GESTÃO E CONSERVAÇÃO DA FLORA E DA VEGETAÇÃO DE PORTUGAL E DA ÁFRICA LUSÓFONA

"In Honorium"
do *Professor Catedrático Emérito*

Ilídio Rosário dos Santos Moreira

ISAPress

GESTÃO E CONSERVAÇÃO DA FLORA E DA VEGETAÇÃO DE PORTUGAL E DA ÁFRICA LUSÓFONA

"In Honorium"
do *Professor Catedrático Emérito* Ilídio Rosário dos Santos Moreira

EDITORES

Ana Monteiro
Fernando Gomes da Silva
Raul Jorge
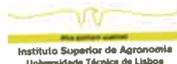
AQUATIC WEED BIOLOGICAL CONTROL: OLFACTORY ATTRACTION OF Weevils *Neochetina bruchi* and *N. eichhorniae* FOR WATER HYACINTH (*Eichhornia crassipes*). A CASE STUDY[1]

Luta biológica contra infestantes aquáticas: a atracção olfactiva dos gorgulhos *Neochetina bruchi* e *N. eichhorniae* por jacinto-aquático (*Eichhornia crassipes*). Um estudo de caso [1]

Maria Teresa Rebelo

University of Lisbon, Faculty of Sciences, Department of Animal Biology, 1749-016 Lisbon, Portugal. CESAM – Center for Environmental and Marine Studies, 3810-193 Aveiro, Portugal.

ABSTRACT

Water hyacinth, *Eichhornia crassipes* (C. Martius) Solms-Laubach, is one of the worst aquatic weeds in many parts of the world. Throughout this naturalized range the most significant biological control agents are two weevil species *Neochetina bruchi* Hustache and *N. eichhorniae* Warner. The use of mass trapping schemes with host-plant volatiles in order to increase the number of *Neochetina* spp. on areas with water hyacinth infestations has been under theoretical analysis. In an attempt to investigate the functional basis of water hyacinth selection by adults of *Neochetina* weevils, the studies of antennal sensory structures and the identification of the host-plant volatiles that mediate the *Eichhornia-Neochetina* attraction were accomplished. Antennal sensilla typology, number and placement analyzed by SEM revealed an increase in sensilla morphological diversity and number from the pedicel to the club in both species and sexes, but in small number when compared with other Coleoptera. Bioassays with a y-tube olfactometer indicated that the volatiles produced by broken leaves and stems of water hyacinth were attractive to both species and sexes of weevils. The collection and analysis of these volatiles by GC-MS indicated the presence of three compounds, (E)-3-hexen-1-ol, (Z)-3-hexen-1-ol, and 2-ethyl-1-hexanol. Olfactometer results indicated that *N. eichhorniae* and *N. bruchi* males and females were attracted to (Z)-3-hexen-1-ol when tested in a y-tube but they did not respond to the other constituents. These results indicate that *Neochetina* weevils are responsive to water hyacinth volatiles, yet, may suggest the importance of other clues (e.g. visual) rather than only olfactory in host selection, which should be further investigate to optimize the water hyacinth integrated control programs.

Key Words: Biological control of freshwater weeds, *Eichhornia crassipes, Neochetina,* antennal sensilla, semiochemicals.

RESUMO

A planta de jacinto-aquático, *Eichhornia crassipes* (C. Martius) Solms-Laubach, é considerada uma das piores infestantes aquáticas a nível mundial. Na sua área de distribuição, os agentes de luta

---

biológica mais significativos são duas espécies de gorgulhos *Neochetina bruchi* Hustache e *N. eichhorniae* Warner. A utilização de voláteis da planta hospedeira em programas de armadilhagem para captura em massa destes insectos e posterior largada em zonas densamente infestadas de jacinto-aquático tem sido alvo de discussões teóricas. Para clarificar as bases funcionais da selecção de jacinto-aquático por adultos de *Neochetina*, procedeu-se ao estudo das estruturas sensoriais das antenas dos gorgulhos e à identificação dos voláteis da planta hospedeira, que medeiam a atracção *Eichhornia-Neochetina*. A tipologia, número e distribuição das sensilas antenais analisadas por MEV revelaram um aumento crescente da diversidade morfológica e do número de sensilas do pedicelo para o bastão da antena, em ambas as espécies e sexos, mas em número reduzido, quando comparado com outros Coleoptera. Os bioensaios realizados com olfatómetro num tubo em Y indicaram que os compostos produzidos por folhas e caules seccionados de jacinto-aquático foram atractivos para ambos os sexos e espécies de gorgulhos. A recolha e análise destes voláteis revelaram a presença de três compostos, (E)-3-hexen-1-ol, (Z)-3-hexen-1-ol e 2-etil-hexan-1-ol. Os resultados do olfatómetro indicam que tanto machos como fêmeas de *N. eichhorniae* e *N. bruchi*, quando testados num tubo em Y, são atraídos para (Z)-3-hexen-1-ol, mas não respondem aos outros constituintes. Estes resultados indicam que os gorgulhos *Neochetina* spp. são sensíveis a voláteis de jacinto-aquático, mas parecem indiciar a importância de outro tipo de estímulos (por ex. visuais), e não apenas olfactivos, na selecção da planta hospedeira, o que deve ser investigado para optimizar os programas de gestão integrada de jacinto-aquático.

Palavras-chave: Luta biológica contra infestantes aquáticas, *Eichhornia crassipes*, *Neochetina*, sensilas antenais, semioquímicos.

INTRODUCTION:

Water hyacinth, (*Eichhornia crassipes*) (C. Martius) Solms-Laubach, a Pontederiaceae, is ranked as one of the world worst invasive water weeds causing widespread problems to millions of users of water bodies and water resources (Penfound & Earle 1948, Gopal 1987). The thick mats of water hyacinth were originally seen mainly as a practical problem for fisheries and navigation, hydroelectric power generation and irrigation schemes. However, water hyacinth is also a major threat to biodiversity, affecting fish and aquatic faunas, plant community structure and diversity, and human health and water supplies (Hill *et al.* 2011).

It is native to the Neotropics and has spread to almost all countries with a suitable climate (Stark & Goyer 1983), and has been introduced around the world as an ornamental plant because of its attractive flowers. Extensive infestations developed in the southern USA (especially Louisiana and Florida), Mexico, Panama, many parts of Africa (especially the Nile and Congo river systems), the Indian sub-continent, South-East Asia, Indonesia and Australia (Holm *et al.* 1977; Gopal 1987; Center 1994; Julien *et al.* 1999). Though water hyacinth is cultivated as an ornamental almost all over Europe, it has naturalized only in Portugal (the highest northern latitude of its ecological range) (Pieterse & Murphy 1990).

Water hyacinth was first noticed in Portugal in 1939 in Tagus bassin, becoming weed in many lowland aquatic systems (Moreira *et al.* 1989). When compared with other countries, the problems caused by this weed are reduced in Portugal, as infestations are mostly in irrigation and drainage channels on the so-called "Lezíria Grande de Vila Franca de Xira", a fertile alluvial plain of the Tagus and Sorraia rivers. Nevertheless, its importance has been increased, mainly on small wetlands

areas that are important biodiversity reserves and particularly valued for their bird populations (e.g. Alverca da Golegã, Lagoas de Santa Margarida, Paúl de Boquilobo, Paul de Madriz, and Paúl da Tornada) (Moreira *et al.* 1989; 1999a).

The maintenance of water hyacinth populations at their lowest "feasible levels" together with the reduction in herbicide use in waterways, due to concerns for the quality of domestic and recreational use water supplies, implies the need for more biorational forms of aquatic weed control. The possible integrated aquatic weed control approaches include: control of nutrient levels, use of booms to control movement of the weed, exploitation of variable water levels, manual removal of the weed from shores and small channels, mechanical removal or destruction by land-based or floating equipment, and use of biological control agents (Center 1994; Julien *et al.* 1999; Moreira *et al.* 1999a).

The first suggestion of using insects to control weeds was made by Asa Fitch in 1855, who suggested the importation from Europe to the USA of insects that fed on toadflax (*Linaria vulgaris*) on the old continent but not in the new one. The first actual use of insects for weed control occurred in 1863 in India when a cochineal insect (*Dactylopius ceylonicus*) was moved from north to south to control the cactus *Opuntia vulgaris* (Van Driesch & Bellows, 1996). Biological weed control has been achieved through two routes: introduction of natural enemies against adventice and native weeds (usually using agents collected from an adventice weed native range), and augmentation of natural enemies, which are released or applied at specific locations where control is needed (Wapshere *et al.* 1989).

While biological control of invertebrates (by predation and parasitism) causes direct mortality of the individuals attacked, biological control of weeds can be achieved by a variety of mechanisms, which does not necessarily include directly-caused mortality of the target plant. Plants that are prevented from successfully reproducing (by flowerfeeders which destroy flowers before they can set seeds, or by seed feeders which destroy the seeds themselves) and then die naturally have been as effectively eliminated as those that are killed outright by herbivore attack (Van Driesch & Bellows 1996).

Nine arthropods and three fungi have been developed and released for biocontrol of *E. crassipes* in more than 40 countries (Guido & Perkins 1975; Harley 1990; Julien & Griffiths 1998; Julien *et al.* 1999; Sosa et al. 2007). The arthropods are the weevils *Neochetina bruchi* Hustache and *Neochetina eichhorniae* Warner (Coleoptera: Erirhinidae); the moths *Niphograpta albiguttalis* (Warren) (Lepidoptera: Pyralidae), *Xubida infusella* (Walker) (Lepidoptera: Pyralidae), and *Bellura densa* Walker (Lepidoptera: Noctuidae); the water hyacinth bug *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae), the grasshopper *Cornops aquaticum* (Bruner) (Orthoptera: Acrididae); the leafhopper *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) and the leaf-mining mite *Orthogalumna terebrantis* Wallwork (Acari: Galumnidae). The fungi are all hyphomycetes: *Acremonium zonatum* (Sawada) W. Gams, *Cercospora piaropi* Tharp and *Cercospora rodmanii* Conway.

The two *Neochetina* weevils have been released into more than 30 countries and are considered the most successful projects for biological control of weeds in the world (Crawley 1989; Julien *et al.* 1999). They have together given significant results in Argentina, Australia, Benin, India, Mexico, Papua New Guinea, South Africa, Sudan, Tanzania, Thailand, Uganda, USA, and Zimbabwe, acting apparently in a complementary fashion (DeLoach & Cordo 1983; Center 1994; Hill & Cilliers 1999; Julien *et al.* 1999). In many other countries where releases have been made, their effectiveness

has been either limited or not yet evaluated. In Portugal, for example, *Neochetina* spp. weevils, imported from Florida in 1995, were preliminary tested in quarantine but did not survived to winter (Moreira *et al.* 1999b). Due to UE restrictions to the use of biological control agents, native from other continents, *Neochetina* weevils were never released in the field to control water hyacinth infestations.

Adult weevils feed on the leaf and petiole surfaces, making distinctive, almost square, feeding scars. This may cause significant loss of functional leaf surface and also may allow entry of pathogens. The most significant damage, however, is caused by the larval stages which develop from eggs laid in the petiole and feed for many weeks inside the petiole tissue, migrating as necessary to new petioles as the tissue dies. This damage to the petiole often results in complete collapse of the leaf and eventually in loss of buoyancy so that the whole plant sinks.

The impact of *Neochetina* spp. in the abundance of water hyacinth has been mitigated by the current weed management practices, which rely heavily on herbicidal control (Center & Durden 1986). Moreover, the lack of effectiveness may be partially attributable to a microsporidiosis that infects Florida populations, which had provided a source of weevils for several countries (Rebelo & Center 2001), before microsporidia-free colonies have been released.

Center *et al.* (2002) suggest that augmentative releases of *Neochetina* weevils quickly suppress regrowth of incipient water hyacinth populations compared with natural infestations. So, the use of mass trapping schemes with host-plant volatiles in order to increase the number of biological control agents on more infested areas has been under theoretical analyses. However, an important factor compromising this augmentative control program is the lack of methods that allow for the large-scale collection or production of sufficient numbers of weevils.

Plant chemistry is likely to affect acceptability (to adults) and suitability (as food). Species of herbivores with narrow host ranges are frequently adapted to respond to specific chemicals found in their host plants. Species with broad host ranges often respond more to nonspecific stimuli coupled with the absence of specific deterrent compounds (Rausher 1992). Thus, chemical communication is emerging as an important component in IPM. Typically, attractants such as pheromones or host-produced volatiles are used to attract pestiferous insects. However, semiochemicals could be used for beneficial insects to monitor the success of biological control releases or for conserving weevils that would be destroyed by herbicide treatments. Once augmentation efforts with semiochemical attractants are feasible and implemented, the biocontrol of this weed should improve dramatically. This would significantly reduce the need for expensive and environmentally damaging weed management practices such as the direct application of herbicides into domestic water supplies.

There is evidence that semiochemicals function to concentrate *Neochetina eichhorniae* around fresh weevil-feeding damage water hyacinth leaves (Del Fosse & Perkins 1977). The weevils are endemic to the Amazon basin and during the wet season and the associated flooding their host plant is patchily distributed over great distances (DeLoach 1975). Under these conditions, host volatile attraction would be highly selective. Host range tests have confirmed that these weevils are highly host specific within the plant family Pontederiaceae and prefer and complete development only on water hyacinth (DeLoach 1975; Gopal 1987; Julien *et al.* 1999).

Semiochemicals may provide a more efficient collecting method, as they have been useful in attracting other weevil species. These include *Metamasius hemipterus sericeus* (Perez *et al.* 1994),

Pissodes nemorensis (Phillips *et al.* 1984), *Rhynchophorus bilineatus* (Oehlschlager *et al.* 1993), *R. cruentatus* (Giblin-Davis *et al.* 1994; Weissling *et al.* 1993; Weissling *et al.* 1994), *R. ferrugineus* (Hallet *et al.* 1993), *R. palmarum* (Oehlschlager *et al.* 1992), *R. phoenicus* (Gries *et al.* 1993), and *R. vulneratus* (Hallet *et al.* 1993).

Although all this considerable work has been done on evaluating weevil and host plant-produced attractants of other weevil-host plant species complexes, and despite the clear and long-standing agricultural pest status of *Eichhornia crassipes* and its biological control agents, the weevils *N. bruchi* and *N. eichhorniae*, little is known about the physiological basis of the sensory aspects of host-plant recognition. While research is underway searching for an aggregation pheromone, host plant volatiles were examined as an additional component of an effective weevil trapping system (Perez *et al.*1997). However, the chemical nature of this activity has never been elucidated.

Identification of water hyacinth chemicals can then be used to understand the behavior and chemical ecology of *N. bruchi* and *N. eichhorniae* and use in a mass trapping scheme to capture large numbers of live weevils for augmentative biological control of the weed. Biological control practitioners attempting to manage these beneficial weevil populations would benefit from trapping methods that monitor populations for abundance, dispersal rates, and seasonality.

How these insects locate and recognize water hyacinth plants and mate presumably depends, at least in part, on mechanical, olfactory, and gustatory sensory receptors. The efficiency of IPM strategies to control *E. crassipes* is dependent on the biological knowledge that can be achieved about the plant-weevil relationships. As the placement of olfactory sensilla on the antennae seems to be a morphological adaptation having an influence on the efficiency or sensitivity odor perception, the specific distribution pattern of olfactory receptors is related to the specific searching behavior of a species (Zacharuk 1985; Bernays & Chapman 1994). Thus, antennal olfactory sensilla description and selective responsiveness of the antennal olfactory system to host-plant volatiles have been the subject of intensive scrutiny (Sass 1978; Alm & Hall 1986; Isidoro & Solinas 1992; Bowen 1995; Merivee *et al.* 1997; Pophof 1997; Merivée *et al*, 1998; Bartlet *et al*, 1999; Merivee *et al*, 1999; Shields & Hildebrand, 1999; Lopes *et al.* 2002). However the ultrastructure of antennal sensilla has never been described for any *Neochetina* spp. The purpose of this study was to fill the gap through an anatomo-ultrastructural study of weevil antennal chemosensilla.

According with Bernays & Chapman (1994), all the leaf-feeding insects that have been examined critically have been shown to be able to smell components of the commonly occurring green leaf volatiles such as hexanol or hexenal. It has also been shown that the number and/or sensitivity of receptors is greater for alcohols and aldehydes with six-carbon-atom chain lengths (the major constituents of the called "leaf odor") than for compounds with shorter or longer chains. Because of this general sensitivity, all phytophagous insects probably have the capacity to smell any plant, whether it is a host or not. In addition to these responses to widely occurring plant volatiles, some insects also exhibit sensory responses to the odors of compounds specific to their host plants. As we hypothesize that *Neochetina* weevils are attracted to host-produced volatiles, we addressed the identification and the behavioral activity of water hyacinth volatiles on *Neochetina* weevils.

Therefore, the research work attempted in this paper was motivated by the potential use of water hyacinth volatiles in mass trapping schemes of *Neochetina* weevils and the need of bio-

logical knowledge on the *Neochetina* spp. sensory structures as well as the determination of water hyacinth volatiles that mediate the weevil-host attraction.

MATERIAL AND METHODS

Neochetina bruchi and *N. eichhorniae* antennae external morphology

The description of *Neochetina* spp. antennal sensilla typology, number and placement was realized by using scanning electron microscopy (SEM) at Faculty of Sciences of Lisbon University, Portugal. The weevils were field-collected from water hyacinth plants located in tanks at University of Florida, USDA/ARS Invasive Plant Research Laboratory at Fort Lauderdale, in South Florida (USA), from 1999 to 2001. To examine the types of sensilla, antennae from freshly killed *Neochetina eichhorniae* and *N. bruchi* adults were excised, dehydrated in a graded ethanol series and air-dried. Then, specimens were attached to alluminium stubs with double stick tape, gold coated with either a Polaron E5350 or JEOL JFC-1200 sputter coater, and viewed with a JEOL JSM 5200 LV scanning electron microscope at accelerating voltage of 15 kV. The types, number and distribution of antennal sensilla were identified from SEM montage micrographs obtained from 3 males and 3 females antenna of each species. Lengths of sensilla were determined by measuring 10 sensilla of each type for both species and sexes.

The sensillar terminology adopted follows that used by Schneider (1964), Dyer and Seabrook (1975), Zacharuk (1980, 1985) and Merivee *et al.* (1997, 1998, 1999, 2002). Long hair-like sensilla were divided into sensilla chaetica and sensilla trichodea according to the way of attachment to the antennal surface. The hairs standing in a wide flexible joint membrane were classified as sensilla chaetica, but long hairs unmovable at their base when touched were classified as sensilla trichodea. Short pegs were classified as basiconic sensilla.

The statistical analyses included mean comparisons, which were performed using a multiple *t*-test with Bonferroni probability adjustment procedure. The software package used was Statistica (version 5).

Attraction of water hyacinth volatiles to *Neochetina bruchi* and *N. eichhorniae*

Insects. Large quantities of *Eichhornia crassipes* root material were collected from southern Florida and examined to locate *Neochetina bruchi* and *N. eichhorniae* cocoons, during 1999 to 2001. The cocoons were carefully removed from the roots and held in Petri dishes with moistened filter paper (to keep high humidity) at approximately 28-30.ºC. Newly emerged adults were collected daily, kept in small vials with filter paper and water hyacinth leaves and their age carefully recorded, until studies were initiated. As weevils younger than 7 days of age were more sensitive to odors than older ones (Weissling *et al.* 1994) 2-5 days old adults were used for behavioral bioassays.

Plant material and volatile collection. Plant samples were collected from the same locations as the weevils and from aquatic tanks at the University of Florida, USDA/ARS Invasive Plant Research Laboratory at Fort Lauderdale, Florida and consisted of freshly cut *E. crassipes* leaves. Fully expanded fresh leaves (40-50g) were cut into 1cm² pieces with scissors and transferred directly to glass volatile collection chambers (4.5x30.5cm; Analytical Research Systems, Micanopy, FL, USA). Aerations were conducted without added moisture by passing filtered air across the leaves and col-

lecting volatiles in collection tubes (7.6x0.6cm) packed with Super-Q adsorbent (30mg; Alltech Associates, Deerfield, IL, USA). Adsorbed volatiles were eluted with CHCl2 (200µl) then frozen (-20°C) until analyzed by GC-MS. Air was filtered with a series of activated carbon filters (Heath and Manukian, 1992). Collections were obtained with air flows of 500 ml/min for 18-24h durations with a 12h photoperiod, ~50%RH and at 25°C. Light was provided by two 100W incandescent bulbs located 30cm above the collection chambers. As a control, volatiles were also simultaneously collected from an identical chamber under the same conditions that lacked plant material. Volatiles were collected from the headspaces of a 5-l flask filled with 500g of chopped water hyacinth leaves. Air was drawn from the flask at 40ml/min for 24h, at 28±2.ºC, over the water hyacinth leaves and through an outlet of Porapak Q (80-100 mesh, 400mg) glass tube (6.6x0.5cm ID) using a vacuum pump. At the end of each adsorption period, the Porapak Q was eluted with solvent (200µl of methylene chloryde) dripping into a 2ml glass vial. These solutions of water hyacinth volatiles were used in GC-MS and behavioral bioassays. When not been used, the solutions were stored in the dark at -3.ºC.

Volatile analysis. The *E. crassipes* foliar constituents were analyzed by GC-MS with an Agilent 6890 instrument fitted with a HP-5MS (Agilent, 30mx0.25mm, 0.25micron film thickness) FSOT column with helium at 36 as a carrier gas. Injections were conducted with an autosampler (HP-7683) split 1:20 at 250.ºC. The mass selective detector (HP 5973) was heated at 250.ºC (source) and 150.ºC (quad) with transfer line 280.ºC and ion source filament voltage of 70eV. Component identification was made on the basis of mass spectral fragmentation, retention index with *n*-paraffins, comparison with authentic constituents, and mass spectral and retention matching. Standards were purchased from commercial sources (e.g., Sigma, St Louis, MO, USA) and were of the highest purity available. Optical isomerism was not investigated.

Behavioral bioassays. Adult weevils were field-collected from south Florida ponds feeding on *E. crassipes* stems and leaves. Weevils were held (27°C; 12h photoperiod) in rearing cages (30x13 cm) and fed fresh *E. crassipes* stems and leaves until tested. As the adults of these weevils are crepuscular, y-tube olfactometer assays were conducted in darkness (red light). All bioassays were conducted in one of three identical y-tubes (Kimble Kontes Vineland, NJ, USA). Each y-tube was composed of a single stem (30cm length x 1cm diameter) joined by two arms (20cm length x 1cm diameter). The y-tubes were oriented horizontally and filtered air (1l/min) was directed through each arm of the tube. Each arm was attached by Teflon tubing to a sample flask (Kimble Kontes Vineland, NJ, USA; 50ml) designated as either a test or a control. Each test sample flask was loaded either with broken *E. crassipes* leaves (5g) or a test compound applied (25µl) to filter paper strips (5x1cm). Test compounds were applied at four concentrations (0.0001, 0.001, 0.01, 0.1mg/µl) in paraffin (Sigma, St. Louis, MO, USA). The control flasks were either empty for the broken-leaves test or contained filter paper treated with paraffin (25µl) as a control for the test compounds. Each weevil was introduced individually and a total of 25 weevils of each sex and species was tested. A positive response was recorded when the weevil crawled to within 2cm of the end of either y-tube; a non-response was recorded when the weevil did not reach this point within 15min. After testing each weevil the y-tube orientation was rotated 180° in order to avoid directional factors. Each tube was washed at the end of the day with hot soapy water, rinsed with deionized water, EtOH (95%), and oven dried (100°C). The two-choice results were analyzed with a *G*-test of independence (SAS/PC; SAS Institute, Inc., 1990; *P* = 0.05) after removal of the non--responsive individuals (9 %).

RESULTS

Neochetina bruchi and *N. eichhorniae* antennae external morphology

General structure of the antennae

Both males and females of *Neochetina eichhorniae* and *N. bruchi* have geniculate antennae formed by 11 antennomeres: a long scape (1st), a pedicel (2nd), a flagellum consisting of a funiculum (3rd-7th) and a relatively big club made of 4 subsegments (I-IV clavomeres, i.e. 8th-11th antennomeres) (Fig. 1).

In both species the antennae are sexually dimorphic in length, with females showing a bigger antennae than males: in *Neochetina bruchi*, pedicels measured an average of 1225µm and 1000µm, and the rest of antennomeres 1325µm and 1175µm, respectively in females and males; in *N. eichhorniae*, pedicels measured an average of 1025µm and 600µm and the rest of antennomeres 1125µm and 1150µm, respectively in females and males.

In both sexes and species, the scape and the antennomeres 3 to 5 are almost glabrous, showed only a few sensilla (Fig. 1). An increase in sensilla morphological diversity and number was observed on the distal region of the pedicel and from antennomeres 6 to 7, with the greatest sensilla density (91% in *N. bruchi* and 83% in *N. eichhorniae*) and variety been observed on the club.

Topography and typology of antennal sensilla

To simplify description and to preliminarily organize the numerous types, sensilla are grouped on the basis of ultrastructural similarity into 5 Types, I to V. Those five different types of sensilla were all found on males and females of *Neochetina bruchi* and *N. eichhorniae*.
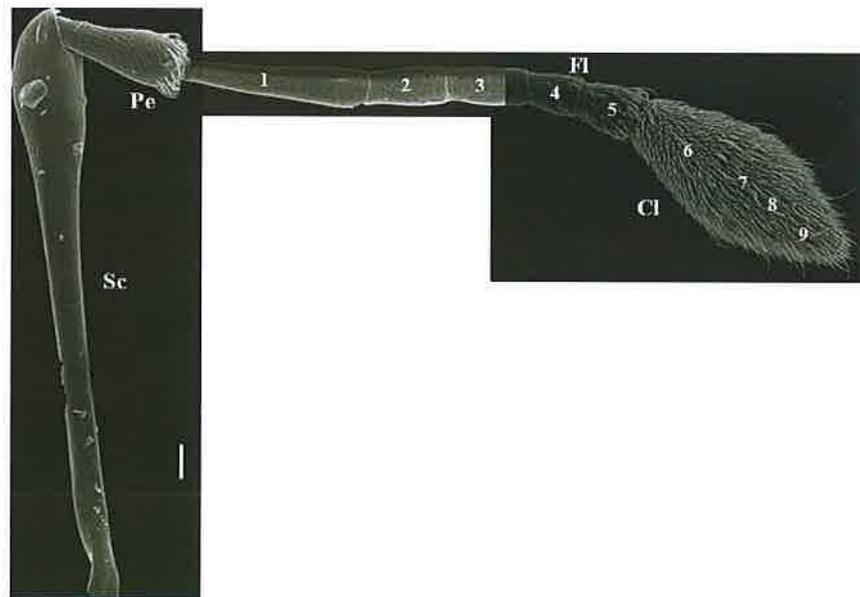

Figure 1. Scanning electron micrograph (SEM) of the antennae of *Neochetina eichhorniae*. The antennae consist proximally of the long scape (Sc) and the pedicel (Pe) and distally of the flagellum (Fl), which in turn is divided into 9 segments. The last four flagellomeres are fused forming the antennal club (Cl). The density of sensilla per antennomere increases distally, being the club densely covered by hairs. The antennae of *N. bruchi* is very similar. Bar = 50 µm.

These weevils showed two distinctive types of sensilla, considered here as Type I and Type II. The other three types, considered as sensilla trichodea (Type III), sensilla chaetica (Type IV) and sensilla basiconica (Type V), were situated on the club only.

Type I: Hair characterized by a tree-like shape, with 2, 3, 4, 5, or 6 branches (subtypes *Ia, Ib, Ic, Id,* and *Ie* respectively) (Figure 2. A, C, D, H). The sensilla range in length from 27 to 47µm. These sensilla are the most numerous type present (62% in females of the two species, 61% and 60% in males, of respectively, *N. bruchi* and *N. eichhorniae*). Type *Ia* occurs in pedicel, 7th antennomere and club of *Neochetina bruchi*. In *N. eichhorniae* occurs in the same antennomeres plus the 6th. Type Ib occurs in all antennomeres except 3-6 in *N. bruchi*. In *N. eichhorniae* this subtype has the same distribution but appears also on antennomer 3-4 in females and 4 in males. Type Ic occurs in pedicel and antennomer 7 in *N. bruchi*. In *N. eichhorniae* occurs in the same antennomeres plus the 6th. Type Id occurs in pedicel in both species and sexes but shows sexual and specific differences in other antennomeres. In *N. bruchi*, occurs also in antennomer 7th in females. In *N. eichhorniae* occurs in antennomeres 6-7 in both sexes, plus antennomeres 3-4 in males. Type Ie occurs only in pedicel of both species and sexes.

Type II: Characterized by a "leaf" shape, can be uniform or have 2, 3 or 4 branches (subtypes IIa, IIb, IIc, and IId, respectively) (Fig. 2. A, B, D, H). The sensilla range in length 42 to 62µm. Type IIa occurs in pedicel, antennomeres 6-7 and first two clavomeres (antennomeres 8-9) in both species and sexes. Males of both species show this subtype of sensilla on the scape. Type IIb occurs in pedicel and antennomer 7th in *N. bruchi*. In *N. eichhorniae* this subtype shows a sexual dimorphism: in females appears in pedicel and antennomeres 6-8th, while in males in addition to those, also occur in antennomeres 1-2. Type IIc shows an interspecific distribution. In *N. bruchi* occurs only in the pedicel, but in *N. eichhorniae* appears also in antennomeres 3-4 and 6-7. There are no sexual differences. Type IId occurs only in the pedicel of *N. bruchi* and males of *N. eichhorniae*. In females of this species also was observed on antennomeres 3-4.

Type III: Sensilla trichodea *sensu* Zacharuk (1985), show a hair shaft 42-72µm long, proximally straight and tapering slightly at their ends (Fig. 2. D, E). They were found over the entire club but mainly on the distal region on both males and females on both weevil species.

Type IV: Sensilla chaetica *sensu* Schneider (1964) and Zacharuk (1980, 1985), show a cuticular apparatus consisting of an outstanding hair shaft 64-80µm long, straight or gently curved, gradually tapering from the base to a blunt tip. They emerge from well-defined sockets and subtend an angle of around 60° with the antennal surface (Figure 2. D, E). These sensilla are relatively few in number but are the longest of all sensilla present. They are distributed symmetrically around the circumference of the club and protude well above all the other sensilla.

Type V: Sensilla basiconica *sensu* Schneider (1964), with hair shaft 14-18µm long, relatively stout, blunt tipped, peg-shaped, rising on a rigid socket, and having abundant porous thin walls (Fig. 2. F, G). Both species and sexes have this type of sensilla interspersed among the sensilla trichodea, over the club.

Cuticular pores: Cuticular circular pits can be found on the club surface, generally associated with sensilla basiconica lying proximally close to the bristle base (Fig. 2. G). These are round, with 0.5-0.6 µm in diameter.
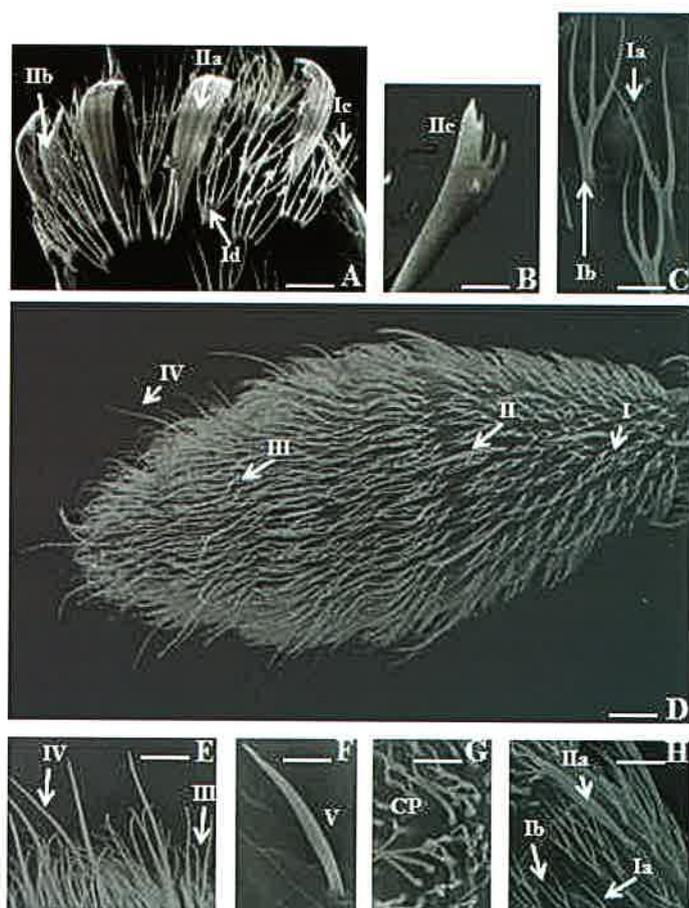
Figure 2. Scanning electron micrographs of antennal sensilla in adult *N. eichhorniae* and *N. bruchi*. A. Detail of distal region of pedicel, showing sensilla types I (c-d) and II (a-b). Bar = 16μm. B. Hair type IIc. Bar = 8μm. C. Hair type I (a-b). Bar = 6.4μm. D. Side view of antennal club showing club regions I-IV and hair types I-IV. Bar = 27μm. E. Hair types III-IV. Bar = 14μm. F. Hair type V. Bar = 3.2μm. G. Antennal club pormenor showing hair type I (a), type V and cuticular pores (arrows). Bar = 8.5μm. H. Antennal club pormenor showing hair types I (a-b) and II (a). Bar = 12.5μm.

Attraction of water hyacinth volatiles to *Neochetina bruchi* and *N. eichhorniae*

Major volatiles extracted from the leaves of water hyacinth

Water hyacinth plants revealed a scarce number of compounds, in extremely low concentrations. The GC-MS analysis of crushed *E. crassipes* leaves and stems indicated that the major volatile components were (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, and 2-ethyl-1-hexanol (Fig. 3). These constituents contributed 18, 68, and 2.5%, respectively of the total amount integrated.
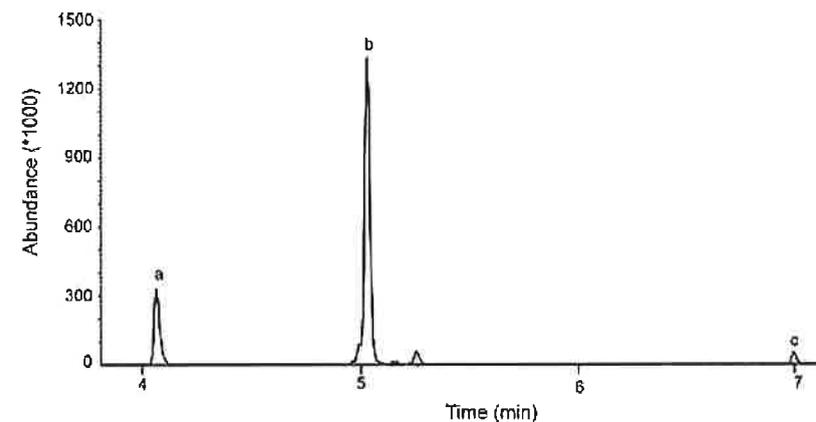

Figure 3. GC-MS profile of volatiles collected for 13h from crushed *E. crassipes* leaves and stems (32.8g). Volatile were adsorbed on Super Q (30mg) and eluted with CHCl2 (200μl). Letters above GC-MS peaks correspond to: (a) (*E*)-3-hexen-1-ol; (b) (*Z*)-3-hexen-1-ol; and (c) 2-ethyl-1-hexanol.

Behavioral bioassays

The Y-tube olfactometer used in this study proved to be a discriminating tool for comparing the response of *Neochetina bruchi* and *N. eichhorniae* adults to several treatments. Periodic tests where no stimulus was introduced into the Y-tube arms revealed no bias for weevils to choose one side over the other, suggesting the lack of volatile absorption onto the plastic Y-tube juncture.

The results of y-tube olfactometer analysis (Table 1) indicate that males and females of both weevil species were attracted to the volatiles produced by crushed *E. crassipes* leaves, with *N. eichhorniae* revealed a slightly stronger attraction (average of 78% – males and females) when compared with *N. bruchi* (76% average of both sexes combined). Analysis of specific volatile constituents with the y-tube olfactometer indicated that neither (*E*)-3-hexen-1-ol nor 2-ethyl-1-hexanol elicited a significant response from either weevil species at any of the concentrations tested and (*Z*)-3-hexen-1-ol was the active component detected by the insects. *N. bruchi* were attracted to lower concentrations than *N. eichhorniae*.

Table 1. Response (%) of *Neochetina* spp. weevils to waterhyacinth leaves or to different concentrations of (Z)-3-hexen-1-ol applied in 25ml of paraffin.

Stimulus	*N. bruchi*		*N. eichhorniae*	
	Males	Females	Males	Females
crushed leaves	87.2 ***	64.0 *	81.6 ***	73.5 ***
Amount (mg/μl)				
0.0001	75.6 ***	62.0	28.6	54.3
0.001	59.1	77.1 ***	57.1	66.7 *
0.01	73.5 ***	55.8	70.1 **	81.3 ***
0.1	71.4 **	62.5	76.2 ***	73.8 ***

*** $P < 0.001$ ** $P < 0.01$ * $P < 0.05$

DISCUSSION

The present study demonstrates that the antennae of *Neochetina eichhorniae* and *N. bruchi* contains twelve morphologically distinct sensillum types. In addition, the two species had similar antennal morphology with regard to the number of segments, types of sensilla and their pattern of distribution on the respective segments of the antenna, with very few exceptions.

Types I and II, are different from any sensilla reported in other insects: *Hylobius abietis* (Mustaparta 1973), *Curculio caryae* (Hatfield *et al.* 1976), *Hypera meles* (Smith *et al.* 1976), *Hypera postica* (Bland 1981), *Conotrachelus nenuphar* (Alm & Hall, 1986), *Ceutorhynchus assimilis* (Isidoro & Solinas 1992), *Semiadalia undecimnotata* (Jourdan *et al.* 1995), *Agriotes obscurus* (Merivée *et al.* 1997), *Limonius aeruginosus* (Merivée *et al.* 1998), *Psylliodes chrysocephala* (Bartlet *et al.* 1999), *Melanotus villosus* (Merivée *et al.* 1999), *Manduca sexta* (Shields & Hildebrand 1999), *Phoracantha semipunctata* (Lopes 2000), *Leptinotarsa* sp. (Sen & Mitchell 2001), *Bembidion properans* (Merivee *et al.* 2002). Even extensively reviews made by Schneider (1964), Zacharuk (1980, 1985), and Gaino & Rebora (1999), showed no such type of sensilla.

Sensilla type III, was considered as trichodea. Sensilla similar to these have been described on several species of Curculionidae. They may be identified with Mustaparta's (1973) "type III hairs" of *Hylobius abietis*, Alm & Hall's (1976) "hairs type IV" of *Conotrachelus nenuphar*, Hatfield's (*et al.* 1976) "sensilla basiconica type II" of *Curculio caryae*, Isidoro & Solinas's (1992) "hair-like sensilla" of *Ceutorhynchus assimilis*. The tubular body at the base of the sensillum is a typical mechanosensitive structure (Zacharuk 1980, 1985). Mustaparta (1973, 1975) on the antennal club of *H. abietis*, considered that they had either a mechanoreceptive function, or no reception function, possibly acting as protective hairs. These sensilla, due to their morphological structure, in *Neochetina* weevils, may have a combined mechanosensory and gustatory function.

Sensilla type IV, considered as chaetica, is very common on weevil's antennae, and may be easily identified with Mustaparta's (1973) "type IV hairs" of *Hylobius abietis*, Alm & Hall's (1986) "hairs type V" of *Conotrachelus nenuphar*, Hatfield's (*et al.* 1976) "sensilla trichodea type I" of *Curculio caryae*, and Isidoro & Solinas's (1992) "sensilla chaetica" of *Ceutorhynchus assimilis*. Accordingly with Bartlet *et al.* (1999) they may have a combined mechanosensory and gustatory function. These sensilla, morphologically typical gustative, have been proven to respond to mechanical stimuli on *Limonius aeruginosus* (Merivee *et al.* 1998). They resemble the one found in the pine weevil (Mustaparta, 1973), "sensillum type IV", which had responses to chemicals in the liquid phase and/or a mechanoreceptive function. Because of their length, in *Neochetina* weevils, they will be the first to come in contact with the substrate. Antennal tapping of leaf surface, prior to feeding, probably exposes these gustatory sensilla to tactile and chemical stimuli.

Sensilla type V, was considered as sensilla basiconica. Similar sensilla have been reported in several species of Carabidae, Cerambycidae, Chrysomelidae, Coccinellidae, Curculionidae, Elateridae, and Scolytidae, and may be identified with Mustaparta's (1973) "type II hairs" of *Hylobius abietis*, Alm & Hall's (1986) "hairs type II" of *Conotrachelus nenuphar*, Hatfield's (et al. 1976) "sensilla basiconica type I" of *Curculio caryae*, Bland's (1981) "sensilla basiconica type II" of *Hypera postica*, Hatfield's (*et al.* 1976) "sensilla basiconica type I" of *Curculio caryae*, Smith's (*et al.* 1976) "sensilla basiconica type I" of *Hypera meles*, Isidoro & Solinas (1992) "peg-like sensilla" of *Ceutorhynchus assimilis*, Jourdan's (*et al.* 1995) "sensilla basiconica" of *Semiadalia undecimnotata*, Merivee's (et al.

1997) "sensilla basiconica" of *Agriotes obscurus*, Merivee's (*et al.* 1998) "sensilla basiconica" of *Limonius aeruginosus*, Bartlet's (*et al.* 1999) "sensilla basiconica" of *Psylliodes chrysocephala*, Merivee's (et al. 1999) "sensilla basiconica type I" of *Melanotus villosus*, Lopes's (et al. 2002) "sensilla basiconica type I" of *Phoracantha semipunctata*, and Merivee's (*et al.* 2002) "sensilla basiconica" of *Bembidion properans*.

Although no histological studies of *N. eichhorniae* and *N. bruchi* antennal sensilla were performed, sensilla basiconica were noted to break off or contact inwardly along their shafts during mounting, demonstrating their thin-walled nature. Comparatively, sensilla chaetida and sensilla trichodea were usually found to be intact. Complementary, their distribution pattern is such that they are protected from mechanical damage by the longer sensilla trichodea and chaetica, also found on *H. abietis* (Mustaparta 1973) and *H. meles* (Smith *et al.* 1976). In electrophysiological and behavioral experiments these type of sensilla have been proven to function as olfactory chemoreceptors in *H. abietis* (Mustaparta 1975), *L. aeruginosus* (Merivee *et al*, 1998), and *P. semipunctata* (Lopes *et al*. 2002). The similarity in location and ultrastructural features of such classical sensilla basiconica, such as a non-flexible base, and a thin, multiporous cuticular wall, which is typical of insect olfactory receptors, with the ability to perceive air-borne stimuli (Schneider 1964; Zacharuk 1980, 1985), allows the assumption that these sensilla are the most probable candidate chemoreceptors on the antenna of *N. eichhorniae* and *N. bruchi*.

No interior sensilla were observed in the pits located on the club surface, generally associated with sensilla basiconica, which indicates they may be epidermal gland ducts. The occurrence of this type of pores is common in other Coleoptera, for example, *C. nenuphar* Alm & Hall's (1986).

Sex pheromone detection is generally attributed to sensillar types disproportionately present in males, for example, the sexual dimorphism in sensillar distribution in *Diabrotica virgifera* (Bartlet *et al.* 1999). The difference in number of sensilla trichodea and basiconica per antenna may be related to their localization and length. There are 2 times (in N. *bruchi*) and 2.5 times (in *N. eichhorniae*) as many of the former as of the latter. On the other hand, the short pegs of sensilla basiconica with the relatively thin walls are more protected by the hair type I, and thereby also less exposed to the air stream than sensilla trichodea. Owing to their length and position the later are more exposed, which could enable them to adsorb odorous molecules from lower concentrations, than sensilla basiconica. That's why sensilla trichodea are usually sex pheromones receptors in insects (Merivee 1992; Merivee *et al.* 1998). In *N. eichhorniae* and *N. bruchi*, however, sexual dimorphism is characterized by slightly differences on the location of type II sensilla on scape. On the other hand, if our speculations on the functional mode of each sensillum are correct, about 27% and 25% of the antennal sensilla of *N. bruchi* and *N. eichhorniae*, respectively, are olfactory. Of these, the majority are probably responsive to host-plant volatiles. Evidence collected so far by the author suggests that there is no intraspecific chemical communication, e.g. sexual or aggregation pheromones, involved in mate location.

These observations together with the behavioral studies have demonstrated that these weevils are primarily responsive to host-plant volatiles. Despite its global distribution, *Eichhornia crassipes* is attacked by few phytophagous generalists. This might indicate that water hyacinth leaves have some deterrents for most species. On the other hand, *Neochetina bruchi* and *N. eichhorniae* not only successfully feed and reproduce on the weed, as also reveal a strong attraction to young leaves (Del Fosse & Perkins 1977; Center & Wright, 1991), they might have co-evolved with the host plant in relation to semiochemicals.

Therefore, the GC analyses of water hyacinth headspace blend revealed very few volatiles when compared with other plant, namely belonging to Myrtaceae family, as *Eucalyptus globulus* (Barata *et al.* 2000). Addicionally, only one compound, (*Z*)-3-hexen-1-ol, showed a significant response in behavior bioassays. This green leaf alcohol has been found in other plant volatiles, has shown activity attracting other species of herbivores (e.g., Ruther 2004), and to have high antennal sensitivity (Hansson *et al.* 1999). It is known that plant surface waxes have characteristics blends of wax components, providing the potential for host-plant selection by phytophagous insects (Bernay & Chapman 1994). The alcohol that elicited a strong response in *Neochetina bruchi* and *N. eichhorniae* is characteristic of the principal constituents of plant epicuticular waxes. As there is some evidence that *Neochetina* is weakly attracted to host volatiles, it is presumed that a mechanism of long-range primary attraction would be required for maintenance of the population.

According with Birch *et al.* (1980), several sympatric species of *Ips* (bark beetles) in the southeastern United States are cross-attracted to infested pine logs. Thus, in addition to the random and host volatile theories, some weevils may find susceptible host by orienting to volatiles produced by competing species during colonization. The volatiles can be host compounds that virtually any weevil would release upon feeding (e.g., monoterpenes). The same mechanism might succeed with *Neochetina* weevils, but needs further research.

In this study, both *Neochetina* species showed no important differences in attraction to water hyacinth volatiles. This might suggest that both species have a similar behavior when concerns the localization of the plant. As Bernay & Chapman (1994) point out, there is no evidence of competition between phytophagous insect guilds. In this case, the competition between water hyacinth weevils would be at level of resources utilization (*Neochetina bruchi* has somehow a quicker larval development than *N. eichhorniae*, for example).

Many studies shown that, for phytophagous insects, the suitability for oviposition and larval development is much more important than for adult feeding (Bernays & Chapman 1994). Center & Wright (1991) studies on *N. eichhorniae* revealed a strong preference for young leaves, with high contents of nitrogen, phosphorous, potassium, magnesium and low levels of calcium. It is possible that the immature instars of *Neochetina* spp. were able to use the amino acids and minerals more efficiently on less calcified (young) leaves.

Neochetina weevils also showed less antennal sensilla than many other insects. This observation, together with the weak response to water hyacinth volatiles might indicate that they use more visual clues rather that olfactory ones to locate the mats of water hyacinth plants. On the other hand, they might find the susceptible water hyacinth by chance interception. The role of sight has been demonstrated in other insects, mainly bark beetles. They are more attracted to traps baited with host odor or pheromone that are placed next to "tree trunk silhouettes" than to traps without such visual stimuli, indicating that beetles orient to the tree trunk during landing (Moser & Browne 1978; Borden *et al.* 1982; Tilden *et al.* 1983; Lindgren *et al.* 1983; Bombosch *et al.* 1985; Ramisch 1986; Chénier & Philogéne 1989). When testing semiochemicals in the field, the spatial and temporal variation of responding insect populations with respect to trap placement may lead to erroneous conclusions. To counter this potential problem, relatively numerous trap replications need to be previously employed (Byers 1989).

For use in a mass trapping scheme to obtain large numbers of live weevils for augmentative biological control of water hyacinth, (*Z*)-3-hexen-1-ol need to be tested in the field. Measurements of its natural release rates are necessary for further understanding of weevil chemical ecology.

FINAL REMARKS

Although there are a number of effective ways to control water hyacinth, it remains as the world's most invasive and damaging aquatic plant. Compared with other methods, biological control is more flexible in its application and is environmentally safe. Furthermore, expenditure ceases after the first few years, but the control achieved continues indefinitely. Biological control using insect natural enemies (e.g. *Neochetina bruchi* and *N. eichhorniae*) which feed only on water hyacinth can effectively control the weed in some areas. However, these insects do not provide a complete solution. Each water body should be considered separately; an ideal combination of measures should be devised for each water body, depending on many factors and in close consultation with all users of the water. In situations where tolerance of the weed is low (e.g. boat docking sites, irrigation and hydroelectric dams), there is a need for a control technology, which combines the environmental safety of biological control with the speed of chemical or mechanical control. Chemical control may be necessary as an extreme measure, for the rapid destruction of large masses of weed, which are seriously impeding access or navigation. All the larvae of *Neochetina* spp. and many adults on the sprayed plants are likely to be lost as a result of complete kill of the weed. This should be considered in deciding the areas to be treated, in addition to the possible problems from deoxygenation when the weed is decomposing. Where *Neochetina* spp. are being introduced, any herbicide treatment should of course be kept well away from the introduction points.

In order to try to speed the effects of biological control agents, augmentative releases of *Neochetina* spp. have been suggested. If large numbers of weevils could be harvested from undisturbed areas or areas targeted for herbicide treatment or held in designated water hyacinth infested areas for redistribution at a later time and then applied to incipient plants or introduced in high densities on seeded water hyacinth, the weed population explosion would be suppressed reducing expenditure of money, time, and risk of environmental contamination. Motivated by the possible use of mass trapping schemes to optimize the control of water hyacinth by *Neochetina bruchi* and *N. eichhorniae*, an attempt to understand the weevil-host attraction was presented.

The study of the antennal fine structure of *Neochetina* spp. revealed twelve morphologically distinct sensillum types, aggregated into 5 main types. Type I, different from sensilla found in other insects, and the most abundant in *Neochetina* weevils, was found on almost all antennal segments. By contrast, type II, also a unique feature, was restricted to very few antennomeres. Comparisons of the cuticular specialization, ultrastructure, and location of these sensilla to those described by other authors suggest that these sensilla are capable of responding to various stimuli, viz. tactile as well as thermo – and/or hygroreception. The distribution patterns of other three sensillar types provided evidence for the importance of the club of *Neochetina bruchi* and *N. eichhorniae* on host-plant reception. All these sensory structures are situated on the antennal club, i.e. 8-11th antennomeres, and consist of three types of chemosensilla, namely sensilla basiconica, chaetica, and trichodea. Sensilla basiconica, are ultrastructurally typical olfactory, comparatively most suitable to detect environmental volatiles present in relatively high concentrations, such as host-plant odours. Sensilla chaetica, externally represented by the most projecting setae from the antennal surface, are ultrastructurally typical contact chemoreceptors strategically located to easy touch and taste host-plant surface. Sensilla trichodea, the second most numerous chemosensilla, distally sickle-shaped, simplest chemoreceptors are ultrastructurally typical olfactory of moderate efficiency, suitable for detecting environmental volatiles present in low concentrations such as pheromones. Therefore, sensilla basiconica and sensilla trichodea are

candidate chemoreceptors based on their close resemblance to sensilla reported to have this function in other insects. However, electrophysiological investigations using single-cell recording are needed to confirm their functions.

The number of sensilla on the antennae of *N. eichhorniae* and *N. bruchi* is not as high as in other weevils. It thus appears reasonable to conclude that a non-polyphagous species has a narrow fit for the neural template coding for feeding behavior than polyphagous feeders. Consequently, a behavioral sequence may only be elicited if a series of peripheral signals indicate a perfect match for the template for feeding behavior.

To determine whether *Eichhornia crassipes* odors play a role in host-finding behavior of *Neochetina* spp., identification of the weed volatiles that elicited behavioral weevil responses was also object of research. The results indicate that the primary volatile produced by crushed *E.crassipes* stems and leaves is (*Z*)-3-hexen-1-ol and this constituent is attractive to both species and sexes of the biological control agents. Additional attractant volatiles may be found that are species--specific and that separate these two weevil species in the field. These volatiles may be additional plant – or weevil-produced compounds that have yet to be elucidated.

As there is no evidence supporting the existence of sexual or aggregation pheromones, or any non-chemical long-range intraspecific communication mechanism (e.g. sound), it is not surprising that both sexes exhibit similar olfactory capabilities. However, the weak attraction revealed to water hyacinth volatiles, might suggest a stronger importance of visual rather than visual clues on host-plant detection. The weevil can determine whether the host tissue is of good quality in terms of nutritional and moisture factors. Presumably, it must not only determine that the plant underneath is the proper host and is suitable for reproduction, but it must also judge potential competition by whether nearby areas have other weevils beginning their attacks. The host--specificity of both species has been demonstrated during extensive host testing and confirmed by observations after their release in numerous countries. Despite being released widely there are no reports of these weevils seeking out and damaging plants other than *E. crassipes*. Further support for their specificity comes from knowledge of *Neochetina* spp. life-history.

In light of all this evidence, releases of these weevils into new countries can now be carried out with very few hosts testing. For Portugal, for instance, we suggest only further testing, including both multiple choice and no choice oviposition, larval development and adult feeding, on *Apium graveolens*, *A. porrum*, *Phaseolus lunatus* and *Pyrus domestica*, to which are no available data, and constitute important agricultural cultures in the country.

AKNOWLEDGEMENTS

REFERENCES

Alm SR, Hall FR 1986 Antennal Sensory Structures of *Conotrachelus nenuphar* (Coleoptera: Curculionidae). *Ann Entomol Soc Am* 79:324-333.

Barata EN, Pickett JA, Wadhams LJ, Woodcock CM, Mustaparta H 2000 Identification of Host and Non-Host Semiochemicals of the Eucalyptus Woodborer, *Phoracantha semipunctata* Fabricius (Coleoptera: Cerambycidae), by Gas Chromatography Coupled Electroantennography. *J Chem Ecol* 26:1877-1895.

Bartlet E, Romani R, Williams IH, Isidoro N 1999 Functional Anatomy of Sensory Structures on the Antennae of *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae). *Int J Insect Morphol Embryol* 28:291-300.

Bernays EA, Chapman RF 1994 *Host-Plant Selection by Phytophagous Insects*. Chapman & Hall, New York. 312 pp.

Birch MC, Svihra P, Paine TD, Miller JC 1980 Influence of Chemically Mediated Behavior on Host Tree Colonization by Four Cohabiting Species of Bark Beetles. *J Chem Ecol* 6:395-414.

Bland RG 1981 Antennal Sensilla of the Adult Alfalfa Weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae). *Int J Insect Morphol & Embriyol* 10:265-274.

Bombosch S, Engler I, Gossenauer H, Herrmann B 1985 On the Role of *Pheroprax* Influencing the Settlement of *Ips typographus* on Spruce. *Z Angew Entomol* 100:458-463.

Borden JH, King CJ, Lindgren S, Chong L, Gray DR, Oehlschlager AC, Slessor KN, Pierce Jr HD 1982 Variation in Response of *Trypodendron lineatum* From Two Continents to Semiochemicals and Trap Form. *Environ Entomol* 11:403-408.

Bowen MF 1995 Sensilla Basiconica (Grooved Pegs) On the Antennae of Female Mosquitoes: Electrophysiology and Morphology. *Ent Exp Appl* 77:233-238.

Byers JA 1989 Chemical Ecology of Bark Beetles. *Experientia* 45:271-283.

Center TD 1994 Biological Control of Weeds: Waterhyacinth and Waterlettuce. In *Pest Management in the Subtropics: Biological Control-a Florida Perspective* (ed. D Rosen, FD Bennet, JL Capinera), 481-521. Intercept, Ltd, Andover.

Center TD, Durden WC 1986 Variation in Waterhyacinth / Weevil Interactions Resulting From Temporal Differences in Weed Control Efforts. *J Aquat Plant Manage* 24:28-38.

Center TD, Hill MP, Cordo H, Julien MH 2002 Waterhyacinth. In *Biological Control of Invasive Plants in the Eastern United States* (ed. RG Van Driesche, S Lyon, B Blossey, MS Hoddle, R Reardon), 41-64. USDA Forest Service, Morgantown, WV.

Center TD, Wright AD 1991 Age and Phytochemical Composition of Water-Hyacinth (Pontederiaceae) Leaves Determine Their Acceptability to *Neochetina eichhorniae* (Coleoptera, Curculionidae). *Environ Entomol* 20:323-334.

Chénier JVR, Philogène BJR 1989 Field Responses of Certain Forest Coleoptera to Conifer Monoterpenes and Ethanol. *J Chem Ecol* 15:1729-46.

Crawley MJ 1989 The Successes and Failures of Weed Biocontrol Using Insects. *Biocontrol News and Information*. 10:213–223.

Del Fosse ES, Perkins BD 1977 Discovery and Bioassay of a Kairomone from Water Hyacinth, *Eichhornia crassipes*. *Fla Entomol* 60:217-222.

DeLoach CJ 1975 Identification and Biological Notes on the Species of *Neochetina* that Attack Pontederiaceae in Argentina (Coleoptera: Curculionidae: Bagoini). *Coleop Bull* 29:257-265.

DeLoach CJ, Cordo HA 1983 Control of Water Hyacinth by *Neochetina bruchi* (Coleoptera: Curculionidae: Bagoini) in Argentina. *Environ Entomol* 12:19–23.

Dyer LJ, Seabrook WD 1975 Sensilla on the Antennal Flagellum of the Sawyer Beetles *Monochamus notatus* (Drury) and *Monochamus scutellatus* (Say) (Coleoptera: Cerambycidae). *J Morphology* 146:513-532.

Gaino E, Rebora M 1999 Larval Antennal Sensilla in Water-Living Insects. Microsc *Res Tech* 47:440-457.

Giblin-Davis RM, Pena JE, Duncan RE 1994 Lethal Trap For Evaluation of Semiochemical Mediated Attraction of *Metamasius hemipterus sericeus* (Olivier) (Coleoptera: Curculionidae). *Florida Entomol* 77:164-177.

Gopal B 1987 *Water Hyacinth*. Elsevier, Amsterdan. 471 pp.

Gries G, Gries R, Perez AL, Oehlschlager AC, Gonzales LM, Pierce HD, Kouda M, Zebeyou M, Nanou N 1993 Aggregation Pheromone of the African Palm Weevil, *Rhynchophorus phoenicis*. *F. Naturwis* 80:90-91.

Guido AS, Perkins BD 1975 Biology and Host Specifity of *Cornops aquaticum* (Bruner) (Orthoptera: Acrididae), a Potencial Biological Control Agent for Waterhyacinth. *Envirommental Entomology* 4(3):400-404.

Hallet RH, Gries G, Gries R, Bordea JH, Czyzewska H, Oehlschlager AC, Pierce HD Jr, Angerilli NPD, Ran A 1993 Aggregation Pheromone of Two Asian Palm Weevils, *Rhyncophorus ferrugineus* (Oliv.) and *R. vulneratus* (Panz.). *Naturwis* 80(7):328-331.

Hansson BS, Larsson MC, Leal WS 1999 Green Leaf Volatile Detecting Olfactory Receptor Neurones Display Very High Sensitivity and Specificity in a Scarab Beetle. *Physiol Entomol* 24:121-126.

Harley KLS 1990 The Role of Biological Control in the Management of Water Hyacinth, *Eichhornia crassipes. Bioc News & Inf* 11(1):11-22.

Hatfield LD, Frazier JL, Coons LB 1976 Antennal Sensilla of the Pecan Weevil *Curculio caryae* (Horn) (Coleoptera: Curculionidae). *Int J Insect Morphol & Embryol* 5:279-287.

Hill M, Coetzee J, Julien M, Center T 2011 Water Hyacinth. In *Encyclopedia of Biological Invasions* (ed D Simberloff, M Rejmánek), 689-692. University of California Press, Berkeley and Los Angeles, CA.

Hill MP, Cilliers CJ 1999 A Review of the Arthropod Natural Enemies, and Factors that Influence Their Efficacy, in the Biological Control of Water Hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae), in South Africa. *African Entomology Memoir* 1:103–112.

Holm LG, Plucknett DL, Pancho JV, Herberger JP 1977 *The World's Worst Weeds*: Distribution and Biology. University Press of Hawaii, Honolulu. 609 pp.

Isidoro N, Solinas M 1992 Functional Morphology of the Antennal Chemosensilla of *Ceutorhynchus assimilis* Payk. (Coleoptera: Curculionidae). *Entomologica* 27:69-84.

Jourdan H, Barbier R, Bernard J, Ferran A 1995 Antennal Sensilla and Sexual Dimorphism of the Adult Ladybird Beetle *Semiadalia undecimnotata* Schn. (Coleoptera: Coccinellidae). *Int J Insect Morphol Embryol* 24:307-322.

Julien MH, Griffiths MW 1998 *Biological Control of Weeds. A World Catalogue of Agents and Their Target Weeds.* 4th Ed. CAB International, Wallingford. 223pp.

Julien MH, Griffiths MW, Wright AD 1999 Biological Control of Water Hyacinth. The Weevils *Neochetina bruchi* and *N. eichhorniae*: Biologies, Host Ranges, and Rearing, Releasing and Monitoring Techniques for Biological Control of *Eichhornia crassipes. ACIAR Monograph*, 60. 87pp.

Lindgren BS, Borden JH, Chong L, Friskie LM, Orr DB 1983 Factors Influencing the Efficiency of Pheromone Baited Traps For Three Species of Ambrosia Beetles (Coleoptera: Scolytidae). *Can Entomol* 115:303-314.

Lopes O 2000 Estudo Ultraestrutural dos Sistemas Sensorial e Glandular de *Phoracantha semipunctata* (Coleoptera: Cerambycidae) Implicados na Comunicação Química. *Tese de Doutoramento*. Universidade de Évora, Portugal.

Lopes O, Barata EN, Mustaparta H, Araújo J 2002 Fine Structure of Antennal Sensilla Basiconica and their Detection of Plant Volatiles in the Eucalyptus Woodborer, *Phoracantha semipunctata* Fabricius (Coleoptera: Cerambycidae). *Arthrop Struct & Develop* 31(1):1-13

Merivee E 1992 Antennal Sensilla of the Female and Male Elaterid Beetle *Agriotes obscurus* L. (Coleoptera: Elateridae). *Proc Estonian Acad Sci Biol* 41:189-215.

Merivee E, Ploomi A, Rahi M, Bresciani J, Ravn HP, Luik A, Sammelselg V 2002 Antennal Sensilla of the Ground Beetle *Bembidion properans* Steph. (Coleoptera, Carabidae). *Micron* 33:429-440.

Merivee E, Rahi M, Bresciani J, Ravn HP, Luik A 1998 Antennal Sensilla of the Click Beetle, *Limonius aeruginosus* (Olivier) (Coleoptera: Elateridae). *Int J Insect Morphol Embryol* 27:311-318.

Merivee E, Rahi M, Luik A 1997 Distribution of Olfactory and Some Other Antennal Sensilla in the Male Click Beetle *Agriotes obscurus* L. (Coleoptera: Elateridae). *Int J Insect Morphol Embryol* 26:75-83.

Merivee E, Rahi M, Luik A 1999 Antennal Sensilla of the Click Beetle, *Melanotus villosus* (Geoffroy) (Coleoptera: Elateridae). Int J Insect Morphol Embryol 28:41-51.

Moreira I, Ferreira T, Monteiro A 1989 Aquatic Weed Bioecology and Controls in Portugal: a Review. In *Portuguese-German Cooperation in Applied Agricultural Activities* (ed. A Bianchi), 71–106. Univ of Vila Real Publications, Vila Real, Portugal.

Moreira I, Ferreira T, Monteiro A, Catarino L, Vasconcelos T 1999a Aquatic Weeds and Their Management in Portugal: Insights and the International Context. *Hydrobiologia* 415:229–234.

Moreira I, Monteiro A, Ferreira T, Catarino L, Franco JC, Rebelo MT 1999b Estudos Sobre Biologia e Combate do Jacinto Aquático (*Eichhornia crassipes* (Mart.) Solms-Laub) em Portugal. *Garcia de Orta (série Botânica)* 14(2):299-304.

Moser JC, Browne LE 1978 A Nondestructive Trap for *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae). *J Chem Ecol* 4:1-7.

Mustaparta H 1973 Olfactory Sensilla on the Antennae of the Pine Weevil, *Hylobius abietis. Z Zellforsch* 144:559-571.

Mustaparta H 1975 Responses of Single Olfactory Cells in the Pine Weevil, *Hylobius abietis* L. (Col.: Curculionidae*). J Comp Physiol* 97:271-290.

Oehlschlager AC, Perez AL, Pierce HD Jr, Gonzalez LM, Gries G, Gries R 1993 Aggregation Pheromone of the Asian Palm Weevil *Rhynchophorus bilineatus. Naturwis* 79:134-135

Oehlschlager AC, Pierce HD Jr, Morgan B, Wimalaratne PDC, Slessor KN, King GGS, Gries G, Gries R, Borden JH, Jiron LF, Chinchilla CM, Mexan RG 1992 Chirality and Field Activity of Rhynchophorol, the Aggregation Pheromone of the American Palm Weevil. *Naturwis.* 79:134-135.

Penfound WT, Earle TT 1948 The Biology of Water Hyacinth. Ecol Mon 18:447-472.

Perez AL, Campos Y, Chinchilla CM, Oehlschlager AC, Gries G, Gries R, Giblin-Davis RM, Castrillo G, Pena JE, Duncan RE, Gonzalez LM, Pierce HD Jr, McDonald R, Andrade R 1997 Aggregation pheromones and host kairomones of West Indian sugarcane weevil, *Metamasius hemipterus sericeus. J Chem Ecol* 23:869-888.

Perez AL, Gonzalez LM, Pierce HD Jr, Oehlschlager AC, Gries G, Gries R, Giblin-Davis RM, Pena J, Duncan RE, Chincifilla C 1994 Aggregation Pheromones of the Sugarcane Weevil, *Metamasius hemipterus sericeus* (Olivier). *Naturwis* 80.

Phillips TW, West JR, Foltz JL, Silverstein RM, Lanier GN 1994 Aggregation pheromone of the deodar weevIl, *Pissodes nemorensis* (Coleoptera: Curculionidae): Isolation and activity of grandisol and grandisal. *J Chem Ecol* 10:1417-1423

Pieterse AH, Murphy KL 1990 *Aquatic Weeds. The Ecology and Management of Nuisance Aquatic Vegetation.* Oxford Science Publications, Oxford. 593 pp.

Pophof B 1997 Olfactory Responses From Sensilla Coeloconica of the Silkmoth *Bombyx mori. Phys Entomol* 22:239-248.

Ramisch H 1986 Host Location by *Trypodendron domesticum* and *Trypodendron lineatum* (Coleoptera: Scolytidae). *Z Angew Zool* 73:159-198.

Rausher MD 1992 Natural Selection and the Evolution of Plant-Insect Interactions. In *Insect Chemical Ecology, and Evolutionary Approach* (ed. BD Roitberg, MB Isman), 20–88. Chapman & Hall, New York.

Rebelo MT, Center TD 2001 *Microsporidia & Neochetina. Water Hyacinth News* 4:5-7.

Ruther J 2004 Male-Biassed Response of Garden Chafer, *Phyllopertha horticola* L., to Leaf Alcohol and Attraction of Both Sexes to Floral Plant Volatiles. *Chemoecology* 14:187-192.

Sass H 1978 Olfactory Receptors on the Antenna of *Periplaneta*: Response Constellations That Encode Food Odors. *J Comp Physiol* 128:227-233.

Schneider D 1964 Insect Antennae. *Annu Rev Entomol* 9:103-122.

Sen A, Mitchell BK 2001 Olfaction in the Colorado Potato Beetle: Ultrastructure of Antennal Sensilla in Leptinotarsa sp. *J Biosci* 26:233-246.

Shields VDC, Hildebrand JG 1999 Fine Structure of Antennal Sensilla of the Female Sphinx Moth, *Manduca sexta* (Lepidoptera: Sphingidae): I. Trichoid and Basiconic Sensilla. *Can J Zool* 77:290-301

Smith CM, Frazier JL, Coons LB, Knight WE 1976 Antennal Sensilla of the Clover Head Weevil *Hypera meles* (F.) (Coleoptera: Curculionidae). *Int J Insect Morphol & Embryol* 5(6):349-355.

Sosa AJ, Cordo HA, Sacco J 2007 Preliminary Evaluation of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), a Candidate for Biological Control of Waterhyacinth. *Biol Control* 42:129–138

Stark JD, Goyer RA 1983 Life Cycle and Behavior of *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) in Louisiana: A Biological Control Agent of Waterhyacinth. *Environ. Entomol* 12:147-150.

Tilden PE, Bedard WD, Lindahl KQ Jr, Wood DL 1983 Trapping *Dendroctonus brevicomis*: Changes in Attractant Release Rate, Dispersion of Attractant, and Silhouette. *J Chem Ecol* 9:311-321.

Van Driesch RG, Bellows TS Jr 1996 *Biological Control*. Chapman & Hall, New York. 539 pp.

Wapshere AJ, Delfosse ES, Cullen JM 1989 Recent Developments In Biological Control of Weeds. *Crop Protection* 8:227–250.

Weissling TJ, Giblin-Davis RM, Gries G, Gries R, Perez AL, Pierce HD Jr, Oehlschlager AC 1994 An Aggregation Pheromone of the Palmetto Weevil, *Rhynchophorus cruentatus* (F.) (Coleoptera: Curculionidae). *J Chem Ecol* 20:505-515.

Weissling TJ, Giblin-Davis RM, Scheffrahn RH 1993 Laboratory and Field Evidence for Male-Produced Aggregation Pheromone in *Rhynchophorus cruentatus* (F.) (Coleoptera: Curculionidae). *J Chem Ecol* 19:1195-1203.

Zacharuk RY 1980 Ultrastructure and Function of Insect Chemosensilla. *Ann Rev Entomol* 25:27-47.

Zacharuk RY 1985 Antennae and Sensilla. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology* Vol. 6 (ed. GA Kerkut, LI Gilbert), 1 – 69. Pergamon Press, Oxford.