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# Defense and carnivory: Dual role of bracts in *Passiflora foetida*

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**Abstract.** Members of the genus *Passiflora* are reported to have evolved modifications which kill insects; they have however never been tested for carnivorous syndrome. The flowers of *Passiflora foetida* consists of highly reticulate bracts which cover and grow along with the buds and fruits. Removal of bracts from developing bud and fruit resulted in higher predatory damage compared to those where the bracts were intact. These bracts also possess a large number of minute glands which ooze sticky secretion. A variety of tiny insects were found trapped by the secretion of the bracts. The secretion of these glands show high proteases and acid phosphatase activity, two common digestive enzymes found in traps of true carnivorous plants. A high quantity of aminoacids were released from freshly freeze killed ants when incubated in buffer extract of bracts· [$^{14}$C] phenylalanine smeared on the glandular surface of bracts was recovered from ovules suggesting potential for absorption of aminoacids. These results suggest a novel role for bracts where primary function is to minimize predatory damage to developing flowers and fruits. The bracts serve as insect traps and also possess the mechanism to digest the trapped insects to obtain free aminoacids.

**Keywords.** *Passiflora foetida;* bracts; proteolytic enzymes; carnivory.

## 1. Introduction

Carnivorous plants are characterized by contrivances to attract and trap insects; mechanisms to kill, digest and absorb useful elements from them· Members of the family Droseraceae, Cephalotaceae, Lentibulariaceae, Nepenthaceae, Sarraceniaceae, Dioncophyllaceae, etc., possess these characters and have been classified as true carnivorous plants (Benzing 1987). Several species of the genus *Passiflora* have long been suspected to possess such syndrome and considered primitively carnivorous (Juniper *et al* 1989). For instance, Gilbert (1971) demonstrated that *P. adenopoda* kills the *Heliconiine* caterpillars falling on the sharp trichomes present on the leaf surface. Flowers of some species of *Passiflora* possess glandular bracts which cover growing buds and fruits. The present study was designed to demonstrate the role of these bracts in *P· foetida*.

### 1.1 *System*

*P. foetida* (Family: Passifloraceae) is an insect pollinated, herbaceous climber usually grown in nutrient poor soils· Each plant produces radiating branches which bear a

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cordate leaf and flower bud at every node· Each flower bud is covered by three highly reticulate green bracts (figure 1). Veins of the bracts end with tiny glandular structures which secrete adhesive oozate· Small insects are found stuck to these bracts. While the bracts completely cover the unopened buds and developing fruits, the open flowers which last for a day, are uncovered ensuring the free movement of pollinators (figure 1). We tested whether bracts of *P. foetida* impart protection from predators to the developing bud/fruits and possess the necessary machinery to digest insects and absorb aminoacids·


**Figure 1.** Diagrammatic representation of habit of *P. foetida.* **(a)**, unopened flower bud covered by bracts; **(b)**, opened flower; **(c)**, developing fruit covered by bracts·

## 2. Methods

Wild plants of *P. foetida* growing in the botanical garden of the GKVK campus of the University of Agricultural Sciences, Bangalore (12° 58′N, 77° 35′E) were used in the present study.

Developmental stages of the flower were classified into (i) bud (1-5 days before flower bloom), (ii) fertilized flowers (1-3 days after flower bloom) and (iii) developing fruits (4th day after flower bloom and later). Observations were made on randomly chosen buds/flowers and developing fruits and the parameters listed in table 2 are recorded. Length of the flower buds and width of the fruits were measured to nearest mm using a Vernier calipers. Surface area of the bracts was measured by tracing out the margins of bracts on a graph sheet. Insects caught in these bracts were identified under microscope up to the family.

To understand the consequences of removal of bracts on various developmental stages of flower, a set of 10 plants were tagged and bracts from all the developing stages of the flower were detached from one of its branches. The adjacent branch on which the bracts were intact served as the control. Developing buds/fruits in 'treated' and 'control' branches was monitored for three consecutive days for damage by predators.

### 2.1 *Estimation of sugars in secretory oozate*

Alcohol soluble sugars were extracted from intact bracts by agitating them with 80% ethyl alcohol and estimated by the method of Dubois *et al* (1956).

### 2.2 *Enzyme assays*

The oozate of floral bracts were assayed for two of the most common enzymes in carnivorous plants *viz.*, protease and acid phosphatase (Lloyd 1942). The undamaged bracts were dipped in phosphate buffer (0·1 M; pH 7·0) and vigorously shaken for 30 min and centrifuged in a refrigerated centrifuge at 5000 *g* for 20 min. The supernatant which formed the secretory gland extract was assayed for protease and acid phosphatase activity, following the methods of Kakade *et al* (1969) and Lowry *et al* (1954) respectively. Soluble casein was used as substrate for protease activity and para nitro phenolphosphate for the acid phosphatase activity. The activity is expressed as standard enzyme units· The proteins in the extracts were estimated by Bradford's dye method (Bradford 1976).

### 2.3 *Gel electrophoretic separation of glandular proteins*

The dilute buffer extract was concentrated for protein by ammonium sulphate precipitation, centrifuged and dialyzed against phosphate buffer. The anionic mini-slab polyacrylamide gel electrophoresis was performed using 7·5% resolving gel and 2·5% stacking gel, with Tris-glycine (pH 8·3) as the reservoir buffer (Davis 1964). The gel was stained for acid phosphatase and Coomassie brilliant blue stain was used for general proteins and destained to get the protein bands (Burk *et al* 1983).

### 2.4 *In vitro digestibility of ants by the glandular extract*

*In vitro* proteolytic activity of glandular extract was tested by incubating freshly freeze killed (undamaged) ants in the buffer extract of the bracts for 90 min at 37°C. The free aminoacids released was estimated by ninhydrin method (Moore and Stein 1948) and compared against the leusine standard.

### 2.5 *Uptake of free aminoacids by the bracts*

To demonstrate the uptake of free aminoacids by the bracts, [$^{14}$C]phenylalanine was smeared onto the bracts· After 1 h, various parts of the flower buds along with intact bracts, buds with detached bracts, buds with calyx and bracts removed, androecium and ovules were separately exposed to X-ray film for autoradiography for three days at –20°C and the film developed according to standard methods (Benzing *et al* 1976).

## 3. Results and discussion

Insects belonging to 10 different families were found to be trapped by the bracts of *P. foetida* (table 1). Most of the trapped insects were smaller and are phytophagous in nature with the exceptions of those belonging to the families Chalcididae and Trichogrammatidae which are insect parasites. Such a phenomenon of catching insects and killing on the flowering stems have been reported in glandular calyx hairs of *Plumbago* (Rachmilevitz and Joel 1976) and some species of *Stylosanthes* (Sutherst and Wilson 1986).

**Table 1.** List of families of insects caught by the bracts *(N* =300) of *P. foetida.*

| Order | | | |
|---|---|---|---|
| Thysanoptera | Homoptera | Hymenoptera | Diptera |
| Thripidae | Membracidae<br>Aphididae<br>Dixidae<br>Psyllidae | Thrichogrammatidae<br>Formicidae | Drosophilidae<br>Unidentified flies |

The alcohol extract of the bracts constituted 0·106% of sugars which impart stickiness to the glandular secretion. The glandular secretion of *Drosera capensis* consists of 4% aqueous solution of acidic polysaccharides (Rost and Schauer 1977) and 0·04% in *Drosophyllum lusitanicum* (Heslop-Harrison 1976).

The surface area of the bracts increases with the developmental stage of the flower. Developing fruits posses larger bracts than the buds. Though bracts of the developing fruits capture more insects than those in the bud stage, the number of insects captured per unit surface area remains same in all three stages (table 2).

Under natural conditions developing fruits showed a greater incidence of insect damage (66·66%) than the developing buds (17·65%)· Removal of bracts resulted

in a sharp increase in damage to buds (55·82%). A similar removal of bracts from developing fruits stage did not result in any significant increase in damage to the fruits (table 5). Such protective function of bracts have been identified in aroids, banana, palms, maize cob and the involucre of bracts in the members of the family Asteraceae (Dutta 1988).

The buffer extract of the bracts showed a very high proteolytic (52 to 47*n* katalas/µg protein) and acid phosphatase (27·3 *n* katalas/µg protein) activity indicating the digestive potentiality of bract secretion (table 4)· Proteases have been reported to be present in the glandular secretion of several carnivorous plants (Heslop-Harrison

**Table 2** Surface area ($mm^2$) of the bracts and the number of insects caught across different stages in *P. foetida*·

| Stage of flower/fruit development | *N* | Surface area of bracts ($mm^2$) (mean ± SD) | Number of insects caught (mean ± SD) | Number of insects caught per $mm^2$ surface area of bracts (mean ± SD) |
|---|---|---|---|---|
| Buds | 27 | 395·7 ± 192·06 | 1·96 ± 2.26 | 0·0049 ± 0.0055 |
| Fertilized flower | 30 | 985·0 ± 261·60 | 5·62 ± 4·04 | 0·0075 ± 0.0086 |
| Matured fruits | 31 | 1591·2 ± 460·11 | 6·29 ± 3·62 | 0·0044 ± 0·0034 |
| Degrees of freedom | | 2, 85 | 2, 85 | 2, 85 |
| *F* Ratio | | 83·82 | 11·70 | 2·11 |
| *P* Level | | < 0·001 | < 0·001 | 0·127 |

**Table 3.** Extent of damage (%) caused by the removal of bracts in *P. foetida*.

| | Treatments | | | | |
|---|---|---|---|---|---|
| | Bracts intact | | Bracts removed | | |
| Stage | *N* | Mean ± SD | *N* | Mean ± SD | *t* value |
| Bud | 92 | 17·65 ± 25·11 | 32 | 55·82 ± 35·82 | 5·95* |
| Developing fruits | 29 | 66·66 ± 35·11 | 19 | 64·50 ± 38·50 | $0·18^{NS}$ |

*$p$ level <0.01
NS, not significant

**Table 4.** Casenolytic protease assay of extract of bract secretory gland in *P foetida.*

| Stage of flower/fruit development | Protein (µg/ml) | Enzyme units (*n* katals) | Specific activity |
|---|---|---|---|
| Buds | 43·75 | 42 | 960 |
| Fertilized flower | 107·50 | 47 | 437 |
| Matured fruits | 122·50 | 32 | 261 |

1975) whereas phosphatases were reported from *Dionaea* (Scala *et al* 1969) and *Pinguicula* (Heslop-Harrison and Knox 1971)· It is likely that several other enzymes are also present in the bract secretion and may be involved in the digestion of insects which are trapped by the bracts.

The specific activity of proteases was highest in the secretion of bracts in the bud stage than that in fertilized flower and matured fruit stages (table 4). The decreased proteolytic activity of bracts in flowers and fruits stage seems to be compensated by higher amount of the protein.

The zymogram pattern of protein showed that there are at least four proteases active against casein (figure 2). A similar banding pattern has also been reported in the crude secretion of mature pitcher of *Nepenthes* (Amagasse 1972) and in *Dionaea muscipula* (Robins and Juniper 1980). Zymogram of acid phosphatase activity showed at least one iso-form in *P. foetida* (figure 2 ). Scala *et al* (1969) showed several phosphatases to be present in the secretion of venus flytrap·

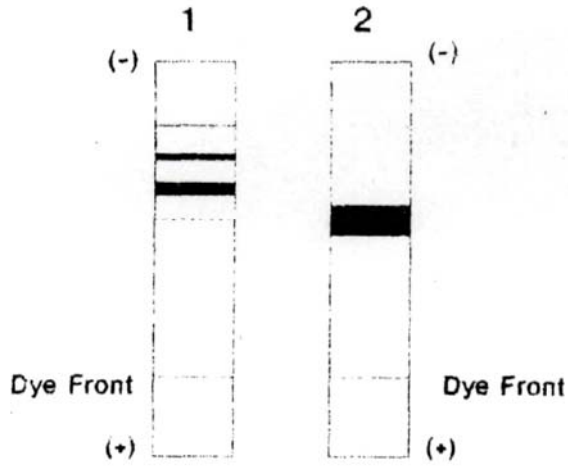


**Figure 2.** Electrophoretic pattern of buffer extract of *P. foetida* bracts· Lane 1, protein patterns; Lane 2, zymogram of acid phosphatase·

The *in vitro* digestion of ants in the presence of glandular extract of *P. foetida* resulted in a higher free amino acid (table 5) release compared to that in its absence suggesting that the crude extract in fact helps in digestion of the trapped insects·

The amino acid uptake experiment indicated that [$^{14}$C]phenylalanine gets absorbed via the bracts to the calyx, corolla, anthers and finally to the developing ovules (figure 3). Heslop-Harrison and Knox (1971) showed that sessile glands of *Pinguicula* absorb [$^{14}$C]-labeI from protein within 4 h of application. This absorption is thought to be energy dependent process (Lloyd 1942). In *Drosera,* the stalked glands absorb [$^{3}$H]aspergine within 2 min of application. Robin and Juniper (1980) have shown that in *Dionaea mucipula* absorption of nutrients is mainly effected through symplast. However, the exact route of absorption in *P· foetida* is not clear from this study.

The above results confirm that the bracts impart protection to the developing buds and supply extra nutrients by attracting, digesting and absorbing the released aminoacids from insects which are trapped.

**Table 5.** *In vitro* digestion of the ants by the *P. foetida* glandular extract.

| Treatment | *N* | Amount of free aminoacids released (μg) |
|---|---|---|
| Buffer | 3 | – |
| Buffer + extract | 3 | 5 |
| Buffer + extract + ant | 3 | 15 |

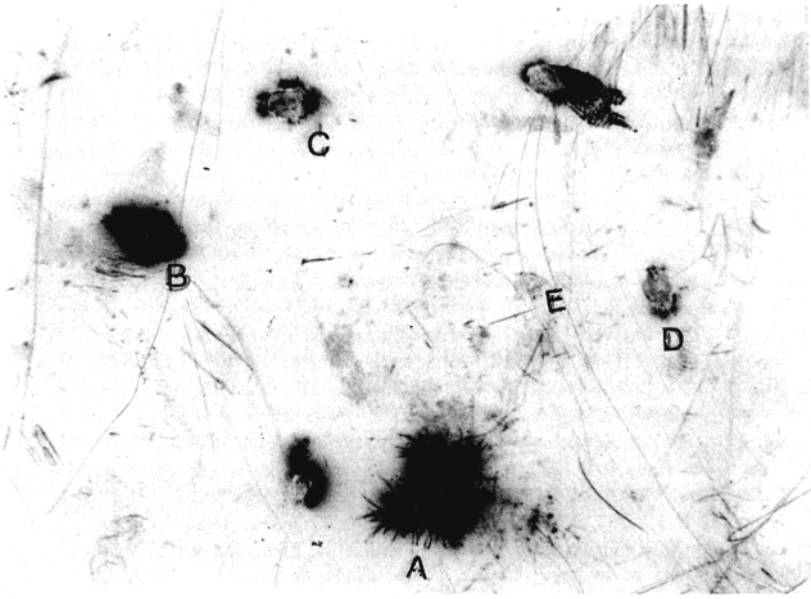


**Figure 3.** Movement of [$^{14}$C]phenylalanine through bracts· (A), Bracts smeared with [$^{14}$C]phenylalanine; (B), flower bud without bracts; (C), flower bud without bracts and calyx; (D), androecium; (E), ovules (faintly developed).

The defensive killing of non-pollinating, phytophagous creeping insects on the flowering stems and the leaves have been demonstrated in several non-carnivorous plants (Juniper *et al* 1989). The absorption of the digested products is the only step that these non-carnivorous plants should evolve to become carnivorous. The surface of stalk cells of certain non-carnivorous plants like *Bromeliads* (Benzing *et al* 1976) and *Hydnophytum formicarum* (Rickson 1979) have been known to absorb the free aminoacids. However, the bracts of *P. foetida* consists of the glands which secrete the digestive fluid which make it different from the non-carnivorous plants.

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