COMPARISON OF *IN VIVO* VERSUS *IN VITRO*-REARED *CATOLACCUS GRANDIS* IN THE FIELD

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Abstract

The movement, searching capacity, and survival under field conditions of *in vitro* and *in vivo* reared *Catolaccus grandis* (Burks) females were compared in Ricardo and Lyford, TX. Dispersal ability and searching capacity was not significantly different within the 30-m radius among parasitoid females reared by the two different methods. However, a significantly higher proportion of stations with parasitism was recorded from *in vivo*- reared *C. grandis* at a 60 m radius. These results indicate that *in vitro*-reared *C. grandis* have a significantly lower dispersal ability, but, their searching capacity is not significantly affected.

Introduction

The potential of the exotic ectoparasitoid *Catolaccus grandis* (Burks) (Hymenoptera: Pteromalidae) as a biological control agent of the boll weevil (*Anthonomus grandis grandis* Boheman) has been studied during the past five years (Morales- Ramos & King 1991, Morales-Ramos & Cate 1992a, 1992b, 1992c, Summy et al. 1992, 1994, 1995, Morales-Ramos et al. 1994, 1995a). Inundative releases of this parasitoid have effectively suppressed boll weevil populations in experimental fields in the Lower Rio Grande Valley (King et al. 1995, Summy et al. 1994, 1995)

Despite the success of experimental augmentative releases of *C. grandis* in controlling boll weevil populations, commercial application of this technology is greatly limited by the high costs of mass propagating this parasitoid. King and Morrison (1984) identified mass propagation as the main constraint in commercialization of augmentative releases of natural enemies. The development of artificial diets for *in vitro*-rearing of natural enemies is considered essential to the commercial application of biological control by augmentation of natural enemies (King & Morrison 1984, King 1993).

Females of *C. grandis* reared on an artificial diet (*in vitro*) developed by Rojas et al. (in press) showed biological attributes comparable to those reared on boll weevil larvae (*in vivo*) (Rojas et al. 1995). Preliminary releases of *in vitro*-reared *C. grandis* in the Rio Grande Valley successfully produced between 39.4 to 55.6% parasitism of boll weevil third instars and pupae (Morales-Ramos et al. 1995b). However, field performance of *in vitro* versus *in vivo*-reared *C. grandis* has not been compared previously. The objectives of this study were to compare mobility, searching ability, and survival in the field of *in vitro* and *in vivo*-reared *C. grandis.*

Materials and Methods

A 34.4-ha (85-acre) cotton field located at Ricardo, Texas was chosen as the experimental field to compare the *in vitro* and *in vivo*-reared *C. grandis*. This field was planted with Stoneville 132 cotton. An additional 48.6-ha (120-acre) field planted to Delta Pine 50 cotton located in Lyford, Texas was chosen as an additional experimental site. However, this field was located within a boll weevil eradication zone and the potential use of insecticide to control the boll weevil was recognized but not anticipated due to a field history of extremely low boll weevil populations.

Parasitoid Rearing

The parasitoids were reared *in vitro* using the Gamma diet developed by Rojas et al. (in press). The diet was dispensed in plastic disposable 128-well bioassay trays (Bio-Ba-128, C-D International, Pitman, NJ). One single *C. grandis* egg was deposited manually (with the aid of a fine brush) in each well. The parasitoid eggs were obtained by stimulating the parasitoid female to oviposit into a petri dish covered with Parafilm$^{\Gamma}$. Approximately 4 ml of the diet were placed into each sterile plastic disposable petri dish. The Parafilm$^{\Gamma}$ cover was coated with a mixture of macerated weevil larvae and the liquid portion of the parasitoid diet. Then the mixture was allowed to dry. These petri dishes were exposed to a colony of 120 females of *C. grandis* over a 4-h period. The parasitoid eggs were collected from the inner wall of the Parafilm$^{\Gamma}$ cover, where the female parasitoids had oviposited them. Once placed in the diet filled bioassay wells, the parasitoid eggs were allowed to develop to the pupal stage at constant 27^{o}C. The parasitoid pupae were then collected and placed inside an emergence cage until they completed development.

The method of boll weevil encapsulation in Parafilm$^{\Gamma}$ (Cate 1987) was used to rear *C. grandis in vivo*. This method has been described in many publications related to *C. grandis* (Morales-Ramos et al. 1992, 1994, 1995a, Summy et al. 1992, 1995).

Release

Parasitoid females were held in the laboratory for 5 to 7 days after emergence before release in the experimental plots. During this time the parasitoid females were exposed to encapsulated boll weevils for 2 to 4 days to stimulate egg production (training).

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The parasitoid females were subsequently aspirated into 1-l paper canisters filled with shredded paper. Seven canisters, each containing 200 females, were prepared weekly. The canisters were taken to the field the day after collection and opened at a previously marked release point.

Experimental Design

An experimental unit consisted of a central release point and 28 field stations. The field stations were distributed around the release point at 3 different distances (Fig. 1). Stations 1 to 4 were located at 15 m from the release point in directions north, east, south, and west respectively. Stations 5 to 12 were located at 30 m from the release point in directions north, northeast, east, southeast, south, southwest, west, and northwest, respectively. Stations 13 to 28 were distributed around a circle of 60 m radius from the release point. Stations 13, 17, 21, and 25 were positioned to the north, east, south, and west respectively (Fig. 1).

Sheets containing 3 encapsulated boll weevil larvae were placed at each of the 28 field stations. The encapsulated weevils were affixed to wooden stakes which were positioned next to each marking flag, with the encapsulated weevils facing east. The encapsulated weevils were collected and replaced daily for a period of 5 to 6 days. The Parafilm capsules were opened in the laboratory to count the *C. grandis* eggs present in each sample.

The surface area of each experimental unit measured 1.13 hectare (2.79 acre). Five experimental units were positioned within the cotton field (Fig. 2). The minimum distance between experimental units (from the outer radius) was 170 m. This distance ensured that most of the parasitism detected within an experimental unit was due to the parasitoids released in the center of that unit and not from parasitoids released in other experimental units (Coleman et al. 1995).

A release of 1400 *C. grandis* females was made at the center of each experimental unit. This release density was equivalent to 500 females/acre within each experimental unit. Each experiment consisted of 4 treatments and a control (one for each experimental unit). The treatments were: 1) *in vitro*-reared and trained females, 2) *in vivo*-reared and trained females, 3) *in vivo*-reared females without training, 4) *in vivo*-reared newly emerged females, and 5) control without release. The position of each treatment within the field was randomly assigned for each experimental run. Only results from treatments 1 and 2 will be presented in this paper. The results of the comparison between the other treatments is presented by Coleman et al. (these proceedings).

Three experiments were completed at Ricardo, Texas: from May 24 to 30, from June 7 to 13, and from June 20 to 26. Three attempts were made at the experimental field at Lyford, Texas on May 2, May 9 and May 31, but only the last was successful due to mortality of parasitoids from insecticidal residues during the first two attempts. The last experiment at Ricardo consisted of a different set of treatments: 1) *in vitro* reared 5 to 7-d old, 2) *in vivo* reared 5 to 7-d old, 3) *in vitro* reared 8 to 10-d old, 4) *in vivo* reared 8 to 10-d old and 5) control.

In addition to the field stations, boll weevil cohorts were placed in the field during two of the experiments at Ricardo. The cohorts consisted of 10 cotton squares infested by third instar boll weevils attached to 1-m strings. Four cohorts were placed in each treatment unit in four directions (North, East, South, and West) from the release point. The cohorts were placed at a distance of 22 and 45 m from the release point on June 7 and June 22, respectively. The cohorts were exposed to field conditions and parasitoids for 2 d. Then the cohorts were recovered and taken to the laboratory for dissection. The number of parasitized boll weevils was quantified and recorded.

Data Analysis

The data were processed to obtain the proportion of stations with parasitism within the 30 m (stations 1 to 12) and the 60 m (stations 13 to 28) radius of each treatment during each of the 6 days after release. The proportion of sites with parasitism for each of the treatments was compared by the *Z*-Test at $\alpha = 0.05$. The *Z* statistic was calculated by the formula:

$$Z = \frac{\pi_1 - \pi_2}{\sigma_{\pi 1 - \pi 2}} \quad \text{for } H_o: \pi_1 - \pi_2 = 0$$

$$\text{where} \quad \sigma_{\pi 1 - \pi 2} = \left[\pi(1 - \pi) \left[\frac{1}{n_1} + \frac{1}{n_2} \right] \right]^{1/2},$$

π_1 and π_2 are the observed proportion of successes of treatment 1 and 2 respectively; π is the proportion of successes common to both treatments; and n_1 and n_2 are the sample sizes of treatments 1 and 2, respectively (Ott 1984).

The *Z*-test was also used to statistically compare the proportion of boll weevils parasitized by *C. grandis* in the cohorts exposed June 7 to 9 and June 20 to 22 at Ricardo. The proportion of parasitized weevils was calculated as number of parasitized weevils divided by the total number of alive and parasitized weevils.

Results and Discussion

In all experiments and locations, both treatments (*in vitro* and *in vivo*-reared) showed a significantly higher proportion of stations with parasitism than the controls. In most experiments, the control units did not show any parasitism at all; however, some parasitoid movement into the control unit was observed in the experiment of May 24-30 at Ricardo, Texas.

During the first experiment at Ricardo (May 24 to 30) *C. grandis* females reared *in vitro* parasitized a significantly higher proportion of sites than females reared *in vivo* within the 30-m radius ($|Z| = 5.05$, $\alpha = 0.05$, $n = 120$). However, no significant differences were observed among the two treatments at the 60-m radius ($|Z| = 1.39$, $\alpha = 0.05$, $n = 132$) (Table 1). The differences observed in proportion of sites with parasitism varied greatly during each of the days after release (Figs. 3 and 4 A).

During the second experiment at Ricardo (From June 7 to 13), *C. grandis* females reared *in vivo* parasitized a significantly higher proportion of sites than females reared *in vitro* within the 30-m radius ($|Z| = 2.75$, $\alpha = 0.05$, $n = 141$) and around the 60-m radius ($|Z| = 3.58$, $\alpha = 0.05$, $n = 145$) (Table 1). When individual dates were analyzed, the differences in proportion of sites parasitized were not significantly different within the 30-m radius for most dates (Figs. 3 and 4 B).

The apparent contradictory results obtained during the first 2 experiments at Ricardo can be explained by the different environmental conditions observed during these two experiments. In the second experiment, the temperatures were considerably higher during the first 4 days after release. This may be an indication that *in vitro*-reared *C. grandis* are less tolerant to high temperatures than the *in vivo*-reared parasitoids.

The third experiment at Ricardo (June 20 to 26), showed no significant differences in proportion of sites parasitized among 8 to 10-d old *C. grandis* females reared by the two methods ($|Z| = 0.22$, $\alpha = 0.05$, $n = 131$) and ($|Z| = 0.448$ $\alpha = 0.05$, $n = 143$) within the 30 and around the-60 m radius respectively (Table 1). A significant difference in proportion of sites parasitized was observed among 5 to 7-d old females within the 30-m radius ($|Z| = 1.99$, $\alpha = 0.05$, $n = 133$), but not around the 60-m radius ($|Z| = 1.25$, $\alpha = 0.05$, $n = 146$) (Table 1). In this case, *in vivo*-reared females of younger age seemed to perform better than their *in vitro*-reared counterparts. No significant differences were observed during each of the days after release (Figs. 3 and 4 C and D).

The only complete experiment at Lyford, Texas (May 31 to June 5) showed no significant differences in proportion of sites parasitized by *C. grandis* among the two rearing methods within the 30-m radius ($|Z| = 1.06$, $\alpha = 0.05$, $n = 98$). However, *in vivo*- reared *C. grandis* parasitized a significantly higher proportion of sites around the 60-m radius ($|Z| = 3.08$, $\alpha = 0.05$, $n = 141$) (Table 1). Individual dates after release did not show significant differences except for day 6 around the 60-m radius (Figs. 3 and 4 E). Low level residual toxicity to malathion may have been responsible for the low survival rate observed at this location in all treatments. This experiment may also be an indication that *in vitro*- reared *C. grandis* are more susceptible to insecticides.

The analysis of all pooled data showed no significant differences in proportion of sites parasitized within the 30-m radius ($|Z| = 0.05$, $\alpha = 0.05$, $n = 623$). However *in vivo*-reared *C. grandis* showed a significantly higher proportion of sites parasitized in the overall analysis around the 60-m radius ($|Z| = 3.96$, $\alpha = 0.05$, $n = 707$) (Table 1). When the data is analyzed by individual days after the release, the only significant differences observed occurred during the first day after release around the 60-m radius (Fig. 5). This is a strong indication that *in vitro*-reared *C. grandis* exhibited a significant reduction in ability to disperse from the release point when compared to *in vivo*-reared parasitoids. However, these data show that the searching capacity of *in vitro*-reared parasitoids is similar to that of *in vivo*-reared *C. grandis*.

The cohort studies revealed no significant difference in mortality induced by parasitism among *C. grandis* females reared by the two methods: (Table 2). ($|Z| = 1.03$, $\alpha = 0.05$, $n = 80$) for the cohorts of June 7-9, ($|Z| = 0.90$, $\alpha = 0.05$, $n = 69$), and ($|Z| = 0.44$, $\alpha = 0.05$, $n = 76$) for the cohorts of June 20-22 younger and older ages respectively. Cohorts of the control units were not parasitized by *C. grandis*. These results indicate that the ability of *C. grandis* to inflict mortality to boll weevil populations is not affected when this parasitoid is reared *in vitro*.

In summary, the searching capacity and the potential to induce boll weevil mortality are not reduced when *C. grandis* is reared *in vitro*. However, *in vitro* reared *C. grandis* had a significantly lower ability to disperse from the release point. Based on results presented in this paper, we conclude that *in vitro*-reared *C. grandis* can be as effective a biological control agent of the boll weevil as those conventionally reared on the natural host. However, it may be necessary to increase the number of release points (with the same release densities recommended for *in vivo*-reared parasitoids) of *in vitro*-reared *C. grandis* to reduce the distance that the parasitoids most disperse to find their hosts.

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Table 1. Differences in proportion of stations showing parasitism by *Catolaccus grandis* reared *in vivo* and *in vitro* within a 30-m radius from the release point.

Release		*In Vitro*		*In Vivo*		
Date	Location	n	P	n	P	Z^a
Within a 30-m Radius						
May 24	Ricardo	60	0.83	60	0.38	5.05*
June 7	Ricardo	69	0.12	72	0.31	-2.75*
June 20[b]	Ricardo	68	0.29	65	0.4	-1.99*
June 20[c]	Ricardo	64	0.42	67	0.40	0.22
May 31	Lyford	50	0.20	48	0.29	-1.06
ALL	Both	311	0.37	312	0.37	-0.05
Around a 60-m Radius						
May 24	Ricardo	64	0.44	68	0.56	-1.39
June 7	Ricardo	84	0.07	61	0.30	-3.58*
June 20[b]	Ricardo	78	0.15	68	0.24	-1.25
June 20[c]	Ricardo	65	0.37	78	0.33	0.45
May 31	Lyford	70	0.10	71	0.31	-3.08*
ALL	Both	361	0.21	346	0.35	-3.96*

Were n is the number of sites, P is the proportion of sites showing parasitism by *C. grandis*.
[a]The value of $Z_{\alpha/2}$ at $\alpha = 0.05$ is 1.96; values of *Z* with the symbol '*' show significant differences.
[b]5 to 7 d old parasitoid females.
[c]8 to 10 d old parasitoid females.

Table 2. Proportion of mortality induced by *Catolaccus grandis* parasitism to cohorts of third instar boll weevils exposed for 2 days to field releases of this parasitoid at Ricardo, Texas.

	In Vitro		*In Vivo*		
Dates	n	P	n	P	Z^a
May 7 to 9[b]	40	0.93	40	0.98	-1.03
May 20 to 22[c]	35	0.57	34	0.61	-0.90
May 20 to 22[d]	39	0.56	37	0.51	0.44

Were n is the number of third instar boll weevils, P is the proportion of weevils parasitized by *C. grandis*.
[a]The value of $Z_{\alpha/2}$ at $\alpha = 0.05$ is 1.96; values of *Z* with the symbol '*' show significant differences.
[b]Cohorts placed 22 m away from the release point.
[c]Cohorts placed 45 m away from the release point of 5 to 7 d old parasitoid females.
[d]Cohorts placed 45 m away from the release point of 8 to 10 d old parasitoid females.

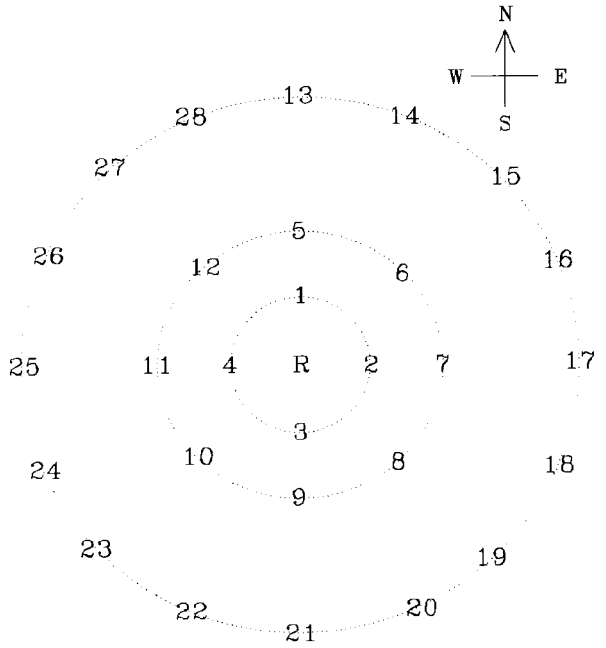

Figure 1. Distribution of sample stations within an experimental unit. 'R' is the release point.

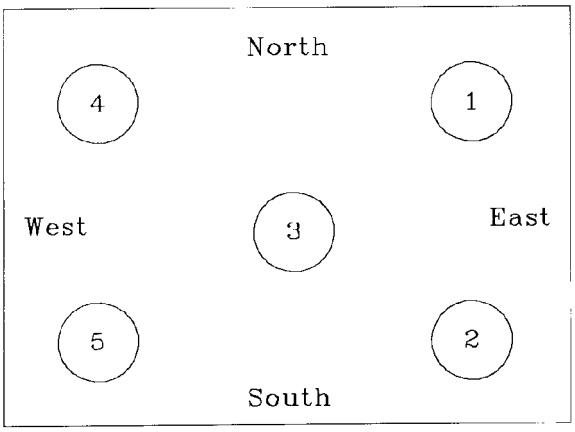

Figure 2. Distribution of the experimental units within the experimental field. Each circle represents an experimental unit as shown in Fig. 1.

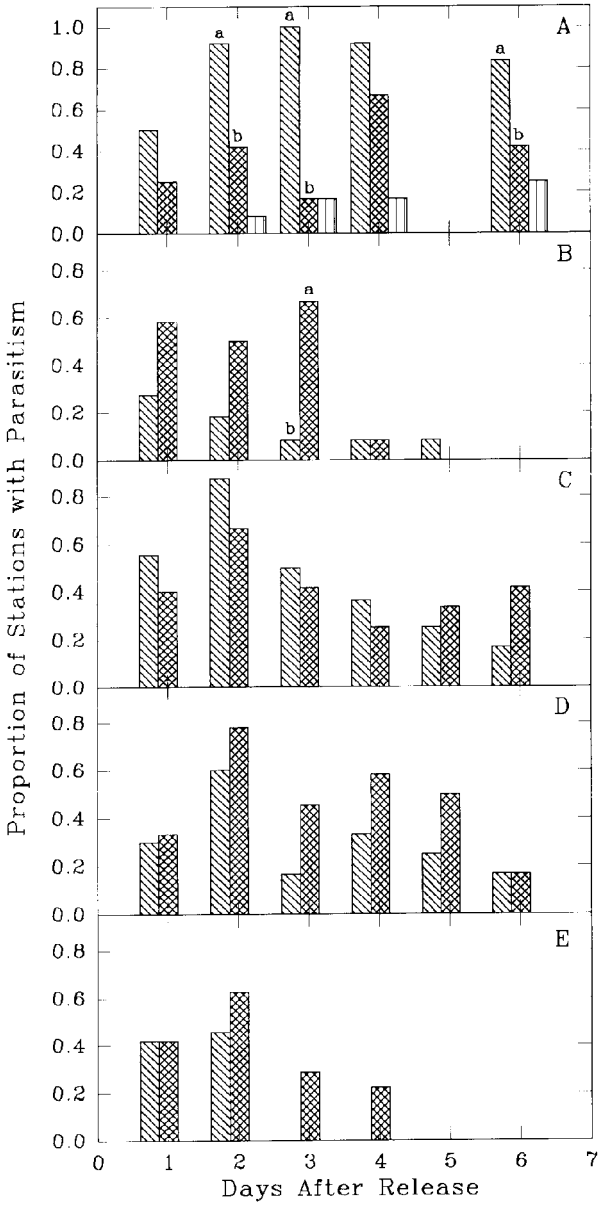

Figure 3. Proportion of sites showing parasitism by *in vitro* and *in vivo*–reared *Catolaccus grandis* and in the control within a 30 m radius from the point of release. A) Ricardo, TX May 24; B) Ricardo June 7; C) Ricardo June 20, 8–10 d old females; D) Ricardo June 20, 5–7 d old females; E) Lyford May 31. Letters appearing on the bars indicate significant differences between treatments.

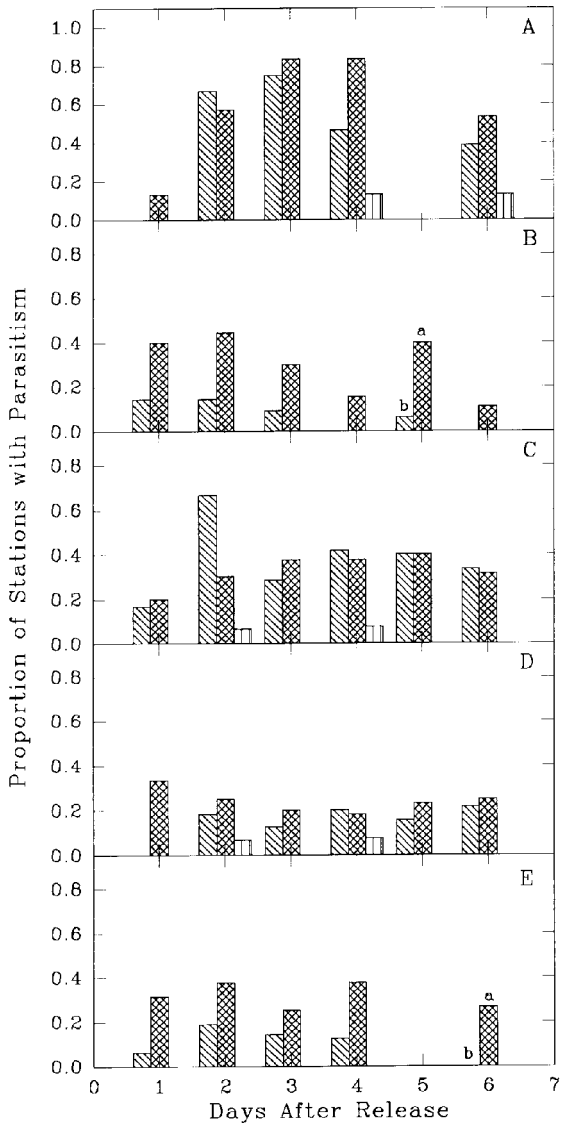

Figure 4. Proportion of sites showing parasitism by *in vitro* and *in vivo*–reared *Catolaccus grandis* and in the control around a 60 m radius from the point of release. A) Ricardo, TX May 24; B) Ricardo June 7; C) Ricardo June 20, 8–10 d old females; D) Ricardo June 20, 5–7 d old females; E) Lyford May 31. Letters appearing on the bars indicate significant differences between treatments.

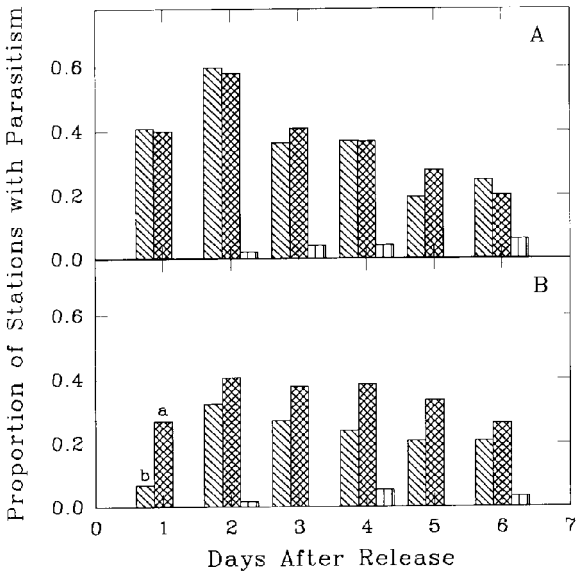

Figure 5. Summary of the proportion of sites showing parasitism of boll weevil larvae by ▨ *in vitro* and ▨ *in vivo*–reared *Catolaccus grandis* and ▥ in the control. A) within a 30 m radius and B) around a 60 m radius. Letters appearing on the bars indicate significant differences between treatments.