







# Enemy Tolerance of *Eupatorium* Plants

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**Keywords:** Natural Enemy, Invasive Plant, Tolerance.


**Abstract:** Alien plants can tolerance the feeding of natural enemy by compensatory growth. In this study, the growth responses of invasive *Eupatorium adenophorum* and native *E. fortune* plants to simulated insect feeding were analyzed. The results showed that the total biomass of *E. adenophorum* had no significant change after simulated insect feeding. The results indicated that *E. adenophorum* rapidly increasanges, but the leaf biomass ratio increased significantly; the total biomass of *E. fortune* decreased signified the biomass of photozygous organs to cope with the simulated leaf loss. As a result, the invasive plant *E. adenophorum* is more tolerant than the native plant *E. fortune*.


## 1 INTRODUCTION


*E. adenophorum* is native to Mexico in North America and Costa Rica in Latin America (Qiang 1998). Due to its strong reproductive capacity, adaptability and fast spread rate, it has caused serious harm to human beings, livestock and even the ecological environment (Xiao 2009). At present, the control methods of *E. adenophorum* mainly include three types: artificial mechanical control, chemical control and biological control (Wang 2004). Among biological control, *Procecidochares utilis* Stone, *Orthezia quadrua*, *Dorylus orientalis* were reported to damage *E. adenophorum*.


*Procecidochares utilis* Stone *Procecidochares utilis* Stone is the specialist enemy of *E. adenophorum*, belonging to Diptera (Diptera: Muscidae), and has certain inhibitory effect on the growth and reproduction of *E. adenophorum* (Gao 2019, Lei 2014). In 1945, the United States introduced *Brasilia sinensis* from Mexico to Maui Island of Hawaii and established the population, and


succeeded in studying its biological characteristics and the feasibility of using it to control the population of *E. adenophorum sinensis* (Ming 2017). In July 1984, the Institute of Ecology of the Chinese Academy of Sciences sent experts to Yadong and Nyalam counties of Tibet, which are on the border with Bhutan, Sikkim and Nepal, and found the *P. utili*, which was introduced to some areas of Yunnan, and then gradually spread to the southwest of Sichuan, Guizhou and Guangxi (Wang 2013). It was found through experiments that the oviposition only on the tender tips of *E. adenophorum* plants, while the hatched larvae crawled to the base of leaves, penetrated the meristems, and entered the stems at the upper part of the shoots, constantly feeding on the young parts at the growth points of *E. adenophorum*, thus impeding the circulation function of the three major vegetative organs of *E. adenophorum* (Xin 1990). People make full use of this habit to inhibit the normal growth and development of *E. adenophorum*, and eventually lead to the wilt of *P. utili* by *E. adenophorum* due to malnutrition, so as to achieve the purpose of biological control. In the course of its development over the next 30 years, it was found everywhere, but its control ability was very limited and it could not achieve the desired effect. On the one hand, *E. adenophorum* has the unique biological characteristics of high seed yield, fast dispersal


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speed, strong ability to regenerate tillers, and vegetative reproduction of plant organs such as roots and stems. On the other hand, it is only an insect, so it takes some time to fully display its biological characteristics. It is impossible to reach the degree that *E. adenophorum* will wither and die immediately once it is parasitic. For these two reasons, the diffusion rate of *E. adenophorum* was much faster than the parasitic rate of *P. utili*, so the expected control effect could not be achieved.

In addition to specialist enemy, the growth of some native generalist enemies is also a threat for *E. adenophorum*. In April 2007, Chinese researchers discovered another insect feeding on *E. adenophorum*, *O. quadra*, on the way from Yingjiang to Tongbiguan Nature Reserve in Yunnan Province. At the time, the researchers observed damage in the field: the *E. adenophorum* had been severely damaged, the whole plant turned brown and withered. This insect mainly concentrates on the stem of *E. adenophorum*, and mostly concentrates on the node of the stem to suck the plant juice (Xu 2011).

It was found that the *D. orientalis*, which would bite the stems and roots of *E. adenophorum*, form holes or eat them completely, and damage the epidermis, cortex, phloem, root cambium and xylem tissues of *E. adenophorum*, thus resulting in the death of *E. adenophorum* due to the nutrient exchange between the broken roots and buds (Yao 2008). *D. orientalis* have a certain selectivity in foraging activities. They usually prefer food with foul or aromatic odor, and the strong and unique odor of *E. adenophorum* is the chemical signal that attracts *D. orientalis* to forage (Chen 2012).

However, plants tend to resist insect feeding through their own defense mechanisms, such as tolerance. Plant tolerance is the ability of a plant to prevent, reduce, or repair damage by compensatory growth. For example, Ellison (1960) believed that the compensatory effect of plants was that the feeding of herbivores was beneficial to the growth of plants (Ellison 1960). Belsky believed that the compensatory effect of plants was a positive response to plant injury, and defined compensatory growth as "the increase of plant biomass and seed yield due to foraging" (Belsky 1986). Therefore, after the research and induction of many scholars, the increased biomass or seed yield of plants under stress was defined as the compensatory effect of plants.

Although several insect have been found and damaged on *E. adenophorum*, we don't know much about how *E. adenophorum* responds to native

generalists. In this study, we investigated the biomass allocation of invasive *E. adenophorum* and native *E. fortune* under natural enemies feeding. The findings of this study are expected to improve our understanding of the compensatory growth of *E. adenophorum* and native plant, and to evaluate the role of native generalist enemies in the process of alienplant invasion.

## 2 MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Seed Collection

Seed collection was carried out one year before the trial. Since the seeds of *E. adenophorum* and *E. fortune* belong to achenes with small and light size, and difficult to collect manually, in order to prevent the early shedding of seeds and the mixing of other unknown seeds, so as to obtain a large number of healthy, complete seeds with high germination rate, the method of isolated seed collection was adopted in this experiment. Isolation method of seed in flowering plants (*E. adenophorum* flowering in November - the following April, *E. fortune* flowering for 7 - November), selected out from the need for seed plants grow strong, no plant diseases and insect pests, variety of pure plant, and then according to the shape of the flowers of the plant to choose appropriate to the size of the yarn pockets of bagged processing.

After the seeds of bagged plants mature and fall off, the collected seeds should be stored in a ventilated and cool place to dry in the shade, and then stored in the envelope bag prepared in advance to prevent the seeds from being dampened and mildewed.

#### 2.1.2 Seedlings

The whole seedling breeding process of this experiment was carried out under the condition of 50% light intensity in the greenhouse. To raise seedlings, the prepared humus soil (0-10 mm Danish Pinclap trophic soil) and sandy soil were evenly mixed in a 3:2 ratio to form a seedling substrate. Then put the mixed seedling substrate into the seedling tray (size: 40 cm \* 40 cm), until it is only about 1 cm away from the hole plate, stop adding soil. Then, continue to fill 19 seedling plates in the same way. The 20 seedlings were divided into 2 groups (*E. adenophorum* group and *E. fortune*

group), with 10 seedlings in each group, and corresponding labels were made. Next, lay a piece of kraft paper on the ground, remove the previously collected seeds from the envelope bag, and gently rub them with your hands so that the seeds are removed from the other non-seed structures and fall onto the kraft paper. After the separation of all the seeds is completed, the separated seeds are evenly dispersed into the seedlings tray of the different groups, and the number of seeds in each seedling tray is controlled at about 100. Finally, the substrate from the sieve is gently sifted evenly into the tray so that the seeds in each tray are thinly coated with the substrate and cannot be blown away by the wind or washed away by water. When all this is done, water the two groups of seeds with a water bottle. After that, water it regularly every two days.

### 2.1.3 Thinning

When the plant had three or four leaf, in order to avoid crowded seedlings, mutual shading, save soil moisture and nutrient, and cultivate strong seedling and guarantee the seedling size is consistent. Thus thinning for plant and dish were required, finally every dish reserved 50-70 seedlings.

### 2.1.4 Transplant

When the height of seedlings were 5 cm, transplanting was started. First, prepare a new nutrient bowl (model: 30 cm \* 35 cm) and an adequate substrate (a mixture of river sand and soil in a 1:2 ratio). Then, the transplanting substrate was placed up to two-thirds of the bowl, and the nutritional bowls were arranged in a pattern of 10 units per row and 4 units per column, with a flowerpot tray under the arranged nutritional bowls. After that, seedlings were raised.

The two plants that transplanted to nutritional bowls should be watered thoroughly one day in advance. When seedlings are raised, the root system of the uprooted plants should be intact and not damaged. One third of the soil at the base of the roots should be removed, and the rotten roots and rotten roots should be removed. In the process of transplanting, the seedlings are held by one hand, and the roots are placed in the dug soil nest. The roots of the seedlings can not be twisted, but should be smoothed, and the spaces around them should be filled and compacted with soil. Transplanting should not be too deep, the root of the plant can be planted. After planting, seedlings should be watered again to ensure the survival of seedlings after transplantation. Since then, watering were taken once every two

days. If the growth of seedlings were slowed, and or did not survive, these seedlings should be timely supplemented. After two weeks of adaptation to 36 % light, the transplanted seedlings began to grow under full light conditions.

### 2.1.5 Fertilization Management

The growth rate of plant seedlings is accelerated after transplanting, then the vigorous growth period begins. At this time, the seedling plant nutrients gradually accumulated, rapid thickening of rhizome, leaf number gradually increased, the demand for fertilizer and water increased significantly. Therefore, in order to promote the growth of the stem and leaves, to meet the growth needs of the plant seedlings, in this period, the corresponding fertilization management should be carried out.

Firstly, according to the dosage of 0.5 g fertilizer (N, P, K available nutrient content  $\geq 35\%$ ) per kg of soil, thus 2 g fertilizer per bowl was weighed. Then dig a hole 5-10 cm deep in each of the four directions about 5 cm from the seedlings. Finally, 0.5 g fertilizer was applied to each hole and then covered with soil to prevent the volatile of fertilizer and reduce waste.

## 2.2 Methods

Twenty sample plants of *E. adenophorum* and *E. fortune* were divided into two groups. One group was treated as simulated insect feeding (MN), in which all leaves (except the top 2-4 young leaves) of a single plant were removed about 50% of the area of the single leaf. Another control treatment (CK) to eliminate natural enemies was to apply a compound insecticide to the leaves of the plants. After the treatment was completed, the whole plant was covered with a gauze net bag of suitable size.

At the end of the experiment, 5 samples out of 10 single plant replicates were randomly harvested in both treatments (MN and CK) of *E. adenophorum*. After the roots, stems and leaves of the plants were thoroughly rinsed and dried, each plant was divided into three parts: root, stem and leaves, and the weight of root, stem and leaves was weighed by an electronic balance (accurate to 0.001g). Then put the weighed roots, stems and leaves into three different envelope bags, and mark the corresponding numbers under different treatment methods on the envelope bags. After completion, all the envelope bags were put into an oven, which was first dried at 105 °C for 1 h, and then baked at 65 °C for 48 h until the

sample was constant weight. Finally, after standing for 10 h, the samples in each envelope bag were weighed again. *E. fortune* did the same thing.

Total biomass (total biomass = root weight + stem weight + leaf weight), root biomass ratio (RMR, root weight/plant total weight), stem biomass ratio (SBR, supporting structure biomass/plant total weight), leaf biomass ratio (LMR, leaf weight/plant total weight), root/shoot ratio (R/C, underground biomass/aboveground biomass), tolerance (BMN-BCK)/BCK).

### 3 RESULTS AND ANALYSIS

#### 3.1 Dry Biomass Allocation

The root biomass ratio and root-shoot ratio of *E. adenophorum* in the control group were significantly lower than those of the same native plant, *E. fortune*. While the leaf biomass ratio was significantly higher than that of *E. fortune*. The root biomass ratio and root-shoot ratio of *E. adenophorum* in the simulated insect feeding group were significantly lower than those of the native plant, while the total dry weight and leaf biomass ratio of *E. adenophorum* were significantly higher than those of the native plant. The results showed that the total dry weight of the native plant *E. fortune* decreased significantly, while the leaf biomass ratio of the invasive plant *E. adenophorum* increased significantly after the simulated insect feeding treatment.

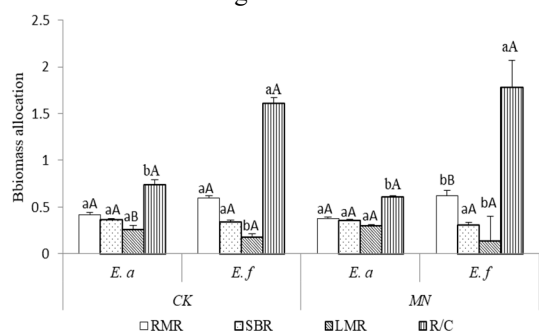


Figure. 1. Dry biomass allocation

#### 3.2 Fresh Biomass Allocation

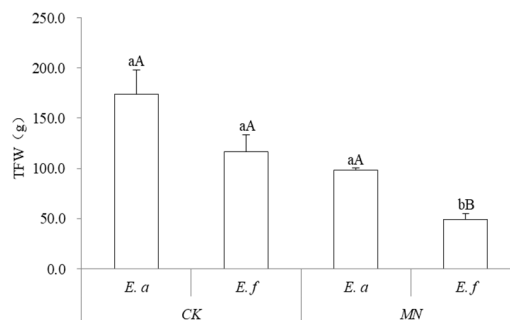


Figure 2: Total fresh weight of biomass (TFW).

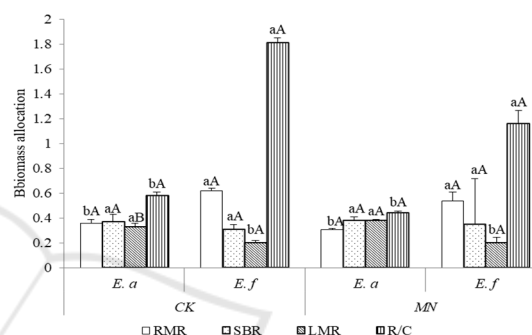


Figure 3: Fresh biomass allocation.

The root biomass ratio and root-shoot ratio of *E. adenophorum* in the control group were significantly lower than those of the same native plant, *E. fortune*. While the leaf biomass ratio was significantly higher than that of *E. fortune*. The root biomass ratio and root-shoot ratio of *E. adenophorum* in the simulated insect feeding group were significantly lower than those of the native plant, while the total fresh weight and leaf biomass ratio of *E. adenophorum* were significantly higher than those of the native plant. The results showed that the total fresh weight of the plant decreased significantly, while the leaf biomass ratio of *E. adenophorum* increased significantly after simulated insect feeding.

#### 3.3 Tolerance

There were no significant differences in the changes of fresh stem water content and root-shoot ratio water content of *E. adenophorum* and *E. fortune*. While the changes of total water content, fresh root water content and fresh leaf water content of *E. adenophorum* were significantly lower than those of *E. fortune*, the tolerance of *E. adenophorum* was significantly higher than that of *E. fortune*.

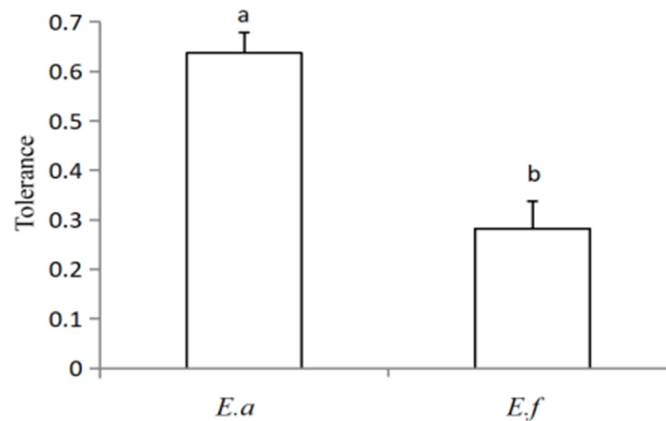


Figure 4: Tolerance in different plants.

#### 4 DISCUSSION AND CONCLUSION

The growth strategies in the biomass allocation were different between *E. adenophorum* and *E. fortune* under herbivory. More biomass of *E. adenophorum* were allocated to leaf compared to *E. fortune*, which can confer its higher carbon income and tolerance for biomass accumulation than native *E. fortune*. thus its total biomass was significantly higher than *E. fortune* after herbivory. Therefore, the biomass allocation pattern made a great contribution to the tolerance and invasion ability of the invasive plant *E. adenophorum*.

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