A comparison of Leaf Processing Rates and Fungal Biomass of Native and Invasive Species

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Abstract

An invasive "alien species" is a non-native organism that causes, or has the potential to cause, harm to the environment, economy, or human health. They successfully establish themselves and overcome existing native ecosystems, out-competing native organisms for food and habitat. The goal of this project is to determine if an invasive species is consumed at a rate similar to native species in an aquatic ecosystem. The invasive plant used in this study was Japanese Knotweed (*Polygonum cuspidatum*), a plant native to Asia that has invaded stream banks in Pennsylvania. This species was compared to Sugar Maple (*Acer saccharum*). Leaves were incubated in Mill Creek, a second order stream, for a period of ten weeks and removed at two week intervals. Water chemistry was analyzed throughout the study. Aquatic hyphomycete spore counts were performed twice to determine the average amount of fungal spores present in the water. Surface area of leaves was measured before incubation and after removal to determine the processing rates. Leaves were weighed and burned in a muffle oven in order to determine the percent of organic content. Fungal biomass was determined by HPLC analysis of ergosterol, a component of fungal membranes. Macroinvertebrates were identified from each leaf pack. Japanese Knotweed was shown to have a faster decomposition rate than the Sugar Maple. The Knotweed also had a lower percent organic content throughout the incubation period. The Japanese Knotweed acquired significantly less ergosterol than the Sugar Maple, indicating less fungal growth. Invertebrates did not show any preference between the two species.

Introduction

There are two sources of primary energy in an aquatic habitat: photosynthesis by algae and higher aquatic plants and allochthonous materials entering from riparian vegetation (Benfield 1996). Small narrow headwater streams of orders 1 to 3 rely almost entirely on allochthonous material to supply energy to the system (Cummins 1975). Leaves entering a stream must be broken down into smaller pieces to be consumed by aquatic organisms. Most of the nutrients from a naturally abscised leaf are taken back into the tree before the leaf falls. The remaining nutrients leach out of the leaf within days of it entering the water. Only hard indigestible structural compounds are left, such as cellulose and lignin. Aquatic fungi break down the cellulose and lignin in leaves. Macroinvertebrates have been shown to prefer leaves that have been colonized by fungi, since these leaves contain more nutrients then sterile leaves (Barlocher 1984). Breakdown is further induced by a group of macroinvertebrates called shredders who reduce the leaves from Coarse Particulate Organic Matter (CPOM) to Fine Particulate Organic Matter (FPOM). The FPOM can then be consumed by filtering invertebrates.

The rate of leaf decomposition depends upon many factors, the leaf species being the most important determinant. Nutrient levels and the species of aquatic fungi present in a stream also affect the decomposition rate. High Nitrate levels in streams cause an increase in leaf decomposition by stimulating microbial growth (Meyer and Johnson 1983), since nitrogen is a limiting nutrient for aquatic fungi (Sridhar and Barlocher 2000). Increased nutrients have also been associated with an increased fungal diversity on leaves (Gulis and Suberkropp 2003). In a study by Cornelissen (1996), a correlation was

found between fall leaf color and decomposition rate. Leaves containing some green color decomposed at a faster rate and lost a greater weight than those that had turned brown or yellow.

Fungal biomass is determined by measuring the amount of ergosterol present on a leaf or in a water sample. Ergosterol is a membrane component found in fungal cell walls that is structurally similar to cholesterol. Its presence can be detected using High Pressure Liquid Chromatography (HPLC). Ergosterol is also present in algae and protozoa in small amounts but the amount is not significant enough to distort data (Mille-Lindblom C., et al 2004). A study by Barajas-Aceves et al (2002) determined that pollution levels do not affect ergosterol measurement, and therefore ergosterol can be used for fungal measurements even in the presence of pollution. However, measurements of ergosterol detect both living and dead fungi, which may interfere with results (Mille-Lindblom C., et al 2004).

Determining leaf processing rates and fungal biomass have been the subjects of previous research projects completed by Lycoming College Students. Honors projects on this research have been completed by Emily Stricker (2001), Christina Panko (2002), and Anthony Sowers (2003).

Stricker investigated two plant species: Sugar Maple (*Acer saccharum*) and River Birch (*Betula nigra*) (2001). Both leaf species were incubated in two different streams of different orders for the summer and the fall. Incubation was performed by attaching leaves to a numbered brick using a rubber band. Leaf packets were removed once a week for five weeks. Stricker concluded that the processing rates of both leaf species were higher in a second order stream than in a third order stream. This is thought to be due to a

lower pH and colder temperatures in the third order stream. Fungal biomass was found to be higher in the fall than in the summer, while leaf processing was found to be lower in the fall than in the summer. Sugar Maple leaves were found to be fast decomposers, compared to the slow decomposing River Birch. Decomposition rate was based on k-values using a method developed by Peterson and Cummins (1974). Fungal colonization was highest for Sugar Maple leaves in the summer and for River Birch in the fall.

The study performed by Panko (2002) involved three different leaf species: Sugar Maple, River Birch, and Pin Oak (*Quercus palustris*). This study also used two different methods for incubating the leaves. The brick method was used along with a method involving attaching small leaf discs to a Plexiglas sheet. The second method was found to be unreliable due to loss of leaf discs. After removal from the stream, some of the leaves were refrigerated and some were frozen. This was done to determine which method was better for preserving fungi on the leaves, but no significant difference in the amount of fungi was found. Panko (2002) came to many of the same conclusions as Stricker (2001). Fungal biomass was found to be higher in the fall and leaf processing was found to be higher in the summer. However, Panko found that Sugar Maple leaves were fast decomposers in the summer but medium in the fall. Both River Birch and Pin Oak were found to decompose at a medium rate in the summer and a slow rate in the fall.

The study performed by Sowers (2003) repeated the study performed by Stricker (2001). However, the bricks were placed in mesh bags before incubation with a mesh size of 2.1 x 2.8 mm. This helped prevent loss of leaf material from abrasion. Sowers incubated the species Sugar Maple and River Birch in the same two streams for the summer and fall. The conclusions were similar to the two previous studies. From this

research, it can be concluded that invertebrate densities are higher in the summer months, which may be due to a lack of allochthonous material in the streams at this time. The invertebrates have fewer choices for food and so the density of invertebrates on leaf material will be high. The incubated leaves would be a prime source of food for invertebrates before leaf abscission occurs in the fall. Fungal spores increase in the early fall and decrease in the late fall and early winter. Invertebrate densities tend to increase along with fungal colonization throughout a season. Leaf processing occurred faster in the summer than the fall, which may be a result of higher water temperatures and higher invertebrate density.

This goal of this project is to determine if an invasive species is consumed at a rate similar to native species in an aquatic ecosystem. An Invasive "alien species" is a non-native organism that causes, or has the potential to cause, harm to the environment, economy, or human health. They successfully establish themselves in and overcome existing native ecosystems, out-competing native organisms for food and habitat. Invasive species are now the second leading cause of species endangerment and extinction, the first being habitat destruction. Of the species listed as invasive by the National Invasive Species Council, more than half are terrestrial or aquatic plants (Invasivespecies.gov).

The invasive plant used in this study is Japanese Knotweed (*Polygonum cuspidatum*). Japanese Knotweed is an invasive plant species native to Japan, China, Korea, and Taiwan (www.invasiveplants.net). It has been in the United States since the late 19th century and is present in all but nine states. Japanese Knotweed prefers moist soils and can be detrimental to waterways. In many areas, knotweed has out competed out

all native vegetation in the riparian zone surrounding a water body. Its shallow roots do little to prevent erosion and it has little value to wildlife.

Japanese Knotweed was compared to the native species, Sugar Maple. I utilized the methods that have been used and improved in the previously mentioned honors projects. However, in order to eliminate experimental bias, I have used only one study site to incubate both species and completed the study entirely during the fall. I also incubated the leaves for ten weeks, removing leaf packets once every two weeks, providing a longer range of data. I will compare my data to that obtained in the previous studies.

Methods

Study Area

The Study area was Mill Creek, a second-order stream, with head waters at Rose Valley Lake (Lycoming County, PA). This Creek enters the Loyalsock Creek at Montoursville, PA. The field work for the study took place between late September and November, 2004. The area of the creek used for leaf incubation is downstream from agricultural and residential areas. An EPA habitat assessment, as described by Plafkin et al (1989), was performed for a 100 meter stretch along the study site. Twelve habitat parameters were measured on a scale of 0 to 20, with 0 being poor and 20 being excellent. The right bank has a score of 140 and the left bank has a score of 145 (Table 1).

Physical and Chemical Assessment

Physical and chemical assessments were performed at the time the leaves were incubated and at each removal of leaves, approximately every two weeks. Dissolved Oxygen (ppm) and Temperature (°C) were measured in the field using a hand-held YSI model 55 DO meter. The pH and alkalinity (ppm CaCO3) were measured using a Corning pH meter. Nitrate (ppm NO3-), nitrite (ppm NO2-), total phosphorous (ppm PO4), and orthophosphorous were measured using a HACH/DR 4000 Spectrophotometer. Nutrients were measured from water samples within 24 hours after removal from the stream.

Leaf Processing Rates

Sugar Maple leaves were collected in late August from a tree on the Lycoming College campus. Japanese Knotweed leaves were collected in late August from a site along the Susquehanna River in Montoursville. All leaves were collected pre-abscission. The surface area of each leaf was measured using a LI-COR Model LI-3000A area meter. The areas were recorded in the order in which each leaf was placed in a pack. Each set of five leaves was weighed using an analytical balance and then attached to a brick and secured with a mesh bag and a rubber band. Forty eight packets were made for each species.

The leaf packets were then incubated in Mill Creek beginning on September 21, 2004. Every two weeks thereafter, four packets of each species were removed from the stream. Invertebrates were removed from the leaves in the lab and preserved in 70% ethanol. The leaves were then rinsed with deionized water to remove debris. The leaves

from two of the packets were again measured for remaining surface area. A processing rate was determined from this surface area in terms of a k-value (Cummins 1975). The k-value can be used to determine the speed of processing as slow, medium, or fast. Sample calculations are included in Appendix I.

Five leaves from one pack for each species were individually dried in an oven at 80 °C for at least 24 hours. The dried leaves were ground up with a pestle and mortar. Each ground leaf was put into a pre-weighed crucible and weighed again to obtain the dry weight of the leaf. The leaves were then heated at 550 °C in a muffle oven for two hours to burn off organic materials. The mass of the leaf was taken again to determine the percent organic content. This was also performed on leaves that had not been incubated to serve as a control.

Fungal Biomass

Fungal biomass was determined using High Pressure Liquid Chromatography (HPLC) to detect the amount of ergosterol present in leaf samples. The HPLC instrument was made up of a Waters 510 pump, a Whatman Partisil 5 OD5-3 25-cm x 4.6mm column, a 100uL sample loop, and a Waters 991 photodiode array detector with Millennium software. The solvent was pure HPLC grade methanol. All HPLC measurements were made at a flow rate of 1.5 mL/min resulting in ergosterol peaks around 5.2 minutes. The wavelength used, that at which maximum absorption of ergosterol occurs, was 282 nm.

Trials were performed using a new method developed by Clark and Franz (Unpublished Independent Study 2004) as part of an analytical chemistry practicum. However, the new method, which was tried on a limited number of samples last winter, did not result in

recovery of ergosterol during field trials this fall. Therefore, for analysis of the incubated leaves in this study, I used the procedure developed by Newell et al (1988), which had been utilized in previous Lycoming Honors projects (Stricker 2001) (Panko 2002) (Sowers 2003).

The method developed by Clark and Franz (2004) was a combination of methods previously developed by Gessner and Schmitt (1996), the Waters Company (2005), and Newell et al (1988). The leaves were rinsed with distilled water after incubation to remove debris and refrigerated over night. Leaves were refluxed in 25 mL of 0.14 M KOH in pure methanol. For the percent recovery trials, leaves were not used. A 1cc 30 mg sorbent Oasis cartridge was conditioned with three volumes of 2.5 mL methanol followed by three volumes of 2.5 mL deionized water. Once the samples were cooled, 5 mL of 0.75 M HCl was added to acidify the solution. The solution was filtered with a 0.45 um Whatman filter to remove debris. The solution was then loaded through the cartridge. The ergosterol should remain on the Oasis cartridge. To ensure that this was happening, the loaded solution was saved to be analyzed by HPLC. Next, the cartridge was washed with 10 mL of 5% methanol in water. This wash was also kept to be tested for ergosterol. Finally, the ergosterol was eluted off with 10mL of 100% methanol. The three solutions, the loaded solution, the wash solution, and the eluted solution, were all filtered with a 13 mm 0.45 um nylon membrane Whatman filter. The samples were then injected into the HPLC in 50uL volumes. Two trials were performed in which ergosterol was injected into the pre-filtered solution. A 0.250 mL Hamilton syringe was used to inject a volume of 0.500mL of a 25.365 ug/mL ergosterol solution. In both trials, no

ergosterol was recovered in any of the three solutions, leading to the conclusion that the ergosterol was not being eluted off the filter.

The method developed by Newell et al (1988) was performed as follows. The leaves were incubated and collected as previously mentioned. After collection, fifteen 10 mm discs were cut out of each leaf species using a cork borer. These discs were then weighed and put into a round bottom flask with 25mL HPLC grade methanol. The flasks were refluxed for 30 minutes at 80 °C. Ten mL of 10% KOH (aq) was added and the solution was refluxed for another 30 minute period. Debris was filtered out through a coarse glass frit 40-60 um, by water aspiration. The filtrate was transferred to a vial where 5 mL of 20% aqueous NaCl was added. Ergosterol was extracted with 10 mL of pentane. The content of the tube was then mixed and the pentane layer was removed. This was repeated with two 5mL portions of pentane. The extracted portions were dried overnight to allow the pentane to evaporate. The residue was dissolved in one mL of HPLC grade methanol. To ensure that all of the ergosterol was dissolved, a sonnicator was used. The solution was then filtered through a 0.45um Whatman filter to ensure the integrity of the HPLC. The solution was injected into the HPLC instrument in volumes of 50uL. The peak areas given by the HPLC were compared to a standard curve and ug of ergosterol was determined. Sample HPLC chromatograms are included in Appendix IV. Ergosterol was converted to fungal biomass using a conversion factor. Two percent recovery trials were performed in which known amounts of ergosterol were used and leaf discs were omitted from the above procedure.

A standard curve was created by injecting solutions with known concentrations of ergosterol (Aldrich, 95%) into the HPLC. The Standard Curve can be found in Appendix

III. Standard solutions of 1.268 ug/mL and 25.365 ug/mL were utilized in different volumes. The results were used to form an equation of the standard curve. This equation was then used to determine the amount of ergosterol from a given peak area of an unknown.

Aquatic Hyphomycetes Spore Analysis

In order to determine the concentration of spores present in the stream, 300mL of water from the stream was filtered through a 0.8 um Millipore filter by vacuum filtration. The filters were dried then flooded with 0.01% Trypan blue in lactic acid. This kills the spores and stains them for counting. The filters were then heated at 55 °C for 55 minutes. The filter was mounted on microscope slide and the spores were counted using a compound microscope. This was performed twice during the study to provide an average spore concentration.

Invertebrate Data

Macroinvertebrates were collected off of the leaves in the packets and preserved in 70% ethanol. They were then identified to the Genus level and the feeding group was determined for each genus among shredders, gatherers, scrapers, filterers, and predators (Hilsenhoff 1987) (Plafkin et al 1989). The number of taxa was counted, where a taxon was considered to be a family. Other statistical analysis included the percentage of taxa that were shredders and the percentage of the dominant taxon. The ratio of Ephemeroptera: Plecoptera: Trichoptera was also determined. Simpson and Shannon Indexes were calculated to determine diversity. A kick sample was taken to determine the

feeding group distribution present in the stream. This is obtained by kicking up sediment in a one meter square area and catching all the invertebrates from the area in a net.

Results

Physical and Chemical Assessment

Throughout the study period, pH remained stable between 6.18 and 7.23 with an average pH of 6.71 (Table 2). Alkalinity was between 1 and 21 ppm, averaging 12.4 ppm. Nutrient levels increased throughout the fall. On October 4, 2004 the first removal of incubated leaves, Orthophosphate averaged 0.1475 ppm. This increased to 1.365 two weeks later. The highest measurement was obtained on November 1, 2004 when orthophosphate averaged 5.5 ppm. Levels declined in the following two weeks to 1.74 ppm. Total Phosphorous and Nitrate levels followed a similar pattern, with low measurements in October, increasing to a peak on November 1 and decreasing afterwards. Nitrite levels peaked on October 18, 2004, the fourth week after incubation. Dissolved oxygen levels were healthy throughout the study and increased as the temperature decreased. The average dissolved oxygen level was 10.58 ppm. Temperature ranged from 14.6°C to 4.1°C.

Leaf Processing Rates

The k-values expressing leaf composition, for Japanese Knotweed were much higher than those obtained for Sugar Maple. The average value for the Knotweed was 0.0147 ± 0.007, while the average value for the Sugar Maple was 0.00278 ± 0.004 (Table

3). Knotweed is a fast decomposer since the k-value is greater than 0.010. Sugar Maple is a slow decomposer since the k-value is below 0.005.

Sugar Maple was found to be a relatively fast decomposer in the summer studies performed previously. However, in the fall decomposition slows. Panko (2002) found that Sugar Maple was a medium decomposer in a fall study. The data obtained by Sowers (2003) agreed with that obtained in this study, showing that Sugar Maple leaves decompose slowly in the fall. However, these studies were performed over a shorter period of time.

The percent organic content differed between the two leaf species. There was less organic content in Japanese Knotweed, which had a value of 54.2 % after two weeks of incubation (Figure 1). Due to technical problems with the temperature of the muffle oven, pre-incubation data was not obtained. The Sugar Maple had an organic content of 78.8 % after two weeks of incubation. Organic content of Knotweed decreased constantly throughout the incubation period. Sugar Maple, however, showed a slight increase between weeks six and eight. The values of the Knotweed were continuously lower than that of the Maple. After eight weeks of incubation, Knotweed had decreased to 23.6 % organic content and Maple had decreased to 58.3 %.

These results show a similar trend to that found in the previous studies, since the percent organic content decreased throughout the season. In the study by Sowers (2003), the Sugar Maple decreased in organic content from 91% to 45% in five weeks during the summer. This study also showed that organic content decreases faster in the fall, which is also displayed in Panko's study (2002). Data was obtained over an eight week period in this study, but organic content of Maple never fell below 55.9 %.

Fungal Biomass

Fungal biomass was determined for leaves that had not been incubated as a control. The Sugar Maple leaves had no ergosterol present on the control leaves. Japanese Knotweed had an average of 0.45 ug fungal biomass/mg detritus on control leaves (Figure 2).

Sugar Maple had the greatest amount of fungal biomass accumulation at four weeks of incubation. This peak was 4.6 ug fungal biomass/mg detritus. The amount fell to 1.9 ug at week six, and then rose to 3.3 at week eight. After eleven weeks of incubation, the fungal biomass was 1.9 ug fungal biomass/mg detritus.

Japanese Knotweed peaked at week six with 0.9 ug; 3.7 ug less than the Maple peak and 0.9 ug less than the Maple value at six weeks. Knotweed decreased after week six to 0.5 ug by week eleven. The lowest value of 0.46 ug occurred at four weeks, the week during which fungal colonization of Sugar Maple had peaked.

The Sugar Maple data obtained in the previous studies differs from that obtained in this study. In Stricker's study (2001), Sugar Maple had a peak value of 1.12 ug fungal biomass/mg detritus. Panko (2002) had a peak fall value of 5.00 ug/mg detritus. In Sowers' study (2003), the average in the early fall was 4.67 ug/mg detritus and the late fall average was 2.30 ug/mg detritus. The second and third studies correlate closely with my data, while the first study had much lower numbers of fungal colonization. All of the data was obtained at the same study site, along Mill Creek.

Percent Recovery tests were run on the HPLC using the method developed by Newell et al (1989). The results averaged 95.5% recovery of ergosterol.

Aquatic Hyphomycetes Spore Analysis

The average spore count was 6708 spores/L. There were 10,316 spores/L in the first sample and 3100 spores/L in the second sample both of which were taken in the November. In Panko's study, the highest number of spores per liter was 6307 spores. This spore count was taken from Mill Creek in the fall. In Sower's study, the late fall average was 4533 spores/L. The first sample was much higher than that found in previous studies while the second sample was lower.

Invertebrate Data

After each collection, invertebrates were collected and identified from four leaf packs. A complete list of invertebrates can be found in Appendix II. There were 67 invertebrates on the Sugar Maple leaf packs after 13 days of incubation, averaging 17 per leaf pack (Table 4). The number increased greatly to 167 invertebrates at 27 days of incubation, averaging 42 per leaf pack. Invertebrates then decreased slightly to 161 by 41 days, averaging 40 per leaf pack. Japanese Knotweed leaf packs had 61 invertebrates after 13 days with an average of 15 per leaf pack. There were 180 invertebrates after 27 days with an average of 45 per leaf pack. After 41 days, there were 147 invertebrates, averaging 10 per leaf pack. Invertebrate density peaked at four weeks, or 27 days, of incubation for both Sugar Maple and Japanese Knotweed. The number of invertebrates per leaf pack is much higher than that found in the 2003 fall study (Sowers) in which

only 6 invertebrates were found on incubated Sugar Maple leaves. These leaves had only been incubated for two weeks. In the 2002 fall study, 22 invertebrates were collected from a Maple leaf pack that had been incubated for 28 days. This is less than half the amount of invertebrates found in the current study at 27 days.

The kick sample data showed that 18% of the invertebrates in the population of Mill Creek are scrapers, 18% are filterers and 4% are shredders (Figure 6). The remaining 60% belonged to other feeding groups.

At 13 days, 60% of the invertebrates found on the Sugar Maple leaves were filter collectors (Figure 3). Of the remaining invertebrates, 26% were collector gatherers, 7% were scrapers and 6% were predators. There were no shredders. On the Knotweed leaves, the highest percentage of invertebrates was also the filter collectors, which made up 69% of the total. Ten percent of the invertebrates were collector gatherers, 3% were scrapers, 3% were shredders and 15% were predators.

At 27 days, the filter collectors were the dominant feeding group, making up 54% of the total invertebrate on the Sugar Maple leaves and 59% of the total invertebrates on the Japanese Knotweed leaves(Figure 4) . The remaining invertebrates on the Sugar Maple were made up of 31% collector gatherers, 3% scrapers, 6% shredders, and 6% predators. The remaining invertebrates on the Japanese Knotweed were made up of 28% collector gatherers, 3% scrapers, 6% shredders, and 5% predators.

At 41 days, the filter collectors were still the dominant feeding group in the Japanese Knotweed leaf packets, making up 46% of the total invertebrates (Figure 5). However, the dominant feeding group in the Sugar Maple leaf packs was collector gatherers, which made up 38%. This feeding group had a high representation in the

Knotweed leaves at 35%. The filter collectors made up 35% of the total Sugar Maple invertebrates. In the Maple, the remaining feeding groups were 12% scrapers, 13% shredders, and 2% predators. On the Knotweed, there were 4% scrapers, 11% shredders, and 4% predators.

The trends in distribution of feeding groups are similar to that found in the previous studies by Sowers (2003) and Stricker (2001). Filter collectors and collector gatherers were the two most common feeding groups. The results of the 2002 fall study (Panko) showed a higher percentage of shredders in Mill Creek than were obtained in this study.

The number of taxa, or families, was counted in each sampling. The numbers of taxa followed the same pattern as the invertebrate densities for both the Maple and the Knotweed. The highest number of taxa occurred after 27 days (Table 5). There were 11 taxa for Sugar Maple after 13 days, 18 taxa after 27 days, and 14 taxa after 41 days. For Japanese Knotweed, there were 13 taxa at 13 days, 19 taxa at 27 days, and 11 taxa at 41 days.

The percentage of the taxa that were shredders was also calculated, since shredders are the most important feeding group for creating Fine Particulate Organic Matter. The percent taxa of shredders at 13 days were 0% for Maple and 8% for Knotweed (Table 6). At 27 days, 11% of the taxa on the Maple leaves were shredders and 21% on the Knotweed. At 41 days, shredders made up 7% of the Maple taxa and 18% of the Knotweed taxa. The percentage of shredders was greater on the Knotweed leaf packs at each sampling.

For both Sugar Maple and Japanese Knotweed, Hydropsychidae was the dominant taxa for all three samplings (Table 7). The lowest percentage was 28% occurring in the Maple leaf pack at 41 days. The highest percentage of Hydropsychidae was 61% occurring in the Knotweed leaf pack at 13 days.

The ratio of Ephemeroptera: Plecoptera: Trichoptera was determined and the number of genera in each family was counted. Trichoptera were the dominant family at all three samplings for both species. However, their dominance decreased. After 13 days, 53 out of 66 invertebrates found on the Sugar Maple belonged to the family trichoptera (Table 8). On Japanese Knotweed, 49 out of 56 invertebrates belonged to the family trichoptera. After 41 days, 60 out of 117 invertebrates found on the Sugar Maple were trichoptera (Table 10). This was a decrease from 80% in the first sample to 51% in the third. Similarly on the Knotweed, 59 out of 94 invertebrates were trichoptera which was a decrease from 89% to 63%. The data after 27 days of incubation fell between these two ranges (Table 9). The number of Ephemeroptera was twice that of Plecoptera throughout the study.

Simpson and Shannon diversity indexes were also determined for invertebrates collected from leave packs. The Simpson Diversity Index ranges from zero to one with higher numbers indicating a higher diversity. The Simpson Index was slightly higher for the Sugar Maple at all three sampling periods (Table 4). The highest diversity was obtained at week six for both leaf species. Sugar Maple had an index of 0.825 while Knotweed had an index of 0.767. The Shannon Diversity Index ranges from zero to infinity although values are usually between one and ten. At week two, the Shannon

Index was higher for Japanese Knotweed. At weeks four and six, the Shannon Index was higher for Sugar Maple.

Discussion

Japanese Knotweed was found to be a fast decomposer according to its k-value. Sugar Maple was found to be a slower decomposer. Since Knotweed decomposes faster, it is available as a nutrient source for a shorter period of time. Sugar Maple decomposed at a slower rate in this study than it had in the Stricker (2001) or Panko (2002) studies. However, these experiments did not use mesh bags for leaf incubation. The use of mesh bags has been found to slow decomposition rate (Cornelissen 1996). My results agreed with Sowers (2003) in which the mesh bags were utilized.

Organic content had a tendency to decrease throughout the incubation period. However, there was a slight increase in Sugar Maple organic content between weeks six and eight from 55.9% to 58.3%. Fungal biomass at week six was 1.8 ug/mg detritus. It increased to 3.3 ug/mg detritus at week eight. Fungal biomass may add to the organic content of the leaf and may have caused this inconsistency in the data. This could also explain the increase in percent organic content for Japanese Knotweed between weeks two and four. Organic content increased from 54.2% to 55.2%. There was no fungal biomass data for week two, so its effects cannot be concluded from the Knotweed data.

Organic content was much higher in Sugar Maple than in Japanese Knotweed. In previous studies, organic content had also been determined for Pin Oak and River Birch. Both of these leaf species contained more organic content than the Sugar Maple. Japanese

Knotweed has the lowest organic content of the leaf species that have been studied in ongoing research. The chemical composition of a leaf affects its rate of decomposition and fungal biomass accumulation. Japanese Knotweed has a very different chemical composition than the previously studied leaves. It is the only leaf species of the four that decomposes at a fast rate in the fall. It also has a low fungal biomass accumulation relative to the Sugar Maple in this study.

There was a greater accumulation of fungal biomass on the Sugar Maple than on the Japanese Knotweed. The Knotweed may be less nutritious for fungi than leaves with higher levels of fungal colonization (Barlocher 1984). These results may have differed if the Knotweed had been incubated in a stream ecosystem that had experienced Knotweed invasion of its banks, which has not occurred on Mill Creek. Leaves entering a water system will affect the species and diversity of the aquatic hyphomycetes living in the system. In a study by Barlocher and Graca (2002), exotic riparian vegetation was found to lower fungal diversity but did not change the number of fungal spores in the stream. Exotic plant species change fungal communities, causing different species to be dominant. Therefore, exotic vegetation may not be colonized by many fungi in a stream that has not experienced a change in fungal community to accommodate the particular plant species. The study by Barlocher and Graca also found that fungi are capable of breaking down uncommon species, which explains the fact that the Knotweed in Mill Creek did show some colonization. The fast rate of decomposition of Knotweed must be due to the chemical composition of the leaves as opposed to fungal decomposition. The non-incubated Knotweed leaves had 0.45 ug of fungal biomass per mg detritus. The fungal biomass on incubated Knotweed leaves peaked at 0.9 ug/mg detritus, only twice

the amount on the control. This indicates that there may have been less aquatic fungi colonizing the leaves than shown in the ergosterol data. Since dead fungi are detected during ergosterol extraction, fungi that colonized the leaves prior to incubation may remain on the leaf and interfere with test results.

The fungal biomass accumulation on Sugar Maple differed from that found in two of the previous studies. The highest value in this study was 4.6 ug/mg detritus obtained after four weeks of incubation. In Stricker's study (2001), the peak value for Sugar Maple fungal biomass accumulation in the fall was 1.12 ug/mg detritus found after 47 days of incubation. Stricker did not complete an aquatic hyphomycete spore analysis, so I cannot determine how the number of fungal spores in the water compares to the levels during my study. However, the levels of nitrate, nitrite, and orthophosphate during the fall 2004 were much higher than those during the fall of 2000. This could have stimulated microbial growth. In Panko's study (2002), the fungal biomass accumulation was slightly higher than that obtained in this study, with a peak of 5.00 ug/mg detritus. Panko (2002) performed aquatic hyphomycete spore analysis and had an average of 4,259 spores/L, while Sowers (2003) had a late fall average of 4,533 spores/L. The average spore count in this study was 6,708 spores/L. The amount of spores found in this study was much greater than that of the previous studies. This may have been a result of the high nutrient levels, as mentioned above.

Total numbers of invertebrates peaked at week four, which is also the week at which fungal biomass accumulation for Sugar Maple was highest. This is consistent with the data obtained in previous studies. Invertebrate densities tend to increase as fungal biomass increases. At week four, the number of invertebrates on the Japanese Knotweed

leaf pack was very close to the number found on the Sugar Maple leaf pack. However, the Knotweed did not experience a peak in fungal biomass accumulation until week six.

Invertebrate collection was performed differently than in the previous studies. I collected invertebrates from the leaves, the bricks, and the bags. In the previous three studies, invertebrates had been collected off of the leaves only. This affected total numbers, making a quantitative comparison difficult. However, tt is still possible to compare feeding group distributions.

There were no distinct patterns in feeding group distribution between the Maple and the Knotweed. Both species had filter collectors as the dominant group after two and four weeks of incubation. The dominant group on Sugar Maple after week six was the collector gatherers, while filter collectors remained dominant on the Knotweed. Both leaf species saw an increase in shredders throughout the study. The Simpson and Shannon Indexes indicated that Sugar Maple had a higher diversity of invertebrates. However, there was not enough data to determine if a significant relationship existed.

Feeding group distribution was similar in this experiment to that in two of the previous studies (2001) (2003), showing the filter collectors and collector gatherers as the dominant groups. In Panko (2002), shredders were found to be the dominant group. However, in that study no mesh bags, which may act to limit the size of invertebrates that have access to the leaf, were used. Shredders tend to be larger than filterers. In an experiment by Barlocher and Schweizer (1983), coarse bags with holes 3mm in size were used to allow invertebrates to enter and fine bags with holes 0.3mm in size were used to prevent invertebrates from entering. In my experiment the bags were 2.8 mm in size,

which should be sufficient to allow invertebrate to enter and would only limit entry of the largest invertebrates.

Filter collectors may have been the dominant feeding group because the mesh bags can act as filters. The mesh may help the invertebrates filter the water with less effort and therefore the bricks may appeal as a more favorable substrate than loose leaves in the water. This is consistent with the kick sample data. This data indicates that only 18% of the invertebrates in Mill Creek are filter collectors. Therefore since such a large percentage of filter feeders were found in the leaf packs, they must prefer them.

There was not enough data gathered to determine statistical significance in any part of this experiment. This was mainly due to high water events inhibiting access to the stream. I was not able to begin incubation until September 21, 2004 due to high water conditions. I had planned to remove leaf packs at ten weeks incubation to complete a full analysis of invertebrates, organic content, and ergosterol. I was not able to enter the water until the eleventh week and was prevented by time constraints from obtaining all of the data.

For future studies involving Japanese Knotweed, leaves should be incubated in a stream whose banks have been invaded by Knotweed. This could determine if the lack of fungal colonization is due to a difference in dominant fungal species. Aquatic hyphomycete spore analysis could be used to determine the differences between fungal communities in water ways invaded by Knotweed and those that are not. It is possible that some fungal biomass is being lost when the leaves are rinsed off. This rinse water could be tested for the presence of ergosterol.

Bibliography

American Public Health Association. 1995. Standard methods for the examination of water and wastewater. American Public Health Association. New York, NY.

Barajas-Aceves, Martha, Mainul Hassan, Ranuel Tinoco, Rafeal Vazquez-Duhalt. 2002. Effect of pollutants on the ergosterol content as indicator of fungal biomass. Journal of Microbiological Methods. 50: 227-236.

Barlocher, Felix and Martin Schweizer. 1983. Effects of leaf size and decay rate on colonization by aquatic hyphomycetes. Oikos. 41: 205-210.

Barlocher, Felix. 1985. The role of fungi in the nutrition of stream invertebrates. Botanical Jounral of the Linnean Society. 91: 83-94.

Barlocher, Felix and Manuel A. S. Graca. 2002. Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams. Freshwater Biology. 47: 1123-1135.

Barlocher, Felix and Miranda Corkum. 2003. Nutrient enrichment overwhelms diversity effects in leaf decomposition by stream fungi. Oikos. 101: 247-252.

Benfield, E. F. 1996. Leaf breakdown in stream ecosystems. Methods in Stream Ecology, 27: 579-589.

Cornelissen, J.H.C. 1996. An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. Journal of Ecology. 84: 573-582.

Dangles, Olivier, Mark O. Gessner, Francois Guerold, and Eric Chauvet. 2004. Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. Journal of Applied Ecology. 41: 365-378.

Ecology and Management of Invasive Plants Program. 2002, December 11. Japanese Knotweed. http://www.invasiveplants.net/invasiveplants/InvasivePlants/Knotweed/Knotweed.asp. Retrieved on April 11, 2005.

Gessner, M.O. and E. Chauvet. 1992. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Applied Environmental Microbiology 59(2): 502-507.

Gessner, M.O. and A.L. Schmitt. 1996. Use of solid-phase extraction to determine ergosterol concentrations in plant tissue colonized by fungi. Applied Environmental Microbiology 62: 415-419.

Graca, Manual A.S. 2001. The role of invertebrates on leaf litter decomposition in streams- a review. International Review of Hydrobiology. 86: 383-393.

Gulis, V. and K. Suberkropp. 2003. Leaf litter decomposition and microbial activity in a nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biology. 48: 123-134.

Gulis, Vladislav, Amy D. Rosemond, Keller Suberkropp, Holly S. Weyers, and Jonathan P. Benstead. 2004. Effects of nutrient enrichment on the decomposition of wood and associated microbial activity in streams. Freshwater Biology. 49: 1437-1447.

Hilsenhoff, W.L. Rapid field assessment of organic pollution with a family-level biotic index. Am. Benthol. Soc 7(1): 65-68; 1988.

Houlahan, Jeff E. and C. Scott Findlay. Effect of invasive plant species on temperate wetland palnt diversity. 2004. Conservation Biology. 18 (4): 1132-1138.

Invasive Species.gov. 2005, April 7. Species Profiles. http://www.invasivespecies.gov/profiles/japktwd.shtml. Retrieved April 11, 2005.

Meyer, Judy L. and Carol Johnson. 1983. The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. Freshwater Biology, 13: 177-183.

Mille-Lindblom, Cecilia, Eddie von Wachenfeldt, Lars J. Tranvik. 2004. Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death. Journal of Microbiological Methods. 59: 253-262.

Newell, S.Y., T.L. Arsuffi, R.D. Fallon. 1988. Fundamental procedures for determining egosterol content of decaying plant material for liquid chromatography. Applied Environmental Microbiology 54: 1876-1879.

Panko, Christina. 2002. The Comparison of Leaf Processing Rates in Streams, Percent Organic Content, and Fungal Biomass in the Summer vs. Fall/Early Winter. Unpublished Honors Thesis.

Pascoal, Claudia, Fernando Cassio, and Pedro Gomes. 2001. Leaf breakdown rates: a measure of water quality? International Review of Hydrobiology. 56: 407-416.

Peterson, R.C. and K.W. Cummins. 1974. Leaf Processing in a woodland stream. Freshwater Biology 4: 343-368.

Plafkin, J.L., M.T. Barbous, K.D. Porter, S.K. Grossi, and R.M. Hughes. 1989. Rapid Bioassessment Protocols for use in Streams and Rivers: Benthic Macroinvertebrates and Fish. United States Environmental Protection Agency, EPA/440/4-89/001.

Raviraja, N. S. K.R. Sridhar, and Felix Barlocher. 1996. Breakdown of introduced and native leaves in two Indian streams. Internationale Revue Der Gesamten Hydrobiologie. 81: 529-539.

Sowers, Anthony. 2003. The Determination of Leaf Processing Rates and Fungal Biomass via a Chemical Index. Unpublished Honors Thesis.

Sridhar, K. R., and Felix Barlocher. 2000. Initial Colonization, Nutrient Supply, and Fungal Activity on Leaves Decaying in Streams. Applied and Environmental Microbiology. 66(3): 1114-1119.

Stricker, Emily. 2001. Leaf Processing in Streams and the Determination of Fungal Biomass via a Chemical Index. Unpublished Honors Thesis.

Suberkropp, Keller. 2001. Fungal growth, production, and sporulation during leaf decomposition in two streams. Applied and Environmental Microbiology. 67: 5063-5068.

Vila, Montserrat and Jacob Weiner. 2004. Are invasive plant species better competitors than native plant species?- evidence from pair-wise experiments. Oikos. 105: 229-238.

Volker, E.J., D. DiLella, K. Terneus, C. Baldwin, I. Volker. 2000. Journal of Chemical Education 77: 1621

Waters. http://www.waters.com (retrieved on April 20, 2005).

Table 1: Habitat Assessment

Parameter	Score for In-stream Characteristics	Bank Characteristics Right Bank	Left Bank
Instream Cover	13		
Epifaunal Substrate	12		
Embededness	16		
Velocity/Depth Regimes	14		
Channel Alteration	12		
Sediment Deposition	10		
Frequency of Riffles	13		
Channel Flow Status	14		
Condition of Bank		16	16
Bank Vegetative Protection		10	10
Grazing/Other Disruptions		8	13
Riparian Zone Width		2	2

Table 2: Water Chemistry

Date	Incubation time	pH	Alkalinity (ppm)	Orthophosphate (ppm)	Total Phosphorous (ppm)	Nitrate (ppm)	Nitrite (ppm)	DO (ppm)	Temp °C
21-Sep	0 days	6.92	15	0.46	0.61	1.78	0.01	10.15	14.6
4-Oct	13 days	6.73	17.5	0.15	0.23	0.9	0.0065	8.42	13
18-Oct	27 days	7.23	16	1.37	2	5.05	0.276	13.5	10.4
1-Nov	41 days	6.64	6	5.5	5.5	4.6	0.066	9.02	11
15-Nov	55 days	6.48	5.5	1.74	4.22	0.85	0.018	11.83	4.1

Additional Chemical and Physical Characteristics
(obtained September 21)

Conductivity (mi	56.83
Total Dissolved	70.25
Biological Oxyge	3.25
Width (m)	9.6
Volume (m^3)	2.03
Percent Oxygen	103%

Table 3: average k-values

Week	Maple avg k-value	decomposition speed
2	0.001212 ± 0.000486	slow
4	0.00076 ± 0.0016	slow
6	0.0085 ± 0.0054	medium
8	0.00068 ± 0.00068	slow
Average	0.00278 ± 0.003815	slow

Week	Knotweed avg k-value	decomposition speed
2	0.018 ± 0.016	fast
4	0.023 ± 0.035	fast
6	0.0081 ± 0.005	medium
8	0.0097 ± 0.0097	medium
Average	0.0147 ± 0.007032	fast

Table 4: Total Number Invertebrates

Date	Species	Total Number
10/4/2004	Maple	69
	Knotweed	62
10/18/2004	Maple	176
	Knotweed	178
11/1/2004	Maple	165
	Knotweed	130

Table 5: Number of Taxa

Date	Species	Number Taxa	Number Individuals	Simpson	Shannon (H')
10/4/2004	Maple	11	69	0.6636	2.212
	Knotweed	13	62	0.6176	2.261
10/18/2004	Maple	18	176	0.7483	2.692
	Knotweed	19	178	0.7038	2.644
11/1/2004	Maple	14	165	0.8252	2.88
	Knotweed	11	130	0.7674	2.586

Table 6: Percentage of Taxa Shredders

Date	Species	% Taxa Shredders
10/4/2004	Maple	0
	Knotweed	8
10/18/2004	Maple	11
	Knotweed	21
11/1/2004	Maple	7
	Knotweed	18

Table 7: Dominant Taxa

Date	Species	Dominant Taxa	Percentage
10/4/2004	Maple	Hydropsychidae	55
	Knotweed	Hydropsychidae	61
10/18/2004	Maple	Hydropsychidae	44
	Knotweed	Hydropsychidae	51
11/1/2004	Maple	Hydropsychidae	28
	Knotweed	Hydropsychidae	42

Table 8: E:P:T ratio at 13 days

	Ephemeroptera	Plecoptera	Trichoptera
Maple			
Total Number	11	2	53
Percentage	17	3	80
Number Genera	5	1	3
Knotweed			
Total Number	3	3	49
Percentage	5.5	5.5	89
Number Genera	2	2	4

Table 9: E:P:T ratio at 27 days

	Ephemeroptera	Plecoptera	Trichoptera
Maple			
Total Number	22	8	96
Percentage	17	6	76
Number Genera	3	6	5
Knotweed			
Total Number	19	4	113
Percentage	14	3	83
Number Genera	7	2	8

Table 10: E:P:T ratio at 41 days

	Ephemeroptera	Plecoptera	Trichoptera
Maple			
Total Number	35	22	60
Percentage	30	19	51
Number Genera	7	2	3
Knotweed			
Total Number	23	12	59
Percentage	24	13	63
Number Genera	1	6	2

Figure 1: Comparison of Percent Organic Content

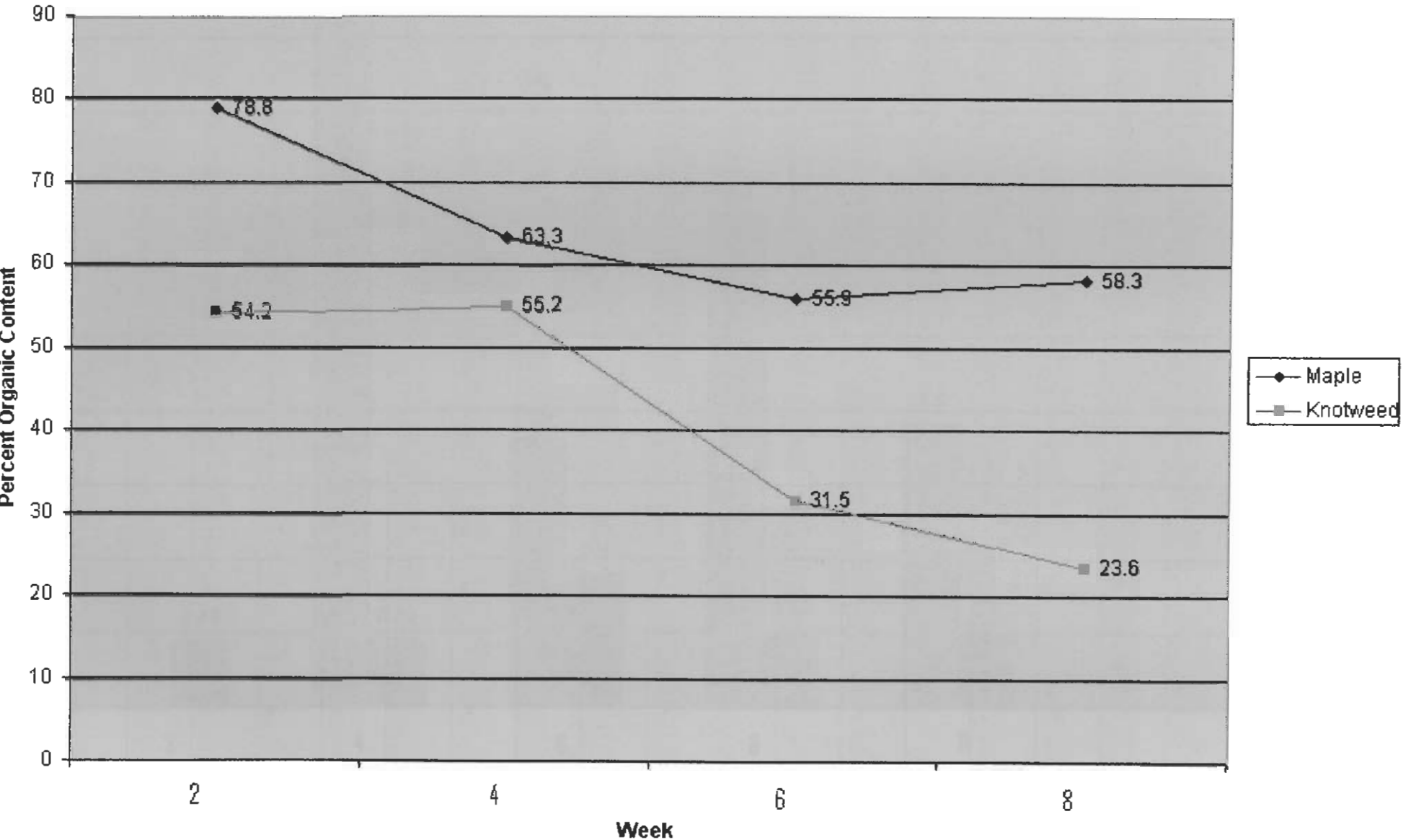


Figure 2: Comparison of Fungal Biomass

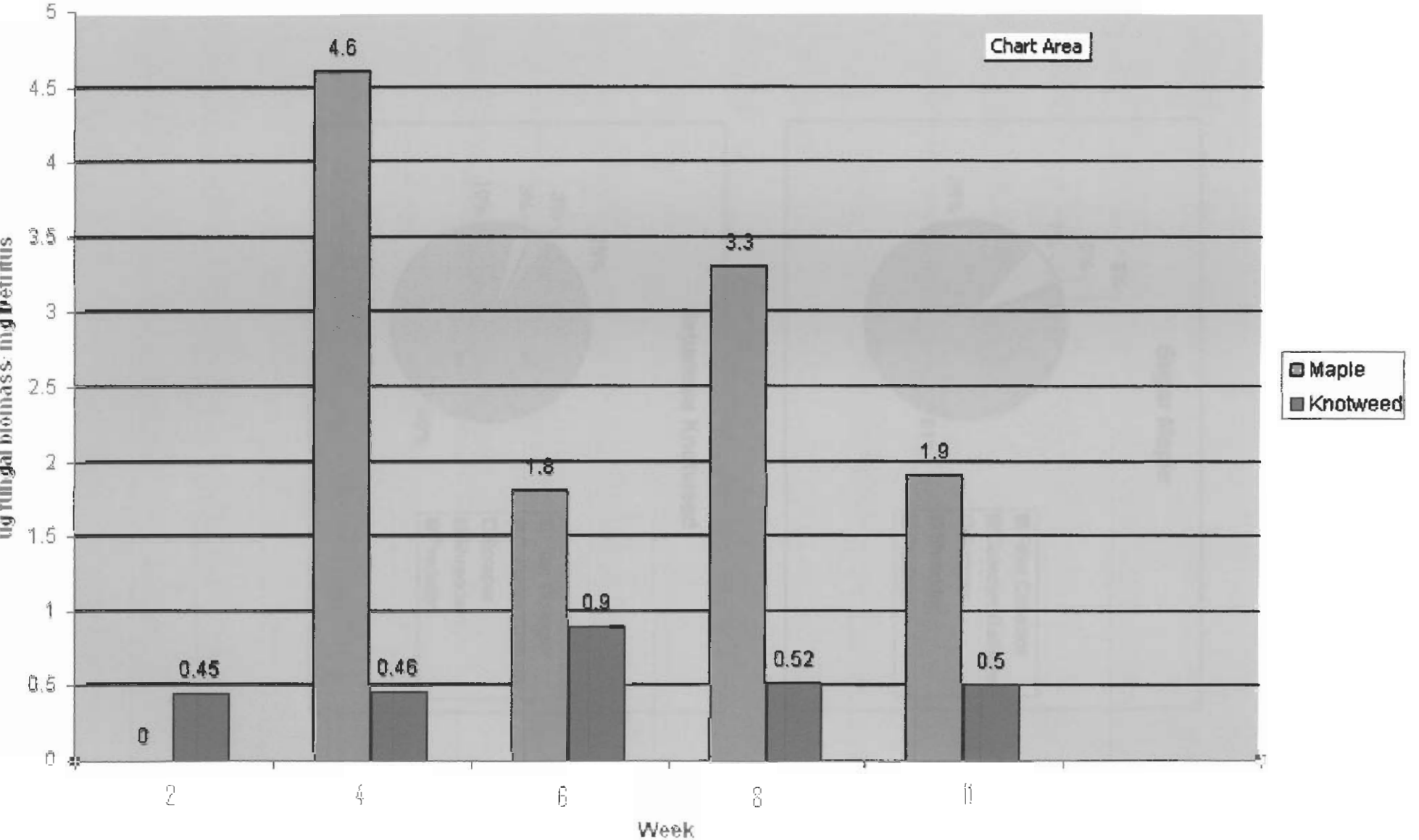
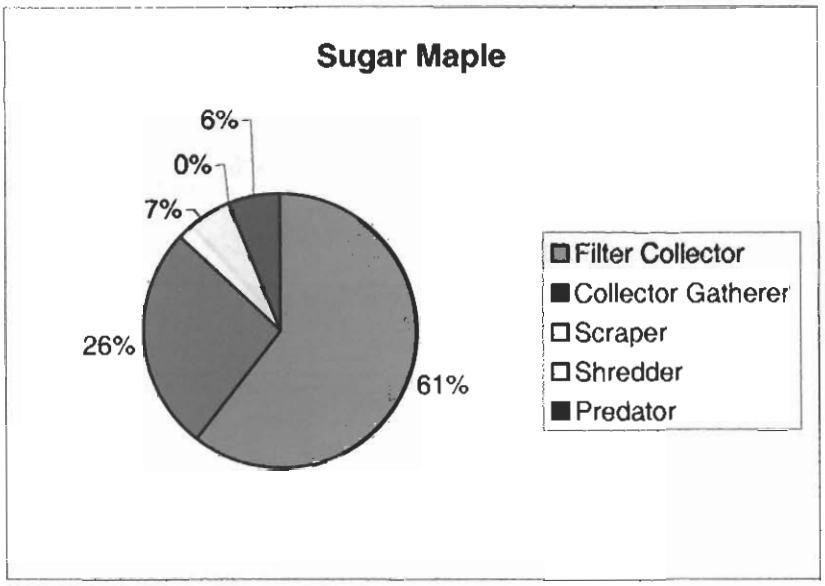


Figure 3: Functional Feeding Groups after 13 days



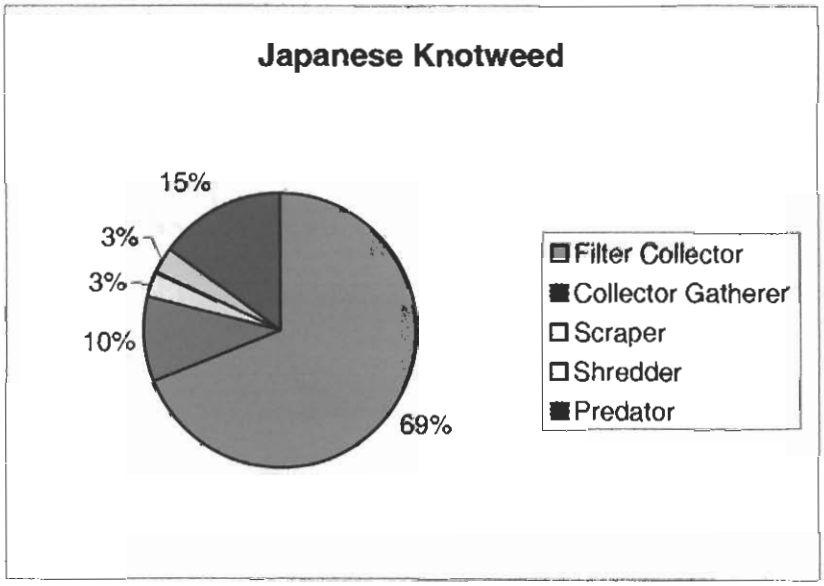
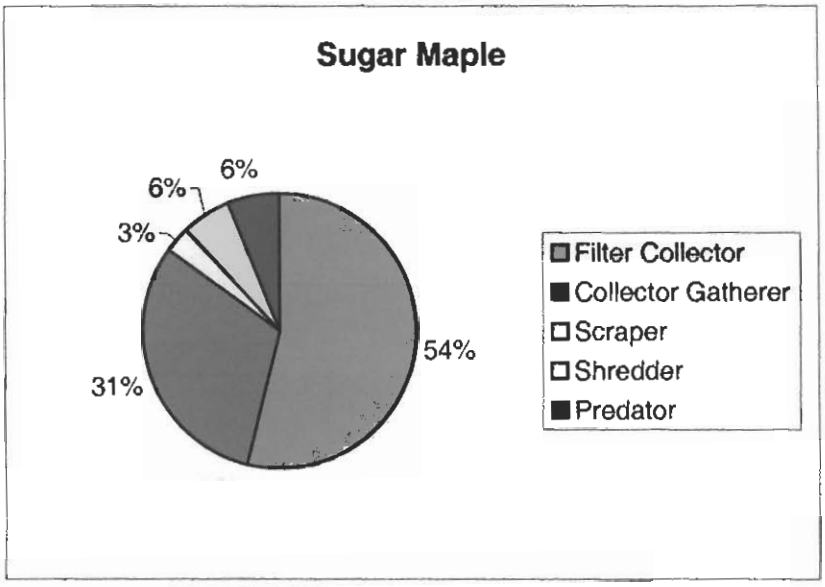


Figure 4: Functional Feeding Groups at 27 days



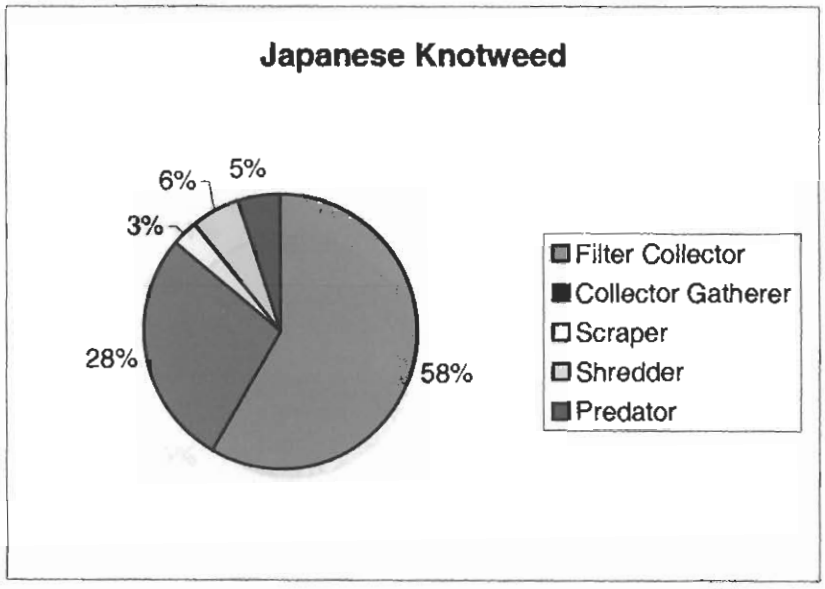
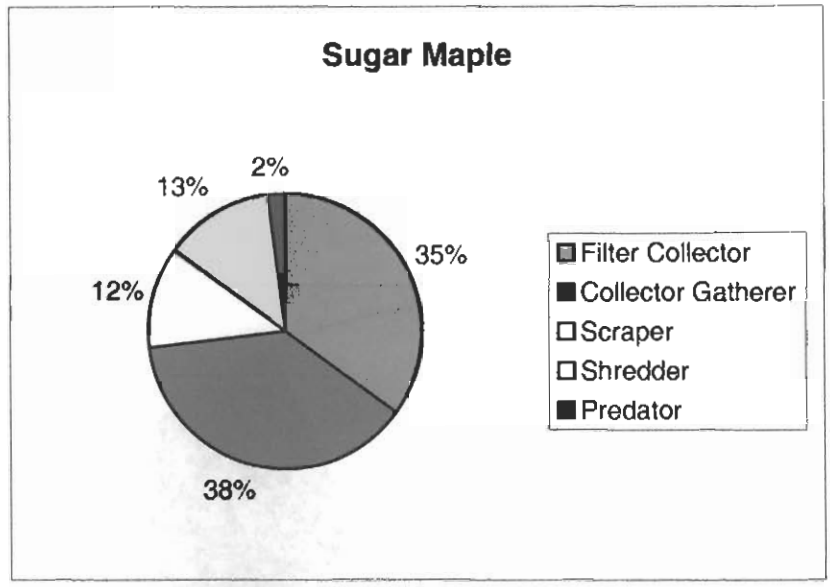


Figure 5: Functional Feeding Groups after 41 days



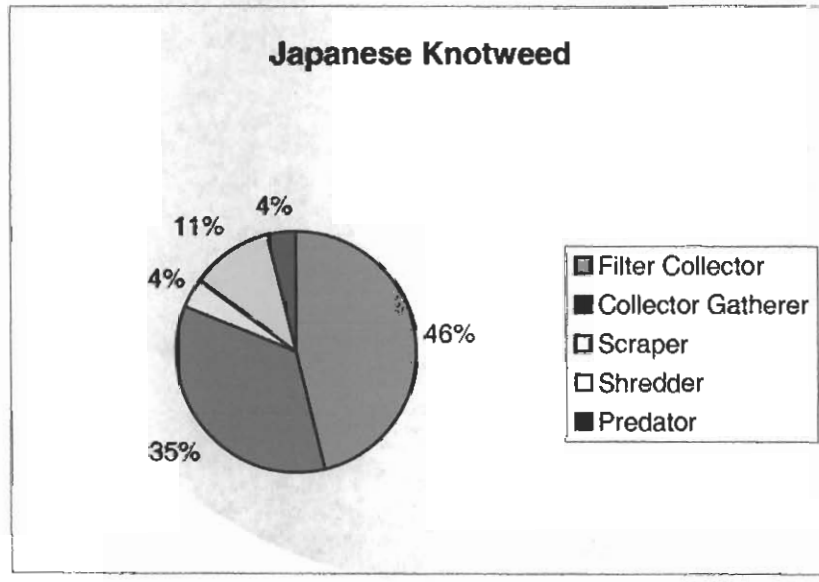
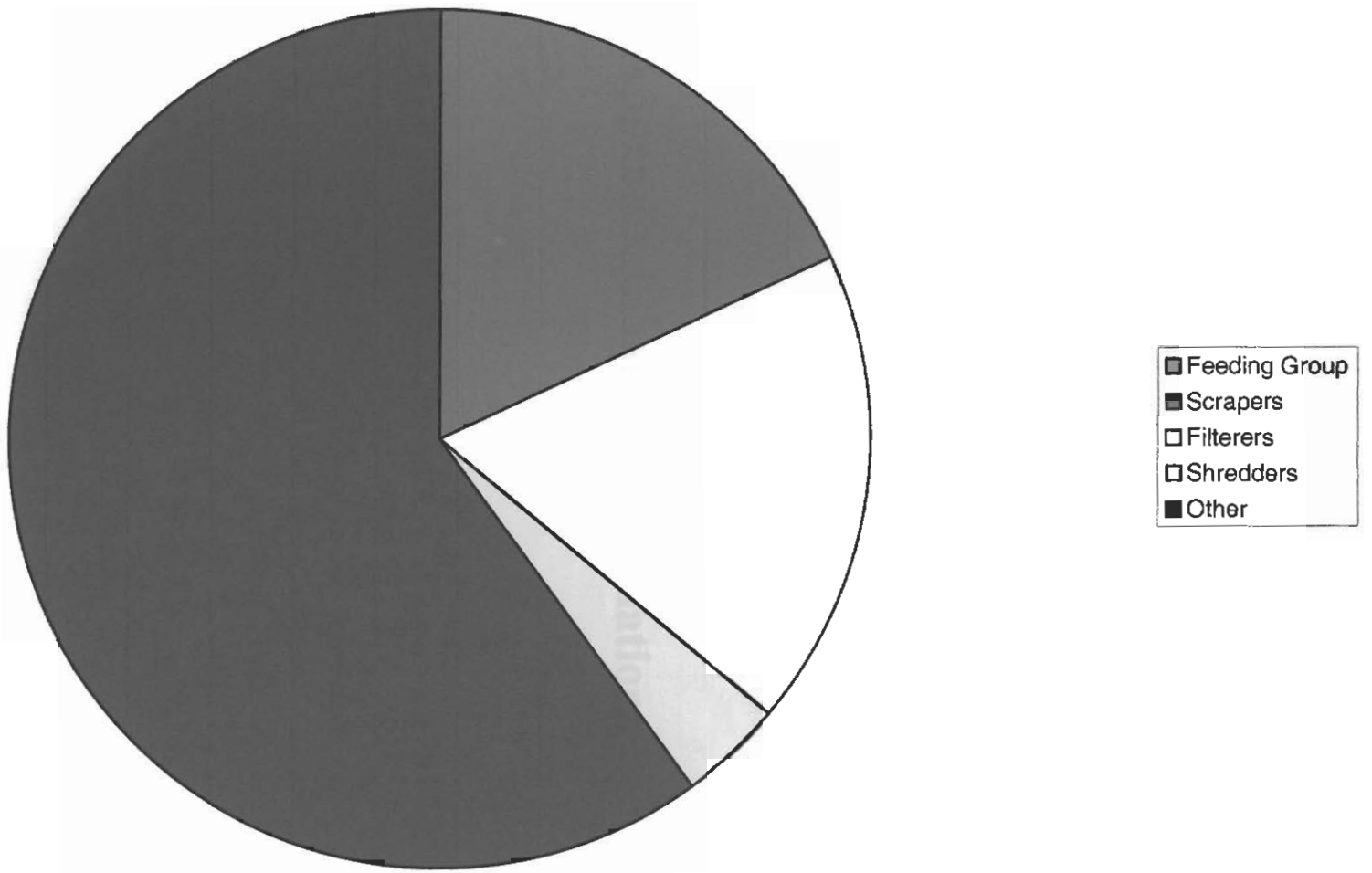


Figure 6: Kick Sample Feeding Groups



Appendix I: Sample Calculations

Sample Calculations

1. Percent Organic Content:

(Crucible weight + pre-burn weight)- post burn weight = ash free dry weight
(ash free dry weight/ pre-burn weight) * 100% = percent organic content

2. Leaf processing Rates:

-[{ln(post-incubation surface area/ pre-incubation surface area}/incubation length]

3. Working Standard curve:
Inject amounts of ergosterol standard to establish a working curve

Injection Volume (uL)	Standard Concentration (ug/mL)	Inj. Vol x Standard vol	Injected amount (ug)
50	1.268	(0.05 mL) (1.268ug/mL)	0.0634
77	1.268	(0.077 mL) ((1.268ug/mL)	0.0976
98	1.268	(0.098 mL) (1.268ug/mL)	0.1243
20	25.365	(0.02 mL) (25.365 ug/mL)	0.5073
42	25.365	(0.042 mL) (25.365 ug/mL)	1.065
50	25.365	(0.05 mL) (25.365 ug/mL)	1.268
60	25.365	(0.06 mL) (25.365 ug/mL)	1.522

4. Equation of a Line
Once the working curve is plotted on the Kaleidagraph, the equation of a line was found by linear fit to be:

ug ergosterol = 1.1977 10^(-6) (Peak Area) - 0.0021235 R=0.99884

5. ug ergosterol in leaf sample:

a.) ug in injected sample from the equation of the line and the peak area

Maple at 6 weeks: 1.1977 10^(-6) (697255) - 0.0021235 = 0.834 ug

b.) injected concentration = $\frac{\text{ug ergosterol}}{\text{Injected volume (0.050 uL)}}$

$\frac{0.83249 \text{ ug}}{0.050 \text{ uL}}$ = 16.68 ug/mL ergosterol

c.) Accounting for dissolution volume

$$\frac{\text{(ug ergosterol)}}{1.00\ \text{mL}} \times 1.00\ \text{mL} = \text{ug ergosterol/ refluxed sample}$$

$$\frac{16.68\ \text{ug}}{1.00\ \text{mL}} \times 1.00\ \text{mL} = 16.68\ \text{ug ergosterol/ refluxed sample}$$

6. Weight % Ergosterol:

$$\frac{\text{Total ug ergosterol/ 15 discs}}{\text{Leaf Weight, mg/ 15 discs}} = \text{mg erg/ mg leaf}$$

$$\frac{16.68\ \text{ug erg}}{168.4\ \text{mg leaf}} = 0.099 \times 100\% = 9.9\%$$

7. Fungal Biomass Determination:

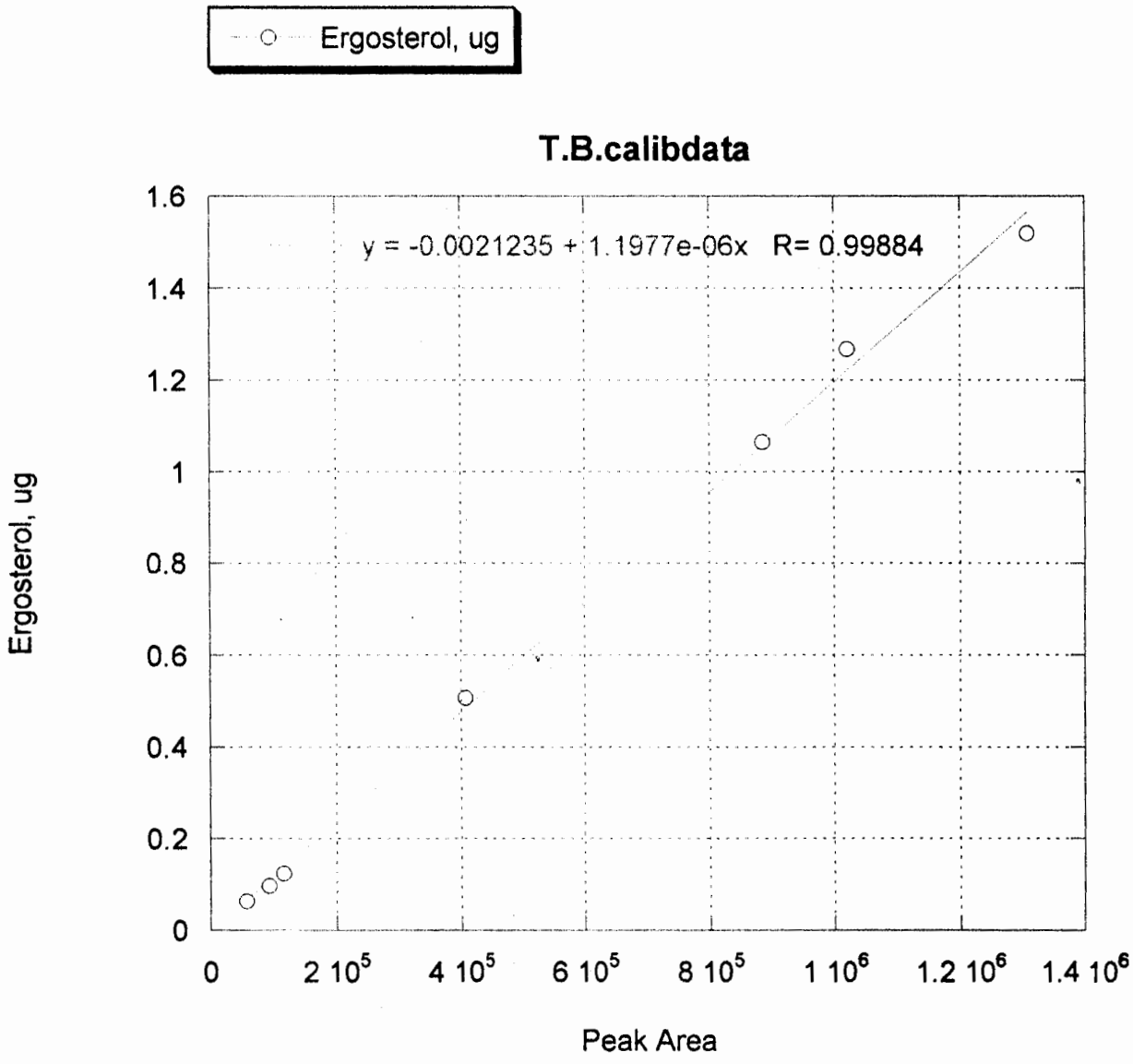
$$\frac{\text{ug ergosterol}}{\text{mg leaf}} \times \frac{182.6\ \text{g fungal biomass}}{\text{g ergosterol}} = \frac{\text{mg fungal biomass}}{\text{mg leaf}}$$

Appendix II: Invertebrate Index

Leaf Species	Incubation (days)	Order	Family	Genus	Feeding Group	Count
Sugar Maple	13	Plecoptera	Chloroperlidae	Haploperla	P	2
Sugar Maple	13	Ephemeroptera	Baetidae	Acentrella	CG	1
Sugar Maple	13	Ephemeroptera	Baetidae	Baetis	CG	3
Sugar Maple	13	Ephemeroptera	Ephemerellidae	Serratella	CG	1
Sugar Maple	13	Ephemeroptera	Heptagenidae	Stenonema	SC	5
Sugar Maple	13	Ephemeroptera	Isonychiidae	Isonychia	FC	1
Sugar Maple	13	Odonata	Gomphidae	Gomphus	P	1
Sugar Maple	13	Trichoptera	Hydropsychidae	Hydropsyche	FC	38
Sugar Maple	13	Trichoptera	Hydroptilidae	Ochrotrichia	CG	12
Sugar Maple	13	Trichoptera	Polycentropidae	Polycentropus	FC	3
Sugar Maple	13	Diptera	Chironomidae		CG	1
Sugar Maple	13	Diptera	Empididae		P	1
Japanese Knotweed	13	Isopoda	Asellidae	Caecidotea	SH	1
Japanese Knotweed	13	Plecoptera	Perlodidae	Isogenoides	P	1
Japanese Knotweed	13	Plecoptera	Taeniopterygidae	Strophopteryx	SH	2
Japanese Knotweed	13	Ephemeroptera	Ephemerellidae	Drunella	CG	2
Japanese Knotweed	13	Ephemeroptera	Heptogeniidae	Cinygmula	SC	1
Japanese Knotweed	13	Megaloptera	Corydalidae	Nigronia	P	1
Japanese Knotweed	13	Trichoptera	Brachycentridae	Brachycentrus	FC	4
Japanese Knotweed	13	Trichoptera	Hydropsychidae	Hydropsyche	FC	38
Japanese Knotweed	13	Trichoptera	Leptoceridae	Oecetis	P	4
Japanese Knotweed	13	Trichoptera	Rhyacophilidae	Rhyacophila	P	3
Japanese Knotweed	13	Coleoptera	Psephenidae	Psephenus	SC	1
Japanese Knotweed	13	Diptera	Chironimidae		CG	3
Japanese Knotweed	13	Diptera	Tipulidae	Antocha	CG	1
Sugar Maple	27	Annelida	Oligocheata			1
Sugar Maple	27	Plecoptera	Perlodidae	Isoperla	P	6
Sugar Maple	27	Plecoptera	Pteronarcidae	Pteronarcys	SH	1
Sugar Maple	27	Plecoptera	Taeniopterygidae	Strophopteryx	SH	1
Sugar Maple	27	Ephemeroptera	Baetidae	Baetis	CG	3
Sugar Maple	27	Ephemeroptera	Ephemerellidae	Ephemerella	CG	8
Sugar Maple	27	Ephemeroptera	Heptagenidae	Stenacron	CG	2
Sugar Maple	27	Ephemeroptera	Heptagenidae	Stenonema	SC	6
Sugar Maple	27	Ephemeroptera	Leptophlebiidae	Leptophlebia	CG	2
Sugar Maple	27	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	CG	1
Sugar Maple	27	Trichoptera	Brachycentridae	Brachycentrus	FC	1
Sugar Maple	27	Trichoptera	Hydropsychidae	Hydropsyche	FC	78
Sugar Maple	27	Trichoptera	Polycentropidae	Neureclipsis	FC	15
Sugar Maple	27	Trichoptera	Psychomiidae	Psychomyia	CG	1
Sugar Maple	27	Trichoptera	Rhyacophilidae	Rhyacophila	P	1
Sugar Maple	27	Coleoptera	Ptilodactylidae	Anchytarsus	SH	7
Sugar Maple	27	Diptera	Athericidae		P	3
Sugar Maple	27	Diptera	Chironomidae		CG	37
Sugar Maple	27	Diptera	Empididae		P	1
Sugar Maple	27	Diptera	Tipulidae	Tipula	SH	1
Japanese Knotweed	27	Annelida	Oligocheata			1
Japanese Knotweed	27	Isopoda	Asellidae	Caecidotea	SH	1
Japanese Knotweed	27	Plecoptera	Perlidae	Eccoptura	P	1
Japanese Knotweed	27	Plecoptera	Perlodidae	Isoperla	P	3
Japanese Knotweed	27	Ephemeroptera	Baetidae	Baetis	CG	1

Japanese Knotweed	27	Ephemeroptera	Ephemerellidae	Ephemerella	CG	4
Japanese Knotweed	27	Ephemeroptera	Ephemerellidae	Serratella	CG	3
Japanese Knotweed	27	Ephemeroptera	Heptagenidae	Stenacron	CG	1
Japanese Knotweed	27	Ephemeroptera	Heptagenidae	Stenonema	SC	4
Japanese Knotweed	27	Ephemeroptera	Leptophlebiidae	Choroterpes	CG	2
Japanese Knotweed	27	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	CG	4
Japanese Knotweed	27	Trichoptera	Brachycentridae	Brachycentrus	FC	4
Japanese Knotweed	27	Trichoptera	Brachycentridae	Micrasema	SH	1
Japanese Knotweed	27	Trichoptera	Hydropsychidae	Hydropsyche	FC	91
Japanese Knotweed	27	Trichoptera	Phryganeidae		SH	2
Japanese Knotweed	27	Trichoptera	Polycentropidae	Neureclipsis	FC	6
Japanese Knotweed	27	Trichoptera	Polycentropidae	Polycentropus	FC	2
Japanese Knotweed	27	Trichoptera	Psychomiidae	Psychomyia	CG	3
Japanese Knotweed	27	Trichoptera	Rhyacophilidae	Rhyacophila	P	3
Japanese Knotweed	27	Coleoptera	Ptilodactylidae	Anchytarsus	SH	2
Japanese Knotweed	27	Diptera	Athericidae		P	2
Japanese Knotweed	27	Diptera	Blephariceridae		SC	1
Japanese Knotweed	27	Diptera	Chironomidae		CG	31
Japanese Knotweed	27	Diptera	Tipulidae	Tipula	SH	5
Sugar Maple	41	Annelida	Oligocheata			1
Sugar Maple	41	Plecoptera	Leuctridae	Leuctra	SH	21
Sugar Maple	41	Plecoptera	Perlidae	Neoperla	P	1
Sugar Maple	41	Ephemeroptera	Baetidae	Baetis	CG	2
Sugar Maple	41	Ephemeroptera	Ephemerellidae	Ephemerella	CG	3
Sugar Maple	41	Ephemeroptera	Ephemerellidae	Serratella	CG	10
Sugar Maple	41	Ephemeroptera	Heptagenidae	Cinygmula	SC	1
Sugar Maple	41	Ephemeroptera	Heptagenidae	Stenacron	CG	1
Sugar Maple	41	Ephemeroptera	Heptagenidae	Stenonema	SC	13
Sugar Maple	41	Ephemeroptera	Leptophlebiidae	Leptophlebia	CG	5
Sugar Maple	41	Trichoptera	Hydropsychidae	Hydropsyche	FC	47
Sugar Maple	41	Trichoptera	Limnephilidae	Apatania	SC	2
Sugar Maple	41	Trichoptera	Polycentropidae	Neureclipsis	FC	11
Sugar Maple	41	Coleoptera	Psephenidae	Psephenus	SC	3
Sugar Maple	41	Diptera	Athericidae		P	2
Sugar Maple	41	Diptera	Chironomidae		CG	41
Sugar Maple	41	Diptera	Tipulidae	Hexatome	P	1
Japanese Knotweed	41	Annelida	Oligocheata			3
Japanese Knotweed	41	Plecoptera	Leuctridae	Leuctra	SH	12
Japanese Knotweed	41	Ephemeroptera	Ephemerellidae	Attenella	CG	1
Japanese Knotweed	41	Ephemeroptera	Ephemerellidae	Ephemerella	CG	9
Japanese Knotweed	41	Ephemeroptera	Ephemerellidae	Serratella	CG	6
Japanese Knotweed	41	Ephemeroptera	Heptagenidae	Heptagenia	SC	2
Japanese Knotweed	41	Ephemeroptera	Heptagenidae	Stenonema	SC	2
Japanese Knotweed	41	Ephemeroptera	Leptophlebiidae	Leptophlebia	CG	3
Japanese Knotweed	41	Trichoptera	Hydropsychidae	Hydropsyche	FC	54
Japanese Knotweed	41	Trichoptera	Polycentropidae	Neureclipsis	FC	5
Japanese Knotweed	41	Coleoptera	Psephenidae	Ectopria	SC	1
Japanese Knotweed	41	Coleoptera	Ptilodactylidae	Anchytarsus	SH	2
Japanese Knotweed	41	Diptera	Athericidae		P	5
Japanese Knotweed	41	Diptera	Chironomidae		CG	25

Appendix III: Ergosterol Standard Curve

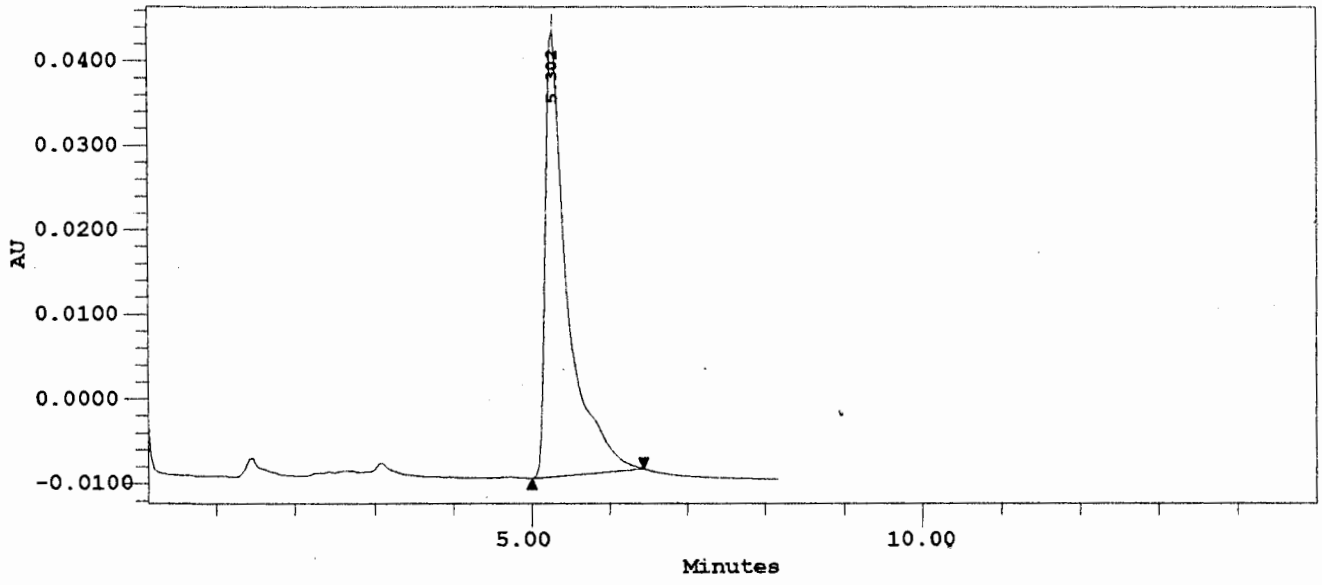

Ergosterol, ug
T.B.calibdata
y = -0.0021235 + 1.1977e-06x R= 0.99884
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2 10^5
4 10^5
6 10^5
8 10^5
1 10^6
1.2 10^6
1.4 10^6
Peak Area
Ergosterol, ug

Appendix IV: Sample HPLC Chromatograms

Millennium Results Report		September 30, 1988		Page: 1 of 1
Report Method:	Default		Version: 2.15	
For Sample:	25.365 std	Vial: 9	Injection: 1	Channel: 991M
Proc Chan:	282		Processed: 09/30/88 04:19:55 PM	
Channel Descr:	PDA 282.0 nm			

Millennium Sample Information

Project Name:	Ergosterol		
Sample Name:	25.365 std - Standard		
Vial:	9	Sample Type:	Unknown
Injection:	1	Volume:	50.00 µL
Channel:	991M	Run Time:	15.0 min
Date Acquired:	09/30/88 04:11:24 PM	Date Processed:	09/30/88 04:19:55 PM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	Ergosterol_MS		
Processing Method:	Ergosterol_PM		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1		5.302	1021087	52936		BB

Report Method: Default Version: 2.15

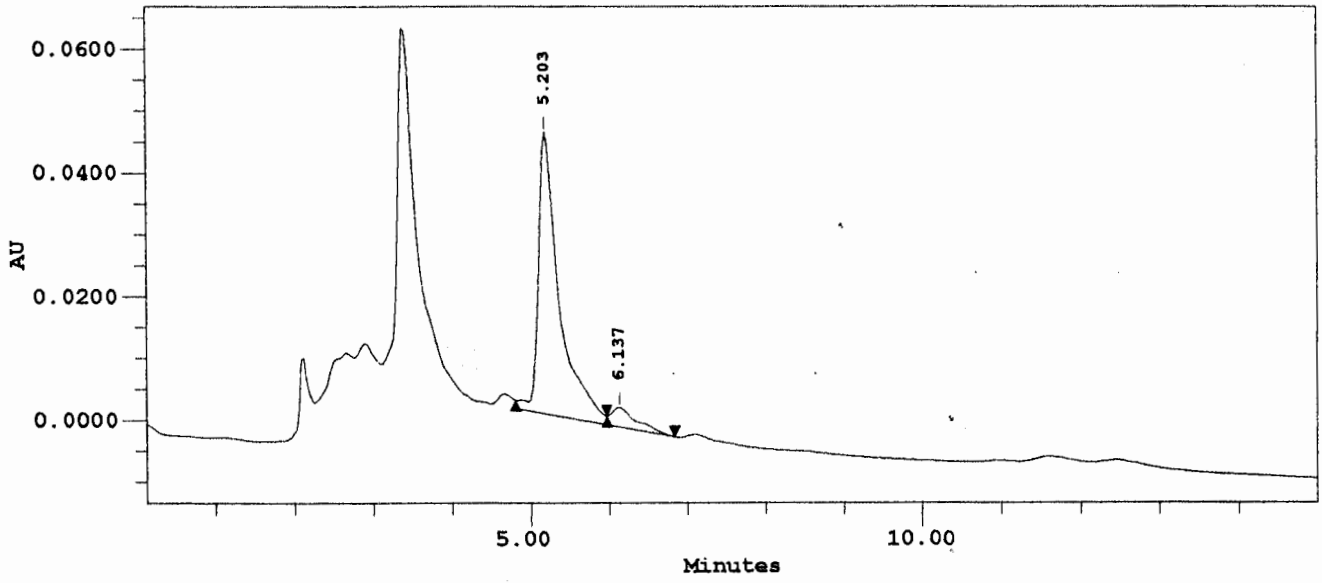
For Sample: Maple1 Vial: 1 Injection: 1 Channel: 991M

Proc Chan: 282 Processed: 10/21/88 11:12:07 AM

Channel Descr: PDA 282.0 nm

Millennium Sample Information

Project Name:	Ergosterol		
Sample Name:	Maple1 at week 4		
Vial:	1	Sample Type:	Unknown
Injection:	1	Volume:	50.00 µL
Channel:	991M	Run Time:	15.0 min
Date Acquired:	10/21/88 10:56:39 AM	Date Processed:	10/21/88 11:12:07 AM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	Ergosterol_MS		
Processing Method:	Ergosterol_PM		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1		5.203	846664	45674		VV
2		6.137	70773	3162		VB

Report Method: Default Version: 2.15

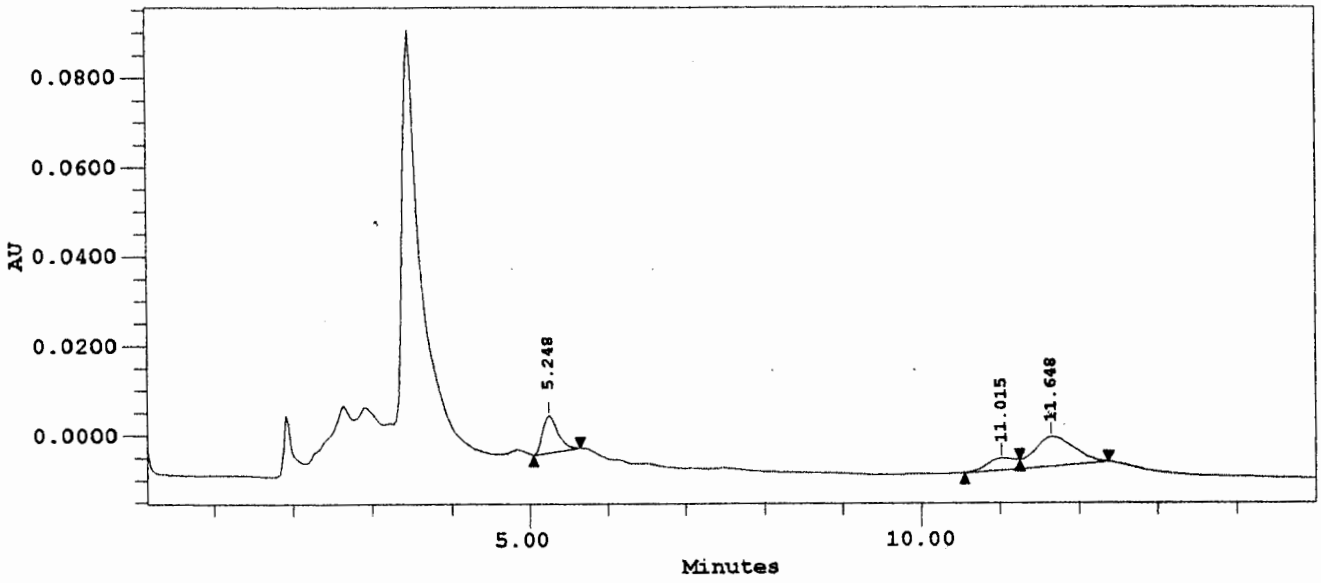
For Sample: Knotweed1 Vial: 4 Injection: 1 Channel: 991M

Proc Chan: 282 Processed: 10/21/88 12:58:33 PM

Channel Descr: PDA 282.0 nm

Millennium Sample Information

Project Name:	Ergosterol		
Sample Name:	Knotweed1 at week 4		
Vial:	4	Sample Type:	Unknown
Injection:	1	Volume:	50.00 mL
Channel:	991M	Run Time:	15.0 min
Date Acquired:	10/21/88 12:26:21 PM	Date Processed:	10/21/88 12:58:33 PM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	Ergosterol_MS		
Processing Method:	Ergosterol_PM		



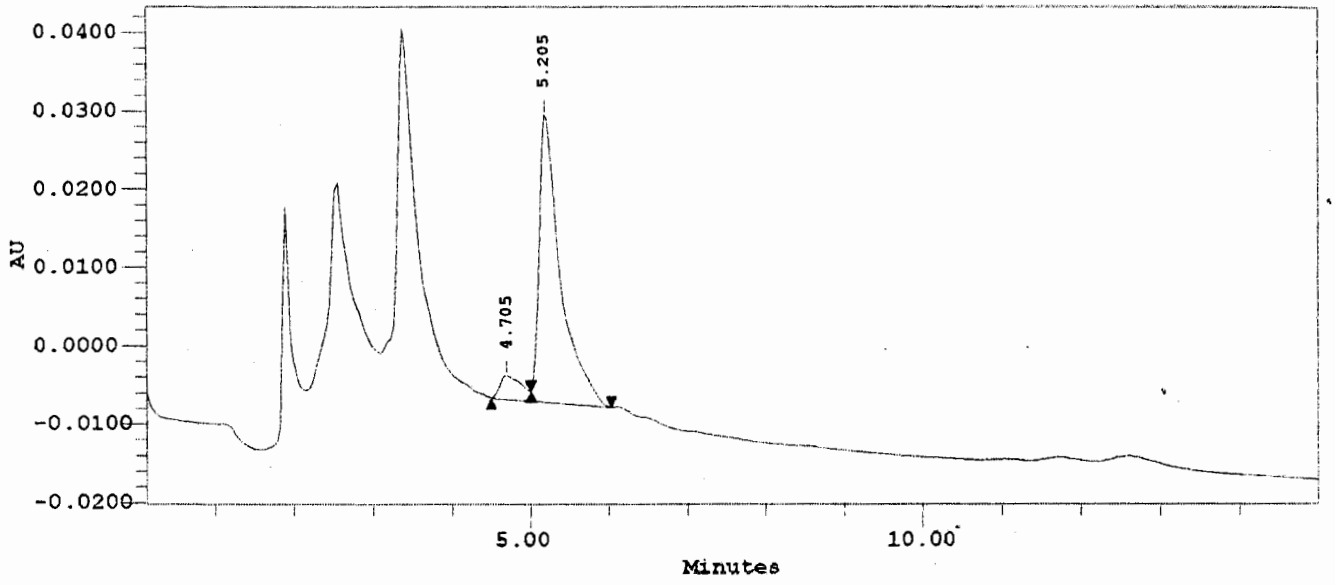
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1		5.248	110780	8321		BB
2		11.015	65653	2663		BV
3		11.648	230092	6500		VB

Millennium Results Report		November 4, 1988			Page: 1 of 1
Report Method:	Default		Version:	2.15	
For Sample:	M1	Vial: 1	Injection:	1	Channel: 991M
Proc Chan:	282		Processed:	11/04/88 11:00:26 AM	
Channel Descr:	PDA 282.0 nm				

Millennium Sample Information

Project Name:	Ergosterol		
Sample Name:	M1 Maple at 6 weeks		
Vial:	1	Sample Type:	Unknown
Injection:	1	Volume:	50.00 µL
Channel:	991M	Run Time:	15.0 min
Date Acquired:	11/04/88 10:44:57 AM	Date Processed:	11/04/88 11:00:26 AM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	Ergosterol_MS		
Processing Method:	Ergosterol_PM		



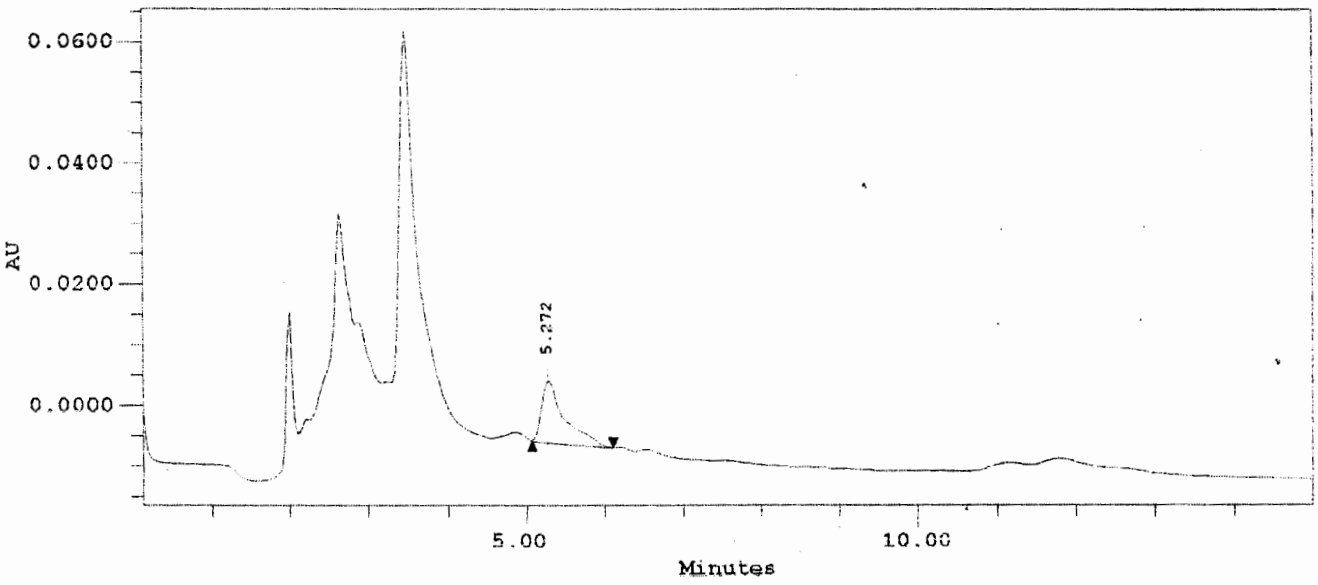
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1		4.705	57779	3023		BV
2		5.205	697255	36769		VB

Millennium Results Report		November 4, 1988		Page: 1 of 1
Report Method:	Default		Version: 2.15	
For Sample:	K2	Vial: 4	Injection: 1	Channel: 991M
Proc Chan:	282		Processed: 11/04/88 11:48:29 AM	
Channel Descr:	PDA 282.0 nm			

Millennium Sample Information

Project Name:	Ergosterol		
Sample Name:	K2 Knotweed at 6 weeks		
Vial:	4	Sample Type:	Unknown
Injection:	1	Volume:	50.00 uL
Channel:	991M	Run Time:	15.0 min
Date Acquired:	11/04/88 11:32:54 AM	Date Processed:	11/04/88 11:48:29 AM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	Ergosterol_MS		
Processing Method:	Ergosterol_PM		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1		5.272	208375	10195		VB

Report Method: Default Version: 2.15

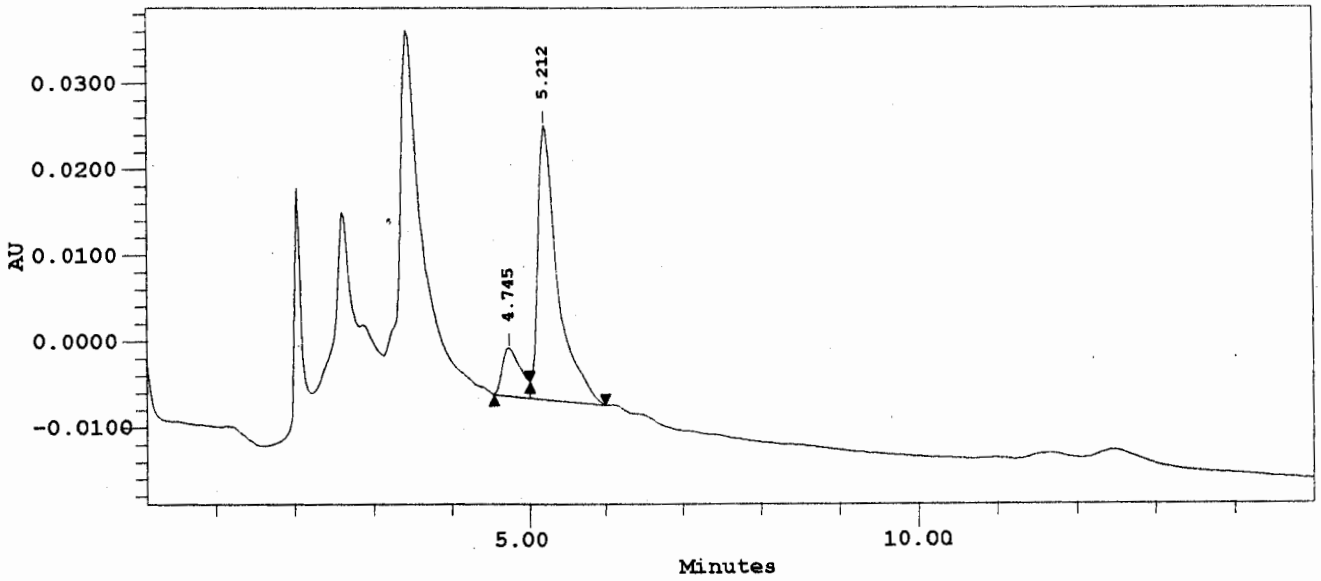
For Sample: m1 Vial: 1 Injection: 1 Channel: 991M

Proc Chan: 282 Processed: 11/18/88 10:24:36 AM

Channel Descr: PDA 282.0 nm

Millennium Sample Information

Project Name:	Ergosterol		
Sample Name:	m1 Maple at 8 weeks		
Vial:	1	Sample Type:	Unknown
Injection:	1	Volume:	50.00 µL
Channel:	991M	Run Time:	15.0 min
Date Acquired:	11/18/88 10:09:08 AM	Date Processed:	11/18/88 10:24:36 AM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	Ergosterol_MS		
Processing Method:	Ergosterol_PM		



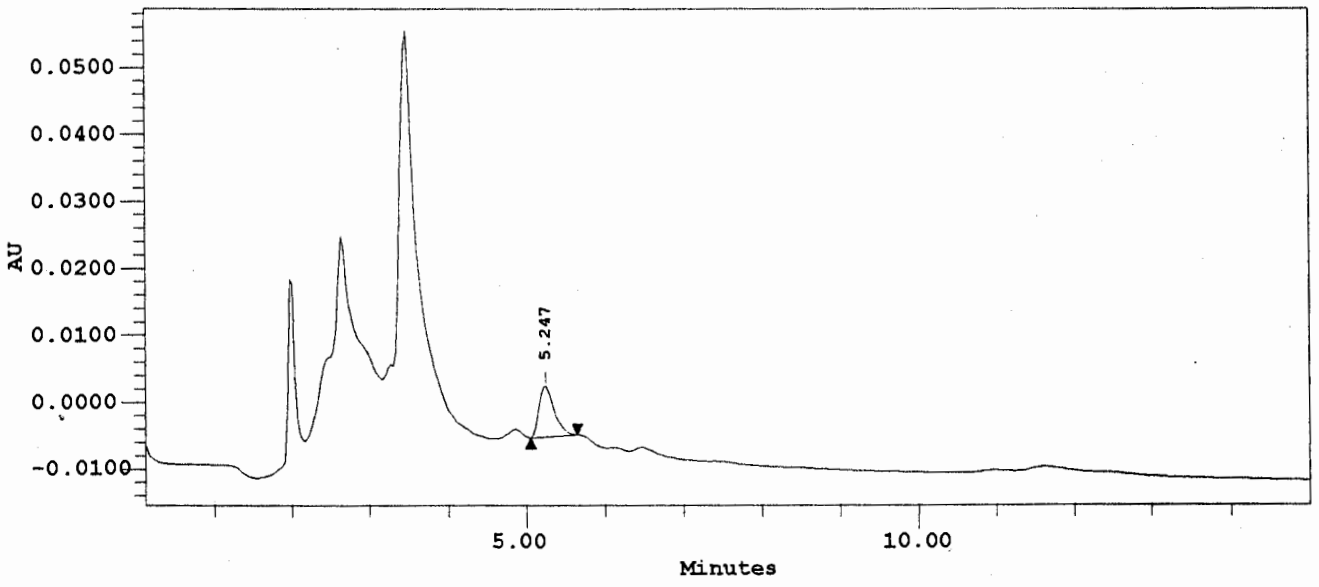
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1		4.745	91540	5542		BV
2		5.212	578891	31832		VB

Millennium Results Report		November 18, 1988			Page: 1 of 1
Report Method:	Default		Version:	2.15	
For Sample:	K1-2nd run	Vial: 4	Injection:	1	Channel: 991M
Proc Chan:	282		Processed:	11/18/88 11:14:54 AM	
Channel Descr:	PDA 282.0 nm				

Millennium Sample Information

Project Name:	Ergosterol		
Sample Name:	K1-2nd run Knotweed at 8 weeks		
Vial:	4	Sample Type:	Unknown
Injection:	1	Volume:	50.00 uL
Channel:	991M	Run Time:	15.0 min
Date Acquired:	11/18/88 10:59:25 AM	Date Processed:	11/18/88 11:14:54 AM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	Ergosterol_MS		
Processing Method:	Ergosterol_PM		



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1		5.247	102366	7526		BB