Insect-Induced Changes in *Chromolaena odorata*

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*Pareuchaetes pseudoinsulata* was introduced to Guam to control the noxious weed *Chromolaena odorata*. Insect-induced biochemical and physiological changes were observed in *C. odorata* when *P. pseudoinsulata* fed on it. The photosynthesis rate and the chlorophyll content were reduced in insect-infested plants. This is a reversible process. When *P. pseudoinsulata* is removed, the leaves return to green. Protein separations were performed using SDS polyacrylamide gel electrophoresis and size exclusion chromatography. Several different proteins were present in the leaves of plants with induced change. Ribulose-1,5-bisphosphate carboxylase is present in reduced quantities in yellow plants. The quantity of flavonoids in yellow leaves is apparently higher than in green leaves.

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**Introduction**

*Chromolaena odorata* (L.) R. M. King & H. Robinson (Asteraceae) is a perennial shrub native to the new world tropics. It is an aggressive weed that has become a pest in many tropical regions around the world. The first documented record of *C. odorata* in the Mariana Islands was from Apra Harbor, Guam in 1963 (Seibert, 1989). *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) was introduced to Guam and other islands in the Mariana Islands at the beginning of 1985 to control *C. odorata*. *P. pseudoinsulata* has been successful in controlling the spread of *C. odorata* and has reduced its dominance as a weed (Muniappan et al. 1989, Seibert 1989).

Various physiological changes occur in *C. odorata* when *P. pseudoinsulata* feeds on it. Leaves of infested plants turn yellow over the entire plant. It is not a localized response. The change in leaf color appears to be the result of defensive mechanisms specifically induced by *P. pseudoinsulata* feeding.

Several attempts were made to induce the yellowing response artificially (Marutani and Muniappan 1991). The color change could not be induced by either cutting or tearing the leaves. Drenching a saturated solution of excreta of caterpillars on leaves, which were either intact or wounded, did not induce leaf yellowing.

Palatability, survivability and feeding behavior of *P. pseudoinsulata* caterpillars was affected by the leaf yellowing (Marutani and Muniappan 1991). Caterpillars placed in containers in the presence of green and yellow leaves favored the green leaves. First instar caterpillars fed solely yellow leaves do not survive. The nocturnal feeding behavior of caterpillars also changed on yellow plants. On green plants, caterpillars migrate to the ground during the day and migrate up the plant towards evening. On yellow plants, the caterpillars show no regular movement pattern. To understand the interactions between these 2 organisms, several studies were conducted to determine what biochemical and physiological responses occur in *C. odorata*.

**Methods and Materials**

**Analytical Procedures**

*Chlorophyll and leaf color measurement.* Leaf color and chlorophyll content were measured by several different methods. Chlorophyll content was estimated using a chlorophyll meter (SPAD Minolta Corp.). A Chroma Meter (Minolta Corp.) was used to measure leaf color in the 3 components: L
(luminance); a (green-red); and b (blue-yellow). To further quantify the observed leaf color changes, leaf chlorophyll was extracted from green and insect-induced yellow leaves by homogenization in acetone (Thammasiri et al. 1986). Leaf tissues of 2-4 g fresh weight were immersed in deionized water for 15 min. Leaves were gently wiped dry and homogenized in 75 ml acetone. A slurry was filtered through glass wool using a Buchner funnel connected to a water aspirator and the residue was extracted repeatedly with acetone until the filtrate was colorless. The amount of filtrate was measured and an equal amount of petroleum ether was added. The entire mixture was placed in a separatory funnel and water (15% of mixture volume) was added. The funnel was inverted several times and then immobilized until 2 distinct layers were present. After the lower layer was drained and discarded, the upper layer was poured into a round flask wrapped with aluminum foil. The solvent was evaporated and the residue was resuspended in 3 ml of acetone and stored at -29°C. Samples were separated in a high performance liquid chromatograph (HPLC) with a reverse phase column. HPLC chromatograms of the chlorophyll extractions were compared at 445 nm.

Photosynthesis (CO₂ uptake). Rate of photosynthesis was estimated from the rate of CO₂ Photosynthetic Photon Flux Density (PPFD) and chamber temperature uptake with an LI-6200 photosynthesis meter (LI-COR).

Water soluble protein analysis. Water soluble proteins were extracted from C. odorata to determine what changes occur in green and yellow leaves. Initially, samples of green and yellow tissues were separated on acrylamide gels. About 1 g plant samples were macerated in extraction buffer solution in a plastic bag. The gels used were 12% SDS polyacrylamide prepared according to Laemmli (1970) and Ausubel et al. (1989). Samples of 40 μl were used per well. The gels were run with 1x SDS buffer at 30 mA for 5 h.

In Experiment 4, pre-cast 12% SDS tris-glycine and 4-20% SDS tris-glycine gels were used. Samples were colorimetrically compared for protein concentration. The volume per well varied depending on the protein concentration to allow uniformity of concentration among lanes. The volume range was 10-20 μl. The gels were run with 1x SDS buffer at 30 mA for 90 min. The gels were stained with Coomassie Blue. Molecular weight standards were included in gel runs. Dried gels were scanned with a densiometer and the molecular weights of unknowns were estimated.

In addition to separating the proteins of C. odorata by electrophoresis, a size exclusion column (SEC) has been used with an HPLC. The column was 4.6 mm ID x 250 mm long and was packed with 5 μm particle size silicon chemically bonded with a hydrophilic polymer layer. The pore size was 300 Å. This column separates the proteins by molecular size.

Flavonoids. Samples (3 g fresh weight) of green and yellow leaves were homogenized in 30 ml of 80% methanol (MEOH). The homogenate was filtered through 45 μm filters and then through SPICE (ANALTECH) cartridges. Samples (20μl) were injected and run with a methanol-acetic acid mobile phase gradient. The samples were run at 2 different wavelengths (280 and 340 nm).

Additional samples were prepared by homogenizing 16 g of leaf tissue in 150 ml of 80% MEOH. The solvent was evaporated and the homogenate was redissolved in 10 ml of 80% MEOH. The samples were filtered through 45 μm filters and then were passed through SPICE columns. The injected 20μl samples were run at 280 and 340 nm with a reverse phase column.

Experimental Procedures

Experiment 1. Roots, young green stems, older stems, young leaves (shoot tips), mature leaves from the middle of plant, and old leaves from the base of C. odorata were sampled for qualitative protein composition. The samples were compared by gel electrophoresis as described below.

Experiment 2. Mature leaves of both uninfested green plants and insect-induced yellow plants were sampled in the field. The samples were compared by gel electrophoresis. Ribulose-1,5-bisphosphate carboxylase was used as a marker. Leaf samples were also taken for flavonoid analysis using HPLC.
Experiment 3. Eight potted plants of C. odorata were individually covered with muslin cages. Five 3rd-5th instar caterpillars of P. pseudoinsulata were placed on each of 4 plants. The plants were compared over time for physiological and biochemical changes due to the insects' feeding. Leaf samples were analyzed for protein content by gel electrophoresis chlorophyll content and CO₂ uptake.

Experiment 4. This experiment was similar to Experiment 3. In addition to qualitative protein comparisons, the plants were measured daily for changes in chlorophyll content and leaf color. After the chlorophyll level in the infested plants reached a stable low level (11-17 mg/cm²), caterpillars were removed and plants were allowed to recover. Leaves were sampled over time as they were consumed by the caterpillars. Carbon dioxide uptake was measured as the chlorophyll levels changed.

Results and Discussion

Chlorophyll and Leaf Color Measurement

It was found that green uninfested plants had chlorophyll levels of 30-45 mg/cm². In the insect-infested plants, the chlorophyll levels dropped as low as 9 mg/cm² (Fig. 1). There was a great reduction in all pigments detected at this wavelength (Figs. 5 and 6). The yellow leaves had reduced quantities of several compounds when observed at 450 nm (Fig. 6). Some variation occurred in the response but the general response was for the infested plants to experience a drop in chlorophyll that returned to normal levels after removal of the caterpillars. The time series plots of chlorophyll levels and leaf color are presented in Figs. 1-4. The chlorophyll levels in the infested plants dropped to readings of 9-17 mg/cm². Once the caterpillars are removed, the chlorophyll level increased to a level similar with the uninfested plants.

Photosynthesis (CO₂ Uptake)

Leaf yellowing is a result of changes in chlorophyll concentration and this results in reduced level of CO₂ uptake or reduced photosynthesis. These changes indicate a degenerative process occurring in yellowing plants. It is a reversible process. When caterpillars were removed, the plants fully recovered.

Water Soluble Protein Analysis

In Experiment 1, differences in the protein profile were found among the various plant parts. Roots and older stems were found to have fewer protein bands. There were no qualitative differences in total proteins among the young, mature or old leaves.

![Figure 1. Time series plot of chlorophyll content in leaves of infested (CLIN) and not infested (CLNI) leaves of C. odorata.](image-url)
Figure 2. Time series plot of leaf color (L) in leaves of infested (LCLIN) and not infested (LCLNI) leaves of *C. odorata*.

Figure 3. Time series plot of leaf color (a) in leaves of infested (LClIN) and not infested (LClNI) leaves of *C. odorata*.

Figure 4. Time series plot of leaf color (b) in leaves of infested (LCbIN) and not infested (LCbNI) leaves of *C. odorata*. 
Figure 5. HPLC chromatogram of chlorophyll content in green leaves.

Figure 6. HPLC chromatogram of chlorophyll content in yellow leaves.
In Experiment 2, leaf tissues of all green plants in the field populations of *C. odorata* were identical whereas leaves of insect-induced yellow plants displayed variation in protein banding. There was also variation within the yellow examples. Major changes were noted in the region of the large and small subunits of ribulose-1,5-bisphosphate carboxylase (Rubisco). The bands became faint in yellow leaves.

In Experiment 3, artificial infestation of *C. odorata* confirmed the results of Experiment 2. Among the 4 plants infested, 2 turned yellow faster than the others. The change in the band for the small subunit of Rubisco occurred to a greater degree in the faster yellowing plants. The plants with no insects displayed the same protein pattern as the green plants in Experiment 2.

Additional samples were run on 10 and 12% tris/glycine gels and trycine 10-20% gradient gels. Rubisco was used as a standard and molecular weight markers were used to estimate the molecular weights of the various bands. There were 2 main bands for the 2 subunits of Rubisco. The large subunit had a molecular weight of about 53,000. The small subunit had a molecular weight of 14,000. It was found that Rubisco was one of the key bands that changed between yellow and green samples. This enzyme plays a key role in the carboxylation and oxygenation of ribulose 1,5-bisphosphatate. It has an important role in photosynthesis and it is generally the most abundant component among water soluble proteins. It is also thought to play a role as a storage protein. The catalytic functions of Rubisco are located on the large subunit. The function of the small subunit is unknown. While a general banding pattern was observed for green and yellow plants, some inconsistencies were observed. Particularly in the yellow plants sampled, the banding pattern was variable. Some green plants also did not produce the expected pattern. When sampling, the plants were categorized as either green or yellow based on chlorophyll readings. Although some of the leaf samples were yellow, the banding pattern was more similar to a green plant and vice versa.

It was realized that there are not 2 discrete classes of yellow and green; there is a continuous gradient between green and yellow. When green plants are chewed, the leaves over the entire plant begin to turn yellow until the chlorophyll content reduces to 9-15 mg/cm². After the leaves are consumed or become less palatable, the caterpillars may migrate to new plants (Marutani and Muniappan 1991). After the caterpillars leave the plant, new leaves, any remaining leaves and portions of chewed leaves return to green color. One phase of the cycle is degradation in response to infestation; the other phase is recovery. The process appears to be an example of reversible senescence. This reversibility would explain some of the discrepancies observed in protein banding patterns. Some of the yellow samples were in the recovery phase having already left the degradation phase.

The peaks observed from protein analysis by HPLC were similar to the results obtained by electrophoresis. Most noteworthy were the apparent changes in the sub-units of ribulose-1,5-bisphosphate carboxylase (Figs. 5 and 6).

### Flavonoids

Chromatograms from leaf samples indicated that there are no qualitative differences between green and yellow plants (Figs. 7-10). There are quantitative differences between green and yellow samples. Yellow samples have consistently shown higher concentrations of compounds. Particularly noticeable are the compounds with retention times of 10 and 22 min.

### Conclusions

The results indicate that many biochemical and physiological changes occurring in *C. odorata* were due to insect-induced changes. The changes indicate that the plant responds with defensive mechanisms resulting in a senescent response. There are biochemical changes, in response to infestation, which cause the plant to undergo a degradative or senescent process. If the insects are removed from the plants, the degradation process reverses.
Figure 7. HPLC chromatogram of water soluble proteins in a green leaf.

Figure 8. HPLC chromatogram of water soluble proteins in a yellow leaf.
Figure 9. HPLC chromatogram of compounds at 340 nm from a green leaf sample.

Figure 10. HPLC chromatogram of compounds at 340 nm from a yellow leaf sample.
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References


