

Sources of Nitrogen and Its Influence on
Sarracenia purpurea L. Plants in a North American Bog
BIOS 569 - Practicum in Aquatic Biology

Amy J. Farmer

Address for Fall 1996 Semester:

33 Leinster Gardens, Flat 4

London, W2 3AN, England

Dr. Richard G. Carlton

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ABSTRACT

Sarracenia purpurea L., known as the American Pitcher Plant, is generally regarded as a carnivorous plant. Most carnivorous plants share a common habitat, i.e. they grow in soils having an acid, boggy, and often peaty nature in which plant nutrients, especially nitrogen, are deficient. Whether the absorption of essential nutrients from insects is essential to growth or simply a luxury is open to debate. The purpose of my field study was to determine if *Sarracenia* plants receiving no supplemental nitrogen and deprived of any nitrogen-uptake from insects would fare less well (e.g. less leaf growth and fewer leaves) than a control group of plants receiving nitrogen from insects. The principal finding in this research is that depriving *Sarracenia* plants of insects as a supplemental source of nutrients does not affect the growth of the pitcher's height, maximum volume, and hood height. However, this research indicated *Sarracenia* plants deprived of insects did grow in a different way, i.e. they produced fewer leaves and their leaves opened sooner than non-deprived plants. Also examined was the relationships between variables in the habitat of *Sarracenia*, i.e. characteristics of the perched water table, pitcher fluid, and the insects captured by the pitcher.

Introduction

Sarracenia, known as the American Pitcher Plant, is generally regarded as a carnivorous plant (Joel, 1978). Most carnivorous plants share a common habitat, i.e. they grow in soils having an acid, boggy, and often peaty nature in which plant nutrients, especially nitrogen, are deficient (Slack, 1979). In general we can say that carnivorous plants grow in nutrient-poor wetlands. Acidic substances, such as decaying plant matter called tannin, can become concentrated in nutrient-poor wetlands and thereby increase the acidity of the water which typically flushes at very slow rates. High concentrations of acid create conditions for two events. First, microorganisms which aid in decomposition of decaying plant matter are inhibited by the environmental acidity. Second, plants attempting to assimilate nutrients are also inhibited by acidic soil. Chronic acidity ensures that certain wetlands remain nutrient-poor. *Sarracenia* seems to thrive in nutrient-poor wetlands not because it is acid-loving but rather because it may have adapted to nutrient-poor environments where many other plants cannot flourish. Confirming this statement, Slack (1979) states: Conditions are extremely acidic in such places [certain wetlands], but acidity itself doesn't seem to be an essential requirement of the plant [*Sarracenia*], for it also grows in alkaline marl bogs around the Great Lakes.

Sarracenia purpurea L., commonly called the Northern Pitcher Plant, is an herbaceous perennial from *Sphagnum*-dominated wetlands that produces pitcher-shaped leaves in a rosette. These pitchers serve to entrap and digest insects (Chapin and Pastor, 1995). Insects can be attracted to the leaf by various means: the pitcher develops from insects is essential to growth or simply a luxury is open to debate. Chapin and Pastor (1995) say that *Sarracenia* must be obtaining sufficient nitrogen and phosphorous from other sources such as rainwater or soil in quantities enough to sustain growth, as the lack of nitrogen and or phosphorous had no effect on above ground biomass, the number of leaves produced in the same year, or average leaf mass. Chapin and Pastor (1995) admit that areas with lower nutrient availability might show greater change. The decomposition of the victims is hastened by the feeding activities of detritivores that often live in the pitchers, such as microbes and larvae of sarcophagid flies, midges and mosquitoes (Cresswell, 1991)

Naturalists, going back to Darwin, have noted that carnivorous plants are most commonly found in nutrient-poor environments (Chapin and Pastor, 1995). Given some of the unique morphology these plants have for the capture of insect prey, it has often been thought that these plants have evolved traits that fulfill nutritional requirements through the capture and digestion of insect prey (Chapin and Pastor, 1995). Prey capture and subsequent decomposition increases the growth rate of plants which obtain an increased supply of nitrogen, mineral ions, and CO₂ from detritivore respiration (Cresswell, 1991). Amino

Sources of Nitrogen and Its influence on *Sarracenia purpurea* L. Plants in a North American Bog acids, peptides. and other nutrients from the insects are absorbed and utilized directly by the pitcher plant leaf (Fish and Hall,1978).

Whether the absorption of nutrients from insects is essential to growth or simply a luxury is open to debate. Chapin and Pastor (1995) report that *Sarracenia* must be obtaining sufficient nitrogen and phosphorous from other sources such as rainwater or soil in quantities enough to sustain growth, as the lack of nitrogen and or phosphorous had no effect on above ground biomass, the number of leaves produced in the same year, or average leaf mass. Chapin and Pastor (1995) admit that areas with lower nutrient availability might show greater changes in the aforementioned variables with added nutrients and/or insects. The purpose of my field study was to determine if *Sarracenia* plants receiving no supplemental nitrogen and deprived of any nitrogen-uptake from insects would fare less well (e.g. less leaf growth and fewer leaves) than a control group of plants receiving nitrogen from insects. I also surveyed the environment to determine if other pertinent relationships would merit further exploration.

Materials and Methods

Glass Wool Blockage Experiment

A 64m² plot in Forest Service Bog, located in the University of Notre Dame Environmental Research Center in Vilas County, Wisconsin, was chosen as the site for the glass wool blockage experiment. This plot was divided into four 16m² quadrats. Each quadrat was marked with white flags and given a unique number. Figure 1. Within each of the four quadrats, five plants were chosen at random and each flagged with a number. Each leaf was given a waterproof tag with an identification letter (e.g. the designation 1-1-A refers to quadrat one, plant one, leaf one). Newly produced leaves were tagged with a number and the date of opening. Figure 2. These four quadrats were then randomly assigned treatments. The treatments were as followed: (1) add glass wool; (2) add ammonium and glass wool; (3) add ammonium. The fourth quadrat served as a control. Treatments 2 and 3 were not executed, however, due to concerns whether or not the proposed concentration of ammonium would be toxic to the plants considering the fragile, dwarfed appearance of the leaves.

Initial measurements of each of the ten plants in the experiment were taken, and leaf height, leaf volume, and hood height were recorded. Leaf height was initially recorded by using calipers, but this was found to be an inaccurate technique. Therefore, a string with a black spot was held at the base of the plant, and the remainder of the string was traced along the curvature of the leaf. The string was then transferred to a ruler and a measurement was

Sources of Nitrogen and Its influence on *Sarracenia purpurea* L. Plants in a North American Bog taken. For the initial and final measurements, the waterproof identification tags were not a factor in measuring the leaf height. However, the small gap of the waterproof tag which circled the base of the leaf may have added error into the second measurement of height. Leaf volume was taken using two unique methods for the quadrats. In the control quadrat (#4), leaves were drained using a large-volume syringe, and the pitcher plant phytolomata was kept in acid-washed jars. Volume was then recorded by measuring the amount of water needed to completely fill the leaf. The leaves were then drained, and the original pitcher plant phytolomata was replaced. Between each volume measurement the syringe was acid-washed using a 5% HCl solution, and the contaminated jar was replaced with a new, acid-washed jar. For the experimental quadrat (#1), the pitcher plant phytolomata and any insects were removed, and the leaf was rinsed and filled with a quantity of glass wool sufficient to block the opening of the pitcher. This method has been tested and proven to work effectively (Chapin and Pastor, 1995). Rinsing the glass wool in distilled water prevented the introduction of additional nutrients to the pitcher. Periodically, distilled water was added to the openings in order to prevent drying of the leaves. Measurements for hood height were also acquired with the use of a marked string. Also noted were the color of the leaf, the degree of damage, the presence of spider webs, and the height of the flower from its base to top. Measurements of the plants were taken three times during the summer on 2 and 3 June, 18 and 20 June, 16 and 17 July 1996.

Water Chemistry

The concentration of NO_3^- in the center of the marked quadrats was measured using the Cadmium Reduction Method on the Hach Kit on 17 June 1996. The NH_3 concentration was measured using the Nessler Method on the Hach Kit on 17 June 1996, 25 June 1996, 8 July 1996, and 17 July 1996. The samples for this test had to be diluted by half as the original reading was over the range. Diluted readings were then multiplied by two. The PhosVer3 (Ascorbic Acid) Method for PO_4^{3-} was also conducted on 25 June 1996 using the Hach Kit. These measurements were used to determine the limiting element in the environment as well as to follow the levels of NH_3 in the site. The pitcher plant phytolomata was also sampled for its NH_3 concentration on 17 July 1996 using the Salicylate Method on the Hach Kit. The pitcher plant phytolomata was filtered completely in order to remove all suspended materials which would affect the reading.

Pitcher Plant Insect Sampling

A general survey of pitcher plants was conducted. The pitcher plant phytolomata

Sources of Nitrogen and Its influence on *Sarracenia purpurea* L. Plants in a North American Bog characteristics (i.e. phytolomata pH, phytolomata volume, and number/order of intact insects) and ground water chemistry (i.e. pH and depth of the water table) at different sites were compared to each other as well as compared across time. The primary purpose of the sampling exercise was to collect intact insects that had recently fallen into the plant and identify them down to order for later experiments. The secondary purpose of this sampling exercise was to discover a general quantitative pattern of influence between the water table, leaf phytolomata, and intact insects in the phytolomata.

Four sites were established at locations surrounding the bog lake (see Figure 1). Each site was sampled on these dates: 11 June, 26 June, and 7 July 1996. At each site the depth and pH of the water table were measured at random locations. Ten plants in each site were drained using a large-volume syringe, and the contents were held in an acid-washed jar. The total phytolomata volume, pH, and number of intact insects were recorded. Each insect was preserved individually in 70% EtOH, measured, drawn and identified to order. Figures 3-13. In total, 120 pitcher plants were sampled. Other ancillary data were recorded such as weather and three days' previous weather.

Capacity for Direct Release of Ammonia

To test the capacity for direct release of ammonia, the most common species of each Order were located in the field. The most common Coleoptera was found on *Nuphar variegatum*, a floating water lily, in Tenderfoot Lake. These leaf-eating beetles are in the genus *Donacia*. The hymenoptera were caught with a sugar trap in the proximity of Forest Service Bog. The dipterans were collected by a sweep net and were frozen in the lab until they could be sorted. The dipterans which had fallen into the plant during the sampling sessions could not be found in the area. Dipterans of the same size and shape as the most common dipteran were used in lieu of the actual species. Fourteen coleopterans, ten hymenopterans, and ten dipterans were soaked in individual jars without agitation in 50mL of distilled water for 24 hours. Three control jars filled with 50mL of distilled water were also allowed to stand for 24 hours. The insects were removed from the jars, and the water was tested for NH₃ using the Salicylate Method on the Hach Kit. Simultaneous with measuring the insect water, the perched water was also measured with the Hach Kit to account for any nitrogen increases in the perched water over the 24-hour testing period.

Results

Glass Wool Blockage Experiment - Treatment Effects

Growth rate in height, volume, and hood height changed significantly over the six-week

Sources of Nitrogen and Its influence on *Sarracenia purpurea* L. Plants in a North American Bog experiment in both the experimental quadrat (1) and the control quadrat (4). Between Date 1 and 3, height changed positively in quadrat 1 ($p = .052$ $n = 41$) and in quadrat 4 ($p = .033$ $n = 41$). Volume changed positively in quadrat 1 ($p = .031$) and in quadrat 4 ($n = 36$ $p = <.001$). For an unknown reason, Systat would not yield hood height statistics comparable to those in the preceding sentence.

Systat was then used to compare interquadrat growth. Findings revealed no significant differences in rates of growth due to treatments. Initial height ($n = 50$ $p = .656$) and volume ($n = 45$ $p = .556$) did not differ between quadrats. No claims can be made regarding hood height. Terminal height ($n = 50$ $p = .556$) and volume ($n = 27$ $p = .19$) also did not differ significantly due to treatments. Again no claims can be made regarding hood height. Because the initial height and the terminal height were not significantly different, one can infer that the treatment had no significant influence on the rate of growth in either height or volume. Figures 14, 15, 16.

Out of the three variables measured (i.e. height of leaf, height of hood, and volume of leaf), the volume of the leaf was the only variable which noticeably differed between quadrats, on average, during the experiment. In the experimental quadrat, the mean growth rate in volume of leaf was 1.92 mL per leaf. In the control quadrat, the mean growth in volume was 12.8 mL per leaf. Both the mean growth rate in height of leaf and the height of hood did not noticeably differ in either the experimental or the control quadrat. The mean growth rate in height of the leaf in the experimental quadrat was 3.46 cm and 3.44 cm in the control quadrat. The mean growth rate in the height of the hood was 1.45 cm per leaf in the experimental quadrat and 1.51 cm per leaf in the control quadrat.

In the experimental quadrat, eight new leaves were produced, and of these new leaves, approximately 37% opened by the end of the experiment. In the control quadrat, the plants produced thirteen new leaves--approximately 22% of these leaves opened by the end of the experiment. Figures 17, 18.

During the six weeks of the experiment, only one plant out of ten in the two quadrats was flowering. This observation may indicate that this season was the non-flowering season of a bi-annual cycle.

Pitcher Plant Sampling

After sampling 40 leaves on three different dates, 38 insects were found that could be identified to Order. This count does not represent the viable larvae which inhabit the leaf--but the insects which had fallen into the leaf and drowned. The average number of dead intact insects per leaf was 0.316.

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- pH of Phytollemata in Plants. Figure 19. Exploring relationships between pH and time reveals that pH increases over time. However, the variance in readings among sites was large, e.g. on one date the average pH level in ten plants at Site 3 was 6.9 whereas on the same day the average pH level in ten plants at Site 4 was 4.1. (Note: the maximal range of pH over all sites over all dates was 3.9 to 6.9.)
- Volume of Phytollemata in Plants. The average phytollemata volume per leaf over all sites over all dates was 14.30 mL. Average phytollemata volume varies widely over time, e.g. Date #1, 16.15 mL; Date #2, 17.22 mL; and Date #3, 9.55 mL. Average phytollemata volume also varied noticeably by site, e.g. Site #2, 11.60 mL; Site #3, 14.43 mL; Site #4, 16.23 mL; and Site #5, 14.97 mL.
- Intact Insects in Phytollemata of Plants and the Influence of pH on the Number of Intact Insects. Figure 20. The average number of intact insects (per ten plants) varied widely over all sites, i.e. from 0 insects per ten plants on one date at Site #4 to 7 insects per ten plants on one date at Sites #2 and #5. From personal observation, when a dipteran was found in a leaf, other dipterans are likely to be found. Across all sites, a slight relationship existed between number of intact insects and pH levels, i.e. the lower the pH of the phytollemata, the higher the number of intact insects. [Note: Included in the insect population were a small number of spiders which were counted as insects.]
- Intact Insects vs. Time. Across all sites, the number of intact insects dropped over time- with the most precipitous drop occurring between Dates #1 and #2.
- pH of Ground Water. Figure 21. With the exception of one 5.1 pH reading at one site on one day, the pH of the ground water across all sites fluctuated within a narrow band, i.e. range of 4.3 pH to 3.6 pH.
- Perched Water Levels. Across all sites and across all dates, the distance between the top of the Sphagnum and the top of the perched water table ranged from 2.3 cm to 7.0 cm.

Nutrient Flow

- Nutrients in the Perched Water. Sampling revealed that the perched water was nitrogen-limited. Specifically, the N to P ratio is less than 16 to 1; it was 11.5 to 1. (NH₃ was measured at 4.36 mg/L average, and P was measured at .16 mg/L average.) Therefore, following the flow of nitrogen would be following the nutrient of highest demand by the

Sources of Nitrogen and Its influence on *Sarracenia purpurea* L. Plants in a North American Bog plants. Furthermore, it was important to distinguish between which form of nitrogen is essential, i.e. distinguish between ammonia and nitrate. Hach Kit testing revealed that no nitrate existed in the perched water. Therefore, following the flow of ammonia would be necessary.

Compared to fresh water lakes where ammonia concentration may be 1.0 mg/L on average, ammonia concentration in the perched water at Site #1 was high, i.e. it ranged from an average of 4.36 mg/L on one date to an average of 2.38 mg/L on another date. Figure 22. Measurement revealed a correlation between ammonia levels and pH levels in the perched water. Higher pH levels correlate with lower ammonia levels. Figure 23.

- Ammonia in the Pitcher Plant Phytolomata. From one sample (with four replicates) using the Hach Kit, ammonia levels in the plant phytolomata averaged 3.41 mg/L.
- Insects' Short-Term Capacity for Direct Release of Ammonia. Using a customized test whereby insects were dropped into distilled water, the short-term capacity for direct release of ammonia was categorized by insect as follows: Hymenoptera, average of 0.0125 mg/L per insect; Coleoptera, average of 0.055 per insect; and Diptera, average of 0.0925 mg/L per insect.

Discussion

G. Cheers's book, *A Guide to Carnivorous Plants of the World*, suggests that *Sarracenia* "all grow at a similar rate" (1992). The key objective of the glass wool blockage experiment was to determine if blocking the supply of insect-based nutrition would impede the rate of growth in selected plants. Experimental measurements of leaf volume and leaf height were not significantly affected by the blockage. Perhaps no difference in leaf volume and leaf height was due to the fact that plants in Site #1 had an ample supply of nitrogen from ground water, i.e. this entire site had a high water table and a high concentration of ammonia. Also no discernible difference may have been due to the limited time frame of the experiment, i.e. six weeks. Another similar cotton blockage experiments (e.g. Chapin and Pastor) support the absence of discernible differences due to the deprivation of secondary nutrients, such as insects. Cresswell (1991) concluded in his research that "the relatively low capture rate that I observed in a northern population of *S. Purpurea* may mean that, in comparison to *S. leucophylla* in southern populations, it relies far more on nutrients from the substrate than from insect captures." Differences of opinion exist, however, because other scientists such as Joel imply that carnivorous plants "can trap small animals with the

Sources of Nitrogen and Its influence on *Sarracenia purpurea* L. Plants in a North American Bog aid of their leaves, and exploit them as a secondary nutrient source" (Joel 1986).

Depriving the plants from insects had a noticeable effect on the pattern of growth, i.e. the blocked plants (the experimental group) produced five fewer leaves than the unblocked plants (the control group). Furthermore, leaves of the blocked plants opened sooner than the leaves of the unblocked plants. One hypothesis for this morphological difference might be that unblocked plants have a natural rate of petiole and leaf development--whereas a blocked plant, sensing in effect that a source of nutrients (i.e. insects) is blocked, compensates by opening leaves sooner with the idea that short-term survival is preferred over longer-term natural development.

An interesting observation is that the types of insects captured in this experiment parallel that of other researchers, e.g. Cresswell found a "capture breakdown" of 71% diptera; 9.7% hymenoptera; 7.3% collemboda; and 5.5% coleoptera. Rather than reflecting typical insect population densities, the plants may have evolved selective entrapment techniques, e.g. nitrogen-rich dipterans might be favored over less nutrient rich insects. Dipterans, as a group, although small in size individually had the highest capacity to release nitrogen directly into the phytolomata.

The principal finding of this research is depriving *Sarracenia* plants of insects as a supplemental insect source of nutrients (in the form of ammonia) does not statistically alter the growth rate of leaves and leaf volume. However, this research indicated *Sarracenia* plants deprived of insects did grow in a different way, i.e. they produced fewer leaves and their leaves opened sooner than non-deprived plants. Therefore, nitrogen in the form of ammonia appears to be supplemental rather than essential to the growth of *Sarracenia*.

Limitations of Research. Any research study has limitations due to resources, time etc. In this research project, three key limitations were apparent to the researcher.

Aside from general macroscopic concerns about the representative quality of the entire site itself, key concerns arose regarding using averages for data points, e.g. insects per leaf and pH of phytolomata. Some studies that tracked individual plants showed results that differed from this research. For example, Fish and Hall (1978) found that pH per leaf dropped over time rather than increased--as in this research project. This discrepancy deserves exploration because it may reveal a flaw on the study's sampling technique, i.e. a larger sample size may be necessary to determine the correct relationship between pH and time. Or both Fish and Hall as well as this study may be valid--with differences suggesting pH/time variations that are sensitive to site selection.

Second, the plants in this research were quite damaged, apparently from frost and insects. Many leaves were browning, and many leaves had holes in them. These initial conditions of the experiment may have provided misleading conclusions, e.g. when measuring

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leaf volumes at the end of the experiment.

Third, the research could be improved by more robust equipment. For example, this research only measured the capacity of direct release of nitrogen through diffusion. A most revealing statistic might be the long-term capacity of insects to release nitrogen. Total nitrogen could be measured by using the Hach Kit to measure the Kjhedal reaction.

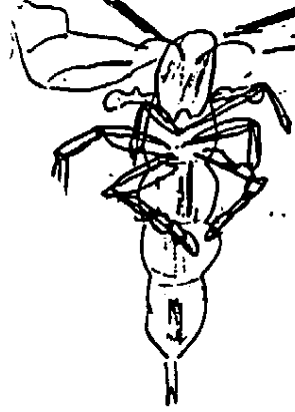


Fig. 1. Photograph of site of experimental plot (four quadrats marked with stakes) in forest, near Bog, Vilas Co., Wisconsin.



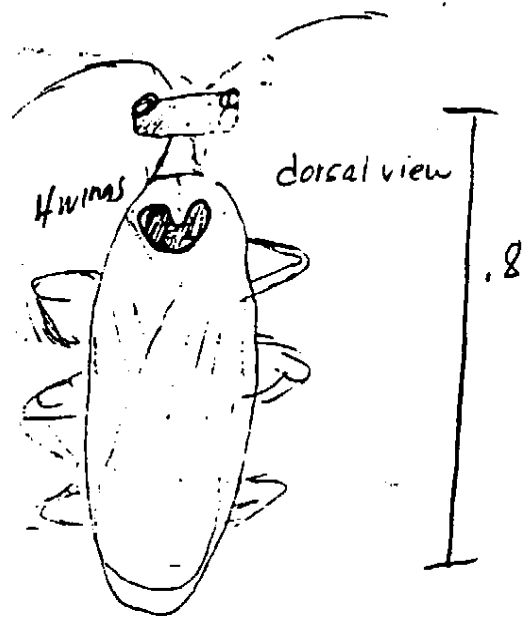
Fig. 2. Photograph of plants in the experimental plot (Fig. 1) with 100% herbicide treatment.

② U.I.



.35 #1
#138.2 #5 → .3
#27B tangled
.35

⑥



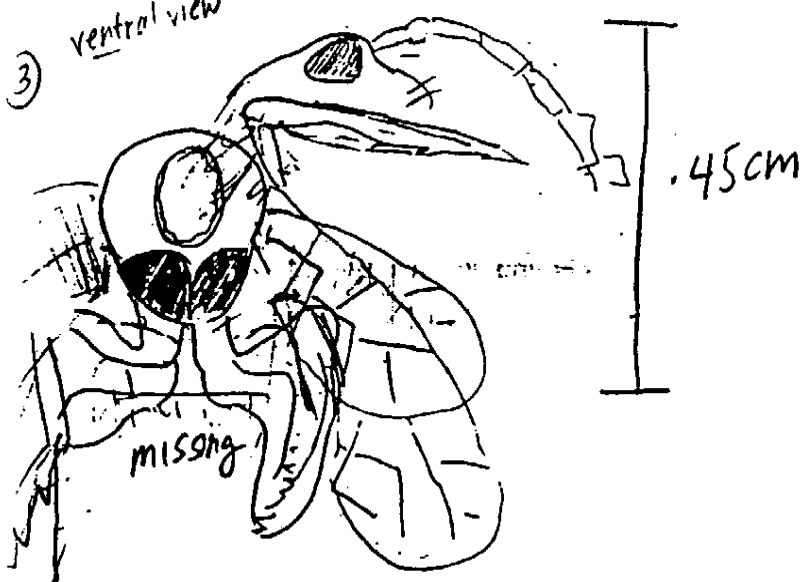
4 wings

dorsal view

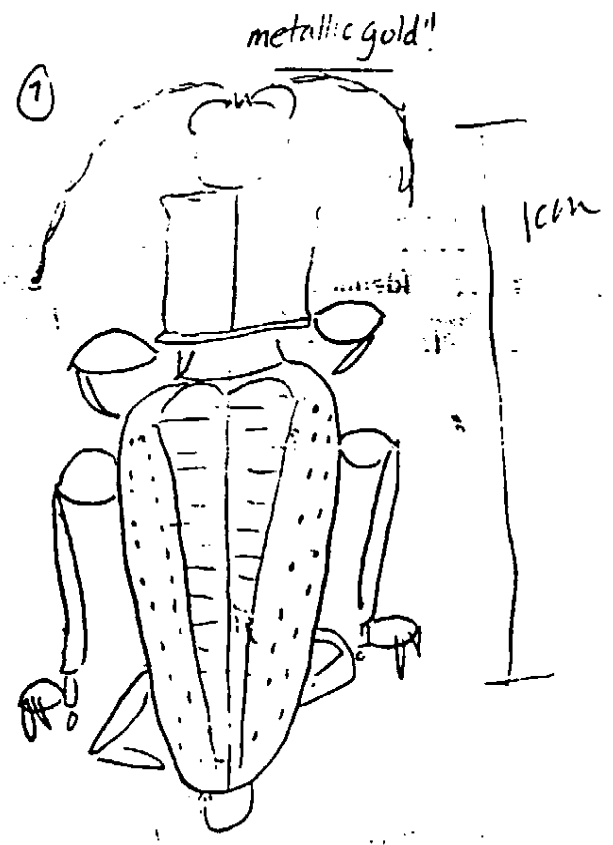
H₂O chemistry

- NO₃⁻ analysis 25 mL
- NH₄⁺ analysis 25 mL

③ ventral view

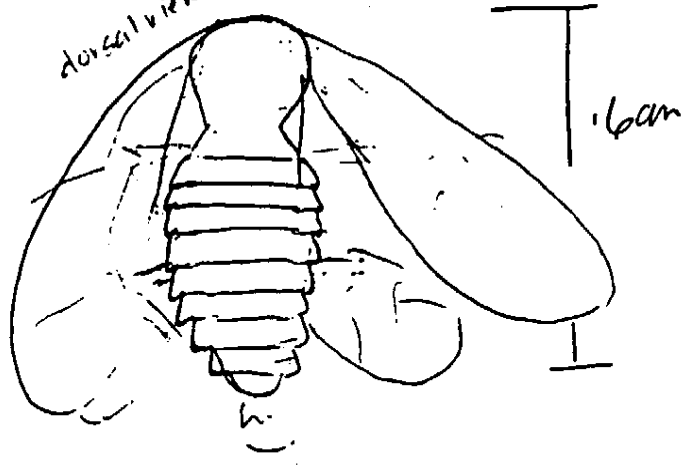


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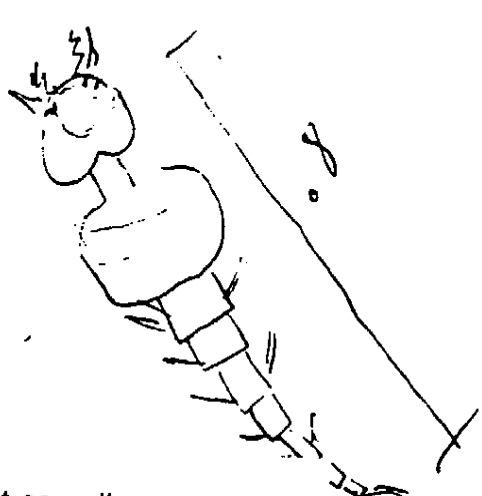


metallic gold!

dorsal view Nohead!

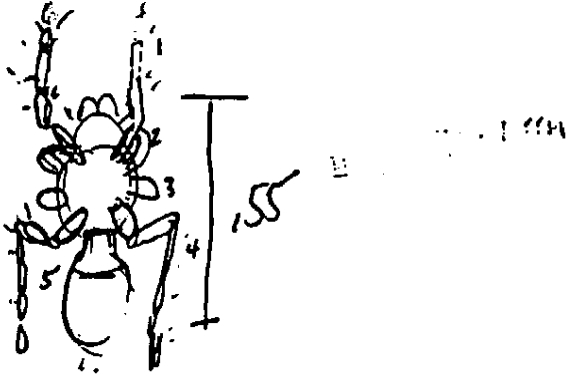


1.6cm

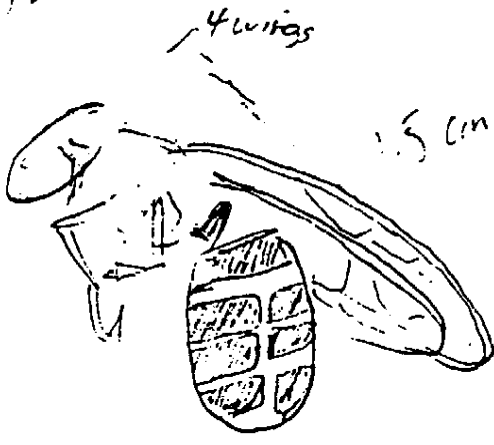


.8

g.3. Drawings of intact insects found during pitcher plant sampling session on 11 June 1996 in Site 40



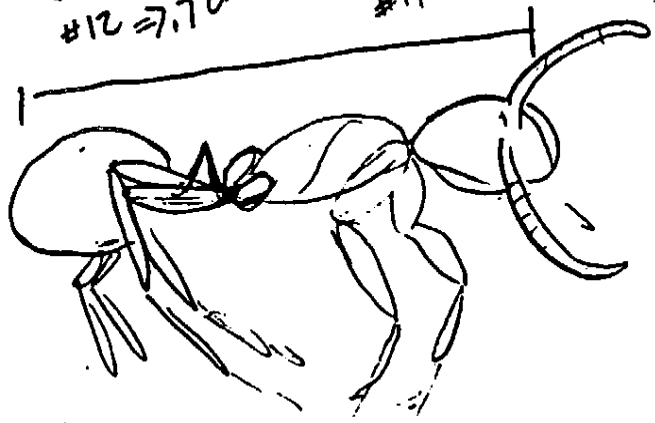
) Lateral



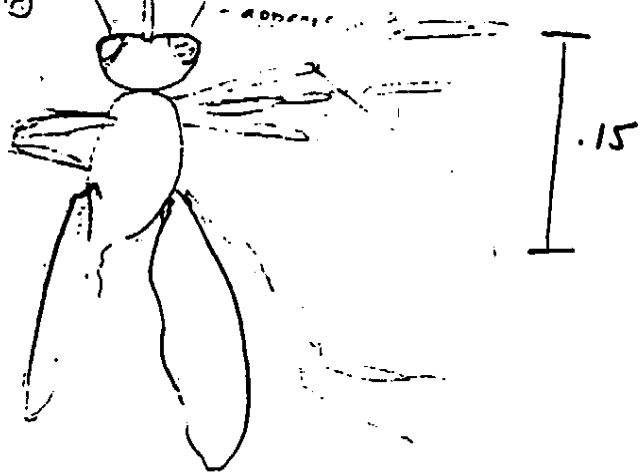
) + (12)

erat

#11 ⇒ .6 cm #14 ⇒ .6 cm #18 - Nim
 #12 ⇒ .7 cm #15 ⇒ .5 cm #29 ⇒ .75
 #17 ⇒ .7 cm #31 ⇒ .3
 (bent over)



g.4. Drawings of intact insects found during pitcher plant sampling session on 12 June 1996 in Site #3.



B) same as #1

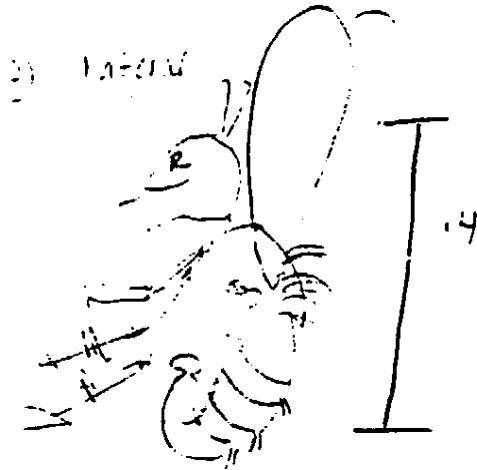
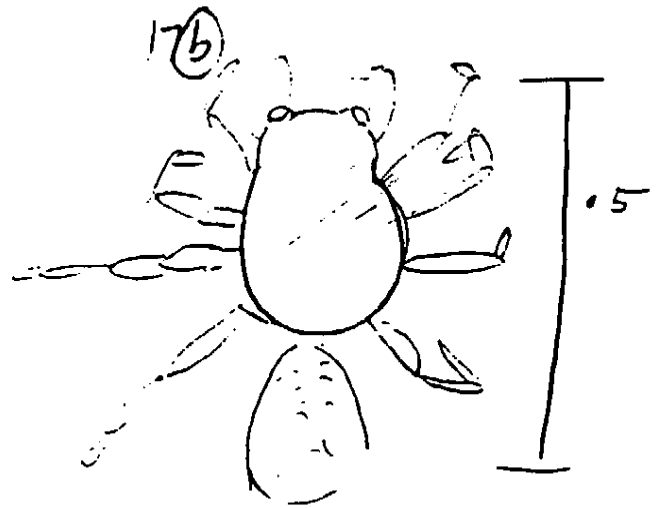
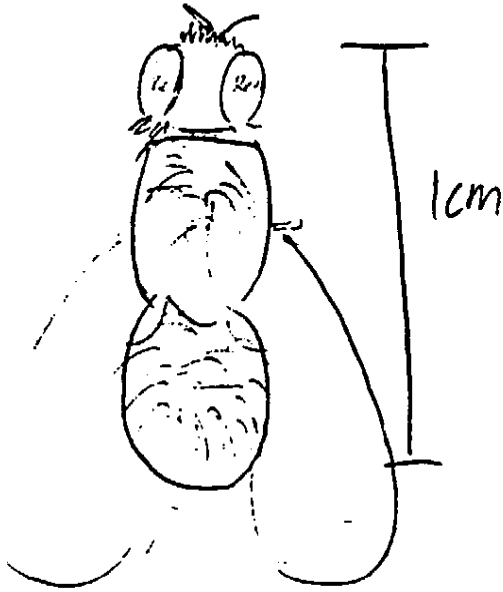
Same as #1 but miss part of abdomen
measurement = .15

Same as #1 measurement = .15



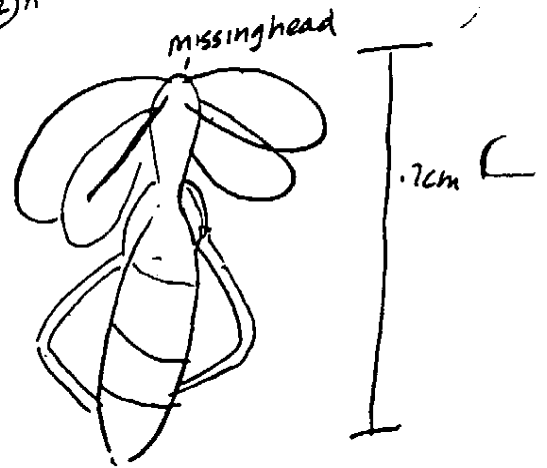
19) Same as #11 (no measurement, curved in last cent stretch)

g. 19

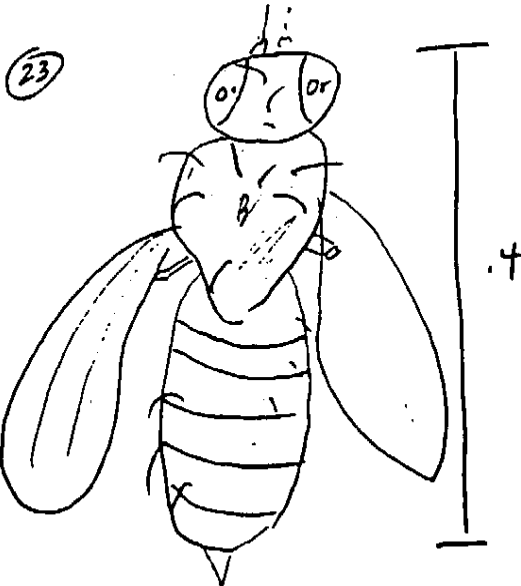


21 ?

22A

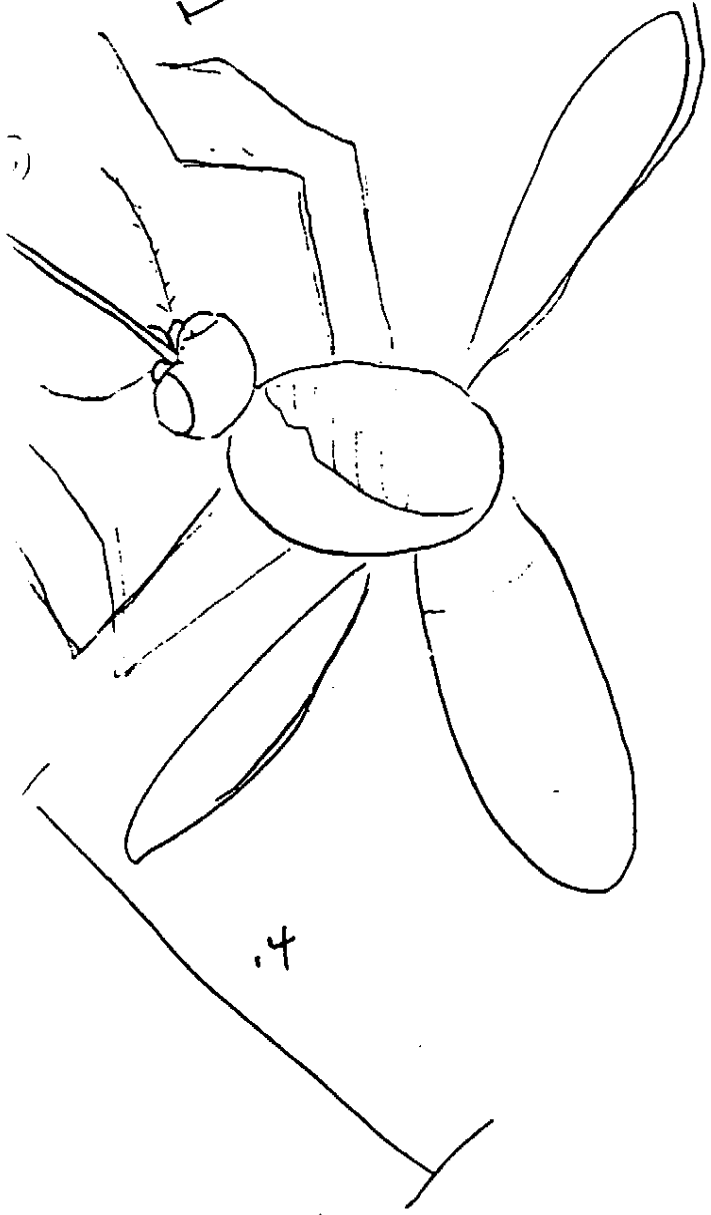
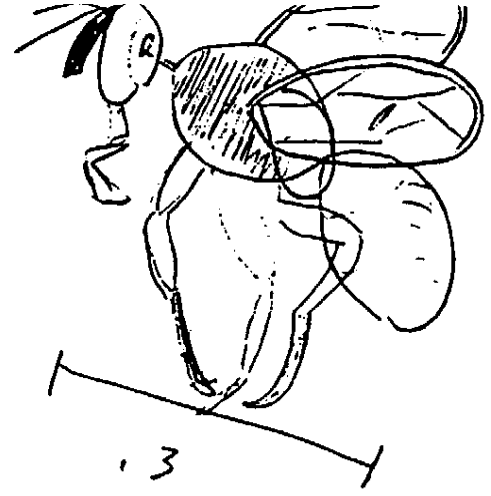
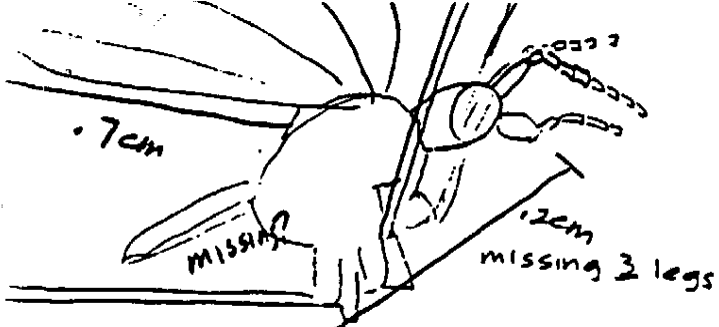


same as #1



23

Fig. 6. Drawings of intact insects found during pitcher plant sampling session on 14 June 1996 in Site #5.



g.7. Drawings of intact insects found during pitcher plant sampling session on 26 June 1996 in Site #2.

1 SA
m. eggs

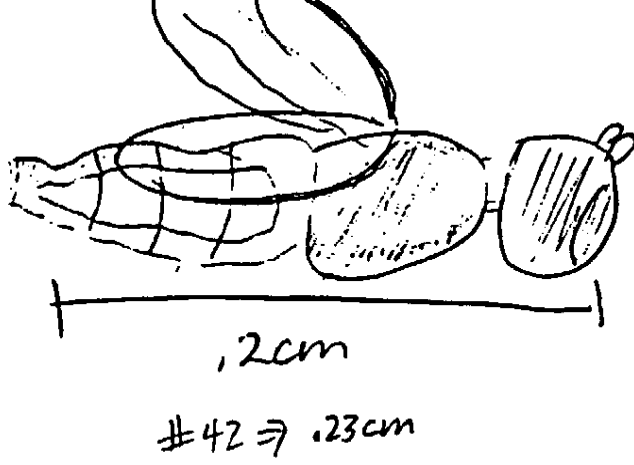


Fig. 8. Drawings of intact insects found during pitcher plant sampling session on 26 June 1996 in Site #3.

30) U1

1) same as #11

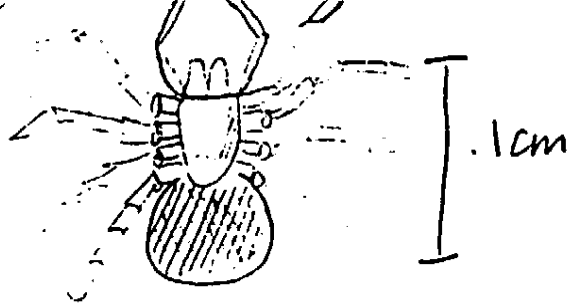
↳ possibly a smaller
ant species

F7

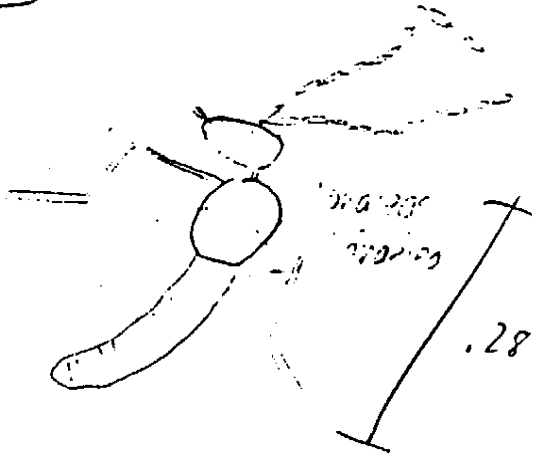
C

G



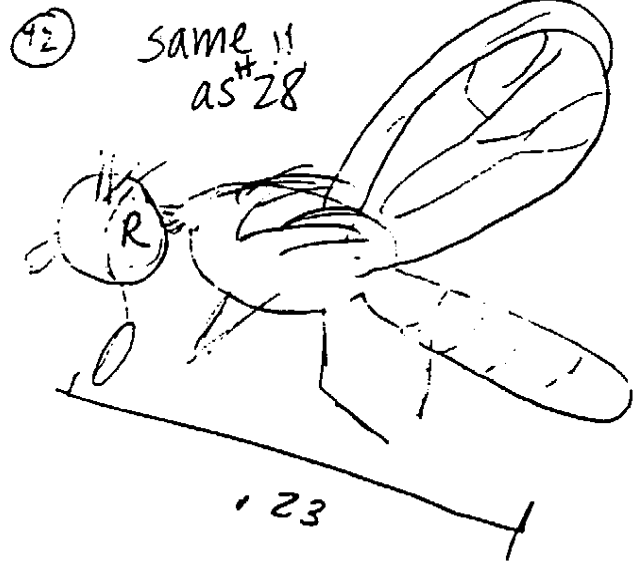
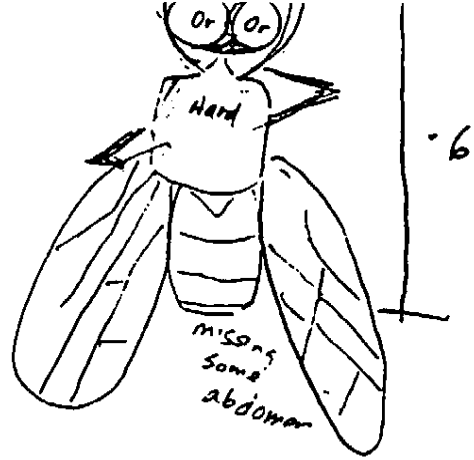
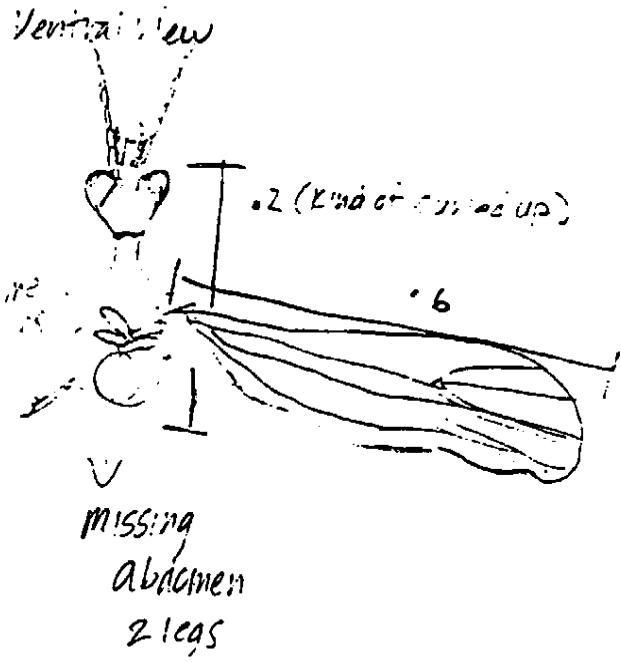


35



UI
UI

3) Pupating larvae of vial #8



g.11. Drawings of intact insects found during pitcher plant sampling session on 7 July 1996 in Site #2.



fig.12. Drawings of intact insects found during pitcher plant sampling session on 7 July 1996 in Site #3.

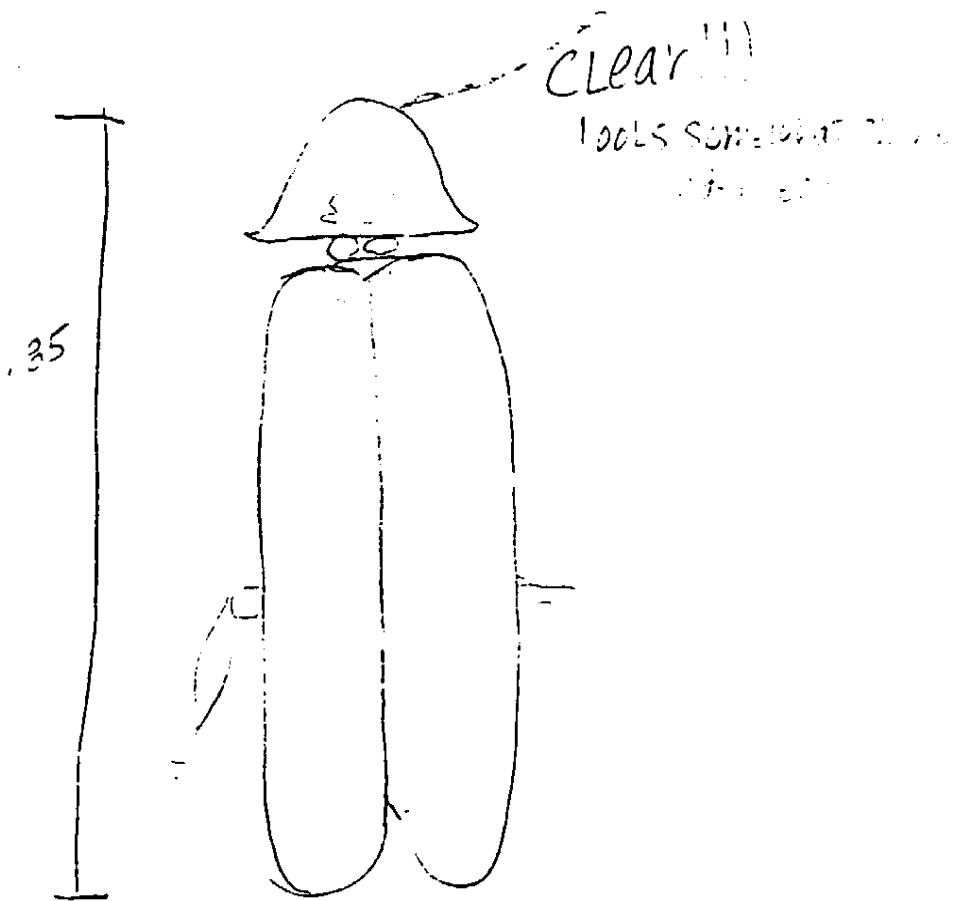


Fig. 13. Drawings of intact insects found during pitcher plant sampling session on 7 June 1996 in Site #5.

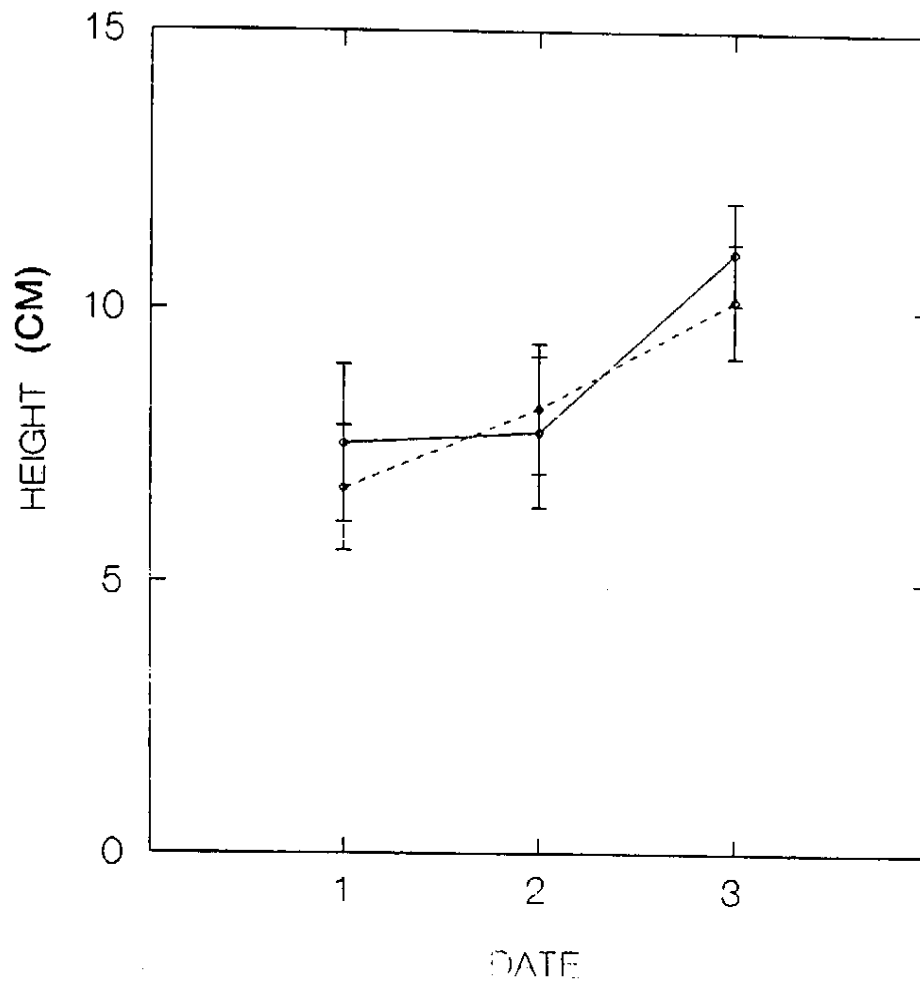


Fig.14. Growth in height (in centimeters) over time in the experimental (---) and control quadrat (—)

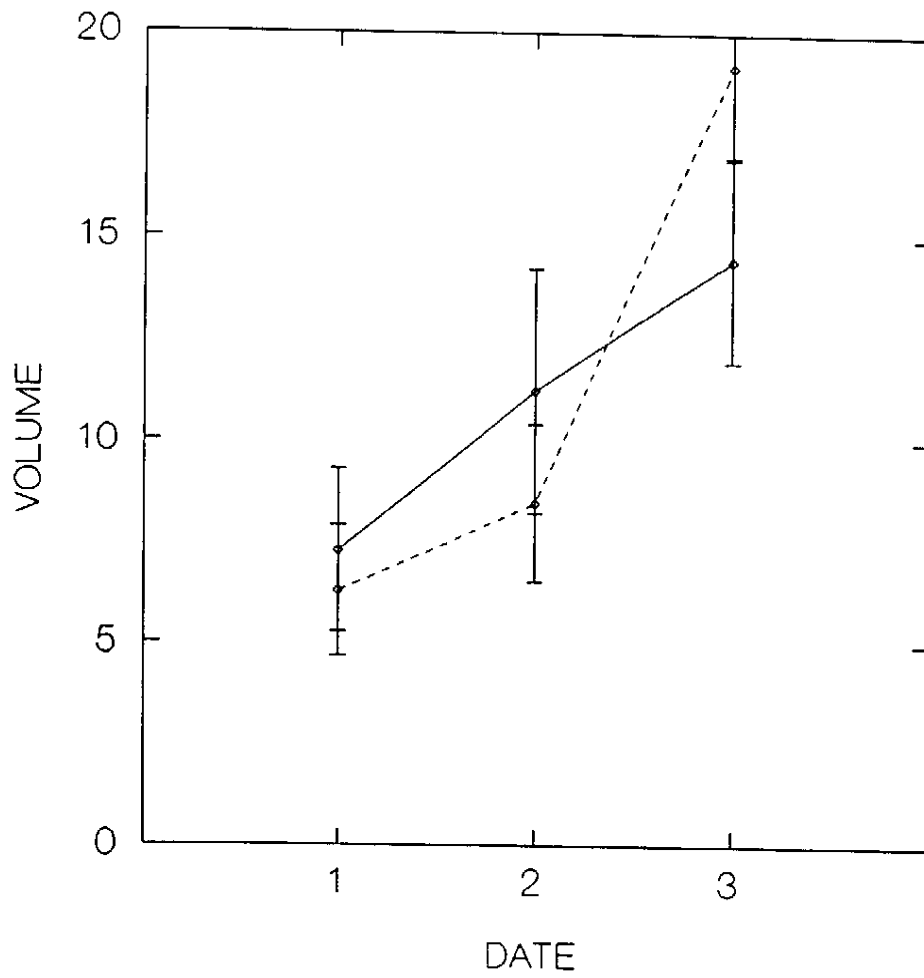


Fig.15. Growth in maximum volume (in milliliters) over time in the experimental(---) and control quadrat (—)

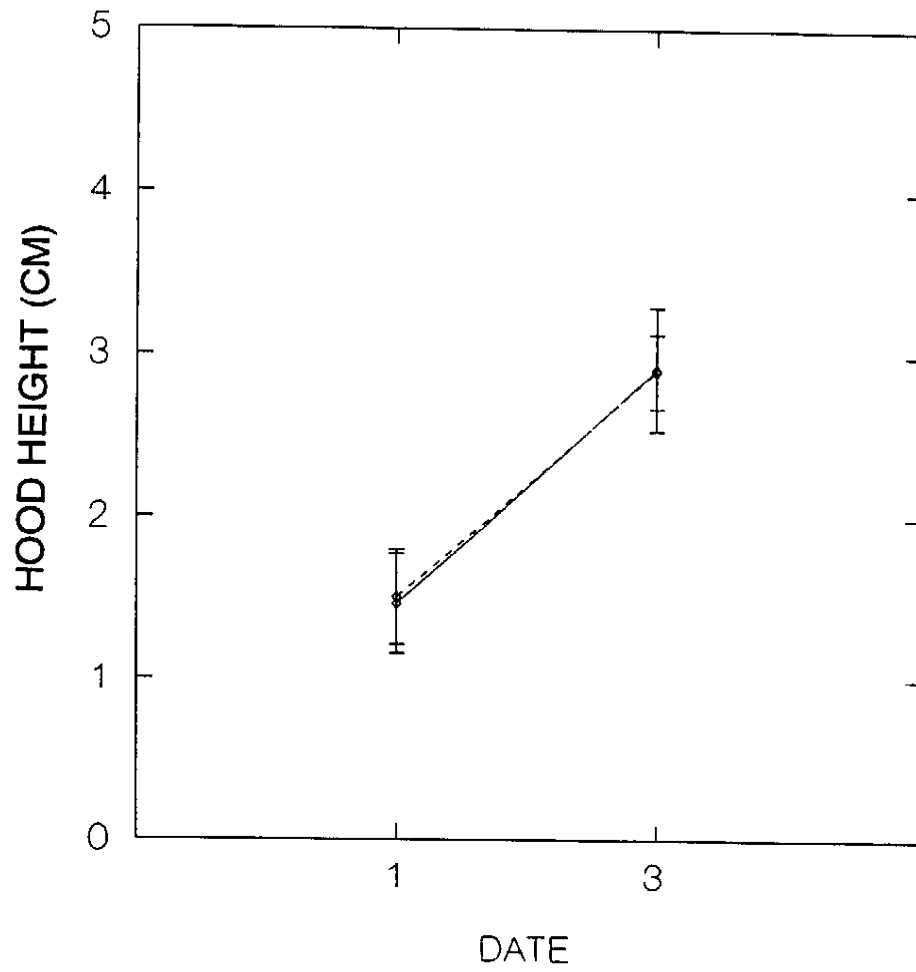


Fig.16. Growth in hood height in centimeters over time in the experimental (---) and control quadrat (—)

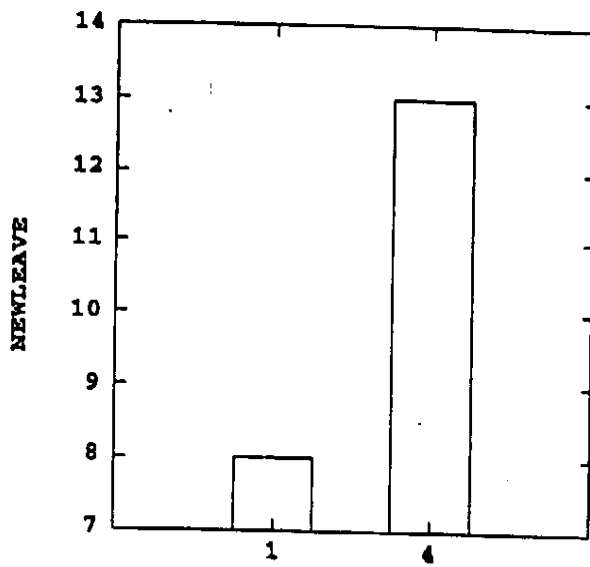


Fig.17. Number of new leaves produced in the experimental quadrat (#1) and control quadrat (#4)

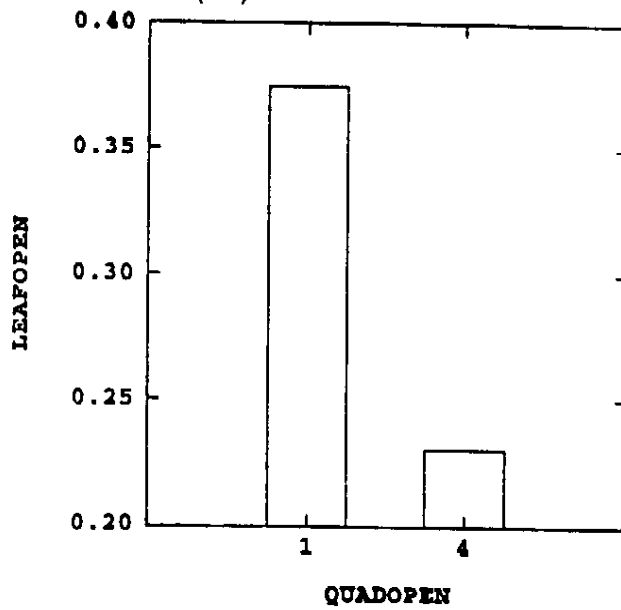


Fig.18. Percentage of pitcher openings of the newly-produced leaves in the experimental quadrat (#1) and control quadrat (#4).

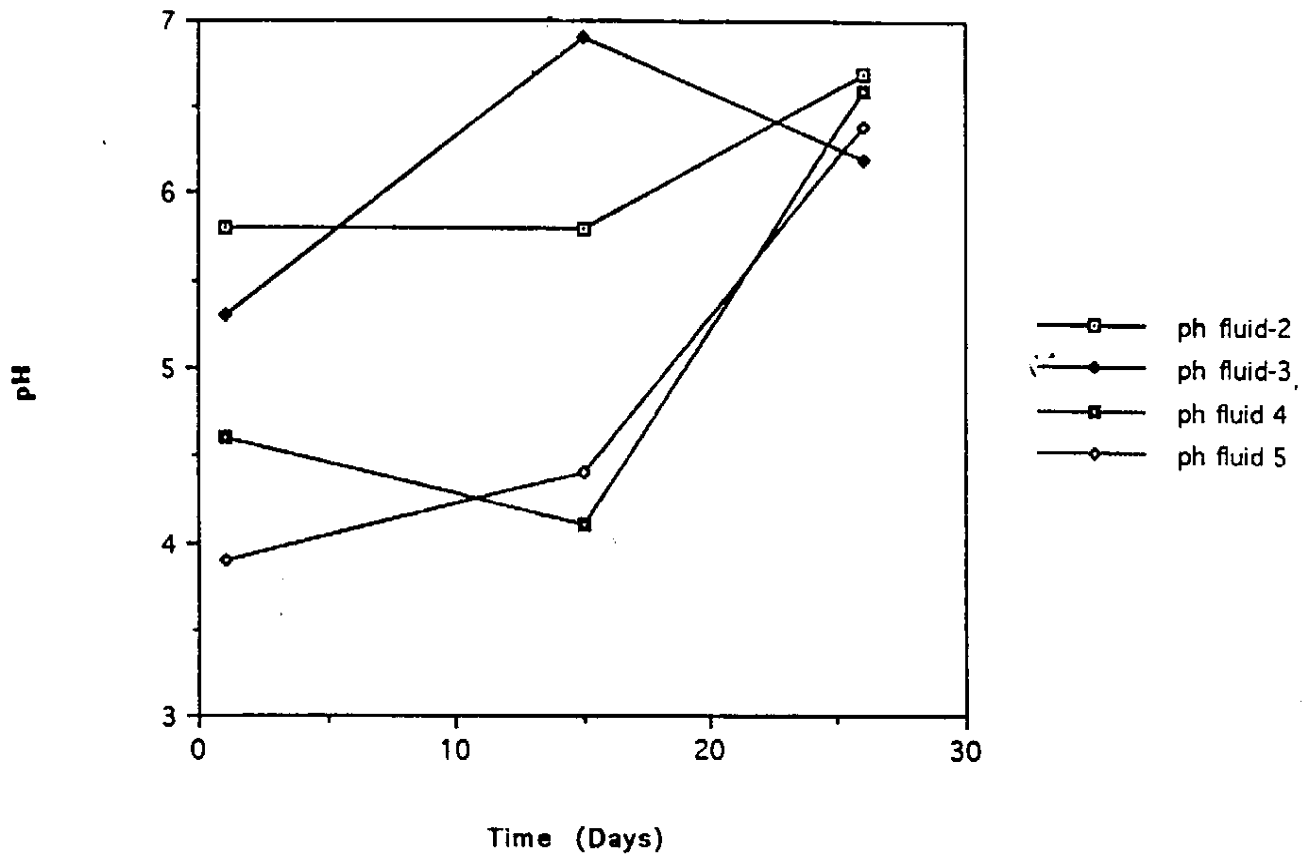


Fig.19. pH of the phytolamata over time in sampling site:

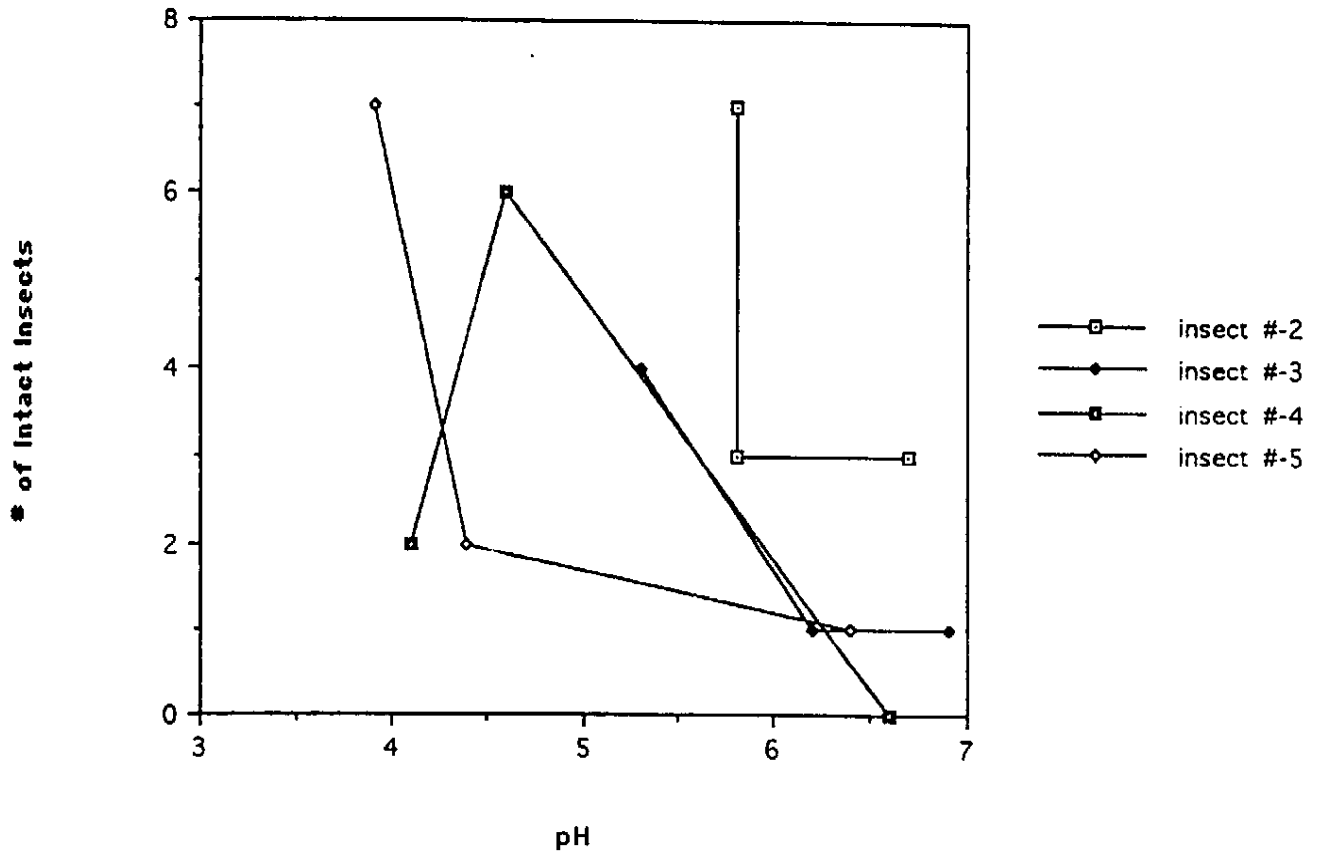


Fig 20. Relationship between number of intact insects found in phytotemata and pH of the phytotemata in sampling sites 2-5.

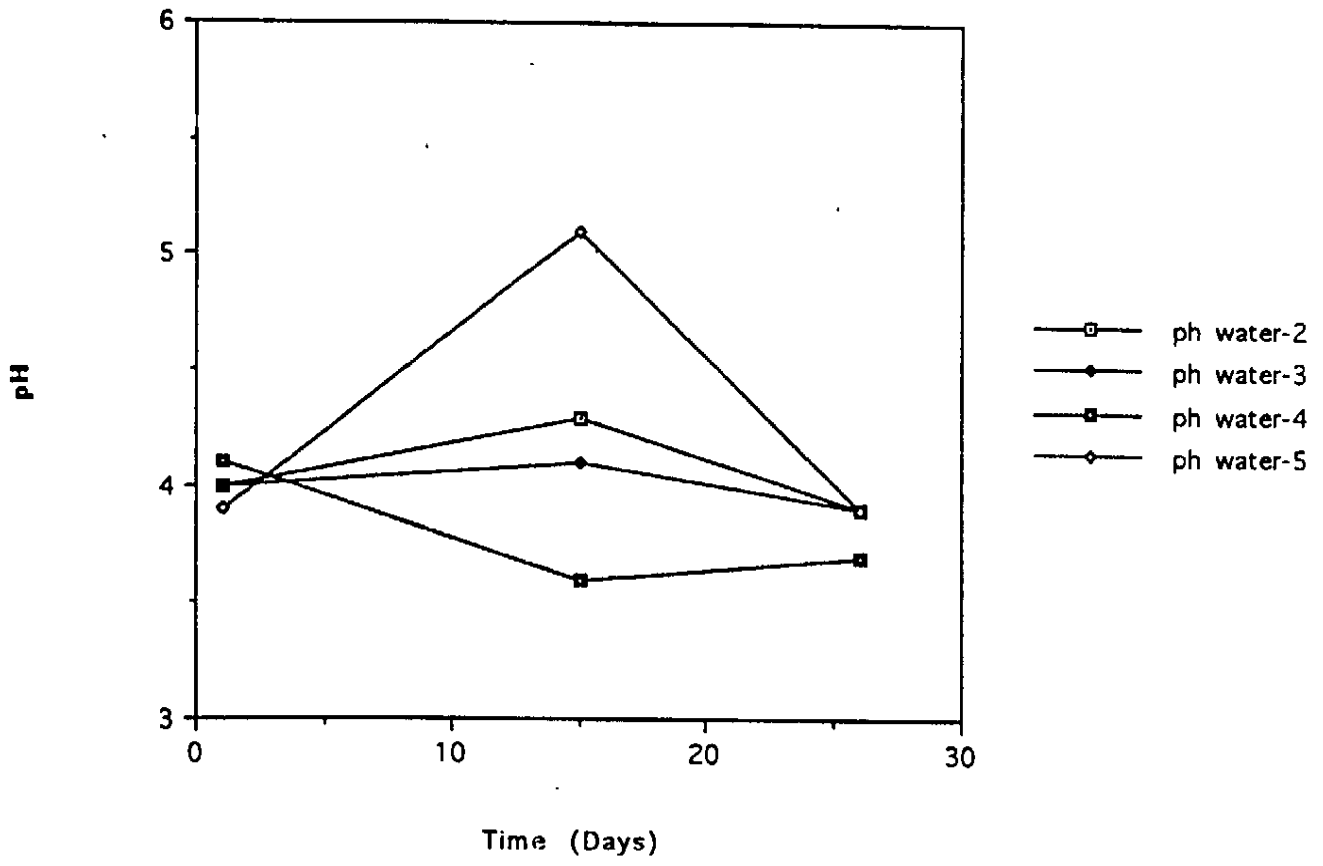


Fig.21. pH of the perched water table over time in sampling sites 2-5.

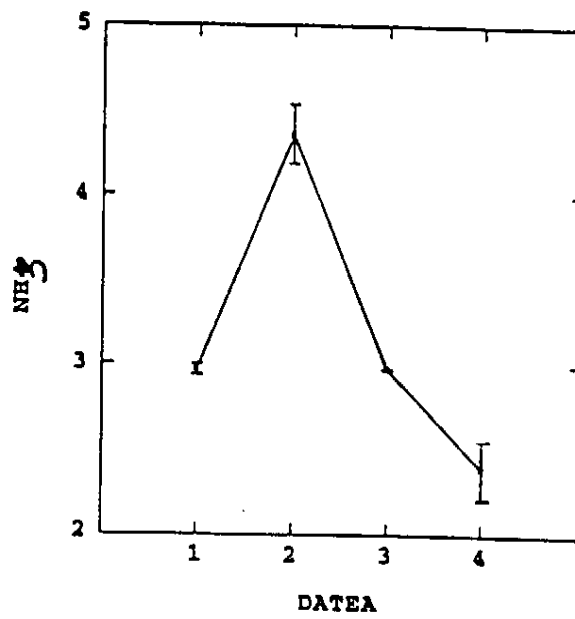


Fig.22. Concentration of NH_3 in the perched water table at the intersection of the four marked quadrats over time.

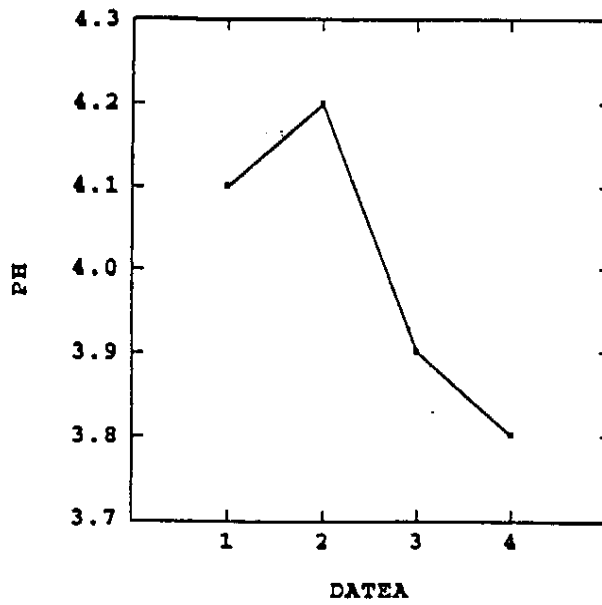


Fig.23. pH of perched water table at the intersection of the four quadrats over time.

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