FEEDING BY *BAGOUS AFFINIS* (COLEOPTERA: CURCULIONIDAE) INHIBITS GERMINATION OF HYDRILLA TUBERS

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ABSTRACT

Bagous affinis Hustache (Coleoptera: Curculionidae) larvae feed inside subterranean turions or tubers of hydrilla (*Hydrilla verticillata* (L.f.) Royle, Hydrocharitaceae) during low water conditions. This results in reduced germination of the tubers. To determine the number of *B. affinis* required to reduce tuber germination, dioecious hydrilla tubers were exposed to various *B. affinis* egg to tuber ratios. The tubers were then held for germination. The number of adults produced and the number of tubers germinating for each treatment and damage category were recorded. In all treatments, tuber germination was significantly reduced compared with the controls. The proportion of tubers germinating tended to decrease with an increase in the number of eggs initially placed in the treatment. This reduction in germination resulted from an increase in feeding damage. The results of this study suggest that *B. affinis* should be released in the field with an egg to tuber ratio of 2:1 or greater.

Key Words: Biological control, aquatic weed control, hydrilla tuber weevil, insect feeding damage

RESUMEN

Las larvas de *Bagous affinis* Hustache (Coleoptera: Curculionidae) se alimentan de los tallos subterráneos (tubérculos) de la elodea de la Florida (*Hydrilla verticillata* [L.f.] Royle, Hydrocharitaceae) cuando el agua es poco profunda, lo que reduce su germinación. Para determinar el número de *B. affinis* requerido para reducir la germinación de los tallos subterráneos de la elodea, fueron expuestos tubérculos dióicos a varias densidades de huevos del insecto y se esperó a que germinaran. El número de adultos producido, el número de tubérculos que germinaron y la categoría de los daños fueron registrados en cada tratamiento. En todas las variantes la germinación de los tubérculos fue significativamente reducida con respecto a los testigos. La proporción de los tubérculos germinados tendió a disminuir con el aumento del número de huevos inicialmente colocados en cada tratamiento. Esta reducción de la germinación fue el resultado del aumento del daño producido por los insectos al alimentarse de los tallos. Los resultados de este estudio sugieren que *B. affinis* debe liberarse en el campo a una proporción de huevos por tubérculo de 2:1 o mayor.

Bagous affinis Hustache (Coleoptera: Curculionidae), the hydrilla tuber weevil, is a biological control agent for hydrilla (*Hydrilla verticillata* (L.f.) Royle; Hydrocharitaceae), a submersed aquatic weed. The life cycle of this weevil is geared to a wet-dry seasonal climate. In the dry season, the weevils feed upon the above-ground portions of the hydrilla plant that are exposed as water recedes from an aquatic site (Baloch et al. 1980, Buckingham 1988). Female weevils oviposit in moist organic matter found in and among the stranded hydrilla plants (Baloch et al. 1980, Buckingham 1988). Upon egg hatch, the larvae burrow through the soil seeking subterranean turions (tubers) of hydrilla. The larvae complete three instars while feeding inside the tubers and then pupate either within the tuber or in the soil (Bennett & Buckingham 1991). The feeding activity of the larvae destroys the tubers by consuming the meristems, or by providing an entryway for other organisms such as fungi or bacteria. Destruction of populations of hydrilla tubers, known as tuber banks, is important in controlling hydrilla because the tubers are a source of new infestations for up to 4 years after formation of the tubers (Van & Steward 1990, L. W. J. A., unpublished data).

Hydrilla is classified as a Category A pest in California and, as such, must be managed with eradication as the objective. In a cooperative program with the California Department of Food and Agriculture, we investigated the use of the hydrilla tuber weevil in an inundative release program to reduce and possibly eliminate tuber banks at selected sites in California. The hydrilla tuber weevil was selected for use in this program because in its native range it infested almost 100% of hydrilla tubers at a site during the dry season. In the following wet season, there was little or no regrowth of the hydrilla at this site (Baloch et al. 1980). In California, some of the water systems infested with hydrilla undergo a seasonal drawdown, thereby potentially exposing hydrilla tubers to attack by *B. affinis*. To estimate the number of weevils to be released at an infested site, the number of weevils and the amount of feeding damage required to cause a reduction in germination of a population of tubers must be determined. In this study, the relationship between *B. affinis* density and reduction in tuber germination was investigated by measuring the amount of germination by dioecious hydrilla tubers after exposure to different numbers of *B. affinis* larvae. This study was conducted in the laboratory because the Category A pest designation of hydrilla would not allow the establishment of field plots.

Materials and Methods

The ratio of *B. affinis* to hydrilla tubers required to reduce tuber germination was investigated in experiments that were conducted at the USDA Aquatic Weed Control Research Laboratory, Davis, California, from 16 December 1992 to 20 May 1994. The insects used in these experiments had been in laboratory culture for 8 to 10 generations. The weevils used to originate this colony were collected outside Bangalore, India in April 1991. They were cultured in quarantine at the Florida Biological Control Laboratory, Gainesville, Florida, for 1 generation before shipment to California in the summer of 1991. The dioecious hydrilla tubers were obtained from the USDA Aquatic Plant Management Laboratory, Ft. Lauderdale, Florida. Known numbers of hydrilla tubers were exposed to different numbers of *B. affinis* larvae. Eggs were used to initiate the experiments because placing eggs on the soil surface more closely reflects actual field conditions in which adults are released and allowed to oviposit. Eggs are also more amenable to transfer to experimental containers than neonate larvae. Of the eggs used in these experiments, approximately 90% hatched (K. E. G., unpublished data).

Fifty replicates of each of the following egg to tuber ratio treatments were established: 1:5 (2 eggs: 10 tubers), 1:4 (2 eggs: 8 tubers), 1:2 (2 eggs: 4 tubers), 1:1 (2 eggs: 2 tubers), 2:1 (4 eggs: 2 tubers), and 5:1 (10 eggs: 2 tubers). These treatments represent the following tuber densities: 10 tubers, 3,306 per m^2; 8 tubers, 2,645 per m^2; 4 tubers, 1,323 per m^2; and 2 tubers, 662 per m^2. The treatments describe the initial experimental conditions. Each replicate consisted of a plastic rearing container (5.5 x 5.5 x 6 cm) filled with a sandy loam soil that had been moistened with a 1% benomyl solution until damp, but friable. The benomyl solution was used to prevent the growth of fungi (Bennett & Buckingham 1991). Hydrilla tubers were weighed individually, and the required number buried approximately 3 cm below the soil surface. *B. affinis* eggs were dissected from water-soaked wood (an oviposition media) that had been placed in a colony cage for 24-48 h. The appropriate number of eggs was placed on moist filter paper on the soil surface, and the container was covered with foil to maintain soil moisture. Controls were set up exactly like the experimental containers, except that no eggs were included. Thirty-five replicates were set up as controls for each of the four tuber densities in the six treatment ratios (i.e., tuber density of 10 for the 1:5 ratio; 8 for the 1:4 ratio; 4 for the 1:2 ratio; and 2 for the 1:1, 2:1, and 5:1 ratios). All containers, both treatments and controls, were held at 27°C for 25 days. The containers were misted 3 times per week with tap water to maintain soil moisture.

To determine germination of the tubers, all *B. affinis* and tubers were recovered and counted. The tubers were then broken in half medially. The interior of each tuber was examined and scored according to the following feeding damage scheme: 0, 1-25, 25-50, 50-75, and 75-100% of the interior damaged. The tubers were then grouped according to treatment, replicate, and feeding damage category, and placed in petri dishes (9 cm diam). The tubers were covered with tap water and placed at 27°C with a photoperiod of 16:8 (L:D) for 7 days. Under these conditions, any non-dormant tubers capable of germinating should have germinated (Spencer & Anderson 1986).

The effect of breaking the tubers in half medially on germination was investigated by examining the germination of 100 tubers, 50 broken, and 50 left entire. The tubers were placed in petri dishes (9 cm diam), covered with tap water, and held for 7 days at 27°C with a photoperiod of 16:8 (L:D). The number of tubers germinating was recorded.

Comparisons of the proportion of tubers in each feeding damage category among ratio treatments were done using X^2 analysis (Steel & Torrie 1960). The effect of tuber size on the amount of feeding damage was investigated by assigning tubers to one of five size classes (0.10 - 0.15 gm, 0.16-0.20 gm, 0.21 -0.25 gm, 0.26 - 0.30 gm, or 0.31 - 0.35 gm) and comparing the proportion of tubers in each feeding damage category among size classes. This comparison was done using X^2 analysis (Steel & Torrie 1960). The proportion of tubers germinating among ratio treatments, between ratio treatments and controls, among feeding damage categories, and between broken and entire tubers were compared using X^2 analysis (Steel & Torrie 1960).

RESULTS

The number of *B. affinis* adults produced increased with an increase in the egg to tuber ratio treatment (Table 1). The treatments were set up with differing numbers of eggs, so the proportion of adults produced were compared among treatments. Significantly lower proportions of adults were produced at the 5:1, 2:1, and 1:1 treatment ratios than at the 1:5, 1:4, and 1:2 treatments (Table 1; X^2=39.02, df=5, P<0.05). This lower production of adults may be due to greater intraspecific competition among the larvae. Such competition could result in greater mortality of the larvae in the higher treatments as compared with the lower treatment ratios, even though the larvae are not cannibalistic (Bennett & Buckingham 1991).

The proportion of tubers fed upon increased with an increase in the egg to tuber treatment ratio (Fig. 1A; X^2 = 91.1, df=5, P<0.01). The proportion of tubers damaged was found to be independent of the weight of the tuber (Table 1; X^2=6.1, df=4, P>0.10), suggesting that the increase in damage was the result of an increase in the number of larvae present. The proportions of tubers withinthe feeding damage categories dif-

TABLE 1. THE MEAN WEIGHT ± STD. ERR. OF TUBERS, THE MEAN NUMBER ± STD. ERR. OF *B. AFFINIS* ADULTS PRODUCED AND THE PROPORTION OF EGGS SURVIVING TO THE ADULT STAGE IN EACH RATIO TREATMENT.

Ratio Treatments	Mean Wt. of Tubers (gm)	Mean No. of *B. affinis* Produced	Proportion of Eggs Surviving To Adult
1:5	0.21 ± 0.003	0.7 ± 0.01	0.35
1:4	0.21 ± 0.003	0.62 ± 0.01	0.31
1:2	0.22 ± 0.004	0.84 ± 0.13	0.42
1:1	0.25 ± 0.007	0.50 ± 0.10	0.25
2:1	0.22 ± 0.005	0.86 ± 0.14	0.22
5:1	0.23 ± 0.007	1.76 ± 0.29	0.18

fered significantly among the egg to tuber treatment ratios (Fig. 1B; X^2=304.6, df=20, P<0.01). At the low treatments (1:5), more of the tubers were in the no or low (0%, 1-25%) feeding damage categories, whereas, at the higher treatments, more tubers were found in the higher feeding damage categories (50-75%, 75-100%; Fig. 1B).

Germination of the tubers was not influenced by breaking the tubers in half medially (X^2=2.38, df=1, P>0.10). Of the tubers that were broken in half, 64% (n = 50) germinated. Of the tubers left entire, 78% (n = 50) germinated.

Comparisons of the proportion of tubers germinating in the treatments with those in the controls summed over all feeding damage categories revealed significant differences (Fig. 2A; 1:5: X^2=45.39, df=1, P<0.01; 1:4: X^2=15.76, df=1, P<0.01; 1:2: X^2=9.91,df=1, P<0.01; 1:1: X^2=14.69, df=1, P<0.01; 2:1: X^2=14.69, df=1, P<0.01; 5:1: X^2=14.96, df=1, P<0.01). In all egg to tuber treatment ratios, except the 1:2 treatment, the proportion of tubers germinating was less in the treatments than in the controls (Fig. 2A). This demonstrated the ability of *B. affinis* to reduce tuber germination. In the 1:2 egg to tuber treatment ratio, a greater proportion of tubers germinated in the treatment than in the control (Fig. 2A). The reason for this difference is unclear. However, in this treatment, the proportion of tubers germinating in all feeding damage categories was greater than in other treatments (Fig. 2B).

In general, there was a reduction in tuber germinationwith an increase in the density of *B. affinis* and the amount of feeding damage (Figs. 2A, 2B, and 3). Comparison of the proportion of tubers germinating among egg to tuber treatment ratios without regard to feeding damage category, revealed a significant decrease in germination as the treatment ratio increased (Fig. 2A; X^2=71.0, df=5, P<0.05). The proportion of tubers germinating in the controls and in each feeding damage category, regardless of ratio treatment, decreased significantly with an increase in damage category (Fig. 3; X^2=101.58, df=5, P<0.01). The proportion of tubers germinating decreased substantially for those tubers in the 25-50 and 50-75% feeding damage categories. No tubers germinated in the 75-100% feeding damage category (Fig. 3).

DISCUSSION

The results suggest that for *B. affinis* to decimate hydrilla tuber banks, they should be released with an egg to tuber ratio of 2:1 or greater. The objectives of the release should dictate the ratio used. For example, if *B. affinis* was used in an inoculative release program where establishment of the weevil was the objective, the egg to tuber ratio for release should be 1:1 or 2:1. These lower ratios should be used because

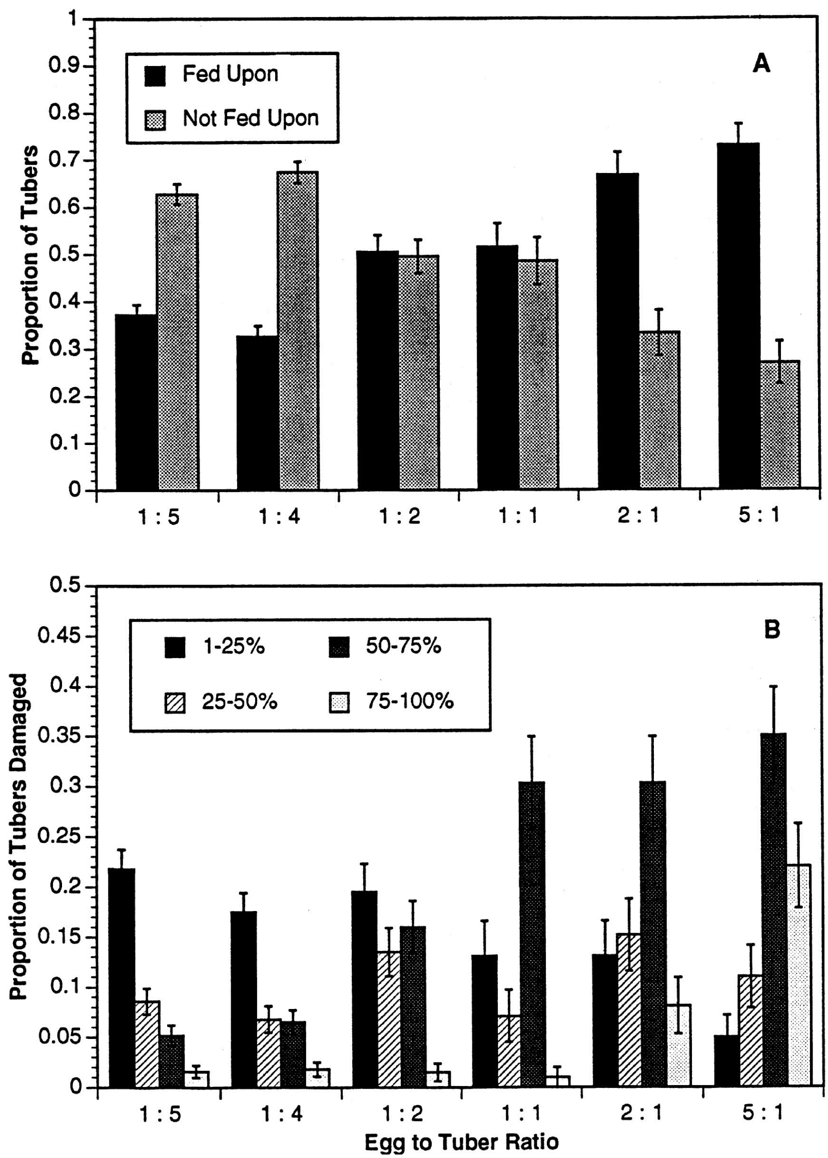

Fig. 1. A.) The proportion of tubers that had been fed upon or not fed upon for the 1:5 (n = 500 tubers), 1:4 (n = 399 tubers), 1:2 (n = 200 tubers), 1:1 (n = 99 tubers), 2:1 (n = 99 tubers), and 5:1 (n = 100 tubers) egg to tuber ratio treatments. Please note 1 tuber was unaccounted for in the 1:4, 1:1, and 2:1 treatments. The proportion of tubers fed upon increased significantly ($P < 0.01$) with an increase in the ratio treatment. B.) The proportion of tubers in each feeding damage category in which feeding damage occurred for each treatment. The proportion of tubers within feeding damage categories differed significantly ($P < 0.01$) among the ratio treatments.

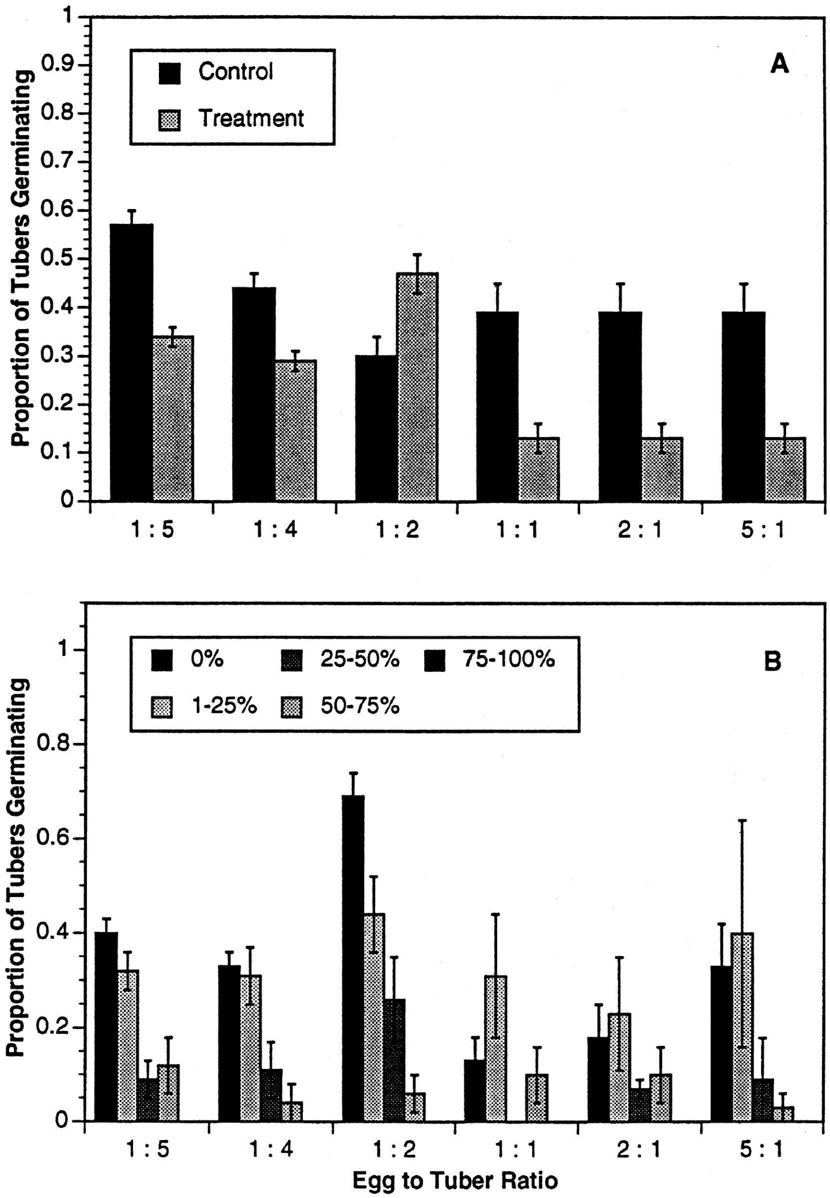

Fig. 2. A.) The proportion of tubers germinating in the controls and in each ratio treatment. There was a significant decrease ($P < 0.05$) in germination with an increase in ratio treatment. Within each ratio treatment, the proportion of tubers germinating differed from that in the controls ($P < 0.01$). (See text for X^2 values). B.) The proportion of tubers germinating in each feeding damage category for each treatment.

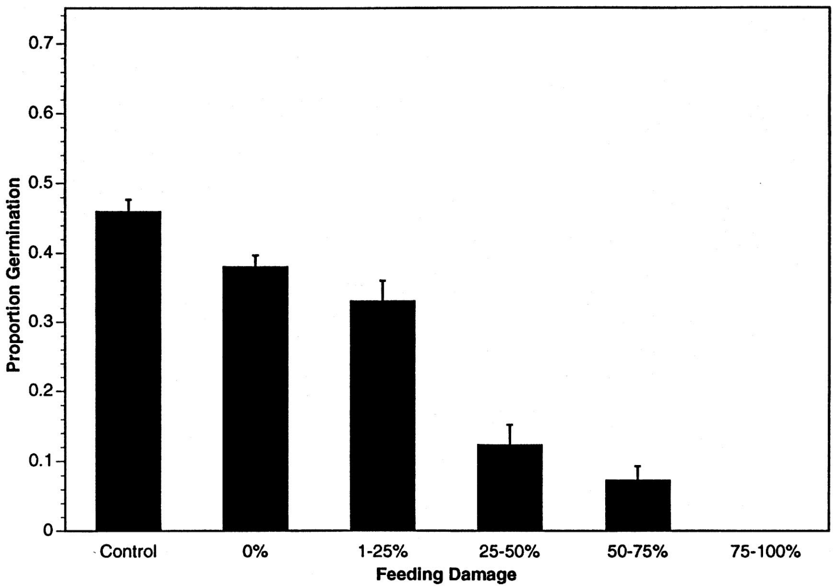

Fig. 3. The proportion of tubers germinating in each feeding damage category summed over the controls and all egg to tuber ratio treatments. There was a significant ($P < 0.01$) decrease in germination with an increase in feeding damage category.

they resulted in proportionally more adults being produced from the eggs than the 5:1 egg to tuber ratio. However, if *B. affinis* was used in an inundative release program where the objective was maximum tuber destruction, then the egg to tuber ratio for release should be 5:1 or greater. The higher egg to tuber ratio should be used because production of adult *B. affinis* would not be a priority.

The egg stage of *B. affinis* may not be the most convenient life stage for release in the field. Conversion of the number of eggs to the number of adults requires knowledge of the mean fecundity, the sex ratio of a population of weevils, and the percent egg eclosion. For *B. affinis* in the laboratory, the mean fecundity is 231.7 eggs per female (Bennett & Buckingham 1991), the sex ratio is approximately 1:1 (Bennett & Buckingham 1991), and approximately 90% of all eggs hatch (K. E. G., unpublished data). To achieve a 2:1 egg to tuber ratio at a site would require 1 weevil for every 52 tubers, assuming that the life history attributes for *B. affinis* given above are representative of those in the field. For the 5:1 egg to tuber ratio, 1 weevil would be required for every 21 tubers.

In hydrilla-infested aquatic sites in Florida and California, tuber densities ranged from 0-510 and 20-1,000 tubers per m^2, respectively (Bowes et al. 1979, Anderson & Dechoretz 1982, Sutton & Portier 1985). Reduction of the tuber banks in infested sites in Florida using *B. affinis* would have required the release of between 0.1 - 10 weevils per m^2 to achieve the 2:1 egg to tuber ratio, and between 0.1 - 25 weevils per m^2 for the 5:1 ratio. In California, between 1 - 20 weevils per m^2 would have to be released for the 2:1 ratio, and between 1 - 48 weevils per m^2 for the 5:1 ratio.

In practice, the number of weevils released should probably be greater than those given above because the weevils may not be as successful in the field as they are in the laboratory. In two other studies where *B. affinis* was released in the field, the percent of tubers attacked was not as great as that in the laboratory. In Florida, *B. affinis* was released at an egg to tuber ratio of about 1:5. In the tubers recovered from these sites, 0 - 16.6% had been fed upon (Buckingham et al. 1994). In California, *B. affinis* was released at an egg to tuber ratio of approximately 1.2:1, and 11.2% of the sentinel tubers (tubers that were placed in the field to monitor the success of a release) were fed upon (Godfrey et al. 1994). In this laboratory study, 37.2 and 52.5% of the tubers had been fed upon in the 1:5 and 1:1 ratios, respectively. The lower rate of larval attack in the field may have been due to a variety of factors such as soil temperature, soil texture, or movement by the adults before oviposition (Buckingham et al. 1994).

The ratios of weevils to tubers required for maximum tuber destruction determined in this study should be viewed as guidelines for release numbers. Many factors influence the ability of *B. affinis* to destroy tubers. However, the results of this study suggest that under favorable conditions, *B. affinis* has the ability to impact hydrilla tuber banks.

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