Texas.

Daniels, N. E. 1977. Seasonal populations and migration of the greenbug in the Texas panhandle. Southwestern Entomol. 2: 20-26.

Daniels, N. E., and L. D. Chedester. 1980. Winter survival of greenbugs in the Texas panhandle. Texas A&M Agr. Expt. Stn. Prog. Rept. MP-1447. College Station, Texas.

Daniels, N. E. 1981. Migration of greenbugs in the Texas panhandle in relation to their biotypes. Texas A&M Agr. Expt. Stn. Prog. Rept. MP-1487. College Station, Texas.

Dixon, A. F. G. 1973. Biology of aphids. Edward Arnold (Publishers) Limited. London.

Dixon, A. F. G. 1985. Aphid ecology. Blackie and Son Ltd. Glasgow and London.

Eagles, C. F. 1967. Variation in the soluble carbohydrate content of climatic races of Dactylis glomerata (Cocksfoot) at different temperatures. Ann. of Bot. 31: 645-651.

Edelman, J., and T. G. Jefford. 1968. The mechanism of fructosan metabolism in higher plants as exemplified in Helianthus tuberosus. New Phytol. 67: 517-531.

Escalada, J. A., and D. N. Moss. 1976. Changes in nonstructural carbohydrate fractions of developing spring wheat kernels. Crop Sci. 16: 627-631.

Harper, A. M., and S. Freyman. 1979. Effect of the pea aphid, Acyrthosiphon pisum (Homoptera: Aphididae), on cold hardiness of alfalfa. Can. Entomol. 111: 635-636.

Harper, A. M., and S. Freyman. 1983. Cold hardiness of 1-, 2-, and 3-year old alfalfa infested with the pea aphid (Homoptera: Aphididae). Can. Entomol. 115: 1243-1244.

Heinze, P. H., and A. E. Murneek. 1940. Comparative accuracy and efficiency in determination of carbohydrates in plant material. Mo. Agri. Expt. Stn. Res. Bull. 314. Columbia, Missouri.

Hogan, M. E., and J. E. Hendrix. 1986. Labeling of fructans in winter wheat stems. Plant Physiol. 80: 1048-1050.

Holt, D. A., and A. R. Hilst. 1969. Daily variation in carbohydrate content of selected forage crops. Agron. J. 61: 239-242.

Housley, T. L., and C. J. Pollock. 1985. Photosynthesis and carbohydrate metabolism in detached leaves of Lolium temulentum L. New Phytol. 99: 299-507.

Housley, T. L., and C. S. T. Daughtry. 1987. Fructan content and fructosyltransferase activity during wheat seed growth. Plant Physiol. 83: 4-7.

Jackson, H. B., L. W. Coles, E. A. Wood, Jr., and R. D. Eikenbary. 1970. Parasites reared from the greenbug and corn leaf aphid in Oklahoma in 1968 and 1969. J. Econ. Entomol. 63: 733-736.

Krotkov, G. 1943. Diurnal changes in carbohydrate of wheat leaves. Can. J. Res. 21: 26-40.

Lamb, K. P. 1959. Composition of the honeydew of the aphid Brevicoryne brassicae (L.) feeding on swedes (Brassica napobrassica DC.) J. Insect Physiol. 3: 1-13.

Levitt, J. 1981. Responses of plants to environmental stress. Chilling, freezing, and high temperature stress. Academic Press, New York.

Lindahl, I., R. E. Davis, and W. O. Shepherd. 1949. The application of the total available carbohydrate method to the study of carbohydrate reserves in switch cane (Arundinara tecta). Plant Physiol. 24: 285-294.

Mackenzie, D. J., and C. B. Wylam. 1957. Analytical studies on the carbohydrates of grasses and clovers. VIII. Changes in carbohydrate composition during the growth of perennial rye-grass. J. Sci. Food Agric. 8: 38-45.

Maxwell, F. G., and R. H. Painter. 1962. Auxins in honeydew of Toxoptera graminum, Therioaphis maculata, and Macrosiphum pisi, and their relation to degree of tolerance in host plants. Ann. Entomol. Soc. Am. 55: 229-233.

McCaig, T. N., and J. M. Clarke. 1982. Seasonal changes in nonstructural carbohydrate levels of wheat and oats grown in a semiarid environment. Crop Sci. 22: 963-970.

Meier, H., and J. S. G. Reid. 1982. Reserve polysaccharides other than starch in higher plants. In Loewus, F. A. and Tanner, W. (Eds.), Encyclo. Plant Physiol. 13A: 435-471. Springer-Verlag, Germany.

Miller, E. C. 1924. Daily variation of the carbohydrates in the leaves of corn and the sorghums. J. Agric. Res. 27: 785-808.

Mino, Y., and K. Maeda. 1974. Metabolism of sucrose and phlein in the haplocrom of timothy. J. Japan. Grassl. Sci. 20: 6-10.

Nakamura, M. 1968. Determination of fructose in the presence of a large excess of glucose. Part V. A modified cysteine-carbazole reaction. Agr. Biol. Chem. 32: 701-706.

Nault, L. R. and P. L. Phelan. 1984. Alarm pheromones and sociality in pre-social insects. In Bell, W. J. and Carde, R. T. (Eds.), Chemical Ecology of Insects. Sinauer Associates, Inc., Massachusetts.

Ojima, K., and T. Isawa. 1968. The variation of carbohydrates in various species of grasses and legumes. Can. J. Bot. 46: 1507-1511.

Olien, C. R., and M. N. Smith. 1981. Protective systems that have evolved in plants. In Olien, C. R. and Smith, M. N. (Ed.), Analysis and improvement of plant cold hardiness. CRC Press, Inc., Boca Raton, Florida.

Olien, C. R., and G. E. Lester. 1985. Freeze-induced changes in soluble carbohydrates of rye. Crop Sci. 25: 288-290.

Ortman, E. E., and R. H. Painter. 1960. Quantitative measurements of damage by the greenbug, Toxoptera graminum, to four wheat varieties. J. Econ. Entomol. 53: 798-802.

Peters, D. C., E. A. Wood, Jr., and K. J. Starks. 1975. Insecticide resistance in selections of the greenbug. J. Econ. Entomol. 68: 339-340.

Pike, K. S., and R. L. Shaffner. 1985. Development of autumn populations of cereal aphids, Rhopalosiphum padi (L.) and Schizaphis graminum (Rondani) (Homoptera: Aphididae) and their effects on winter wheat in Washington state. J. Econ. Entomol. 78: 676-680.

Pollard, D. G. 1973. Plant penetration by feeding aphids (Hemiptera: Aphidoidea): a review. Bull. Ent. Res. 62: 631-714.

Pollock, C. J., and P. A. Ruggles. 1976. Cold-induced fructosan synthesis in leaves of Dactylis glomerata. Phytochem. 15: 1643-1646.

Pollock, C. J., and T. Jones. 1979. Seasonal patterns of fructan metabolism in forage grasses. New Phytol. 83: 9-15.

Pollock, C. J. 1982. Oligosaccharide intermediates of fructan synthesis in Lolium temulentum. Phytochem. 21: 2461-2465.

Pollock, C. J., E. J. Lloyd, J. L. Stoddart, and H. Thomas. 1983. Growth, photosynthesis and assimilate partitioning in Lolium temulentum exposed to chilling temperatures. Physiol. Plant. 59: 257-262.

Pomeroy, M. K., and D. B. Fowler. 1973. Use of lethal dose temperature estimates as indices of frost tolerance for wheat cold acclimated under natural and controlled environments. Can. J. Plant Sci. 53: 489-494.

Pontis, H. G. 1970. The role of sucrose and fructosyl-sucrose in fructosan metabolism. Physiol. Plant. 23: 1089-1100.

Pontis, J. G., R. A. Wolosiuk, and L. M. Fernandez. 1972. The role of sucrose and sucrose synthetase in Helianthus tuberosus. In Piras, R. and Pontis, H. G. (Eds.), Biochem. Glycosidic Linkage. Academic Press, New York.

Pontis, H. G., and E. del Campillo. 1985. Biochemistry of storage carbohydrates in green plants. Academic Press, London.

Porter, K. B., G. L. Peterson, and O. Vise. 1982. A new greenbug biotype. Crop Sci. 22: 847-850.

Preece, I. A. 1957. Cereal carbohydrates. R. Inst. Chem.

Refai, F. Y., E. T. Jones, and B. S. Miller. 1955. Some biochemical factors involved in the resistance of the wheat plant to attack by the Hessian fly. Cereal Chem. 32: 437-451.

Rocher, J. P. 1967. Les levanes de Lolium italicum. Synthese dans les organes vegetatifs. Physiol. Veg. 5: 71-80.

Rutherford, P. P., and A. E. Flood. 1971. Seasonal changes in the invertase and hydrolase activities of Jerusalem artichoke tubers. Phytochem. 10: 953-956.

Scott, R. W., T. G. Jefford, and J. Edelman. 1968. Sucrose fructosyltransferase from higher plant tissues. Proc. Biochem. Soc. p. 23-24.

Smith, A. E. 1976. B-Fructofuranosidase and invertase activity in tall fescue culm bases. J. Agric. Food Chem. 24: 476-478.

Smith, D., G. M. Paulsen, and C. A. Raguse. 1964. Extraction of total available carbohydrates from grass and legume tissue. Plant Physiol. 39: 960-962.

Smith, D. 1967. Carbohydrates in grasses. II. Sugar and fructosan composition of the stem bases of bromegrass and timothy at several growth stages and in different plant parts at anthesis. Crop Sci. 7: 62-67.

Smith, D. 1968. Carbohydrates in grasses. IV. Influence of temperature on the sugar and fructosan composition of timothy plant parts at anthesis. Crop Sci. 8: 331-334.

Smith, D. 1972. Carbohydrate reserves in grasses. The Biology and Utilization of Grasses. Academic Press, New York.

Smith, D. 1973. The nonstructural carbohydrates. In Butler, G. W. and Bailey, R. W. (Eds.), Chemistry and Biochemistry of Herbage. Academic Press, London.

Smith, D. 1981. Removing and analyzing total nonstructural carbohydrates from plant tissue. Wisconsin Agric. Exp. Stn. Res. Rep. 41.

Starks, K. J. and R. L. Burton. 1977. Preventing greenbug outbreaks. USDA-ARS Leaflet No. 309. Hyattsville, Maryland.

Starks, K. J., R. L. Burton, and O. G. Merkle. 1983. Greenbugs (Homoptera: Aphididae) plant resistance in small grains and sorghum to biotype E. J. Econ. Entomol. 76: 877-880.

Trent, J. D., and S. Christiansen. 1986. Determination of total nonstructural carbohydrates in forage tissue by p-hydroxybenzoic acid hydrazide flow-injection analysis. J. Agric. Food Chem. 34: 1033-1037.

Tsusue, Y. and Y. Fujita. 1964. Mono-and oligosaccharides in the blue-green alga, Tolypothrix tenuis. J. Gen. Appl. Microbiol. 10: 283-293.

Tyler, J. M., J. A. Webster, and E. L. Smith. 1985. Biotype E greenbug resistance in wheat streak mosaic virus-resistant wheat germplasm lines. Crop Sci. 25: 686-688.

Wagner, W., F. Keller, and A. Wiemken. 1983. Fructan metabolism in cereals: induction in leaves and compartmentation in protoplasts and vacuoles. Z. Pflanzenphysiol. Bd. 112: 359-372.

Waite, R., and J. Boyd. 1953. The water-soluble carbohydrates of grasses. I. Changes occurring during the normal life-cycle. J. Sci. Food Agric. 4: 197-204.

Weinmann, H., and L. Reinhold. 1946. Reserve carbohydrates in South African grasses. J. South Afr. Bot. 12: 57-73.

Weinmann, H. 1947. Determination of total available carbohydrates in plants. Plant Physiol. 22: 279-290.

Weinmann, H. 1948. Underground development and reserves of grasses. A review. J. Brit. Grassld. Soc. 3: 115-140.

Wellso, S. G., C. R. Olien, and R. P. Hoxie. 1985. Winter wheat cold hardiness and fructan reserves affected by Rhopalosiphum padi (Homoptera: Aphididae) feeding. Great Lakes Entomol. 18: 29-32.

Wellso, S. G., C. R. Olien, R. P. Hoxie, and A. S. Kuhna. 1986. Sugar reserves and cold hardiness of winter wheat reduced by larval feeding of the Hessian fly, Mayetiola destructor (Say) (Diptera: Cecidomyiidae). Environ. Entomol. 15: 392-395.

White, L. M. 1973. Carbohydrate reserves of grasses: a review. J. Range Manage. 26: 13-18.

Wiese, M. V. 1977. Compendium of wheat diseases. Amer. Phytopath. Soc., St. Paul.

Wood, E. A., Jr. 1971. Insecticidal control of the greenbug. J. Econ. Entomol. 64: 704-707.

APPENDIXES

APPENDIX A

REAGENTS USED IN CYSTEINE-CARBAZOLE COLOR TEST

TABLE I

REAGENTS USED IN CYSTEINE-CARBAZOLE COLOR TEST

µg	Water (µl)	Fructose (µl)	Sulfuric Acid (ml)	Cysteine (µl)	Carbazole (µl)
Standards:					
0	420	0	2.5	85	85
1	417	2.5	2.5	85	85
5	408	12.5	2.5	85	85
10	395	25.0	2.5	85	85
Samples:					
1st Ext:	370	50.0*	2.5	85	85
2nd Ext:	370	4.0*	2.5	85	85
3rd Ext:	370	4.0**	2.5	85	85

*Concentrations used in second fructan experiment.

**4.0 µl of a ten-fold dilution

APPENDIX B

ROOT AND SHOOT DATA FROM TNC EXPERIMENT

TABLE II

ROOT DRY WEIGHT MEANS (GRAMS)

TNC EXPERIMENT/TAM-W-101

Days After Greenbug Removal	Treated Means	Control Means
0	0.041a	0.057
7	0.125a	0.169
14	0.252a	0.359
21	0.451a	0.590
28	0.794a	0.930
35	1.152	1.177

a=Significantly different from control ($P<0.05$; df=31)

TABLE III

SHOOT DRY WEIGHT MEANS (GRAMS)

TNC EXPERIMENT/TAM-W-101

Days After Greenbug Removal	Treated Means	Control Means
0	0.055a	0.071
7	0.197a	0.249
14	0.414a	0.520
21	0.889a	1.052
28	1.428a	1.652
35	1.946	2.060

a=Significantly different from control ($P<0.05$; df=31)

TABLE IV

PER CENT TNC IN ROOT DRY WEIGHT

TNC EXPERIMENT/TAM-W-101

Days After Greenbug Removal	Treated Means	Control Means
0	3.433	3.042
7	3.922	4.255
14	2.485	2.797
21	6.707	7.947
28	10.295	11.022
35	12.275	11.015

($P<0.05$; df=31)

TABLE V

PER CENT TNC IN SHOOT DRY WEIGHT

TNC EXPERIMENT/TAM-W-101

Days After Greenbug Removal	Treated Means	Control Means
7	9.742	10.240
14	6.202	6.977
21	8.212	8.540
28	11.100	10.802
35	9.965	9.450

($P<0.05$; df=31)

APPENDIX C

FRESH WEIGHTS OF LEAF, STEM, AND ROOT SECTIONS/TEST I AND TEST II/ FRUCTAN EXPERIMENTS

TABLE VI

FRESH WEIGHTS OF LEAF, STEM, AND ROOT

SECTIONS/TEST I/FRUCTAN

EXPERIMENT/TAM-W-101

	EXTRACTION I 3 DPI*		EXTRACTION II 14 DPI		EXTRACTION III 1 WPR**	
	FRESH WEIGHT (GRAMS)					
	***B	NB	B	NB	B	NB
LEAF	0.183a	0.167	0.892	1.096	1.743a	2.224
STEM	0.089	0.081	0.328a	0.443	0.748a	1.043
ROOT	0.319	0.293	0.639	0.792	1.628a	2.257

*DPI=Days Post Infestation

**WPR=Week Post Removal

***B=Greenbugs NB=No Greenbugs

a=Significantly different from control ($P<0.05$; df=15)

TABLE VII

FRESH WEIGHTS OF LEAF, STEM, AND ROOT

SECTIONS/TEST II-FRUCTAN

EXPERIMENT/TAM-W-101

and CENTURY

TAM-W-101

	EXTRACTION I 3 DPI*		EXTRACTION II 1 WPR**	
	FRESH WEIGHT (GRAMS)			
	***B	NB	B	NB
LEAF	0.097	0.118	0.503a	0.885
STEM	0.031a	0.039	0.118a	0.219
ROOT	0.153	0.172	0.415a	0.659

CENTURY

	EXTRACTION I 3 DPI*		EXTRACTION II 1 WPR**	
	FRESH WEIGHT (GRAMS)			
	***B	NB	B	NB
LEAF	0.123	0.132	0.759	0.911
STEM	0.039	0.046	0.197	0.195
ROOT	0.197	0.210	0.635	0.712

*DPI=Days Post Infestation

**WPR=Week Post Removal

***B=Greenbugs NB=No Greenbugs

a=Significantly different from control (P<0.05; df=15)

APPENDIX D

TOTAL KETOSE SUGAR LEVELS FROM LEAF, STEM, AND ROOT SECTIONS/TEST I AND TEST II/ FRUCTAN EXPERIMENTS

TABLE VIII

TOTAL KETOSE SUGAR LEVELS OF LEAF, STEM,

AND ROOT SECTIONS/TEST I/FRUCTAN

EXPERIMENT/TAM-W-101

	EXTRACTION I 3 DPI*		EXTRACTION II 14 DPI		EXTRACTION III 1 WPR**	
	KETOSE SUGAR LEVEL (mg/g fresh weight)					
	***B	NB	B	NB	B	NB
LEAF	0.947a	0.163	0.550	0.660	2.513	2.847
STEM	1.823	1.400	2.237	2.083	7.047	9.157
ROOT	0.507a	0.307	0.433	1.057	1.160	1.657

*DPI=Days Post Infestation

**WPR=Week Post Removal

***B=Greenbugs NB=No Greenbugs

a=Significantly different from control ($P<0.05$; df=15)

TABLE IX

TOTAL KETOSE SUGAR LEVELS OF LEAF, STEM,

AND ROOT SECTIONS/TEST II/FRUCTAN

EXPERIMENT/TAM-W-101

and CENTURY

TAM-W-101

	EXTRACTION I 3 DPI*		EXTRACTION II 1 WPR**	
KETOSE SUGAR LEVEL (mg/g fresh weight)				
	***B	NB	B	NB
LEAF	2.562a	3.679	10.160	8.451
STEM	1.043	1.101	10.949a	7.061
ROOT	0.483	0.663	3.818a	3.044

CENTURY

	EXTRACTION I 3 DPI*		EXTRACTION II 1 WPR**	
KETOSE SUGAR LEVEL (mg/g fresh weight)				
	***B	NB	B	NB
LEAF	1.962	2.126	10.512	8.668
STEM	2.035	2.354	5.577	3.719
ROOT	0.950	1.023	2.102	2.404

*DPI=Days Post Infestation

**WPR=Week Post Removal

***B=Greenbugs NB=No Greenbugs

a=Significantly different from control ($P<0.05$; df=15)

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