

# Ecological Studies of *Neochetina bruchi* and *N. eichhorniae* on Waterhyacinth in Argentina<sup>1,2</sup>

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## ABSTRACT

Both *Neochetina bruchi* Hustache and *N. eichhorniae* Warner had three generations a year near Buenos Aires, Argentina. Peak populations of adults occurred in September, January, and April to May. Both species overwintered as adults, larvae, and pupae. The maximum rate of oviposition occurred in October and November, and the rate declined thereafter through the season. *Neochetina bruchi* was more abundant in spring and summer and *N. eichhorniae* in fall and winter. The two species oviposited and rested on different parts of the plant. The weevils damaged waterhyacinth [*Eichhornia crassipes* (Mart.) Solms.] throughout the year, but maximum damage was done during the summer, when an average of 130 feeding spots per leaf were made by the adults and 30% of the petioles were damaged by tunneling of the larvae. The two species may be able to co-exist because of a shift in the abundance of their preferred ovipositional sites, caused by the seasonal development of the plants. The two species would probably complement each other if introduced into another country for biological control of waterhyacinth.

## INTRODUCTION

In Argentina, waterhyacinth occurs in slow moving streams, canals, lakes, and lagoons in the humid region from the northern border of the country south to the delta of the Río Paraná at Buenos Aires. The plant usually grows only a few meters out from the shoreline though it is occasionally abundant enough to block small waterways. The lush growth that completely covers bodies of water in the southeastern United States does not usually occur in Argentina because of the combined attack of its natural enemies. Severe damage is done by several species of insects that feed on the leaves or bore in the petioles and crowns, and by mites and snails.

The two species of weevils, *Neochetina bruchi* and *N. eichhorniae* are among the four or five most promising organisms for introduction into the United States to control waterhyacinth (1, 2). The two species are very similar in appearance, but *N. bruchi* is slightly larger and lighter brown than *N. eichhorniae* and often has a tan chevron across the elytra; DeLoach (3) and Warner (11) give descriptions and keys for their identification. Perkins (7, 10) made earlier observations on the biology of both species; he found that *N. eichhorniae* would not attack economically important plants and subsequently released it in the field in Florida (8). DeLoach (4) and Perkins and Maddox (9) subsequently found that *N. bruchi* was also sufficiently

<sup>1</sup>Coleoptera: Curculionidae: Bagoini.

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host-specific for introduction, and this species has also been released in Florida.

DeLoach and Cordo (5) reported that *N. bruchi* and *N. eichhorniae* have very similar life histories. The adult weevils of both feed on the leaves, and the females oviposit in the petioles of waterhyacinth. The larvae of both species tunnel in the petioles and crown where they do extensive damage and then pupate underwater attached to living roots of waterhyacinth. The eggs of *N. bruchi* required 7 to 8 days to develop, larvae ca. 30 days, and pupae ca. 30 days; ca. 78% survived through the immature stages. Adults lived an average of 138 days, and a generation required 96 days at 25 C in the laboratory. *Neochetina eichhorniae* had a lower rate of increase, a longer generation time (ca. 120 days), and required longer to kill waterhyacinth plants than did *N. bruchi* (5).

The question, how could two species with such similar biologies co-exist in the same area on the same host plant and apparently occupy identical ecological niches, was of major concern in the introduction program. We also needed to know how the two species would interact if both were introduced into the U. S. Part of this question was answered by DeLoach and Cordo (5) who reported that *N. bruchi* preferred to oviposit in the older, bulbous petioles, and *N. eichhorniae* preferred the slender petioles of the young equitant leaves of the central bud; they also found differences in response of the two species to temperature. In the present study, we made extensive observations in the field in Argentina from December 1971 to May 1974 to find how the two species are able to co-exist. We also measured seasonal history of the weevils and the amount of damage inflicted to waterhyacinth through the year in an effort to predict their effectiveness as control agents when established in the United States.

## METHODS AND MATERIALS

Insect and plant populations were sampled throughout each of 2 years, usually every 2 weeks, in a small lagoon of ca. 3 ha that adjoined the Río Paraná de las Palmas, across from the Balneario Municipal of the city of Campana, 70 km NW of Buenos Aires. The lagoon was divided into eight general areas and on each date one sample was taken from each area; some samples were taken near the bank, some near the border of the floating mats of plants or in the center of the mats. Samples were taken by placing a frame 0.5 m<sup>2</sup> over the plants; the area encircled was then searched quickly for insects that might crawl or fly out of the marked area; then the plants were removed, examined again for insects, and placed in large plastic bags. The day after collecting the samples, we examined the roots for pupae, dissected all petioles and examined them for larvae, and measured the leaves and counted the feeding spots in a subsample of one typical plant from each bag. We did not search specifically for eggs. Offshoots were counted as plants if they had fully expanded leaves and fibrous roots that reached the water. Less frequent samples were taken from a small canal ca 7 m wide near the Río Paraná at the town of Dique Luján, 40 km NW of Buenos Aires and from a roadside canal 5 m wide between

the river and the town of Escobar, 15 km NW of Dique Luján.

Adults of both *N. bruchi* and *N. eichhorniae* were collected for laboratory tests each time waterhyacinths were sampled at Campana. On each date, we held a group of 10 females of each species for 2 weeks after collection to measure the rate of oviposition. These females were placed in 0.5-liter jars, provided with petioles and leaves of waterhyacinth, and held at a temperature of 25 ± 2 C with a 16-hr photophase. The plant material was replaced and the eggs were collected twice a week. The total number of adults collected on each date, often several hundred, was used to determine the proportion of each species in the population.

Collections of adult weevils were made on six dates (February to April) to measure the degree of separation of the two species based on three sizes of sampling units: (1) different sites on the same plant, i.e., the central petiole and bud, between two central petioles, under ligules covering a young offshoot, under ligules of outer leaves, and on the pseudolaminae or petioles, (2) entire plants, and (3) 0.25 m<sup>2</sup> samples consisting of seven to ten plants each. A Chi-square test was used to compare observed and expected ratios of all possible combinations of the two species in the three sizes of sampling units.

## RESULTS AND DISCUSSION

### Damage to waterhyacinth

The seasonal rate of growth of waterhyacinth plants was measured and correlated with the seasonal damage inflicted by its natural enemies (especially *Neochetina* spp.) throughout 2 years in the lagoon at Campana.

Waterhyacinth plants in the winter typically are small, with short spreading leaves and bulbous petioles (6); the petiole and pseudolamina together usually were less than 30 cm long and 20 cm high above the water surface (Figure 1A). These plants had only ca. 4 to 6 leaves per plant but the density of leaves was high (400 to 600 leaves per m<sup>2</sup>, 80 or more plants per m<sup>2</sup>). During the rapid spring growth in early December, the new leaves were less bulbous, more elongate, and more upright. By midsummer, plants with bulbous petioles were found only at the border of the floating mats, and plants within the mats had only slender, upright, equitant leaves growing nearly 1 m high. These plants were much larger, and had 8 to 10 leaves per plant but the density was low (200 to 250 leaves per m<sup>2</sup>, 25 to 30 plants per m<sup>2</sup>). The plants were surrounded by 4 to 8 offshoots of various sizes still attached to the central plant by stolons, all inter locked with neighboring plants to form dense mats of floating plants. Offshoots from the large summer plants produced only slender equitant leaves. The tall plants gradually died back in the late fall and the leaves were of the short, bulbous overwintering type (Figure 1A).

Damage from *Neochetina* and other arthropods was lowest (10 to 20 feeding spots per leaf) in the spring (September to November) (Figure 1B), but increased rapidly in December and January, about 2 to 3 weeks after rapid

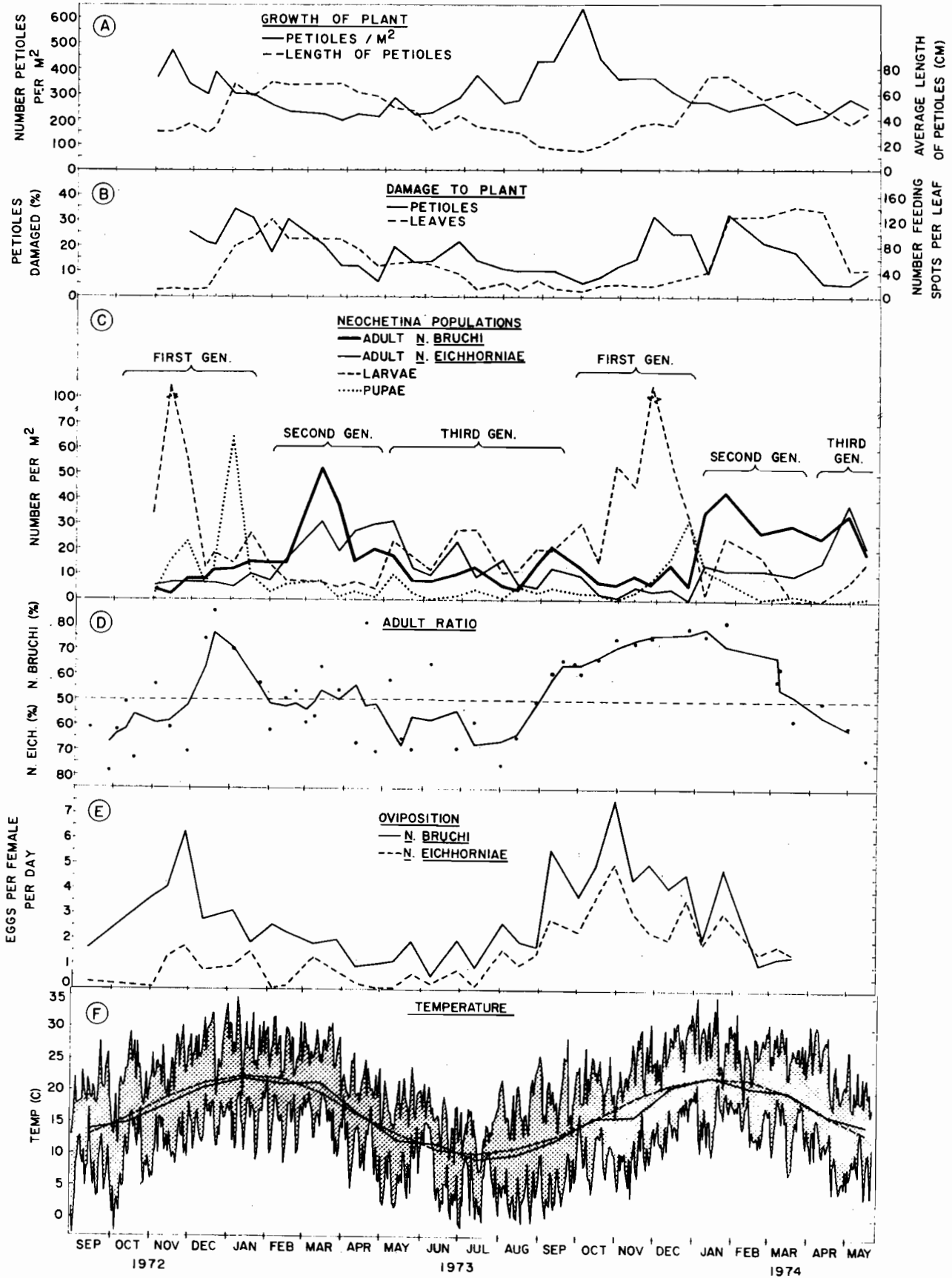


Figure 1. Seasonal abundance of *Neochetina* and waterhyacinth: A) Growth of waterhyacinth plants, B) Damage caused to waterhyacinth plants by adult *Neochetina* feeding on the leaves and by larvae tunneling in the petioles, C) Seasonal abundance and number of generations of *Neochetina bruchi* and *N. eichhorniae* (larvae and pupae of both species together), D) Ratio of adults of *N. bruchi* and *N. eichhorniae* (points are observed ratio, line is a 3-point moving mean), E) Oviposition by field-collected *Neochetina* females collected on each date and held for 2 weeks in the laboratory, and F) Daily temperatures during the study period (shaded area is daily range, — is monthly, - - - is 22-year monthly mean).

plant growth began, to a summer average of 120 to 140 feeding spots per leaf. Damage by larvae tunneling inside the petioles began in mid-November with the large first generation of larvae, and 25 to 35% of the petioles were damaged during early summer. The percentage of damaged petioles decreased to a low in late April and increased again in the fall (May and June) when the third generation of larvae was produced.

### Seasonal abundance of *Neochetina*

Both *N. bruchi* and *N. eichhorniae* produced three generations a year at Campana during the 2 years of the study, though the sequence of generations was more distinct in the 1973 to 1974 season (Figure 1C). The small first generation of adults emerged in September and October, apparently from overwintering larvae and pupae; populations increased gradually as more adults emerged from the pupae. The young females oviposited at a high rate in October and early November and produced a large population of larvae that reached a peak of 105.5 per m<sup>2</sup> on 14 November, 1972 and of 114.5 per m<sup>2</sup> on 28 November, 1973. The subsequent peak of pupae occurred in late December.

A large second generation of adults emerged in January 1974, but in 1973 this peak was unaccountably delayed and did not occur until March (Figure 1C). The adult population of the second generation reached a peak of 75 to 85 adults per m<sup>2</sup> in both years (*N. bruchi* and *N. eichhorniae* together), or about two adults per plant. However, the distribution was not uniform: many plants had no weevils, 4 to 6 per plant was common, and a maximum of 17 adults were found on one plant. The peak population of second-generation larvae occurred in late January.

A small third generation of adults was produced in late April 1974, but in 1973 it did not appear until June. The third generation of larvae and pupae appeared in May and overwintered. All stages were found throughout the winter, and reproduction and development of the immatures apparently continued on days when the weather was warm enough. (Temperatures of 15 to 20 C were not uncommon throughout the winter (Figure 1F). Adult populations gradually declined through the winter to a seasonal low in October.

Population peaks of the last two generations were not as distinct as that of the first. Two reasons were apparent: first, the generations of adults tended to overlap considerably. Field-collected adults lived an average of over 4 months and a maximum of 6 months in the laboratory, so adults from one generation could live over into the next generation or, possibly, throughout the summer and fall. Second, generations of larvae and pupae appeared to overlap because *N. bruchi* developed faster than *N. eichhorniae* and the immature stages of the two species could not be distinguished. Earlier laboratory studies (5) indicated that a generation of *N. bruchi* at optimal conditions required an average of slightly more than 3 months, and a minimum of 2 months from eggs to first reproduction by the next generation females; a generation of *N. eichhorniae* required an average of ca. 4 months. This pattern fits the 1973 to 1974 field populations well since the first generation occurred

in September, the second generation 4 months later in January (delayed by the cool spring weather), and the third generation 3 months later in April (Figure 1C).

A similar pattern of seasonal abundance was observed in the small canal at Dique Luján, except that in the 1973 to 1974 season, populations were much greater than at Campana. Larval populations reached a peak of 322 per m<sup>2</sup> on 6 November and 368 per m<sup>2</sup> on 20 November 1973, or three times the number at Campana. Very large pupal populations followed: on 19 January 1974, 251 pupae were collected from 80 plants, or an average of 3.14 pupae per plant (or 138 pupae per m<sup>2</sup>). However, this population occurred only in small areas of large plants: other areas with smaller plants, that had been heavily attacked by larvae of the pyralid moth *Sameodes* (= *Epipagis*) *albiguttalis* (Warren), had fewer pupae. At Escobar, 15 km NW of Dique Luján, peak larval populations were nearly as great as at Dique Luján; they reached 340 per m<sup>2</sup> on 7 November and 298 per m<sup>2</sup> on 19 December 1973.

### Segregation of *N. bruchi* and *N. eichhorniae* in the field

During the periodic population sampling in the lagoon at Campana, we often observed adults of both *N. bruchi* and *N. eichhorniae* on plants in groups of two to six or more, as well as singly. Sometimes the groups contained only one species, but many groups contained both species. When we analyzed the species composition of different sized groups of weevils in specific sites on a plant (Table 1), we found a statistically significant separation of species for two of the five group-sizes (two to six adult weevils per group). However, when only two weevils occurred in a group, the species were not segregated; also, two males or two females were in a group about as often as a male and a female and a male *N. bruchi* and a female *N. eichhorniae*, or vice versa, were together in a group as often as a male and female of the same species. *Neochetina bruchi* preferred the area beneath the basal ligules of the outer leaves where 52% of the adults were found (1.7 times as many *N. bruchi* as *N. eichhorniae*). *Neochetina eichhorniae* preferred the area beneath the small, tender leaves wrapped around the central petiole where 40% of the adults were found (2.5 times as many *N. eichhorniae* as *N. bruchi*) (Table 2). The separation of adult *N. bruchi* and *N. eichhorniae* that we found in different sites on plants in the field corresponded closely to the preference for oviposition sites reported by DeLoach and Cordo (5).

A similar segregation was found when the sampling unit was an entire plant (Table 1); a statistically significant separation of species appeared in the groups of three or five weevils per plant, indicating that *N. bruchi* tend to congregated on certain plants and *N. eichhorniae* on other plants.

Although only two of the five group-sizes (two to six weevils per group) in each of the two types of sampling units were significantly different from the expected ratios, the difference was always in the direction of greater segregation of species than expected by chance. There was also a trend toward segregation in several other group-sizes, but this was not statistically significant. Thus, the weevils tend-

TABLE 1. NUMBER OF ADULT *N. bruchi* AND *N. eichhorniae* FOUND IN DIFFERENT SIZES OF GROUPS ON PLANTS IN THE FIELD.

Number weevils per group	Number of times each combination of species was found <sup>a</sup> <i>N. bruchi</i> ← → <i>N. eichhorniae</i>	Chi <sup>2</sup>
<u>Each site on a plant<sup>b/,c/</sup></u>		
1	137 - 114	2.11
2	25 - 49 - 37	4.12
3	8 - 4 - 17 - 20	43.22***
4	4 - 4 - 4 - 7 - 5	15.95***
5	2 - 2 - 1 - 6 - 5 - 2	7.84
6	1 - 0 - 1 - 3 - 2 - 2 - 1	4.16
<u>Entire plant<sup>b/</sup></u>		
1	47 - 31	3.28
2	17 - 16 - 13	4.96
3	6 - 4 - 12 - 9	12.96***
4	2 - 7 - 4 - 8 - 4	7.59
5	1 - 2 - 1 - 10 - 5 - 4	11.92*
6	2 - 2 - 1 - 2 - 5 - 3 - 1	10.02
<u>Expected ratio<sup>d/</sup></u>		
1	1 : 1 (1-0) (0-1)	
2	1 : 2 : 1 (2-0) (1-1) (0-2)	
3	1 : 3 : 3 : 1 (3-0) (2-1) (1-2) (0-3)	
4	1 : 4 : 6 : 4 : 1 (4-0) (3-1) (2-2) (1-3) (0-4)	
5	1 : 5 : 10 : 10 : 5 : 1 (5-0) (4-1) (3-2) (2-3) (1-4) (0-5)	
6	1 : 6 : 15 : 20 : 15 : 6 : 1 (6-0) (5-1) (4-2) (3-3) (2-4) (1-5) (0-6)	

<sup>a</sup> In the Chi<sup>2</sup> analysis, values were grouped so that expected values were greater than 1.

<sup>b</sup> Observed values from the field; numbers of times the various combinations of *N. bruchi* and *N. eichhorniae* were found. For example, on entire plants, three weevils were found together 31 times: six times there were three *N. bruchi*, four times two *N. bruchi* and one *N. eichhorniae*, 12 times one *N. bruchi* and two *N. eichhorniae*, and nine times three *N. eichhorniae*. Since the expected ratio was 1:3:3:1, the difference between the expected and observed ratios was highly significant.

<sup>c</sup> Sites on a plant in which weevils were found were the central petiole and bud, between two central petioles, under ligules covering young offshoots, under ligules of outer leaves, and on the pseudolaminae and petioles.

<sup>d</sup> The expected ratio is the appropriate expansion of the binomial. Numbers in parenthesis indicate numbers of *N. bruchi* and *N. eichhorniae*, or both, in each class; for example, with two weevils per sampling unit, we would expect to find two *N. bruchi* on one occasion, one *N. bruchi* and one *N. eichhorniae* on two occasions, and two *N. eichhorniae* on one occasion.

ed to segregate themselves by species at specific sites on a plant and on different plants; however, the segregation was not complete, and there was also considerable mixing.

When the sampling unit was 0.25 m<sup>2</sup> (three to nine plants), we found little evidence of segregation of the species. Nevertheless, the plants in certain areas of the lagoon were inhabited predominantly by one species or the other for periods of several weeks or months, while plants in other areas were equally inhabited by both species. For example, a small partly shaded canal on one side of the lagoon was usually inhabited predominately by *N. bruchi*, and an area of ca. 20 m diam in the northeast corner of the lagoon was inhabited mostly by *N. eichhorniae*.

#### Rate of oviposition

The rate of oviposition by field-collected *N. bruchi* varied four to five fold through the year (Figure 1E). It was highest when the young females of the first generation were present during late October and early November and decreased thereafter through the year. Oviposition by *N. eichhorniae* followed a similar pattern but was nearly always at a lower rate. In the spring of 1973 (September to November), the maximum rate for *N. bruchi* was 7.6 eggs per female per day, and for *N. eichhorniae* 5.0 eggs per female per day. Also, oviposition by *N. eichhorniae* was much less during the spring of 1972 (September to November) than during the same period of 1973 to 1974, for no apparent reason.

The effect of the high rate of oviposition by young females in November was seen in the very large peak of larvae and pupae that followed. However, the much larger second generation of adults which emerged in February and March oviposited at a low rate in the laboratory and produced only a small increase in larval and pupal populations in the field, and that only after an unaccountably long time (Figure 1C).

If reproduction during the second generation were equal to that of the first generation, the resulting large second generation of larvae would have inflicted extremely heavy damage to waterhyacinth. One possible explanation is that some natural enemy attacked the adults at this time of year, reducing the fecundity of the females. No insect parasitoids were ever reared from the several thousand adult weevils held in the laboratory; however, the adults were rather heavily parasitized by an unidentified species of nematode<sup>4</sup> that might have reduced fecundity. Also, increased predation on second-generation larvae could be partly responsible for the reduced populations, and some larvae and pupae may have drowned when the old central plants occasionally died and sank into the water.

#### Ratio of *N. bruchi* and *N. eichhorniae*

*Neochetina bruchi* was more abundant through the summer and early fall (until March or April) than *N. eichhorniae* at Campana, but *N. eichhorniae* was more abundant in late fall and winter in both 1972 to 1973 and 1973 to 1974 (Figure 1D). In mid-summer, 75 to 80% of the population was *N. bruchi*, but in the winter, only 25

<sup>4</sup>DeLoach, C. J. 1975. Unpublished data.

TABLE 2. LOCATION OF *N. bruchi* AND *N. eichhorniae* ON WATERHYACINTH PLANTS IN THE FIELD.

Site on plant	<i>N. bruchi</i>			<i>N. eichhorniae</i>		
	Male	Female	Total	Male	Female	Total
Central petiole and bud	16	16	32	44	36	80
Between 2 central petioles	11	13	24	14	6	20
Ligule covering young offshoot	18	13	31	11	17	28
Under ligule of outer leaves	67	41	108	37	26	63
On pseudolaminae or petioles	9	5	14	8	3	11
Totals	121	88	209	114	88	202

to 40% was *N. bruchi*. Adult *N. bruchi* reached the peak annual population earlier in both years than did *N. eichhorniae* (Figure 1C).

The ratio was different at Escobar and Dique Luján; occasional samples taken there during the summer of 1973 were almost entirely *N. bruchi*. Also, in the spring and summer of 1973 to 1974, 85 to 90% of the population was *N. bruchi*, but *N. eichhorniae* increased to 30 to 40% of the population in March at Dique Luján.

#### Co-existence of *N. bruchi* and *N. eichhorniae*

An explanation for the seasonal shift in the relative abundance of *N. bruchi* and *N. eichhorniae* may hold the key to explaining how the two species can co-exist and occupy such apparently similar ecological niches. The most important biological characters separating the two species seem to be differences in ovipositional behavior, rate of increase and generation time, and temperature tolerances (5). The following hypothesis relating the interaction of these characteristics with the seasonal growth stages of the waterhyacinth plant is offered to explain the seasonal alternation in abundance of the weevils.

The small first generation of adult weevils in the spring (September) is predominantly *N. bruchi*; the females oviposit at a high rate in waterhyacinth plants that are mostly small with the bulbous petioles favored by *N. bruchi* for oviposition. The combination of favored oviposition sites and the greater rate of increase and shorter generation time of *N. bruchi* allow it to outproduce *N. eichhorniae*.

A large second generation of adults produced in mid-summer (January), is also mostly *N. bruchi*. The waterhyacinth plants now are large with slender petioles and have very few of the bulbous petioles preferred by *N. bruchi*; since *N. eichhorniae* favors the central bud for oviposition, it has a relatively constant source of oviposition sites. The shortage of its favored oviposition site now restricts the increase of *N. bruchi*, allowing *N. eichhorniae* to overtake it. If nematodes or other enemies preferentially attacked one species of weevil, they would also influence the species ratio in the next generation; however, evidence of such an effect has not been obtained.

A small third generation of adult weevils occurs in the fall (April to May); it is mostly *N. eichhorniae*. During May and June the tall, slender leaves of waterhyacinth gradually die back and are replaced with small, bulbous leaves that overwinter. The abundance of its favored oviposition site again favors *N. bruchi*, and this together with

its greater rate of increase allows it to outproduce *N. eichhorniae*, resulting in the higher percentage of *N. bruchi* characteristic of spring populations. Although oviposition by both species is low, the greater tolerance of *N. bruchi* to cold temperatures (5) also allows it to survive the winter better than *N. eichhorniae*.

#### CONCLUSIONS

*Neochetina bruchi* and *N. eichhorniae* produce damage throughout the year in Argentina, but damage is most severe during the summer; however, the degree of suppression of waterhyacinth by its natural enemies is still less than desired. We need to obtain better control in the United States from the introduced insects that was observed in Argentina; this is possible by eliminating nematodes or other natural enemies of the insects before releasing them in the field in the United States.

*Neochetina bruchi* and *N. eichhorniae* alternate in abundance through the year, apparently dependent on the interaction of their ovipositional behavior, rates of increase, and temperature tolerances with the seasonal changes in the growth form of the waterhyacinth plant. This alternation in abundance probably allows the continued co-existence of the two weevil species on waterhyacinth in the same area. Indeed, *N. bruchi* and *N. eichhorniae* would appear to complement each other in a control program by attacking plants in different growth stages and at different times of the year. Optimum control of waterhyacinth would therefore be achieved by introducing both species of weevils. However, the new balance achieved between the two weevil species and waterhyacinth may not be the same in the United States as in Argentina since other organisms in the system are different. The balance probably will shift toward lesser abundance of one of the weevil species at the expense of the other.

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