POTENTIAL SEMIOCHEMICALS OF WHEAT (*TRITICUM AESTIVUM* L.) INDUCED BY OVIPOSITION AND FEEDING OF THE WHEAT STEM SAWFLY, *CEPHUS CINCTUS* NORTON (HYMENOPTERA: CEPHIDAE)

by

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ABSTRACT

Cephus cinctus Norton (Hymenoptera: Cephidae), the wheat stem sawfly, is currently the most devastating insect pest of wheat production in Montana. Currently, no effective controls are in place to check its damage and spread throughout wheat fields in the northern Great Plains. Natural biological control of sawflies occurs primarily in the form of larval parasitoids which attack the sawfly larva in the stem; however, these parasitoids are not reliably effective in controlling sawfly populations. Insect damage induces chemical changes in plants, and often these changes are part of a defensive response to the insect injury. Some of these chemical changes are apparent in the volatile chemicals produced by the plants and may include semiochemicals used by sawflies and parasitoids. Identifying the changes in volatile production could enhance the understanding of sawfly-wheat plant-parasitoid interactions and lead to more effective control measures for the wheat stem sawfly. I investigated the differences in the volatile chemicals produced by sawfly-infested and uninfested wheat plants and endeavored to determine if those differences were qualitative or quantitative. Additionally, I wanted to determine if changes in volatile production induced by the wheat stem sawfly could be mimicked by wounding coupled with the application of sawfly cuticular wax to wheat stems or by the injection of frass-treated water into the internodes of wheat stems. Volatiles of infested and uninfested wheat plants were collected and compared, with the results indicating that sawfly damage induces quantitative changes in some volatile chemicals produced by wheat. These results are discussed regarding their context within sawfly-wheat plant-parasitoid interactions and implications for better sawfly control. Volatiles from sawfly-infested, uninfested, frass-water-injected, and pin-pricked/wax-treated plants were also collected, and differences in 11 compounds selected from the results of the 1st experiment were compared. The results of this experiment found that pin-pricked/wax-treated plants came closer to mimicking the volatile production changes induced by sawfly infestation, but neither frass-water injection nor pin-pricked/cuticular wax application reliably induced the same changes in wheat volatiles that sawfly infestation did. There was, however, a definite response of the wheat to the application of the sawfly cuticular wax, and its significance is discussed.

INTRODUCTION

Herbivory of insects has been shown to cause changes in plant chemistry in numerous studies. These changes often result in new or increased production of volatile compounds; these compounds can attract insect predators, discourage insect feeding, and encourage or discourage oviposition by insects. If these volatile compounds can be identified, it may be possible to use them on a broader scale to reduce insect herbivory.

Cephus cinctus Norton, the wheat stem sawfly, is currently the most destructive insect pest to the wheat industry of Montana, and it has been a devastating pest of wheat for more than a century (Weiss and Morrill 1992). The wheat stem sawfly has caused significant wheat crop damage not only in Montana, but throughout much of the northern Great Plains including western Minnesota, North Dakota, northern South Dakota, eastern and central Montana, western Manitoba, Saskatchewan, and eastern Alberta. Losses in Montana are estimated to exceed $25 million (Montana State University Extension Service, 1997), and annual region-wide losses including several states and 3 Canadian provinces may reach $100 million (Hartel et al., 2003). Sawflies remain a pest in wheat because they are extremely difficult to control. Adult sawflies have a short lifespan coupled with a prolonged emergence, and the eggs, larvae, and pupae are enclosed in the stem of their host plant. All of these characteristics combine to make modern insecticides with short residue times virtually useless in controlling the sawfly population.

The type of damage caused to wheat production by the sawfly is both direct and indirect. The direct damage caused by the sawfly comes from the feeding that the larva does while inside the wheat stem. The feeding of the sawfly on the parenchyma and

nodes of the stem can reduce wheat yield substantially, with an estimated range of 2.8-26% yield loss (Holmes et al., 1977; Morrill et al., 1992, 1994). Indirect damage from the sawfly begins when the larva tunnels down to the bottom of the wheat stem and chews a ring to effectively cut the wheat when it is nearing time for harvest. The wheat stem then often falls over and the head of the wheat may be lost during harvest. Grain is then lost, and the ground where it falls may need to be sprayed with herbicide to prevent volunteer wheat that may develop during the fall or the following year. Even if the head remains intact, lodged wheat is very difficult to harvest. The header on the combine must be lowered in order to attempt to pick up the fallen stems. Lowering the header results in the possibility of contacting rocks and inconsistencies in the ground that will damage the header, necessitating equipment repairs and harvest delays. One further impact of lowering the header on the combine is that more parasitoids of the wheat stem sawfly will be destroyed since they often form their cocoons in the lower parts of the wheat stem.

Several methods of controlling wheat stem sawfly infestations exist, but none of them are very effective. Sawfly-resistant, solid-stem wheat varieties have been available since 'Rescue' was introduced in the 1940's, but these varieties usually produce lower yields than hollow-stem varieties, and their solidness is very dependent upon environmental factors (Platt, 1941). Predators and parasitoids of sawflies do exist, but they are not reliably effective against the sawfly population and have yet to be mass-reared for re-introduction into areas infested with wheat stem sawflies. Although tillage of sawfly-infested stubble has been extensively tested as a method to kill diapausing sawflies, the results have been mixed as to effectiveness in killing overwintering larvae. Most often, the results have shown that overwintering larvae are not extensively harmed,

and the population of adults the following year is almost totally unaffected; in addition, tillage has also been found to damage populations of sawfly parasitoids (Runyon et al., 2002), thus hindering the naturally-occurring biological control of sawflies.

It would be highly beneficial to learn if wheat produces volatile compounds in response to feeding by wheat stem sawfly larvae – if wheat does produce sawfly induced volatiles, knowledge of their respective identities and amounts could lead to new strategies for the management of sawflies.

Test Hypotheses

The principal test hypotheses investigated in my research are the following:

- Test Hypothesis 1: Wheat plants that are infested by sawfly larvae produce volatile chemicals that differ either quantitatively or qualitatively from the volatile chemicals produced by wheat plants uninfested by sawflies.
- Null Hypothesis 1: Wheat plants infested by sawfly larvae produce the same volatile compounds in the same amounts as wheat plants uninfested by sawflies.
- Test Hypothesis 2: Chemical compounds in the cuticular waxes of wheat stem sawflies or in their frass can be coupled with physical damage to induce volatile-production changes similar to those caused by sawfly infestation.
- Null Hypothesis 2: Volatile production changes in sawfly-infested wheat plants are induced by some other means than cuticular wax compounds or frass compounds coupled with physical damage.

LITERATURE REVIEW

Herbivore-induced Volatile Production Changes

In response to herbivory, plants emit a blend of volatiles that may differ quantitatively and qualitatively from those volatiles emitted by an intact plant (Dicke and van Loon, 2000). These volatiles often negatively impact the pest population that induced them. Induced resistance is well documented, having been reported for over 100 plant species (Karban and Baldwin, 1997). Wheat is known to give off volatiles under normal growing conditions (Buttery et al., 1985), and to differ in its volatile production due to herbivory and stress (Anderson and Peters, 1994). Anderson and Peters (1994) infested wheat seedlings with *Schizaphis graminum* Rondani (Homoptera, Aphididae) and found that volatile production increased when the number of *S. graminum* per shoot increased.

Most of the herbivore-induced volatiles produced by plants can be grouped into two main categories, direct and indirect responses. However, these categories are fairly superficial since the response of plants to infestation is virtually always a direct defense that also indirectly affects the insects (Turlings et al., 1991b, 1992b). Direct responses are plant defenses that affect the pest insect or pathogen directly, such as the formation of neoplasm, production of oviposition deterrents, and the production of toxins (Hilker and Meiners, 2002). Secondary plant metabolites can have a significant effect on the organisms that feed on plants (Turlings and Benrey, 1998). Examples of direct defenses include the production of toxic secondary metabolites, such as nicotine or furanocoumarins which can kill attacking herbivores outright, and defensive proteins,

such as protease inhibitors or polyphenol oxidases, which decrease nutrient availability and slow the growth of herbivores (Baldwin and Preston, 1999). At the very least, toxic secondary metabolites can force herbivores to invest limited resources in detoxification (Baldwin et al., 2001), and these costs can deter herbivores from feeding on the plant. Herbivore-induced volatiles were found by Bernasconi et al. (1998) to repel aphids from feeding on corn. Bernasconi et al. (1998) theorized that repellency might be due to the fact that plant odors could indicate: 1) the plant has initiated the production of toxic compounds; 2) potential competitors are present on the plant; 3) the plant is attractive to parasitoids and predators. Whatever the reason, the repellency of an insect pest is greatly beneficial to a plant. The oviposition behavior of herbivorous insects can also be influenced by the chemical response of plants to infestation (Anderson and Alborn, 1999; Jonsson and Anderson 1999). Anderson and Alborn (1999) determined that plants infested by *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) larvae of different developmental stages were readily differentiated by the adult seeking an oviposition site. Chemical cues may enable female sawflies to be able to determine when a stem has a sawfly larva already inside. There is significant selection pressure for this ability since only one sawfly larva survives within a stem in a normal system. While there are many examples of direct responses to insect herbivory, indirect responses also play an important role in the trophic interactions of plants and insects.

Indirect responses are plant defenses that attract parasitoids and predators of various life stages of the herbivore. Herbivore-induced plant volatiles can serve to attract predatory insects to the location of their prey (Turlings et al, 1990; Dicke et al., 1990; Vet and Dicke, 1992; Stowe et al., 1995; Takabayashi and Dicke, 1996; Mayland et al., 2000;

Van Poecke et al., 2001; Bertschy et al., 2001; Maeda et al., 2001). Colazza et al. (2004) found that oviposition by *Nezara viridula* L. (Hemiptera: Pentatomidae) induced synomones which attracted the egg-parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae). Induced volatiles can be highly specific in attracting the correct parasite or parasitoid to attack their host insect (De Moraes et al, 1998; Meiners et al., 2000; Dicke 1999); that is, some plants can give off an herbivore-specific blend of volatiles. Because of this, it is possible that parasitoids of the wheat stem sawfly are being attracted by sawfly-herbivory-induced volatiles of wheat. This could be exploited through artificial lures or genetic manipulation of wheat lines to attract more parasitoids to fields experiencing problems with sawfly infestation – if the mechanism of induction was better understood.

Mechanism of Induction

Most often, mechanical damage alone does not induce the same response as insect herbivory does in plants. From several studies, it appears that chemicals produced by herbivorous insects are more responsible for inducing the plant response. Chemical compounds within an insect's oral secretions that come into contact with damaged plant tissue have been found to induce the synomones that attract parasitoids of the herbivore (Turlings et al., 2000; Alborn et al., 1997, 2000). The inducement of volatiles is not limited to oral secretions only; oviposition by *Bruchus pisorum* L. (pea weevil) (Coleoptera: Bruchidae) and *Callosobruchus maculates* F. (cowpea weevil) in pods of specific lines of pea (*Pisum sativum* L.; Fabaceae) stimulates the formation of tumor-like

growths which can impede the progress of a weevil larvae (Doss et al., 2000). Analysis of whole-body extracts from these weevils has led to the isolation of some specific long-chain α,ω-diols, which, when applied to pods of *P. sativum*, stimulate the formation of these same, tumor-like growths. Some similar chemical compounds have been identified in the cuticular hydrocarbons of the wheat stem sawfly (Bartelt et al., 2002). Frass, too, can contain chemicals that attract parasitoids (Turlings et al., 1991a). The potential pheromones of wheat-stem sawfly (Cosse et al., 2002) include compounds that have been isolated from wheat, thus showing that wheat volatiles are likely one of the cues used by sawflies for host plant selection.

Wheat and Other Host Volatiles

The main parasitoids which attack sawflies present a unique system in the respect that they attack sawfly larvae at two different ages, and thus they are responding to cues from their host or the infested wheat plant at two very different growing periods. *Bracon cephi* Gahan and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae), the most common parasitoids of wheat stem sawfly, attack the larva both when the host plant is green and still developing, and when it is drying down and the wheat kernels are near ripening. Since the sawfly larva is enclosed within the stem of the host plant, it is most likely that the cues used by the larval-seeking parasitoids are emitted by the host plant rather than from the larva. Recorded volatiles produced by wheat and oats include groupings of aliphatic aldehydes and ketones, aliphatic alcohols and esters, terpenoids, and aromatic compounds (Buttery et al., 1982, 1985). Buttery et al. (1982, 1985)

obtained volatiles on Tenax (60-80 mesh, Applied Science Laboratory) traps from wheat and oat plants that had been recently harvested. The method I used to collect volatiles is similar, but is performed on intact plants. Hamilton-Kemp and Anderson (1984, 1986) also described some volatile components of wheat; however their method of obtaining the volatiles utilized steam distillation-extraction, which can result in both enzymatic and physical oxidation of chemicals to forms that do not normally occur in the plant. Some chemical compounds associated with wheat appear in greater amounts at different stages of the plant's life such as during the ripening of the wheat kernels. These compounds are found primarily in the plant tissue associated with the head of the wheat plant such as those found by Kato et al. (2002) and Gatford et al. (2002). Because the volatiles produced by wheat and other host plants changes quantitatively over time, the volatiles which attract the parasitoids may also change over time and could even include some of the ripening compounds associated with wheat.

Plant Communication Using Volatiles

Herbivory-induced volatile cues are not used by insects alone; they can constitute airborne information that can be transferred among plants (Arimura et al., 2000). Arimura et al. (2000) found that by exposing uninfested lima bean plants to herbivory-induced volatiles, they could achieve a defensive reaction in the plant that would result in its becoming less suitable a food source for *Tetranychus urticae* Koch (Acari: Tetranychidae) (spider mites). Baldwin and Schultz (1983) theorized that an airborne cue was responsible for increased levels of defensive compounds in potted poplar and maple

seedling that had not been damaged, but were near seedlings which had been damaged. Guerrieri et al. (2002) conversely found that plants apparently needed more than volatiles in the air to induce changes in an uninfested neighbor, but could induce changes in the neighboring plant if there was root contact between the two. Such properties could mean that relatively small amounts of herbivory-induced volatiles could trigger a plant response for an entire field that would make it less attractive to some herbivores. At the very least, there has been conclusive evidence presented by researchers that, whether or not plants transfer information to each other, they do transfer information to other parts of the same plant; that is, the induction of volatiles is not limited to one site but is systemic (Turlings et al., 1992a, b; Baldwin, 1994; Stowe et al., 1995; Bowles, 1998).

Current and Potential Uses of Volatile Chemicals

Induced volatiles have been used on a small scale to manage populations of pest insects but have the potential to be used on a much grander scale for insect control. Bartelt et al. (2002) discussed the use of pheromones in trapping wheat stem sawfly, and these trials have been further modified (ongoing research) to include known plant volatiles in order to better attract sawflies to the traps. These traps can be used to monitor sawfly populations and do reduce the number of sawflies that enter a wheat field, though not enough to reduce the population for the following season. Combining induced plant volatiles with pheromone components would likely enhance the attractiveness in many pheromone-mediated trapping systems. Annihilation programs that use a toxin with pest-attracting pheromones, such as those programs cited by Tumlinson et al. (1976), could

instead use induced synomones with the pheromones to reduce the environmental hazards while still reducing pest populations by attracting predators and parasitoids. This is a variation on the suggestion of Tumlinson et al. (1976) to use a kairomone in place of the toxin. As far back as the late 70's, a compound now known to be an induced volatile was found to attract a predatory insect (Flint et al., 1979). James (2003a, b) researched and was successful in using several different synthetic herbivore-induced plant volatiles to attract parasitoids. A further development along the lines of parasitoid attraction which has had little research up to now is that of genetically manipulating or breeding plants which will respond more vigorously and/or more rapidly to pest damage; as was noted by Powell and Picket (2003), this development would encourage the proper synchrony of pest presence and parasitoid arrival.

Repelling pests would be even more effective than attracting parasitoids. If female sawflies are able to distinguish between plants with a sawfly larva in them, such as suggested earlier, such a trait could be exploited to cause females to reject stems that had been treated with the proper "repellents". Repellency of some pests due to induced plant volatiles has been established in studies by Birkett et al. (2000), Bruce et al. (2003), De Moraes et al. (2001), and the previously noted Bernasconi et al. (1998). Birkett et al. (2000) and Bruce et al. (2003) determined that the induced plant volatile cis-jasmone repelled aphids from plants in laboratory and field trials; in addition, Birkett et al. (2000) established that just the application of cis-jasmone further induced chemical changes in the plants which rendered them more attractive to parasitoids – the benefits of the application of the induced volatile were two-fold. Jasmonates and their precursor, jasmonic acid, appear to be a particular group of compounds which act as elicitors of

plant resistance across species lines. Constabel et al. (1995) were able to induce an increased production of polyphenol oxidase, a defensive protein, in young tomato plants (*Lycopersicon esculentum* Miller; Solanaceae) by exposing them to methyl jasmonate vapor. Thaler et al. (1996, 1999a, 1999b) utilized methyl jasmonate and jasmonic acid to induce plant resistance in tomato plants against corn earworm, *Helicoverpa zea* Boddie, and beet armyworm, *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae). Thaler (1999a) further found that the exogenous application of the jasmonates did not affect the yield of the test plants. Baldwin (1998) used methyl jasmonate application to roots to elicit plant defenses in coyote tobacco (*Nicotiana attenuata* Torrey; Solanaceae). Lu et al. (2004) used jasmonic acid application to induce resistance in Chinese cabbage (*Brassica campestris* L.; Brassicaceae) to the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae). Conversely, Lu et al. (2004) also found that jasmonate application to common cabbage (*B. oleracea* L.) induced susceptibility to the diamondback moth; such a result encourages further research into the interactions of plants and induced volatiles as elicitors of further plant defense. The fact that jasmonates are biologically active for so many different plant-insect systems also indicates that advances in the understanding of one plant species' volatile production could lead to advances for science in multiple plant-insect systems.

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MATERIALS AND METHODS

Plants and Infestation

Wheat (*Triticum aestivum* variety 'McNeal') used in each experiment was grown in 13x13x13.5 cm, tapered, square pots under temperature-regulated greenhouse conditions. The photoperiod was 15 hours, which was maintained throughout the duration of the experiments with the aid of supplemental lighting (GE Multi-Vapor MVR1000/C/U) to account for shortening hours of daylight. The temperature setting for the greenhouse was 22°C during the day and 20°C at night with a 1.5°C range above or below these temperatures before the heating or cooling systems began running. The soil consistently used was approximately 1/2 (by volume) a mixture of peat, perlite, vermiculite, starter nutrient charge, wetting agent, and dolomitic lime (unknown proportions, pH~6-7) and the remaining 1/2 was a mixture composed of 1/3 sand, 1/3 soil, and 1/3 Canadian sphagnum peat moss (pH~5.5-6). Soils were obtained from a local commercial provider and were thoroughly mixed together by hand prior to planting. The plants were watered as needed, usually 2-3 times weekly until the plants reached the 3 leaf stage; at this time, all but one of the plants were removed so as to give the remaining plant no stress due to crowding (plants were seeded 2-4 seeds per pot to ensure that at least one plant grew and to give more plants to select from for similar sizes and stages). At this stage, fertilizing was also initiated, with Peters® General Purpose 20-20-20 at 100 ppm nitrogen in 250ml of water, twice a week, continuing until the infested plants were cut by the infesting larvae. When the plants reached boot stage and began to head (Zadoks stage 49; Zadoks et al., 1974), their water demands were greater, and they

were watered more frequently, up to once a day. After a sawfly larva cut an infested plant, fertilizing and watering was stopped for all plants since the cut stem could no longer take up water. Plants were grown in batches of 15-16 pots with only 11 plants selected for each experiment immediately prior to sawfly infestation; having a larger than necessary number of plants to select from gave better chances for choosing plants at or very near the same size and stage. Plants were selected when they had reached a Zadoks scale stage between 32 and 45. All 11 selected plants were then carefully fitted with a small, plastic cylinder around the main stem (See Figure 1).

Figure 1. Infesting Cylinder

Two female sawflies were introduced into the cylinders on 7 of the plants (infested plants; treatment), and the cylinders were sealed with tape to prevent the sawflies from escaping; the other 4 cylinders on the remaining plants were left empty (uninfested/control plants). The unequal number of treatment versus control plants was set up with the hope of getting at least 4 plants truly infested by sawflies; not all plants

exposed to the sawflies were truly infested. Infestation could only be confirmed when the stems were "cut" by the sawfly larva, or were cut and split by myself to look for signs of infestation (larvae and/or frass); all stems were cut and split at the end of all volatile collections.

Sawflies were reared together (male and female) in batches of 1000 from stubs collected from a heavily infested field in the fall or spring prior to experimentation. Stubs were kept in a refrigerator at 5-7°C for greater than 90 days (required diapause) until removing to room temperature for 3-6 weeks at which point sawfly adults would begin to emerge. After the 1 to 2 days allowed for sawfly oviposition, each plastic cylinder was removed and any living sawflies were killed.

Plant-wounding Experiment

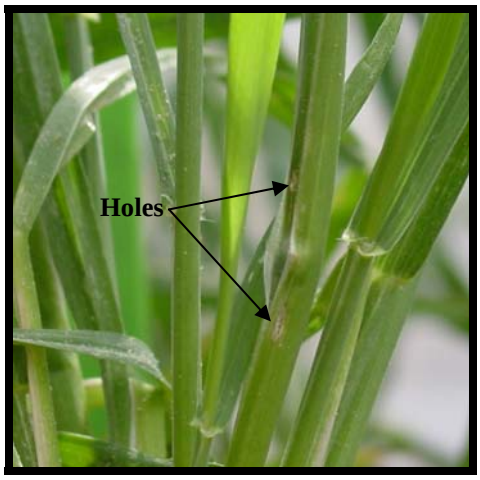
Because plants injured by wheat stem sawflies are uniquely damaged, special methods were devised to simulate the wounding to the stem caused by the adult and larva. Specifically of interest were insect-produced chemicals that might be contributing to volatile chemical induction; therefore, cuticular wax obtained from the bodies of adult female sawflies was used in conjunction with some of the treatments, and water in which sawfly larva frass had been soaked was used in other treatments. The wax from the females was obtained by first soaking 85 female sawflies in hexane for several days and then removing the liquid, which was then placed under nitrogen to remove all solvent from the extract. The larval frass was obtained by splitting approximately 75 infested stems during the previous summer and collecting the frass, which was then stored in a

freezer at -27°C. This frass was suspended in 37.5 ml of deionized water and allowed to soak overnight, and then the water was withdrawn and filtered through a 0.2 μm syringe filter.

Plants were grown as in the manner listed previously, with the exception that they were initially in a sealed growth chamber; this chamber had a daytime temperature of 22°C and a nighttime temperature of 19°C, with a 15 hr light, 9 hr dark cycle. Plants in the first replicate were removed from this growth chamber 1 week prior to applying the treatments. Those in the second replicate remained in the growth chamber until the treatments were applied in order to limit damage by an infestation of thrips in the greenhouse. Four different treatments were used on 11 total plants:

1) Three of the plants (infested controls) were exposed to 2 female sawflies as in the manner described previously;
2) On the day the plastic cylinders were removed, three other plants (pin-pricked) were pricked just above and below the 2nd node(See Figure 2), first with the tip of a size #11 Exacto® blade which had been dipped in the sawfly wax and then with a size #1 insect pin which had been dipped in the sawfly wax (approximately 0.5 mg of wax were applied in the treatment, this is less than 0.1 mg greater than the amount of wax calculated to come from one adult female sawfly based on the amount of wax extracted from all 85 females);

Figure 2. Pin/blade Holes in Wheat



3) Three weeks after the first volatile collection, three other plants (frass-treated) were injected with 200 µl of frass-treated water in the 1st and 2nd internodes (100 µl per internode) using a syringe equipped with a 25 gauge needle – volatiles were collected from these plants during the 3 weeks prior to this treatment, and these collections were analyzed as uninfested plants up until the point of treatment.
4) All 11 of the plants were equipped with the same plastic cylinders during the time allowed for sawfly infestation, and this was the only treatment applied to the uninfested plants (5 uninfested plants prior to frass-water treatment, 2 uninfested plants after).

The infested plants are described as controls because they are the plants of interest for comparison to the wounded treatments; the uninfested plants are included simply to note whether the infested controls have statistically significantly different chemical production than these plants. Unfortunately, when this experiment was completed, and the plants were split to verify sawfly infestation, it was determined that only one of the infested controls was truly infested. In order to balance out the experiment, results from infested

plants and control plants from the first experiment were grouped with these experiments; these plants had similar Zadoks stages (at the time of collection) to those in the replicate they were grouped with. An analysis of variance (ANOVA) was run for one of the weeks to determine if there was a statistically significant difference between the control plants in the two experiments, and the *p*-value was not statistically significant at $\alpha = 0.05$ (actual *p*-value = 0.6889 and 0.3404 for the two replicates); from this test, it was assumed that the plants would not have statistically significant differences in any of the weeks, and therefore it was valid to include some of the results from the 1st experiment in the 2nd experiment.

Volatile Collections and Analysis

A glass volatile collection chamber (VCC) supported by a ring stand and attached to an automatic volatile collection system (VCS, Analytical Research Systems, Inc., Gainesville, FL) was placed over the main stem of each of the 11 plants immediately after the plastic cages were removed (See Figure 3). The VCS is essentially the same as that described by Heath and Manukian (1992), with the exception that the VCC's have one open end in order to place them over and collect volatiles from living plants. The VCC's measured 800 mm in length and had an inner diameter of 40 mm.

Figure 3. Automatic Volatile Collection Chambers and System

A Teflon® sleeve was taped shut around the base of the stem and taped to the glass tube to avoid introducing outside air into the system. Filtered, humidified air was pumped into the tube through a no. 7 ChemThread fitted inlet sealed with a cap and rubber o-ring at a rate of 1 liter per minute (lpm). Air was pulled out at the same rate through an identical inlet at the top of the VCC. A volatile collector trap (6.35 mm OD, 76 mm long glass tube; Analytical Research Systems, Inc.) containing 30 mg of Super-Q adsorbent (Alltech Associates, Inc., Deerfield, Illinois) was inserted into this port and sealed with a cap and rubber O-ring. Super-Q is a cross-linked polymer composed of divinylbenzene and ethylvinylbenzene capable of collecting volatiles through cohesion due to its large surface area (500-600 m^2/g). A Teflon® sleeve was also taped to one empty tube which was placed over an empty pot, and volatiles were collected from this tube as a blank (See Figure 4).

Figure 4. Collection Tube on Plant and Blank Tube

Volatiles were collected for 10 or 12 hours during the day on one filter and 6 or 8 hours during the night on another filter without changing the tube covering the plant. Only data collected from the plants during the day period were analyzed for this thesis. Plants were covered by the tube for approximately 24 hrs only on the collection days to minimize any stress on the plant caused by the collection setup. Volatiles were collected from the plants and blank at 5-10 day intervals after the first collection until the plants had been cut by sawfly larvae or reached a stage suitable for harvest (Zadoks 92-93). After each collection, all glass tubes and manifolds were rinsed with hot tap water, followed by acetone and then hexane, prior to being placed in an oven set at 150°C where they remained until the next collection time.

To prepare the collections for analysis, volatile chemicals were first eluted from the volatile collector traps with 200 µl of certified ACS Spectranalyzed® hexane (Fisher Scientific, Fair Lawn, New Jersey), which was collected in a 200 µl conical insert within a 2 ml autosampler vial. To each sample was then added 10 µl of a 0.73 ng/ul solution of decane in hexane and/or trans-2-nonene in hexane (internal standards), and then 3 µl of

the sample was transferred to the gas chromatograph (GC) column (J&W Scientific HP-5MS 30m × 0.25mm ID, 0.25 μm film thickness) by the autosampler (Agilent 7683 series injector). The system analyzing the volatiles was a GC (Agilent 6890 instrument) coupled to a mass selective detector (MSD, Agilent 5973 instrument). The samples were injected onto the column in pulsed-splitless mode, with an initial pressure of 12.00 psi for 1 min. The inlet temperature of the GC was set at 250°C. The column temperature was held at 50°C for 4 minutes and then increased in temperature at a rate of 5°C/min until it reached 160°C; at this temperature the rate of increase changed to 25°C/min until the final temperature of 280°C was reached. A temperature of 300°C was maintained for the transfer line to the MSD. The flow rate in the column was set at 1.2 lpm. Some samples were run with a slightly different method that held the oven at 50°C for 12 min and then followed the same temperature regime as the previous method. Comparisons of chromatograms from infested plant samples and control plant samples to chromatograms from blank samples allowed for elimination of many contaminant peaks. Comparing infested plant and control plant chromatograms revealed any statistically significant changes in the volatile-production of wheat that the oviposition and feeding of *C. cinctus* might induce. The identities of volatile compounds of interest were determined from the comparison of the mass spectrogram of the peak of interest to those within the NIST mass spectral library (Rev. D.02.00), and by comparison to retention times for authentic standards when available. Peaks in the chromatograms of the volatiles collected from the plants in the first experiment were integrated by the ChemStation integrator in the data analysis program of Agilent Technology's ChemStation software package; the integrator had an initial threshold value of 15 and all other parameters were set to 0. Each

chromatogram usually had over 150 peaks. Figure 5 is an example of the chromatograms typically obtained from the GC-MSD runs of the volatiles collected from the plants; this particular chromatogram is from an infested plant in the 3rd week of collections. The results of these integrations were transferred to a Microsoft® Excel© spreadsheet where they could be corrected for differences in retention times for the same compound. All peaks being considered for grouping into the same retention time across samples were examined for purity while verifying their identity, and any peaks found to contain irresolvable mixtures or identified to be compounds of non-plant origin (e.g. column degradation, septa bleed) were cut from the analysis. Because many extraneous peaks remained, restrictions were instituted to eliminate more "noise". Peaks used for analysis occurred in more than half of the control or infested plants, and also had an area indicating plant production of that compound was equal to or greater than 0.25 ng/hr. From examining many of the chromatograms, it was determined that the vast majority of the peaks at or below this threshold were either noise or were mixtures of several components that could not be reliably separated.

Figure 5. Chromatogram from Infested Plant 3 Weeks after Infestation

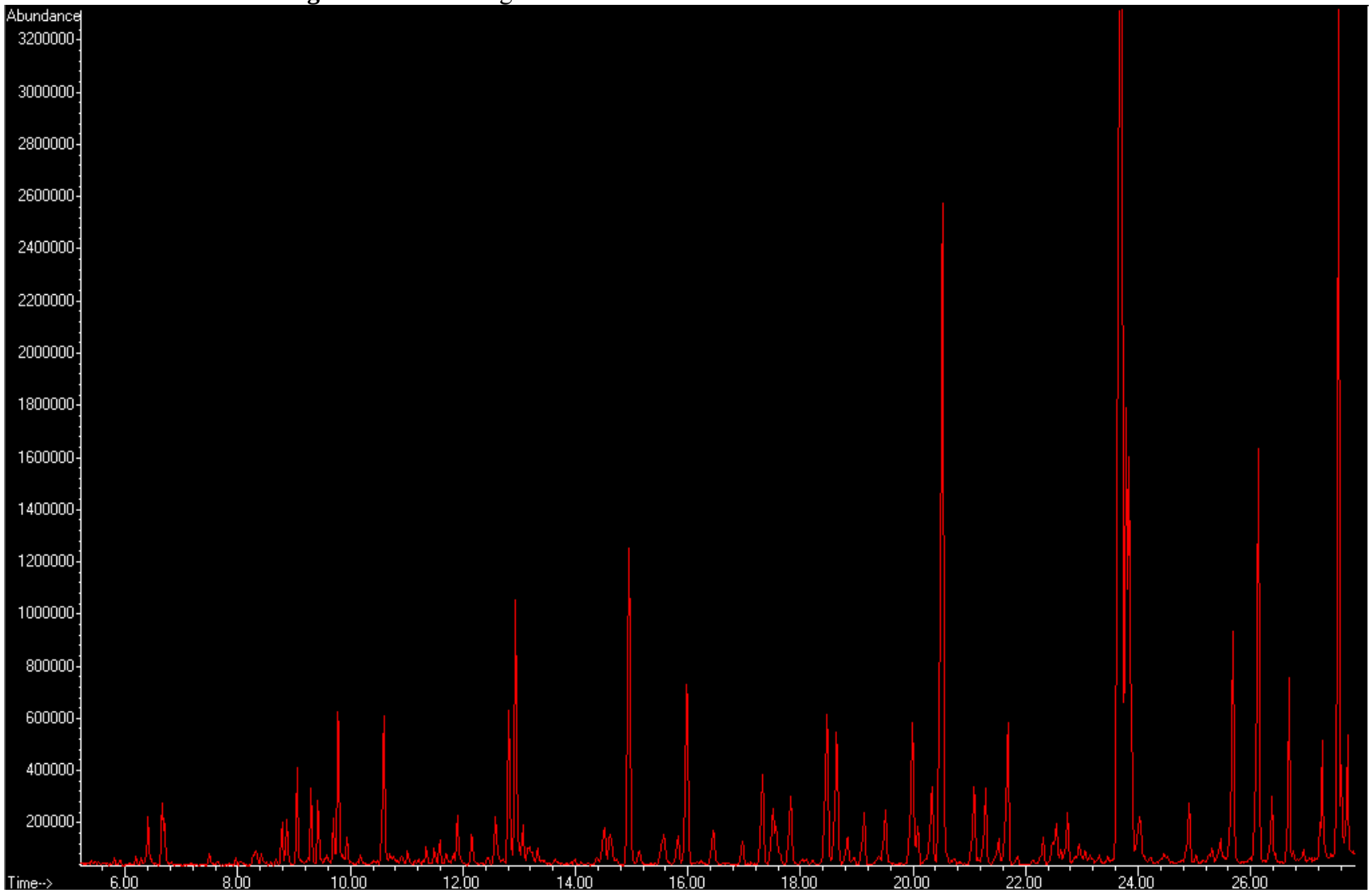
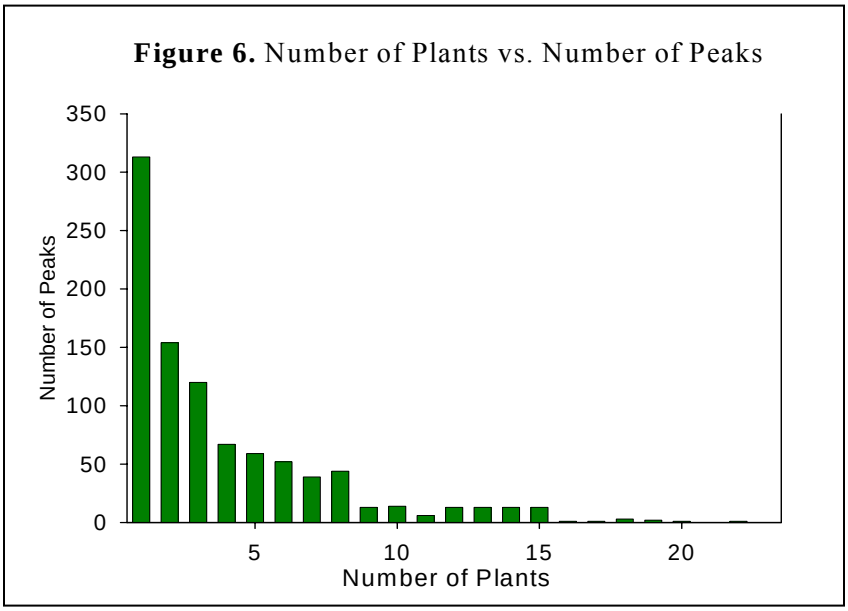
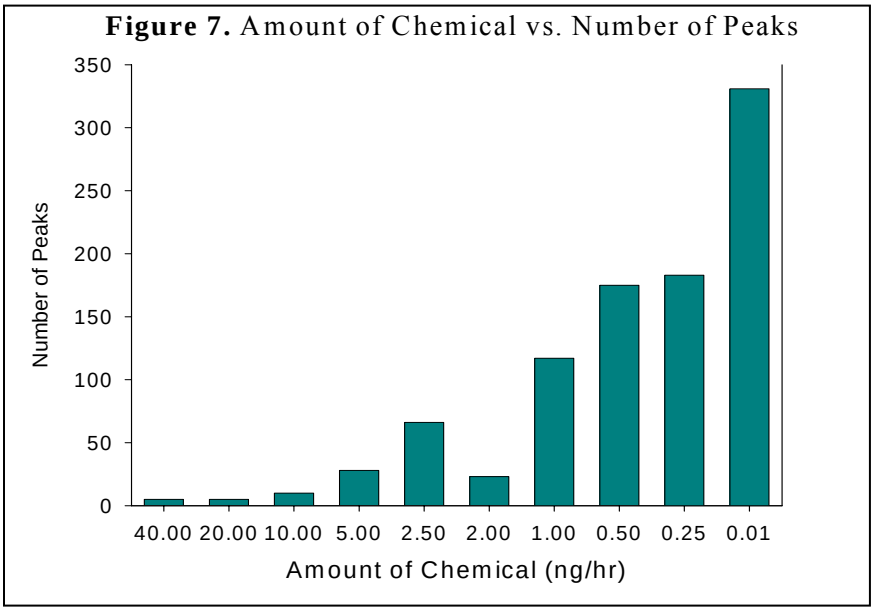


Figure 6 illustrates that, although there are many peaks, a large number occur in only one



plant, and thus cannot be used accurately in the analysis. Figure 7 illustrates that many of the peaks occur at or below the threshold of 0.25 ng/hr and can also be cut to further reduce the number of peaks analyzed for differences between infested and uninfested plants.



In order to ensure that the data being examined would show any effects from

larval feeding, only data from the 3rd collection week onward were examined in the 1st experiment; the larva does not hatch until approximately one week after oviposition, and it does very little feeding for the first week of its life.

RESULTS

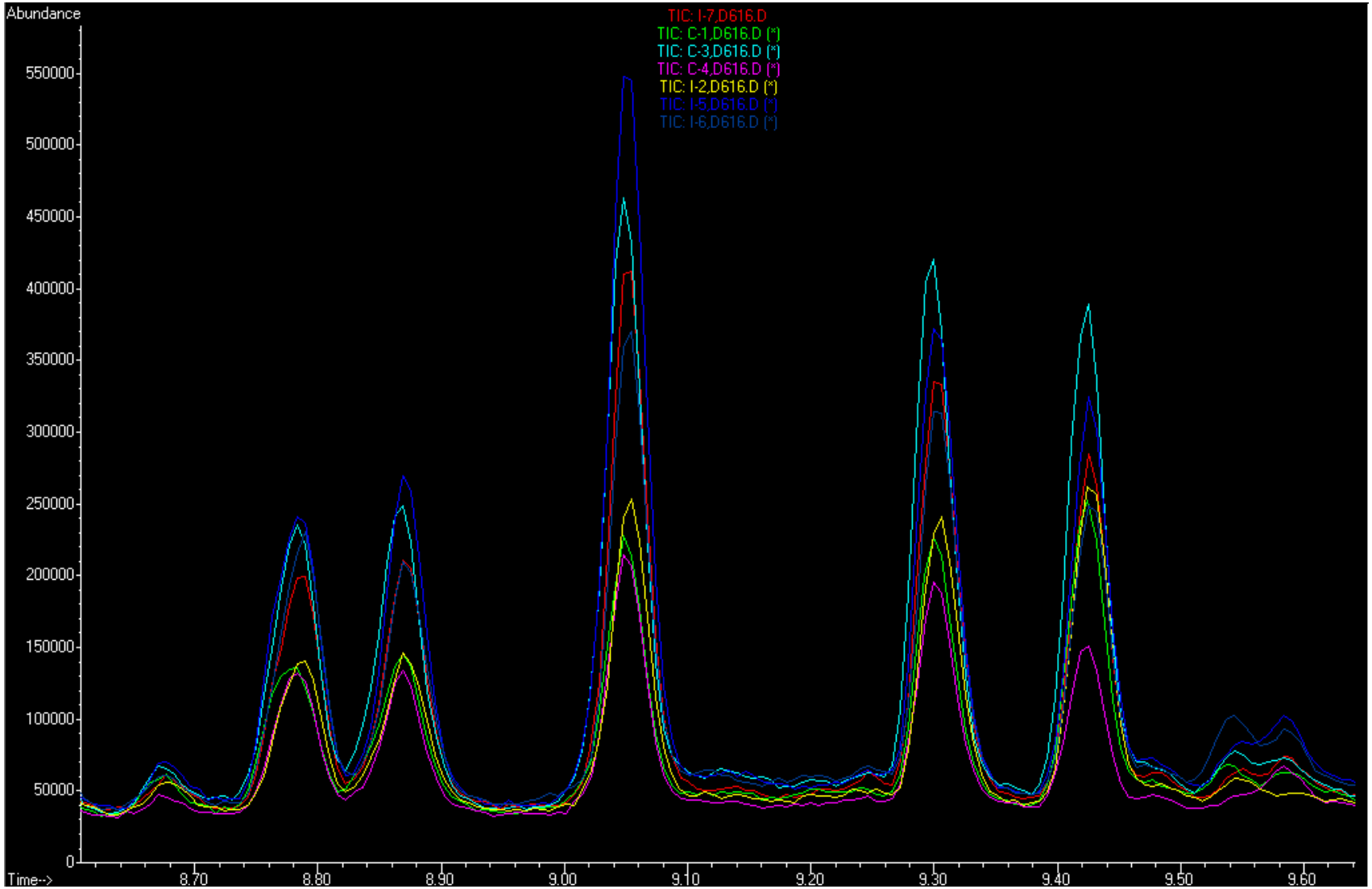
Infested vs. Uninfested Experiment

The first attempt to find differences between infested plants and control plants was to view overlaid chromatograms of the two and then see if there were readily apparent differences. There were no readily apparent differences between the two, as can be seen in Figure 8, which shows a section of overlaid chromatograms of 4 infested plants and 3 control plants (chromatograms are designated C-1, -3, and -4 for control plants and I-2, -5, -6, and -7 for infested plants) 3 weeks after infestation – this overlay is centered on 1-octen 3-ol, a compound whose average amounts were later found to be statistically different between the infested and control plants. Since no readily apparent differences were seen, it was decided that the areas of all the peaks should be imported into a spreadsheet for a more in depth analysis. After extensively "cleaning" all of the data for contaminant peaks, the results were first visualized by plotting a scaled average. This scaling was done for each chemical according to the following equation:

(Average amount of chemical produced by infested plants - Average amount of chemical produced by uninfested plants) / (Average amount of chemical produced by infested and uninfested plants)

In this way, if the infested plants on average produced more of a compound than the controls, then the value would be positive; if less, then the value would be negative, and if equal, the value would be 0.

Figure 8. Chromatographic Overlay Centered on 1-Octen-3-ol



From a graph of these values (See Figures 9-12), one would be interested in the points that deviated farthest from 0. Also of interest is the overall trend throughout the weeks.

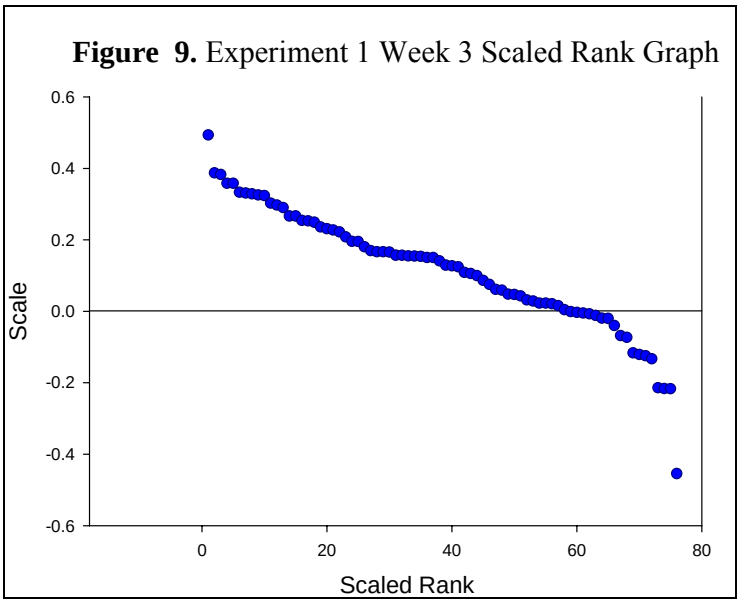


Figure 9. Experiment 1 Week 3 Scaled Rank Graph

Weeks 3 and 4 (Figures 9 and 10) seem to have a very similar set of data, with a fair number of points falling above the 0 axis, indicating that the infested plants are usually producing larger amounts of most chemicals, on average.

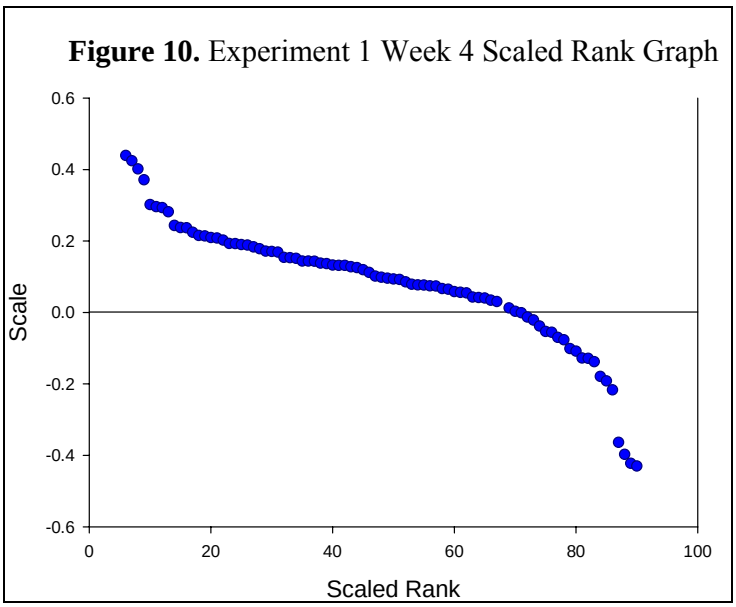
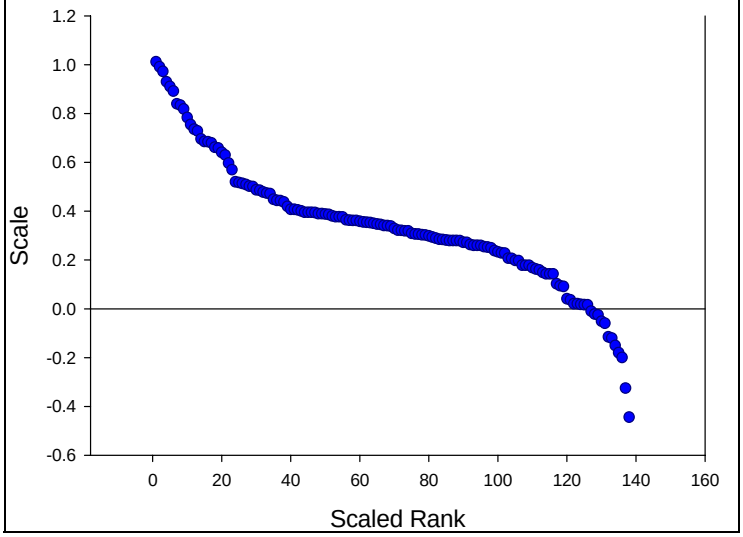


Figure 10. Experiment 1 Week 4 Scaled Rank Graph

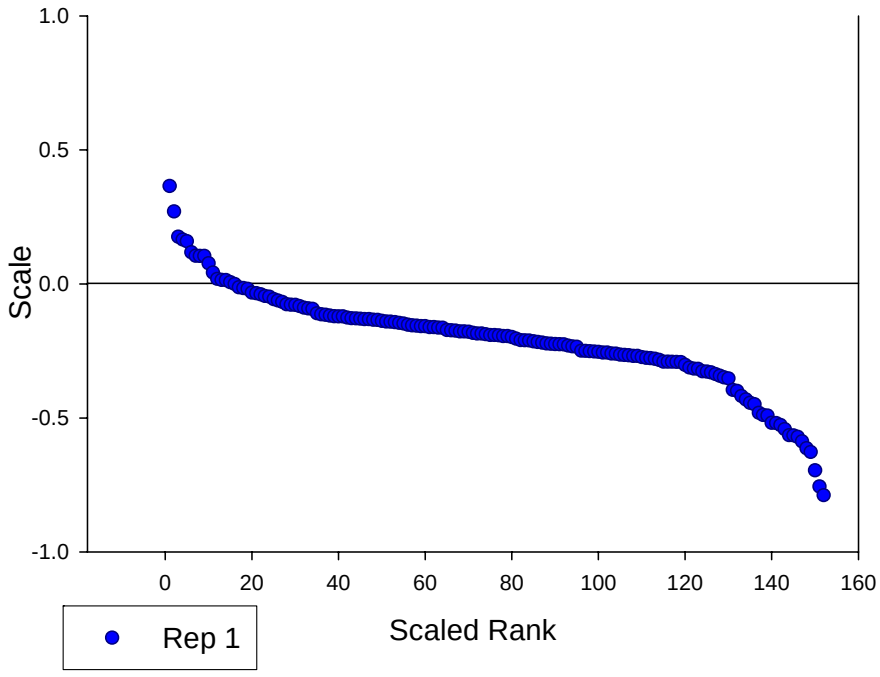
In week 5 (Figure 11) there are even greater numbers of points falling above the 0 axis, indicating that there is a marked difference in the average amount of chemicals produced by infested and uninfested wheat plants.

Figure 11. Experiment 1 Week 5 Scaled Rank Graph



While this visualization can be useful as a cursory analysis, it can be very biased by large variances within the data. Upon looking at the data contributing to many of the points farthest from zero, such as the ones marked with a red arrow in Figure 12, it was discovered that there often was only one plant that was producing a very large or very small amount of chemical relative to the other plants (See Table 1).

Figure 12. Experiment 1 Week 6 Scaled Rank Graph, Replicates 1 and 2



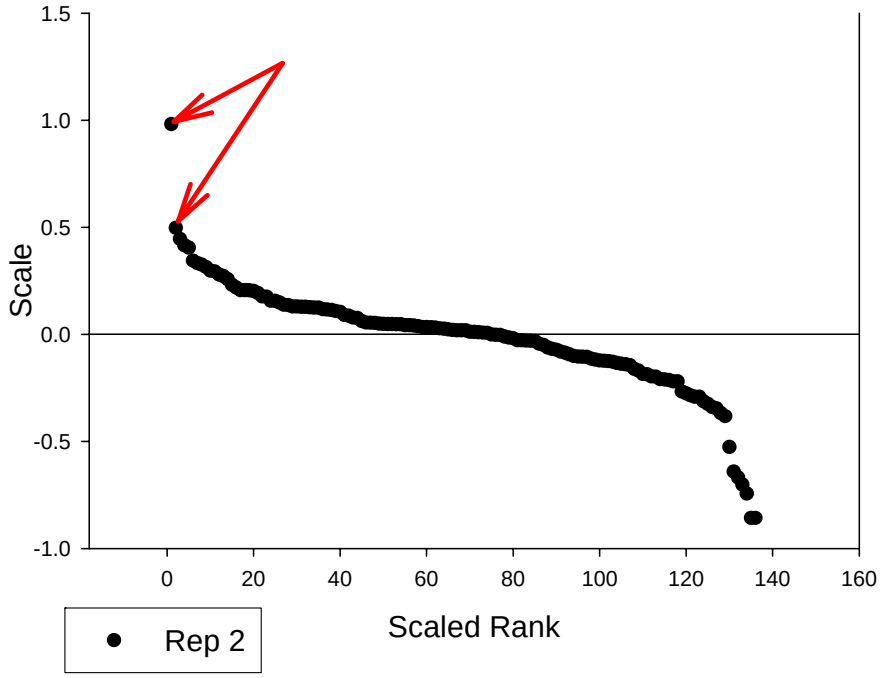


Table 1. Examples of Large Variance Skewing Data Points

				Plants					
Retention Time	Blank	C-1	C-2	C-3	C-4	I-1	I-3	I-4	I-6
29.7	0.0000	0.6623	0.0000	0.0000	0.7214	0.0000	0.8292	0.0000	**3.2211**
38.14	0.0000	0.3491	0.6711	0.4923	0.3339	0.9305	0.3667	**1.0753**	0.6956

After recognizing this problem, I decided to use a Student's *t*-test (two-sample unequal variance) to determine if there were statistically significant differences between mean chemical production in the uninfested and infested plants; this test is robust to problems associated with large variances; however, it does not give reliable results when there are significant interaction effects. In order to test for interaction effects, an ANOVA was run in R (R language and environment version 1.7.1, copyright 2003, The R Development Core Team) on the data collected in each week; an interaction plot of the means was also run in R to test for interaction effects. Weeks 3 and 4 had no significant interaction effects between replicates and treatments and thus the replicates could be tested together by the *t*-test without any problems. Weeks 5 and 6 did show significant interaction effects between the replicates and treatments, so the *t*-test had to be conducted on the individual replicates. Each collection week had at least two replicates.

Graphs of statistically significant compounds in each week are displayed in the next several pages. In week 3, at $\alpha = 0.08$, 3 compounds are statistically significant: 1-octen-3-ol, 2-undecanone, and tridecane (Figure 13). All compound identities listed hereafter with an asterisk are only tentative because no commercial standard was available to verify their particular retention times. In Week 4 there are again 3 compounds which are statistically significant at $\alpha = 0.08$, but their identities are hexahydrofarnesyl acetone, 2-pentadecanone*, and 2-tridecanone (Figure 14).

Figure 13. Week 3 Significant Compounds

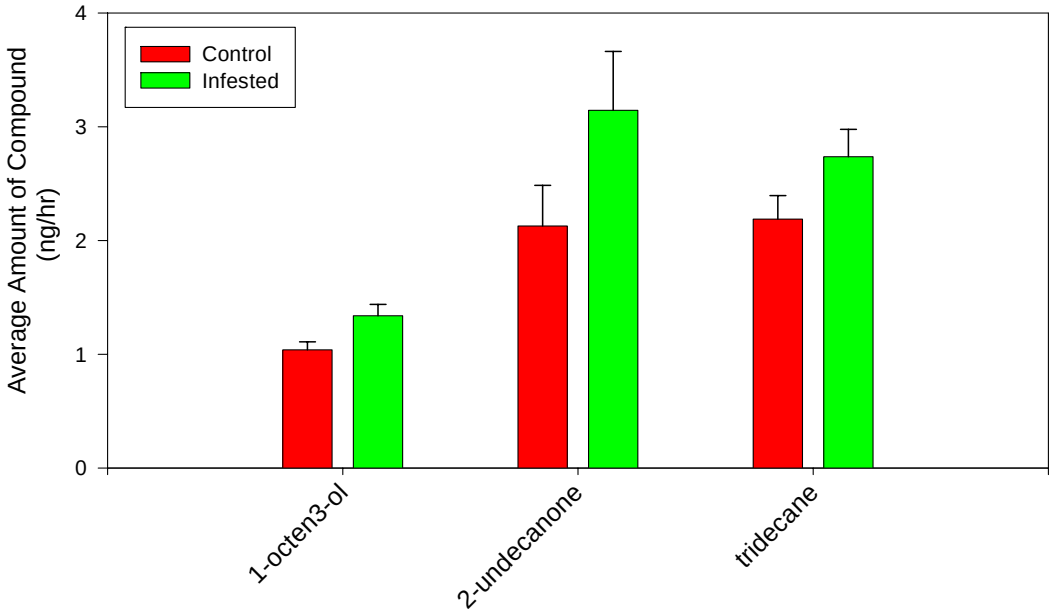
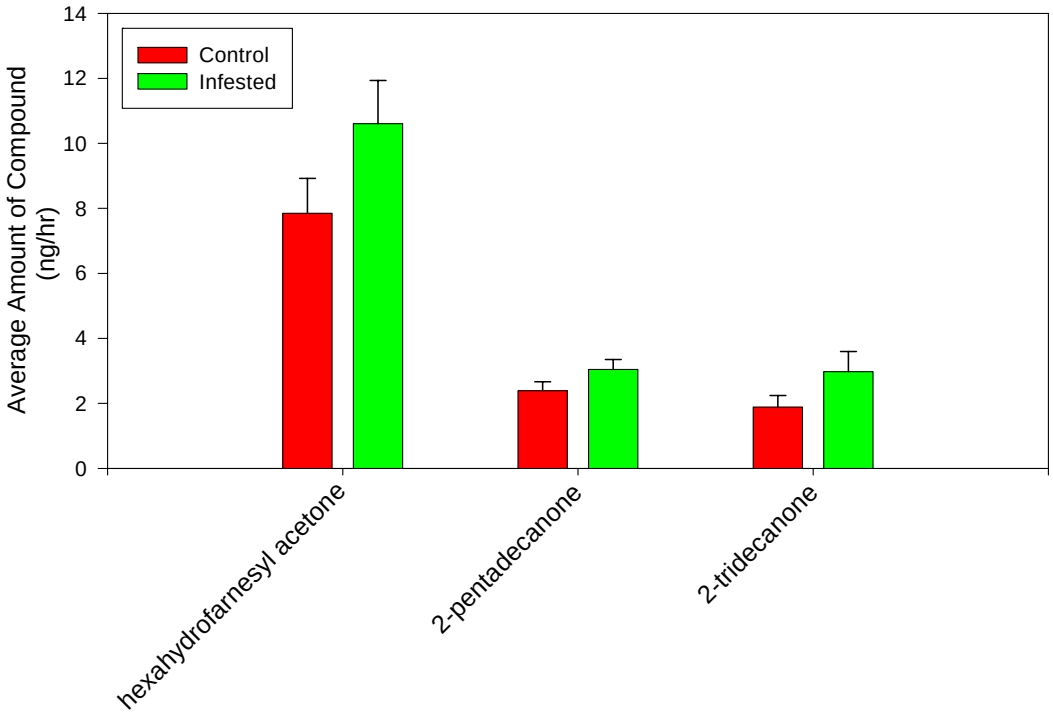


Figure 14. Week 4 Significant Compounds



In weeks 3 and 4, the statistically significant compounds for each week are all produced in greater amounts on average by the infested plants when compared to the control plants. The statistically significant ($\alpha = 0.05$ for all further results) compounds for replicate 1 of week 5 are benzyl alcohol, pentadecanal and hexahydropseudoionone* (Figure 15). In replicate 2 the statistically significant compounds are vanillin, hexahydropseudoionone*, 6-methyl-2-heptanone*, geranyl acetone, 4-oxoisophorone, benzyl alcohol, 2-pentadecanone*, dihydroactinidiolide*, 6-methyl-5-hepten-2-one, and 2-tridecanone (Figure 16). Both 2-pentadecanone* and 2-tridecanone retained their significance from the collections done in week 4 to those done in week 5. Benzyl alcohol and hexahydropseudoionone* were consistent in their statistical significance between both replicates of week 5. While the overall trend of infested plants producing greater average amounts of the statistically significant compounds holds true for week 5, it is evident that at least some of the statistically significant compounds are now being produced in greater amounts by the control plants compared to the infested plants. For week 6, the statistically significant compounds of replicate 1 are 1-tetradecene, 2,6-dimethyl-2,6-octadiene*, an unknown compound, phenylethanal, and hexahydropseudoionone* (Figure 17). Replicate 2 of week 6 has 1-hexanol, pentadecanal, and hexahydrofarnesyl acetone as statistically significant compounds (Figure 18). The structures of the chemicals referred to in the text can be seen in Figure 19. In this final collection, it is now evident that the overall trend of greater average amounts produced by the infested plants has reversed, and the control plants are now the ones usually producing greater average amounts of the significant compounds. This result is consistent with the scaled rank graphs for the final week of analysis.

Figure 15. Week 5 Rep 1 Significant Compounds

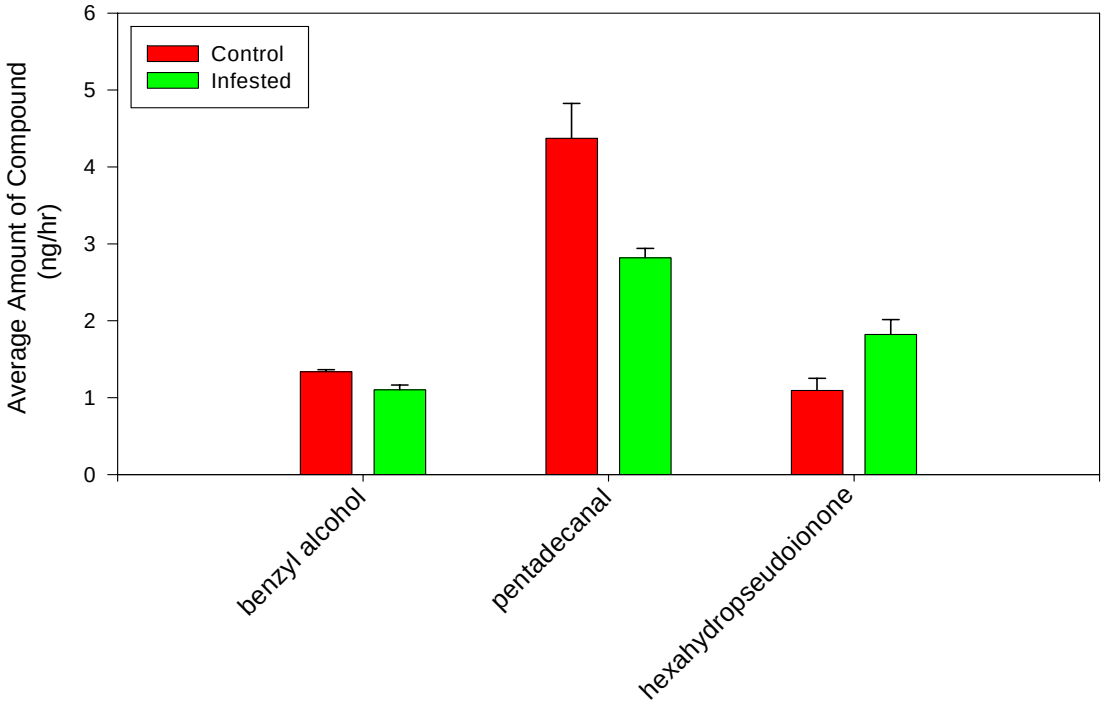


Figure 16. Week 5 Rep 2 Significant Compounds

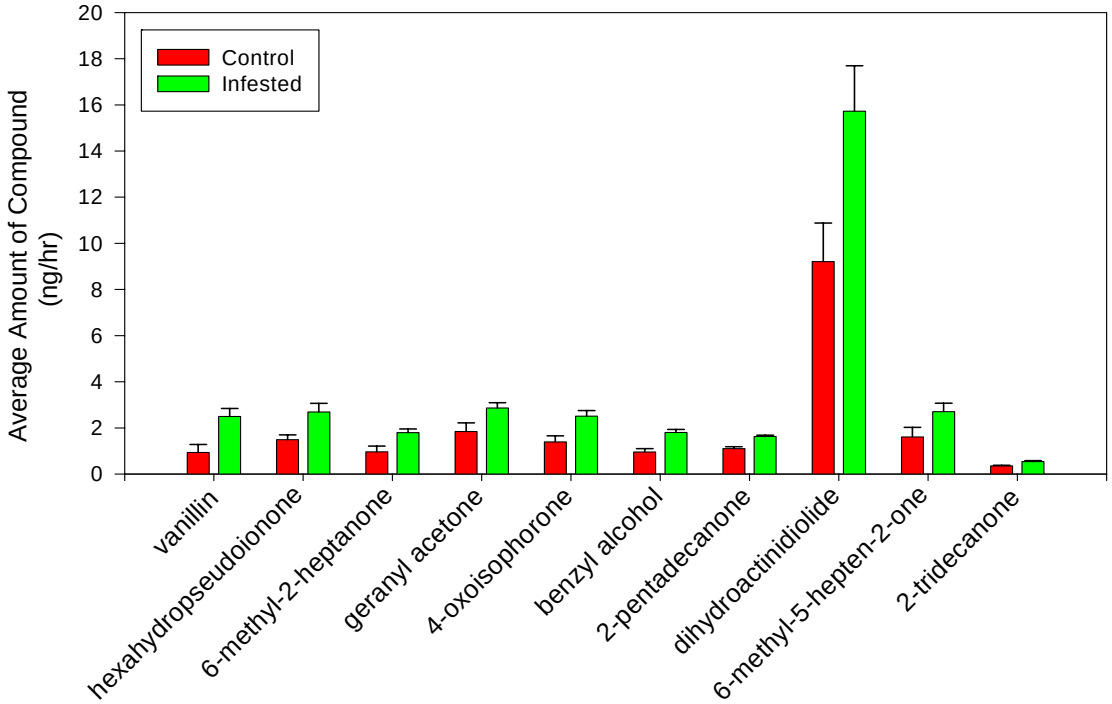


Figure 17. Week 6 Rep 1 Significant Compounds

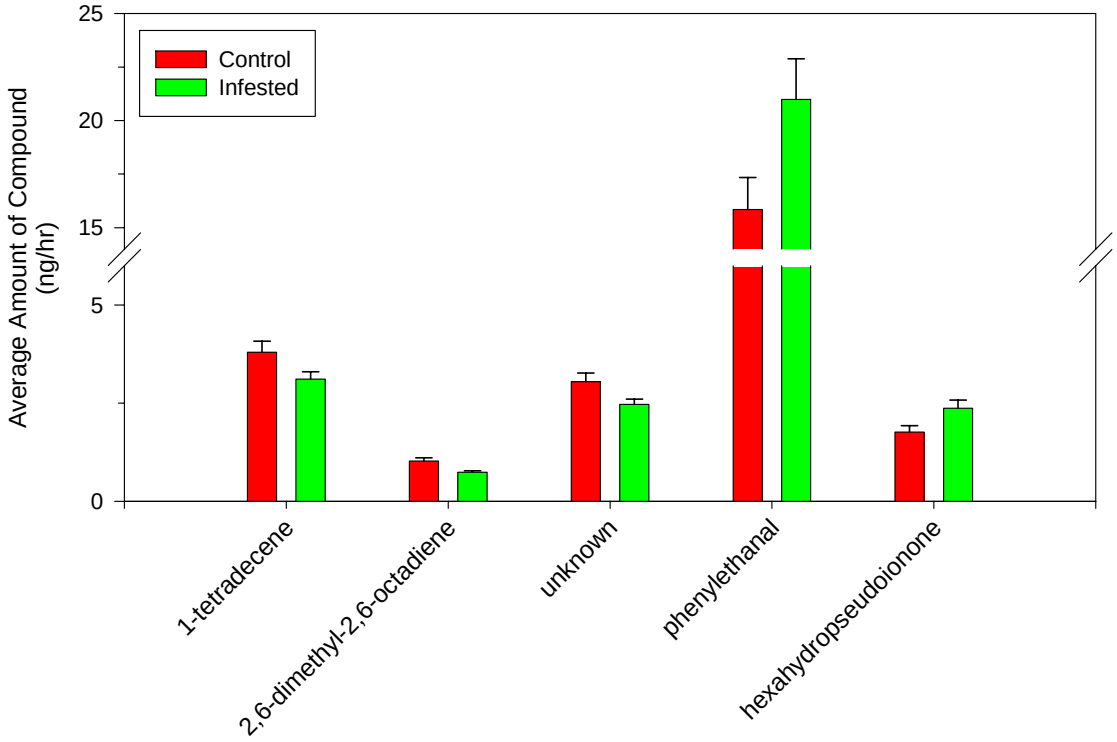


Figure 18. Week 6 Rep 2 Significant Compounds

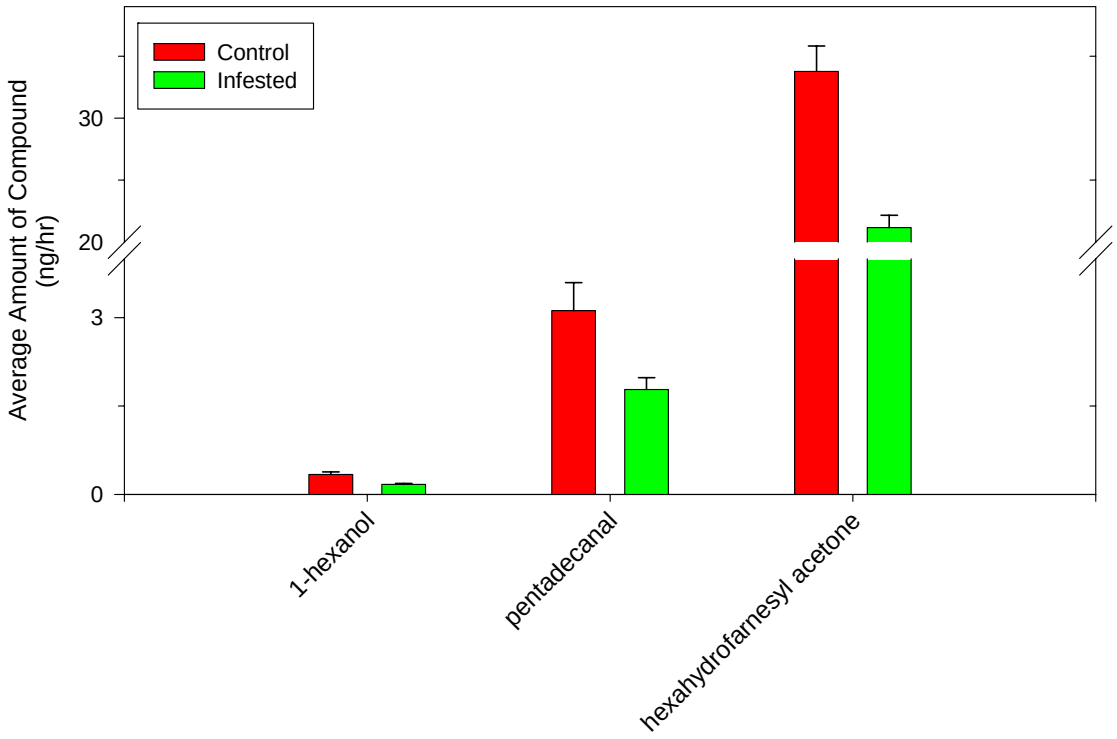
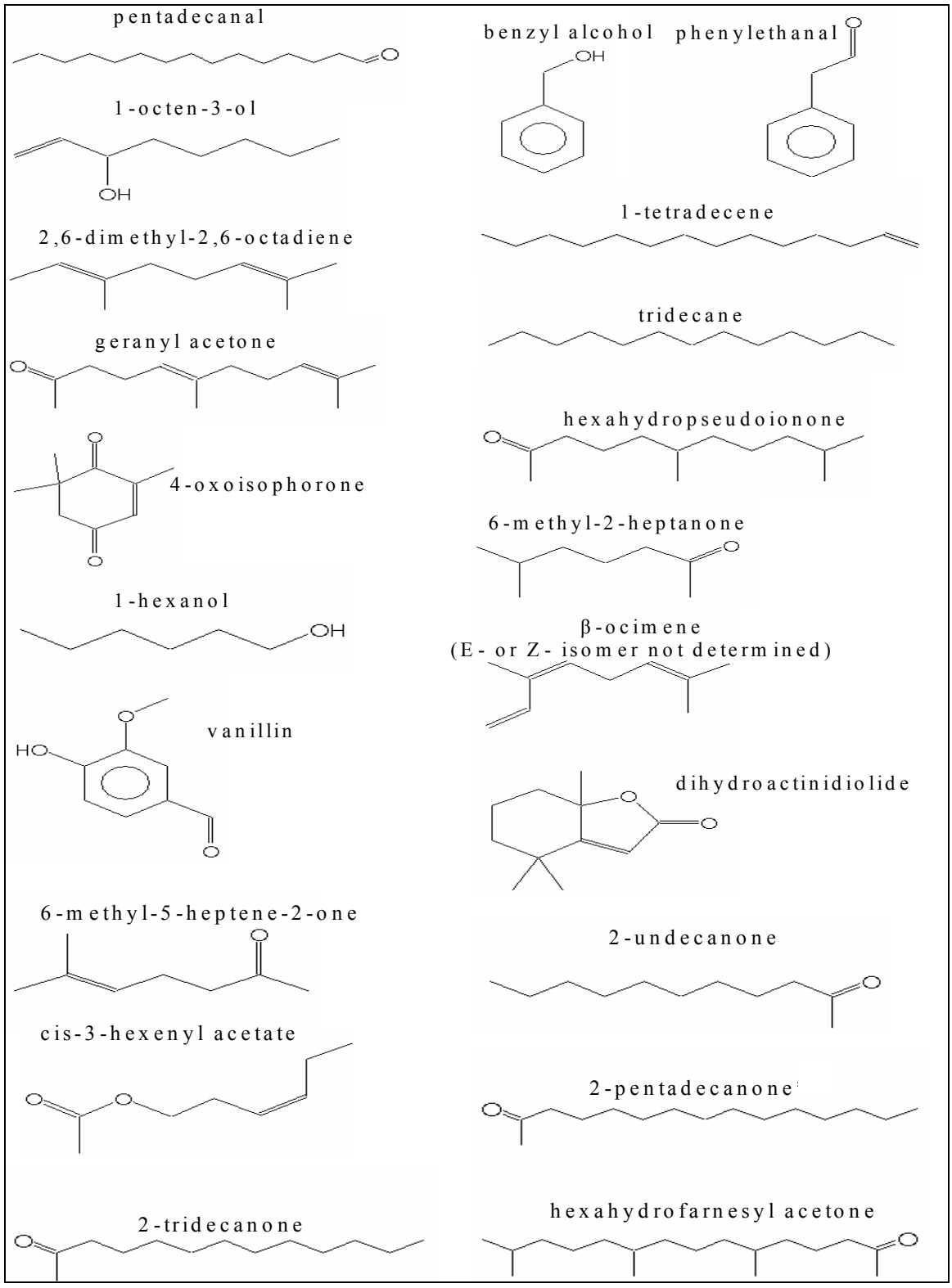


Figure 19. Structures of Chemicals Referred to in the Text



Plant-wounding Experiment

Eleven plant compounds seen in the 1st experiment were selected to analyze in the 2nd experiment. These compounds were selected based on purity, peak size (often large chromatographic peaks) and consistency in the first experiment, as well as known biological activity. Chromatographic peak size was used as a criterion because it was not apparent from the 1st experiment that there was 1 particular class of compounds that was giving evidence of difference between uninfested and infested plants, so it was assumed that selecting compounds based on size would not greatly bias results; moreover, larger peaks tended to be more pure. The 11 compounds chosen are: 1-octen-3-ol, cis-3-hexenyl acetate, phenylethanal, β-ocimene* (cis- or trans- isomer not determined), 1-tetradecene, hexahydropseudoionone*, 2-tridecanone, dihydroactinidiolide*, 2-pentadecanone*, pentadecanal, and hexahydrofarnesyl acetone. In particular, cis-3-hexenyl acetate and β-ocimene* were added to the analysis because I was interested in studying the plant response to sawfly oviposition, and many studies have shown these 2 compounds to be associated with initial plant-wounding responses.

There are two replicates in time in this experiment, and the data collected from them has been divided into two logical groupings based on one of the treatments. The plants injected with the frass-treated water were analyzed as uninfested plants up until the 3rd week of collection (the point at which they were injected with the frass-treated water). In order to perform a correct doubly-multivariate repeated measures analysis of variance (RMDMANOVA), it is necessary to analyze the volatiles in the first two weeks of collections as one group (with the frass-treated plants being designated the same as

uninfested plants), and to analyze the volatiles in the 3rd, 4th, and 6th weeks as another group (the fifth week of collections was excluded from the analysis due to the fact that not enough infested plants collected from in the first experiment were at the same stage as the plants in this experiment at this collection time). The RMDMANOVA was run using the general linear model of the SAS ® program (SAS Institute, 1988). The data were transformed to stabilize normality using a power of 0.15, as was indicated by a plot of the log-likelihood function versus the Box-Cox parameter λ. The results of the analysis indicated that there was a significant experiment effect, but no significant treatment by experiment interaction effects. Graphs of the results are therefore shown separately for each experiment in order to see the variation between replicates. Only weeks where significant differences between treatment average amounts were found in at least one of the replicates are reported in the graphs that follow. The Zadoks stage reported is the average stage of all the plants in a particular replicate for the day following the collections. The legend for the graphs uses a "U" to designate the bar for uninfested plants, an "I" to designate the bar for the infested controls, a "P" to designate the bar for the pin-pricked plants, and an "F" to designate the bar for frass-treated plants (only seen in weeks 3, 4 and 6). A single asterisk under the graph title designates significance for the treatments at $\alpha = 0.1$; a double asterisk designates significance for the treatments at $\alpha = 0.05$. Examining the graphs in Figures 20 through 27, it is evident that the volatile chemical amounts in the pin-pricked and frass-treated plants are more often similar to those collected from the uninfested plants than to those collected from the infested controls. In some cases, though, it does appear that the pin-pricked plants mimic the infested plants' volatile production response to sawfly infestation.

Figure 20. Cis-3-Hexenyl Acetate, Phenylethanal, and 1-Tetradecene: Week 1 Collections, Replicates 1 and 2

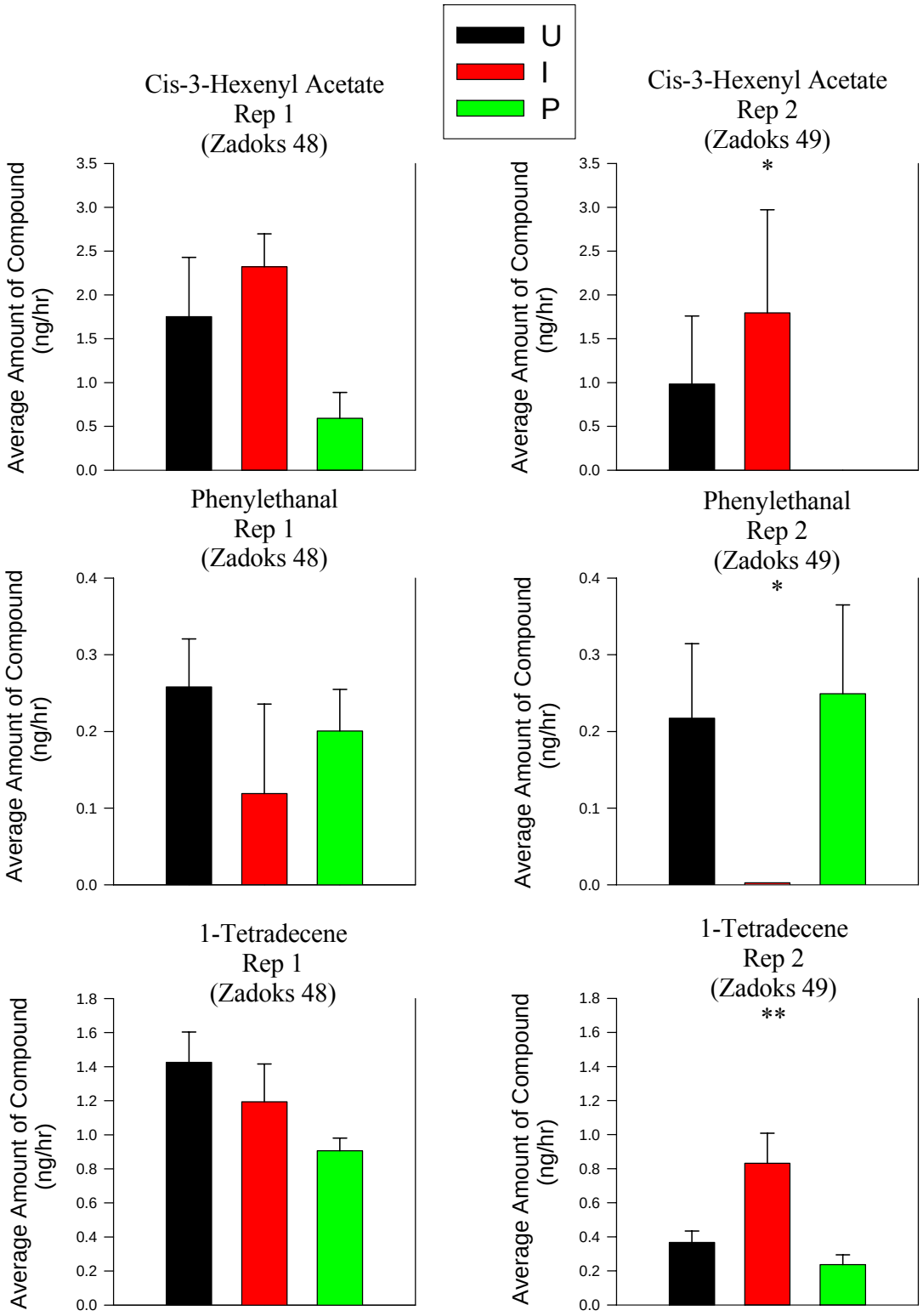


Figure 21. 1-Octen-3-ol, Pentadecanal, and Hexahydrofarnesyl Acetone: Week 2 Collections, Replicates 1 and 2

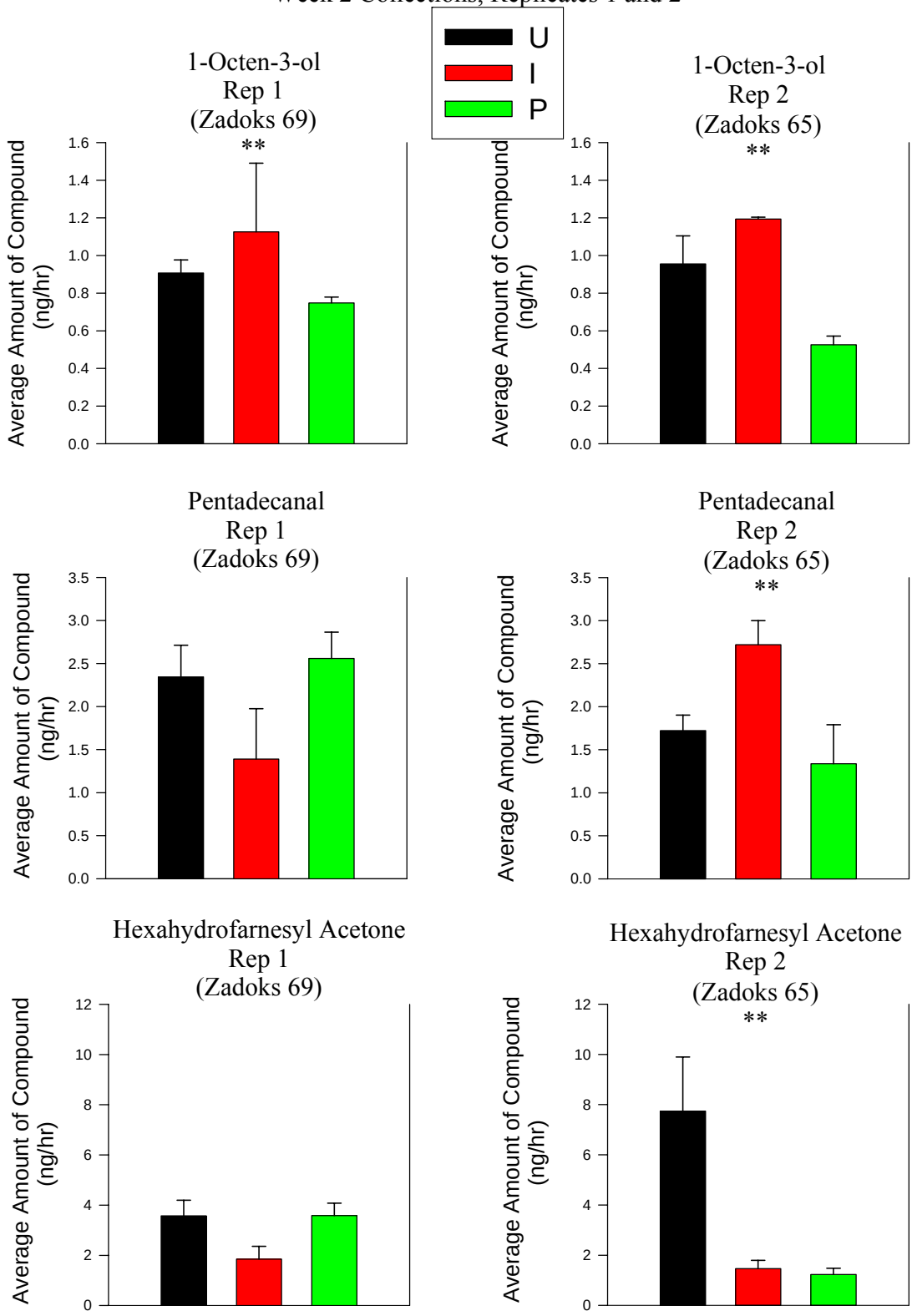


Figure 22. 1-Tetradecene, Hexahydropseudoionone, and Dihydroactinidiolide: Week 2 Collections, Replicates 1 and 2

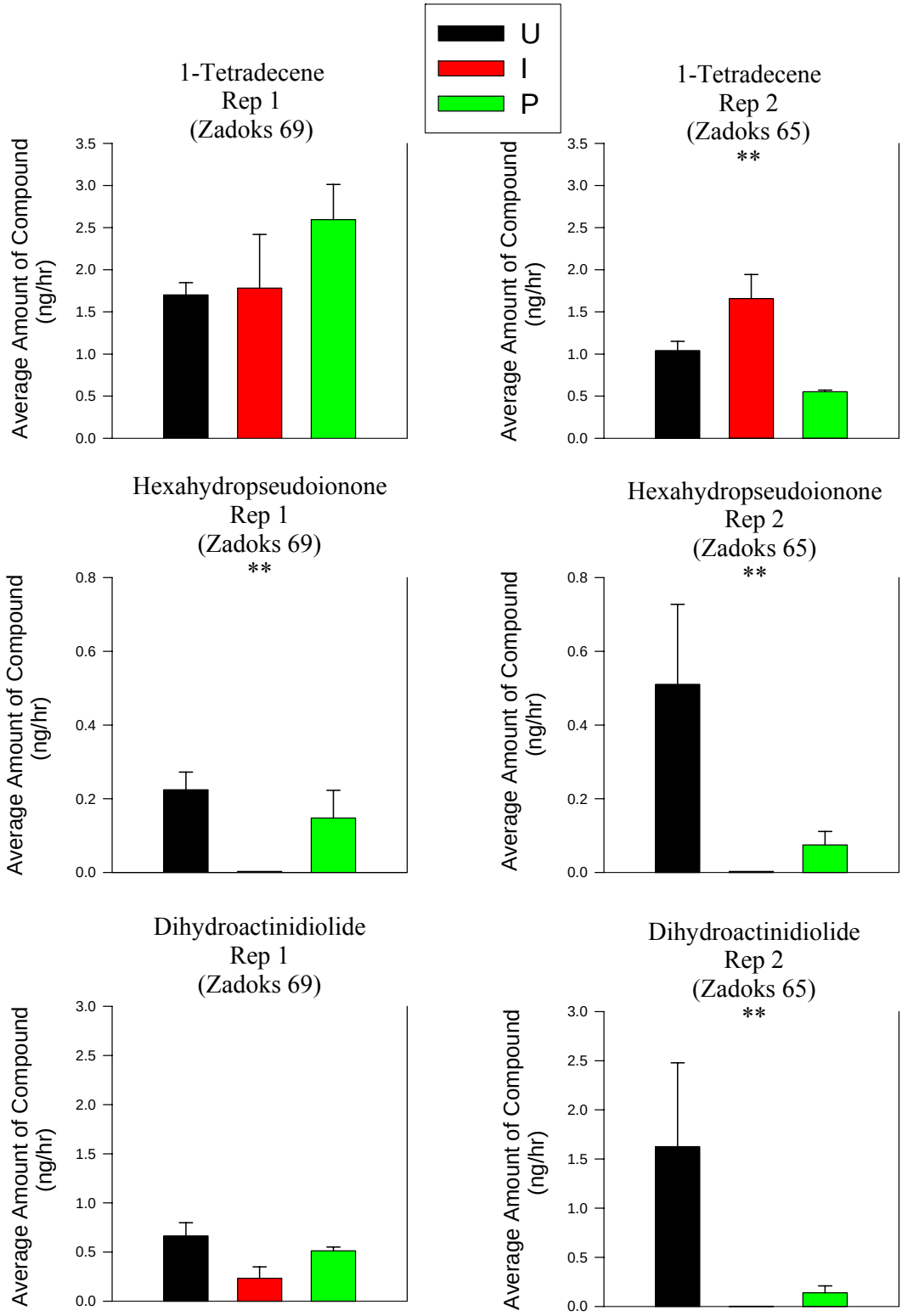


Figure 23. Hexahydrofarnesyl Acetone, ß-Ocimene, and Hexahydropseudoionone: Week 3 Collections, Replicates 1 and 2

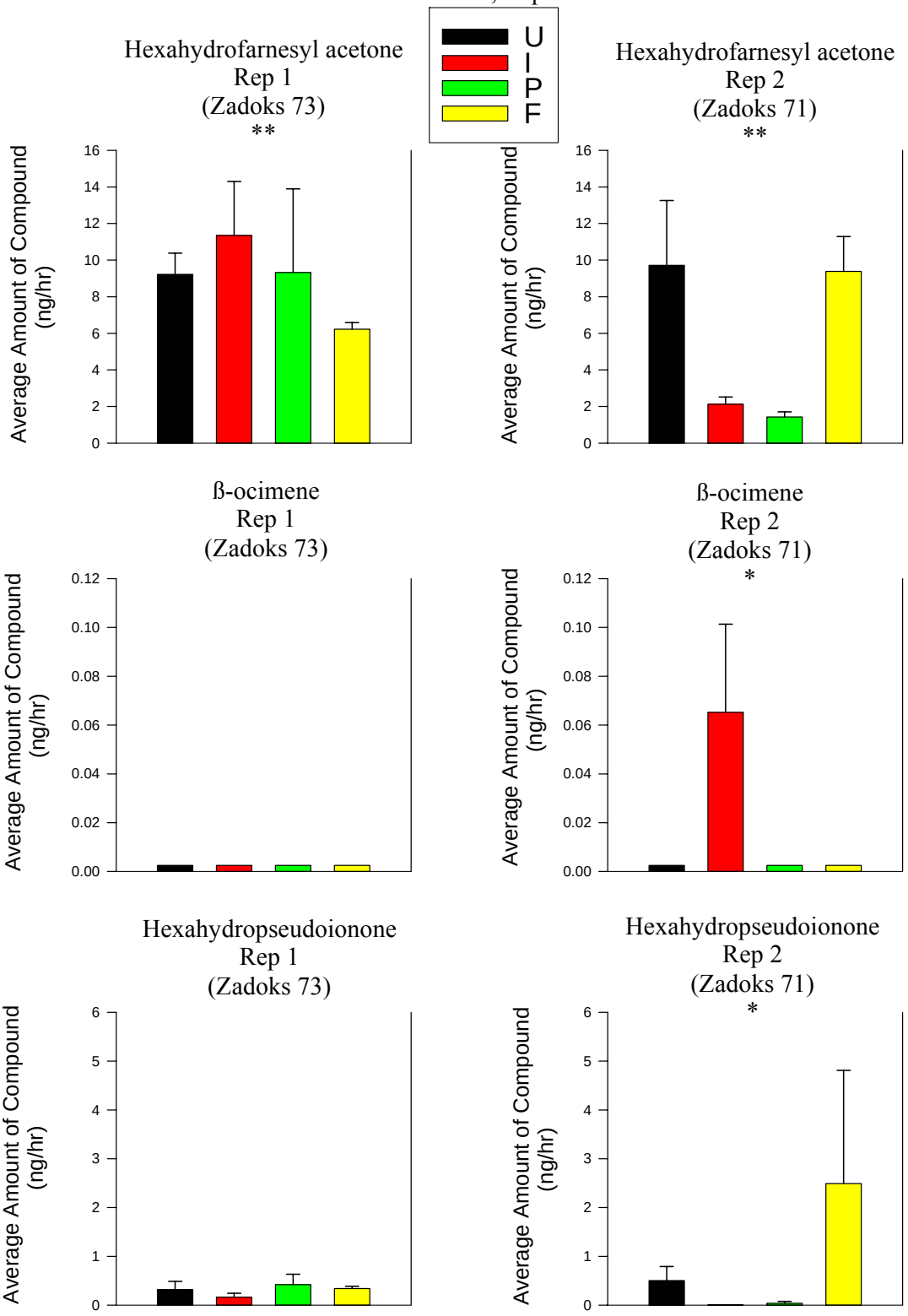


Figure 24. Pentadecanal, Hexahydrofarnesyl Acetone, and Phenylethanal: Week 4 Collections, Replicates 1 and 2

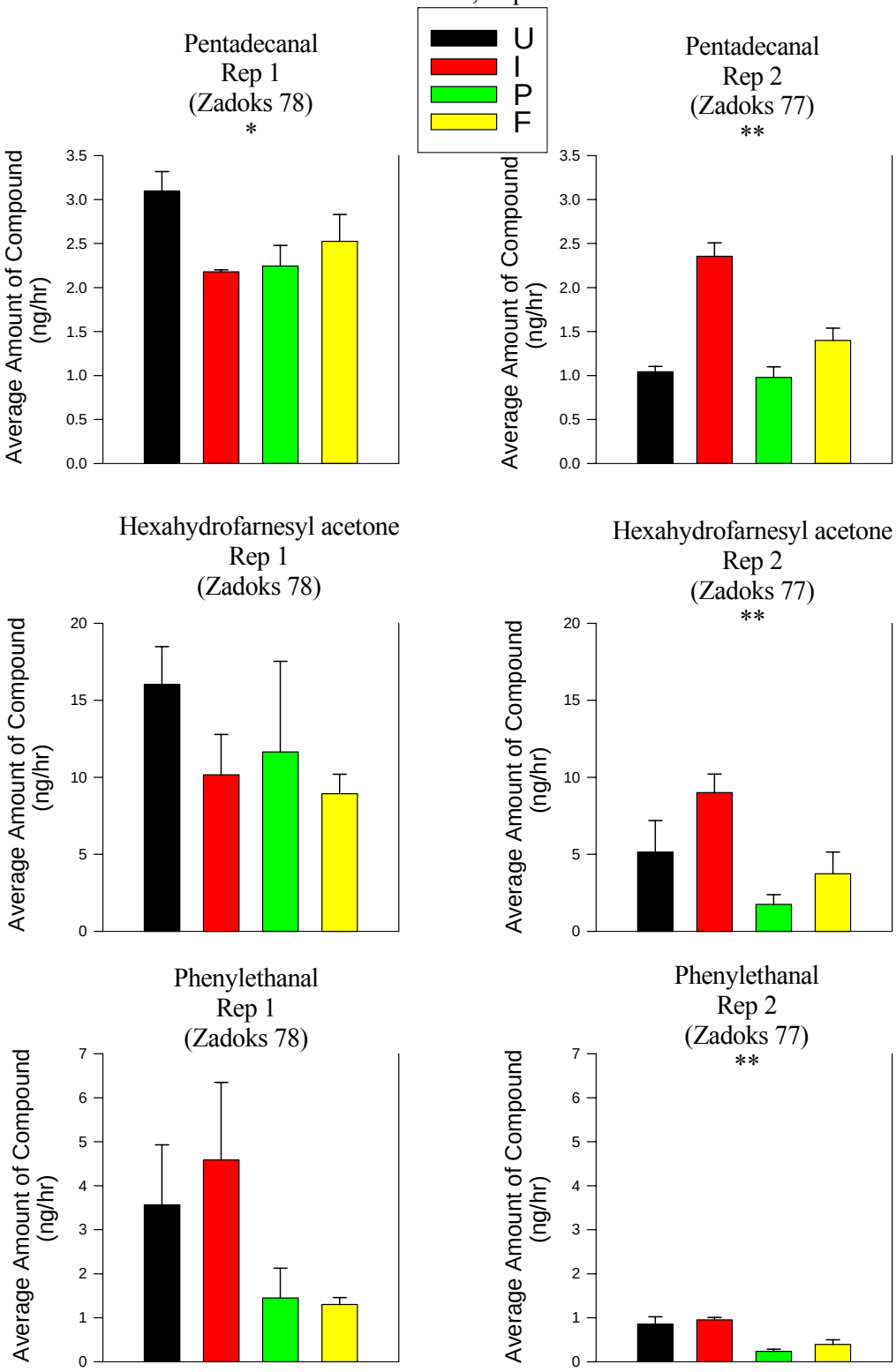


Figure 25. 1-Tetradecene, Dihydroactinidiolide, and 2-Tridecanone: Week 4 Collections, Replicates 1 and 2

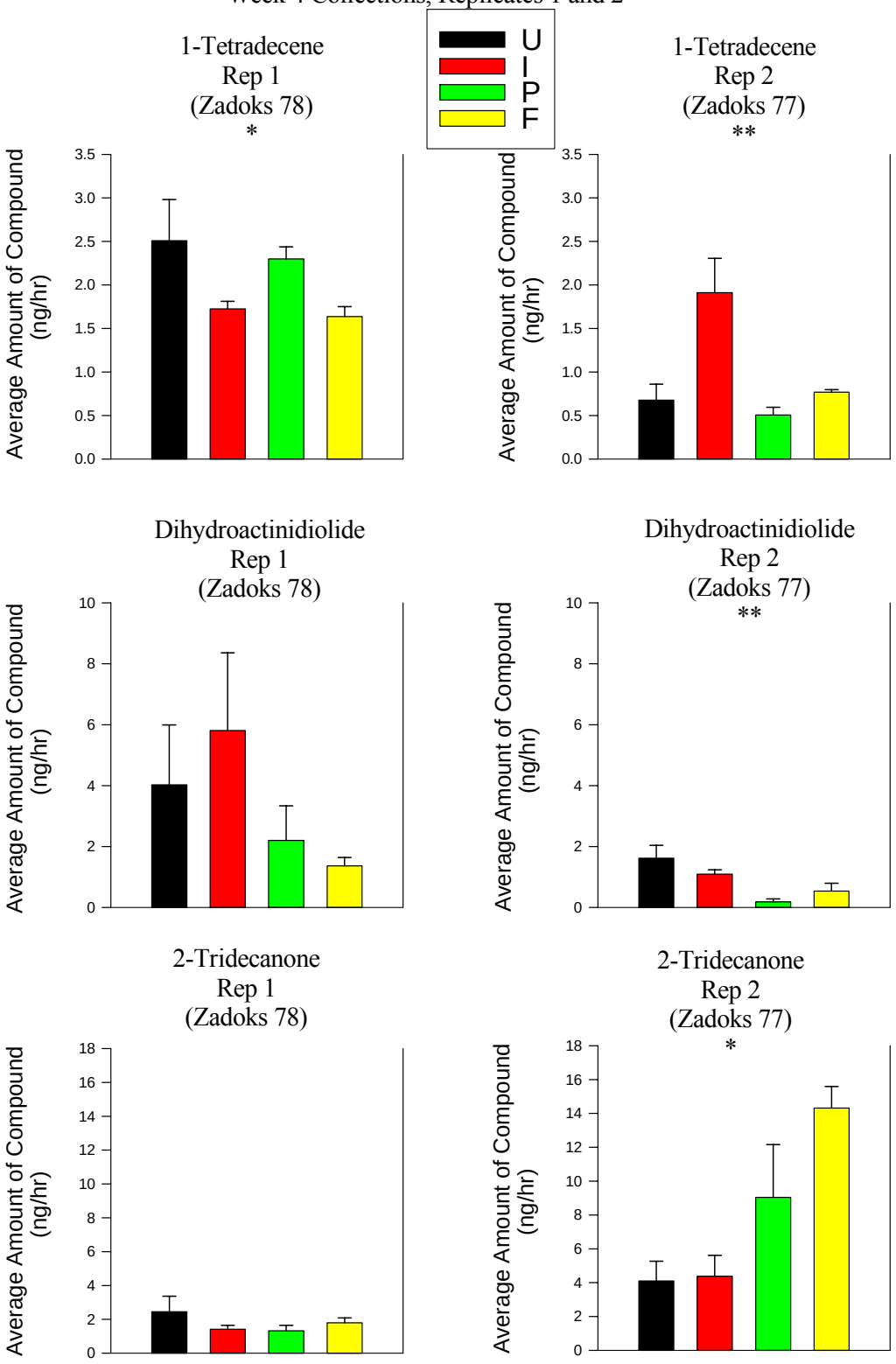


Figure 26. 2-Pentadecanone, Hexahydrofarnesyl Acetone, and Phenylethanal: Weeks 4 and 6 Collections, Replicates 1 and 2

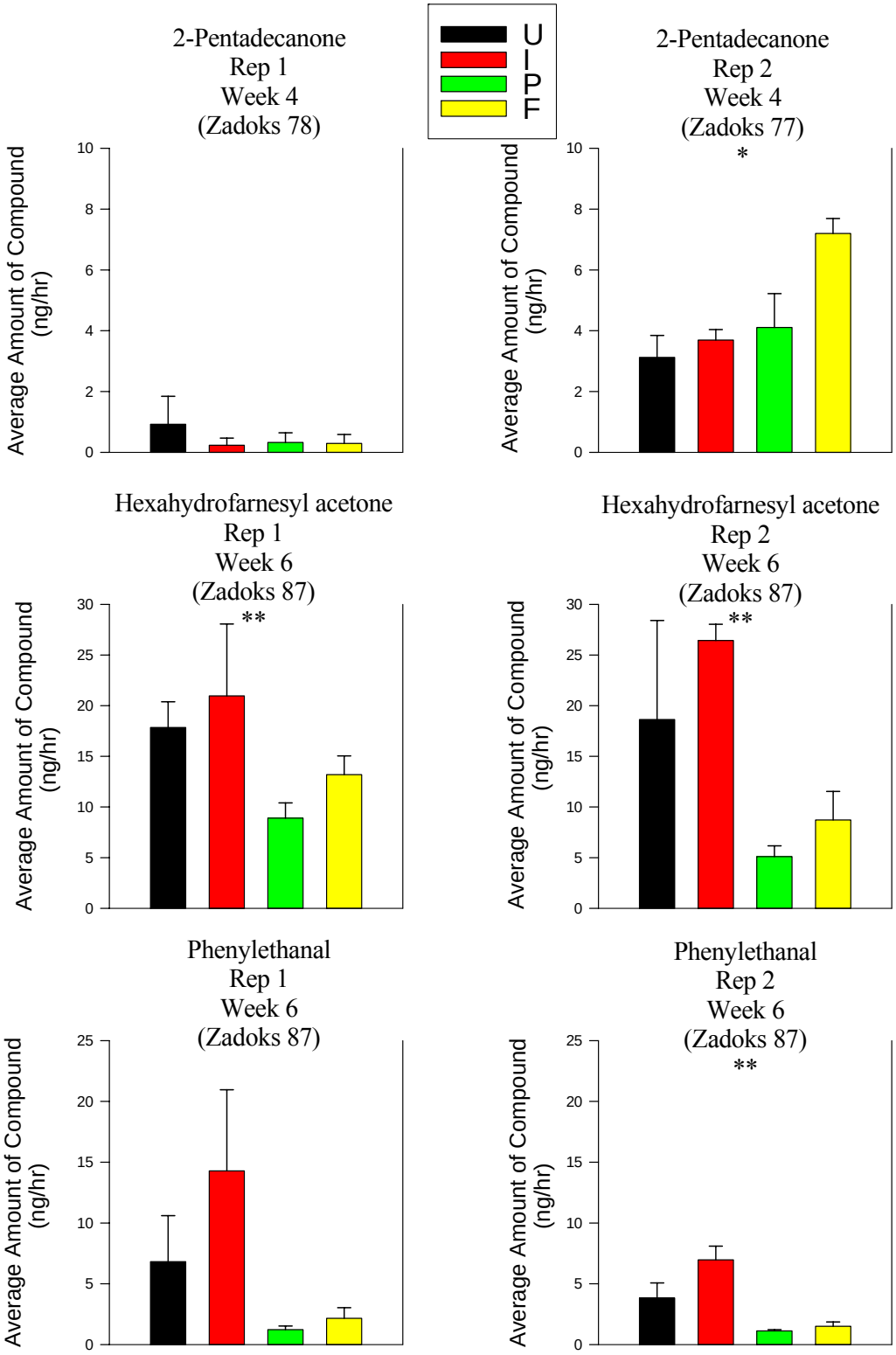
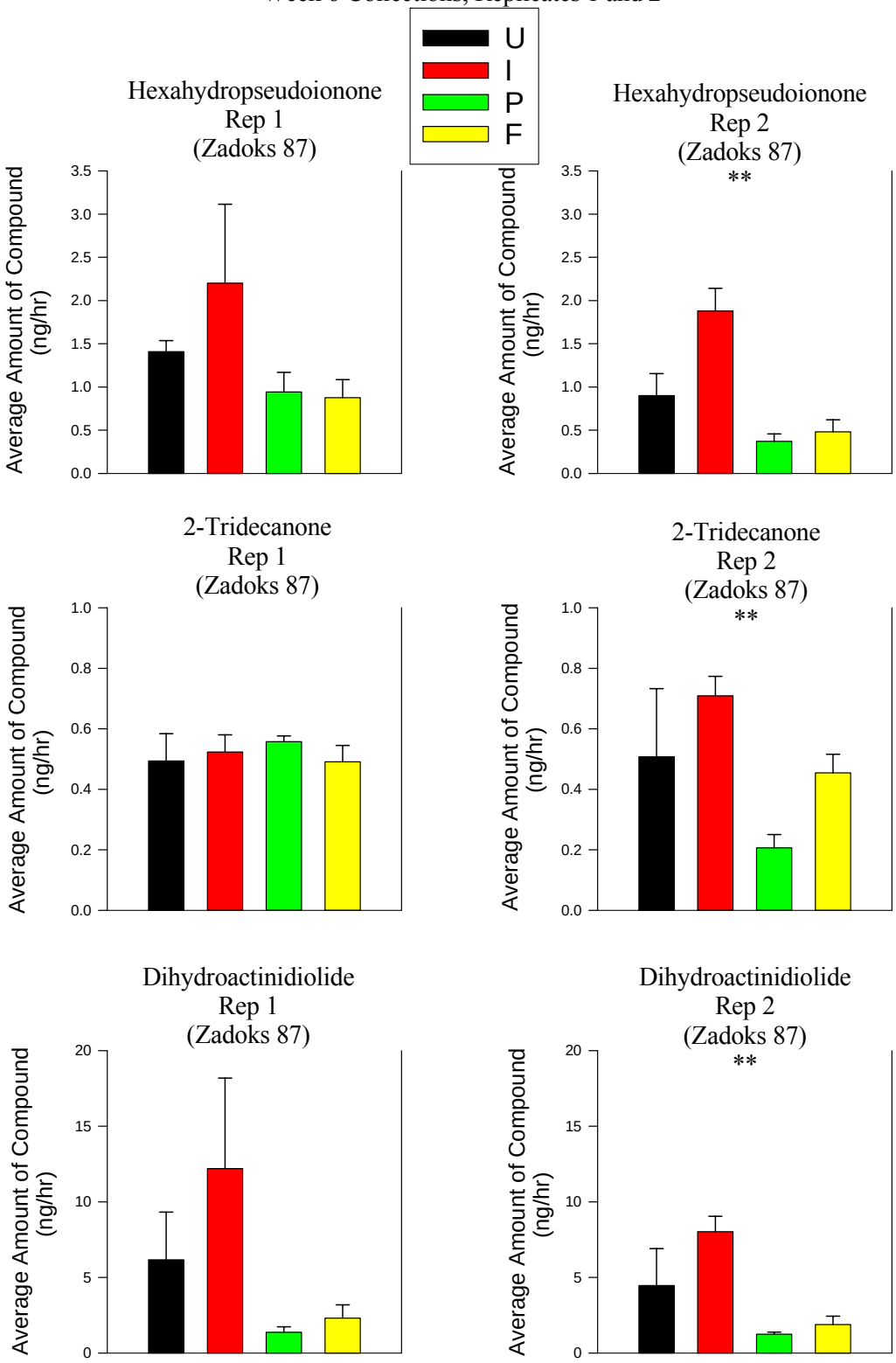
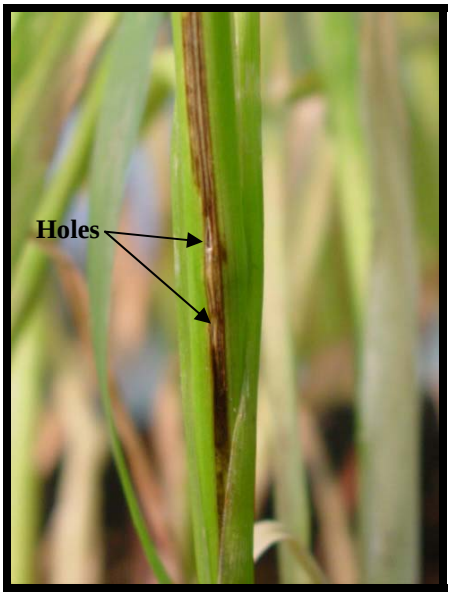


Figure 27. Hexahydropseudoionone, 2-Tridecanone, and Dihydroactinidiolide: Week 6 Collections, Replicates 1 and 2



In Figure 21, the level of hexahydrofarnesyl acetone in week 2, replicate 2 is very similar between the infested plants and the pin-pricked plants. In Figure 22, hexahydropseudoionone* and dihydroactinidiolide*, replicate 2, and Figure 23, hexahydrofarnesyl acetone and hexahydropseudoionone*, replicate 2, are several more cases where the chemical levels are similar between the infested plants and the pin-pricked plants. The overall trends are usually similar between the replicates, although the level of significance of their treatments varies considerably. It is likely that the discrepancies between the two replicates are due in part to damage caused by thrips, which was more extensive for plants in replicate 1 than in replicate 2. In addition to the volatile chemical production responses associated with the pin-pricked plants, there was also an apparent hypersensitive response in the plants that could be seen in the form of necrosing tissue surrounding the pin-pricked/Exacto© blade holes (See Figure 28).

Figure 28. Hypersensitive Response of Wheat to Sawfly Cuticular Wax



DISCUSSION

Infested vs. Uninfested Experiment

The results of the experiment involving only the infested and uninfested plants show no novel compounds produced by the plants infested by wheat stem sawflies; however, there are significant differences in the average quantities of certain chemicals between the two treatments. Although the identity and number of these compounds varies over time, for each collection there are significant differences in volatile production between the treatments. The entire list of compounds whose average amounts had a statistically significant difference between the treatments in at least one collection is 1-octen-3-ol, 2-undecanone, tridecane, 2-tridecanone, 2-pentadecanone*, hexahydrofarnesyl acetone, benzyl alcohol, pentadecanal, hexahydropseudoionone*, vanillin, 6-methyl-2-heptanone*, geranyl acetone, 4-oxoisophorone, dihydroactinidiolide*, 6-methyl-5-heptene-2-one, 1-tetradecene, 2,6-dimethyl-2,6-octadiene*, an unknown compound, phenylethanal, and 1-hexanol. The biological activity of most of these compounds has already been well established by other researchers. Many previous studies for several different insect species have found 1-octen-3-ol to have biological activity, often as an attractant for haematophagous insect species (Ingvarsdottir et al., 2002; Omura et al., 2002; Sant'Ana et al., 2002; Nojima et al., 2003; Rueda and Gardner, 2003; Barrozo and Lazzari, 2004; Syed and Guerin, 2004); though this kind of an attraction does not fit very contextually into the situation with sawflies, it is notable that 1-octen-3-ol spans so many different orders of insects in its known biological activity. The 3 ketones 2-tridecanone, 2-undecanone, and 2-

pentadecanone* have all been shown to have insecticidal properties (Williams et al., 1980; Antonius, 2001). The compounds 1-octen-3-ol and 2-tridecanone were previously found by Birkett et al. (2004) to be present in the panicles of wheat. 2-undecanone and 2-pentadecanone* were previously found in wheat by Buttery et al. (1985) and Cervantes et al. (2002), respectively. I could not find any reference to tridecane being associated with wheat in the literature, other than in cases where mites were infesting stored wheat (Curtis et al., 1981); it may be that tridecane was present due to the presence of predatory mites used to control thrips in the greenhouse, or it may simply have not yet been reported in the literature as a wheat volatile. Vanillin has not been associated with living wheat plants, but has been found in wheat flour by Czerny et al. (2002). The compound 6-methyl-5-hepten-2-one is an induced volatile of wheat caused by the infestation of *Rhopalosiphum padi* L. (Homoptera: Aphididae) (Quiroz et al., 1997), and could therefore be induced by other insect infestations as well. Hamilton-Kemp and Andersen (1984) identified 1-hexanol as one of the volatile chemicals found in wheat. Of the remaining 12 compounds, I could find only dihydroactinidiolide* (Kato et al., 2002, 2003) and pentadecanal (Hamilton-Kemp and Andersen, 1986) reported in the literature as being associated with wheat. The studies by Kato et al. (2002, 2003) found that dihydroactinidiolide* had great activity as a germination inhibitor in wheat seeds. Dihydroactinidiolide* has also been reported as a component in the venom gland secretions of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Rocca et al., 1983), which leads me to think that it might have biological significance in other insect systems as well. Dihydroactinidiolide*, hexahydrofarnesyl

acetone, and 4-oxoisophorone were all listed by Schulz et al. (1993) as being found in the secretions of scent organs of some species of African butterflies.

Although not all of the 20 compounds have been previously reported from wheat, all of them have been reported as chemical components of other plant species. Hexahydrofarnesyl acetone was found in the fern *Elaphoglossum spathulatum* (Bory) Moore (Lomariopsidaceae) by Socolsky et al. (2003), in *Gingko biloba* L. (Gingkoaceae) by Cheng-zhang et al. (2000), and in the primrose *Lysimachia microcarpa* Handel-Mazzetti ex C.Y. Wu, (Primulaceae) by Hui et al. (1993). Hexahydropseudoionone* has been identified in the essential oils of the flowering tree *Goniothalamus malayanus* Hook. f. and Thomson (Annonaceae) (bin Jantan and bin Ahmad, 2002). 4-oxoisophorone was found in field mustard (*Brassica rapa* L.; Brassicaceae) by Doughty et al. (1996). Benzyl alcohol was found in several species of tobacco plants by Raguso et al. (2003), and benzyl alcohol and phenylethanal were both found in alfalfa by Tava et al. (2000). Zhu et al. (1984) found 6-methyl-2-heptanone* in the headspace volatiles of Arabian jasmine (*Jasminum sambac* L. Aiton; Oleaceae). Geranyl acetone was found in oats (*Avena sativa* L.; Gramineae) – another plant species that sawflies oviposit in – by Buttery et al. (1982). El-Kayati et al. (1998) identified 1-tetradecene in the volatiles of peanuts. Rose flowers contain the compound 2,6-dimethyl-2,6-octadiene* (Knapp et al., 1998). From looking at the structural information obtained from the mass spectrogram of the unknown compound, it is most likely a furan-based compound – furan-related compounds are found in a multitude of plant species and some have even been associated with wheat straw (Klinke et al., 2002). Associating these compounds with wheat does

not appear to be unfounded, just based on the fact that the literature shows their association with plant systems is already in evidence.

Plant-wounding Experiment

The graphs chosen to display results from this experiment were selected based on the statistical analysis indicating that there were significant differences in volatile production between some of the treatments. Cases where the treatments did not show significance – while indicating that the artificial manipulations were similar to the other treatments – were not included because this lack of significance also indicated that no real difference in volatile production existed between the infested and uninfested plants.

There were 2 compounds added to the plant-wounding experiment which were not found to be statistically significant in the 1st experiment: cis-3-hexenyl acetate and β-ocimene*; these 2 compounds were analyzed in the 2nd experiment because the compounds are known to be present in greater amounts when plants are younger (personal observations), and their biological significance has been established (Turlings et al., 1998; Halitschke et al., 2000). Both the studies of Turlings et al. (1998) and Halitschke et al. (2000) make particular reference to the fact that β-ocimene* and cis-3-hexenyl acetate are induced shortly after plant wounding. These compounds were included to help elucidate any differences or similarities between the treatments and the infested controls in the early collection periods. Cis-3-hexenyl acetate does show significance in the first collection period (Figure 20), likely because this collection

immediately followed the plant wounding caused by sawfly oviposition and the pin-pricked/wax treatment; this result further justifies the insertion of these two compounds.

CONCLUSIONS

Infested vs. Uninfested Experiment

My first hypothesis may be partially accepted, since the main conclusion that may be drawn from the 1st experiment is that sawfly infestation induces changes in volatile production in wheat, but these changes are quantitative, not qualitative. A further interpretation of this main conclusion might be that sawfly infestation increases the rate of senescence and decreases the length of life of the wheat plant; this can be evidenced by examining the identities of the volatile compounds which differ significantly between infested and uninfested plants over their respective lifetimes. No novel compounds were seen in the experiment, but quantitative differences were able to be observed from the data. The case where quantitative changes in volatile production are induced by insect herbivory is mentioned briefly by Dicke and van Loon (2000). The decrease in lifespan of the wheat plant is no surprise to anyone studying wheat stem sawflies, since the larva often cuts a wheat plant – effectively killing that stem – before the plant dries down naturally. The volatiles in some cases, however, support the idea that this "increased ripening (senescence)" or "harvesting" by the sawfly might be an ongoing process during the life of the plant infested by a sawfly larva. Some of the compounds which are increased on average in the infested plants in a given week are compounds which we find increased in the uninfested plants a week or two later, or are simply known to be associated with ripening. Hexahydrofarnesyl acetone levels in week 3 of the 1st experiment are higher for in the infested plants, but this difference reverses for week 6. Hexahydrofarnesyl acetone appeared in the greatest amounts for either infested or

uninfested plants in the latter weeks of infestation, and could therefore be considered a ripening compound. Dihydroactinidiolide was reported as a ripening compound in wheat by Kato et al. (2002, 2003), and its average level in week 5 was significantly higher in the infested plants than the controls. Had more collections been made with shorter intervals between collections, and had fewer greenhouse pest problems occurred, it may have been possible to better elucidate the changes which were taking place between infested plants and uninfested plants over time; nevertheless, the results obtained are a valuable starting point to understanding possible mechanisms whereby parasitoids of the wheat stem sawfly can differentiate between infested and uninfested wheat plants, as well as mechanisms which might repel ovipositing sawfly females. A possible mechanism by which parasitoids could differentiate between infested and uninfested plants is explained by the data from this experiment. Differences in rate of senescence and also volatile quantities between infested and uninfested plants are present during the 2 periods when parasitoids attack the sawfly larvae in the stems. This fact is important since the wheat plant has almost fully senesced at the time (harvest) when the 2nd generation of parasitoids attacks the sawfly, and it is therefore unlikely that the plant will invest resources to produce a novel compound which might attract the parasitoids. While this study does not find any novel induced compounds associated with the insect damage as much of the literature suggests should be the case, this system and study are unique and bear further scrutiny. In most of the insect-plant systems studied by other researchers, the insect does damage which may result in the death of the plant before it can reproduce. Sawfly damage to wheat has never been shown to kill a wheat plant before reproduction takes place; therefore, there is little selection pressure on wheat to have the ability to

produce a novel compound that negatively affects the sawfly. However, while most induced-volatile studies involve insects causing plant damage in the open where their presence can be seen and their odors are readily available to the environment, the wheat stem sawflies are enclosed in the stem and do minimal damage, and yet their parasitoids are able to detect them amongst thousands of stems, so it is very likely that there is some type of external chemical cue that the parasitoids can detect. One further difference between this study and that of other volatile collection research is that these collections were for longer periods of time than most researchers use. It is possible that the compounds suggested to be novel are actually present in the volatiles of the undamaged plants being collected from, and that the only novel property of these compounds is their increased production. Further research in this area should incorporate the compounds that are statistically significant in this experiment into behavioral studies (e.g. olfaction and oviposition studies), and GC-electroantennogram detection (GC-EAD) studies.

Plant-wounding Experiment

The plant-wounding experiments revealed that, of the pin-pricked and frass-treated treatments, the pin-pricked treatment more often had similar average chemical amounts compared to the infested controls; however, neither treatment was very good at mimicking the volatile production response of the infested plants to sawfly infestation. In regards to my second hypothesis, I can partially accept it, but not fully. It does appear that the pin-pricked/wax treatment is mimicking some of the effects that sawfly infestation has on the volatile chemistry of wheat, but the frass-water injection doesn't

seem to be causing a plant response similar to that of infestation. More research into the combination of both the frass-water injection and the pin-pricked/wax treatment is needed, because both cuticular wax chemicals and frass chemicals are present when sawflies infest a wheat stem. The response of the wheat plant to the cuticular wax of sawflies is notable, and merits further study; this response is a good example of the hypersensitive response mentioned by Baldwin and Preston (1999) and Hilker and Meiners (2002). The necrotic tissue seen on the plant in Figure 28 shows clearly that the plant is very responsive to the wax, even to the point that tissue is necrosing which was never contacted by the wax; only the tissue which can be seen as white in the picture surrounds the pin/blade holes and had actual contact with the cuticular wax of the sawfly. It is likely that if this response had accompanied an actual oviposition event by a wheat stem sawfly, the eggs deposited inside the stem would likely have not survived due to the death of the tissue on which they were laid. Genetically manipulating wheat or selectively breeding it to enhance this response could lead to more sawfly-resistant and possible even sawfly-repellant strains of wheat.

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