# ENHANCEMENT OF EFFECT OF NEOCHETINA EICHHORNIAE FOR BIOLOGICAL CONTROL OF WATERHYACINTH

by
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## INTRODUCTION

Although specific examples will be taken from studies with a particular insect species, the principles discussed here could be applied to biological control programs utilizing other insects and other target weeds. Techniques will include studies completed, preliminary results from studies underway, anticipated future studies, and theoretical studies which may never be undertaken.

Many scientists and laymen conceive of biological control using imported insects as simply shipping the tested and cleared species to the country of introduction, releasing it, usually passing through quarantine in the process, and taking some data on its effect and population size in the field. If the insect can bring about adequate control using this approach, it may be as well to follow this procedure. Often, however, the economic threshold is too low for the introduced insect alone to reduce weed abundance sufficiently.

Limitations of the weevil, *Neochetina eichorniae* Warner, in control of waterhyacinth, *Eichhornia crassipes* (Mart.) Solms, were recognized by myself (Perkins 1973) and DeLoach and Cordo (1976) in Argentina. In addition, from the studies by Vickers and Charba in the United States (O'Keefe 1976), it was estimated that 300 adult weevils would be needed to consume only 1 pound of waterhyancinth material in a year. This would be a tiny fraction of the overall waterhyacinth biomass within a square meter. We knew from laboratory and field studies, however, that smaller numbers of weevils than this could stress waterhyacinth notably. Indeed, in some weevil release sites in the U.S., open water actually appeared where formerly a cover of waterhyacinth had been present for years. The insect, although the new agent introduced into the biocoenose, obviously did not act alone. Recognizing this fact, we began a series of studies in collaboration with various institutions and individual collaborators to determine what interactions exist and methods for manipulation to enhance the effect of introduced species. Besides interactions, manipulation methods to increase the health or population size of *N. eichhorniae* were considered. Enhancement methods, outlined in Table 1, include: (1) improvement of the release colony, (2) combination with other arthropods, (3) combination with vertebrates, (4) combination with plant pathogens, (5) chemical enhancement, and (6) mass collecting or rearing, and distribution. Papers describing results of these studies are already published or in preparation, and I will not dwell on them in detail here. I will attempt to present the enhancement methods being tried and rate them, in my opinion, as to the most promising.

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## IMPROVEMENT OF RELEASE COLONY POTENTIAL

Theoretically, simply importing the insect, thus removing it from its native natural enemies, can improve the potential of the insect colony released. Likewise, elimination of natural enemies in the country of introduction could help in establishment of the original colony and in enhancing the effect of future generations. In South Africa, for example, scale insects were rendered more effective against cactus by controlling their parasites, using DDT (Anneke *et al.* 1969).

The species introduced into a new land is able to take advantage of possible loss of resistance in the target weed due to years of separation of insect and host plant and the resultant lack of selection pressure from the insect and loss of resistance in the plant. Of course, we need to consider the potential development of resistance by the plant due to selection pressure by the introduced species in the future, even though some speculation refutes this assumption (Green 1975). Similarly, genetic improvement of the introduced insect species has been suggested, using artificial selection to develop

Table 1. Potential Methods for Enhancement of Effect of *Neochetina eichhorniae* on Waterhyacinth.

| Category | Method | Technique or Specific Agent(s) | Rating[a] | Reference or[b] Investigator(s) |
|---|---|---|---|---|
| Improvement of Release Colony | (1) Importation Alone | | E | P |
| | (2) Genetic | | C | ——— |
| | (3) Pathogen Elimination | See Table 2 | G | ——— |
| Combination with other Arthropods | (1) Imported | (1) *Orthogalumna terebrantis* Wallwork | G | D |
| | | (2) *Neochetina bruchi* Hustache | G | De |
| | (2) Native | (1) *Tetranychus tumidus* Banks | S(F) | (P) |
| | | (2) *Arzama densa* Walker | F | V |
| Combination with vertebrates | Fish | *Ctenophanyngodon idella* Val. | G | (DSP) |
| Combination with Plant Pathogens | Fungus | (1) *Acremonium zonatum* (Saw.) Gams | F to G | CPL |
| | | (2) *Cercospora* spp. | F | CO |
| Chemical Enhancement | (1) Kairomones | ——— | S | DP |
| | (2) Herbicides | 2,4-D[c] | G | (P) |
| | (3) Nutrient Deprivation | Fe, Ca, P, N | G | (P St) |
| Population Distribution and Abundance | (1) Mass Rearing | Artfiicial Diet | P | (P) |
| | (2) Mass Collecting | Kairomones | G | (PDL) |
| | (3) Distribution to new sites | From Broken Host Plant Mass Release | S | (P) |

[a] Rating: C—Considered but not yet tried E—Excellent; F—Fair; G—Good; P—Poor; S—Study in progress (Rating is subjective opinion of author) See text for explanation of reasoning.

[b] References: CPL—Charudattan, Perkins, Littell 1976; CO—Conway; D—Del Fosse; De—DeLoach; DP—Del Fosse and Perkins; DSP—Del Fosse, Sutton, Perkins 1976; PDL—Perkins, Durden, Lovarco; V—Vogel and Oliver. Investigators (unpublished work in progress): (P)—Perkins; (PSt) —Perkins and Steward.

[c] 2,4-dichlorophenoxyacetic acid (triethanolamine salt)

an insect with desirable attributes. We attempt to discover these attributes to some extent when we search for a strain of an introduced insect species from a particular climatic region in the insect's native region. We have not yet selected for an improved strain of *N. eichhorniae*, although we have considered such a study.

Of particular concern with *N. eichhorniae* were insect pathogens. Table 2 indicates the precautions taken to insure release of a healthy colony.

*N. eichhorniae* was successfully imported and established on waterhyacinth in the United States and has begun to damage waterhyancinth notably in the field. Pathogens known from Argentina were successfully eliminated from the *N. eichhorniae* colony released in the field, and continual collection and observation has turned up no serious pathogen enemies in the U.S. This could be one reason the U.S. populations of *N. eichhorniae* exceed (200 adults/m$^2$) populations in Argentina (50-100 adults/m$^2$).

COMBINATION WITH OTHER ARTHROPODS. A few other arthropods, imported and native, are known to attack waterhyancinth. (Perkins 1974). Two host specific arthropods, besides *N. eichhorniae*, are already in the U.S. *Orthogalumna terebrantis* Wallwork, apparently introduced to the U.S. with the first waterhyacinth, was studied in laboratory and field to determine interaction with waterhyacinch (Del Fosse 1976). *Neochetina bruchi* Hustache, first released in 1974 (Perkins and Maddox 1976), has been released with an equal number of *N. eichhorniae* to determine interactions and relative numbers after a given time. Results of this study are still incomplete. DeLoach and Cordo (1976) have studied these two species and their relative effects on waterhyacinth in the laboratory and field in Argentina.

Native U.S. arthropods studied include the red spider mite, *Tetranychus tumidus* Banks, and the moth, *Arzama densa* Walker. These arthropods are

Table 2. Steps in Elimination of Pathogens of *Neochetina eichhorniae* Warner at Time of Introduction

Step No.

1. Adult weevils were received in sealed container by post.
2. Container was opened in room with controlled access.
3. Container, packing material, enclosed plant material were placed inside plastic bag and incinerated.
4. Adult weevils were placed in pie pan covered with glass pane and this pie pan was placed inside wooden sleeve cage.
5. Eggs were dissected from waterhyacinth tissue daily for 2 weeks and semi-weekly thereafter or after decline in egg production.
6. Eggs were placed on a moist paper towel and washed in 0.5% hypochlorite solution to surface sterilize them.
7. Eggs were placed in waterhyacinth plants in greenhouse or in field by splitting waterhyacinth petioles, placing eggs inside and taping petiole closed.
8. Weevils with disease symptoms in imported adult culture were sent in sealed tubes to insect pathologists for identification.[a]
9. Dead weevils from imported adult culture were placed in 70% ethyl alcohol.
10. Adults and larvae were examined by insect pathologists for organisms such as microsporidia which could be transmitted transovarially.[b]
11. All plant material and associated material from imported adult culture were placed inside plastic bags and incinerated after use.
12. Glassware and cages were cleaned with detergent and hypochlorite solution after last adult had died.
13. Importation of adult weevils was halted after field population reached adequate levels.
14. Field populations were monitored to assure that diseases known from Argentina did not appear.[c]

[a] Fungus, *Beauveria brongniartii* and *Aspergillus* sp. *flavus* group found (G. Allen, pers. comm.)

[b] A native (to U.S.) nematode, order Rhabditida, was found in adult *N. eichhorniae* from the field in theU.S. but no microsporidia.

[c] The fungus, *Beauveria bassiana*, which is native to the U.S. was found in one specimen of thousands observed.

not host specific and therefore may be better able to exploit favorable circumstances and possibly be more devastating to waterhyacinth than host-specific species under certain conditions. Harris (1973) has stated this principle in selecting biological control agents. Studies are still underway with *T. tumidus* combined with *N. eichhorniae* in field sites.

Results. In laboratory studies, Del Fosse (1976) confirmed that a synergistic effect took place when the mite, *O. terebrantis,* and *N. eichhorniae* occurred together or were placed on waterhyacinth in combination. Waterhyancinth abundance was only 50% the amount in the control. The combination was also more effective than the mite or weevil alone. DeLoach and Cordo (1976) stated that the weevil, *N. bruchi,* should augment the damage produced by *N. eichhorniae* based on studies they conducted in Argentina with both species. These two species would be tied closely to waterhyacinth and would be rated as good combination control agents.

The arthropods *Tetranychus tumidus* Banks and *Arzama densa* Walker would rank only as fair, based on preliminary results which indicate sporadic occurrences only, depending on conditions. *T. timidus* requires hot, dry conditions and is affected by fungus and coccinelid predators. *A. densa* apparently adapted to waterhyacinth from pickerelweed, *Pontederia cordata* L., and has been difficult to find in the absence of pickerelweed.

## COMBINATION WITH VERTEBRATES

The white amur fish, *Ctenopharyngodon idella* Val., although a general feeder, can be effective if only waterhyacinth is available. The fish feeds on the roots and occasionally on leaves that enter the water. Laboratory pool studies were conducted in Fort Lauderdale, with 3 pools as control, fish alone, weevil alone, and fish plus weevil, respectively. Comparisons were made of plant abundance in each pool (Del Fosse, *et al.* 1976).

Results. *C. idella* and the weevil together reduced plant increase most (Del Fosse, *et al.*), Plants in these pools had the roots eaten by fish and the upper plant damaged by insects. Waterhyacinth abundance was reduced 20 to 38% using the combination as compared with the control pools. It should be noted that this was a controlled laboratory experiment, and similar results would only be expected under conditions of waterhyacinth monoculture and confinement of the fish to a limited area. Under these conditions, control using the combination should be good. Other vertebrates would probably be less effective than the white amur in combination with the weevil; the manatee, for example, would consume the entire plant, including the weevils.

## COMBINATION WITH PLANT PATHOGENS

It has long been recognized that pathogen attack has followed damage by *N. eichhorniae.* Studies were initiated in laboratory pools and field sites to determine which species of pathogens were present and to evaluate how the most promising species could be applied as a biological herbicide in areas of high *N. eichhorniae* population to enhance the control effect (Charudattan *et al.* 1976).

Results. Among the most promising fungi for combination with *N. eichhorniae* was *A. zonatum* due to its virulence and ubiquitous occurrence. In nature, however, it was so widespread that results of fungal application as a biological herbicide was masked. It was recovered from larval tunnels, from adult feeding spots (under very moist conditions), and from tunnels of *O. terebrantis.* Conditions of high temperature and humidity limited use of the fungus. Del Fosse (1976), for example, did not find the fungus in weevil feeding spots during field studies in Fort Lauderdale. Therefore, the rating in combination with *N. eichhorniae* would be fair. Under proper conditions, the combination would rate as good, however.

## CHEMICAL ENHANCEMENT

Kairomone. The presence of a kairomone was suspected when it was discovered that injured field plants were more attractive to adult *N. eichhorniae* than uninjured plants (Perkins *et al.* 1976). Studies were conducted using an olfactometer to confirm the presence of this attractant (Del Fosse and Perkins 1976).

Results. The kairomone from waterhyacinth, though of an unknown chemical nature at this writing, is a powerful attractant. It exists naturally in waterhyacinth and is liberated by injuring the plant. The kairomone is useable as a tool in enhancement of control by attracting large numbers of the weevil (see below).

**Nutrients.** Studies with nutrient deficient waterhyacinth plants was untaken to determine whether degree of feeding by *N. eichhorniae* was affected by lack of a particular mineral.* The feasibility of using nutrient deprivation to stress the plant without diminishing feeding stress by *N. eichhorniae* was one of the objectives. Cut portions of plants deficient in calcium, iron, phosphorous, nitrogen, and all minerals, were exposed to adult *N. eichhorniea,* and the degree of feeding was assessed.

Results. Nutrients in canals in Florida are seldom limiting to waterhyacinth growth. However, in laboratory studies on plants deficient in iron, calcium, phosphorus, nitrogen, and all nutrients, results indicated continued normal feeding on plants deficient in iron and calcium. If these minerals could be limited to the weed, perhaps through chelation, the plants could be stressed by nutrient deficiency without affecting insect feeding. The potential I would rank as good only if the technology of deprivation of nutrients could be developed.

**Herbicide.** The herbicide 2,4-D can kill waterhyacinth if a high enough dosage is used. Concern by organizations and individuals, however, regarding chemicals being applied in the waterways, makes reduced quantities or frequencies of application desirable. Plots were established in a field site with a population of *N. eichhorniae* of approximately 3 adults/plant. Three plots were treated, and 3 plots were left untreated as controls. Plants were sampled inside and outside the treated plots and inside and outside the untreated plots. Data taken included number of adult weevils, degree of adult feeding, larval tunnels, plant size and density, associated organisms, and degree of herbicide damage.

Results. When applied to waterhyacinth in less than normal dosage (2 lb/acre), 2,4-D initially kills some of the plants, but stimulates rapid regrowth in surviving plants. This regrowth is very attractive to *N. eichhorniae* adults, which in preliminary studies have attacked the regrowth so voraciously as to kill the plants completely.* The plant hormone 2,4-D has also been shown to attract insects to crop plants (Oka and Pimentel 1976), increasing damage. Based on these results, the use of 2,4-D with the weevil would rate as good.

## POPULATION DISTRIBUTION AND ABUNDANCE

**Artificial diet.** Mass rearing, using an artificial diet, was considered even prior to introduction of

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*Perkins, B. D. and K. K. Steward. 1976. Feeding by *Neochetina eichhorniae* adults on waterhyacinth deficient in certain nutrients. J. Aquatic Plant Management (In ms.).

*N. eichhorniae.* Diets were developed using a modified Van der Zant's medium. Adult and larval acceptance and oviposition were tested.

Results. It is axiomatic that greater damage can be inflicted to the weed using greater numbers of *N. eichhorniae* than would normally occur. Artificial diet has been studied as a technique for producing quantities of *N. eichhorniae.*** The weevil, however, is too closely tied to its host in life cycle for the diet to be more than a brief adult food supplement. Since the normal host plant suffices and is simpler to obtain and prepare, such an artificial diet would be needless to produce quaniities of weevils.

**Mass collecting**. Mass collecting was attempted after weevil release, since quantities of adults were needed to establish additional sites. The weevil was present in low numbers and would hide during the day, making collection very slow and tedious. Methods tried included light traps, a vacuum machine, sweepnets, and extraction in the laboratory using a Berlese funnel. None of these methods was effective. Plant modification, however, did concentrate the insects and improve collection efficiency.

Results. Mass collection from the field, using the kairomone (see chemical enhancement, above), is feasible. Injured plants attract 8 to 16 times as many adult insects as uninjured plants, thus improving collecting efficiency. A cooperating agency collected 33,000 adult weevils from a Fort Lauderdale field site using this technique (pers. comm. C. Zeiger, U.S. Army Corps of Engineers, Jacksonville, FL).

**Mass Release.** Relatively low numbers of adult weevils can kill a waterhyacinth plant under the proper conditions. In laboratory studies, using as few as 5 adults in a closed aquarium, a waterhyacinth plant may be killed in time by weevil feeding and the accompanying tissue deterioration due to plant pathogens and saprophytes. Field plants have been found large and healthy with more than 20 adults, however, indicating the importance of proper conditions in affecting the weed.

Mass release has not yet been attempted in the field due to limitations in manpower to do the collecting. Numbers needed to begin field studies are estimated, hypothetically, at double the normal high field population of ca. 250 adults/m$^2$. At an anticipated collecting rate of 1,000 adult *N. eichhorniae*/person/day, one person a day would be needed for each 2 m$^2$ treated. A field plot measuring 8m$^2$ would require 32 person days (32,000 adults), and replicated 3 times, 96 person days. Needless to say, more personnel, time, and an even more efficient collection method are needed.

**Distribution.** Distribution by man can supplement the natural spread of *N. eichhorniae* which moves only a few miles each year. Adult weevils are responsible for spreading the species, but spread is slow, and the population increases slowly, even after the new colony is established by individual flying weevils. Cooperators have been supplied infested waterhyacinth sites for collection of this weevil for their own use, and new release sites have been established by them and by researchers to examine weevil response under different conditions. Currently, more than 100 sites have been established with *N. eichhorniae* and natural spread is beginning to fill the gaps between sites. No waterhyacinth mat can be found in Broward County, Florida that does not have *N. eichhorniae* present, and it is spreading.

## CONCLUSION

Now that the useable organisms and techniques have been listed and discussed, the reader will be interested in how they could be utilized in a future control program. Table 3 indicates a series of hypothetical steps which could be utilized. The table is not a recommendation, but a hypothetical example. Techniques listed and discussed here are not exhaustive for enhancing control by the weevil, *N. eichhorniae.* It is clear, however, that tools exist for manipulating the degree of control. As man determines the economic threshold level of the weed, he must likewise determine the degree of effort and techniques to be employed to reach this goal.

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*Perkins, Unpublished data

**Perkins, B. D. 1976. Towards an artificial diet for rearing *Neochetina eichhorniae* for waterhyacinth control. In mis.

Table 3. Hypothetical Steps in Control of Waterhyacinth in a Lake Using Enhancement of Effect of *N. eichhorniae*.[a]

| Step No. | Techniques | Limitations |
|---|---|---|
| 1. | Release healthy *Neochetina eichhorniae* adults in sufficient numbers for insect establishment; allow 3-12 months for population increase. | Slow population increase, maximum weevil density may be too low for control. |
| 2. | Establish other host-specific arthropods (if not already present): | |
| | *Orthogalumna terebrantis* | Hot, dry conditions needed. |
| | *Neochetina bruchi* | Slow population increase. |
| 3. | Apply fungus, *Acremonium zonatum* as biological herbicide. | Hot, humid conditions needed. |
| 4. | Treat limited areas of high economic value (boat trails, fishing areas, etc.) with low dosage (less than 2 lb/A of 2,4-D) | Application regulated by established legislation. |
| 5. | Release mass numbers of adult *N. eichorniae* (500 /m² along fringes of herbicide treated area. | Much time may be needed for weevil collection prior to release. |
| 6. | Release white amur fish at norm[a]l stocking rate for this body of water. | Waterhyacinth monoculture necessary. Government approval for release necessary. |

[a] This table is entirely hypothetical, and in practice, some steps above would probably be eliminated or placed in different order. The above table should *not* be construed as a recommendation, since research still underway may prove some of these steps unworkable.

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