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# Influence of a biotic stress (leaf curl viral infection) on the sex ratio and resource allocation in *Momordica tuberosa* (Roxb.) Cogn. — A monoecious perennial herb

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We have examined the effect of a biotic stress, leaf curl viral infection, on the floral sex ratio and resource allocation patterns in a monoecious herb, *Momordica tuberosa*. Diseased plants had significantly less number of female flowers and consequently exhibited a predominant male biased floral sex ratio. Nearly 26 per cent of the diseased plants were completely males. Resource allocation pattern to the vegetative and reproductive units remained unaltered in diseased plants, however, a greater proportion of reproductive resources was allocated to male function. We discuss the adaptive significance of such shift in the floral sex ratio and resource allocation between the sexes.

MONOECIOUS plants alter their sex ratio (number of male to female flowers) and allocation of resource to reproductive structures as an adaptive response to their resource status[1–4], habit[5], alterations in environmental conditions such as soil moisture[6], light[6] and to altitude[7]. Such adaptive sex ratio changes in response to abiotic stresses have also been confirmed under carefully controlled conditions of light and soil moisture regimes[6].

Very little is known about the sex ratio variations in response to biotic stresses such as pest and disease incidence. Bazzaz[8] reported a male biased sex ratio of tall *Ambrosia trifida* plants compared to short as a response to a higher degree of seed predation by insects. Infection of a fungal pathogen, *Ustilago spp.* has long been known to reverse the sex in a dioecious *Lychenis* and monoecious *Zea mays*[9]. To the extent that biotic stresses also affect the resource status of the plant, it may be expected that the response of plants and their

resource allocation patterns between sexes is similar to that observed under abiotic stresses. In this article, we report the effect of biotic stress induced by a viral infection (leaf curl) on the sex ratio and resource allocation to vegetative and reproductive structures in *Momordica tuberosa* (Roxb.) Cogn., in its natural habitat.

## System

*Momordica tuberosa* (Roxb.) Cogn. (Syn: *Luffa tuberosa*) (Cucurbitaceae) is a monoecious, insect-pollinated, prostrate, tuber forming perennial herb. It colonizes the fallow and cultivated lands of arid regions of southern India. Many scandant branches radiate from a common tuber. Both primary and secondary branches bear at each node either male (in 2–5 flowered raceme) or solitary female flowers. The light yellowish male flowers are larger ($n = 74$, $\bar{X} = 2.05 \pm 0.03$ cm) compared to females ($n = 64$; $\bar{X} = 1.41 \pm 0.02$ cm; $P < 0.01$) in diameter and possess a dark yellow spot at the base of the petals. Female flowers produce four to five ovules arranged linearly. About 2 to 3 per cent of the plants, including seedlings, are generally infected by leaf curl virus and transmitted through white flies (*Bemisia tabacii*) (personal observation). The infected plants are stunted with pale yellowish crinkled leaves and are thus readily distinguishable from healthy plants.

The study was conducted at the Regional Research Station, University campus, Raichur (16° 15′ N, 77° 20′ E; 389 m above MSL), Karnataka, India.

## Methods

The data were collected from randomly chosen, similar aged healthy ($n = 38$) and diseased ($n = 27$) plants growing adjacent in the habitat. On every plant, the length of primary and secondary branches was measured and number and sex of the flowers borne at each node were recorded. From these data, the nodal sex ratio (NSR) for each branch was computed as the ratio of the number of nodes bearing male flowers to those bearing female flowers. Further, for each plant the floral sex ratio (FSR) was computed as the ratio of total male to total female flowers. The success of female flowers to develop to fruit at any node was recorded by either direct observation of the developing fruit (as in younger nodes) or by the persistent fruit wall after the seeds have dispersed (as in older nodes). From these data, the fruit set percentage was computed for healthy and diseased plants. The seed number per fruit and seed set was also recorded.

To analyse the biomass allocation pattern, male, female and vegetative structures (above ground parts) of healthy ($n = 45$) and diseased ($n = 27$) plants were separated, oven dried for 3 days at 60°C and the weights recorded.

Insect pollinator visitation rates to male and female flowers in healthy plants were recorded for 5 minutes ($n = 47$) between 06.00 and 09.30 hrs. Number of male and female flowers available in one square meter area was counted, and total pollinators, and number of male and female flowers visited by them in 5 minutes were recorded. From this the visitation rate was calculated separately for male and female flowers. One-day-old stigmas were excised and pollen grains on the stigmas were counted using a light microscope for both healthy and diseased plants. The viability of pollen grains from diseased and healthy plants was assessed using acetocarmine staining technique.

## Results

Primary and secondary branches of diseased plants exhibited NSR of 6.61:1 and 4.19:1 respectively and was significantly male biased compared to that of healthy plants (1.67:1 and 0.95:1 respectively; Table 1), however in the latter the NSR was significantly different from 1:1 (primary branches : $\chi^2 = 21.76$; $P < 0.001$, $df = 1$; secondary branches : $\chi^2 = 25.08$, $P < 0.001$, $df = 1$).

The diseased plants had a highly male biased FSR (9.44:1) compared to the healthy plants (3.53:1; $P < 0.001$; Table 2). This appears to be due to the significant reduction of female flowers per plant under diseased condition (Table 2).

Diseased plants exhibited significantly reduced fruit set (26.50 per cent) and seed set (51.90 per cent) compared to healthy plants (Table 2). Stigmas captured significantly greater number of pollen grains ($\bar{X} = 27.50$)

**Table 1.** Length of, and number of nodes bearing male and female flowers in primary and secondary branches of healthy and diseased plants

| | Healthy | | Diseased | | t test significance |
|---|---|---|---|---|---|
| | $n$ | Mean ± SE | $n$ | Mean ± SE | |
| **Primary branch** | | | | | |
| Length (cm) | 128 | 96.41 ± 4.05 | 128 | 20.04 ± 1.08 | $P < 0.001$ |
| Nodes bearing male flowers | 128 | 16.23 ± 0.48 | 128 | 12.42 ± 0.35 | $P < 0.001$ |
| Nodes bearing female flowers | 128 | 13.96 ± 0.60 | 128 | 1.86 ± 0.20 | $P < 0.001$ |
| Nodal sex ratio[†] | 128 | 1.67 ± 0.19 | 75* | 6.61 ± 0.54 | $P < 0.001$ |
| | (128 | 0.55 ± 0.012 | 128 | 0.89 ± 0.011 | $P = 0.001$) |
| **Secondary branch** | | | | | |
| Length (cm) | 103 | 30.64 ± 2.34 | 55 | 9.33 ± 0.75 | $P < 0.001$ |
| Nodes bearing male flowers | 103 | 5.40 ± 0.30 | 55 | 5.80 ± 0.29 | NS |
| Nodes bearing female flowers | 103 | 7.15 ± 0.38 | 55 | 0.69 ± 0.16 | $P < 0.001$ |
| Nodal sex ratio[†] | 103 | 0.95 ± 0.07 | 18** | 4.19 ± 0.68 | $P < 0.001$ |
| | (103 | 0.43 ± 0.016 | 55 | 0.91 ± 0.024 | $P = 0.001$) |

NS = Non-significant.
* = 53 primary branches were not included for computation of NSR as they did not contain nodes with female flowers.
** = 37 secondary branches were not included for computation of NSR as they did not contain nodes with female flowers.
† = NSR was also expressed as proportion of male flower[17] producing nodes to avoid omission of data points. The primary and secondary branches of healthy and diseased plants were compared employing Wilcoxon-Mann-Whitney Test[21] (shown in parentheses).

Table 2. Number of male and female flowers, floral sex ratio, percentages of fruit and seed set, pollen grain/stigma (Pg/St) and pollen fertility in healthy and diseased plants

| | Healthy | | Diseased | | t test signi-ficance |
|---|---|---|---|---|---|
| | n | Mean ± SE | n | Mean ± SE | |
| Male flower number plant | 44 | 119.31 ± 17.17 | 24 | 99 71 ± 8.33 | NS |
| Female flower number plant | 44 | 37.18 ± 3.68 | 24 | 9.25 ± 1.95 | $P < 0.001$ |
| Floral sex ratio[†] | 44 | 3.53 ± 0.30 | 17* | 9.44 ± 5.10 | $P < 0.001$ |
| | (44 | 0.73 ± 0.02 | 24 | 0.91 ± 0.01 | $P = 0.001$) |
| Fruit set (%) | 35 | 40 50 ± 2.00 | 25 | 26.50 ± 5.00 | $P < 0.001$ |
| Seed set (%) | 237 | 68 63 ± 1.77 | 64 | 51.90 ± 2.70 | $P < 0.001$ |
| Pg St | 30 | 27.50 ± 4.27 | 26 | 7.31 ± 2.43 | $P < 0.01$ |
| Pollen sterility | 50 | 1.91 ± 0.51 | 44 | 2.79 ± 1.01 | NS |

* Seven plants were not considered for computation of floral sex ratio as they did not possess female flowers.

[†] = FSR was also expressed as proportion of male flowers in a plant to avoid omission of data points. The FSR of healthy and diseased plants were compared employing Wilcoxon–Mann–Whitney test (shown in parentheses).

in healthy plants compared to those in diseased plants ($\bar{X} = 7.31$); there was no difference in pollen grain fertility between the healthy and diseased plants (Table 2). The frequency distribution of seeds per fruit of the diseased and healthy plants differed significantly (Figure 1; KS test $P < 0.01$; $D = 0.207$). A higher proportion of fruits of healthy plants had more number of seeds while those of diseased had less number of seeds.

Disease incidence significantly retarded the growth of primary and secondary branches and reduced the number of nodes (Table 1) and consequently the total vegetative biomass (Table 3). The proportion of resources allocated to vegetative structures remained unaltered in diseased ($\bar{X} = 86.19 \pm 10.04\%$) and healthy ($\bar{X} = 83.11 \pm 6.28\%$) plants ($t = 1.61$; $P > 0.05$). However, healthy and diseased plants exhibited contrasting patterns for resource allocation to different sexes (Figure 2); diseased plants allocated a higher fraction (11.87%) of the total resources to male parts compared to healthy plants (2.95%) (Table 3). The prominent insect pollinators visiting Momordica plants were bees (Apis sp., Halictus sp.), butterflies, hoverflies and skippers. Male flowers attracted significantly greater number of pollinators per unit time compared to female flowers (Table 4).

## Discussion

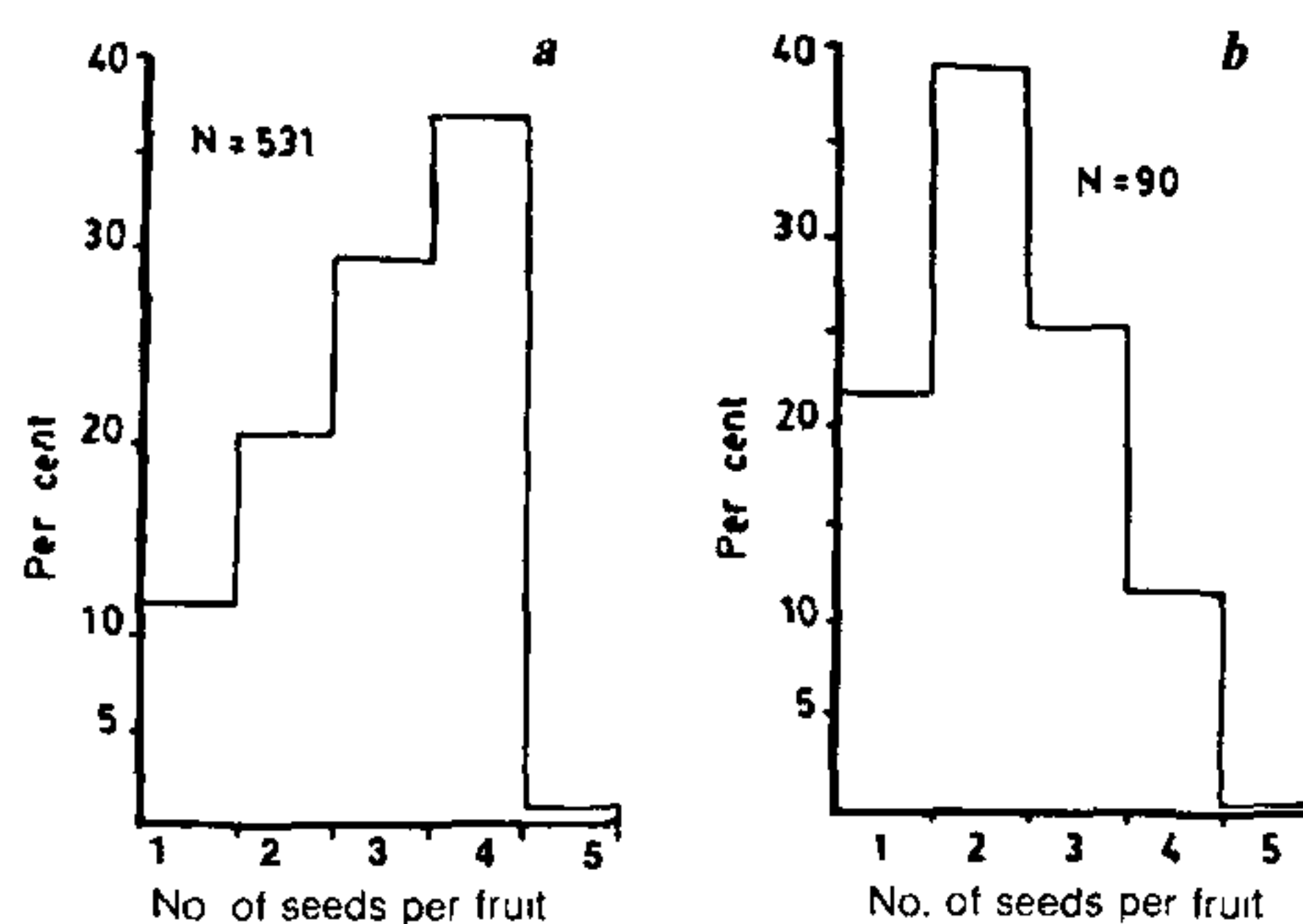The findings suggest that diseased plants of Momordica



Figure 1. Distribution of seed number per fruit in (a) healthy and (b) diseased plants.

tuberosa function predominantly as males as evident by the following: (a) among the diseased plants ($n = 27$), 26 per cent did not produce even a single female flower, indicating a complete shift from monoecy to maleness, (b) both primary and secondary branches of diseased plants showed significantly more male biased NSR compared to those of healthy plants, (c) diseased plants also showed a higher male biased FSR for the whole plant. Several workers[2,4,6,10-12] have also observed such male biased sex ratio under abiotic stress conditions in

Table 3. Biomass (g) allocation pattern in healthy and diseased plants

| | Healthy | | Diseased | | t test signi-ficance |
|---|---|---|---|---|---|
| | n | Mean ± SE | n | Mean ± SE | |
| Vegetative structures | 49 | 2.399 ± 0.18 (83.11 ± 6.28) | 24 | 1.373 ± 0.16 (86.19 ± 10.04) | $P < 0.001$ |
| Male structures | 49 | 0.087 ± 0.01 (2.95 ± 0.25) | 24 | 0.165 ± 0.02 (11.87 ± 0.86) | $P < 0.03$ |
| Female structures | 49 | 0.377 ± 0 04 (13.94 ± 1.05) | 24 | 0.055 ± 0.02 ( 1.94 ± 0.25) | $P < 0.001$ |

Values in parentheses indicate percentage.

**Table 4.** Pollinator visitation rates to flowers in healthy plants

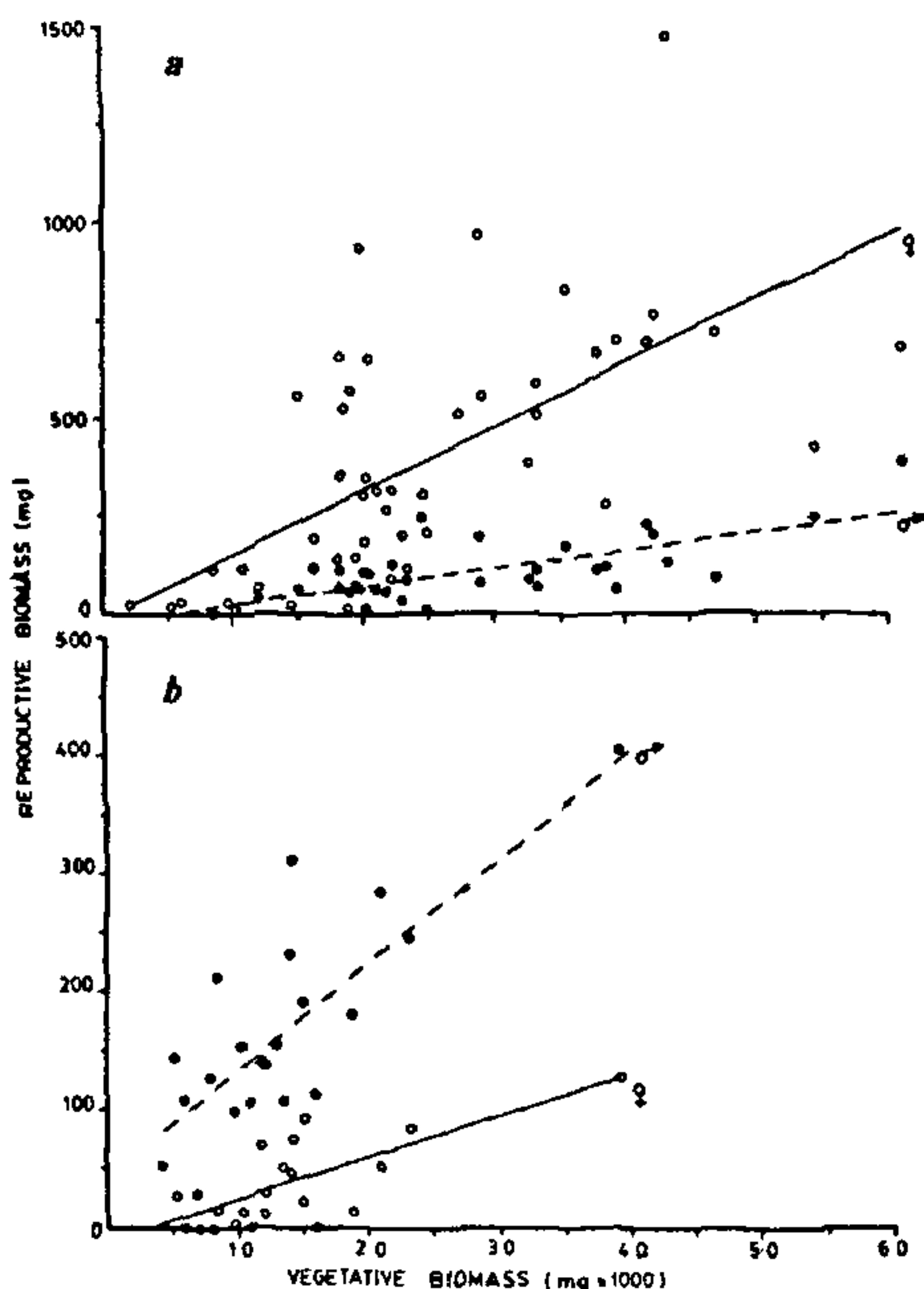| | Male flower | | Female flower | | t test signi-ficance |
|---|---|---|---|---|---|
| | n | Mean ± SE | n | Mean ± SE | |
| No. of pollinators visited per 5 min | 47 | 2.300 ± 0.27 | 47 | 0 790 ± 0.12 | $P < 0.01$ |
| No. of pollinators visited per flower | 47 | 0.311 ± 0.04 | 47 | 0.098 ± 0.02 | $P < 0.001$ |
| Total no. of flowers/grid | 47 | 27.936 ± 1.23 | 47 | 13.830 ± 0.85 | $P < 0.001$ |



**Figure 2.** Scatter of biomass allocation between sexes (males: closed circles; females: open circles) and vegetative structures among: (a) Healthy plants: female, $n = 44$; $Y = 0.167X + 0.126$; $R^2 = 64\%$ and male, $n = 44$; $Y = 0.040$ X $+0.376$; $R^2 = 57\%$, (b) Disease plants: female, $n = 24$; $Y = 0.031$ X $-0.012$; $R^2 = 48\%$ and male: $n = 24$, $Y = 0.080$ X $+0.038$; $R^2 = 65\%$.

monoecious plants. A few workers have reported a shift in gender expression from female to maleness due to severe winter[13,14], low soil moisture[15], and high temperature[16].

Significant reduction in fruit and seed set in diseased plants is indicative of a reduced reproductive success accrued through female flowers. Post-fertilization abortion of flowers and seeds under highly reduced resource status, might have shaped the reduced female success in diseased plants. The theory of sex allocation[17] predicts

that in the face of such decreased success through females, it pays for the plant to allocate more of its resource to male flowers. The observed reduction in female success and the highly male biased sex ratio of diseased *Momordica* plants is in conformity with this prediction. Though, the small frequency of diseased plants did not permit us to estimate the success through their male gametes, it could be argued that the success through male in diseased plants would be high compared to that through female for the following reasons: (a) the cost of producing pollen grain is considerably less than that of ovules (and seeds). Therefore, even with limited success of pollen grains reaching the stigma, diseased plants can gain fitness by investing their limited resource into males, (b) attractiveness of the whole plant can be expected to increase if diseased plants invest more on male function, as male flowers are borne in 2-5 flowered racemes, consequently the pollinator visitation might also increase[18,19]. In fact, the male flowers of healthy *Momordica* plants attracted a greater number of pollinators with higher rate of visitation compared to female flowers. Kohn and Casper[20] have demonstrated recently that gene flow in *Cucurbita foetidissima* (Cucurbitaceae) is mediated to a greater extent through pollen. It would be expected under these conditions, that selection should favour diseased plants to allocate more of their resources to male function than female. Such alterations in gender expression may proximally be achieved by increasing the levels of plant growth regulators under diseased conditions. In fact, fungal and bacterial infections in plants are known to increase the levels of plant growth regulators especially of gibberelins, a key hormone in promoting maleness among cucurbits (H. Y. Mohan Ram, pers. commun.).

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