Pacific Science (1979), vol. 33, no. 3

The Reproductive Biology of *Cyrtandra grandiflora* (Gesneriaceae) on Oahu[1]

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ABSTRACT: In *Cyrtandra grandiflora* flowering is correlated with a relatively dry period or with the associated increased sunlight that occurs about 3 months prior to flowering. Fruits are mature about 5 months after flowering. *Cyrtandra grandiflora* is self-compatible and requires pollination for seed set. Anthesis takes 7 days and exhibits marked protandry. The pollen is viable for the entire 7 days; however, the gynoecia are receptive from the opening of the stigma lobes on the fourth day until senescence. This overlap of sexual maturity in the last 3 days of anthesis allows for animal-mediated autogamy. No pollinator was observed, but a crawling, precinctive pollinator is suggested which results in inbreeding, selfing, or the stimulus for agamospermy. Seed set is prolific, with high germination rates, but seedlings are small and slow-growing and mortality is high in the field. *Cyrtandra grandiflora* studied here is shown to have a stable, reproducing population.

THE GENUS *Cyrtandra* (Gesneriaceae) is comprised of 169 known species for the Hawaiian Islands in general and 127 taxa for Oahu alone, all of which are endemic (St. John 1966). Eighty-six species are listed by Fosberg and Herbst (1975) as endangered and 29 are listed as extinct or probably or possibly extinct. This investigation of *C. grandiflora* was initiated to provide more information on the breeding system, pollinators, and survival potential of a representative of the genus *Cyrtandra*.

MATERIALS AND METHODS

Two populations of *Cyrtandra grandiflora* on Tantalus in the Koolau Mountains were studied. Site 1, consisting of 40 mature plants, was used for the phenology study. The number of flowers and fruits was counted at approximately monthly intervals for 14 months from June 1977 through July 1978 to determine any periodicity in these reproductive phases. Flowering and rainfall near the site were recorded to determine whether rainfall is correlated in any way with flowering. Flowers were tagged and the period of subsequent fruit development was observed.

All plants were measured and grouped into size classes according to height. These size classes were graphed to show their relative proportions as an indication of stability of the population.

Site 2, consisting of approximately 100 mature plants, was used for experimental purposes to avoid interfering with the phenology study at site 1.

Black-and-white photography was used to document the morphological changes associated with anthesis.

Pollen at anthesis was stained in acetocarmine, mounted in Hoyer's mounting medium, covered with a cover slip, and warmed gently over a flame (Brewbaker 1967) to determine its nuclear condition. Percent germination of pollen in vitro from flowers on day 1 through day 7 of anthesis was determined by placing the pollen in a drop of distilled water in a vaseline ring positioned on a glass slide. The slide was then incubated in a moist petri dish for 2 hr. At least 200 grains from each of three flowers of each age were then scored for

[1] This paper is part of an M.S. Thesis submitted to the University of Hawaii. Manuscript accepted 31 May 1979.

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TABLE 1

GERMINATION RATE OF *Cyrtandra grandiflora* POLLEN OF FLOWERS OF VARIOUS AGES AFTER 2 HR IN DISTILLED WATER

AGE OF FLOWER*	% GERMINATION PER FLOWER IN VITRO			% GERMINATION, $\bar{X}$
	A	B	C	
Day 1	26	21	4	17
Day 2	4	17	24	15
Day 3	17	6	11	11
Day 4	47	3	28	26
Day 5	16	12	19	16
Day 6	9	7	43	20
Day 7	16	3	16	12

*Three new flowers of each age were tested.

germination. Only those grains with pollen tubes at least as long as the diameter of the grain were scored as germinated. Percent viability as indicated by stainability in cotton blue [1 percent analin blue in lactophenol (Radford et al. 1974:219)] is shown for comparison (Table 1).

The timing of stigma receptivity was tested by hand pollinating flowers. Flowers were bagged with fine-mesh nylon bags (hole size = 35 μm) in the field before maturation of the stigmas; then pollinations were performed with a camel hair brush at various stages of maturity. The flowers were rebagged for 24 hr before picking them for microscopic examination. The fresh gynoecia were sliced longitudinally and stained in lacmoid in 30 percent ethyl alcohol on a slide. Microscopic examination revealed the extent of germination of pollen and growth of pollen tubes.

Nectar was collected and measured for volume in 10 μl microcapillary tubes. All nectar samples were taken in the morning, but environmental conditions varied from day to day. Samples were taken from flowers that had been bagged for 5 days and from flowers of various ages that were not bagged. The percent soluble solids (sucrose equivalents) of the nectar was measured on an Agago refractometer in the field. The presence of amino acids was ascertained by spraying a spot of nectar dried on filter paper with 0.2 percent ninhydrin in 95 percent methanol, developing it in a 93°C oven for 10 min, and noting the resultant color (Lehninger 1970:79).

Aspects of the breeding system of *Cyrtandra grandiflora* were tested by bagging floral buds, as described above, to exclude pollinators. Various hand pollinations were done with a brush on the fourth, fifth, or sixth day of anthesis when the stigma was receptive. Flowers were then rebagged until senescence. Subsequent fruit set was compared to that of a comparable number of flowers that were unbagged and thus open pollinated. Two sets of data for open pollination were taken—one in October 1977; the other in May 1978, to see if there was seasonal variation in fruit set.

Autogamy was tested by bagging flowers for the entire period of anthesis and comparing subsequent fruit set to a comparable number of unbagged flowers. Self-compatibility was tested by the above bagging regime and hand pollinating with pollen from the same flower or with pollen from a flower on the same plant. Controlled outcrossing was done by hand pollinating previously bagged flowers with pollen from other plants in the same population. Interspecific compatibility was tested by using the above bagging regime and hand pollinating *Cyrtandra grandiflora* stigmas with *C. sandwicensis* pollen.

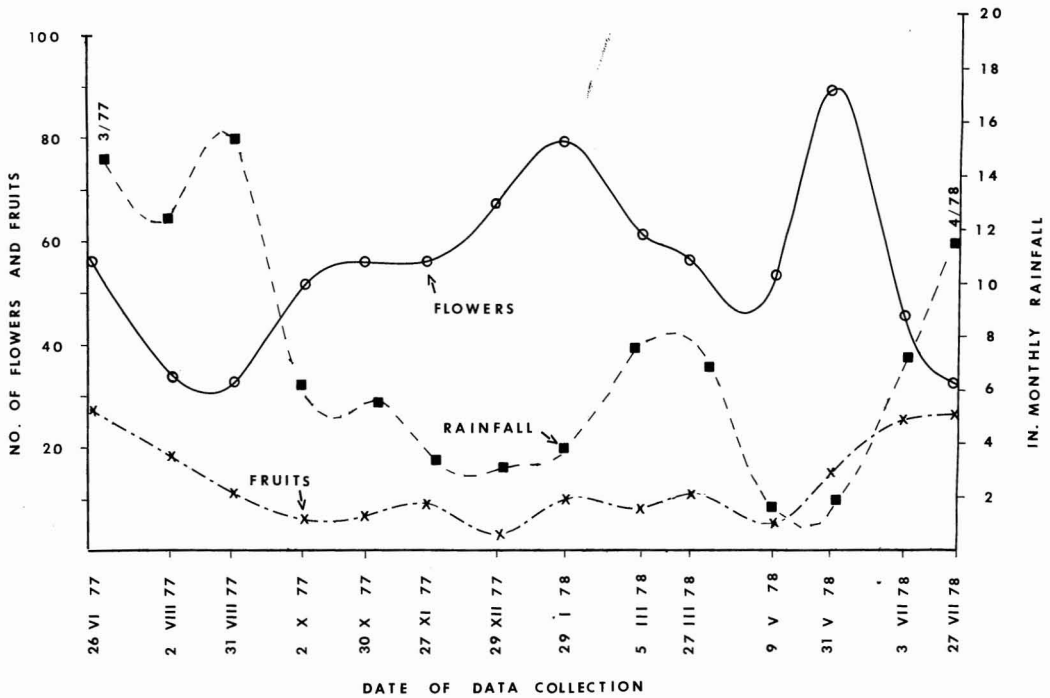

FIGURE 1. Phenology of *Cyrtandra grandiflora*—number of flowers and number of fruits counted on 40 plants at approximately monthly intervals from June 1977 to July 1978. Dashed line with squares indicates total monthly rainfall that occurred 3 months prior to the flower counts.

Observations and collections of insects were made during every field trip. Several types of insect trapping compounds were applied to corolla tubes and stems to catch visitors to the plants and the corolla tube. One observation was made at night on Tantalus to watch for possible nocturnal pollinators.

Seed viability was estimated by the germination rate on wet filter paper in a petri dish and on cindery soil, a porous rock and ground-up leaf litter from Tantalus in pots on a mist bench in the greenhouse. The number of full and shrivelled seeds per fruit was counted to give an indication of seed development and production.

Attempts were made to propagate *Cyrtandra grandiflora* vegetatively by rooting leaf and stem cuttings in vermiculite on a mist bench in the greenhouse. Field observations of vegetative propagation were also made.

RESULTS AND DISCUSSION

Phenology

The number of flowers and fruits counted during the 14-month period (Figure 1) shows no pronounced seasonal variation. Correlations were made between monthly flower counts and the 4-week interval of rain that occurred from 0 to 22 weeks prior to the date of each flower count. A significant negative correlation (Figure 2) between flowering and the interval of rain that occurred from 12 to 19 weeks prior to the flower count is shown. This correlation can be seen by plotting the monthly total rainfall (delayed 3 months) with the monthly flower counts (Figure 1). This may indicate that flowering is initiated by dry periods and that bud development takes from 12 to 19 weeks. Adequate moisture is seldom lacking in the upland rain forest habitat preferred by

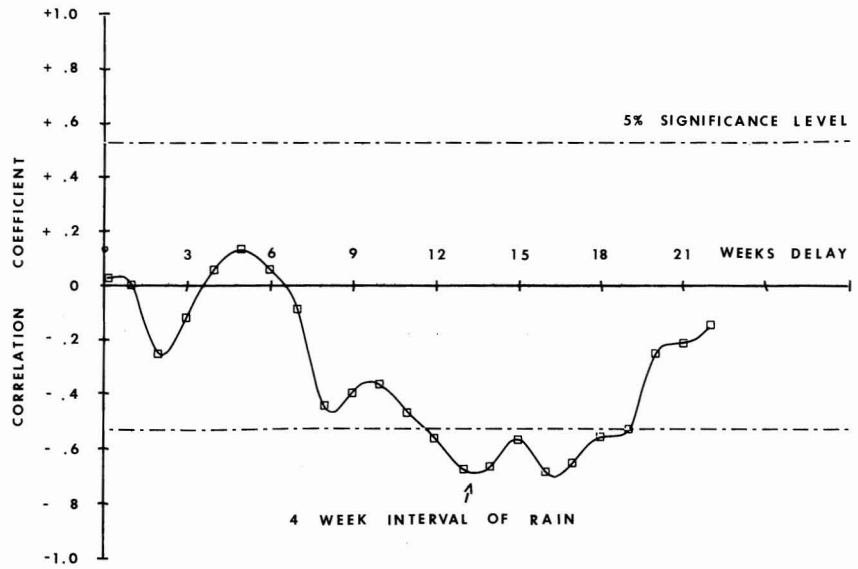

FIGURE 2. *Cyrtandra grandiflora* flowering correlated with a 4-week interval of rain delayed 0–22 weeks prior to the flower counts.

Cyrtandra, so perhaps it is the increased sunlight that accompanies dry periods that triggers flowering, rather than the dry period itself. Fruit development was observed to take about 5 months.

Population Characteristics

The population structure at site 1 (Figure 3) shows a typical inverse J-shaped curve indicative of a stable population that has good regeneration by seedling growth and adequate representation of all other size classes (Mueller-Dombois and Ellenberg 1974:96–98).

Anthesis

The sequence of events from opening of the bud until senescence of the flower takes about 7 days.

DAY 1: Corolla bulges out of enclosing calyx and bracts; lobes begin to open. Thecae of the two fertile anthers appressed against the top lip of corolla tube opening outward. Stigma lobes closed, about 3 mm below anthers.

DAY 2: Corolla fully open; thecae fully expanded and releasing pollen. Stigma still immature, below anthers.

DAY 3: Stigma lobes opening. Anther filaments twisting and moving thecae from top of corolla tube facing out (Figure 4, left).

DAY 4: Stigma lobes fully open. Anthers erect in middle of corolla tube (Figure 4, middle).

DAY 5: Anthers face down against bottom of corolla tube (Figure 4, right).

DAY 6: Anthers senescing (Figure 5, left).

DAY 7: Whole flower senescing (Figure 5, middle).

LATER: Corolla tube with epipetalous stamens fallen off. Ripening ovary surrounded by calyx tube and bracts (Figure 5, right).

Protandry is evident in the first 4 days of anthesis, followed by maturation of the stigma in the later stages of anthesis. Normally, the anthers and stigma do not touch, but I have observed the anthers in contact with the stigma in several flowers. This may be due to delay in senescence of the anthers or to early maturation of the stigma under the influence of environmental

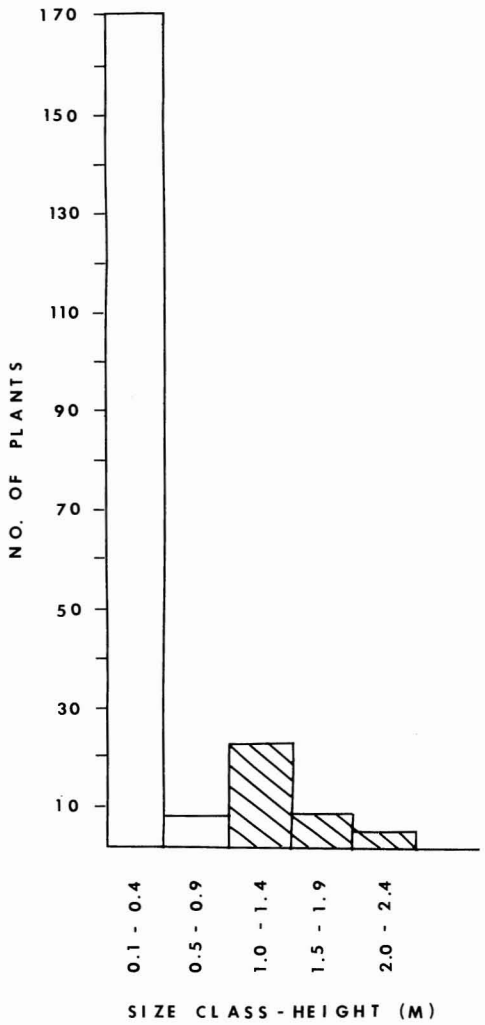

FIGURE 3. Number of plants of *Cyrtandra grandiflora* in size classes according to height of plant. Shaded bars indicate size classes that contained flowering plants.

or hormonal factors. The result is self-pollination in this self-compatible species (Table 2). This mechanism may ensure some fruit set in the absence of pollinators. In normal anthesis, however, the position of the anthers and stigma in their mature stages is such that a large pollinator (for example, a sphingid) would contact the anthers or stigma on the dorsal surface of its thorax and effect pollination.

Pollen

The pollen of *Cyrtandra grandiflora* is tricolpate with reticulate patterning of the exine (Figure 6). Fresh pollen is covered with a sticky *pollenkitt*, which causes masses of pollen grains to stick together. This is characteristic of many entomophilous species of plants but is lacking in those that are wind-pollinated (Knoll 1930). The pollen is binucleate at anthesis (Figure 7), which conforms to Brewbaker's (1967) classification of the Gesneriaceae. Binucleate pollen is more viable in vitro and has greater longevity in storage than trinucleate pollen. *Cyrtandra grandiflora* pollen is viable for the entire period of anthesis (Table 1), making it available for pollination over the lifetime of the flower. Viability as indicated by stainability in cotton blue averages 98 percent. This comparison was made to show that pollen viability in vivo is much higher than in vitro.

Gynoecium

Microscopic examination of longitudinal sections of the gynoecia in days 4, 5, and 6 of anthesis, when the stigmas were expanded, revealed almost 100 percent germination of pollen on all the stigmas. The callose plugs in the pollen tubes stained bright blue and were clearly visible where the tubes grew through the styles into the ovaries. Pollen tubes were visible among the ovules but none was seen actually entering a micropyle. The stigmas thus appear to remain receptive from day 4 until senescence.

Nectar

Nectar is produced by an annular disk at the base of the ovary. Nectar production ranged in volume from 1 to 73 μl ($\bar{X} = 9$ μl) in 23 bagged flowers and 1 to 110 μl ($\bar{X} = 5$ μl) in 22 unbagged flowers. The average total soluble solids (sucrose equivalents) in the nectar of 15 flowers was about 9 percent. This is at the low end of a range of sugar concentrations of nectar of 43 species of flowering plants reported by Percival (1961). The ninhydrin test for amino acids was positive, as a definite bluish-purple color developed (Baker and Baker 1973:103–104).

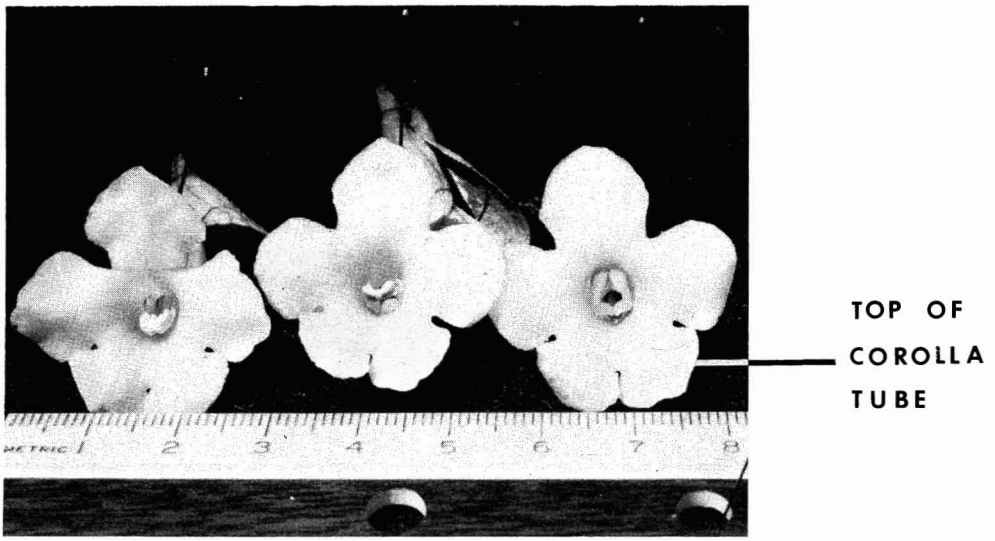

FIGURE 4. Days 3, 4, and 5 of anthesis in *Cyrtandra grandiflora*.

FIGURE 5. Days 6, 7, and later of anthesis in *Cyrtandra grandiflora*.

Breeding System

The absence of fruit set when flowers were bagged to exclude pollinators for the entire period of anthesis (Table 2) indicates that *Cyrtandra grandiflora* does not normally self-pollinate without outside help. The high (69 percent) fruit set that resulted when flowers were self-pollinated by hand indicates that *C. grandiflora* is self-compatible. It is also compatible with other plants of the same species, as one would expect, but the controlled outcrossing produced only 48 percent fruit set (Table 2). The interspecific cross

TABLE 2

FRUIT SET IN *Cyrtandra grandiflora* UNDER DIFFERENT POLLINATION REGIMES

CATEGORY	NUMBER OF FLOWERS	NUMBER OF FRUITS FELL	NUMBER OF FRUITS SET	FRUITS SET (%)
Bagged	20	20	0	0
Controlled self-pollination*	39	12	27	69
Controlled outcrossing*	31	16	15	48
Cross-pollination with*				
C. sandwicensis♂	29	12	17	59
Open pollination, Oct. 1977†	38	26	12	32
Open pollination, May 1978†	33	20	13	39

*Average fruit set for controlled pollination = 59.6%.
†Average fruit set for open pollination = 35.2%.

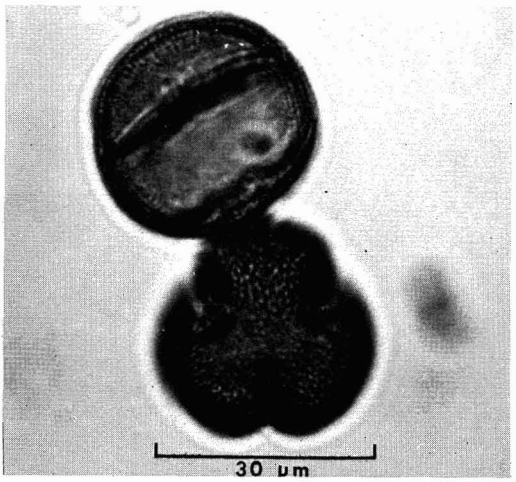

FIGURE 6. *Cyrtandra grandiflora* pollen showing the exine.

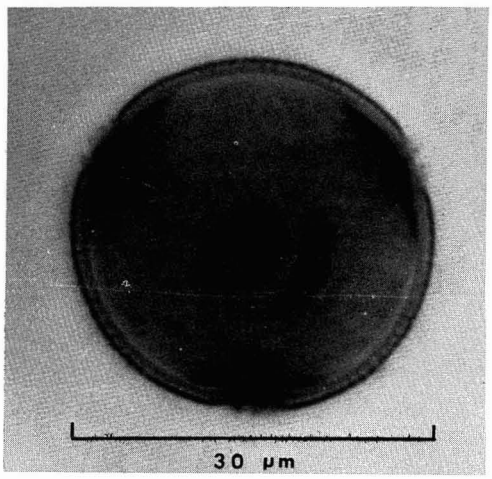

FIGURE 7. *Cyrtandra grandiflora* pollen showing the nuclei at anthesis.

between *C. grandiflora* ♀ and *C. sandwicensis* ♂ produced 59 percent fruit set (Table 2). The average fruit set with hand pollination ($\bar{X}$ = 59.6 percent) is significantly different from the average fruit set with open pollination ($\bar{X}$ = 35.2 percent).[3] This may be due to more efficient pollination by hand. Perhaps the agent that pollinates *Cyrtandra* in nature visits only about one third of the flowers.

It is possible that the seeds are produced apomictically, by development of various unreduced cells of the female gametophyte such as an embryo sac mother cell, a somatic cell of the ovule or a 2*N* egg. It could be that pollination is necessary to stimulate hormone production for fruit development but that fertilization of the cell developing into the embryo does not necessarily occur. The result would be populations of plants with the same genetic composition. This repro-

[3]The hypothesis that $u_x = u_y$ can be rejected at the 97.5 percent confidence level (t = 3.19 with d.f. = 168) when testing the independent means obtained by hand pollination ($\bar{X}$) against those obtained with open pollination ($\bar{Y}$), according to the method of Minium (1970, pp. 304–305).

TABLE 3

SEED VIABILITY, NUMBER PER FRUIT, AND GERMINATION OF *Cyrtandra grandiflora*

REPLICATE NUMBER	SEEDS WITH EMBRYOS (%)	SEEDS WITHOUT EMBRYOS (%)	TOTAL NUMBER OF SEEDS PER FRUIT	GERMINATION IN WATER (%)*	GERMINATION IN SOIL
1	33	67	2,638	10	—
2	99	1	3,834	0	—
3	99	1	3,341	1†	—
4	100	0	3,278	1†	—
5	98	2	476	0†	—
6	33*	67*	4,000*	0†	—
7	—	—	—	90	Yes
8	—	—	—	1†	Yes
9	—	—	—	10†	Yes
10	—	—	—	1†	Yes

* Estimation.
† Fungus infection.

ductive mechanism could account, in part, for the wide speciation in the genus *Cyrtandra* by perpetuating apomictically any new types that arise. The potential for year-round, prolific seed production is present whether due to apomixis, selfing, or outcrossing, but it seems to be limited by the necessity of an outside vector to facilitate pollination.

Pollinators

No native pollinators were observed or captured on *Cyrtandra grandiflora* either during the day or at night. A recently introduced *Macroglossum* sp. sphinx moth was seen taking nectar and in the correct position to collect pollen from young flowers and to deposit it on the stigmas of older flowers. It may help perpetuate the species in the future but was not the agent in the past. The bagging experiments and the observed fruit set indicate that a pollinator is active on *C. grandiflora* and probably has been since the arrival of *Cyrtandra* in the Islands, presuming that protandry was present in the ancestral species and not newly derived since establishment in the Hawaiian Islands. The incidental selfing that I observed is not adequate to produce the abundant fruit set recorded. The fairly uniform fruit production throughout the year (Figure 1) indicates a nonseasonal pollinator. A precinctive crawling insect that pollinates while wading around in the corolla tube would cause selfing and would explain the many small, localized populations and species of *Cyrtandra* and the lack of hybrids.

Seed Viability

The germination rate of seeds on moist filter paper varies from 0 to 90 percent (Table 3) depending, in part, on whether the dishes became infected with fungus. The germination rate on Tantalus cindery soil and on a porous rock appeared to be high. Two weeks after sowing the seed, the soil surface was covered with small (1-mm tall) seedlings. Only three seedlings emerged on the ground-up leaf litter and these soon died, probably because this is not the normal substrate for seedling establishment. Seed set is potentially around 4000 seeds per fruit (Table 3). Seed development ranged from 33 to 100 percent, perhaps due to the efficiency of pollination, pollen fertility, or environmental conditions. Seeds and seedlings are small (average seed length = 284 μm) and have a high mortality rate in the greenhouse and the field.

Vegetative Propagation

Adventitious rooting of leaves and stems is common in the Gesneriaceae. *Cyrtandra*

grandiflora roots prolifically from the stem in the field and in vermiculite in the greenhouse. No adventitious rooting of leaves was observed in the field or achieved in the greenhouse. In nature, *C. grandiflora* is capable of vigorous vegetative and reproductive growth even when partially uprooted and procumbent. It characteristically roots along the horizontal portions of the stem and the new growth assumes a positive phototropic posture. Breakage of stems or partial uprooting seems to result in cauliflory and more abundant flowering than normal.

Cyrtandra grandiflora is typical of many Hawaiian plants in having a prolonged flowering period with greater flowering following the dryer season (C. H. Lamoureux, personal communication). Reproduction occurs both sexually and asexually. Self-compatability and self-pollination are common in plants successfully established on isolated islands, allowing for population buildup from the one or few founding individuals (Baker 1955). Plants closely coevolved with a biotic pollinating agent would have less chance of survival, since the pollinator would probably not arrive with the plant. Such relationships would tend to develop over a long period of mutual coadaption of plant and animal. Thus, founding plants on isolated islands tend to be generalists in growth and reproduction. Those that are "plastic" may go on to speciate widely as *Cyrtandra* appears to have done in the Hawaiian Islands.

ACKNOWLEDGMENTS

I wish to thank C. H. Lamoureux, G. E. Carr, D. J. C. Friend, and E. A. Kay for suggestions and criticism throughout this study.

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