OVERWINTERING AND ESTABLISHMENT POTENTIAL OF *BAGOUS AFFINIS* (COLEOPTERA: CURCULIONIDAE) ON *HYDRILLA VERTICILLATA* (HYDROCHARITACEAE) IN NORTHERN CALIFORNIA

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ABSTRACT

Bagous affinis Hustache (Coleoptera: Curculionidae) has potential as a biological control agent for subterranean turions (also called tubers) of hydrilla (*Hydrilla verticillata* (L.f.) Royle; Hydrocharitaceae). The ability of *B. affinis* to overwinter and establish was investigated at 2 sites in northern California; a pond in Calaveras County and the Chowchilla River in Madera County. In cage studies conducted at the pond, *B. affinis* survived the winter for 2 seasons. After releases at the pond, *B. affinis* successfully reproduced and survived during the summer of 1992. Weevil larvae had damaged both sentinel and native tubers at the site. In the spring, following the release of *B. affinis* at the pond, a light trap and baited shelter traps were used to detect weevils that had overwintered naturally at the site. No weevils were recovered. At the Chowchilla River site, *B. affinis* failed to establish, possibly due to a lack of tubers in the section of the river where the releases were made.

Key Words: Aquatic weed control, biological control, field biology, noxious weeds.

RESUMEN

Bagous affinis Hustache (Coleoptera: Curculionidae) tiene potencial como agente de control biológico, al atacar los tallos subterraneos (tubérculos) de la Elodea de Florida (*Hydrilla verticillata* (L.f.) Royle; Hydrocharitaceae). La habilidad de *B. affinis* para invernar y establecerse fué investigada en dos sitios del norte de California: un estanque en el condado de Calaveras y el río Chowchilla en el condado de Madera. En estudios en jaulas, llevados a cabo en el estanque, *B. affinis* sobrevivió el invierno a lo largo de dos estaciones más. Después de las liberaciones en el estanque, *B. affinis* se reprodujo exitosamente y sobrevivió durante el verano de 1992. Las larvas del picudo dañaron tanto los tuberculos secundarios como los tuberculos primarios. En la primavera, luego de la liberación de *B. affinis,* se utilizó una trampa de luz y trampas con cebo para detectar los picudos que habían imvernado en el estanque. No se recuperaron picudos. En el río Chowchilla B. affinis no se estableció, posiblemente debido a la falta de tubérculos en la sección del río donde se efectuaron las liberaciones.

Hydrilla verticillata (L.f.) Royle (Hydrocharitaceae) has been a serious weed in ponds, lakes and waterways since its discovery in California in 1976 (California Department of Food and Agriculture 1991). As a part of a state-mandated eradication program designed to reduce the spread and density of *H. verticillata* (hydrilla), infested aquatic sites are quarantined, or closed to fishing and recreation. The hydrilla then is treated with herbicides, physically removed by dredging, and/or controlled by release of sterile triploid grass carp, *Ctenopharyngodon idella* (Cuvuer and Valenciennes; Cyprinidae). The fish have only been released in canals in the Imperial Irrigation District. These tactics are very effective against the above-ground portions of the plant, however, subterranean vegetative propagules, called subterranean turions or tubers, have a greater survival rate when using these tactics than the above-ground portions of the plant. Tubers are the primary cause of reinfestations. Currently, densities of hydrilla tubers are reduced by attrition, use of soil fumigants, or dredging. Chemical and physical methods are often disruptive to the aquatic environment. Thus, in an attempt to reduce disruption to wetland environments, biological control of hydrilla tubers was investigated.

In the 1970's and 1980's, a search was initiated for organisms that fed upon hydrilla tubers on the Indian subcontinent (Baloch et al. 1980). A weevil, *Bagous affinis* Hustache (Coleoptera: Curculionidae), was found feeding on hydrilla tubers in central Pakistan (Baloch et al. 1980). The weevil had infested 90-100% of the exposed hydrilla tubers as waters receded during the dry season. No regrowth of hydrilla occurred at this site in the following wet season (Baloch et al. 1980). *Bagous affinis* was imported into quarantine in the United States in the mid 1980's to study its biology and host plant range (Buckingham 1988). The life cycle in the laboratory was determined, and it was found that the weevil could not withstand submergence [not longer than 2.5 days (larvae), and not longer than 5 days (adults); Bennett and Buckingham 1991]. From studies of host plant range, *B. affinis* was found to feed and complete development only on hydrilla (Buckingham 1988).

B. affinis was imported into California in the summer of 1991 to determine if it could be used to reduce densities of hydrilla tubers biologically. The weevil seemed well suited to conditions in northern California because in some of the water systems infested with hydrilla, drawdown conditions exist naturally or may be created artificially. The suitability of *B. affinis* as a biological control agent for hydrilla tubers in California was investigated by examining its ability to overwinter and to establish after release.

Materials and Methods

All *B. affinis* used were the progeny of weevils collected outside Bangalore, India, in April 1991. The weevils were cultured in quarantine at the Florida Biological Control Laboratory, Gainesville, Florida, for 1 generation before shipment of the weevils to California. The weevils were maintained in laboratory culture at the USDA Aquatic Weed Control Research Laboratory, Davis, California, for 2-10 generations before use in the experiments. Voucher specimens were deposited at the Bohart Museum, Department of Entomology, University of California, Davis, California.

The ability of *B. affinis* to overwinter in northern California was determined for 2 consecutive fall-spring seasons with caged weevils placed along the bank of a hydrilla-infested pond near Mountain Ranch, California, in Calaveras County (approximate lat. 38°N, long. 120°W). In the first fall-spring season, the studies were initiated by placing 35 cages (28.5 cm diam x 35.5 cm height) at the pond on 23 September 1991. Each cage contained 10-15 cm of soil in which 50 hydrilla tubers had been buried at a depth of about 5 cm. Only dioecious hydrilla tubers were used in these experiments because dioecious hydrilla is the predominant type of hydrilla found in California. On the soil surface, approximately 20 hydrilla stems covered a 2-cm cube of water-saturated floral foam. The hydrilla stems served as a food source, and the floral foam, an oviposition substrate. [Floral foam was found to be an acceptable substrate for oviposition, and more convenient to work with in field studies than water-soaked wood, the natural oviposition substrate (Bennett & Buckingham 1991).] Ten *B. affinis* adults were placed in each cage. Five randomly selected cages were returned to the laboratory on the following dates: 15 October, 1 November, and 18 December 1991; 7 February, 3 March, 9 April, and 6 May 1992. On each sample date, fresh hydrilla stems were added to the cages remaining in the field. The contents of the retrieved cages were sorted by examining the hydrilla stems to detect *B. affinis* adults, and the soil was washed through a 5-mm sieve to recover tubers. The tubers were examined for evidence of feeding by *B. affinis* larvae and to recover any *B. affinis*. The number and life stage of any *B. affinis*, and the number and condition (i.e., fed upon or not fed upon) of the tubers were recorded.

In the second fall-spring season, the 35 cages were placed at the pond on 21 September 1992. The contents of each cage was similar to that in the first season, except that 25 tubers, and 5 *B. affinis* adults were placed in each cage. In addition, each cage contained two 20-ml vials filled with water and stoppered with sponges. Food and water were replenished every 2 weeks for the first 2 months. For the remainder of the study, fresh food and water were provided on the following sampling dates: 19 October, 16 November, and 14 December 1992; 19 January, 17 February, 15 March, and 19 April 1993. The procedures for sorting the contents of the cages were the same as those used in the first season. The trend in percent of tubers fed upon by *B. affinis* was regressed against time for both years using PROC REG (SAS Institute 1982).

During both seasons, the air and soil temperatures within the cages were monitored using thermistors connected to a Li-Cor LI-1000 Data Logger® (Lincoln, NE). Within the cage, the thermistor for the air temperature was positioned approximately 10 cm above the soil surface, and that for the soil, buried approximately 5 cm below the soil surface. The hourly mean air and soil temperatures were recorded.

The establishment potential of *B. affinis* was investigated at 2 sites in northern California. The first site was the pond in Calaveras County used for the overwintering studies. Releases of *B. affinis* eggs and adults were made in 1991 and 1992, respectively. The other site was along the Chowchilla River near Raymond, California, in Madera County (approximately lat. 37°N, long. 120°W) where adult weevils were released in 1991 only. For releases at the pond, the water level had to be drawn down, and this was done from 21-26 August 1991 and from 18-21 May 1992. Drawdown was necessary to insure access to tubers by *B. affinis* larvae. On 30 August 1991, the pre-release tuber density in the exposed pond bottom was estimated by taking three 15-cm diam core samples (sample depth = 10 cm) from each of 15 transects that were arranged in a spoke-like fashion around the pond (total number of samples = 45). Each transect ran from the edge of the water to the bank of the pond. The first core in each transect was taken next to the edge of the water, the second, 1.5 m from the edge of the water, and the third, 3 m from the edge of the water. On 8 September 1992, after all *B. affinis* releases had been completed, the post-release density of tubers was estimated as described above except that 25 transects were sampled (total number of samples = 75). The area of exposed pond bottom sampled for the post-release estimate was approximately the same as that for the pre-release estimate of tuber density. Soil cores were bagged, returned to the laboratory, and washed through a 5-mm sieve. The number and condition (i.e., fed upon or not fed upon) of the tubers were recorded. The mean pre- and post-release densities were compared using a t-test (Steel &Torrie 1960).

The first set of releases at the pond was made on 24 September 1991 and consisted of 3 releases of 50 *B. affinis* eggs each. The eggs were placed near the edge of the water at sites thought to have the greatest probability of having tubers. At each site, pieces of water-soaked wood containing eggs were placed on the surface of the soil, and covered with a 5-cm layer of moist sphagnum moss. The release site was covered with a wire-mesh cage (mesh size 2.5 x 3.5 cm; cage diam 75 cm, height 1.2 m) to exclude cattle that used the pond for water. On 15 October 1991, the soil around (diam of area removed = 40 cm) and under (depth = 10 cm) the release site was placed in a plastic bag and returned to the laboratory. The soil was washed through a 5-mm sieve to recover tubers, and the number and condition of any tubers recovered were recorded.

In the second set of releases, 500 *B. affinis* adults were released on the following dates: 21 May (200), 17 June (100), 15 July (100), and 11 August 1992 (100). On each release date, the weevils were divided among 4 randomly chosen sites near the edge of the water. At each release site, weevils were provided hydrilla stems, a 2-cm cube of water-saturated floral foam, and a source of free water. To monitor the success of each release, we buried a mesh bag (1 x 1 cm mesh) containing 25 tubers 5 cm below each release site. These tubers were used as "sentinel" tubers to monitor feeding activity by *B. affinis* larvae. The release site was covered with a screen made of Saran® screening (Lumite-Synthetic Industries, Gainesville, GA; 32 x 32 mesh) to keep birds and other small animals out of the release site. The sentinel tubers were recovered 25-30 days after release. The tubers were broken in half to determine the presence of *B. affinis*. The number and life stage of any *B. affinis* found, and the number and condition of the sentinel tubers were recorded. During the releases, the mean hourly ambient air temperature and soil temperature (5 cm below the soil surface) were monitored using thermistors connected to a Li-Cor LI-1000 Data Logger® (Lincoln, NE).

To determine if any *B. affinis* produced as a result of the releases in 1992 survived the winter, 2 types of traps, black light and baited shelter trap, were deployed around the pond during the spring of 1993. The black light (UV, 365 nm) trap, suspended by a frame 1.5 m above the soil surface on the bank of the pond, was used to attract emerging weevils in "flight mode" (i.e., energy resources directed toward the development of indirect flight muscles; Bennett & Buckingham 1991). The insects attracted to the light were collected in a jar (1 liter) containing a 1:1 mixture of ethylene glycol and water. The trap was operated for 3 consecutive days per week beginning 19 April and ending 26 May 1993. The contents of the jar were sorted under 10X magnification.

The baited shelter trap was used to attract weevils that were in “reproductive mode” (i.e., energy resources directed toward the reproductive system, not the indirect flight muscles; Bennett & Buckingham 1991). Each shelter trap consisted of a 1 liter container (diam = 12 cm; height = 12 cm) that contained soil and 25 tubers buried 5 cm below the soil surface. A 2-cm cube of water-saturated floral foam and a mesh bag (1 x 1 cm mesh) containing hydrilla stems were placed on the soil surface. Each trap was covered with a screen (2.5 x 3.5 cm mesh) to exclude other animals. Each week of light trapping, 5 baited shelter traps were buried in the soil around the light trap such that the top rim of the container was flush with the surrounding soil surface, much like a pitfall trap. The shelter traps were left in the field for 1 week. Upon return to the laboratory, the hydrilla stems from the tops of the traps were examined to recover any adult weevils. The soil within the trap was covered with moist sphagnum moss, and the traps were held for 30 days under standard rearing conditions [27^{o}C, 14:10 (L:D)] to allow any *B. affinis* immatures to develop to the adult stage. The contents of the traps were examined, and the number of *B. affinis* recovered was recorded.

The second *B. affinis* release site in California was along the Chowchilla River. This site was selected because the river goes through a natural low water period. Most of the river bed was exposed from mid summer through early fall. The density of hydrilla in the river was low because the river had been treated with herbicides for the past 5 years in an attempt to eradicate hydrilla. The release site was a small pool that had a few hydrilla plants and tubers. The density of hydrilla tubers within the pool could not be quantitatively estimated without destroying the site. *Bagous affinis* adults were released on 11 October (200) and 25 October 1991 (103). The site was left undisturbed until 12 November 1991 when the site was sampled for tubers. Standard tuber sampling (i.e., using a 15-cm diam core sampler) was attempted, but abandoned because the soil in the bottom of the pool was a very fine sand that did not hold together as the sampler was pulled up through the 60 cm of water covering the site. Instead, a shovel was used to remove soil from the bottom of the pool. The soil was washed through a 5-mm sieve to recover tubers. The number and condition of tubers recovered were recorded.

To determine if *B. affinis* had overwintered and established at the Chowchilla River site, we used black light trapping and tuber sampling. Black light trapping (as described above) was done continuously from 23 - 27 April and from 30 April - 4 May 1992. The ambient air temperature at the site was recorded during trapping with a recording thermometer (PTC Instruments Los Angeles, CA).

The river bed was sampled during the summer, the natural low water period. If *B. affinis* had established at this site, then feeding damage should have been evident on the tubers. Tubers were sampled by taking fifty 15-cm diam core samples (depth of sample = 10 cm) on 9 July and 21 August 1992 (total number of samples = 100) from a 30-m section of the river. The midpoint of this section of river was the release site. Transects traversed the river bed at 3 m intervals beginning at the release site and running up and down river. Five cores were taken from the release site and from each transect. The core samples were washed through a 5-mm sieve to recover tubers. The number and condition of the tubers recovered were recorded.

RESULTS

Bagous affinis survived the winter in cages at the pond in Calaveras County in 1991 - 1992 and in 1992 - 1993 (Table 1). In the first season, 2 adults were recovered on the 9 April 1992 sampling date (Table 1). These individuals were in all likelihood the F_1 progeny of the adults originally placed within the cages because the mean number of tubers fed upon and the percent of tubers fed upon increased with the length of time the cages were in the field (Table 1). Linear regression of the percent of tubers fed upon against time resulted in a significant positive regression ($F = 54.2$; df = 1,5; $P < 0.05$; $R^2 = 0.92$). Much of this feeding damage was characteristic of that produced by *B. affinis* larvae. This trend in feeding activity suggested that *B. affinis* was successfully reproducing within the cages. In addition, in the laboratory under optimal conditions, *B. affinis* adults survive a mean of 127.5 days (range 55 - 225 days; Bennett &Buckingham 1991). The 9 April 1992 sampling date was 231 days after the cages were placed in the field.

In the second season, 4 adults were recovered, 3 from the samples taken on 17 February, and 1 from the 15 March 1993 sampling date (Table 1). These individuals also were in all likelihood the progeny of adults originally placed within the cages because as in the first season, the percent of tubers that had been fed upon increased with the length of time the cages were in the field (Table 1). Linear regression of the percent of tubers fed upon against time resulted in a significant positive regression ($F = 40.3$; df = 1,5; $P < 0.05$; $R^2 = 0.89$). In addition, the 17 February and the 15 March sampling dates are 150 and 177 days after the initial placement of the cages in the field, respectively. The 3 adults recovered from the 17 February sampling date began ovipositing about 7 days after being brought into the laboratory and produced 44 progeny when held under rearing conditions. These results show that *B. affinis* can successfully reproduce after overwintering in northern California.

The mean number of tubers recovered in both seasons decreased with the length of time the cages were in the field (Table 1). This decrease was due to the degradation of tubers either naturally or after having been fed upon by *B. affinis* larvae. Van & Steward (1990) found that some tubers will degrade naturally through time. The reason for this degradation is not known. In the degradation of tubers through the feeding activity of *B. affinis*, the larvae provide an opening for other organisms to enter and attack the tuber. In some cases, entire tubers were destroyed, whereas in others, whole tubers and pieces of tubers were recovered.

TABLE 1. TOTAL NUMBER OF *B. AFFINIS* RECOVERED, THE MEAN NUMBER (STD. DEV.) OF TUBERS RECOVERED, THE MEAN NUMBER (STD. DEV.) OF TUBERS FED UPON BY *B. AFFINIS* LARVAE, AND THE PERCENT OF TUBERS FED UPON FROM THE OVERWINTERING STUDIES CONDUCTED AT THE POND IN CALAVERAS COUNTY IN 1991-1992 AND 1992-1993. THE MAXIMUM NUMBER OF TUBERS RECOVERABLE WAS 50 IN 1991-1992 AND 25 IN 1992-1993.

Date	No. of *B. affinis* Recovered[1]	Mean No. of Tubers Recovered	Mean No. of Tubers Fed Upon	Percent of Tubers Fed Upon
		1991 - 1992		
15 October	2A	48.2	3.2	6.6
		(1.64)	(2.17)	
1 November	1A	37.8	4.2	11.1
		(7.69)	(2.68)	
18 December	1A	32.0	8.6	26.9
		(15.87)	(2.19)	
7 February	0	26.8	7.4	27.6

TABLE 1.(CONTINUED) TOTAL NUMBER OF *B. AFFINIS* RECOVERED, THE MEAN NUMBER (STD. DEV.) OF TUBERS RECOVERED, THE MEAN NUMBER (STD. DEV.) OF TUBERS FED UPON BY *B. AFFINIS* LARVAE, AND THE PERCENT OF TUBERS FED UPON FROM THE OVERWINTERING STUDIES CONDUCTED AT THE POND IN CALAVERAS COUNTY IN 1991-1992 AND 1992-1993. THE MAXIMUM NUMBER OF TUBERS RECOVERABLE WAS 50 IN 1991-1992 AND 25 I N 1992-1993.

Date	No. of *B. affinis* Recovered[1]	Mean No. of Tubers Recovered	Mean No. of Tubers Fed Upon	Percent of Tubers Fed Upon
		(10.33)	(3.29)	
3 March	0	23.8	7.2	30.3
		(13.61)	(4.92)	
9 April	2A	17.8	9.4	52.8
		(5.79)	(7.92)	
6 May	0	28.4	14.4	50.7
		(12.36)	(6.31)	
		1992 - 1993		
21 October	2L	18.8	10.4	55.3
		(4.21)	(3.21)	
16 November	1L	16.4	9.8	59.8
		(4.51)	(2.86)	
14 December	1A	9.4	6.6	70.2
		(3.05)	(2.97)	
19 January	0	10.4	8.2	78.9
		(5.37)	(4.15)	
17 February	3A	4.6	4.4	95.7
		(2.61)	(2.51)	
15 March	1A	3.8	3.6	94.7
		(2.39)	(2.30)	
19 April	0	2.6	2.4	92.3
		(4.77)	(4.34)	

[1]L = larva, A = Adult.

The soil and air temperatures within the cages were similar for both fall-spring seasons. At no time during these time periods did the soil temperatures within the cage drop below 1^{o}C or rise above 29^{o}C. The air temperatures within the cages were more variable. The lowest air temperatures ranged from -8.5 to -13.2^{o}C in mid-winter, and the highest air temperatures ranged from 43.6 to 45.3^{o}C in the fall.

Releases of *B. affinis* at the pond resulted in successful reproduction and survival by *B. affinis* in the summer of 1992. For the first set of releases in which *B. affinis* eggs were released, establishment of the weevil could not be monitored because no tubers were found at the release sites. For the second set of releases, however, sentinel tubers were used to monitor establishment. From the weevils released on 21 May 1992, 5 pupae and 2 adults were recovered from sentinel tubers (Table 2). For the other release dates, no weevils were recovered from the sentinel tubers, however, some tubers had been fed upon by *B.affinis* larvae (Table 2). The air and soil (5 cm depth) temperatures during the releases were in a range suitable for *B. affinis* reproduction and development.

To further investigate the establishment of *B. affinis* at this site, the density of native tubers was estimated before and after releases of *B. affinis*. Before the release of any *B. affinis*, the native tuber density within the pond was estimated as 0.82 tubers per 15-cm core (Table 3). After all releases were complete, the native tuber density within the pond was estimated as 0.12 tubers per 15-cm core (Table 3). This apparent decrease in native tuber density was not statistically significant ($P > 0.05$). However, 22% of the native tubers recovered had feeding damage that resembled that imparted by *B. affinis* larvae. We assume that this feeding damage had been caused by *B. affinis* because no native organisms are known to feed inside hydrilla tubers in the United States (Balciunas & Minno 1985).

TABLE 2. THE MEAN NUMBER (STD. DEV.) OF SENTINEL TUBERS RECOVERED, THE MEAN NUMBER (STD. DEV.) OF SENTINEL TUBERS FED UPON, AND THE TOTAL NUMBER OF *B. AFFINIS* RECOVERED FROM SENTINEL TUBERS FROM RELEASES OF *B. AFFINIS* ADULTS MADE AT A POND IN CALAVERAS COUNTY IN 1992.

Sampling Date	Mean No. of Sentinel Tubers Recovered	Mean No. of Sentinel Tubers Fed Upon	Total B. affinis Recovered[1]
21 May	23.0	6.0	5P,2A
	(0.82)	(2.71)	
17 June	24.33[2]	1.33	0
	(1.15)	(0.58)	
15 July	24.5	2.75	0
	(0.58)	(1.26)	
11 August	25.0	0.75	0
	(0.82)	(0.96)	

[1]P = pupa, A = adult.
[2]One release site destroyed by cattle. Maximum total recoverable sentinel tubers = 75.

Light and baited shelter traps operated in the spring of 1993 did not capture any overwintering progeny from *B. affinis* released at the pond in the summer of 1992. However, 6 of the 125 tubers (4.8%) examined in the last set of baited shelter traps (placed in the field 24 May 1993), had damage resembling that caused by *B. affinis* larvae.

Releases of *B. affinis* made along the Chowchilla River did not result in establishment of the weevil. Six tubers were recovered from samples collected approximately 30 days after release. Of these tubers, 2 had damage characteristic of *B.affinis* larval feeding, suggesting that the weevils initially released at this site were successful in reproducing. In subsequent monitoring of the site, no weevils were captured by the light trap, and no tubers (i.e., tuber density = 0 tubers per 15 cm-core) were recovered in the tuber sampling of the river. The absence of hydrilla tubers in this section of the river was due to hydrilla plant removal and treatment of the river with herbicides in previous years. Given the lack of tubers in this section of the river, it seems unlikely that *B. affinis* could have survived without food resources even if it could overwinter at the site.

TABLE 3. The mean number (std. dev.) of hydrilla tubers recovered in 15-cm core samples from the exposed pond bottom in Calaveras County.

	Transects			
Sampling Date	Water's Edge	1.5 m from Water's Edge	3 m from Water's Edge	Overall Mean No. of Tubers
30 August 1991	0.13	0.40	1.93	0.82
	(0.35)	(0.83)	(2.60)	(10.67)
8 September 1992	0.12	0.16	0.08	0.12
	(0.44)	(0.47)	(0.28)	(3.52)

Discussion

Bagous affinis demonstrated an ability to adapt to the climate in northern California. Adult weevils were able to survive the winter within cages despite soil temperatures dropping to near 1°C and air temperatures approaching -14°C. These cold temperatures did not appear to reduce the reproductive success of the weevils because weevils recovered from overwintering cages produced progeny in numbers equivalent to those weevils in the colony when placed under normal rearing conditions.

The ability of the weevils to survive the winter within the cages suggested that the weevils may also be able to overwinter naturally. However, no naturally-overwintering weevils were trapped at either release site in light traps. This is not surprising considering that the greatest attraction to a light trap by a particular species of insect occurs within a narrow range of environmental conditions. For *B. affinis*, the environmental conditions for maximum attraction to a light trap include temperatures at sunset at or above 20°C and little or no wind (Buckingham et al. 1994). For both release sites, the temperature at sunset was at or above 20°C for about half of the trapping days; however, on all trapping days light to moderate winds prevailed. In addition, for *B. affinis* to be attracted to the light trap, the weevils would have to be in "flight mode", not "reproductive mode". The density of weevils that may have overwintered naturally also would not have been large, thereby making it difficult to detect weevils using light traps.

The baited shelter traps should have been more attractive to any naturally-overwintering *B. affinis* that were in reproductive mode than the light traps. No weevils were recovered from the traps, but a small amount of feeding damage was detected in the tubers from the traps. This feeding damage suggests that the density of overwintering weevils was probably very low.

Given its demonstrated ability to overwinter, it is not surprising that the weevil successfully reproduced using native tubers at 1 of the 2 release sites. Its failure to establish at the Chowchilla River site was probably due to the lack of tubers in the part of the river where it was released. In order for *B. affinis* to establish, it must have a food resource. At the pond in Calaveras County, the weevil reproduced successfully and survived through the summer of 1992. Evidence of its reproduction and survival was found in both sentinel and native tubers. In the sentinel tubers, *B. affinis* immatures were recovered after the first release, and tubers damaged by *B. affinis* were recovered in subsequent releases (Table 2). The lack of recovery of *B. affinis* in the later releases can be explained by the developmental rate of *B. affinis*. For the first set of releases, the soil temperature at 5 cm (the depth of the sentinel tubers) was near 21°C. At 21°C, the development of *B. affinis* from larva to adult requires approximately 30 days (K.E.G., unpublished data). Given a short amount of time for oviposition and egg hatch, it was expected that the sentinel tubers recovered about 30 days after release would contain *B. affinis* immatures. For the remaining releases, the soil temperature at 5 cm increased to 25 - 28°C. The development of *B. affinis* from larva to adult requires approximately 20 and 12 days at 25 and 28°C, respectively (K.E.G., unpublished data). Again, given a short period of time for oviposition and egg hatch, it was expected that *B. affinis* could have completed development leaving only damaged tubers.

Of the native tubers recovered from the pond after all *B. affinis* releases were complete, 22% had been fed upon by *B. affinis*. This level of tuber damage was within the range of tuber damage found by Buckingham et al. (1994) at a lake in Florida where *B. affinis* had successfully survived and reproduced for 3 months. They found from 0.57 - 32% of the tubers recovered from the lake had been fed upon by *B. affinis*. In addition, they found degradation of the tubers after *B. affinis* feeding as we did in this study.

Bagous affinis has potential as a biological control agent for hydrilla tubers in California. This weevil could be used as one element in the long-term management strategy to reduce numbers of hydrilla tubers. To do this, *B. affinis* should be released at sites that undergo annual drawdowns and have tuber densities sufficient to sustain populations of the weevil from year to year. In addition, *B. affinis* could also be used in more intensive, short-term management of hydrilla tubers through inundative releases at sites where more rapid reductions of tuber numbers are required.

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