OBSERVATIONS ON THE POLLINATION OF Rhabdodendron macrophyllum (Spruce ex Benth.) Huber.

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SUMMARY

The species ***Rhabdodendron macrophyllum*** *(Spr. ex Benth.) Hub. (Rhabdodendraceae) was observed in order to determine its pollination mechanism. Although it flowers around the year, there are flowering peaks when it is visited by several species of pollen-gathering bees. The principal floral visitors are two species of trigonid bees and one* ***Melipona****. The* ***Melipona*** *and one other visitor used the buzz method to extract pollen from the longitudinally dehiscent anthers. The Trigonid bees collected pollen without buzzing. The flowers open around 6:00 a.m. and are available to the bees until about 10:30 a.m. Pollinator exclusion experiments showed that this species sets fruit equally well by self fertilization.*

INTRODUCTION

The genus **Rhabdodendron** is one of the curiosities of Amazonia which has been placed variously by different systematists. For example, in the Chrysobalanaceae (under the name **Lecostemon**) by Bentham (1853), in the Rutaceae by Gilg and Pilger (1905) and as a separate family, Rhabdodendraceae by Prance (1968, 1972). The genus consists of two Amazonian species — **R. amazonicum** (Spr. ex Benth.) Hub. and ***R. macrophyllum*** *(Spr. ex* Benth.) Hub. — and one species from northeastern Brazil, **R. gardnerianum** (Benth.) Sandw., which is known only from the type collection. Because the systematic position of this small group is still debated it is of interest to collect as much data as possible about it from many different aspects. This paper presents the details of a study of the pollination of one species, **R. macrophyllum** (Spr. ex Benth.) Huber, which is of restricted range in white sand habitats around Manaus. It is quite common in those areas and colonizes other open spaces, such as sandy fields and roadsides.

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MATERIALS AND METHODS

This study was made at a site 14 km northwest of Manaus near an abandoned sand pit on the unexcavated fringe. It is located near to the Tarumã Baixa falls. At this locality there was a dense colony of **Rhabdodendron macrophyllum** which contained about 200 individuals. Observations were made in February 1982 and 1983, which corresponds to one of the peak flowering periods of this species.

Samples of each flower-visiting insect were collected and are deposited in the entomological collection of INPA:

B. Nelson	1222	small beetle
	1227	caterpillar
	1228	small yellow flies
	1229	orange **Trigona** bee
	1230	Hymenoptera
	1231	black Trigona (= BN 1233)
	1232	**Melipona** (= BN 1238, GTP 28175)
	1233	black **Trigona** (= BN 1231)
	1234	**Epicharis**?; large irridescent green bee
	1235	black **Trigona**; short abdomened
	1236	black **Trigona**; long legged
	1237	Coleoptera
	1238	**Melipona** (= BN 1232, GTP 28175)
	1239	Diptera (= BN 1240)
	1240	Diptera (= BN 1239)
	1241	Diptera
	1242	Diptera
G. T. Prance	28175	**Melipona** (= BN 1232, 1238)
	28176	**Xylocopa**
	28186	black **Trigona**
	28187	green Diptera

The herbarium voucher material of **Rhabdodendron macrophyllum** is B. Nelson 1290 deposited at INPA and NY.

Observations of flower visitors were made on eight days, and their behavior was noted. Flower phenology, complete insect activity over short periods, length of insect visits, and number of flowers visited on each shrub were recorded.

Four hundred twenty-nine flower buds were bagged for the exclusion of insects to observe the rate of self-fertilization. These were compared with 532 control flower buds that were marked but not bagged. The bags were installed on February 20, 1982. Each bag covered the terminal end of an erect branch with nearly mature buds. Leaves were clipped so as to permit the bag to fit over the branch. Non-bagged control branches with nearly mature flower buds were tagged and leaves clipped in the same way. Most bagged flowers opened during the days of February 24, 25 and 26, and the branches were

clipped for off counting on March 12. The young fruits were counted and preserved in FAA.

RESULTS

1. Flower phenology

At the beginning of this study on February 1, 1982, a few shrubs had isolated open flowers, but there were a large number of nearly mature flower buds. Most observations were made three weeks later when this large crop of buds began to open (February 24, 25 & 26). At that time, on any single day about 15% of the buds were open, showing that there is a definite peak flowering period of this species. In the region of Manaus it flowers around the year, but for much of the year there are only a few isolated open flowers on a few shrubs. However, several times during the year there is a burst of flowering. Our observations were planned coincide with one of these flowering periods.

The flowers begin to open at first light around 5:45 a.m. and by 6:00 a.m. many are opening. They burst open with an impressive force as the pressure of the expanding mass of anthers causes the thick coriaceous sepal-like petals to fall off. At this time of day during a flowering peak one can hear a rain of petals falling to the ground. A flower generally opens fully over a ten minute period, and the movement of the expanding stamens can be clearly seen. The petals are caducous leaving the white mass of linear anthers fully exposed. At this time the flowers have a distinct smell reminiscent of Vicks Vaporub. Anthers begin to dehisce soon after the flowers open and by 8:45 a.m. all anthers are fully dehiscent.

By 10:30 a.m. most of the flowers have begun to wilt and shortly afterwards anthers begin to fall off. By 11:00 a.m. most anthers have fallen off the flowers and any that remain are knocked off easily by any flower visitors.

The flowers, therefore, are open and available to pollinators for a comparatively short time of approximately four and one half hours from 6:00 a.m. to 10:30 a.m.

2. Bee visitors to flowers

The effective pollinators of **Rhabdodendron macrophyllum** are various species of small pollen-gathering bees. Their activities in the flowers are described below separately from those of other flower visitors. Observations made on February 25, 1982 — the middle of a three day flowering peak — are as follows:

6:35 - 7:00 a.m. The first orange **Trigona** bee (BN 1229) arrived, and two other individuals of this species were observed nearby. This bee crawled about over the flowers, frequently touching both the anthers and the stigma. It flew from shrub to shrub spending a few minutes at each shrub. It removed pollen from the anthers by an interesting method, by running its mouth parts down the still closed line of dehiscence and opening it in a way that resembles the shelling of peas.

7:00 - 8:00 a.m. A brief rain reduced bee activity between 7:00 and 7:15 a.m. but immediately afterwards, between 7:00 and 7:45 a.m. the dominant flower visitors

were orange (BN 1229) and black (BN 1231, 1233) Trigonid bees. There were up to five bees in each bush. Usually the two species remained segregated in groups of their own, perhaps as a result of calling others from the hive to the same bush. During this period on larger Meliponid bee (**Melipona**, BN 1232) was observed collecting pollen. Its large pollen sacs were filled with **Rhabdodendron** pollen. This bee moved quickly from flower to flower visiting up to 15 in a single minute, unlike the small Trigonids which remained much longer on each flower.

8:00 - 8:30 a.m. Several hundred black **Trigona** (BN 1231, 1233) were working the bush that had only five bees earlier. All bees were filling their pollen sacs with **Rhabdodendron** pollen. Their work caused them to touch frequently against the linear stigmas. The black **Trigona** bees appeared to concentrate on certain bushes leaving the rest free for other species of bees. During the same time period a large irridescent green bee (BN 1234, possibly **Epicharis**) was observed extracting pollen by the buzz method. This bee hovered in front of a flower for one to two seconds, landed in the center of the flower, touching the stigma, and buzzed for one half second, which covered the venter with pollen from dehiscent anthers. At about the same time a shortabdomened black **Trigona** (BN 1235) was collecting pollen on a shrub 15 meters from the three plants dominated by the more numerous black **Trigona** species (BN 1231, 1233). This bee had clearly visible large pollen sacs.

8:30 - 8:45 a.m. Large numbers of black **Trigona** (BN 1231, 1233) remained on the same three bushes and a few of this species were now visiting other flowering bushes. Some other plants had up to ten of the orange **Trigona** (BN 1229) and ocasional fast-flying **Melipona** (BN 1232, 1238). At 8:40 a.m. a long-legged **Trigona** bee was collected (BN 1236) which was observed collecting pollen but was never a frequent visitor. It did not move about from bush to bush but its pollen sacs were full. By 8:45 a.m. the anthers were all fully dehiscent and bee activity continued. A **Melipona** (BN 1232, 1238) buzzed a flower and was coated with a cloud of pollen. Buzzing of **Rhabdodendron** flowers caused a clearly visible shower of pollen. The leaves below the flowers that were buzzed by **Melipona** and by BN 1234 were coated with a clearly visible dust of pollen.

8:45 - 11:00 a.m. The fairly common **Melipona** (BN 1232) became quite active at 8:50 a.m. This bee dusted itself with pollen by grasping the anthers, its weight causing the flower to bend downward, then buzzing briefly. The buzzing movement of this bee caused a great cloud of pollen to cover it. The bee immediately landed on a leaf and combed pollen from its head, venter and abdomen. Pollen was transferred to the sacs while hovering and the sacs became large and full. This bee visited up to 15 flowers per minute and frequently moved from shrub to shrub. The action of this bee leads it to touch the stigmas frequently. By 9:45 a.m. the **Melipona** was much more common: up to four individuals could be observed on a single plant. This bee was probably an effective pollinator since it created a cloud of pollen in each flower, was coated with pollen and visited most flowers on each plant. It moved from bush to bush spending no more than five minutes on each. Sometimes one would return to a bush which it previously had visited. At the same time, several hundred black Trigonids (BN 1231, 1233) remained

active on their three main bushes and in lesser numbers (five to ten individuals per plant) on scattered other bushes.

By 11:00 a.m. anthers were falling off the plants and activity by the common black **Trigona** (BN 1231, 1233) had almost ceased. The **Melipona** (BN 1232, 1238) however, continued to forage, but knocked off most of the anthers when it tried to buzz them.

Similar observations were made the following day when the activity of the black **Trigona** (BN 1231, 1233) began at 6:36 a.m., one minute different from the first bee arrival of the previous day. By 7:05 a.m. there were thirty of these bees on their favorite bush. Anthers were too wet for effective buzzing early in the morning, which may account for the later activity of the buzzing bees.

The only other bee visitor observed was a single casual visit by one **Apis melifera**.

It is interesting to note that little or no bee activity occurred on the first day of the three day flowering peak in February, 1982, even though 15% of the bud crop opened on this first day. Presumably the hives did not discover this food source until the second day.

On a bright sunny day of observation (February 24, 1983) bee activity ended and flower wilting began much earlier (by 9:00 a.m.) than on the cloudy day described above. Also in 1983 a species of **Xylocopa** (GTP 28176) was observed buzzing the flowers, and a fifth species of **Trigona** (GTP 28186) was a frequent visitor to the flowers.

3. Census of bee activity

Two types of census were made in order to quantitatively compare the effectiveness of different bee species as pollen transfer agents. Table 1 is a census of the number of individuals of each bee species found on, or making a visit to, each of 24 different **Rhabdodendron macrophyllum** plants. Each plant was observed for 30 seconds shortly after 10:00 a.m. on February 25, 1982. Table 2 records the length of time spent by different individuals of the three most important bee species on a single plant before moving on to another plant. The data for table 2 were obtained between 7:00 and 8:30 a.m. on February 26, 1982.

The small black **Trigona** (BN 1231, 1233) was the dominant flower visitor in the population of shrubs studied, but confined most of its visits to a few preferred shrubs. Both the orange **Trigona** (BN 1229) and **Melipona** (BN 1232, 1238) were found on as many plants as the dominant black **Trigona** during the fifteen minute census, despite lower numbers of individual bees. Data from table 2 suggest that the **Melipona** has the most potential as an effective pollinator. It visited an average of 9.6 flowers per minute while the orange and black **Trigona** visited 2.1 and 0.5 flowers per minute, respectively. The **Melipona** also moved quickly to other plants, spending an average of only 1.7 minutes on each plant. The orange and black **Trigona** were more sedentary, spending much more time on each plant: 13,5 minutes and 12.3 minutes respectively.

The **Melipona** collected pollen by the buzz method ensuring a good powdering of pollen over its venter. On subsequent flower visits the venter of this larger bee generally contacted the stigma, permitting pollen transfer. The buzz collecting

technique was not used by the **Trigona** species nor were their smaller bodies as densely covered with pollen.

When taken in conjunction with the difference in pollen collecting techniques, the data from tables 1 and 2 suggest that the **Melipona** bees (BN 1232, 1238) are the most effective potential cross-pollination agents, despite their lower numbers.

4. **Other insects observed**

Since several other insects were observed and collected during this study they are briefly described. However, they do not seem to be important as pollinators.

A small beetle (BN 1222) was observed on open flowers on February 1, 1982, well before the flowering peak. It scraped the surface of the ovary with its mandibles and sat motionless on the ovary or receptacle. It was not observed eating pollen or flower parts, but there were beetles in five of the ten flowers found open that day.

On February 2, 1982 a caterpillar (BN 1227) was collected which bores a single hole in near-mature buds and eats the anthers inside. Young caterpillars are the size shape of the buds and older caterpillars develop yellowish white processes which apparently mimic the anthers of the open flowers. The change from bud mimicking to flower mimicking in this caterpillar is striking.

Though there were no bee visitors on February 24, 1982 (first day of the three day flowering peak) many small yellow flies (BN 1222) about half the size of fruit flies, were present from 6:00 a.m. to 12:30 p.m. They were also present on February 25 at 6:00 a.m. but were disturbed by the visiting bees later that day. These flies apparently eat pollen and land on both the anthers and stigmatic surface. As many as 15 flies per flower were observed at 11:30 a.m. on February 24, the day before great numbers of black **Trigona** discovered the flower crop. The flies confined their activity to shaded flowers. These flies were not very active and probably are not effective in transference of pollen from one shrub to another since they remained on a single shrub for a long time. A larger fly collected at 10:00 a.m. (BN 1239) eats pollen by running its proboscis up the anther. It was not dusted with pollen and is unlikely to be an effective pollinator.

5. **Exclusion experiment**

429 flower buds were bagged of which a total of 343 set fruit (80%). 523 control flower buds were observed of which 267 set fruit (50%); see table 3.

The difference in fruit set can be attributed to the loss of three control branches, possibly due to predation by herbivores or being broken off by alighting birds. The inflorescences that were protected by bags were not subject to such damage. It is likely, therefore, that fruit set is about equal in bagged and unbagged flowers. This clearly shows that **R. macrophyllum** is capable of self fertilization and that selfing does not reduce the number of fruits formed.

DISCUSSION

This study has shown that **Rhabdodendron macrophyllum** is not dependent on cross fertilization for reproduction. However, since there is a great deal of bee activity within the flowers a considerable amount of cross pollination between different shrubs is likely to occur. It seems to be a plant that is highly attractive to a variety of bees. It has not developed a close relationship with any particular bee. The rather unusual flower structures such as the linear anthers and long lateral stigma are not therefore, the result of any close pollinator coevolution.

Rhabdodendron macrophyllum, although a local endemic to Manaus, has become quite common as a colonizer species in felled areas on white sand, in fields and along roadsides. This colonization ability is in keeping with its ability to self pollinate.

ACKNOWLEDGEMENTS

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RESUMO

A espécie ***Rhabdodendron macrophyllum*** *(Spr. ex Benth.) Huber (Rhabdodendraceae) foi observada a fim de se determinar o seu mecanismo de polinização. Ela floresce durante o ano inteiro, porém, a época de maior floração é na estação chuvosa. Neste período de máxima floração, ela é visitada por diversas espécies de abelhas que coletam pólen. Os principais visitantes são duas espécies de Trigonini e uma do gênero* ***Melipona****. A* ***Melipona*** *e uma espécie rara, e maior, usam o método de vibração para extrair o pólen das anteras rimosas. As abelhas Trigonini colecionam pólen sem vibrar. Apesar de ser menos numerosa a* ***Melipona*** *é o agente polinizador mais eficiente. Ela visita mais flores por minuto, e mais plantas por minuto do que as Trigonini, e fica mais coberta com pólen devido à técnica vibratória de coleta de pólen. As flores de* ***R. macrophyllum*** *se abrem as 06:00 horas e estão disponíveis aos polinizadores até mais ou menos 10:30, quando murcham e as anteras começam a cair. Experimentos para excluir os insetos dessa possibilidade mostraram que a planta pode ser autofecundada porque havia igual número de frutos nas inflorescências sem polinizadores.*

Table 1. Census of bees visiting 24 individuals of *Rhabdodendron macrophyllum*, 10:00 - 10:15 a.m. on February 25, 1982. Thirty seconds of observation at each shrub.

	Visitor					Comments
Shrub N°	A	B	C	D	E	
01	10	--	02	--	--	
02	--	01	--	--	--	Few flowers on shrub
03	08	--	02	--	--	One visitor "C" moved from shrub N° 01
04	--	--	--	01	--	
05	01	--	--	--	--	
06	--	--	--	--	--	
07	--	01	04	--	--	
08	--	02	02	--	--	
09	--	--	01	--	--	
10	--	--	--	01	--	
11	--	02	--	--	--	
12	--	--	01	--	--	
13	--	04	01	--	01	
14	--	03	--	--	01	
15	--	15	--	--	--	
16	--	--	--	--	--	Few flowers on shrub
17	15	--	--	--	--	
18	04	--	--	--	--	Many more "A" here earlier
19	07	--	--	--	--	Many more "A" here earlier
20	07	--	--	--	--	Many more "A" here earlier
21	50	--	01	--	--	Many nore "A" here earlier
22	12	--	--	--	--	
23	--	08	--	--	--	
24	--	15	02	--	--	
	114	**51**	**16**	02	02	Total number of individual bees in plant population.
	09	**09**	**09**	02	02	Number of plants visited by each bee species

KEY: "A" - Black *Trigona* (BN 1231, 1233)
"B" - Orange *Trigona* (BN 1229)
"C" - *Melipona* (BN 1232, 1238)
"D" - Black *Trigona*, long-legged (BN 1236)
"E" - Black *Trigona*, short-abdomened (BN 1235)

Table 2. Length of visits by three bee species to Rhabdodendron macrophyllum, 7:00 - 8:30 a.m. on February 26, 1982.

A. Black Trigona (BN 1231, 1233)

Replicates (different bees)	Time on plant	Number of flowers visited
01	8.0 min.	10
02	4.0 min.	06
03	25.0 min.	04
Averages:	12.3 min./plant	6.7 flowers/visit

Average number of flowers visited per minute = **0.54**

B. Orange Trigona (BN 1229)

Replicates (different bees)	Time on plant	Number of flowers visited
01	13.5 min.	28

Average number of flowers visited per minute = **2.07**

C. Melipona (BN 1232, 1238)

Replicates (different bees)	Time on plant	Number of flowers visited
01	0.3 min.	02
02	1.5 min.	25
03	0.3 min.	01
04	1.7 min.	11
05	1.0 min.	06
06	3.3 min.	25
07	3.9 min.	45
Averages:	1.7 min./plant	16.4 flowers/visit

Average number of flowers visited per minute = **9.64**

Table 3. Fruit formation with and without pollinator exclusion nets.

A. Exclusion Net Results

Net N?	Number of buds on Feb. 20, 1982	Number of young fruits on Mar. 12, 1982	Percent fruit set
01	39	36	92%
02	60	51	85%
03	51	32	63%
04	56	50	89%
05	133	113 (of these only about 60% are maturing)	85%
06	31	18 (of these only 08 are maturing)	58%
07	59	43	73%
Totals:	429	343	**Average: 80%**

B. Control Results (Without Excluson Net)

Tag N?	Number of buds on Feb. 20, 1982	Number of young fruits on Mar. 12, 1982	Percent fruit set
01	37	34 (of these only about 28 are maturing)	92%
02	56	00 (entire infl. broken off)	00%
03	95	77	81%
04	46	00 (entire infl. broken off)	00%
05	213	109 (of these only about 50% are maturing)	51%
06	35	28	80%
07	30	04 (rest of infl. broken off)	13%
08	20	15	75%
Totals:	532	267	**Average: 50%**

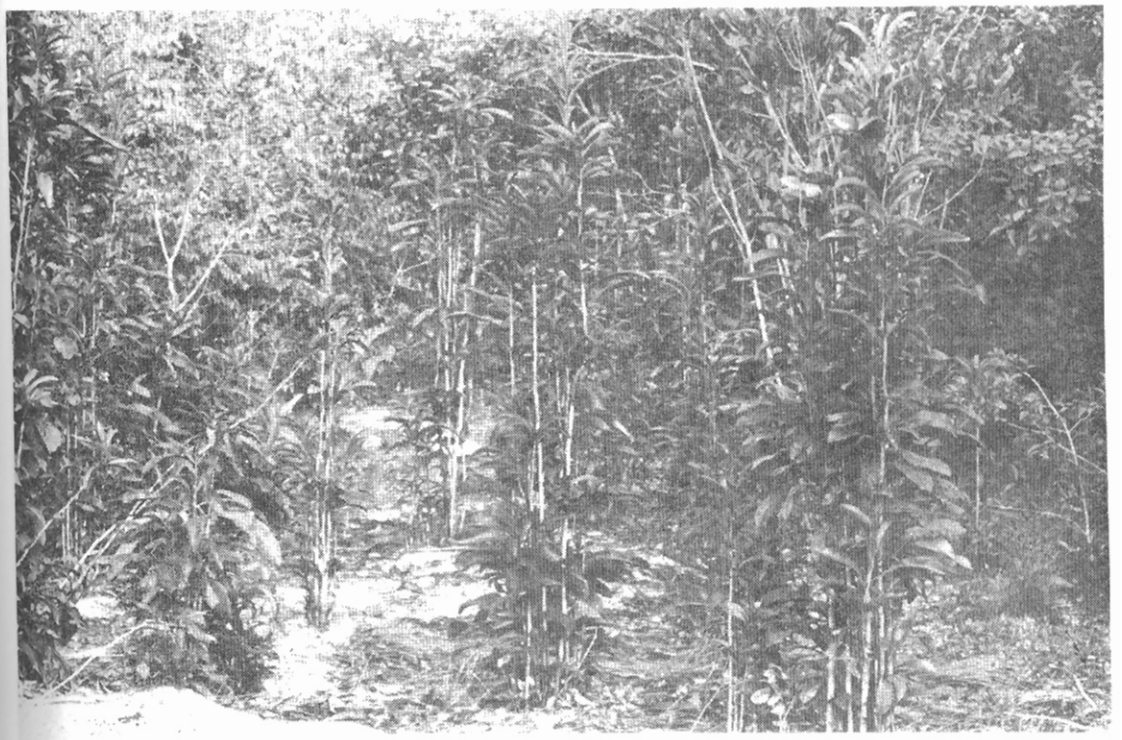

FIG. 1. **Rhabdodendron macrophyllum** at study site.

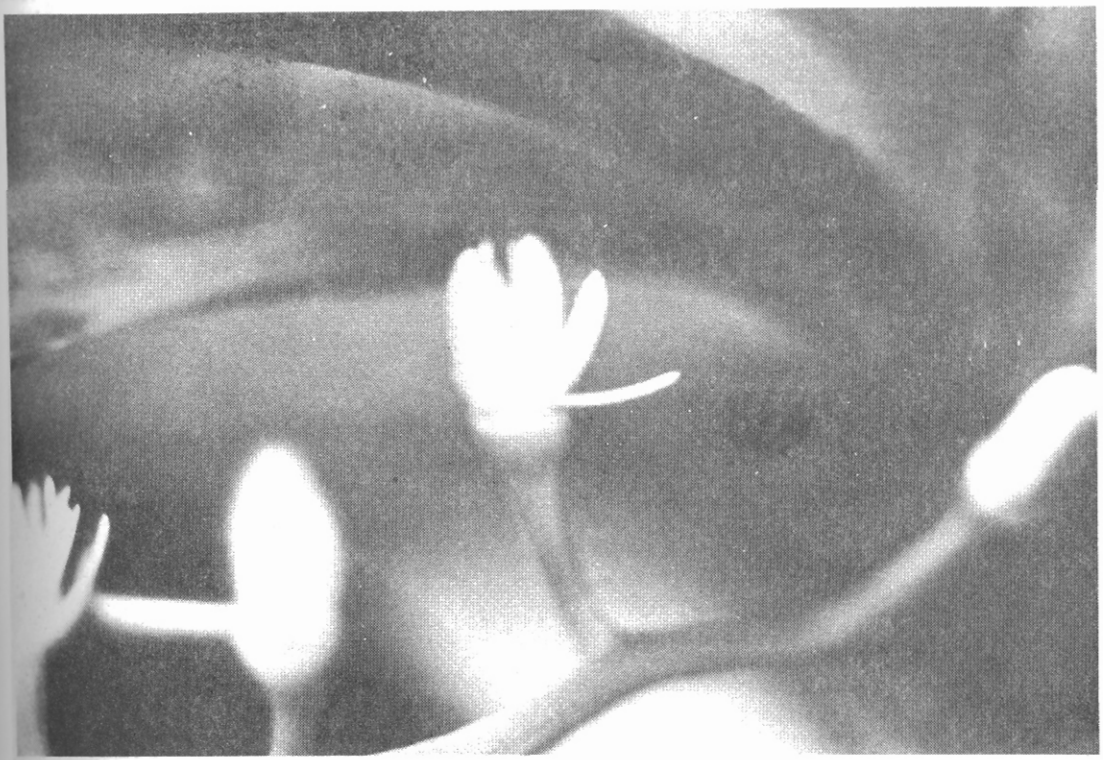

FIG. 2. Early morning bud of **R. macrophyllum** as it bursts open.

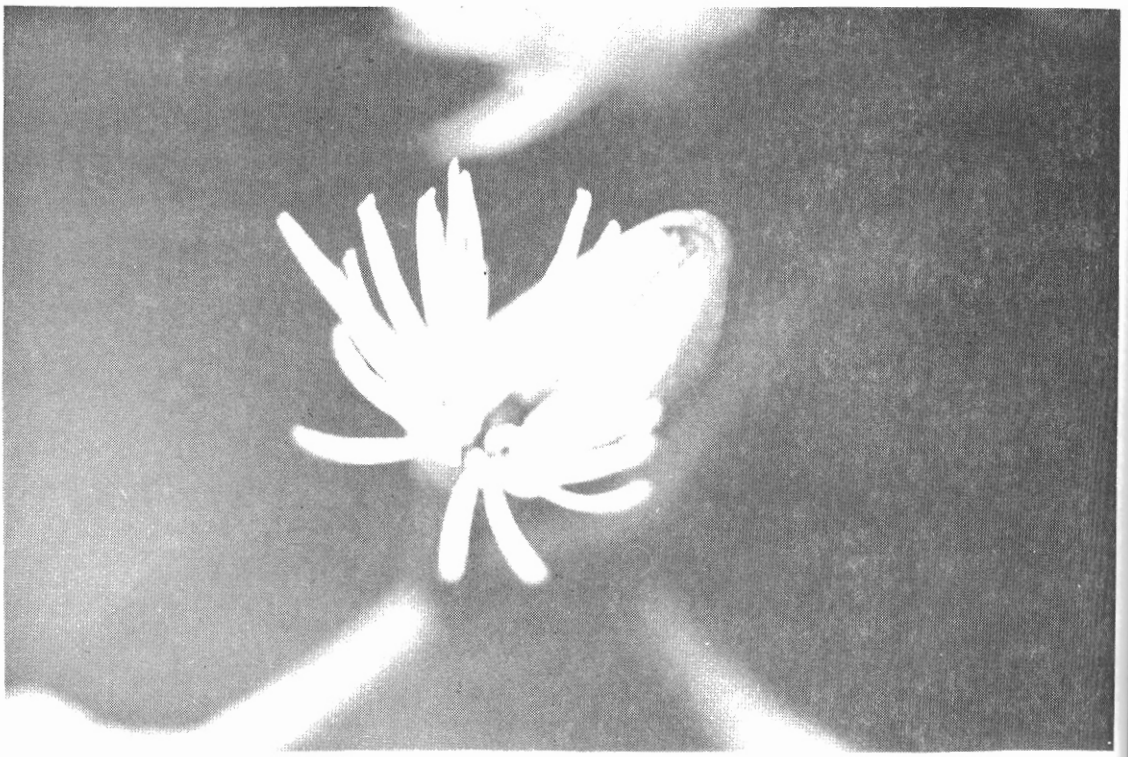

FIG. 3. Opening flower of **Rhabdodendron macrophyllum** with two petals still attached.

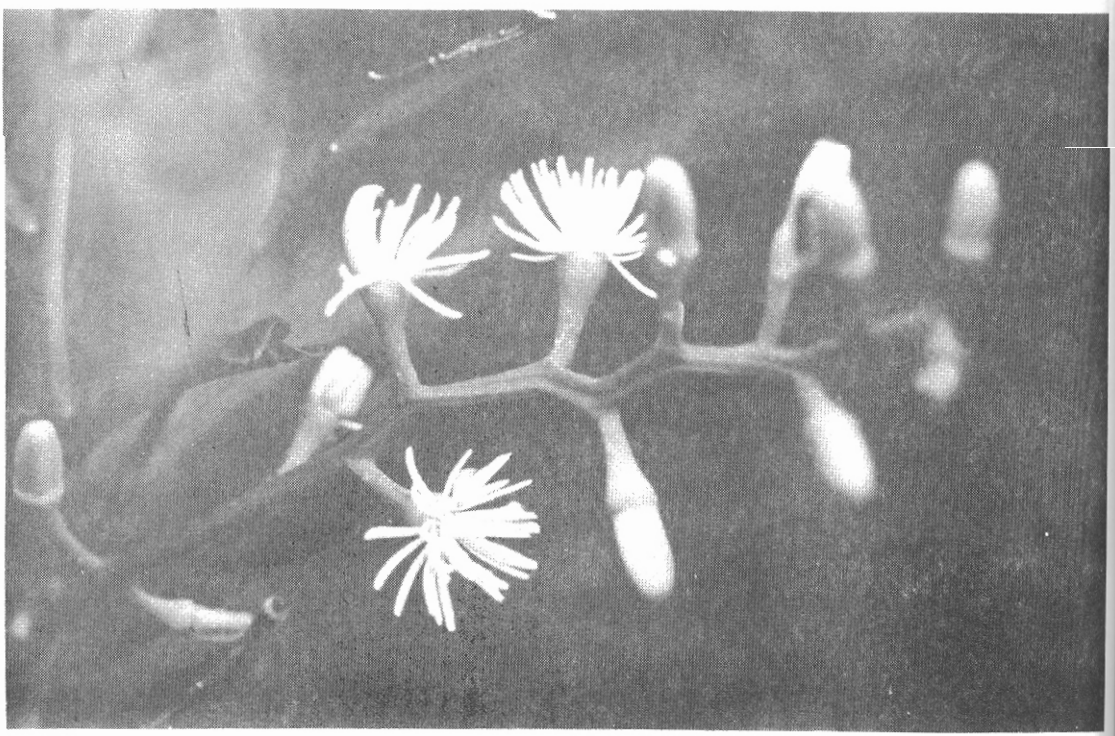

FIG. 4. Inflorescence of **Rhabdodendron macrophyllum**.

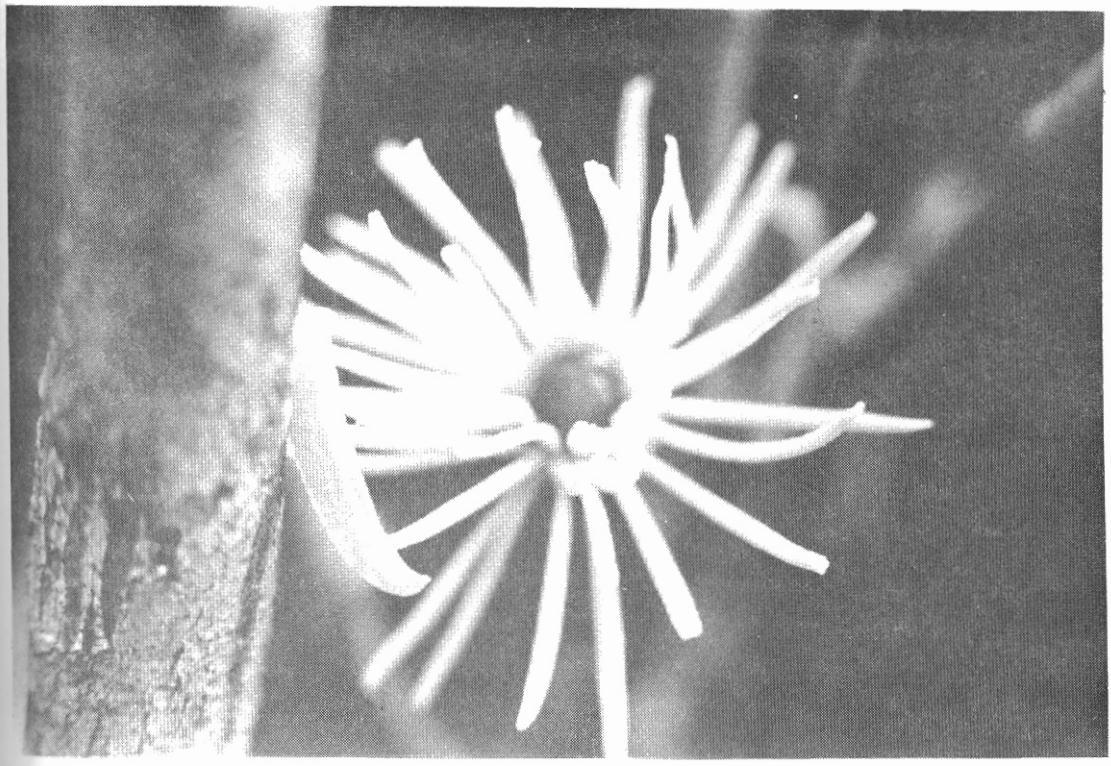

FIG. 5. Fully open flower of **Rhabdodendron macrophyllum** showing the linear anthers.

FIG. 6. Bee visiting flower of **Rhabdodendron macrophyllum**.

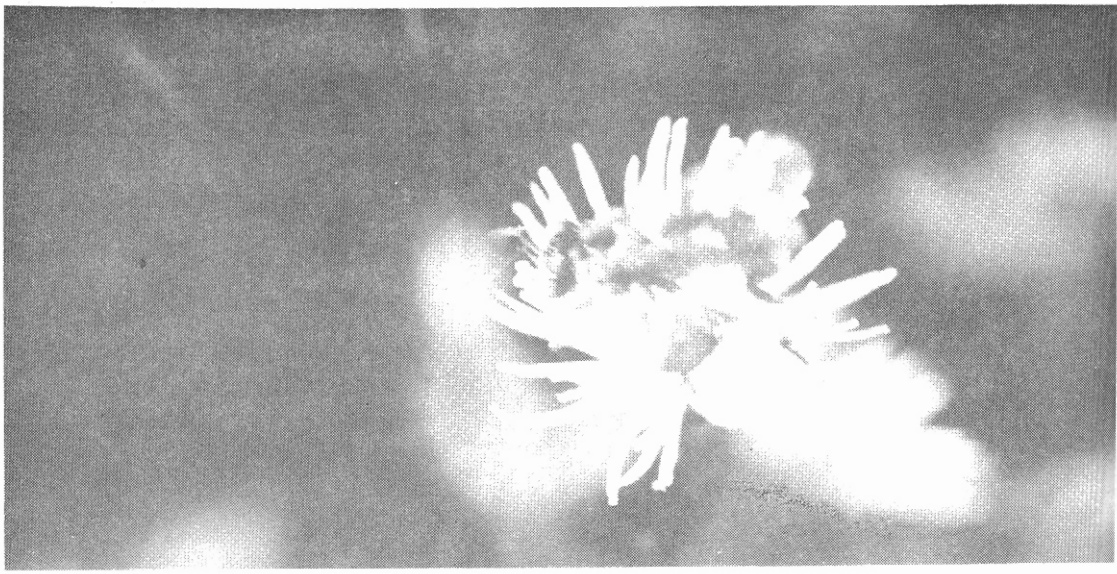

FIG. 7. Bee visiting flower of **Rhabdodendron macrophyllum**.

FIG. 8. Mid-morning shot of **Rhabdodendron macrophyllum** showing dehisced anthers fallen on a leaf.

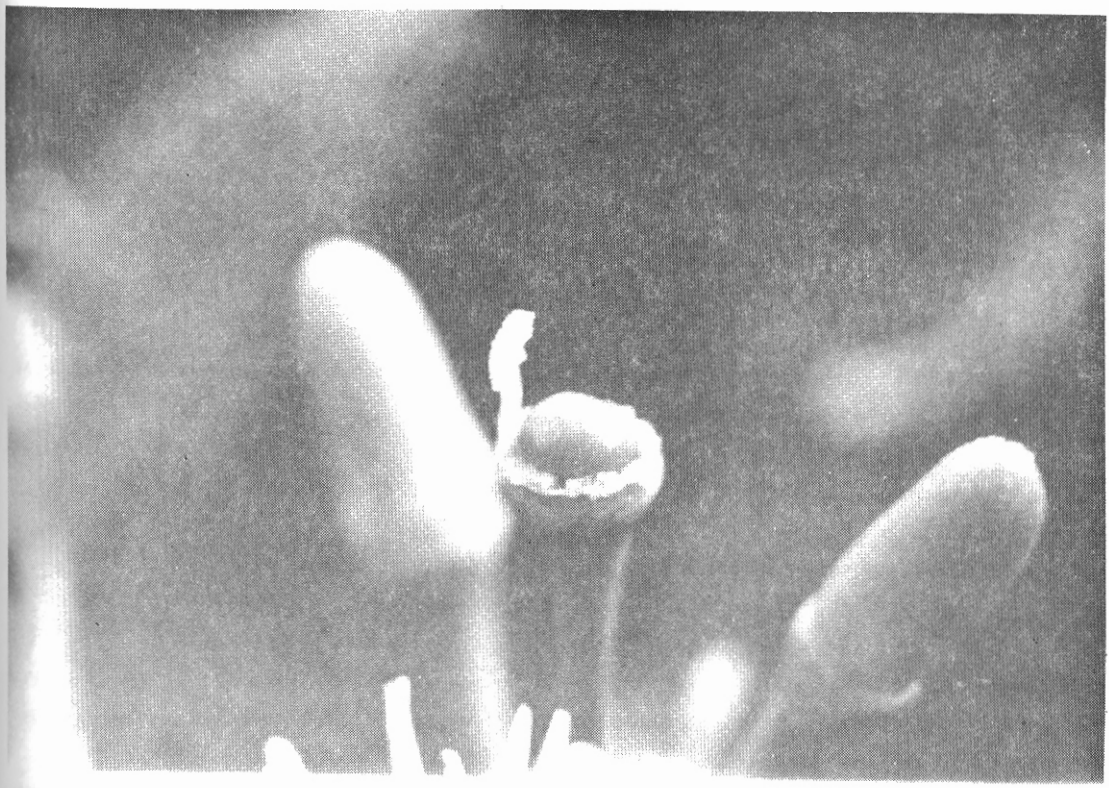

FIG. 9. Afternoon flower of **Rhabdodendron macrophyllum** when all anthers have dehisced and ovary and style remain.

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