Evaluating the interaction between buckwheat, *Fagopyrum esculentum* (Polygonaceae) and wireworm *Agriotes sputator* L. (Coleoptera: Elateridae)

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Abstract

Laboratory experiments were carried out to determine if buckwheat, *Fagopyrum esculentum*, produces phytochemicals that act as deterrents, anti-feedants, or toxins against *Agriotes sputator* wireworms. Choice assays were conducted to test the attractiveness of germinating, branching and flowering buckwheat, red spring wheat (*Triticum* spp) and island barley (*Hordeum vulgare*) to the larvae. There was no evidence that the wireworms were deterred by buckwheat and the germinating stage of all three crops was the most attractive. Twenty-one day, no choice feeding assays were conducted to determine change in mass and mortality of *A. sputator* larvae when fed buckwheat or barley; no differences between hosts were observed. However, while wireworm herbivory significantly reduced the growth of barley, it did not affect buckwheat, suggesting that this species may produce anti-feedants. Longer feeding assays and field trials are required to confirm this possibility.

Keywords: wireworms, *Agriotes sputator*, buckwheat, *Fagopyrum esculentum*, rotation crop, anti-herbivore, repellence, anti-feedant activity, choice test, six-arm underground olfactometer, no choice test, root feeding assay.
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Chapter 1

1. Background & Introduction

1.1 Resource Acquisition

Organisms must acquire essential resources such as food and mates at different stages of development, and their success at doing so will determine if they are to pass on their genes to the next generation. The efficiency with which resources are acquired will depend on the individual’s traits, such as specialized appendages or structures, and the ability to detect and respond to cues and signals in the environment under a range of biotic (such as intra and interspecific competition) and abiotic (such as temperature, moisture) conditions. Furthermore, in many species different stages in the life cycle live in markedly different habitats (tadpole and frog, mosquito larva and mosquito adult) and thus will have stage-specific adaptations and cues for effective resource acquisition.

Focusing on nutritional resource acquisition in animals, it is evident that the relative importance of the different senses (olfaction, tactile, visual, auditory, and gustation) used when foraging will depend on a variety of factors, including the life stage, daily activity patterns and habitat in which the organism lives. For example, as noted above, species that go through metamorphosis may live in markedly different habitats at different stages in their life cycle, such as tadpoles and frogs. In other cases the same life stage of different species may live in the same habitat and have similar feeding habits although one is diurnal and the other nocturnal (e.g hawks and owls preying on small mammals). Similarly, while aquatic organisms by definition live in water, their habitats may vary from stagnant water, fresh water streams, to marine environments. Thus, a good understanding of these parameters is required when investigating the senses and cues used when foraging for food.

These principles certainly hold for insects. For example, bees are terrestrial and diurnal, and so they can use vision to detect flower color contrast, olfaction to detect chemical gradients, as well as taste and touch contact to assess plant quality (1, 2). In contrast, for soil dwelling insects such as termites, wireworms, or grubs, visual cues are of minor importance and foraging behaviours are
more dependent upon chemical, moisture, and temperature gradients (3). Even though chemicals are used in both cases discussed, the manner in which cues move through the different media differs considerably.

1.2 Soil Dwelling Insect Herbivory

Soil dwelling insect species have different feeding habits and even within a species these may vary between developmental stages. As previously mentioned, navigation towards a food source is heavily dependent upon olfactory cues but this process will also be affected by soil properties, such as particle size, soil moisture, and temperature (4). In herbivorous insects, the first step in foraging for host plants is a response to carbon dioxide (CO₂), whereby the insects move up the concentration gradient until they find more specific infochemicals that indicate the host plant quality/acceptability (4). Once the potential plant host is located insects accept or reject the root system depending on the presence of feeding stimulants or repellants (4).

1.3 Plant Defensive Mechanisms

Plants have evolved a variety of means to defend themselves against herbivory or attack from other organisms. These may be constitutive (always present) or induced (produced in response to the appropriate stress). Structural features such the thickness, fiber content and waxiness of leaves, and the presence of spines, prickles, thorns, and trichomes serve to deter feeding and oviposition, as may the presence of secondary metabolites, often phenols, including flavonoids and tannins (5). Plants also use defensive proteins such as toxic lectins, proteinase inhibitors that reduce protein digestibility, and reactive oxygen species (ROS) inducing peroxidases, polyphenol oxidases and lipoxygenases that cause gut lesions (5, 6, 7).

In all cases, defences may be constitutive and/or induced in response to attack. For example, following defoliation, the trichome density on new leaves of *Rhapanus raphanistrum* and *R. sativus*, are significantly higher than on control plants (8). Similarly, while *Nicotiana attenuata* plants always contain low levels of nicotine, higher levels are observed following herbivory (9).
The production of induced defences is a temporal cascade, initiated by cues from both the herbivore and the plant, including phytohormones such as jasmonic acid, salicylic acid, and ethylene (5, 10). Phytohormones are involved in the regulation of a wide variety of genes responsible for growth and reproduction of a plant. They are also involved in the upregulation of metabolic genes, in order to increase the biosynthesis of secondary metabolites (such as alkaloids, phenylpropanoids, and terpenoids) which are essential for anti-herbivory effects (10, 11). Since phytohormones co-regulate many genes in a biosynthetic pathway, changes may within a few hours (10).

The defences mentioned above are generally classified as direct defences, although, plants may also benefit from indirect defences. For example, herbivore induced plant volatiles (HIPV) emitted following defoliation may not only cause the upregulation of defenses in other parts of the same plant, but also in either conspecific or heterospecific neighbouring plants (5, 12, 13). Furthermore, there are now many examples in the literature showing that volatiles from damaged plants are used by the herbivore’s natural enemies as a reliable cue when searching for suitable food/oviposition sites (5, 14, 15). For example, the production and exudation of the volatile (E)-β-caryophyllene in Zea mays in response to root damage by larvae of the Western corn rootworm, Diabrotica virgifera virgifera, attracts the entomopathogenic nematode Heterorhabditis megidis (14). Similarly, as a response to the compound volicitin found in the regurgitant of Spodoptera exigua larvae, maize releases volatiles which attract the parasitoid wasp Microplitis croceipes (16).

Plants can also protect themselves against herbivores by engaging in compensatory growth (17). As herbivores feed, the growth of energy acquiring structures (foliage, and roots) increases, leading to the plant obtaining the nutrients necessary for regenerating the biomass lost (18). As this occurs, nutrient stores may also be strategically displaced to areas of the plant not accessible to herbivores, but returned once herbivory has declined and the biomass has been restored (17, 18). Plants will deploy the strategy that best accommodates their energetic needs and provides the best defense outcomes for the least cost. Consequently, defensive compound production and compensatory growth are usually on opposing sides of a trade-off (18), where defensive compounds are effective at lessening herbivore damage in a relatively quick time period. However, in cases of prolonged herbivory, it is not energetically favorable to produce defensive compounds,
and a plant could shift from defensive compound production to nutrient stores/energy retention, and increased nutrient acquisition/energy conversion while it waits out the herbivory attack (18).

Clearly natural defensive mechanisms can make some plants less susceptible to damage by certain herbivores, which opens the door to the use of plant defenses in non-insecticidal pest management (see below).

1.4 Wireworms

Wireworms, the soil dwelling larvae of click beetles (Coleoptera: Elateridae) (Fig. 1), may be saprophagous, carnivorous, herbivorous, or a combination of these (19, 20). In North America, females oviposit in late May to early June and the eggs hatch 4-6 weeks later (21). Larval development may take 2-5 years, passing through 8-13 instars, depending on the species (21), and it is during this period that they are considered pests due to the damage caused by feeding on the root systems of agricultural crops (20, 21). At the end of larval development wireworm pupate in early fall, with the resulting adults hibernating in the soil over winter and emerging the following spring (21).

Figure 1 Agriotes sputator larva.
There are about one-hundred economically important Holarctic wireworm species (20), of which 30 occur in Canada and cause reduced crop yield and consequent economic losses in potato, maize, strawberry and cereal crops (22). However, three exotic species, *Agriotes obscurus* L., *A. lineatus* L., and *A. sputator* L. introduced from Europe in the 1850s, are the most pestiferous where they have established (20). These are responsible for annual losses of 6 million dollars in potatoes alone (23).

1.5 Wireworm Control

1.5.1 Insecticides

The application of the organochlorines Aldrin and Lindane, prior to seeding, was the primary control means against wireworms as their long residual activity made them suitable for treating the long lasting larval development stages (20). However, because of their adverse environmental effects, they were deregistered in the 1990s in North America (21, 24). Subsequently, organophosphates and carbamates were used but caused lower larval mortality and, therefore, were less reliable for pest control (20, 21, 24). The inconsistent performance of these insecticides together with their high toxicity to humans (20) resulted in them being withdrawn as an acceptable control option. More recently, pyrethroids, neonicotinoids, and phenyl pyrazoles have been used for wireworm control in affected regions (20, 21, 24) and although none are very effective they provide some protection against crop damage, generally by acting as feeding deterrents (20, 24). It is clear that at present pesticides are not particularly effective as a means of wireworm control and their use does cause significant ecological problems; consequently alternative means are needed to reduce the impact of wireworms.

1.5.2 Cultural Control Approaches

The need to move away from a reliance on synthetic insecticides for the control of insects has resulted in an approach called integrated pest management (IPM), where different methods are used in combination to provide economically, socially and environmentally acceptable control
One option could be the use of cultural practices that may render the habitat unsuitable for wireworm. For example, as wireworm densities increase proportionally to the number of years that a field has been used to grow grasses, pasture, and cereals (20, 21), leaving a field fallow for 3 to 4 years effectively eliminates the populations (21, 26). However, leaving a field empty for such a long time is neither practical nor economically feasible for farmers. Disruption of the soil (cultivation) by tilling, diskng, or ploughing, has also been considered, as this can injure different developmental stages of the insect, as well as expose them to desiccation and predation (20, 21). However, for this technique to be effective the soil must be worked when most wireworm are located in the top 10 cm, but wireworms may descend as far as 30 cm below the surface in response to soil moisture and temperature, limiting the usefulness of this approach (20, 27, 28).

Field flooding can also reduce wireworm densities but the efficacy of this approach is affected by the temperature and salinity of the soil. Furthermore, flooding must occur for extended periods during the summer growing months, limiting the attractiveness of this control method (29).

Crop rotation, alternating between growing crops unfavorable to wireworm populations with susceptible crops, could also be a means of reducing losses of yield due to wireworms (20, 30, 31). The unfavourable plants may affect wireworms by the production of phytochemicals from the roots that are repellent, act as anti-feedants, or may lack phagostimulant properties. For example, crucifers are not generally attacked by wireworms, in part due to the production of glucosinolates. These hydrolyze into toxic feeding deterrents in the soil and adding these to the substrate proved effective against one wireworm species under laboratory conditions (20, 32, 33). However, the quantities required to mitigate losses under field conditions makes this approach economically unfeasible (20).

Trap crops, which are more attractive to the wireworm than the cash crop, have also been explored for wireworm control (34, 35). However, this method only provides short term protection as it does not decrease wireworm densities.

Biological control, using parasitoids, predators or pathogens, is another component of IPM. However, wireworms appear to have very few natural enemies that could be reliably used within an IPM program (20). Given that most options considered as alternates to insecticides have not
proved particularly effective and wireworm densities continue to increase, additional research is needed if farmers are to have a long-term, reliable program for wireworm control.

1.6 Field Observations during Crop Rotation Trials

Preliminary field studies in Nova Scotia, using buckwheat, mustard, or flax seed in rotation with potato and carrot, found that all three rotation crops resulted in a reduction of wireworm populations. However, the subsequent crop yield in fields where mustard or flax seed had been used was significantly lower that when buckwheat was used as the rotation crop (31). Buckwheat, *Fagopyrum esculentum* (Polygonaceae), is a valuable plant world-wide as it not only has medicinal properties due to its high antioxidant and flavonoid content (36) but because the grain can be stored for long periods of time without significant reduction in its nutritional value (37). Buckwheat has known allelopathic effects against other plant species (36, 38) and releases flavonoids and phenols into the rhizosphere (36, 37). These compounds, such as eugenol, isoeugenol and methyleugenol may impair larval development of various soil dwelling herbivores species (39, 40, 41). Additionally, the plant has been used as a cover crop to control scarab beetle *Melolontha* spp. grubs in Scots pine stands in Poland (42).

1.7 The Buckwheat Project

Given the above-mentioned field observations a research project was initiated by Agriculture & Agri-Food Canada (AAFC) to investigate (i) the impact of buckwheat on soil chemistry, (ii) interactions between buckwheat and soil microbiota, and (iii) the effect of buckwheat on wireworms. My contribution to this project was to determine if allelopathy is responsible for the reduction in wireworm populations when buckwheat was used as rotation crop.

My hypothesis is that phytochemicals exuded by the buckwheat root system interfere with normal wireworm foraging and/or feeding behavior. This may result from the phytochemicals acting as repellents so the insects avoid the roots, as anti-feedants that reduce feeding, and/or as toxins when ingested. My specific objectives were (i) to determine if buckwheat produced compounds that are
repellant to wireworms, and (ii) to determine how the interaction with the buckwheat root system affects wireworm viability. The presence of a repellent would result in the wireworms not moving toward buckwheat roots, while a deterrent effect would result in wireworms moving toward the roots but subsequently not feeding. If neither repellent nor deterrent activity is observed, then a decline in viability may occur due to toxins ingested during larval feeding resulting in either increased mortality or morbidity.

This research will not only broaden our understanding of wireworm foraging behavior but could also provide valuable information that will be useful in the development of a sustainable, non-insecticide alternative, for the control of wireworms.
1.8 References


Chapter 2

2. Examining the Repellent Effect of Buckwheat on Wireworms

2.1 Introduction

The behaviours required for efficient resource acquisition by organisms have been selected for, and fine-tuned over evolutionary time as adaptations for survival in their environment. As mentioned in chapter 1, how an organism detects environmental cues will depend on the medium in which resource acquisition takes place and the traits of the organism that allow it to detect these cues. Consequently, above-ground organisms are able to rely on most, if not all, of their senses and therefore are able to detect a wider variety of cues to locate sources of nutrition. In contrast, soil-dwelling organisms live in an environment where certain senses, such as vision, are of little use; other senses, such as olfaction, are more developed due to the heavy reliance on chemical cues for navigation towards food sources (1).

Soil-dwelling insects generally follow a common pattern in their foraging behaviour. Initially, they move randomly through the soil until they encounter cues indicating the presence of a potential food source. In herbivorous species, organisms may initially respond to increased levels of carbon dioxide (CO₂), as this could originate from a potential host root system. They navigate up the CO₂ concentration gradient until they encounter more specific plant exudates, which provide more precise chemical information on the suitability of the plants as a food source. In the absence of suitable cues oriented behaviour will stop, otherwise navigation towards the root system continues. Acceptance and active feeding will occur if the plant contains the appropriate phagostimulants, or the plant will be rejected if it is an unsuitable host (2, 3, 4). However, both the emission of, and response to, cues affecting foraging behaviours may be modified by prevailing abiotic conditions, (e.g. soil temperature and humidity, soil particle size) (2, 5). Similarly, foraging behaviour may be affected by biotic factors including the developmental stage of the herbivore, the phenology of the plant and the presence of other herbivores and/or microbes (1).

Wireworms generally aggregate in patches and tend to move longitudinally in the soil column in response to changes in soil moisture and temperate, while travelling laterally in response to chemical attractants (3, 4, 5, 7). Their locomotion and survival is greatly affected by soil moisture,
as the larvae are immobilized in very saturated soil and are unable to feed. On the other hand, wireworms are highly susceptible to desiccation in soil volumetric water content below 10% (3). Wireworms in soils of smaller particle size are able to travel faster due to the decreased friction and lack of barriers (5, 6). Larvae in the genus *Agriotes* exhibit the general pattern of foraging behaviour mentioned above. For example, their strong affinity to CO₂ results in greater attraction to geminating plants, especially grains, pasture, and wild grass (7, 8, 9) but a moulting individual exhibits a lower response to cues than a non-moulting individual (10). *Agriotes* larvae use a wide range of volatile and contact semiochemical cues to locate and initiate feeding on hosts, including some volatile organic compounds (VOCs), as well as certain amino acids, carboxylic acids and sugars, all of which may vary as a function of plant phenology (4, 11). In contrast, phenols and flavonoids generally deter soil dwelling insect herbivores (4, 12).

MacKenzie, et al. (2010), reported that following the use of buckwheat, *Fagopyrum esculentum*, as a rotation crop, populations of wireworm decrease significantly in Nova Scotian potato and carrot fields (13). Buckwheat root exudates contain both phenols and flavonoids that increase in concentration with plant age, and have a negative allelopathic effect against other plant species (14, 15). It is hypothesized that buckwheat produces a repellent that causes *A. sputator* wireworms to avoid the plants. In this chapter, wireworm foraging behaviour is examined in a six-arm underground olfactometer used for other soil insects (16) as a function of both type (buckwheat, barley *Hordeum vulgare* (var. Island), and wheat *Triticum* spp. (var. Red Spring) and phenological stage (germinating, branching and flowering) of the plants.

### 2.2 Materials and Methods

#### 2.2.1 Insects and Soil

Soil from the AAFC Harrington Research Farm in Charlottetown, Prince Edward Island (PEI) was used to grow plants and for the bioassays at the AAFC research center in London ON. This was to ensure that the experimental conditions reflected those where the use of buckwheat as a rotation crop resulted in a decline in wireworm densities. Soil in this area is classified as fine, loamy, sand and has been subjected to barley/soy or barley/potato rotations without insecticide or fertilizer
treatment for 5 years. The soil was filtered through 2 cm and 1 mm sieves to extract debris and most macro-organisms, and then was stored in sealed plastic bags at 4°C until needed. The *A. sputator* wireworms collected at AAFC Harrington were stored at 15 °C in plastic containers filled with moist PEI soil and potato slices added as a food source every 3 weeks. To identify the wireworms, all specimens were examined with an Olympus SZX16 Stereomicroscope (x100 magnification, Lumenera Infinity 2 microscope camera, and Lumenera Analyze Imaging program version 6.1). Only the *A. sputator* larvae were found, and they could be identified by the characteristic brownish juxtaposed plates on the sternum between the coxae (17). Wireworms may spend a substantial amount of time in a fasting state (8), so when required for assays larvae were moved from the holding conditions to containers at 20°C with a feeding bait composed of 11 ml wheat seed and 11 ml of corn seed layered in 50 ml of moist, medium grade Vermiculite (18) placed in the centre. This was changed every 4-5 days, and over a two week period all wireworms observed feeding were collected and were separated into early (1st-3rd), mid- (4th-6th), and late- (7th-8th) instars based on the number of thoracic spiracle teeth with a Nikon SMZ25 Stereomicroscope (x300 magnification, Nikon DS-Ri2 microscope camera, NIS Elements Stereo Imaging Software version 4.40) (19); only late-instar larvae were available for the olfactometer assays since only 4 mid-instar, and no early instar larvae were found in this specific shipment of wireworms.

2.2.2 Plants

Buckwheat and the positive controls (barley and wheat) were germinated for 2 days in a growth cabinet (22±2°C, 16L: 8D, and 40-60% humidity). The germinated seeds were then transplanted and grown in 10 cm diameter pots filled with PEI soil with 20% volumetric water content, the optimal condition for wireworms (20) and microbial diversity (21) in a greenhouse under 16L: 8D, at 23-27°C, and 40-60% relative humidity.

2.2.3 Underground Olfactometer

Three six-arm underground olfactometers (16) (Fig. 2) were used to assay wireworm responses to various plant type/plant phenological stage combinations (see below) in a growth cabinet at 22±2°C, 16L: 8D, and 40-60% humidity. All the arms of the olfactometer were wrapped in aluminum foil to simulate the darkness below ground, while the mid chamber and the end pots
remained exposed to light. The mid chamber had a parafilm seal with small perforations to direct airflow from the arms to the middle. Three plants at the same growth stage, along with the soil they were grown in, were transplanted into each arm of the olfactometer 24 h before an assay was carried out. Preliminary trials determined that this number of plants was required in order to generate a response by the wireworms within 24 h. For any given plant species (buckwheat, barley, and wheat), two arms contained germinating seeds, two contained plants at branching stage (axillary buds grow into primary branches), and two contained plants at the flowering stage (blooming) (Fig. 3). Ten larvae were placed in the mid chamber and their positions were determined 24 h later. There were 10 replicates each for wheat, barley, and buckwheat.

Figure 2 Set up of the six-arm underground olfactometers used in wireworm foraging choice assays.
Figure 3 Schematic of the six-arm underground olfactometer assays ran to determine the relative preference of *Agriotes sputator* for germinating (G), branching (B), and flowering (F) plants for buckwheat (*Fagopyrum esculentum*), barley (*Hordeum vulgare*), and wheat (*Triticum spp.*).
2.2.4 Statistics

The data were modelled with a generalized linear model (GLM) of binomial distributions using the statistical analysis program R (version 3.2.5) and the tool Rstudio, (Appendix 1). Differences in the number of wireworms in each arm of the olfactometer were analyzed using a one-way ANOVA with chi square test after saturating the model (Appendix 2.1) and significant pairwise comparisons were identified with Tukey’s Post Hoc analyses (Appendix 2.2). The assumption of a saturated model was met, and over-dispersion was checked using a dispersion parameter for quasibinomial family; it was found to be negligible (Appendix 3).

2.3 Results

Plant phenological stage had a significant effect on larval movement [$\chi^2(2, N=90) = 8.97$, $P=0.01$], but plant type [$\chi^2 (2, N=90) = 0.37$, $P=0.83$] or plant type x plant stage interaction [$\chi^2 (4, N=90) = 4.56$, $P=0.34$] did not. A post hoc Tukey test showed that plants in the germinating stage were significantly more attractive to the larvae than those in the branching stage ($P=0.02$) (Fig. 4) However, for buckwheat and wheat, larvae showed no preference between germinating and flowering plants ($P=0.0629$), or between flowering and branching plants ($P=0.853$).
Figure 4 Preference of *Agriotes sputator* wireworms in the arms of a six-arm underground olfactometer. Plant phenological stage had a significant effect (*p*<0.05) on larval movement with plants in the germinating stage being more attractive to the larvae than those in the branching stage. •- Significant difference (*p* <0.05).

2.4 Discussion

The finding that the germinating stage is the most attractive to *A. sputator* larvae is consistent with findings for other wireworm species (8). The fact that the branching stage was less attractive, even though concentrations of certain volatiles, acids, and sugars increase with age (4, 11) suggests that CO₂, which is highest in the germinating stage (4, 8, 9), serves as the major longer distance chemical cue for foraging herbivorous wireworms. Interestingly, this significant difference in attraction of wireworms is not found when comparing germinating plants to flowering plants, or branching plants to flowering plants. This could potentially be due to insufficient of power of this experiment to detect small effect sizes. However, it can also be attributed to the delicate balance between CO₂, root volatiles, and other exuded compounds that can affect the attraction (or lack thereof) of herbivores to plant root systems (6, 8, 11). Other known wireworm attractants that occur at higher concentrations in older plants, such as hexanal, (E)-hex-2-enal, (E)-non-2-enal, and (E,Z)-nona-2,6-dienal, as well as certain acids and sugars (4, 11) would serve as cues when
the herbivore is in closer proximity to the potential host; especially as the olfactometer used is a closed system. There is a consistent pattern for greater attraction to germinating plants demonstrated by many insect herbivores, including wireworms, in part due to the nutritionally advantages of feeding on that plant tissue. Though the biomass in the roots of a germinating plant is of lower quantity than that of a mature plant, the cotyledons and seed from which the plant is germinating contain a higher concentration of nutritious stores that are used for the rapid growth of the plant (22).

In agroecosystems there is little variability in crop phenology and plant diversity is usually limited to one species. Thus, wireworms do not need to select for the most suitable species of host plant but rather have to feed on what is available. Earlier in the growth season they may be able to rely more on the long range CO₂ emissions exuded by young plant roots, and as the season progresses, they may have to rely more the short range attractants exuded by older plants to locate the preferred food.

Based on the results of a power analysis of the current data set (Appendix 4), it is clear that a larger number of replicates would have been preferable, but as we had to rely on field collected material, this was not possible in the time available for the project. However, the results clearly show, contrary to the initial hypothesis, that wireworm larvae are not repelled by buckwheat, regardless of the phenological stage tested. Therefore it is unlikely that the decline in wireworm populations observed in the field was due to larvae starving as a result of avoiding the buckwheat. Alternate explanations for the decline in wireworm populations are the absence of appropriate phagostimulants once the larvae have located the buckwheat plant, or that the roots contain toxic defence compounds. These possibilities were tested in the experiments described in the following chapter.
2.5 References

Chapter 3

3. Investigating the Direct Interaction between Buckwheat Roots and Wireworms

3.1 Introduction

Soil-dwelling insect herbivores initially use carbon dioxide (CO2) gradients as the first non-specific attractant to locate suitable host plants, but once in close proximity they use more specific semiochemicals to determine the location and suitability of the root system (1). Once physical contact has been made with the plant acceptance or rejection is determined by the phagostimulants and/or anti-feedants encountered (1, 2). It is evident from the results of the olfactometer assays presented in Chapter 2 that there are no differences in the response of A. sputator larvae to buckwheat when compared with other cereal crops. Therefore, the hypothesis that the observed decline in wireworm larvae following the use of buckwheat is due to the host plant producing chemical repellants is not supported. However, plants produce various defensive compounds in response to herbivory that may act as anti-feedants, or toxins (3, 4).

Wireworms of the genus Agriotes accept a wide variety of plant species as sources of food, responding to non-specific phagostimulants in the form of sugars, fats, and amino acids (2, 5). Only quinine, and allyl-iso-thiocyanate, have been found to cause wireworms to reject a food source but these are not produced by many plant species (6), and so it is unlikely that wireworm populations declined because they do not actually accept buckwheat as a food source. However, buckwheat does produce, in response to herbivory (7, 8), a suite of phenols and flavonoids that negatively affect a number of different coleopteran herbivores (9, 10).

Accordingly, a series of feeding assays were conducted to test the hypothesis that compounds in the buckwheat roots result in decreased feeding and eventually lead to wireworm starvation, or are toxic to larvae. Mid- and late-instar A. sputator larvae were provided either buckwheat, F. esculentum, or barley, H. vulgare, that were either germinating or in the flowering stage. It was predicted that (i) wireworm mortality would be higher while gain in body mass would be lower on buckwheat than on barley, (ii) mid-instar larvae would be affected more negatively than late instar
wireworms, and (iii) the effects would be greater on flowering than germinating buckwheat due to the presence of more defensive compounds.

3.2 Materials and Methods

3.2.1 Insects and Soil

As described in the previous chapter, soil and *A. sputator* wireworms were collected from the AAFC Harrington Research Farm in Charlottetown, PEI, and sent to the AAFC research center in London ON. The soil had been subjected to barley/soy or barley/potato rotations without insecticide or fertilizer treatment. The soil was filtered through 2 cm and 1 mm sieve to extract debris and most macro-organisms, and then stored in sealed plastic bags at 4°C until needed. The wireworms were stored in plastic containers filled with moist PEI soil at 15°C and potato slices were added as a food source every 3 weeks. When larvae were required, individuals were moved from the holding conditions to containers held at 20°C with a feeding bait composed of 11 ml wheat seed and 11 ml of corn seed layered in 50 ml of moist, medium grade Vermiculite (11) placed in the centre. The bait was changed every 4-5 days over a two week period and all wireworms observed feeding were collected and were separated into early (1st-3rd), mid- (4th-6th), and late- (7th-8th) instars based on the number of thoracic spiracle teeth observed under a Nikon SMZ25 Stereomicroscope (x300 magnification, Nikon DS-Ri2 microscope camera, NIS Elements Stereo Imaging Software version 4.40) (12). Only mid- and late-instar larvae for these feeding assays, as no early instar larvae were found in this shipment.

3.2.2 Plants

Similar to the experiment described in chapter 2, barley and buckwheat were germinated for 2 days in a growth cabinet (22±2°C, 16L: 8D, and 40-60% humidity), and then transplanted and grown in 10 cm diameter pots filled with sieved PEI soil maintained at 16-20% soil moisture, the optimal condition for wireworms (13) and microbial diversity (14). Plants were grown in the AAFC London greenhouse under 16L: 8D, 23-27°C, and 40-60% relative humidity. Once the plants reached the desired growth stage, the plants and soil were transplanted to 24 cm diameter plastic
pots to begin the feeding assay. Germinating plants were transplanted after emergence from the soil, and flowering plants were transplanted once flower buds were visible (buckwheat), or when the head of the plant had emerged from the boot (barley). The feeding assays were carried out for 21 days as previous laboratory studies had shown that this was sufficient to detect potential effects (15, 16). Therefore, only germinating and flowering plants were used as the initiation stages in order to avoid overlap of the plant phenological stages during the tests, as buckwheat requires approximately two weeks to grow from germinating to branching, and from branching to flowering.

3.2.3 Feeding Assay

All pots contained of 8 plants of the same type and growth stage in approximately 800g soil at 16-20% moisture, and were kept in the greenhouse at 16L: 8D, 23-27°C, and 40-60% relative humidity (Fig 5).

The experimental pots were infested with 5 wireworms of the same instar to simulate densities per surface area of a highly infested field (17) and these were placed in individual nylon cages (BugDorm-44545F insect rearing cage 47.5x47.5x47.5 cm) in order to avoid losing escaping larvae or emergent adult click beetles. For my experimental design, each of the 8 combinations of plant type, plant growth stage, and wireworm instar were to be replicated 10 times. However, due to seasonal and technical setbacks beyond my control, not enough larvae were collected to achieve 10 replicates for all combinations. Table 1 outlines the number of repetitions per treatment combination for which there were sufficient larvae. Control pots (un-infested plants) were replicated 10 times per plant type and plant stage, but were not placed in nylon cages.
Figure 5 Set up of no choice feeding assays when *Agriotes sputator* wireworms were fed buckwheat, *Fagopyrum esculentum*, or barley, *Hordeum vulgare*, for 21 days.

Table 1 Number of feeding assays using mid- or late-instar *Agriotes sputator* wireworms, feeding on buckwheat, *Fagopyrum esculentum*, or barley, *Hordeum vulgare*.

<table>
<thead>
<tr>
<th></th>
<th>Buckwheat</th>
<th></th>
<th>Barley</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Germinating</td>
<td>Flowering</td>
<td>Germinating</td>
<td>Flowering</td>
</tr>
<tr>
<td>Mid-Instar</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Late-Instar</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
At the start of each feeding assay, the 5 wireworms were individually weighed and the mean weight calculated. The shoot length (from soil to the tip of the plant) of all 8 plants in both the infested and un-infested pots was measured. After 21 days, the final shoot lengths were measured and the difference used to calculate the change in length (shoot growth). The wireworms were extracted from the soil, and the proportion surviving and their weight recorded. The average change in wireworm weight was calculated by subtracting the initial average wireworm weight from the final average weight within each pot. The fresh weight of both root and shoots, as well as root length of each plant in each pot were recorded. Then the roots and shoots of each plant were oven dried for 48 h at 60°C (SMO5 SHEL LAB Forced Air Oven, Sheldon Manufacturing Inc.) and the dry root and shoot weights recorded.

3.2.4 Statistics

The plant variables measured (i.e. shoot growth, final root length, fresh shoot and root weight, dry shoot and root weight) were analysed using a Two-way ANOVA (R version 3.2.5 and the tool Rstudio). Data for both host plants and the different phenological stages were analysed separately, testing the variables: treatment (infested/un-infested), and wireworm instar group (Appendix 5.1, 5.2). The wireworm variables (i.e. proportion surviving and change in mass) were also analysed using a multi-way ANOVA (R version 3.2.5 and the tool Rstudio) testing the variables: plant type, plant stage, and wireworm instar (Appendix 5.4). Tukey’s Post Hoc Analysis was used to identify pairwise significant differences (Appendix 5.3). All models were tested for normality with a QQ-plot and residual homogeneity with a Residual plot. Power analyses were conducted for all data sets (Appendix 6).

3.3 Results

3.3.1 Shoots

Regardless of instar, wireworms caused no significant decline in growth, or fresh and dry mass of gminating (Fig. 6 A-C) or flowering buckwheat (Fig. 6 D-F). In contrast, the presence of either wireworm larval stage resulted in a significant decline in shoot growth (Fig. 7 A) and fresh weight
(Fig. 7 B) of germinating barley compared with controls (Table 2). However, dry shoot weight was not significantly different between germinating barley treatments and controls (Fig. 7 C) and no significant effects were detected when barley was in the flowering stage (Fig. 7 D-F).

**Figure 6** The effect of wireworm feeding on shoot growth and fresh/dry shoot weights of germinating (A-C) and flowering (D-F) buckwheat, *Fagopyrum esculentum*. 
Figure 7 The effect of wireworm feeding on shoot growth and fresh/dry shoot weights of germinating (A-C) and flowering (D-F) barley, Hordeum vulgare. ● - Significant difference ($p < 0.05$).
Table 2 Statistical values of Two-factor ANOVA analyses looking at the effects of wireworm defoliation on shoot variables of buckwheat, *Fagopyrum esculentum*, and barley, * Hordeum vulgare*.

<table>
<thead>
<tr>
<th></th>
<th>Average Shoot Growth</th>
<th>Fresh Shoot Weight</th>
<th>Dry Shoot Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infestation Treatment</td>
<td>Wireworm Instar</td>
<td>Infestation Treatment</td>
</tr>
<tr>
<td><strong>Germinating</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buckwheat</td>
<td>$F_{1,23}=0.00$</td>
<td>$F_{1,23}=0.94$</td>
<td>$F_{1,23}=1.46$</td>
</tr>
<tr>
<td></td>
<td>$p=1.00$</td>
<td>$p=0.34$</td>
<td>$p=0.24$</td>
</tr>
<tr>
<td><strong>Flowering</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buckwheat</td>
<td>$F_{1,23}=1.65$</td>
<td>$F_{1,23}=1.49$</td>
<td>$F_{1,23}=0.37$</td>
</tr>
<tr>
<td></td>
<td>$p=0.21$</td>
<td>$p=0.24$</td>
<td>$p=0.55$</td>
</tr>
<tr>
<td><strong>Germinating</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>$F_{1,23}=14.34$</td>
<td>$F_{1,23}=0.02$</td>
<td>$F_{1,23}=9.82$</td>
</tr>
<tr>
<td></td>
<td>$p&lt;0.001$</td>
<td>$p=0.90$</td>
<td>$p&lt;0.01$</td>
</tr>
<tr>
<td><strong>Flowering</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>$F_{1,25}=3.95$</td>
<td>$F_{1,25}=0.02$</td>
<td>$F_{1,25}=2.28$</td>
</tr>
<tr>
<td></td>
<td>$p=0.06$</td>
<td>$p=0.89$</td>
<td>$p=0.14$</td>
</tr>
</tbody>
</table>
3.3.2 Roots

A rather similar pattern was seen with the root systems, with no significant effect of treatment on final root length, or fresh and dry root weight of germinating or flowering buckwheat plants exposed to either age class of wireworm (Fig. 8 A-F). However, exposure to either stage of wireworm resulted in a significant decrease in all parameters measures compared to controls for both phenological stages of barley (Fig. 9 A, C, D-F), except for fresh root weight of germinating plants (Fig. 9 B).

![Graphs showing the effect of wireworm feeding on root length and fresh/dry root weights of germinating (A-C) and flowering (D-F) buckwheat, Fagopyrum esculentum.](image-url)

Figure 8 The effect of wireworm feeding on root length and fresh/dry root weights of germinating (A-C) and flowering (D-F) buckwheat, *Fagopyrum esculentum.*
Figure 9 The effect of wireworm feeding on root length and fresh/dry root weights of germinating (A-C) and flowering (D-F) barley, *Hordeum vulgare*. ● - Significant difference (p < 0.05).
Table 3 Statistical values of Two-factor ANOVA analyses looking at the effects of wireworm defoliation on root variables of buckwheat, *Fagopyrum esculentum*, and barley, *Hordeum vulgare*.

<table>
<thead>
<tr>
<th></th>
<th>Average Root Length</th>
<th>Fresh Root Weight</th>
<th>Dry Root Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infestation</td>
<td>Wireworm Instar</td>
<td>Infestation</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>Treatment</td>
</tr>
<tr>
<td>Germinating Buckwheat</td>
<td>F₁,₂₃=1.31, p=0.26</td>
<td>F₁,₂₃=0.13, p=0.72</td>
<td>F₁,₂₃=2.69, p=0.11</td>
</tr>
<tr>
<td>Flowering Buckwheat</td>
<td>F₁,₂₃=2.95, p=0.1</td>
<td>F₁,₂₃=0.63, p=0.44</td>
<td>F₁,₂₃=2.21, p=0.15</td>
</tr>
<tr>
<td>Germinating Barley</td>
<td>F₁,₂₃=6.36, p=0.02</td>
<td>F₁,₂₃=0.00, p=0.96</td>
<td>F₁,₂₃=3.31, p=0.08</td>
</tr>
<tr>
<td>Flowering Barley</td>
<td>F₁,₂₅=8.84, p&lt;0.01</td>
<td>F₁,₂₅=1.16, p=0.29</td>
<td>F₁,₂₅=10.64, p&lt;0.01</td>
</tr>
</tbody>
</table>
3.3.3 Wireworms

Neither the proportion of wireworm surviving (Fig 10) nor larval weight change over the 21 day assay (Table 4) were significantly affected by either the species or phenological stage of host plants.

Figure 10 The proportion of *Agriotes sputator* larvae surviving after 21 days of exposure to buckwheat, *Fagopyrum esculentum* (A) and barley, *Hordeum vulgare* (B).
Table 4: The average weight change of *Agriotes sputator* wireworms after 21 days of exposure to buckwheat, *Fagopyrum esculentum*, and barley, *Hordeum vulgare*.

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Plant Stage</th>
<th>Larval Instar</th>
<th>Initial Weight (mg)</th>
<th>Final Weight (mg)</th>
<th>Weight Change (mg)</th>
<th>Change from initial weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>Germinating</td>
<td>Mid</td>
<td>10.9±1.7</td>
<td>10.9±1.5</td>
<td>0.0±2.2</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late</td>
<td>17.2±2.0</td>
<td>16.4±5.2</td>
<td>-0.8±4.5</td>
<td>-4.6</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>Mid</td>
<td>11.7±1.4</td>
<td>9.5±2.5</td>
<td>-2.2±2.4</td>
<td>-18.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late</td>
<td>16.6±2.0</td>
<td>13.7±6.1</td>
<td>-2.9±5.6</td>
<td>-17.4</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>Germinating</td>
<td>Mid</td>
<td>10.9±1.2</td>
<td>11.8±4.0</td>
<td>0.9±4.8</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late</td>
<td>17.1±1.6</td>
<td>14.9±3.0</td>
<td>-2.2±2.6</td>
<td>-13.1</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>Mid</td>
<td>11.7±1.1</td>
<td>10.2±1.7</td>
<td>-1.5±1.8</td>
<td>-12.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late</td>
<td>17.0±1.6</td>
<td>15.3±5.0</td>
<td>-1.6±4.2</td>
<td>-9.5</td>
</tr>
</tbody>
</table>

3.4 Discussion

The prediction arising from my hypothesis, that chemicals present in buckwheat roots would result in increased mortality of wireworm larvae when compared with barley was not supported. While body mass changes were not significant there was considerable variability in the observed changes in larval mass, and it seems that the larvae lost more weight while feeding on flowering plants. However, wireworms go through bouts of feeding and fasting that may last several weeks that could influence the amount of weight lost or gained (18). Furthermore, even during fasting individuals may increase their mass by absorbing water though the cuticle and changes in weight have also been associated with the onset of moulting (18). Consequently, given that the larvae used were field collected, and of similar ages, they may not have been in the same physiological state; caution should be used when drawing any conclusions about the observed patterns in weight change. The power analysis of the wireworm data (Appendix 6.2) showed that at least 180 observations would be required in order to detect a large effect on the wireworms (Appendix 6.3); nearly three times the number actually used.

As predicted wireworm feeding caused significant reductions in most of the barley growth parameters measured but this was not the case for buckwheat. One possible explanation is that buckwheat roots produce anti-feedants and consequently the level of herbivory was significantly reduced. Lack of apparent feeding was not associated with increased mortality possibly because
mid-instar larvae, that require live plant tissue, may survive several weeks without feeding while more advanced instars are able to feed on decaying organic matter in the soil (18). Furthermore, as noted above larvae may have begun periods of fasting after the commencement of the assays, thus it is possible that a three week feeding assay, while sufficient to detect the impact of highly toxic compounds (15, 16) is of insufficient duration to measure anti-feedant effects. Under field conditions, when buckwheat is used in crop rotation, it is grown throughout the summer so there would be a larger time window for any anti-feedant effects to be manifested. Furthermore, as it is plowed in as a green fertilizer at the end of the season the anti-feedants could affect the larger larvae if subsequently this decaying material remains unpalatable.

Plants may also compensate in response to herbivory (19) by increasing the growth of foliage and roots in order to acquire the nutrients necessary for regenerating the biomass lost (20) or by strategically storing nutrients in areas of the plant not accessible to herbivores (19, 20). Thus, the reduced impact of wireworm on buckwheat when compared with barley could be the result of compensatory growth.

Plants not only produce various defensive compounds as a direct chemical defense in response to herbivory (3, 4), but may also have indirect chemical defenses, whereby volatiles emitted following herbivory are used as foraging cues by the herbivore’s natural enemies (3, 21). Though there is not a great of knowledge about the natural enemies of wireworms, if volatiles from defoliated buckwheat result in increased predation and/or parasitism, this could have contributed to the observed population decline in the field.

While the results suggest that buckwheat roots may contain anti-feedants or mild toxins, it is still unclear if they play a role in the decline of wireworm populations when buckwheat is used as a rotation crop in a management scheme under field conditions. Additional assays of longer duration, need to be carried out to determine if there are active anti-feedants in buckwheat roots. If yes, the next step will be to test specific compounds in feeding assays, and to investigate the possibility that these direct chemical defenses affect switching between feeding and fasting bouts during molting and subsequently affect long term survival of the wireworms. Furthermore, the possibility that volatiles emitted by buckwheat following defoliation serve as foraging cues deserves attention.
The results do not allow me to eliminate the possibility that buckwheat exhibits compensatory growth in response to herbivory, but such a defense strategy would not cause the observed decline in wireworm numbers in the field. However, examining compensatory growth would be a useful component to include in new assays, for more biomass is produced would be positive when disking the crop into the soil as a green fertilizer.
3.6 References


Chapter 4

4. Conclusion

The use of buckwheat, *F. esculentum*, as a rotation crop in potato and carrot fields results in a reduction of wireworm populations, something that was not observed when other crops, such as barley or wheat were used. The causes for such an effect were unknown and the goal of my thesis was to determine if buckwheat released allelopathic chemical defenses into the rhizosphere that may affect normal foraging behavior or directly impair larval development. I was specifically interested in testing if there was (i) an antixenosis effect, with phytochemicals acting as repellents to keep wireworm away from the roots, and (ii) an antibiosis effect, where compounds present in the roots acted as anti-feedants and/or toxins once ingested.

In order to test for antixenosis, I carried out choice assays using a six-arm underground olfactometer to determine the relative attractiveness of germinating, branching and flowering buckwheat, red spring wheat (*Triticum* spp) and barley (*H. vulgare*). The responses of *A. sputator* wireworms to the three different phenological stages of buckwheat were similar to those of the other host plants. Germinating plants were the preferred stage for all three plant species, likely due to higher amounts of carbon dioxide (CO₂) exuded by younger plant roots, as this is a very attractive foraging cue for soil dwelling herbivores (1). The results from the olfactometer assays, did not support the original hypothesis that buckwheat exudates would act as repellents for wireworms.

I assessed the impact of root herbivory by comparing different growth parameters of buckwheat and barley when exposed to mid- and late-instar *A. sputator* larvae and tested for the antibiosis effect by quantifying mortality and changes in larval weight when wireworms were fed both plant species for 21 days. Contrary to the predictions arising from my working hypothesis, there was no clear evidence that buckwheat had better chemical defenses than barley; mortality was similar in the two cases and any relative weight loss was greater in barley-fed larvae. However, for both host plants weight loss was greater for larvae that fed on flowering stages compared with those that fed on germinating plants, suggesting that older plants are more chemically defended.
The impact of wireworm defoliation was considerably less on buckwheat, regardless of phenological stage, than on barley. These results lend at least partial support to the hypothesis that buckwheat roots contain compounds that act as feeding deterrents. It is possible that the absence of any significant decline in most buckwheat growth parameters measured could be due to compensatory growth in response to herbivory rather than reduced herbivory (2).

The combined results of my experiments do not provide any clear explanation for the observed decline in wireworm densities following the use of buckwheat as a rotation crop with potatoes and carrots in the field. However, the possible presence of feeding deterrents certainly merits further attention. Wireworms can sustain quite lengthy periods without food, may feed on decaying matter and show alternating periods of feeding and fasting (3), so feeding bioassays should be run for extended periods as any anti-feedant effects (or even mild toxicity) may not be readily detectable with 21 days. It would also be advisable to run large scale field trials, comparing the rotation of potato with either barley or buckwheat. In this way, one could actually monitor temporal changes in wireworm density and the relative abundance of different larval stages as any impact may occur during the growing season or after the crop has been plowed under as green fertilizer. In addition, field trials could also be used to determine if buckwheat volatiles serve as form of indirect chemical defense by attracting natural enemies of wireworm. If important, then the incidence of either parasitism or predation would be higher in buckwheat than barley plots. Since wireworms are an economically relevant pest, it is important to investigate all avenues that can potentially lead to the advancement of a sustainable, non-insecticide alternative as a means of control.
4.1 References


Appendix 1. Generalized linear model (GLM) of binomial distributions of olfactometer assay data

1.1 Beginning with all variables tested and their interactions: Generalized linear model of binomial distributions includes the variables “Plant.type”, “Chamber”, and the interactions between these variables

```{r}
mymodel1<-glm(cbind(Count, Total-Count)~Plant.type*Chamber, family=binomial, data=Olfactometer.trial.results)

summary(mymodel1)
```

```
# Call:
# glm(formula = cbind(Count, Total - Count) ~ Plant.type * Chamber,
#     family = binomial, data = Olfactometer.trial.results)
#
# Deviance Residuals:
#     Min       1Q   Median       3Q      Max
# -2.3987  -1.1527  -0.3742   0.6870   3.9881
#
# Coefficients:
#             Estimate Std. Error z value Pr(>|z|)
# (Intercept) -1.51635    0.26029  -5.826 5.69e-09  ***
# Plant.typeBW -0.06928    0.37232  -0.186   0.852
# Plant.typeW -0.21825    0.38232  -0.571   0.568
# ChamberF    -0.47608    0.40303  -1.181   0.238
# ChamberG     0.57189    0.34257   1.669   0.095
# Plant.typeBW:ChamberF  0.79604    0.53998   1.474   0.140
# Plant.typeW:ChamberF  0.88576    0.54875   1.614   0.106
# Plant.typeBW:ChamberG -0.08487    0.49149  -0.173   0.863
# Plant.typeW:ChamberG  0.06410    0.49911   0.128   0.904

# Signif. codes:  ^ 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
# (Dispersion parameter for binomial family taken to be 1)
# Null deviance: 179.13  on 89  degrees of freedom
# Residual deviance: 165.24  on 81  degrees of freedom
# AIC: 355.28
# Number of Fisher Scoring iterations: 4
```
1.2 Saturated model: A saturated model is required for a meaningful analysis of a generalized linear model. Only the significant variable group (ie. Chamber) is present in the model

```r
mymodel3<-glm(cbind(Count, Total-Count)~Chamber, family=binomial, data=Olfactometer.trial.results)
```

Appendix 2. Hypothesis testing for olfactometer choice assays

2.1 One-way ANOVA with chi square test of GLM: Chi square is used in ANOVA due to count data

```r
anova(mymodel3, test="Chisq")
```

```
## Analysis of Deviance Table
## Model: binomial, link: logit
## Response: cbind(Count, Total - Count)
## Terms added sequentially (first to last)
##
## Df Deviance Resid. Df Resid. Dev Pr(>Chi)
## NULL 89 179.13
## Chamber 2 8.9627 87 170.17 0.01132 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```
2.2 Post-Hoc Tukey’s Test of pairwise comparisons

```r
summary(glht(mymodel3, linfct=mcp(Chamber="Tukey")))
```

```r
## Simultaneous Tests for General Linear Hypotheses
## Multiple Comparisons of Means: Tukey Contrasts
##
## Fit: glm(formula = cbind(Count, Total - Count) ~ Chamber, family = binomial, 
## data = Olfactometer.trial.results)
## Linear Hypotheses:
## Estimate Std. Error z value Pr(>|z|)
## F - B == 0 0.1155 0.2151 0.537 0.8529
## G - B == 0 0.5635 0.2033 2.772 0.0153 *
## G - F == 0 0.4480 0.1990 2.251 0.0628 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

Appendix 3. Assumption testing for model of olfactometer data

3.1 Over-dispersion: In addition to being saturated, the model must not have over-dispersion. Over-dispersion was checked using a dispersion parameter for quasibinomial family; it was found to be negligible (below 10)

```r
model3.quasi<-update(mymodel3,.~,family=quasibinomial)
summary(model3.quasi)
```

```r
## (Dispersion parameter for quasibinomial family taken to be 1.836179)
##
## Null deviance: 179.13 on 89 degrees of freedom
## Residual deviance: 170.17 on 87 degrees of freedom
## AIC: NA
```
Appendix 4. Power analysis for olfactometer trials

4.1 Cohen estimate of large effect size

```r
cohen.ES(test="anov", size="large")
```

```
##
## Conventional effect size from Cohen (1982)
##
test = anov
size = large
effect.size = 0.4
```

4.2 Calculating actual power of the olfactometer study: Power to which a large effect size is detected in this study

```r
f.hat<-sqrt(0.4^2/(2*9))
pwr.anova.test(k=9, f=f.hat, n=30) # n is total sample size of all groups combined/df of each group + 1
```

```
##
## Balanced one-way analysis of variance power calculation
##
## k = 9
## n = 30
## f = 0.0942809
## sig.level = 0.05
## power = 0.1472343
##
## NOTE: n is number in each group
```
Appendix 5. Hypothesis testing for feeding assays

5.1 Two-way ANOVA testing for buckwheat plant variables

5.1.2 Germinating buckwheat

```r
model.1 <- aov(avg.shoot.growth ~ Treatment * ww.instar, data = GBW)
model.3 <- aov(avg.fresh.shoot.weight ~ Treatment * ww.instar, data = GBW)
model.4 <- aov(avg.dry.shoot.weight ~ Treatment * ww.instar, data = GBW)
model.5 <- aov(avg.fresh.root.weight ~ Treatment * ww.instar, data = GBW)
model.6 <- aov(avg.fresh.root.length ~ Treatment * ww.instar, data = GBW)
model.7 <- aov(avg.dry.root.weight ~ Treatment * ww.instar, data = GBW)

summary(model.1)

## Df Sum Sq Mean Sq F value Pr(>F)
## Treatment 1 0.00 0.00 0.00 0.999
## ww.instar 1 48.9 48.85 0.94 0.342
## Residuals 23 1195.8 51.99

summary(model.3)

## Df Sum Sq Mean Sq F value Pr(>F)
## Treatment 1 0.510 0.5098 1.462 0.239
## ww.instar 1 0.194 0.1936 0.555 0.464
## Residuals 23 8.023 0.3488

summary(model.4)

## Df Sum Sq Mean Sq F value Pr(>F)
## Treatment 1 16729 16729 1.891 0.182
## ww.instar 1 3815 3815 0.431 0.518
## Residuals 23 203432 8845

summary(model.5)

## Df Sum Sq Mean Sq F value Pr(>F)
## Treatment 1 0.00647 0.006467 2.692 0.114
## ww.instar 1 0.00002 0.000022 0.009 0.925
## Residuals 23 0.05525 0.002402

summary(model.6)

## Df Sum Sq Mean Sq F value Pr(>F)
## Treatment 1 3.08 3.0841 1.312 0.264
## ww.instar 1 0.30 0.2995 0.127 0.724
## Residuals 23 54.05 2.3502

summary(model.7)

## Df Sum Sq Mean Sq F value Pr(>F)
## Treatment 1 450.8 450.8 3.299 0.0824
## ww.instar 1 10.9 10.9 0.080 0.7801
## Residuals 23 3142.8 136.6
```

---

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0
5.1.3 Flowering buckwheat

```r
model.1 <- aov(avg.shoot.growth ~ Treatment*ww.instar, data=FBW)
model.3 <- aov(avg.fresh.shoot.weight ~ Treatment*ww.instar, data=FBW)
model.4 <- aov(avg.dry.shoot.weight ~ Treatment*ww.instar, data=FBW)
model.5 <- aov(avg.fresh.root.weight ~ Treatment*ww.instar, data=FBW)
model.6 <- aov(avg.fresh.root.length ~ Treatment*ww.instar, data=FBW)
model.7 <- aov(avg.dry.root.weight ~ Treatment*ww.instar, data=FBW)

summary(model.1)
#
## Df    Sum Sq  Mean Sq     F value  Pr(>F)
## Treatment     1   141.4  141.38      1.653    0.211
## ww.instar     1     127.2  127.21      1.487    0.235
## Residuals    23     1967.6   85.55

summary(model.3)
#
## Df     Sum Sq  Mean Sq     F value  Pr(>F)
## Treatment     1     0.316   0.3157      0.367    0.551
## ww.instar     1     0.160   0.1600      0.186    0.670
## Residuals    23     19.787   0.8603

summary(model.4)
#
## Df     Sum Sq  Mean Sq     F value  Pr(>F)
## Treatment     1   2608.8  2608.8       0.084    0.774
## ww.instar     1     61.6    61.6       0.002    0.965
## Residuals    23    71364.0  31028

summary(model.5)
#
## Df    Sum Sq  Mean Sq   F value  Pr(>F)
## Treatment     1  0.01370  0.01370     2.212    0.151
## ww.instar     1  0.00022  0.00022     0.036    0.851
## Residuals    23  0.14245  0.006194

summary(model.6)
#
## Df     Sum Sq  Mean Sq     F value  Pr(>F)
## Treatment     1    10.76    10.756      2.946    0.0995
## ww.instar     1     2.28     2.281      0.625    0.4374
## Residuals    23    83.97     3.651
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(model.7)
#
## Df    Sum Sq  Mean Sq     F value  Pr(>F)
## Treatment     1     410.4  409.46      0.883    0.357
## ww.instar     1      19.8    19.80      0.040    0.842
## Residuals    23    10678  464.2
```
5.2 Two-way ANOVA testing for barley plant variables

5.2.1 Germinating barley

```r
model.1 <- aov(avg.shoot.growth~Treatment*ww.instar, data=GBar)
model.3 <- aov(avg.fresh.shoot.weight~Treatment*ww.instar, data=GBar)
model.4 <- aov(avg.dry.shoot.weight~Treatment*ww.instar, data=GBar)
model.5 <- aov(avg.fresh.root.weight~Treatment*ww.instar, data=GBar)
model.6 <- aov(avg.fresh.root.length~Treatment*ww.instar, data=GBar)
model.7 <- aov(avg.dry.root.weight~Treatment*ww.instar, data=GBar)

summary(model.1)

# Df  Sum Sq   Mean Sq   F value  Pr(>F)
# Treatment  1  436.0     436.0  14.3355  0.000955 ***
# ww.instar  1   0.5       0.5   0.0170  0.898559
# Residuals 23 699.5     30.4
# Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(model.3)

# Df  Sum Sq   Mean Sq   F value  Pr(>F)
# Treatment  1  0.191     0.191   9.821  0.00466 **
# ww.instar  1  0.026     0.026   1.314  0.26339
# Residuals 23 0.447     0.019
# Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(model.4)

# Df  Sum Sq  Mean Sq   F value Pr(>F)
# Treatment 1  4840   4840   3.328  0.0811
# ww.instar 1  2411   2411   1.658  0.2107
# Residuals 23 33448  1454
# Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(model.5)

# Df  Sum Sq   Mean Sq   F value Pr(>F)
# Treatment 1  0.00987  0.00987  3.309  0.0819
# ww.instar 1  0.00570  0.00570  1.939  0.1771
# Residuals 23 0.06863  0.00298
# Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```
5.2.2 Flowering barley

model.1 <- aov(avg.shoot.growth ~ Treatment*ww.instar, data=FBar)
model.3 <- aov(avg.fresh.shoot.weight ~ Treatment*ww.instar, data=FBar)
model.4 <- aov(avg.dry.shoot.weight ~ Treatment*ww.instar, data=FBar)
model.5 <- aov(avg.fresh.root.weight ~ Treatment*ww.instar, data=FBar)
model.6 <- aov(avg.fresh.root.length ~ Treatment*ww.instar, data=FBar)
model.7 <- aov(avg.dry.root.weight ~ Treatment*ww.instar, data=FBar)
```
summary(model.4)

## Df Sum Sq Mean Sq F value  Pr(>F)
## Treatment     1  749  748.7  0.247 0.624
## ww.instar     1  263  262.7  0.087 0.771
## Residuals    25 75891  3035.6

summary(model.5)

## Df  Sum Sq Mean Sq  F value  Pr(>F)
## Treatment     1  0.04062  0.04062 10.643 0.00319 **
## ww.instar     1  0.00026  0.00026  0.068 0.79676
## Residuals    25  0.09541  0.00382
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(model.6)

## Df  Sum Sq Mean Sq  F value  Pr(>F)
## Treatment     1  36.52  36.52  8.840 0.00644 **
## ww.instar     1   4.81   4.81  1.164 0.29090
## Residuals    25 103.29   4.13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(model.7)

## Df  Sum Sq Mean Sq  F value  Pr(>F)
## Treatment     1  508.1  508.1  5.040 0.0339 *
## ww.instar     1  11.4  11.40  0.113 0.7400
## Residuals    25 2520.2  100.8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```
5.3 Post-Hoc Tukey’s Test of pairwise comparisons for barley plant variables

5.3.1 Germinating barley

TukeyHSD(model.1)

```r
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = avg.shoot.growth ~ Treatment, data = GBar)
##
## $Treatment
## diff   lwr   upr   p adj
## T-C -8.288371 -12.71304 -3.863702 0.0007385
```

TukeyHSD(model.3)

```r
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = avg.fresh.shoot.weight..g. ~ Treatment, data = GBar)
##
## $Treatment
## diff   lwr   upr   p adj
## T-C -0.1735828 -0.2886501 -0.05851551 0.0047321
```

TukeyHSD(model.4)

```r
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = avg.dry.shoot.weight..mg. ~ Treatment, data = GBar)
##
## $Treatment
## diff   lwr   upr   p adj
## T-C -27.6154 -59.28372 4.052923 0.0844838
```
5.3.2 Flowering barley
5.4 Multi-way ANOVA testing for wireworm variables

model.1<-aov(Weight.Diff~plant.type*plant.stage*ww.instar, data=ww)
summary(model.1)

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Appendix 6. Power analysis for Feeding assays

6.1 Power analysis for plant variable testing using Cohen’s large effect size

```r
f.hat <- \sqrt{(0.4^2 / (2*6))}
pwr.anova.test(k=6, f=f.hat, n=13) # n is total sample size of all groups combined/df of each group + 1
```

```r
## Balanced one-way analysis of variance power calculation
##
##k = 6
##n = 13
##f = 0.1154701
##sig.level = 0.05
##power = 0.09670868
```
6.2 Power analysis for wireworm variable testing using Cohen’s large effect size

```r
f.hat <- sqrt((0.4^2)/(2*8))
pwr.anova.test(k=8, f=f.hat, n=29.5)#n is total sample size of all
    groups combined/df of each group + 1
```

```r
##
## Balanced one-way analysis of variance power calculation
##
## k = 8
## n = 29.5
## f = 0.1
## sig.level = 0.05
## power = 0.153055
##
## NOTE: n is number in each group
```

6.3 Calculating the number of observations (n) required in order to detect an large
effect size on wireworm parameters.

```r
pwr.anova.test(k=8, f=f.hat, power=0.8) #number of samples in
each group needed for a power of 0.8
```

```r
##
## Balanced one-way analysis of variance power calculation
##
## k = 8
## n = 180.2626
## f = 0.1
## sig.level = 0.05
## power = 0.8
##
## NOTE: n is number in each group
```
# Curriculum Vitae

<table>
<thead>
<tr>
<th>Name:</th>
<th>Yeritza L. Bohorquez Ruiz</th>
</tr>
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<td><strong>Post-secondary</strong></td>
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