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GROWTH AND BRANCHING OF DAMAGED *SALVINIA MOLESTA*

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Abstract: The floating aquatic weed *Salvinia molesta* in tropical fresh waters is being biologically controlled by the weevil, *Cyrtobagous salviniae*. Damage due to predatory activities was simulated by selective removal of leaves, roots and severing of stolons and the subsequent growth and branching were studied by measuring the number of nodes and length of stolons, number of branches and percentage branching, and dry weight of plants. The leaf and root removal resulted in significantly decreased growth and branching, whilst severing stolons into smaller pieces led to significantly increased branching.

Key words: Aquatic weeds, *Salvinia molesta*

INTRODUCTION

Predators prefer to feed on plant parts of particular ages in definite positions because of variations in toughness, nutrient content and defensive chemicals. Stolons can be damaged directly by feeding and indirectly through trampling by grazing animals. Predation of leaves, buds, shoot parts or stolons and roots reduces the photosynthetic area, transpiration surface and sites for nutrient uptake. Damage may also disturb developmental processes and can change the architecture of plants. It may stimulate the growth of dormant buds as a result of hormonal change or weaken the plant reducing the growth and branching. These effects depend on the severity of damage or grazing. Enhancements of branching in plants following lenient damage by predators have been observed in many species. e.g. *Calluna vulgaris*[1] ; *Trifolium repens* L.[2,3] and in *Lolium perenne* L .[4] There is a compensatory growth following damage. If the damage is lenient, it leaves more residual parts which can support compensatory growth immediately. But if the grazing intensity and frequency exceed the capacity for regrowth, the productivity may decline.[5] Death of root and root nodules and hence reduced nitrogen fixation, after excessive defoliation have been observed in *T. repens*.[6,7] Reduced yield and branching when root formation was prevented have also been observed in *T. repens*.[3] Hatto and Harper[8] showed a significant loss in yield due to defoliation by a surface feeding slug on the grass *L. perenne*. The knowledge on the effect of damage to various parts of plants is helpful for devising strategies for the biological control of problematic weeds.

The floating aquatic fern *Salvinia molesta* Mitchell is a problematic weed in many tropical fresh water bodies including those in the Mahaweli project in Sri Lanka .[9] *S. molesta* only propagates vegetatively.[10] Therefore it is a single genet and the fitness of the entire species depends on branching, growth and fragmentation. The plant grows rapidly and can double in size in less than three days. The plant passes through three morphologically different stages (primary, secondary and tertiary) during its growth. Each ramet of *S. molesta* consists of a pair of leaves which float on the water surface, a dissected leaf which hangs down and functions as a root, a horizontal segment of stolon, an apical bud which develops into a new ramet and three lateral buds which develop into new branches. These buds arise on alternate sides of the stolon at successive nodes. There is a sequence of development of all lateral buds in each ramet and they are ranked as first, second and third. However, under natural conditions, it is the first rank buds that develop into branches while the second and third rank buds only develop when ample nutrients are present.[11]

Biological agents are used to control *S. molesta* in many countries e.g. Australia, Papua New Guinea, Namibia and India.[12] The insects that are natural predators used in control of *S. molesta* are a curculionid weevil, *Cyrtobagous salviniae* Calder and Sands, an acrilid grasshopper, *Paulinia acuminata* De Geer, and a pyralid moth, *Samea multiplicalis*. Gn. *C. salviniae*[12] has been used successfully in Sri Lanka (Fernando I. unpublished observation) for partial control of *S. molesta*. Both adults and small larvae of *C. salviniae* feed preferentially on apical buds, young roots and young leaves of the plant. Young larvae of *S. multiplicalis* preferentially feed on buds and young leaves.[13] Large larvae burrow tunnels through stolons which leads to breakage of stolons.

We describe here the results of experiments to study the effects of damage of leaves, roots and stolons on the growth and branching of *S. molesta*, where damage due to predatory activities was simulated by selective removal of leaves and roots and severing of stolons.

METHODS AND MATERIALS

The effect of selective removal of leaves: Three cement tanks (125x60x30 cm) were placed in the botanical garden of the Department of Botany. These were filled upto 15 cm with mud (brought from a pond where *S. molesta* was growing) then filled upto 27 cm with water and this level was maintained throughout the experiment. Six wooden frames (quadrats) of 25x25 cm were placed afloat in each tank. Uniform (in age and size) cuttings consisting of four ramets of *Salvinia molesta* at their tertiary stage were selected and ten plants introduced into each wooden frame. After two days, the following treatments were performed on each plant in each frame using three replicate quadrats for each treatment: T1-normal plants (control), T2-one of the leaves of every other leaf pair on stolons was removed, and T3-one of the leaves of all the leaf pairs was removed (the excised leaves were always leaves of axils to which buds were attached).

After four weeks of growth, the number of nodes on the main stolon, and on primary stolons (stolons arise from the main stolon), number of branches on the main stolon, length of the main stolon and primary stolons were measured. Then the plants were washed carefully and each plant was placed in a separate paper bag, oven dried at 70^{0}C and the final constant weight was recorded. The percentage branching was calculated.

The effect of selective removal of roots: Tanks were prepared as described above. Uniform ramets were selected and four ramets were introduced into each wooden frame floating in water taking three replicate treatments. After one week of growth the following treatments were allocated to individual plants. These treatments were given from the third node onwards on the main stolon of each plant: T1 - normal plants (control), T2 - every other developing root on the main stolon was removed and T3 - all the developing roots on the main stolon were removed. After four weeks of growth the development of the plants were determined as described above.

The effect of severing of stolons: Tanks were prepared as described above. Uniform cuttings bearing four ramets of *S. molesta* of its tertiary stage were selected and ten plants were introduced into each wooden frame floating in water in the tanks. After two days the following treatments were allocated to each plant in each frame. Five replicate frames were used for each treatment: T1 - normal plants (control), T2 -stolons were severed into two parts and T3 - stolons were severed into single ramets (five parts). After five weeks of growth, the development of the plants were determined as described above. All the treatments in each experiment were allocated according to a completely randomized design.

RESULTS

The effects of selective removal of leaves

Table 1 shows the mean values of parameters for each plant in each treatment. The number of nodes on the main stolon did not show any significant difference between treatments. Plants bore the highest number of nodes on the primary stolons in the control treatment. The number decreased significantly in damaged plants. The reductions were 32% and 37% of the control in T2 and T3 respectively. The length of stolons also followed a similar pattern, although the differences were not significant for main stolons. However, the length of primary stolons was greatest in control plants, whilst there was a significant ($p<0.05$) decrease in length with increased removal of leaves. The stolon length of the less damaged and in more damaged plants was reduced by 25% and 30% respectively.

The number of branches on the main stolons also showed a similar pattern to that of number of nodes and length of stolons. The highest number of branches were found in the control plants and the number reduced significantly ($p<0.05$) by 33% in the damaged plants. The removal of leaves reduced the percentage significantly ($p<0.05$). The reductions in percentage branching were 30% and 35% in the less and the more damaged plants respectively. The dry weight of plants was also affected significantly by the removal of leaves. The dry weights were 31% and 24% of the control in the more damaged and less damaged plants respectively.

Table 1: The effect of selective removal of leaves on growth and branching of *Salvinia molesta*.

	Treatments		
	T1	T2	T3
Number of nodes:			
Main stolon	8.63 ± 0.18[a]	8.40 ± 0.27[a]	9.20 ± 0.09[a]
Primary stolons	9.40 ± 0.20[a]	6.43 ± 0.09[b]	5.93 ± 0.07[b]
Length:			
Main stolon (cm)	11.10 ± 0.28[a]	10.60 ± 0.41[a]	9.90 ± 0.30[a]
Primary stolons (cm)	9.30 ± 0.31[a]	7.00 ± 0.13[ab]	6.46 ± 0.06[b]
Number of branches:			
Main stolon	4.80 ± 0.12[a]	3.23 ± 0.04[b]	3.23 ± 0.05[b]
Percentage branching	55.70 ± 0.62[a]	38.98 ± 1.30[b]	35.78 ± 0.04[b]
Dry weight (g)	0.86 ± 0.01[a]	0.65 ± 0.01[b]	0.59 ± 0.01[b]

Each value is a mean of three replicates. T1 - normal plants (control), T2 -one of the leaves of every other leaf pair on stolons was removed. T3 - one of the leaves of all the leaf pairs was removed. Means were compared with Tukey's HSD value. Means ± SE bearing the same letter within each row do not differ significantly ($p<0.05$).

The effect of selective removal of roots

Table 2 shows the mean values for each plant for each treatment. The removal of roots reduced the number of nodes by 5% and 22% in the less and the more damaged treatments respectively. The number of nodes on primary stolons also followed a similar trend, but the effect was greater than on the main stolons. The highest number was found in the control plants, whilst it was reduced significantly ($p<0.05$) by 52% and 76% in the less and the more damaged plants respectively. The length of stolons also showed a similar trend in suppression of growth. The length of the main stolons was reduced by 25% and 41% in the less damaged and in the more damaged treatments respectively. Primary stolons showed the same pattern but the differences were greater than those of the main stolons. The primary stolons were reduced by 58% and 81% in the less and more damaged plants respectively.

Control plants bore the highest number of branches whilst the numbers were reduced significantly ($p<0.05$) by 46% and 67% in the less damaged and in the most damaged plants respectively. There was a significant decrease in percentage branching with increased intensity of root damage. The dry weights were reduced significantly ($p<0.05$) by 52% and 68% of the control in the less and more damaged plants respectively.

Table 2: The effect of selective removal of roots on growth and branching of *S. molesta*.

	Treatments		
	T1	T2	T3
Number of nodes:			
Main stolon	7.160 ± 0.29^a	6.830 ± 0.15^a	5.58 ± 0.08^b
Primary stolons	15.080 ± 1.40^a	7.250 ± 0.58^b	3.66 ± 0.25^b
Length:			
Main stolon (cm)	15.750 ± 0.88^a	11.750 ± 0.33^b	9.29 ± 0.12^b
Primary stolons (cm)	31.750 ± 3.12^a	13.410 ± 1.24^b	6.04 ± 0.42^b
Number of branches:	4.000 ± 0.26^a	2.160 ± 0.08^b	1.33 ± 0.10^b
Percentage branching	57.090 ± 3.80^a	32.200 ± 1.90^b	23.78 ± 2.24^b
Dry weight (g)	0.507 ± 0.01^a	0.245 ± 0.02^b	0.16 ± 0.01^b

Each value is a mean of three replicates. T1 - normal plants (control), T2 - every other developing root on the main stolons was removed, T3 - all the developing roots on the main stolons were removed. Means were compared with Tukey's HSD value. Means ± SE bearing the same letter within each row do not differ significantly ($p<0.05$).

The effect of severing of stolons

Table 3 shows the overall mean values for each quadrat. Main stolons did not possess significantly different number of nodes. However, primary stolons showed a significant increase ($p<0.05$) in the number of nodes in more damaged plants. Thus branching of stolons was significantly increased with increased intensity of severance of stolons. The number was increased by 30% on the main stolon in the most damaged plants. The percentage branching on the main stolons was also increased significantly by 18% and 42% ($p<0.05$) in the less and more damaged plants respectively. The dry weight however was not affected by these treatments.

DISCUSSION

Growth and branching are the main ways of increasing its population size in *S. molesta*. Branching increases the number of leaves and adventitious roots. All the parameters considered were direct or indirect expressions of branching. Leaf removal and root removal showed a reduction in growth and branching of the plant.

Table 3 : The effect of severing of stolons on growth and branching of *S. molesta*.

Variable	Treatments		
	1	2	3
Number of nodes:			
Main stolon	104.80±3.62[a]	91.60±3.82[a]	95.40±3.72[a]
Primary stolons	150.40±4.59[a]	142.60±7.67[a]	169.80±5.30[b]
Secondary stolons	32.20±4.02[a]	31.20±6.24[a]	34.00±6.42[a]
Length			
Main stolon (cm)	148.60±7.96[a]	128.40±8.33[b]	111.40±4.34[b]
Primary stons (cm)	169.20±9.83[a]	158.20±9.61[a]	186.40±7.10[a]
Secondary stolons (cm)	33.80±4.40[a]	30.30±5.50[a]	35.80±6.95[a]
Number of branches			
Main stolon	50.00±1.79[a]	52.00±1.67[a]	64.80±2.29[b]
Primary stolons	21.00±1.92[a]	19.20±3.62[a]	23.20±3.50[a]
Percentage branching			
Main stolon	48.16±3.24[a]	57.02±2.07[b]	68.40±3.83[b]
Dry weight (g)	7.12±0.49[a]	6.51±0.44[a]	6.14±0.38[a]

T1 - normal plants (control), T2 - stolons were severed into two parts, T3 - stolons were severed into single ramets (five ramets). Means were compared with Tukey's HSD value. Means ± SE bearing the same letter within each row do not differ significantly ($p<0.05$).

Generally the removal of parts returns the plant to an earlier stage of growth as it needs time to replace the lost parts. Regrowth after damage is influenced by the level of residual tissues. If there are no major reserves for the regrowth after damage, the current assimilates cannot meet the demand from newly developing tissues. *S. molesta* has relatively large but few leaves, and the removal of one leaf may have reduced a great proportion of photosynthetic and transpiratory surface of the whole plant.

Removal of roots reduces the surface area of nutrient uptake.[14] Therefore the root system and its morphology determines how much nutrient is absorbed. Root growth is dependent on resources from shoot and *vice versa*. The importance of nodal roots on the persistence of a plant has been reported by many authors. e.g. winter survival of white clover was reported to be due to a higher proportion of rooting.[15] In *S. molesta* every ramet roots regularly. The presence of these adventitious roots which absorb resources from several sites simultaneously may reduce the death risk of the genet, and each nodal root may have a great influence on the growth of each leaf pair and the associated axillary bud which further develops into a branch. Such positive correlations between the frequency of branching and the growth of root have been shown in *T. repens*.[3,16,17] Thus the survival is greater in branches from rooted nodes. These suggest that the development of a branch may partially depend on the presence of the root at that node which may influence the growth and survival of the branch. Thus if a root at a node is damaged or supplied with poor resources, the chance of supporting further growth of its own axillary bud and the subsequent nodules is reduced. In the present experiment roots were removed from newly formed nodes resulting in a cumulative disadvantage and hence growth and branching could have declined.

The effect of severing of stolons was to enhance branching. When the ramets were interconnected some buds remained dormant and when the physiological link was lost due to severance of stolons the potential buds developed into branches. This suggests that as in higher plants there is a correlative inhibition of axillary bud development in *S. molesta*. Similar results have been reported for *Agropyron repens* where about 95% of the buds remain dormant if the plant is undisturbed.[18] In the present study when ramets were disconnected, they spread more and the bud sites may have been exposed more to light than when they were intact. This may also have stimulated the bud development.

The results show that if biological controlling agents damage stolons producing disconnected ramets, the population size will increase due to a stimulation of branching. Furthermore if they are present in flowing water or in a water body exposed to strong winds the population increase will be greater as the plants disperse. Therefore it is important to devise a way to prevent dispersal of *S. molesta* during biological control efforts. Similar studies to those presented here are important before introducing other biological agents to control weeds. Furthermore, it is important to determine the severity of damage that can eliminate the weed populations most successfully.

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