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BIOLOGICAL CONTROL OF WATERHYACINTH IN ZIMBABWE

BELLAH MPOFU

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND

RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Department of Plant Science

Macdonald College of McGill University

Montréal, Québec, Canada.

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Abstract

Bellah Mpofu Ph.D. Plant Science

In a survey conducted in Zimbabwe in 1993, waterhyacinth was present in seven out of the eight provinces. No control measures were imposed on 35% of the infested dams and 61% of the infested rivers, while in 47% of the infested dams and 11% of the infested rivers control of waterhyacinth was being attempted with a combination of 2,4-D and mechanical control methods. The population of *Neochetina eichhorniae* and *N. bruchi* declined during the period 1993 to 1995 in the Hunyani River system. Several fungi were isolated from diseased waterhyacinth, and *Fusarium moniliforme* (isolate 2ex12), *F. solani (isolates 5a ex25 and 2a3)* , *and F. pallidoroseum* (isolate 3ex1) were found to be the most pathogenic. Large numbers of viable conidia were produced in shake-flask liquid fermentation with modified Richard's medium and in solid fermentation with food grains. Conidia production in straw was poor with the exception of waterhyacinth straw. Host range studies conducted in pots and in the field indicated that *Commelina benghalensis* was moderately susceptible to both isolates of *F. solani* in the field, while *Setaria verticilata* grown in pots was moderately susceptible to isolate 2a3. *Brassica rapa* and *Crotalaria juncea* grown in pots were moderately susceptible to *F. moniliforme* but they showed no infection in the field. Fifty-nine additional plant species of ecological and agricultural importance were not susceptible to the *Fusarium* species. When *F. solani* , *F. pallidoroseum* and *Neochetina* spp. were used individually in ponds, they did not control waterhyacinth. When the fungi were combined with *Neochetina* spp., the area covered by waterhyacinth and the volume of waterhyacinth were significantly reduced.

Resumé

Bellah Mpofu Ph.D Phytotechnie

Une enquête conduite au Zimbabwe en 1993, a revelé la présence de la jacinthe d'eau dans sept des huit provinces. Aucune methode de lutte ne s' était imposée à 35% les reservoirs infestés et 61% les rivièresinfestées, tandis que une combinaison de 2,4-D et des méthodes de lutte mécanique avait été entreprise dans 47% des reservoirs infestés et 11% des rivières infestées pour le contrôle de la jacinthe d'eau. La population de *Neochetina eichhorniae* et *N. bruchi* a decliné pendant la periode 1993-1995 dans le réseau de la rivière Hunyani. Plusieurs champignons étaient isolés des plants malades de jacinthe d'eau, et *Fusarium moniliforme* (isolat 2ex12), *F. solani* (isolats 5a ex 25 and 2a3), et *F. pallidoroseum* (isolat 3ex1) étaient decouverts être les plus pathogéniques. Un grand nombre de conidies viables étaient prodiuts en sécouant des facons en fermentation liquide avec un milieu nutritif modifié de Richard et en fermentation solide avec des graines. La production de conidies sur des morceaux de tige etait pauvre excepté les morceaux de tige de la jacinthe d'eau. Des études de la gamme des hôtes conduites en pot et dans le champ ont montré que *Commelina benghalensis* était modérement susceptible aux deux isolats de *F. solani* dans le champ, tandis que *Setaria verticilata* cultivé en pot était modérement susceptible à l'isolat 2a3. *Brassica rapa* et *Crotalaria juncea* cultivés en pot étaient modérement susceptibles à *F. moniliforme* mais ils n'ont montré aucune infection au champ. En plus 59 espèces de plante d'importance ecologique et agricole n'étaient pas susceptibles aux espèces de *Fusarium*. Quant *F. solani*, *F. pallidoroseum* et *Neochetina spp.* étaient utilisés individuallement dans des bassins, ils n'ont pas contôlé la jacinthe d'eau. Quant les champignons étaient combinés avec *Neochetina spp.* la surface couverte par la jacinthe d'eau et le volume de jacinthe d'eau étaient significativement réduits.

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1. GENERAL INTRODUCTION

1.1. Taxonomy and importance of waterhyacinth

Waterhyacinth, *Eichhornia crassipes* (Mart.) Solms is a monocotyledon in the family Pontederiaceae, order Pontederiales. It is presumed to be a native of Brazil and is considered to be the most noxious of all aquatic weeds. It is ranked eighth amongst the world's worst weeds (Holm, Pucknett, Pancho and Herberger 1977).

1.2. Ecology of waterhyacinth

Waterhyacinth is a cosmopolitan, perennial, mat-forming aquatic plant species, which can tolerate a wide range of habitat conditions (temperature, illumination, pH, salinity, winds, current and drought) (Baruah 1984). It is primarily a fresh water plant but can survive up to 13 days in sea water (Anonymous 1980). Optimal growth conditions are a pH of 7.0, a phosphorus concentration of 20ppm and adequate nitrogen (Chadwick and Obeid 1966, Haller and Sutton 1973). Increase in the nutrient content of the water causes a corresponding increase in biomass of the fresh plant. The largest infestations are thus found in waters enriched by sewage and industrial effluent or by run-off from fertilized agricultural land.

Plants will grow in mud and can survive for months on a substrate of low moisture content. The range of waterhyacinth appears to be limited by cold temperatures to tropical or subtropical regions, although little experimental information exists on the cold-tolerance of the plant. It can withstand near freezing temperature of less than 5C for a limited period of time, but exhibits a steady decline in regrowth

potential (Penfound and Earle 1948, Owens and Madsen 1995). Plants whose foliage is severely damaged by frost, will regrow provided that the upper part of the rhizome has not been frozen (Mitchell 1978). In any case, seeds can survive cold conditions (Ueki and Oki 1979). The optimum temperature range for growth is 25C to 30C (Knipling, West and Haller 1970, Harley 1993a).

The plant is morphologically very plastic with rapid vegetative propagation, features that make it well adapted for long distance dispersal and successful colonization of diverse habitats. Vegetative reproduction occurs by the formation of ramets (vegetatively produced plants) at the apex of the stolons which are attached to the parent plant. However, propagation by seed, especially with regard to a primary infestation or a reinfestation following successful control with herbicides, is also very important. Plant doubling time can be as short as five days (Perkins 1978). *Azotobacter chroococum*, a bacterium which is capable of fixing nitrogen, was found in large numbers on the leaves of waterhyacinth and it was suggested that a symbiotic relationship with this microorganism perhaps partly accounts for the prolific growth of waterhyacinth (Iswaran, Sen and Apte 1973).

1.3. Distribution of waterhyacinth

Before the interference of humans, the distribution of waterhyacinth was restricted to tropical South America and perhaps parts of Central America and the larger Caribbean Islands (Sculthorpe 1971). The aesthetically pleasing appearance of waterhyacinth with its large lilac blooms was largely responsible for its being spread

around the world by humans during the 1800s and early 1900s.

Due to its floating habit, phenomenal powers of vegetative reproduction, ability to withstand saline waters for short periods, long lived seeds, and relative freedom from attack by insect pests and diseases *E. crassipes* has now spread through most of the warmer regions of the world (Matthews 1967, Scott, Ashton and Steyn 1979).

1.4. Ecological impacts and economic consequences

The primary ecological impacts of successful invasions of aquatic plants are brought about by the reduced water movements, decreased oxygenation from the atmosphere and an almost complete loss of light penetration into the water. The production and accumulation of large quantities of detritus and organic matter also promote bacterial activity with the consequent transformation of food webs to a detritus base. This transformation is accelerated with increasing levels of eutrophication. Secondary effects include stabilization and compaction of plant mats and their colonization by other aquatic and terrestrial species, leading to further compaction and the development of floating islands of secondary vegetation (Mitchell 1974, Ashton, Steyn and Wells 1979).

The major economic impacts of invasive aquatic plants arise from river blockages and interrupted water flows, evapotranspiration losses, difficulties in the purification of water to attain potable standards, development of increased breeding sites for vectors and intermediate hosts of human diseases such as malaria and bilharzia, and inhibition of recreational uses of water bodies (Mitchell 1974). To these

can be added the costs of eradicating or controlling the infestation, plus the costs of actions taken to ameliorate the ecological effects of the infestation and of control measures. In most cases, inadequate data preclude effective assessment of the financial implications. This is particularly true of attempts to quantify the aesthetic value of uninfested systems and is evident in the uncertainties surrounding estimates of the degree to which recreational activities have been inhibited (Viljoen and Haynes 1985).

Waterhyacinth infestations can have severe environmental effects by changing whole, often unique ecosystems. Mats of floating weeds reduce oxygen and light, and deplete the plankton which form the basis of the food chain. Native fish and aquatic plants and other wildlife are killed off, and balanced ecosystems that have taken millions of years to evolve are destroyed in a matter of years. The physical weight of the weed biomass can also threaten structures such as dams, bridges and pipelines.

Southern African rainfall patterns are highly seasonal. Most areas receive their highest rainfalls from individual storms during the austral summer (Schulze and McGee 1978). As a result most rivers have seasonal flow patterns. The growing needs for water have largely been met by the construction of numerous reservoirs and inter-catchment transfer schemes adjacent to developing areas. These have regulated and stabilized the natural hydrological regimes of most river systems in Southern Africa. However the increasing use of waterborne sewage and industrial effluent disposal systems has caused a progressive deterioration in the water quality in most of the rivers (Toerien, Hyman and Bruwer 1975). This in turn has aggravated the

problems associated with the provision of adequate supplies of potable water.

The indigenous aquatic biota of Southern Africa are adapted to the natural hydrological fluctuations that occur prior to river regulation (Mitchell 1974). Relatively stable open water habitats created by reservoir construction represent an entirely new environment for colonization. Most indigenous plant species are unable to occupy large areas of relatively deep, open water habitats and are confined to shallower marginal zones where they form a continuum between terrestrial and aquatic habitats (Twinch and Ashton 1983). Open water habitats that are underexploited by indigenous species are particularly prone to invasion by alien species (Mitchell 1974). The colonization of reservoirs and regulated rivers by exotic plant species that prevent or inhibit optimal utilization of the scarce water resources has immense financial and ecological implications.

Aquatic plants provide some of the most spectacular examples of successful invasions when introduced into habitats with which they are not in ecological equilibrium (Mitchell 1974). The competitive ability of alien species is manifest in rapid population growth leading ultimately to complete dominance of the available habitat at the expense of indigenous species. In Southern Africa, this sequence of events is particularly evident in those cases where free-floating alien plant species have been introduced into man-made reservoirs.

In Southern Africa, evapotranspirative water loss from *E. crassipes* plants usually varies between 1.2 and 2.4 times the evapotranspirative loss from an open water surface (Ashton, Steyn and Wells 1979). At Hartbeespoort Dam (in the

Republic of South Africa) water quality improved at first because *E. crassipes* prevented the development of excessive algal blooms. However, this had a negligible effect on costs because of subsequent increases in taste and odour problems due to high concentrations of detritus in the water (Ashton et al. 1979).

Four species of alien floating aquatic plants, waterhyacinth (*E. crassipes*), water fern [*Azolla filiculoides* Lam. (Salviniaceae)], Kariba weed [*Salvinia molesta* D.S. Mitchell (Salviniaceae)] and water lettuce *[Pistia stratiotes* L. (Araceae)], are of particular concern in Zimbabwe. *E. crassipes, S. molesta*, and *P. stratiotes* were deliberately imported by humans during the first half of this century as ornamental plants for fish ponds and aquaria (Jacot-Guillarmod 1979). The exact mode of entry of *A. filiculoides* into Zimbabwe is uncertain.

When introduced into suitable habitats in Zimbabwe, population explosions of these plants parallel invasions in other parts of the world (Mitchell 1974). The high degree of success attained by these species is due to their ability to modify their morphology to suit environmental conditions, regenerate from small pieces of vegetative material, and sustain very high rates of vegetative reproduction at low nutrient concentrations (Mitchell and Tur 1975, Musil and Breen 1977).

The rapid production of large areas of photosynthetic tissue by all four species enables them to shade out competing species. Free floating species possess a significant additional advantage in an almost complete independence of substrate conditions and water level fluctuations (Mitchell 1974). This, and their mobility due to wind and water movements, allows rapid occupation of the available water surface.

When several species of water plants compete for the same habitat, the largest and most vigorous species eventually dominates. This was shown in the Cahora Bassa reservoir, Mozambique, where *E. crassipes, Salvinia molesta, Pistia stratiotes* and *Azolla nilotica* were in competition (Bond and Roberts 1978). The small species *A. nilotica*, and then *S. molesta* were eliminated first and *E. crassipes*, the largest species, eventually dominated the flora.

1.5. Utilization of waterhyacinth

Waterhyacinth is not entirely without its virtues and the sheer biomass of plant material in waterhyacinth infestations has prompted investigation of various schemes for its utilization (Wolverton and Mcdonald 1979). It has limited application in the manufacture of poor quality paper, generation of biogas, effluent treatment and in certain handicrafts.

At an international conference on waterhyacinth held in India in 1983, papers on utilization for food and feed, paper and boards, biogas, waste water treatment, water quality management, fertilizer, and use as a source of carbon were presented (Thyagarajan 1984). It is unfortunate however, that the conference did not address the cost/benefit ratio of these proposals to utilize waterhyacinth or the practicality of putting them into commercial operation (Harley 1990).

Although in theory waterhyacinth can be used for a variety of purposes, before commercial production is undertaken, or even before fostering a cottage industry, a number of factors must be considered. Waterhyacinth is 95% water and economical

ways of harvesting and processing large masses of plants with such a high water content are difficult to achieve. As many infestations of waterhyacinth occur in relatively inaccessible regions, transport of unprocessed hyacinth and finished products must be considered (Harley 1990).

Numerous attempts to feed aquatic plants to animals have failed. This is mainly due to the high moisture content of aquatic weeds and their high mineral content (sodium, iron, potassium, and calcium are usually 3 to 100 times higher than comparable levels in terrestrial forages). However, when the plants are partly dewatered and ensiled, they are readily acceptable to both cattle and sheep. The aquatic plants' acceptability by animals is also increased when the plants are used as a supplement or mixed with other fodder (Pieterse 1974).

The products of waterhyacinth are of low value and seldom justify the costs of processing, and world-wide experience is that commercial utilization of waterhyacinth is not economically viable (Marshall 1993). Even if utilization was a viable option, the fact is that a commercial enterprise would require a continuous supply of raw material (waterhyacinth) and the associated problems would not be alleviated and waterhyacinth would continue to be a noxious weed (Irving and Beshir 1982, Phillip, Koch and Koser 1983). The world cannot tolerate the environmental cost of not dealing with waterhyacinth as a serious problem, it must be contained and effectively controlled (Gopal and Sharma 1981).

There is absolutely no doubt that the detrimental effects of waterhyacinth far outweigh its usefulness. Even maximum utilization will remove only a small amount

and does nothing to reduce the detrimental effects. Any conflicts of interest between advocates of utilization and those of unmitigated control may be avoided by applying a weed management scheme which allows small scale utilization while controlling problem infestations (Wright and Center 1984).

1.6. Utilization in Zimbabwe

In Zimbabwe most effort has been directed at improving methods of control and utilization of the weed has received little attention. When several local companies sought permission from government to utilize waterhyacinth for biogas production as well as purification of water, government turned them down citing the fact that waterhyacinth was a gazetted noxious weed and therefore it was illegal to move it. Large scale use would require a constant supply, something to be avoided. Utilization was considered to be very damaging to the environment because it required the establishment of `industrial' plants (and associated developments) in a National Parks area (Marshall 1993).

1.7 CONTROL METHODS

1.7.1. Mechanical control

Mechanical means of waterhyacinth control are relatively expensive but they have the advantage of being free from pollution causing action and consequently may be important for drinking water reservoirs. In general, the use of hand pulling, dung forks, nets, dredging mills, draglines, floating booms, or specially designed machines

for harvesting waterhyacinth are only of local significance (Harley 1990). Small infestations of waterhyacinth may be controlled by these methods but they require a high level of labour and mechanical equipment and are expensive. Manual removal can be useful in regions where there is an abundance of inexpensive labour and where the size of the infestation to be controlled is small. Furthermore, an infestation will regenerate from scattered plants and seeds unless regular inspection, coupled with further treatment, is continued indefinitely. The long term commitment required is difficult to maintain and very expensive (Harley 1988). Because of the occurrence of bilharzia in many tropical areas, long-handled tools must be used, as these make it possible for labourers to cut the weeds from the banks without entering the water (Pieterse 1974).

Permanent drainage to dry out a pond or lake will control waterhyacinth (Smith, Williams, Shaw and Green 1984). Seeds of waterhyacinth remain viable for up to 20 years and should the area again fill with water, seeds of waterhyacinth will germinate and reinfestation occurs (Forno and Wright 1981). Permanent drainage is a useful method of control in appropriate situations where loss of the water does not, for example, inconvenience villages, deny water to livestock, or destroy a local food source (eg. fish).

1.7.2. Chemical Control

Chemical herbicides are currently the principal means of control when an immediate solution to a waterhyacinth problem is needed. Preventive maintenance

programs also rely on chemicals to keep the weed populations at acceptable levels, and to prevent weed migration into unwanted areas.

Control of waterhyacinth is almost exclusively done with one herbicide, 2,4-D (2,4-dichlorophenoxyacetic acid). It is very effective and relatively inexpensive. Treated plants die and decompose in a few days, to a few weeks, and the level of control is usually in the range of 60 to 100 percent. At any time of the year, mats of waterhyacinth can be killed and sunk within two to three months after spraying (Pieterse 1974). The effective concentration is 15-30mg per kg waterhyacinth and it is applied at the rate of 2.0 to 11.2 kg(a.i.)/ha (Meadly 1954, Khanna 1959). Amine and ester forms of the herbicide are widely used.

The chief advantage of chemical control is the practical possibility of large scale application at a relatively low cost. Furthermore in an emergency situation where thick growth of waterhyacinth has to be cleared, it is perhaps the only practical method.

Despite the effectiveness of herbicides, reinfestation of the weed occurs from seeds or clonal multiplication of surviving plants (Gopal and Sharma 1981). Under favourable conditions waterhyacinth plants can grow faster than they can be killed with chemicals (Harley 1993a). Although chemical control may be used to suppress a large infestation, the weed grows back at such a rate that no headway is made (Harley 1988). A control strategy that relies on chemicals will require a high and continuing input of labour and mechanical equipment. Although waterhyacinth can be controlled with herbicides, this method is expensive and is least affordable in those developing

countries in which waterhyacinth creates the most problems. Using this method, long term control will not be achieved except for small infestations which are marginal for growth of waterhyacinth, and surveillance and control must continue indefinitely, or the infestation will regenerate from scattered plants and seeds.

There is also an environmental cost to using herbicides. If the water is used as a potable supply or in agriculture, then any consequences of applying chemicals to control waterhyacinth must be carefully considered. Residues of herbicide in the water and sediments affect the aquatic environment, and kill fish directly, or by reduced levels of dissolved oxygen caused by decaying weed biomass. Drift of the herbicide can affect nearby broadleaf crops.

In practice, then, mechanical and herbicidal control methods usually temporarily reduce waterhyacinth infestations rather than provide long-term control of them. The result is an increase in free water surface, less crowding of floating plants and a return to exponential growth. This is an expensive, temporary alleviation of the problem, and the remaining plants may grow until the infestation returns to its former size (Wright and Center 1984).

1.7.3 Biological control

Biological control of aquatic weeds can be defined as activities aimed at decreasing their populations to acceptable levels by means of living organisms (Pieterse 1977). Biological control is a proven, effective method for managing growth of floating aquatic vegetation. Successful programs have been implemented against

Alternanthera philoxeroides (Mart.) Griseb. (Coulson 1977), *Salvinia molesta* D.S. Mitchell (Room, Harley, Forno and Sands 1981) and *Pistia stratiotes* L. (Harley and Wright 1984).

In its native range in South America, waterhyacinth occurs in the coastal lowlands, along the margins of lagoons and in slowly moving water, along the edges of rivers and streams. It tends to be just one member of a mixed community of floating and anchored plants. Where mats do form they are often quickly destroyed by a combination of biological and hydrological forces, which keep the waterhyacinth population much below the nuisance level (Forno and Wright 1981).

Biological control of weeds can be accomplished by one of two main strategies: the classical (inoculative) strategy or the inundative (bioherbicide) strategy. The classical approach involves the importation and release of one or more natural enemies that attack the target weed in its native range, into areas where the weed is introduced and is troublesome and where its natural enemies are absent (Watson 1991). This is because an exotic weed is likely to have been introduced into a new area free of its normal natural enemies, creating an ecological imbalance which enables it to reproduce and spread much more successfully than in its native range, where it is attacked by a range of natural enemies reducing its competitiveness. It is the introduction of these natural enemies from the weed's area of origin into its exotic range, which leads to successful control of the weed by restoring a natural balance (Cook 1994). Classical biological control is particularly well suited to introduced, perennial weeds of uncultivated areas (Harley and Forno 1992). Classical biological

control does not pollute the environment and, as control agents are chosen for their inability to survive or reproduce on any plant except the target weed, this method of weed control is entirely compatible with responsible environmental management. The objective of classical biological weed control is generally not eradication of the weed species but the self-perpetuating regulation of the weed population at acceptable low levels (Watson 1991). Expenditure ceases after the first few years but the control achieved continues indefinitely (Harley 1990).

In the inundative approach, inoculum is prepared from axenic cultures of an indigenous pathogen and disseminated by artificial means to specific infestations in often precisely described geographic areas (TeBeest 1991). The application of an inundative dose of inoculum and its proper timing shortens the lag period for inoculum build up and pathogen distribution essential for natural epiphytotics (Charudattan 1990).

Unlike the classical agent, a pathogen to be used as a microbial herbicide is cultured in vitro on a large scale and applied in fairly high concentrations to the weed. The need for culturing makes facultative saprophytes and facultative parasites the agents of choice for this strategy. If necessary, microbial herbicides can be applied repeatedly during the growing season or annually using conventional pesticide application techniques. The classical approach differs from the inundative or bioherbicide approach primarily in its ecological rather than technological response to a weed problem (Wapshere 1982). The bioherbicide approach artificially increases the effectiveness of a candidate organism, whereas the classical approach relies on the

innate ability of the introduced biocontrol organism to become established and flourish in its new habitat.

Commonly the classical strategy is regarded as being more suitable for controlling aquatic weeds than the inundative strategy (Charudattan 1984). Many of the important aquatic weeds are exotics in areas where they cause problems, and exotic weeds are good targets for classical biological control. Furthermore, aquatic weeds usually infest large and inaccessible areas. In such situations, the classical biological control agents, with their capacity for active dispersal into remote areas of weed infestation, are generally more practical than microbial herbicides which must be applied in the target areas. The typical magnitude of aquatic weed problems also imposes a cost consideration that would favour classical biological control over microbial herbicides. However, aquatic weeds have high growth rates which are triggered by changes in water chemistry and weather, allowing them to outgrow pressures from biocontrol agents. Although neither a classical nor a microbial herbicide agent may maintain its effectiveness when the host population increases suddenly, a microbial herbicide, rather than a classical agent, can be more easily augmented through re-application of the inoculum to produce a rapid epidemic (Charudattan 1984). The use of indigenous pathogens also ensures that they are well adapted to the local environment (Boyette, Templeton and Smith 1979).

1.8. Biological control of waterhyacinth

Research into biological control of waterhyacinth began in 1961, and more than

70 species of arthropods capable of feeding on waterhyacinth have been found in different parts of the world (Perkins 1974). Six arthropods and three pathogenic fungi have been identified as biocontrol agents of waterhyacinth. Arthropods which feed on waterhyacinth include the mottled waterhyacinth weevil *Neochetina eichhorniae* Warner (Coleoptera:Curculionidae), the chevroned waterhyacinth weevil *N. bruchi* Hustache (Coleoptera: Curculionidae), the moths *Sameodes albiguttalis* Warren (Lepidoptera:Pyralidae), *Haimbachia infusella* (formerly *Acigona infusella*) Walker (Lepidoptera:Pyralidae), and *Bellura densa* Walker (Lepidoptera:Noctuidae), as well as a mite *Orthogalumna terebrantis* Wallwork (Acarina:Galamnidae). The fungi are *Acremonium zonatum* (Sawada) Gams, *Cercospora piaropi* Tharp, and *C. rodmanii* Conway (Waterhouse 1994). Of these nine species, the most effective control agents are the weevils *N. eichhorniae* and *N. bruchi*, the moth *S. albiguttalis*, and the fungus *C. rodmanii*.

1.8.1. Neochetina eichhorniae Warner (Coleoptera : Curculionidae)

N. eichhorniae adults are nocturnal. They feed preferentially on the narrow upper third of the petiole and on the upper surface of the lamina where they remove the epidermal layer and a few layers of the underlying cells to form small sub-circular scars with a diameter of 2-4mm (Delfosse 1978). One adult produces 20 feeding spots/day, and five adults can kill a waterhyacinth plant in the laboratory in about 10 days (Perkins 1974). Younger leaves are more commonly attacked than mature leaves (Stark and Goyer 1983). Weevils often feign death after being disturbed. The larva

burrow within the leaf tissue, causing extensive damage to the petioles, the stem, and the crown. Larval tunnels usually become necrotic and rot due to secondary microbial attack. The leaf may wither under severe larval attack. Final instar larvae create a cocoon out of cut waterhyacinth root hairs through which an oxygen connection may be maintained to the plant (Delfosse 1978).

Maximum oviposition in *N. eichhorniae* is 7.3 eggs/ female/day and eggs are usually placed just beneath the epidermal layer in the tender central leaf, or sometimes in the tender tissue at the base of other leaves and in ligules (Harley 1990). Females produce a maximum and average of 300 and 50 eggs, respectively during their lifetime (Delfosse 1978). The durations of developmental stages of *N. eichhorniae* are:

egg	7 -14 days
larva	75-90 days
prepupa and pupa	14-20 days
generation time	120 days and

a 1:1 sex ratio occurs in the field (DeLoach and Cordo 1976).

Adults are capable of dispersing at least 25km by flight in summer (Harley 1982). Starter colonies of *N. eichhorniae* may be obtained from the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia, the United States Department of Agriculture (USDA) in the United States of America and the International Institute of Biological Control (IIBC) in Trinidad (Harley and Forno 1990).

1.8.2. Neochetina bruchi Hustache (Coleoptera: Curculionidae)

The durations of the developmental stages of *N. bruchi* are:

egg	7.6 days
larva	32 days (approximately)
prepupa	7 days
pupa	23 days (approximately)
and generation time	96 days (Deloach and Cordo 1976)

Although the effects of *N. eichhorniae* on waterhyacinth are similar to those of *N. bruchi* there are several important differences in their behaviour and ecology. In addition to differences in life cycle, *N. eichhorniae* lays fewer eggs and its larvae develop more slowly than *N. bruchi*. *N. eichhorniae* prefers young central leaves for oviposition whereas *N. bruchi* prefers older bulbous leaves. In Argentina *N. bruchi* was more abundant in spring and summer whereas *N. eichhorniae* was more abundant in autumn and winter (Deloach and Cordo 1976).

Although sudden collapse of waterhyacinth mats due to weevil damage has been reported (Wright 1981), usually the weevils produce subtle changes that result in limiting of the dispersal of floating plants, decrease in plant size, suppression of growth in established infestations, a decline in the peak standing crop, a delayed regrowth in the spring, and a steady weed population level in the place of fluctuating annual levels (Forno 1981, Center, Steward and Bruner 1982, Stark and Goyer 1983).

1.8.3. Sameodes albiguttalis (Warren) (Lepidoptera: Pyralidae)

S. albiguttalis female moths lay an average of 300 eggs each, usually in injuries, on the leaves of waterhyacinth. The larvae feed inside the petioles and buds and pupate in white cocoons, usually in bulbous-type petioles (DeLoach and Cordo 1978). Attack may be heavy but is sporadic as this moth shows a preference for tender, often bulbous plants. The durations of the developmental stages are:

egg	4 days
larva	21 days
pupa	7 days
generation time	34 days (Deloach and Cordo 1978).

S. albiguttalis discriminates between different growth forms of waterhyacinth (Center 1984, Wright and Center 1984). Consequently moths disperse from areas of waterhyacinth where the plant form is unfavourable and concentrate in areas where it is favourable, resulting in a patchy distribution. Young larvae are unable to enter leaves with a hard cuticle and attack is predominately on young plants with bulbous petioles found in areas of low plant density, but may also occur on lush larger plants (Wright and Center 1984). *S. albiguttalis* is more active during cooler months (Center 1984).

1.8.4. Cercospora rodmanii Conway, Moniliales

Cercospora rodmanii, a fungal pathogen native to Florida was discovered in 1973 (Charudattan 1984). The fungus *C. rodmanii* is closely related to *C. piaropi*

which also attacks waterhyacinth (Conway 1976). The fungus produces small punctate spotting and chlorosis of the laminae and petioles followed by tip necrosis of the laminae and a spindly appearance of the petioles. Other microorganisms may invade the root area and cause rotting (Conway and Freeman 1977). Abbott Laboratories, Chicago, USA developed a wettable formulation of *C. rodmanii* and this has been extensively tested. *C. rodmanii* can severely affect waterhyacinth growth, especially in conditions that favour a reduced growth rate of the plant (Conway, Freeman and Charudattan 1978). Although the greatest effect of *C. rodmanii* was determined to be on the height of the waterhyacinth plant, death of waterhyacinth and the appearance of open water following the application of *C. rodmanii* to dense waterhyacinth mats has been reported (Conway *et al.* 1978).

C. rodmanii was released in South Africa as a classical biological control agent for waterhyacinth (Morris and Cilliers 1992). This is the first deliberate and authorized release of a foreign pathogen against waterhyacinth anywhere in the world (R. Charudattan, personal communication).

1.8.5. Vertebrates

In addition to attack by invertebrates and fungi, waterhyacinth is also eaten by manatees *Trichechus manatus* (Sirenia: Trichechidae) (Anonymous 1973) and the white amur or grass carp *Ctenopharyngodon idella* (Valenciennes: Cyprinidae) (Baker, Sutton and Blackburn 1974, Delfosse, Sutton and Perkins 1976). These vertebrates do not prefer waterhyacinth to many other aquatic plants and do not cause much damage

to dense stands of the weed (Waterhouse 1994). Furthermore management of grass carp is difficult as the fish is strongly influenced by ecological factors, such as temperature, water pollution and predation. Moreover, in densely populated areas it is not always possible to prevent over fishing and poaching. The manatee is an endangered species with a very low reproduction rate.

1.8.6. Integrated control of waterhyacinth

A multidisciplinary integrated control approach rather than a single control method offers the best prospect for long-term management of waterhyacinth (Pieterse 1977, Charudattan 1986). Integration of control is also imperative if society is to maximize the benefits from the biological control agents that are already in the field.

The particulars of waterweed infestations are highly location specific and therefore emphasis on each of the control methods will vary according to the circumstances, and over time. An integrated control programme is managed to avoid interference between different control methods, while maximising use of the water or the waterways for humans and animals. Physical and chemical control are phased out as soon as is practicable, as total reliance on biological control is the long term objective.

There are a number of ways to integrate weed control agents (Andres 1982, Shaw 1982, Smith 1982). One consideration for such a scheme would be the development of chemical control strategies to complement biological control (Charudattan 1986). Integrating herbicides with weed biocontrol insects may provide

the most satisfactory control by reducing weed density below the economic threshold more quickly than a biocontrol insect alone, or by increasing success with biocontrol insects where they would be marginally effective alone (Messersmith and Adkins 1995). Such a scheme might employ low rates, optimal timing and strategic placement of herbicides (Center *et al.* 1982).

Perkins (1977) conducted preliminary studies on integrating chemical and biological control of waterhyacinth and suggested the use of low dosage herbicide treatments in combination with insects. Reduced plant growth could allow time for populations of biological control agents to build up and sustain control (Center *et al.* 1982). Mortality of waterhyacinth weevils, *N. eichhorniae* and *N. bruchi* was not affected when the weevils were either sprayed or dipped directly in 2,4-D, diquat (6,7-dihydrodipyrido[1,2-α:2',1'-*c*]pyrazinediium ion), glyphosate (N-[phosphonomethyl]-glycine), or additives including a surfactant and a polymer (Haag 1986). Of the herbicides tested by Wright and Skilling (1987) 2,4-D was found to be virtually non-toxic to *N. eichhorniae* although other herbicides were toxic. When applied to waterhyacinth at reduced rates, 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.

Kairomones are allelochemicals of favourable adaptive value to the organism receiving them (Messersmith and Adkins 1995). A natural kairomone from waterhyacinth is a powerful insect attractant for *N. eichhorniae* and the waterhyacinth mite *O. terebrantis.* The kairomone was liberated when waterhyacinth was injured by

wounding or 2,4-D treatment (Perkins 1977). The kairomone enhanced control by attracting large numbers of *N. eichhorniae* weevils to waterhyacinth.

A negative aspect of chemical herbicide usage from a weed management standpoint is the herbicides' effects on the habitat of biocontrol agents (Center *et al.* 1982). When a large population of waterhyacinth is killed within a short period, a large proportion of the arthropods may also die of starvation (Wright and Center 1984). Any surviving adults and immature stages may not be able to migrate to untreated populations of the weed and thus face adverse physical conditions in the dying weed mats. The normal cyclic increase in arthropod populations would be disrupted (Wright and Center 1984). Where a bioherbicide is integrated with herbicides, the herbicides should not prevent pathogen infection by killing entire leaf tissues nor interfere with host susceptibility or pathogen virulence (Charudattan 1986).

The arthropod biocontrol agents of waterhyacinth appear to share life cycle and feeding behaviour traits that are complementary to one another, and beneficial interactions have been found under experimental conditions between an arthropod and a herbivorous fish (Delfosse et al. 1976). Waterhyacinth weevils could be integrated with bioherbicides. In nature, interactions between arthropods and several saprophytic and parasitic fungi and bacteria are common on arthropod-damaged waterhyacinth and there is often an increase in the incidence and impact of microorganisms following arthropod attacks (Charudattan *et al.* 1978).

1.9. Waterhyacinth in Africa

Infestations of aquatic weeds are now found throughout most of Africa. Waterhyacinth in West Africa is endemic in Nigeria and the Ivory Coast, its rapidly spreading through Ghana and is also found in Niger on the River Niger. It is found in every country of Eastern Africa and in all countries except Botswana in Southern Africa (de-Groot 1993).

1.10. Eichhornia crassipes in Zimbabwe

1.10.1 Historical perspective

Waterhyacinth was first observed in the Mukuvisi and Hunyani Rivers in 1937 (Edwards and Musil 1975). In the period 1941-1943 the plant became a serious pest in both rivers. In 1943 the Government added waterhyacinth to the schedule of noxious weeds in the Noxious Weed Act 22 of 1926. Lake McIlwaine (now Lake Chivero), was created by the damming of Hunyani River in 1952 and 1953. The lake was created for the primary function of supplying domestic and industrial water to the City of Salisbury (now Harare). The lake was later declared a Recreational Park and became part of the Parks and Wildlife estate (Gibbs-Russell 1977, Jacot-Guillarmod 1979). The lake is an important overwintering area for migrant waders and other water-birds.

Following the filling of the lake in 1953 there was almost immediate biological reaction. Waterhyacinth, which had been present in limited quantities in the Hunyani River system, particularly in the Mukuvisi River prior to the construction of

the lake, rapidly invaded the lake and was widespread on the lake surface by the end of 1953 (Jarvis, Mitchell and Thornton 1982). There was a marked growth of the plant between 1953 and 1962. Limited spraying with 2,4-D began in 1953 to control the spread of the macrophyte. It was finally brought under control through the use of 2,4-D by 1956, and a Weed Control Officer was appointed to prevent reinfestation. For about a decade from the late 1950s, the growth of waterhyacinth remained at fairly low levels. Regular inspection patrols of the Mukuvisi River upstream of the lake were undertaken, and any plants that were found were removed and destroyed.

However, following reorganisation of responsibilities within government departments, the patrols were discontinued, and within about three years waterhyacinth populations had increased to cover in excess of 30% of the lake surface, with the biggest concentration being in Tiger Bay where the plants were swept by the wind. By 1970, extensive deoxygenation of the waters of the lake, became a common feature. This was because a) control measures had been relaxed, b) seeds from the earlier infestation had been exposed and allowed to germinate in 1968 when the lake level fell to 3.5m below full supply level after the 1967-68 drought and c) the plant's inherent capacity for rapid vegetative growth. In the spring of 1970 it was estimated that the population was doubling in size every 10 to 12 days (Thornton 1982).

A number of management options were considered to control the spread of waterhyacinth on Lake Chivero (Mitchell 1979). The two alternatives considered were mechanical control and chemical control. Control by management of lake level was ruled out as the impoundment was the primary water supply for the City of Salisbury.

Biological control was not considered feasible due to the lack of a suitable indigenous parasite (Mitchell and Rose 1979).

Initially mechanical control was employed to remove the plants from their shoreline habitats. The Medical Officer of Health for Salisbury opposed spraying, on health grounds, and in subsequent years about 2.5 million Zimbabwe dollars were spent on physical control with little sign that the problem would be overcome. These measures employed power boats to push the weed into areas of shallow water where a net, pulled by a tractor, was used to haul the weed onto the shore from where it was removed for disposal. It was estimated that up to 100 tonnes of wet plant material were removed daily from the Tiger Bay area of Lake Chivero alone (Jarvis *et al.* 1982).

There was extensive deoxygenation of the water in the lake, and extensive fish kills at this time caused widespread public outcry. The increasing concentration of nutrients and inorganic ions combined with the deoxygenation and massive algal and macrophyte blooms, led to the lake being described as hypereutrophic in 1971 (Salisbury Sewerage Disposal Environmental Impact Statement Committee, 1979). Extensive publicity resulting from the popularity of the lake as a tourist resort and recreational facility brought about the first effective water pollution control legislation (Burke and Thornton 1982).

Efforts to remove the weed manually failed, and after a sometime acrimonious public debate, chemical control was carried out in September and October 1971 (Marshall 1993). The herbicide 2,4-D amine was selected as the chemical control

agent. Experiments were carried out to investigate the break down time of the active agent in the water and in the bottom muds (Jarvis et al. 1982). Aerial spraying of heavily infested sections of the lake was undertaken in stages. The City of Salisbury placed activated carbon filters on line in the water works as a precaution against contamination of water supplied for potable and irrigation usage (Jarvis et al. 1982).

The extensive use of 2,4-D helped to bring the waterhyacinth problem under control during 1971 and municipal waste water was beginning to be diverted to the irrigation schemes (Thornton 1982). Thus by 1972, there were no more floating mats of waterhyacinth, but only few plants trapped in vegetation along the shore and a recurring problem with the germination of seedlings on exposed mud banks. Another Weed Control Officer was appointed and the control measures that were instituted kept the weed under control for several years (Jarvis et al. 1982).

During the 1972-73 drought, the lake level fell to about 6m below full supply level and exposed many of the seeds deposited during 1971 (Marshall 1993). The plants that germinated in the exposed mud were destroyed before the lake level rose again and there were no floating plants on the lake at this time. These control measures were so successful that they gave the illusion that the problem had been solved and the control measures were abandoned in the early 1970s (Jarvis et al. 1982). Effluent diversion continued in stages through to 1977 when nearly 100% of the municipal wastewater was being treated to tertiary standards. The last fish kill was reported in January of 1976 and although periodic algal blooms still occurred, Lake Chivero was bordering on mesotrophy (Thornton 1980).

The next outbreak followed the 1983-84 drought which caused the lake level to fall by 12m in late 1984 (Marshall 1993). This exposed many seeds which had been lying on the lake bottom since 1971, which germinated and became floating plants once the lake level rose again. Extensive weed mats appeared on the lake in 1985.

Measures to control this outbreak of waterhyacinth began in January 1986 and both chemical and manual removal were employed. The use of 2,4-D (or any other herbicide) was prohibited by the government in 1987 (Marshall 1989). That same year the situation at Lake Chivero was declared a national disaster. Mechanical control was employed, but this was expensive (2.5 million ZWD were used) and ineffective (Marshall 1993). The weed mat continued to spread, despite the increased manual control efforts, and by August 1989 it covered about 15% of the lake's surface (Marshall 1989). In October 1989 a symposium organized by the Research Council of Zimbabwe recommended that the weed be sprayed with glyphosate (a compromise reached because of opposition to 2,4-D). However, this decision was not implemented (Greathead and deGroot 1993).

Lake Chivero overflowed in 1990 and huge quantities of weed were swept over the spillway, completely blocking it, and on the night of 9 April 1990, the weed destroyed Harare's two main water intake pipelines that draw water from Lake Chivero. One effect of the resulting lack of water was a considerable reduction in the area sown to wheat (Marshall 1993). At the same time it was feared that the structure of the dam itself, or downstream structures including rail and road bridges, could be damaged. It is estimated that some 50 000-100 000 tonnes of weed were washed over

and it took eight months to remove it; the cost of the damage and weed removal have never been publicly revealed but estimates suggest that it might be as much as 7 000 000 ZWD (Marshall 1989).

The President of Zimbabwe visited the lake after the catastrophy, after which 2,4-D use was again authorized. The first spraying operation, carried out on 7 August 1990, was inadequately planned and had no lasting effect on the weed mat, which by January 1991 covered about 25 % of the lake (Marshall 1991). An intensive spraying campaign begun in February 1991 reduced the size of the weed mat.

However, to this day the weed has continued to reinfest the lake. This happens in spite of legislation passed against re-infestation of Lake Chivero which is controlled by the provisions of the Parks and Wildlife (General) Regulation, 1975 (Rhodesia Government Notice 965 of 1975) which states that it is illegal to import, grow or to fail to destroy aquatic weeds namely *Salvinia molesta* D.S. Mitchell (Kariba weed), and *Eichhornia crassipes* (waterhyacinth) if they occur on ones property. Although the Noxious Weed Act is administered by the Ministry of Agriculture, activities directed at control of floating aquatic weeds are fragmented over several government and municipal departments. There appears to be no co-ordination of activities and no common policy (Harley 1993b).

1.10.2. Ecology of Lake Chivero

Munro (1966) defined the shoreline habitats of Lake Chivero in terms of the presence or absence of aquatic macrophytes, and the major species of macrophyte

where the plants were present. Relatively few areas of the lake shore were free of macrophytes, most of these being granite outcrops or steep, sand and gravel shores in the main lake basin. Munro (1966) identified the major species of aquatic plants in the impoundment as *Phragmites mauritianus* Kunth, *Typha latifolia* L., *Aponogeton desertorum* Spreng.f., and *Nymphea caerulea* Savigny. In the more riverine upper reaches of the lake, he noted extensive beds of *Polygonum senegalense* Meisn which extended some 30 to 40m out into the lake. These stands were often associated with *Lagarosiphon major* (Ridl.) Wager, forming dense mats, although the latter was also distributed through other areas of the lake.

The distribution of aquatic macrophyte species changed considerably since 1963, possibly as a result of measures taken to control the spread of *Eichhornia crassipes* using 2,4-D (which kills a variety of broadleaf plants). *Typha* spp. stands in the lake basin were greatly reduced following implementation of waterhyacinth control measures in 1971 and *Nymphea* beds were decimated. *Phragmites* spp. stands, on the other hand became slightly more abundant and *Polygonum* increased (Thornton 1982).

Associated with these changes were a number of changes in the avifauna (Jarvis et al. 1982). Some species increased in occurrence after 1971 when the aquatic vegetation had declined. These included *Alopochen aegyptiacus* (Linnaeus 1766) the Egyptian Goose, *Dendrocygna bicolor (*Vieillot 1816) the Fulvous Duck, *Anas hottentota* (Eyton 1838) the Hottentot Teal, *Sarkidiornis melanotos* (Pennant 1769) the Knob-bill Duck, *Netta erythrophthalma* (Wied 1832) the Red-eye Pochard, *Anas erythrorhyncha* (Gmelin 1789) the Red-bill Teal, *Plectropterus gambensis*

(Linnaeus 1766) the Spurwing Goose, *Dendrocygna viduate* (Linnaeus 1766) the White-face Duck and *Fulica cristata* (Gmelin 1789) the Red-knobbed Coot. Since 1971, all birds that utilized floating vegetation for food, either directly or by feeding on life forms in the vegetation, declined which included *Nettapus auritus* (Boddaaert 1783) the Pygmy Goose, *Thalassornis leuconotus* (Eyton 1838) the White-back Duck, *Tachybaptuis ruficollis* (Pallas 1764) the Dabchick, *Porphyrio porphyrio* (Linnaeeus 1758) the Purple Gallinule, *Porphyrio alleni* (Thomson 1842) the Lesser Gallinule, *Gallinula chloropus* (Linnaeus 1758) the Moorhen and *Actophilornis africanus* (Gmelin 1789) the African Jacana (Thornton 1982).

The elimination of floating vegetation must also have resulted in a large reduction in snail and other life forms, thus reducing available food for several bird species. Since light penetration and wave action would also have increased, this probably produced changes in the planktonic flora and fauna. Some freshwater lamellibranch species were apparently absent from the lake in 1973 whereas they were abundant in 1962-63, and although it is likely that drought and water fluctuations produced these anomalies it could be worth considering the possible effects of herbicide application (Tinker 1971, Marshall 1975).

1.10.3 Water Pollution

Zimbabwe lies within the tropics. Its average rainfall ranges from 1700 mm in the east to 320 mm in the south-west. Most of this rain falls between December and February during the rainy season and hence most of the rivers are non-perennial and

cease flowing during the dry season. There is considerable variation from year to year in the run off (Munzwa 1982). The present position is that most of the major urban centres are supplied from man-made lakes. As many of these centres lie along the central watershed, cities are situated upstream of their sources of water supply. This is true of Harare which is located upstream of Lake Chivero and hence any waste products from the city re-enters its source of supply. These waste products would include urban run-off, sediments, and domestic and industrial effluents (Munzwa 1982). Though raw sewage is broken down with biological and chemical treatment before it reaches the lake, enormous quantities of phosphates and nitrates are washed into the lake (Williams 1991).

Water was first drawn from Lake Chivero in November 1953 and until 1959 little change was observed in the quality of the raw water. From 1960, periodic algal blooms appeared in the lake and caused purification difficulties at the works. Public complaints about water pollution increased considerably due to the expansion of urban, industrial and mining activity. Several lakes were showing signs of eutrophication and in particular Lake Chivero was giving cause for concern. Eutrophication is the enrichment of an aquatic ecosystem with plant nutrients (specially phosphorus and nitrogen) resulting in an increased production at all trophic levels (Robarts 1982). In most non-eutrophic lakes phosphorus is usually the nutrient which limits algal growth (Robarts and Southwall 1977) and therefore the higher trophic levels (Melack 1976). Intensive investigations were made at this time to ascertain the cause of this intensive algal bloom in the lake (Munro 1966, Marshall and Falconer 1973a, 1973b; Robarts

1979, Thornton 1980). These investigations revealed that the major contributing factor causing this condition in Lake Chivero was the drainage from the Harare urban area and in particular the sewage effluents which, although of high quality, contained high concentrations of nitrogen and phosphorus (Marshall and Falconer 1973a, 1973b). The drainage had caused rapid eutrophication of Lake Chivero resulting in a typical eutrophic lake with algal activity confined exclusively to the epilimnion and a reservoir of available nutrients in the hypolimnion. The normal ecology had been disturbed with the prolific development of blue-green algae, mainly species of *Microcystis* and *Anabaena* (Thornton 1982).

At about the same time as these studies were being carried out, the Government promulgated two sets of regulations to control pollution and to protect the existing and future water resources of Zimbabwe. The Water Pollution Control (Waste and Effluent Water Standards) Regulations, 1971 (subsequently replaced by the Water (Effluent and Waste Standards) Regulations, 1977) dealt with the standards of effluents that may be discharged into natural water courses. In addition the Public Health (Effluent) Regulations, 1972 dealt with the standards required for the re-use of effluents by irrigation.

Up until this time the research being carried out by the City Engineer's Department was aimed at reducing the amount of nutrients entering Lake Chivero in the hope that this could be done economically and improve the quality of the water in the lake. The advent of these regulations made it necessary to irrigate all the effluent arising in the City of Salisbury at all times of the year so that the regulations would be

complied with.

Considerable success was achieved in controlling nutrient supplies to the lake (Thornton 1982) but population growth in Harare and the relatively new town of Chitungwiza during the last decade seem to be negating these achievements. The fact that diffuse source storm water run-off can potentially supply sufficient nutrients to lakes such as Lake Chivero to maintain a eutrophic state is cause for concern particularly when the continued expansion of urban centres such as Chitungwiza is considered (Munzwa 1982). It suggests that despite the effective control of point source discharges through comprehensive water pollution control legislation, Zimbabwean lakes may continue to be or become eutrophic. To prevent such occurrences in the future it will be necessary to control, through legislation if necessary, the entry of storm water run-off into natural water courses. The problem of eutrophication is, of crucial importance to the problem of waterhyacinth as it is a factor contributing to its rapid growth on Lake Chivero and other water impoundments in Zimbabwe. Any strategy to reduce aquatic weeds must include identification and reduction (preferably elimination) of sources of nutrient enrichment (Harley 1993a).

1.11. Objectives of the thesis

In view of the problems that have been caused by waterhyacinth in Zimbabwe for more than fifty years, and the unsuccessful attempts to control the weed, using mechanical and chemical methods, this project was initiated. Several issues pertaining to the current status of waterhyacinth in Zimbabwe are not known, and these include:

a)the present extent of waterhyacinth infestation, b)the status of biological control agents reported to have been released in Zimbabwe, and c)whether there are any indigenous natural enemies of waterhyacinth in Zimbabwe.

Therefore the objectives of the thesis were:

1) to determine the extent of the waterhyacinth problem in Zimbabwe,

2) to determine whether waterhyacinth weevils have been established and have dispersed in the waterhyacinth populations in Zimbabwe,

3) to form a baseline of data on the fungi associated with diseased waterhyacinth plants in Zimbabwe, and to indicate which have potential as mycoherbicides,

4) to determine media suitable for spore production for the most virulent pathogens,

5) to determine the host range of the most virulent pathogens, and

6) to evaluate the combined effect of waterhyacinth weevils and the most virulent pathogens.

2. EXTENT OF WATERHYACINTH, ITS NATURAL ENEMIES, AND CONTROL EFFORTS IN ZIMBABWE

2.2.1 Introduction

2.1.1. Problems caused by waterhyacinth

Waterhyacinth is currently the worst aquatic weed in Zimbabwe. This is as a result of high rates of growth and reproduction, high competitive ability relative to other floating aquatic plants, movement of plants by wind and water currents, and because of its attractive flowers, spread by humans (Harley 1994). In many areas, waterhyacinth clogs rivers, drainage and irrigation channels, and accelerates silting up of the channels reducing their discharge capacities. It obstructs navigation canals and limits recreational facilities such as fishing, hunting, boating, and swimming.

Waterhyacinth cover is detrimental to fish due to lowered dissolved oxygen, inhibition of phytoplankton production and restriction of the movement and feeding of larger fish (Baruah 1984). It provides both the habitat and food for several vectors of diseases including malaria, encephalitis, and schistosomiasis. Spread of mosquito species *Anopheles cruciana*, and *Anopheles quadrimaculatus*, which are vectors for malaria, is encouraged by waterhyacinth, which provides protection to larvae from predators, and also through facilitation of surface breathing of larvae by restricting the movement of the water (Baruah 1984). It is difficult to control these species of mosquitoes without controlling the aquatic vegetation.

Waterhyacinth vegetation provides habitat for fresh water snails, which are intermediate hosts for schistosomiasis (bilharzia), which is one of the most critical and

insidious diseases of the tropics and subtropics. It has been claimed that the cholera organism concentrates around the roots of waterhyacinth (Harley 1994). This weed also provides ideal sites for the growth of molluscs which impart undesirable taste and odour to water.

One of the most insidious effects of waterhyacinth infestation is the loss of water through evapotranspiration. Loss from waterhyacinth covered water has been reported to be 1.26 to 9.84 times higher than evaporation from open water (Timmer and Weldon 1967). Water loss due to waterhyacinth infestation in the Nile has been estimated to amount to 7.12 $x10^9$ m^3/year which represents one tenth of the average yield of the Nile (Pieterse 1979). This water loss may reach serious proportion in areas of water shortage.

Waterhyacinth also reduces the water storage capacity of reservoirs by displacing large volumes of water. In El Salvador 405 hectares of waterhyacinth displaced 1.22 x 10^6 m^3 of water in Lake Rio (Gopal and Sharma 1981). The direct impact of waterhyacinth on the quality of fresh water is also an important aspect. By reducing the penetration of light and affecting growth of phytoplankton, it lowers the pH and the concentration of dissolved oxygen and increases the level of carbon dioxide.

Waterhyacinth interacts with native vegetation and may significantly reduce the population density of individual species by shading and competition for essential resources. Prolonged competition by a dense growth of waterhyacinth may be expected to reduce the floral biodiversity of a water body (Harley 1994).

2.1.2. Control of waterhyacinth in Zimbabwe

Before national independence, 2,4-D was used to control waterhyacinth in Zimbabwe and during the period 1953 to 1979, 2,4-D was widely used on Lake Chivero (Table 1). After independence the use of 2,4-D in water bodies was banned, and manual control was employed to no avail.

The decade from 1982 to 1992 was dry with the country receiving 78.5% of normal rainfall, based on a 30-year average (Mheen 1995). Many waterhyacinth seeds were exposed, as water levels receded, leading to an increase in waterhyacinth infestations (Marshall 1993). Use of 2,4-D was resumed in 1990 and has continued to date, with no end in sight to the problem of waterhyacinth infestations.

Biological control has been used in other countries to control waterhyacinth with various degrees of success (Forno 1981, Center et al. 1982, Charudattan 1984, Galbraith and Hayward 1984, Waterhouse 1994). In its native environment, waterhyacinth is not a pest, but is kept in check by natural enemies including insects and fungal pathogens (Galbraith and Hayward 1984).

A decision to initiate a biological control project for a weed must be based on factual information. Ideally foreign exploration should be preceded by field surveys in the area of introduction (Schroeder and Goeden 1986). These should determine the distribution of the weed and assess the possibility of further spread,

Table 1. The use of 2,4-dichlorophenoxyacetic acid at Lake Chivero in pre-independence Zimbabwe.

Year	Litres Used	Comments
1953	14 080	Waterhyacinth widely spread
1954-55	14 080	Waterhyacinth present
1955-56	14 080	
1956-57	14 080	
1957-58	7 500	
1958-59	7 040	
1959-63	no record	No records available
1963-64	1 267	Waterhyacinth still abundant
1964-65	nil	Waterhyacinth greatly reduced
1965-66	619	
1966-67	1 408	Waterhyacinth still widespread
1967-68	1 408	
1968-69	2 323	
1969-70	1 232	Aerial spraying
1970-71	3 801	Extensive aerial spraying
1971-72	1 480	Minimum aerial spraying.
1972-73	no record	Shoreline patches sprayed
1973-74	no record	Shoreline patches sprayed
1974-75	290	Minimum manual spraying.
1975-76	290	Less used than last year
1976-77	no record	Very little used
1977-78	nil	
1978-79	302	Patches on shore

Source: Jarvis, Mitchell and Thornton 1982.

make observations on any interaction with the native flora, describe the ecological characteristics of the area of introduction, identify and resolve any conflict of interests, estimate economical loss attributable to the weed, prepare an inventory of arthropods and pathogens attacking the weed in its introduced range, and prepare a historical account of its introduction and spread.

Extensive general surveys have been conducted to find potential pathogen biological control agents for waterhyacinth (Naj Raj and Ponnappa 1970, Freeman 1977, Hettiarachchi, Gunasekera and Balasovriya 1983, Abdel-Rahim 1984). Several pathogens have been evaluated for biological control of waterhyacinth (Table 2).

2.1.3. Attempts at biological control in Zimbabwe

Attention has also turned to biological control of waterhyacinth in Zimbabwe. There is a fledgling project on biological control of waterhyacinth in the Plant Protection Research Institute of the Department of Research and Specialist Services in the Ministry of Agriculture, but it has limited funds and resources. *Neochetina eichhorniae* was first taken to Zimbabwe from South America in 1971 (Julien 1992) but is not known to have been released (Harley 1993a). In 1988, 700 adult weevils of *N. eichhorniae* and 400 adult weevils of *N. bruchi* were obtained from the Agricultural Research Services in Florida, USA by the Plant Protection Research Institute (Chikwenhere 1994).

Table 2. Distribution of some virulent pathogens of waterhyacinth

Pathogen	Geographical area	Ref[1].
Acremonium zonatum (Sawada) Gams	Central and S. America U.S.A. Australia	2 2 5
Alternaria eichhorniae Naj Raj and Ponnappa	India	1
Bipolaris stenospila Drechs.	Central and S. America U.S.A.	2 2
Cercospora piaropi Tharp.	U.S.A. India Sri Lanka Australia South Africa	2 1 3 5 6
Cercospora rodmanii Conway	U.S.A.	2
Phoma sorghina (Sacc.) Boerema, Dornbosch and Van Kesteren	Sudan	4
Rhizoctonia solani	Central and S. America U.S.A. India	2 2 1
Uredo eichhorniae Gonz.-Frag. and Cif.	Central and S. America	2

[1] References

1. Naj Raj and Ponnappa 1970
2. Freeman 1977
3. Hettiarachchi, Gunasekera and Balasooriya 1983
4. Abdel-Rahim and Tawfig 1984
5. Galbraith and Hayward 1984
6. Cilliers 1991

The weevils were released onto the waterhyacinth populations on the Hunyani River system, at Lake Chivero, Pension Farm, Skyline Motel, Riverside Farm and St Mary Township, from January 1990 to October 1991 (Chikwenhere 1994). Unfortunately, the release sites were sprayed with herbicide, killing the plants and preventing the agents establishing (Harley 1993a). Releases were continued in 1992, but the impact of the weevils on waterhyacinth has not been assessed (Chikwenhere 1994).

2.1.4. Objectives

The objectives of this part of the study were:

1. to determine the extent of the waterhyacinth problem in Zimbabwe,
2. to determine whether waterhyacinth weevils have been established in the waterhyacinth populations in Zimbabwe, and if they have established, whether they have spread, and
3. to form a baseline of data on the fungi associated with diseased waterhyacinth plants in Zimbabwe, and to indicate which have potential as mycoherbicides.

2.2. Materials and Methods

2.2.1. Questionnaires

Questionnaires were sent to personnel of the Department of Agricultural, Technical and Extension Services (Agritex) in all the eight provinces of Zimbabwe in

January 1993. The questionnaire was structured as shown in Appendix A. Officers from the different provinces were requested to list the dams and rivers that were infested with waterhyacinth in the different provinces. They were also requested to record the extent of the waterhyacinth infestation. The rating system used was a) 50-100% water cover, b) 20-50% water cover, c) less than 20% water cover (generally in floating mats), and d) less than 20% water cover (growing mainly along the banks). The last question requested information on methods that were being used to control the weed. After responses were received from the provinces, visits were made where possible, to the different water impoundments to confirm the information received, and to obtain additional information from local people and government officers working in the area.

2.2.2. Monitoring of waterhyacinth weevils

During the survey, as visits were made to the different water bodies to confirm presence of waterhyacinth, plants were examined for the presence of typical *Neochetina* spp. feeding marks as well as for the presence of adult *Neochetina* weevils.

In 1993 initial monitoring was done twice at Marimba camping ground on 2 and 30 March. Thirty plants were sampled on both occasions. However further monitoring was not possible because it was not possible to maintain monitoring sites, since they were being sprayed with 2,4-D almost fortnightly. Furthermore the continuous Hunyani River system was the only one which contained the weevil, and this whole system was being sprayed with 2,4-D. Monitoring was resumed in January

to June 1994 and then again in February to August 1995.

In 1995 the weevils were present in very low numbers and would hide during the day making collection very slow and tedious. Wright and Center (1984) studied interactions between *N. eichhorniae* and waterhyacinth and found a constant relationship between the number of weevil feeding marks and the number of adult *N. eichhorniae* on the plants. Because *N. eichhorniae* and *N. bruchi* as well as their feeding scars, are difficult to distinguish, weevil feeding marks were used to monitor the trend in the combined population of both waterhyacinth weevils. Monitoring visits were made once in two months. One hundred plants were randomly selected in the waterhyacinth population growing on the Hunyani River, behind the barrier at Skyline Bridge, as this area was sprayed less often than those nearer Lake Chivero.

During the survey waterhyacinth plants were also examined for any other arthropods that were feeding on the weed. Arthropods were collected and transported to the laboratory in glass jars with perforated tops in which was a waterhyacinth plant. The arthropods were then identified by T. Marange, an Entomologist with the Plant Protection Research Institute in Harare.

2.2.3. Collection of Pathogens

During the survey information on occurrence of pathogens attacking waterhyacinth was collected. Waterhyacinth plants with leaf spots, blights and chlorosis were collected from all sites that were visited during the period January to April 1993. A total of 14 collection visits were made to different sites on different

water bodies. Diseased plants were placed in paper bags and transported to the laboratory.

Isolation of causal organisms was done using standard isolation techniques (Tuite 1969). Diseased leaves and petioles were carefully washed several times in running water to remove soil particles adhering to the leaves. Small pieces of tissue sections (0.5 x 0.5cm) were dissected from the margins of the lesions and surface disinfected by placing the sections in 70% ethanol for 3 seconds, followed by 0.5% sodium hypochlorite for 10 seconds and rinsed in sterile distilled water three times. Five to six pieces were cut from each plant from the leaves as well as the petioles. The surface sterilized pieces were dried between two sterile filter papers, and then placed on both potato dextrose agar (PDA) and water agar (WA) plates which were incubated in continuous fluorescent and incandescent light at an intensity of 300μEm^{-2}s^{-1}, at 25C for one week.

Mycelial growth was observed originating from the tissue sections and subcultures were made by transferring hyphal tips to PDA plates. Single spore isolates were then obtained from these cultures by streaking a needle bearing conidia on PDA plates. Hyphal tip and single spore isolation techniques were employed to obtain pure cultures of fungal isolates. Pure cultures were maintained at 4C on PDA slants.

2.2.4. Plant propagation

Waterhyacinth plants were grown in 100L oil drums cut in half and filled with 40L of water. They were fertilized with 2ml/L of Groesia liquid plant food (5N,

$6P_2O_5$, $7K_2O$, Mg (0.1%), Zn (0.03%), minimum S 0.15%) (a commercial liquid fertilizer used in rose production and prepared by Marlborough Nurseries (Private) Limited, Harare). Drums with waterhyacinth were maintained outdoors. A fresh supply of the groesia solution was added every fortnight. A healthy stock of waterhyacinth was started by removing all but the first leaf from large plants brought in from dams in Mutoko and Masvingo and from the Hunyani River. Healthy daughter plants, (ramets) were selected for experimental work.

Koch's postulates were verified for each fungal isolate. Each of the fungal isolates was established on PDA plates (five plates each) using mycelium plugs from the stock culture. The plates were incubated at 25C for 14 days and observed daily for signs of fruiting bodies and conidia. Prior to inoculation with fungi, two leaf blades on each plant (three to four leaf stage) were scraped lightly with a sterile inoculating needle, to simulate weevil feeding scars. Mycelial discs taken from the edges of the colony, were placed on injured and uninjured portions of the lamina. Plants used as controls were injured in a similar way. Each plant, in each pot was covered with a clear polythene bag for 24 hours, and left in the shade, in order to maintain conditions of high humidity around the inoculated tissue. After the plastic bags were removed the plants were left in the shade where temperature ranged between 17 and 22C. They were watered twice a day with tap water to which 2ml/L of groesia plant food had been added. Plants were examined weekly for up to four weeks for the presence of disease symptoms. Fungi which were non-pathogenic to waterhyacinth were discarded.

At the end of four weeks, leaf discs from diseased plants were surface sterilized, and plated again onto PDA plates, using standard isolation techniques, described above, in order to complete the verification of Koch's postulates. The fungal colonies were then observed to see if they possessed characteristics similar to those of the original colonies that had been used to inoculate waterhyacinth. The 30 remaining isolates were tested again to verify pathogenicity and another 11 were discarded.

Stock cultures of the remaining 19 isolates on PDA slants were subsequently prepared and stored in the refrigerator. Stock cultures on agar slants as well as in sterilized soil were sealed using parafilm and then sent with the appropriate import permits to the quarantine laboratory in the Macdonald Campus of McGill University by air freight. On arrival, soil from the permanent storage tubes was sprinkled onto two petri dishes (for each culture) of PDA, and placed in a growth chamber at 25C for seven days. A mycelial plug was then transferred into a test tube with sterile distilled water and the test tube was shaken, after which 0.2ml of the suspension was transferred to a petri dish of water agar + 100ppm chloramphenicol, and then spread out using a sterile glass rod. After 24 hours, germlings of each fungus were transferred from water agar to four PDA plates, and these were placed in a growth chamber at 24C and continuous fluorescent and incandescent light at an intensity of 300$\mu Em^{-2}s^{-1}$, for seven days. Permanent soil cultures were then made using suspensions of the fungi grown on PDA.

2.2.5. Testing pathogenicity of the fungal isolates

2.2.5.1. Plant Production

These preliminary tests were conducted in growth chambers in the quarantine laboratory at Macdonald Campus of McGill University. Waterhyacinth plants collected from dams in Mutoko and Masvingo and from the Hunyani River were sent with the appropriate import permits to the quarantine laboratory on the Macdonald Campus. Upon arrival the plants were grown in Kassulke's nutrient solution (Galbraith and Hayward 1984), in germination trays in a growth chamber set at 28C day temperature and 20C night temperature with a 14 hour day length, and light intensity of $300\mu Em^{-2}s^{-1}$. A healthy stock of waterhyacinth was started by removing all but the first leaf, from large plants sent from Zimbabwe. Healthy daughter plants (ramets), which developed were selected for experimental work.

2.2.5.2. Identification of fungi

Isolates were grown on potato sucrose agar (PSA), tentatively identified from microscopic examination, and then candidates for future study as mycoherbicides were sent to the International Mycological Institute (IMI) in the United Kingdom where the identity of these fungi was verified.

2.2.5.3. Preparation and application of spore suspension

Nineteen fungal isolates were used to inoculate plants that were grown in plastic containers. Leaves from the different plants were used as the experimental

units, and there were four replicates. On each plant there was a control leaf, a leaf injured on the top surface by pricking with a flamed needle and then inoculated, and an inoculated leaf that was not injured.

Spore suspensions were made from the plates that were exhibiting greatest sporulation, by adding sterile distilled water to these plates, after which they were scraped, to collect the conidia. Spore concentration was determined with the aid of a haemocytometer. Spore suspensions were spread onto the leaf surfaces of healthy waterhyacinth plants using a glass rod. Controls were treated with sterile distilled water. The plants were then placed in a dark dew chamber at 24C for 24 hours after which they were transferred to the growth chamber. The plants were examined weekly for up to four weeks for the presence of disease symptoms.

The pathogenic response was rated according to the length of the lesion as follows: +++>10mm, ++2-10mm, +<2mm, -no infection. Ten of the more pathogenic fungi were reisolated, and their pathogenicity to waterhyacinth confirmed in Zimbabwe using the procedure described above, except that the plants were individually covered with a clear plastic bag after inoculation, and then left in the shade for four weeks.

2.2.6. Effect of inoculum density

These studies were conducted at the Henderson Research Station in Zimbabwe. Healthy waterhyacinth plants at three to four leaf stage were grown in pots (12cm diameter and 7cm deep), and fertilized with 2ml/L of groesia. The plants were inoculated with 0, 10^6, 10^7, and 10^8 conidia/ml of *Fusarium pallidoroseum*, *F.*

moniliforme, and isolates 2a3 and 5aex25 of *F. solani*. The fungi were grown in potato dextrose broth (PDB), in 2L glass jars on a rotary shaker (250rpm), for ten days. Conidia were harvested by passing through a soil sieve onto which two layers of cheesecloth were placed. The fungal material remaining on the cheesecloth and inside the glass jar was rinsed with water. The conidia suspensions were adjusted using water. Application of pathogens was done using an atomizer, and all the plants were sprayed to runoff. The plants were individually covered with transparent plastic for 24 hours and placed in the shade for 28 days after which assessments of weed dry weights were made.

Dry weight of waterhyacinth plants was determined by drying whole waterhyacinth plants in paper bags for four to five days at 60C. Dry weights were recorded as gram per pot. Every fungal treatment was applied to five pots in a completely randomized design. The experiment was performed twice. Results were pooled after testing for homogeneity of variances using Bartlett's test (Steel and Torrie 1980). The experiment was analyzed with a factorial analysis of variance (ANOVA) considering the effect of each factor individually and their interaction.

2.3. Results

2.3.1. Incidence of waterhyacinth infestation.

Waterhyacinth is now present in seven of the eight provinces of Zimbabwe (Figure 1). The highest prevalence of the weed is in Mashonaland East where seven dams and nine rivers were infested (Table 3).

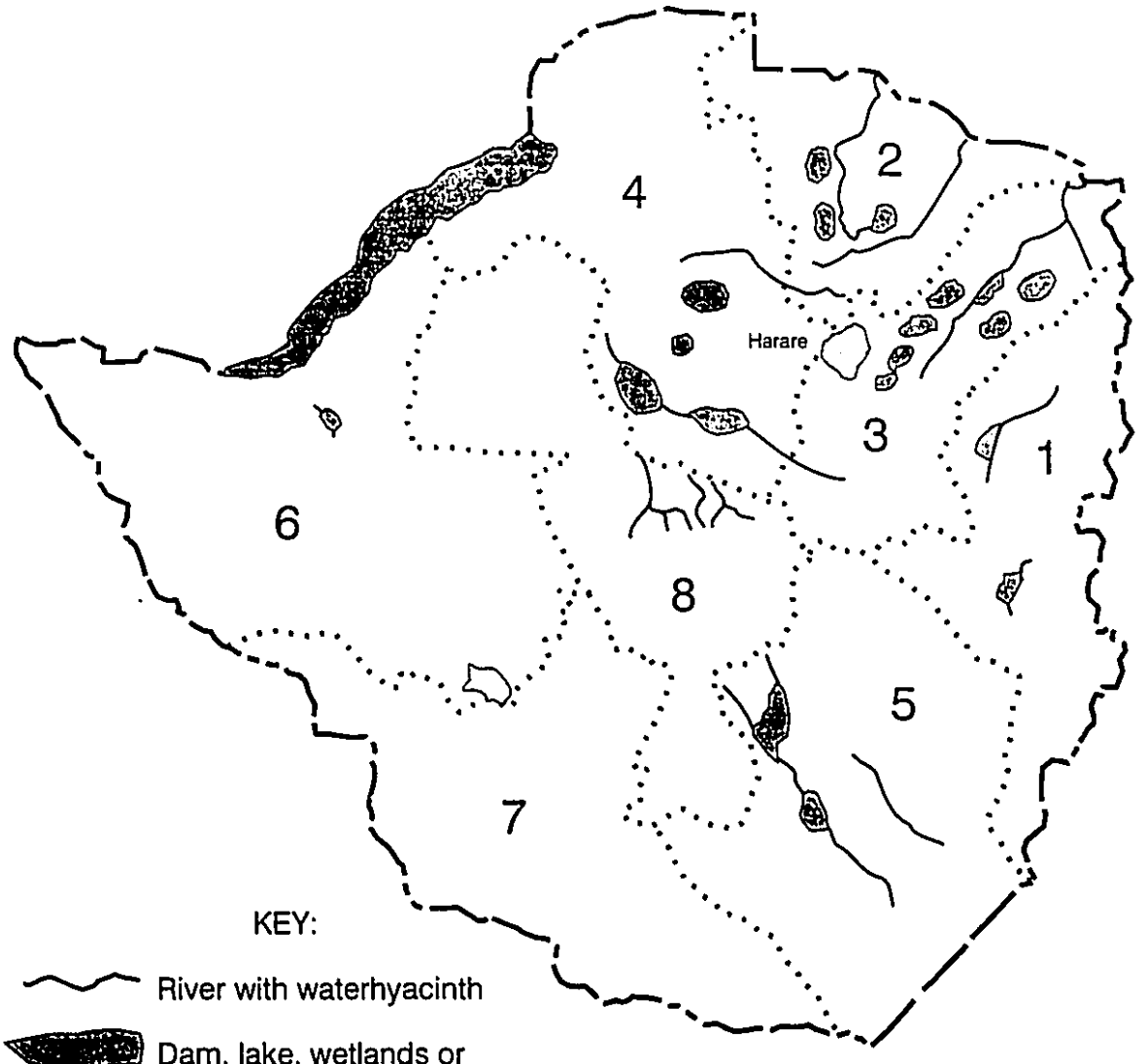


Figure 1. Distribution of waterhyacinth in Zimbabwe in 1993.

Table 3. Location, altitude, mean annual maximum temperature and mean annual minimum temperature, of the different water bodies on which waterhyacinth was growing.

Province	Water body	Altitude (m)	Mean annual maximum temp. C	Mean annual minimum temp. C
Manicaland	Clifton Dam	1200	24.2	10.7
	Nyamapemb-ere River	1200	24.2	10.7
	Rusape River	1430	24.2	10.7
Mashona-land Central	Arrowan Dam	1530	24.2	12.0
	Nyamanetsa Dam	1218	24.2	12.0
	Sharon Dam	1448	24.2	12.0
	Dora River	1481	24.2	12.0
	Mazowe River	702	26.5	10.4
	Musengezi River	1288	24.2	12.0

Table 3. (continued)

Province	Water body	Altitude (m)	Mean annual max. temp. C	Mean annual minimum temp. C
Mashonaland East	Chisamvi Dam	745	25.6	13.9
	Lake Chivero	1382	25.5	12.3
	Dandara Dam	420	25.5	12.3
	Darwendale Dam	1351	25.5	12.3
	Kudzwe Dam	700	25.6	13.9
	Manyame Dam	1380	25.1	11.5
	Seke Dam	1479	25.1	11.5
	Shavanhowe River	1350	25.1	11.5
	Hunyani River	1479	25.1	11.5
	Katiyo River	900	25.6	12.3
	Mukuvisi River	1422	25.1	11.5

Table 3. (continued)

Province	Water body	Altitude (m)	Mean annual maximum temp. C	Mean annual minimum temp. C
Mashonaland East	Nyadiri River	546	25.6	13.9
	Nyakabawo River	900	25.6	13.9
	Nyatsime River	1500	25.1	11.5
	Zaranyika River	1200	25.6	13.9
	Zhombwe River	1200	25.6	13.9
	Chinhamora wetlands	1500	25.5	10.4
	Chingwena wells	840	25.5	12.3
	Madyavava wells	1120	25.6	13.9
	Makwengura wells	1280	25.5	12.3

Table 3. (continued)

Province	water body	Altitude (m)	Mean annual maximum temp. C	Mean annual minimum temp. C
Mashonaland East	Shambanha-ka wells	1322	25.6	13.0
Mashonaland West	Lake Kariba	518	30.7	18.5
	Mana Pools	360	30.7	18.5
Masvingo	Chiredzi	580	29.9	15.7
	Mutirikwi	1094	26.2	12.4
	Triangle	429	29.9	15.7
	Mucheke River	1050	26.2	12.4
	Mushagashe River	1204	25.8	12.5
	Matova River	1094	26.2	12.4
Matebele-land North	Lungwalala Dam	617	30.2	20.0
Midlands	Mvuma streams	1458	25.0	15.7

Wells in seven villages of Mashonaland East were also infested. Waterhyacinth is widespread in the Mudzi, Mutoko, Mrewa, and U[illegible]nba -Maramba -Pfungwe districts (Figure 2). In Mtoko it was introduced into three streams by local individuals who mistook waterhyacinth for the indigenous water plant, Makarara (*Nymphaea caerulea* Savigny). *Nymphaea* spp. are used in these communities for burial rituals, and the rhizomes are also used as food (especially in times of drought). Some farmers reported that in the past they had lost their livestock which drowned in Chisamvi Dam and Kudzwe Dam, below the mats of waterhyacinth after having been browsing on the weed.

In Mashonaland Central, the weed was mainly found in the Centenary district. Reports from Guruve, Mazowe, Mt Darwin, Rushinga and Shamva districts of Mashonaland Central indicated that the weed was not found in those areas. In this province it was present in three farm dams as well as in three rivers. The weed was introduced into the three rivers by villagers who mistakenly believed that it was capable of conserving water.

In Masvingo province, the weed was present in three rivers. It was observed in several pools along Mucheke river and reports from local officers indicate that the weed has been in Mucheke as far back as 1982. The weed was also found growing extensively on Lake Mutirikwi where plants were as high as 1.2m. The favourable high temperatures (mean annual maximum temperature: 26.2C; mean annual minimum temperature 12.4C) experienced in this province for most of the year encouraged prolific growth of the weed.

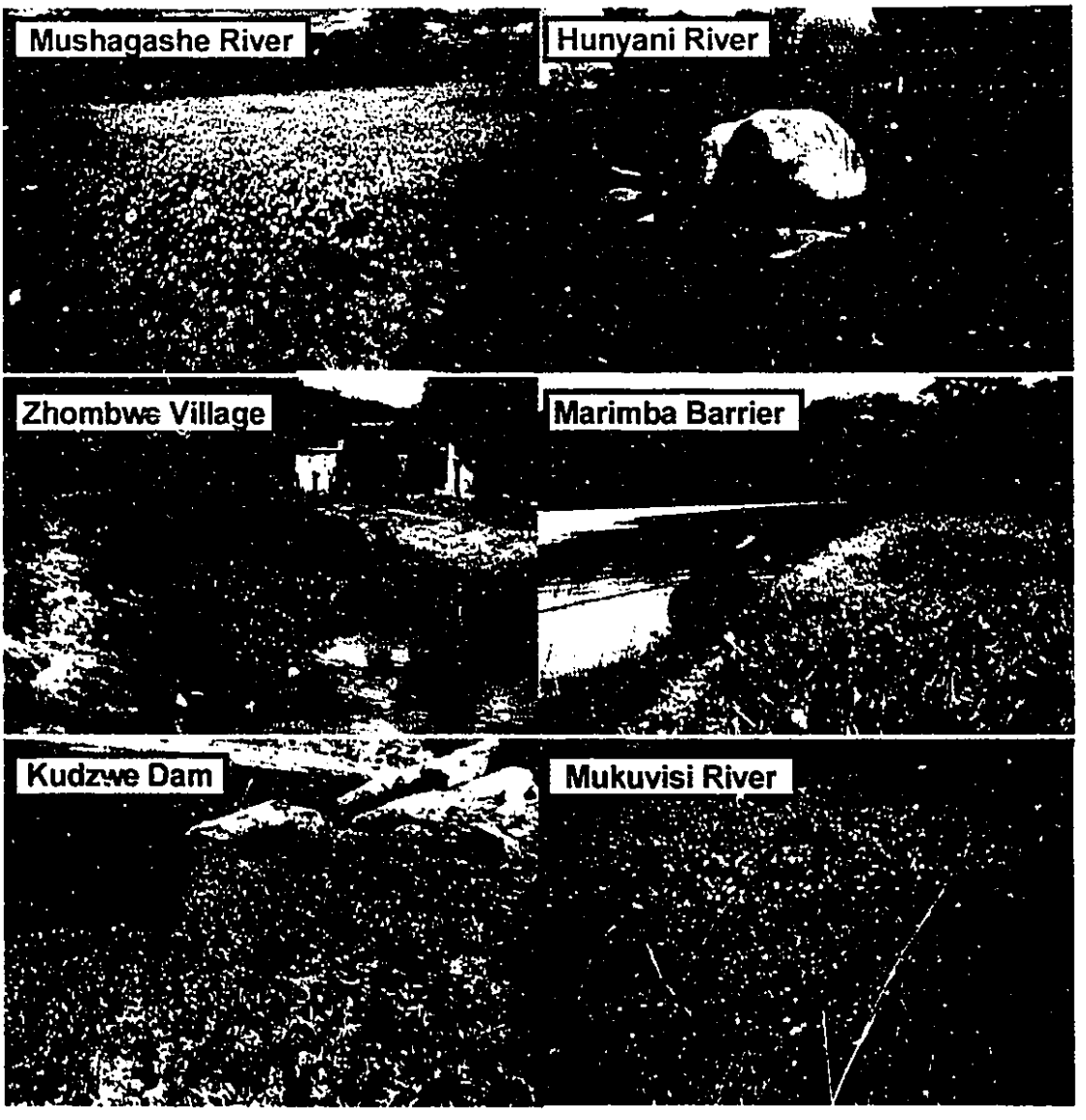


Figure 2. Some sites where waterhyacinth was found growing during the survey.

This was also the case in Triangle and Chiredzi (mean annual maximum temperature: 29.9C; mean annual minimum temperature: 15.7C) where dams on the sugar estates were infested with the weed.

In Mashonaland West, the weed was reported to be present at Mana Pools (on the Zambezi River) as well as on Lake Kariba, where it was spreading extensively in bays and along the shoreline. There was extensive germination and seedling establishment of the weed on the shoreline. Water level had receded by more than five metres due to persistent droughts experienced in Zimbabwe during the last five years, exposing a large dormant waterhyacinth seed reservoir to conditions optimum for germination. The weeds on Lake Kariba were flowering extensively and were often seen floating in mats being moved about with currents.

In Manicaland, whose temperatures are considerably lower than those for the rest of the country (mean annual maximum temperature 24.2C; mean annual minimum temperature 10.7C), the weed was absent in seven (Rusape North, Mutasa, Chimanimani, Nyanga, Chipinge, Mutare and Buhera) of the eight districts of the province. The only district that reported the existence of waterhyacinth was Rusape South. It was found on the Clifton farm dam as well as in Nyamapembere and Rusape Rivers.

In the Midlands Province, waterhyacinth was found growing in streams in Mvuma. In Matebeleland North it was recently reported to be present in Lungwalala Dam in Binga, where it has hampered use of the dam for irrigation. The only

province that reported a complete absence of waterhyacinth was the very arid Matebeleland South with a mean annual rainfall of 477.7mm.

2.3.2. Extent of Infestation

Waterhyacinth growing in 55% of all the infested rivers and 53% of the infested dams was in floating mats which covered less than 20% of the water (Table 4). In 17% of the infested dams as well as the infested rivers, waterhyacinth was found growing mainly along the banks. In these rivers and dams in which the weed was growing along the banks, intensive control measures were being applied, and the weed infestations were mainly due to new germination (from seed) along the shoreline, where formerly submerged seed had been exposed due to receding water levels. Waterhyacinth growing in wells and wetland areas was mainly found in Mashonaland East, where it rapidly formed a complete cover over these small areas. In Mashonaland Central, Nyamanetsa Farm Dam was almost completely covered by waterhyacinth, while in Matebeleland North, Lungwalala Dam was also reported to have an almost 100% infestation of the weed.

2.3.3. Control Measures

Weeds in 47% of the infested dams and 11% of the infested rivers were being controlled by a combination of the herbicide 2,4-D, and mechanical control methods in 1993 (Table 5).

Table 4. Number of waterhyacinth infested water bodies and the extent of infestation, expressed as percentages in brackets.

Extent of infestation	Dams	Rivers	Wells and wetlands
50-100% water cover	2 (11)	0 (0)	7 (100)
20-50% water cover	3 (17)	5 (28)	-
Floating mats covering less than 20% water.	9 (53)	10 (55)	-
Growing along banks covering less than 20% of water.	3 (17)	3 (17)	-

Table 5. Number of dams and rivers on which different control methods were imposed to control waterhyacinth, expressed as percentages in brackets.

Control methods	Dams	Rivers
Chemical and mechanical	8 (47)	2 (11)
Mechanical only	3 (18)	4 (22)
None	6 (35)	11 (61)
Biological, chemical and mechanical	0 (0)	1 (6)

This was mainly in areas that fall under the jurisdiction of the Department of National Parks and Wildlife Management, in Mashonaland East and in Masvingo, as this department was allocated funding to purchase chemicals and sprayers and to hire personnel to do the spraying.

Chemical spraying was often augmented with mechanical and manual removal which involved the use of boats, tractors and lorries which were used in conjunction with nets to pull the weed out of water. The weed was spread out to dry, and later burnt. Manual removal with forks was also commonly implemented especially during periods when weather conditions were adverse, and did not allow spraying, and when herbicide supplies ran out.

In 18% of the infested dams, and 22% of the infested rivers, waterhyacinth was controlled mechanically without the use of 2,4-D. The Department of Natural Resources which also has a mandate to clear noxious weeds, managed to get prison labour involved in manual removal of the weed in Masvingo. Barriers had also been erected across rivers using wire fencing, used oil drums and chains especially at places where they enter dams and lakes, so as to trap the weed and prevent any further infestation of the lakes and dams down stream. The empty drums were attached to the fence and acted as floaters, moving the fence up or down depending on the water level. Although these barriers were reinforced by chains, they often broke because of the pressure from the weed especially during heavy rains.

In some rural areas attempts had been made to remove waterhyacinth manually by local people including school children, but work had since stopped in some of the

areas because the communities wanted to be paid for the work and those involved faced risk of crocodiles, poisonous snakes and drowning.

In Mutoko there were very active fishing co-operatives, whose fishing was severely affected by waterhyacinth. Members of the co-operatives were involved in manual removal of the weed. In many rivers the presence of grass and reeds made complete manual removal of the weed impossible. Where communal (village) people were mobilized to remove the weed they were often hampered by lack of tools. In most situations where local communities cleared the weed in village rivers and dams, they often gave up before the job was completed mainly because they were not paid for their efforts.

No control measures were being applied to the weed in 35% of infested dams and in 61% of the infested rivers. This included Lake Kariba, which lies in a national park, is a very important fishery, and is a major tourist attraction whose waterhyacinth infestations were increasing rapidly. The absence of a control program was mainly because fishermen were lobbying against herbicide spraying. When the weed appeared earlier on Lake Kariba, it had been virtually eradicated by the use of 2,4-D. However this had led to a lot of plant material sinking into the lake, depleting oxygen, and this was followed by extensive death of fish. When we visited the lake, there were no control measures being imposed on the weed, although elephants and hippopotami could be seen eating waterhyacinth from the banks, and this was the only control being imposed on the weed.

2.3.4. Monitoring waterhyacinth weevils

In 1993 *Neochetina* weevils were present in large numbers on the plants that survived spraying with 2,4-D in Hunyani River, Mukuvisi River and Nyatsime River. In Darwendale Dam there were light infestations of waterhyacinth which also showed typical weevil damage. Plants growing along the shoreline in Lake Chivero, also exhibited symptoms of weevil damage. All these rivers and dams belong to the continuous Hunyani River system (Figure 3). Although the weevils had been released on five selected sites, most of which were in the upper catchment area of the Hunyani River system , the results indicated that the weevils had established by natural spread throughout the system. Neither *Neochetina* weevils, nor their feeding marks were found on any of the other rivers and dams that were visited.

Results of initial monitoring done at Marimba Camping Ground showed that weevil populations ranged from 0 to 10 per plant on 2 March, 1993. All the plants that were sampled showed weevil feeding marks. The average number of adult weevils was 2.9 per plant. On 30 March, 1 to 12 adult weevils (average 3.02) per plant were observed.

In 1994 there were very few waterhyacinth plants in the Hunyani River system in the period between January and June. Most of the weed had been flushed out by the summer rains, and those that remained were being intensively sprayed with 2,4-D in a renewed effort to eradicate the weed. The few plants that were encountered did not have any weevil feeding marks.

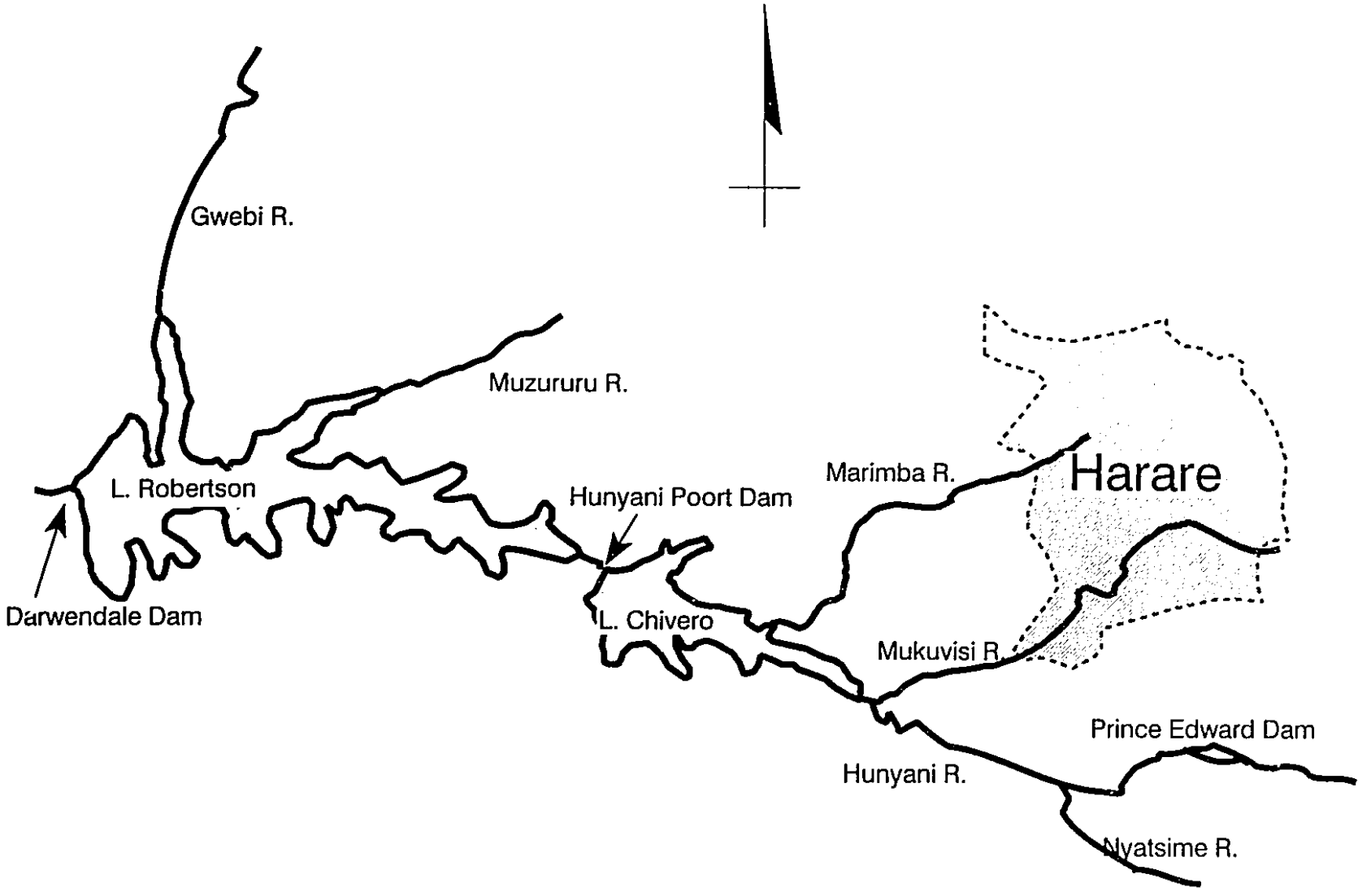


Figure 3. The Hunyani River system, onto which *Neochetina* weevils were released.

In February 1995, weevil feeding marks were found on weed that was growing behind the physical barrier which had been erected at Skyline bridge, but were not found on waterhyacinth growing on the rest of the Hunyani system where they had been found previously. At Skyline bridge, number of weevil feeding marks reached an average of 10.36 marks per plant and these were reduced to 1.44 marks per plant by August 1995 (Figure 4). This decrease in number of feeding scars per plant from February to August 1995 reflected a decrease in adult weevil density.

Other arthropods found feeding on waterhyacinth were the red spider mite (*Tetranychus* spp.) and *Mylothris* spp. whose cylindrical larvae with alternate black and red transverse bands, fed on the weed. *Tetranychus* spp. is not host specific as it is a pest of many crops including cotton and tomatoes. The larvae of *Mylothris* spp. are known to feed on Cruciferae and Capparidaceae. Adults are normally found amongst reeds or papyrus in streams or swamps. In Zimbabwe their distribution is widespread.

2.3.5. Fungi associated with waterhyacinth

The older leaves of waterhyacinth invariably had dark brown punctate spots on the laminae, and at senescence began to die back at the leaf tip. Disease on plants, as judged by the symptoms of leaf spots and necrosis was most severe in the Hunyani River system where waterhyacinth weevils *Neochetina eichhorniae* and *N. bruchi* had been released (Figure 3).

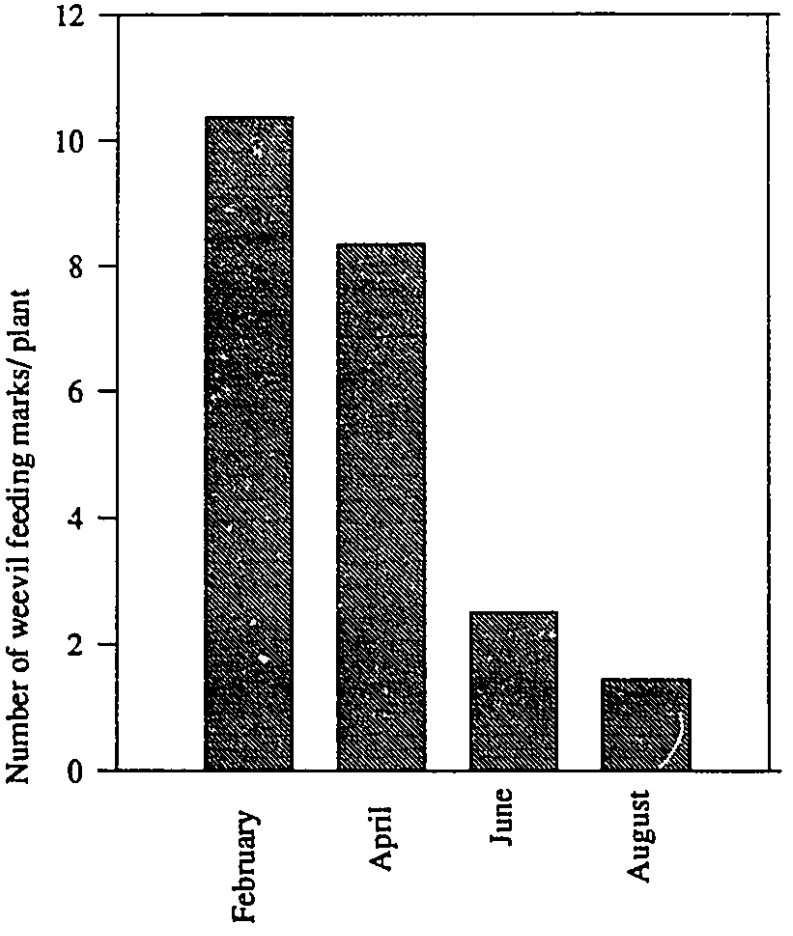


Figure 4. Number of weevil feeding marks on waterhyacinth plants in Hunyani River in 1995.

Ninety three isolates were collected from waterhyacinth growing in this system while 42 isolates were collected from all the other water bodies visited.

Plants growing where the waterhyacinth weevils were absent were generally healthy to moderately diseased. The saprophyte, *Aspergillus* was isolated at high frequency from the foliage of arthropod-infested plants as opposed to non-infested ones. In all 135 accessions of fungi were used to verify Koch's postulates. Most of these were not pathogenic to waterhyacinth and were discarded leaving 30. On repeating pathogenicity tests 19 isolates were found to be consistently pathogenic to wounded plants. About half of these isolates were *Fusarium* spp. On further testing in Canada another nine isolates were discarded and further pathogenicity testing was done on the remaining ten isolates (Table 6). Disease symptoms were observed on both pricked and uninjured leaves, and the inoculated pathogens were reisolated, thus confirming their pathogenicity to waterhyacinth and verifying Koch's postulates. Identification to species level has not yet been possible for every isolate.

2.3.6. Pathogenicity Tests

The fungi from waterhyacinth can be grouped broadly into two categories according to the results of the pathogenicity tests in the glasshouse. Two to four isolates of most species were tested. Weak pathogens did not infect the laminae and petioles when the plant was not wounded (Table 6). These weak pathogens were *Alternaria alternata* (Fr.) Keissler, *Cladosporium cladosporoides* (Fresen) de. Vries, *Bipolaris* spp. *Chaetomium* spp., *Mucor* spp. and *Nigrospora* spp..

Table 6. Fungi isolated from and their pathogenicity to *Eichhornia crassipes*.

Pathogen	Unwounded leaf	Wounded leaf
Alternaria alternata	-	++
Bipolaris spp.	+	++
Cnaetomium spp.	+	+
Cladosporium cladosporiodes	+	++
Mucor spp.	_	+
Nigrospora spp.	++	++
Fusarium moniliforme	++	+++
Fusarium pallidoroseum	++	+++
Fusarium solani isolate 2a3	++	+++
F. solani isolate 5aex25	++	+++

Key

- = no infection
+ = lesion <2mm
++ =lesion 2-10mm
+++=lesion >10mm

The lamina infection by these weak pathogens was commonly flare-shaped, narrowing from the point of the inoculum to the leaf tip, or small yellowish brown spots on the leaves.

The species of *Fusarium* were more virulent, and were notable in causing water-soaked areas of various sizes around the site of inoculation to the petiole. Unwounded laminae were also infected, but at a slower rate. The *Fusarium* isolates were identified as *Fusarium moniliforme* Sheldon (isolate 2ex12) (IMI 360956), *F. solani* (Martius) Sacc. (isolate 5aex25) (IMI 364361), *F. solani* (Martius) Sacc. (isolate 2a3) (IMI 364362), and *F. pallidoroseum* (Cooke) Sacc. (previously known as *F. semetectum* Berk. & Ravi.) (isolate 3ex1) (IMI 364360) (Figure 5).

Three days after inoculation both isolates of *F. solani* produced reddish brown spots starting from the leaf margins, on the older leaves. Twenty to 22 days after inoculation, the upper surface of the petioles began to wither, and subsequently the whole plant died. All the *F. solani* isolates tested produced two types of spore; the small kidney-shaped, single-celled microconidia, and the sickle shaped 4-5 septate macroconidia in culture. Chlamydospores were abundant on PDA and these were formed in chains. Cultures of isolate 5aex25 were cream in colour while those of isolate 2a3 were blue on PSA.

Inoculation with *F. pallidoroseum* caused browning starting from the leaf margins, which affected the youngest leaf first. During the second week the leaves began to wither, and later the petioles also withered on the bottom surface.

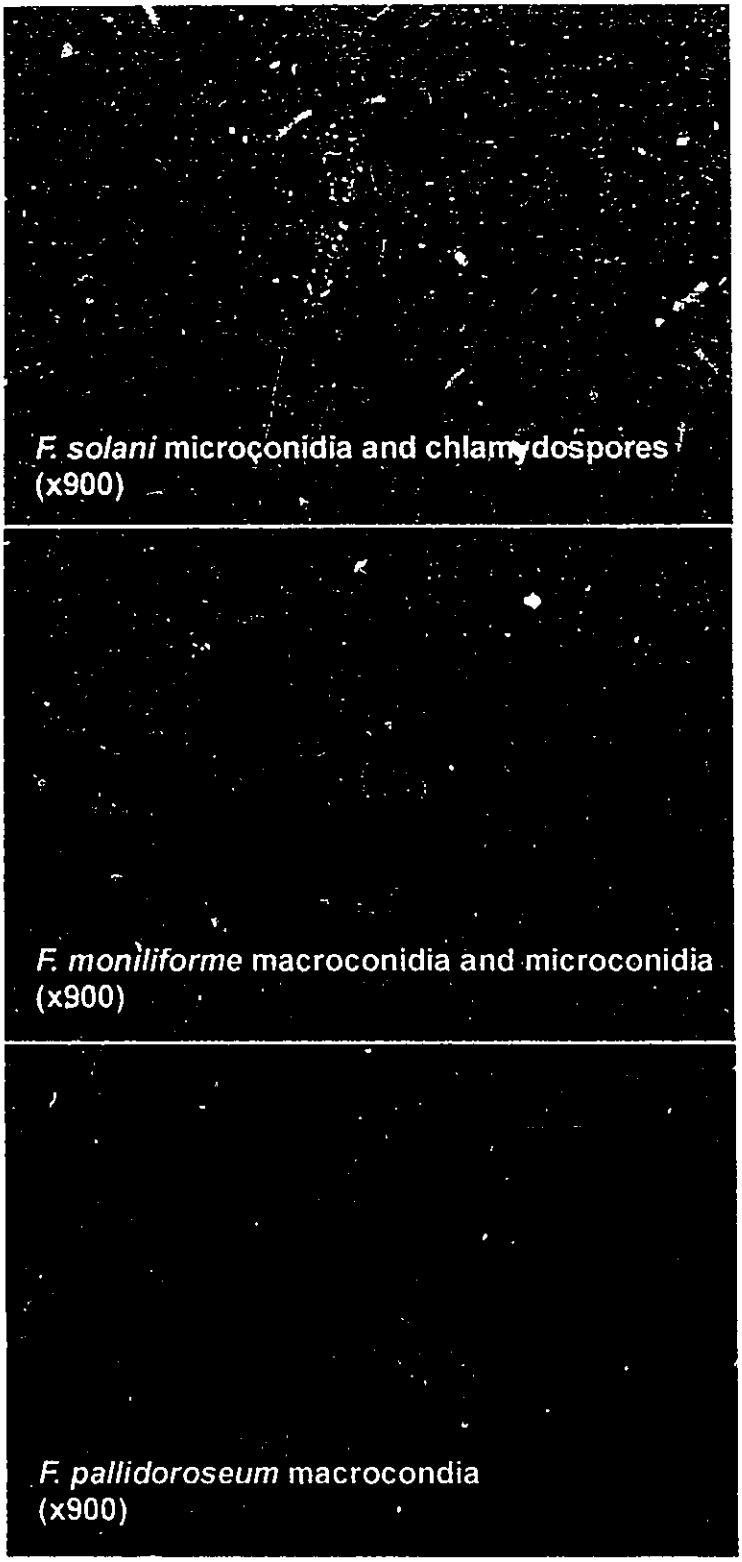


Figure 5. Conidia of ***Fusarium solani,*** ***Fusarium moniliforme,*** and *Fusarium* ***pallidoroseum.***

F. pallidoroseum did not produce microconidia on PDA, but produced macroconidia which were variable in size. The culture on PDA was brown in colour. Inoculation with *F. moniliforme* resulted in browning of both old leaves as well as new leaves, but older leaves were affected more. In the second week after inoculation, chlorosis of the leaves set in and this was followed by withering, after which the leaves dried up. It did not affect petioles. *F. moniliforme* produced abundant single celled microconidia that were oval in shape. Macroconidia were slightly sickle shaped. The white aerial mycelium was tinged with purple when grown on PDA.

2.3.7. Effect of inoculum density

F. moniliforme did not significantly reduce weed biomass when it was applied at conidial densities less than 10^8 conidia/ml (Table 7). Both isolates of *F. solani* reduced weed weight when a conidial suspension of density, 10^7 conidia/ml was applied. There was no significant further reduction in weed weight when the density of the *F. solani* isolates was increased to 10^8 conidia/ml. *F. pallidoroseum* reduced weed weight when a conidial density of 10^6 was applied, and weed weight continued to decline as the density of conidia in the suspensions applied, increased.

Table 7. Weed weight (g) of waterhyacinth as affected by *Fusarium* spp. applied using different densities of conidia.

Density of conidia	*Fusarium solani* 2a3	*Fusarium solani* 5aex25	*Fusarium moniliforme*	*Fusarium pallidoroseum*
0	1.524a[1]	0.984a	0.897a	0.957a
10^6	1.426a	0.971a	0.749a	0.850a
10^7	0.699b	0.465b	0.766a	0.648b
10^8	0.552b	0.438b	0.525b	0.479c

[1]Means followed by the same letter in the column are not significantly different at P=0.05, according to Duncan's multiple range test.

2.4. Discussion

In Zimbabwe waterhyacinth infestations are now found throughout most of the country, in lakes, dams, rivers, wells and wetlands. Most infestations occur south of latitude 16° at varying elevation. The climate is not typically tropical because it has a very definite cool season at one time of the year, and is not like northern temperate or Mediterranean climates because the rainy season is in summer in Zimbabwe rather than in winter. Temperatures do not favour rapid growth during winter and waterhyacinth may be frosted. However, during summer, favourable temperatures coupled with high nutrient levels promote rapid growth of waterhyacinth in Lake Chivero, the Manyame River and elsewhere in the country.

Zimbabwe's natural river systems are not, in general suitable for the development of large populations of floating aquatic plants. This is because the rivers are highly seasonal with many drying out completely during the dry season, and there are no flood plains or swamps like those in East or Central Africa. However the construction of numerous reservoirs has changed the nature of the rivers and provides a suitable habitat for floating plants. Shallow, stagnant water and high nutrient content of the water which is often connected with human activities encourages proliferation of waterhyacinth infestations.

In many communal areas there was no control imposed on the waterhyacinth infestations, and this was due to the lack of awareness in the village communities of the threat caused by waterhyacinth. Furthermore, land and water in Zimbabwe are communally owned and therefore the responsibility to remove the weed does not fall

squarely on any individual. It is often presumed that responsibility to control the weed lies with the state, but no government ministry receives adequate funding to manage the weed. Because of the importance of tourism in Zimbabwe, the Department of National Parks and Wildlife Management has been allocated funds for weed removal from lakes and rivers which lie within the national parks areas. However, there are no weed scientists in the waterhyacinth control teams.

Local councils are also expected to remove weeds within their areas, but this has rarely been done because of the lack of resources. Minimal weed removal has been done by Harare City Council on their portion of the Hunyani River System. The Department of Natural Resources has a mandate to remove all noxious weeds in areas other than those covered by the local councils and the Department of National Parks and Wildlife Management. However, the department is hampered by the lack of funding, equipment, and manpower and hence has resorted to using prisoners as well as attempting to motivate and assist local citizens.

Monitoring of a floating aquatic weed presents obvious difficulties. Plants can change position under the influence of wind and water currents, and can grow in situations which are difficult or hazardous for the research worker (Harley 1994). With floating aquatic weeds, monitoring spread of agents is confounded by movement of weed mats in response to the action of water currents and/or wind (Harley and Forno 1992).

Frequent use of herbicides causes a rapid and extensive loss of habitat for the waterhyacinth weevils. Adult weevils are mobile, but eggs, larvae and pupae are not,

and these life history stages are reduced drastically as a secondary effect of herbicide application programs. Weevil populations have a much slower rate of increase than waterhyacinth populations and as a result, regrowth of a weed mat after spraying will be favoured until the insect population can once again reach effective levels (Center and Durden 1986).

Relatively few fungi attacking waterhyacinth were found, and most of these caused little damage, confined to one or a few leaf spots, and failing to invade the petiole or rhizome. Leaf infections encountered in this study were usually found on the older leaves, and production of healthy new leaves continued unabated.

Several features contribute to this presence of few diseases on waterhyacinth. Its prodigious growth rate in mid-season allows plants to outgrow modest infections. The high physiological capacity of diseased plants allows them to compensate for damaged leaves with a supply of healthy young leaves which carry on the normal metabolic reactions to support further leaf production (Caunter and Mohamed 1990). The cuticle is a poor surface for infection by fungal conidia. Its low wettability means that water droplets roll over the leaf surface, which would be expected to reduce the retention of conidia, dispersed in water, and to limit the germination and penetration of those conidia that are on the leaf due to lack of free water (Charudattan, Perkins and Littell 1978). The waterbodies from which diseased plants were collected in Zimbabwe, were highly eutrophicated because they receive sewage and industrial effluent from the surrounding urban areas. This increased nutrition may impart a higher degree of immunity to the plants either through an increased growth rate or an

altered metabolism (Conway et al. 1978). The presence of phenolic compounds in the leaf is another means of resistance to fungal disease. There are two morphologically distinct types of idioblasts (phenol-storing cells) in the leaves of waterhyacinth, which contain four phenolic acids, compounds implicated in plant resistance to microbial attacks (Martyn and Cody 1983). These effect fungal growth and the natural infection and spread of disease. Hyphae of *Acremonium zonatum* (Sawada) Gams which penetrated the phenol cells appeared dead (Martyn et al. 1983).

Frequent use of chemical herbicides against waterhyacinth results in a reduction in the overall level of biocontrol pressure on weed regrowth. The drastic reduction in waterhyacinth populations following herbicide treatment eliminates the habitat for insect biocontrol agents and delays the subsequent buildup of insect populations and biocontrol pressure on rebounding weed populations (Wright and Center 1984). Microbial attacks that normally follow insect damage are also diminished (Charudattan et al. 1978). Thus it is common to find the healthiest waterhyacinth plants in areas of frequent chemical herbicide use (Charudattan et al. 1990).

There are however, factors which predispose waterhyacinth to infection. Two of these are damage by insects, illustrated by the fact that more fungal isolates were isolated from waterhyacinth growing where *Neochetina* weevils had been released, and weather conditions (Galbraith and Hayward 1984). However, there were few waterhyacinth weevils in the Hunyani River system and thus few wounds on the weeds, and this partly explains why there were few diseases on the weed. Furthermore, the sporadic nature of the infestations in non-perennial water bodies, a

result of droughts, as well as the incidence of frost during winter which retards waterhyacinth growth, are not conducive to the development of diseases.

The majority of the fungi which have been isolated from waterhyacinth in this study are ubiquitous species on decaying plant material. This applies to the weak pathogens like *Alternaria alternata* isolated in this study. An isolate of *A. alternata* isolated from waterhyacinth in India was highly virulent to waterhyacinth under some conditions, and was considered to have biocontrol potential (Aneja and Singh 1989). However, *A. alternata* was also isolated in Egypt where it was found to be a weak pathogen that induced, small, zonate, yellowish brown spots on the leaves (Mansour, Zahran and Shady 1980). Although *Nigrospora* was not identified to species level, it is worth noting that the weak pathogenicity of *N. sphaerica* (Sacc.) Mason to waterhyacinth was enhanced when used in combination with *Neochetina* (Conway, Freeman and Charudattan 1974).

Reduction of waterhyacinth biomass by the *Fusarium* spp. increased as the density of conidia applied increased. Application of high levels of inoculum may compensate for possible constraints preventing a disease epidemic such as environmental conditions, low pathogen virulence, or host resistance (Templeton and TeBeest 197[illegible],

Galbraith and Hayward (1984) noted that *Fusarium* could only be considered for use in biological control with extreme caution because of the disease and crop loss caused by so many members of the genus. This genus is also known to produce a large number of mycotoxins (Auld and Morin 1995). However, several Fusaria have

been evaluated as bioherbicides. *F. oxysporum* Schlecht var. *cannabis* was evaluated for biological control of illicit marijuana (*Canabis sativa* L.) in Carlifornia (Hilderbrand and McCain 1978). *F. oxysporum* Schlecht var. *orthoceras* provides control for broomrape (*Orobanche aegyptica* Pers.) in watermelon (*Citrullus vulgaris* Schrad.) fields in the Astrakhan region of the U.S.S.R. (Boyette, Templeton and Oliver 1984). *Fusarium solani* App. & Wr. f. sp. *cucurbitae* Snyd. & Hans. has been evaluated for biological control of Texas gourd [*Cucurbita texana* (A.) Gray] (Boyette, Templeton and Oliver 1984). *F. oxysporum* Schlecht emend. Snyd. & Hans and *F. nygamai* Burgess and Trimboli have been evaluated for the control of *Striga hermonthica* (Del.) Benth. in Africa (Abbasher 1994, Ciotola, Watson and Hallett 1995).

F. pallidoroseum is a secondary invader of plant tissue. It is often found associated with a disease complex (Booth 1971). *F. solani* attacks hosts weakened by unfavourable conditions or following nematode damage or virus infections (Booth 1971). However there are examples of physiological specialisation within the latter species, so the waterhyacinth isolate may deserve further investigation, since it was one of the few fungal isolates which caused soft rot in petioles. *F. moniliforme* is a major parasite of several Gramineae. It occurs on a very wide range of other hosts represented by 31 families in which it may cause diseases such as seedling blight, scorch, foot rot, stunting and hypertrophy (Booth 1971). It also produces mycotoxins which include fumonisins, fusarin c and moniliformin (Nelson, Tousson and Cook 1981). However *F. moniliforme* (Sheldon) isolated from jimsonweed (*Datura*

stramonium L.) caused damage to jimsonweed and other weed species and was considered for development as a herbicide (Abbas, Boyette, Hoagland and Vesonder 1991). That isolate produced fumonisin B_1 [(propane)-1,2,3-tricarboxylic acid diesters of long-chain aminopentals] in large amounts and some related fumonisin compounds as minor metabolites (Abbas, Vesonder, Boyette, Hoagland and Krick 1992). Fumonisin B_1 was shown to be responsible for the fungal toxicity of jimsonweed and other weeds (Abbas et al. 1991, Abbas et al 1992, Tanaka, Abbas and Duke 1993).

There are other reports of *Fusarium* species which are pathogenic to waterhyacinth. An unidentified species of *Fusarium* has been found in the larval tunnels of plants infested with *Neochetina eichhorniae* and the mite *Orthogalumna terrebrantis* (Charudattan et al. 1978). *F. roseum* (LK) was only a weak foliar pathogen of waterhyacinth (Rintz and Freeman 1972) although it was able to kill hydrilla (*Hydrilla verticilata* (L.f.) Royle [Hydrocharitaceae]). In spite of its pathogenicity to some terrestrial plants, *F. roseum* was still considered to have potential in biological control in an aquatic environment, but has been rejected on the basis of the poor results of large scale pilot tests (Freeman et al. 1981; Charudattan et al. 1983). Leaf spots caused by *F. equiseti* (Cda) Sacc. have been reported in India, but the rate of new leaf formation allows the plants to survive the infection (Banerjee 1942). Snyder and Hanson (1945) considered this species to be synonymous with *F. roseum*. *F. chlamydosporum* was also observed to cause disease on waterhyacinth in India (Aneja et al. 1993). Small young leaves were less susceptible to infection than larger and older leaves both in the field and in experimental ponds.

The chief aim of this project was the exploration of the indigenous fungi for potential mycoherbicides, in order to avoid the unnecessary introduction of an exotic fungus to the biocontrol programme. The native fungi are poorly known, and this limited study is unlikely to have detected all the fungi associated with waterhyacinth in Zimbabwe.

F. solani and *F. pallidoroseum* were previously isolated in Australia. This also applies to *A. alternata* which was isolated in Egypt and India (Mansour, Zahran and Shady 1980; Galbraith and Hayward 1984; Aneja and Singh 1989). *Mucor* spp. and *Bipolaris* spp. were isolated in Australia and in the USA while *Cladosporium cladosporoides* was isolated from waterhyacinth in Egypt (Mansour et al. 1980). *Chaetomium* spp. and *Nigrospora* spp. were isolated in the USA (Charudattan et al. 1978).

3. MASS PRODUCTION OF POTENTIAL MYCOHERBICIDES

3.1. Introduction

3.1.1. Inoculum production

Production of large amounts of infective propagules of fungi is a requirement for the development of potential bioherbicides. This is due in part to the need to increase efficacy of these microbes, as current methods for increasing efficacy rely mostly on increasing the inoculum (Baker and Henis 1990). Production methods for large quantities of conidia should be economical, relatively simple, require no special equipment or handling, and the inoculum produced should retain its viability and pathogenicity for long storage periods (Hildebrand and McCain 1978).

Several methods and media have been used to produce sufficient amounts of inoculum of various fungi studied or used as bioherbicides. Liquid cultures in shake flasks or small fermentation vessels supported sporulation in vitro of *Fusarium solani* f.sp. *cucurbitae* (Boyette, Templeton and Oliver 1984) in modified Richards medium with V-8 juice. Inexpensive agricultural products are also commonly screened for economic production of fungal inoculum. Cornmeal/sand medium was used to produce fungus-infested granules of *F. solani* (Boyette et al. 1984).

In general, solid state fermentations do not require sophisticated formulation procedures prior to use (Connick, Lewis and Quimby 1990). However, there are several inherent problems with solid state fermentation. The preparations are generally bulky, they may be subject to a greater risk of contamination, and they may require extensive space for processing, incubation and storage (Connick et al. 1990).

Controlling pH and using monitoring devices to determine moisture and pH is also a problem in solid state fermentation (Aidoo, Hendry and Wood 1982).

3.1.2. Granular formulation

Granular formulations are often better suited for use as postemergence bioherbicides, than are spray formulations because the granules provide a buffer from environmental extremes and can serve as a food base for the fungus (Abbasher 1994). Wheat straw was used to control marijuana (*Cannabis sativa* L.) with *F. oxysporum* f.sp. *cannabis* (Hilderbrand and McCain 1978). Oat seeds infested with *Fusarium solani* f.sp. *cucurbitae* were used to control Texas gourd (*Cucurbita texana* L.) (Boyette et al. 1984). The same weed was controlled with the same fungus using a cornmeal-sand formulation in which mycelium and a mixture of microconidia, macroconidia and chlamydospores of the fungus were produced (Boyette et al. 1984). Barley grains and wheat straw mixed with crushed maize grain infested with *Fusarium oxysporum* f. sp. *orthoceras* were used to control *Orobanche cumana* on sunflower fields in Bulgaria (Bedi and Donchev 1991).

3.1.3. Objectives

The objectives of this study were to evaluate solid agricultural products and commonly used complex or defined liquid media for spore production of *F. solani* (isolates 5aex25 and 2a3), *F. moniliforme* and *F. pallidoroseum*, and to evaluate the virulence of the inoculum produced.

3.2. Materials and methods

3.2.1. Seed inoculum preparation:

Soil cultures of the original single-conidium isolates of *F. solani*, *F. moniliforme*, and *F. pallidoroseum* were sprinkled onto fresh PDA in petri dishes (9cm diameter). Plate cultures were incubated at 25C in continuous fluorescent and incandescent light (intensity of 300$\mu Em^{-2}s^{1}$) for four weeks. Agar disks with mycelium (6mm diameter) from the margin of these colonies were used to seed liquid and solid media.

3.2.2. Solid substrates

Seeds of maize (cv. R201), soybean (cv. Roan), wheat (cv. Sengwa), barley (cv. Nata), and food beans (cv. Natal Sugar) were evaluated as solid substrates for *F. solani*, *F. moniliforme* and *F. pallidoroseum* conidia production. Twenty grams of a substrate were moistened with 30ml of deionized water. Barley, maize and wheat straw were obtained from the same varieties mentioned above, while groundnut straw was obtained from plants of the variety Plover, and waterhyacinth straw was collected from the Hunyani River. After harvesting, the straw was left in the sun to dry after which it was cut into pieces 1 to 2cm in length. Because of the differences in bulk of the different straws, different amounts of straw and water were used in 250ml Erlenmeyer flasks. Three grams of waterhyacinth straw were mixed with 30ml water, 10g of maize straw were mixed with 90ml water, and 10g of the wheat, barley and groundnut straw, were mixed with 60ml of water.

3.2.3. Solid substrate fermentation:

All the flasks of solid media were autoclaved for 40 minutes (100kPa and 120C). Flasks of cooled medium were shaken by hand and seeded with an agar block of inoculum (6mm in diameter), under aseptic conditions. Inoculated flasks were incubated on a laboratory bench in a controlled temperature room maintained at 25C under continuous fluorescent and incandescent light (intensity of 300$\mu Em^{-2}s^{1}$) for 14 days. Flasks were shaken by hand every two to three days throughout the incubation period to prevent aggregation of solid particles and to improve aeration.

Conidia from solid media were harvested by adding 50ml of deionized water to each flask, shaking the flasks on a rotary shaker at 250rpm for 5-10 minutes, and pouring the contents through two layers of cheesecloth supported by a 250μm soil sieve. Fungal material remaining in the flask and on the cheesecloth were rinsed with water. Conidia production was determined with the aid of a haemocytometer.

3.2.4. Liquid media

Eight 250ml Erlenmeyer flasks containing 100ml of potato dextrose broth (PDB) (200g potatoes, 20g dextrose, H_2O to make up 1000ml), potato sucrose broth (PSB) (200g potatoes, 20g sucrose, H_2O to make up 1000 ml), Tochinai solution (10g peptone, 0.5g KH_2PO_4, 0.25 $MgSO_4.7H_2O$, 20g maltose, 1000ml H_2O), and modified Richards medium (10g sucrose, 10g KNO_3, 2.5g $MgSO_4.7H_2O$, 5.0g KH_2PO_4, 0.02g $FeCl_3.6H_2O$, 150ml V8 juice, H_2O to make 1000ml) (Tuite 1969, Walker 1980), were seeded with an agar block of inoculum (6mm in diameter) under aseptic conditions,

and incubated on a rotary shaker (250rpm) for seven days under laboratory conditions as described previously.

Conidia were harvested by filtration through two layers of cheesecloth supported by a 250µm soil sieve. The fungal material (hyphae and conidia) remaining on the cheesecloth and inside the flask was rinsed with 50ml water. Conidia production was determined with the aid of a haemocytometer.

3.2.5. Assessment of viability

Droplets of conidia suspension ($1x10^5$ conidia/ml) in deionized water were sprayed onto three 9mm petri dishes with water agar, using an atomizer under aseptic conditions, and incubated at 25C for 24 hours. Plates were examined with the aid of a dissecting microscope, and conidia were considered to have germinated when the germ tube was greater than the width of the conidium.

3.2.6. Pathogenicity tests

Healthy waterhyacinth plants, at the 3 to 4-leaf stage, with a well developed root system were selected from clones that had been collected from dams in Masvingo and Mutoko and from Hunyani River. Twenty plants were grown in germination trays (51cm long, 25cm wide and 6cm deep), in tap water to which was added 2ml/L of groesia fertilizer.

Pathogenicity tests were conducted using inoculum generated from whole soybeans and crushed soybeans, which had been inoculated with *F. solani* isolate

5aex25 and *F. pallidoroseum* and incubated for two weeks as described earlier. Eight 250ml Erlenmeyer flasks were used for each substrate, and of these four were used for the wet treatments while the remainder were used for the dry treatments. After two weeks, media used in the dry treatments were placed on separate sheets of paper for 24 hours, to dry under aseptic conditions.

Application of inoculum was done by broadcasting the contents of each flask (both wet and dry media) onto the surface of the weeds in each tray. The trays were then left in the shade for four weeks.

3.2.7. Data analyses

A completely randomised design was used for all the experiments. All experiments were performed twice. Count data were transformed using logarithmic transformation (Steel and Torrie 1980), prior to an analysis of variance (ANOVA). Dry weight of waterhyacinth plants was determined four weeks after inoculation by drying whole waterhyacinth plants in paper bags for four to five days at 60C. Dry weights were recorded as gram per tray. Results were pooled after testing for homogeneity of variances using Bartlett's test (Steel and Torrie 1980) and also when no significant difference due to the experiment was detected.

3.3. Results

3.3.1. Solid substrates

Conidia were produced on all the solid substrates tested (Table 8). *F. solani* isolate 5aex25 produced the largest number of conidia on beans, while *F. solani* isolate 2a3 sporulated well on barley, maize and on soyabeans. There was very little mycelial growth on both beans and soybeans by all the fungi tested. The conidia produced by both isolates of *F. solani* were mainly microconidia, with less than one percent being macroconidia, on all the grains and pulses tested. *F. moniliforme* produced mainly microconidia on the grains and pulses, while about 30% of the conidia produced on groundnuts, beans and barley were macroconidia. On soybeans, *F. moniliforme* produced microconidia and macroconidia in approximately equal amounts while only 2% of the conidia produced on maize were macroconidia. *F. pallidoroseum* produced the highest number of conidia on wheat and these were mainly microconidia, while it produced macroconidia on all the other grains and pulses tested.

Conidia yield from the straw was generally lower as compared to that from the grains and pulses. Both isolates of *F. solani* and *F. moniliforme* sporulated well on waterhyacinth straw. The conidia produced by both isolates of *F. solani* were about 10% macroconidia. *F. moniliforme* produced mainly microconidia on the waterhyacinth straw, while it produced both microconidia and macroconidia on the other straws.

Table 8. Total number of conidia (x10^7) produced by *Fusarium* isolates per gram of solid substrate.

Substrate	*Fusarium* 5aex25	*solani* 2a3	*Fusarium moniliforme*	*Fusarium pallidoroseum*
maize	0.16c[1]	8.60a	0.33c	0.71b
soybean	1.05c	8.30a	8.40a	0.01b
beans	8.15a	2.54b	2.27b	0.01b
wheat	0.39c	2.14b	0.32c	7.45a
barley	5.35b	8.80a	0.47c	0.21b
maize straw	0.01c	0.28c	0.29c	0.02b
waterhyacinth straw	0.56c	1.83b	1.83b	0.10b
groundnut straw	0.55c	0.87c	1.45b	0.46b
wheat straw	0.1c	0.1c	0.11c	0.05b
barley straw	0.43c	0.20c	0.36c	0.80b

[1]Means followed by the same letter in the column are not significantly different at P=0.05, according to Duncan's multiple range test.

F. pallidoroseum produced mainly macroconidia on all the straw substrates tested except groundnut straw in which it yielded approximately equal amounts of macroconidia and microconidia.

3.3.2. Liquid media

Complex media composed of a natural plant substrate and defined chemicals (modified Richard's medium and PDB) supplied essential nutrients in a balance which favoured good production of conidia for those media tested (Table 9). Both isolates of *F. solani* produced the highest number of conidia in modified Richard's medium, while *F. moniliforme* and *F. pallidoroseum* produced the highest number of conidia when grown in PDB.

F. solani isolate 5aex25, *F. moniliforme* and *F. pallidoroseum* produced more conidia in PDB than in PSB, while *F. solani* isolate 2a3 produced more conidia when PSB was used. Conidia production by all the *Fusarium* isolates in Tochinai solution was poor under submerged liquid conditions. Conidia produced in PSB and PDB were mainly microconidia with some macroconidia, while conidia produced in modified Richard's medium were mainly macroconidia.

3.3.3. Assessment of viability

All the fungi germinated on water agar within 24 hours after spraying. Conidia produced in submerged culture were morphologically similar to conidia produced on the grains and pulses as well as on the straw.

Table 9. Total number of conidia ($x10^7$) per ml of liquid media produced by different *Fusarium* isolates.

Media	*Fusarium* 5aex25	*solani* 2a3	*Fusarium moniliforme*	*Fusarium pallidoroseum*
Potato dextrose broth	7.42a[1]	8.79b	18.80a	2.01a
Potato sucrose broth	0.28b	13.00a	3.20c	0.30b
Modified Richards medium	9.84a	13.30a	10.90b	1.48a
Tochinai solution	0.03c	0.81c	0.16d	0.01c

[1]Means followed by the same letter in the column are not significantly different at P=0.05, according to Duncan's multiple range test.

3.3.4. Pathogenicity test

The soybean production system was demonstrated to be very efficient in terms of number of conidia produced, and was further investigated in the pathogenicity test, using the most promising bioherbicide candidates, *F. solani* isolate 5aex25 and *F. pallidoroseum*. The lowest dry weight of 12.9g was obtained from waterhyacinth treated with wet crushed soybeans inoculated with *F. solani* isolate 5aex25, and this was followed by wet whole soybean inoculated with *F. pallidoroseum* (Table 10).

Table 10. Dry weight of waterhyacinth treated with different inoculated soybean media per tray in grams.

Media	Control	*Fusarium pallidoroseum*	*Fusarium solani* 5aex25
wet whole soybean	20.13a[1]	13.95c	15.41b
dry whole soybean	20.13a	22.65a	19.70a
wet crushed soybean	21.84a	15.95bc	12.93b
dry crushed soybean	22.41a	16.62b	18.46a

[1]Means followed by the same letter in the column are not significantly different at P=0.05, according to Duncan's multiple range test.

3.4. Discussion

All the *Fusarium* isolates produced conidia on all the solid media tested in varying degrees. Both isolates of *F. solani* and *F. moniliforme* sporulated profusely on soybeans and beans, but *F. pallidoroseum* produced few conidia on these substrates. Mycelial growth was limited on beans and on soybeans. A good culture medium supports high sporulation and low mycelial growth (Dhingra and Sinclair 1995). The concentration of medium constituents determines the quality and quantity of growth and whether sporulation or vegetative growth will dominate and generally sporulation is favoured by nutritional exhaustion (Dhingra and Sinclair 1995).

Hilderbrand and McCain (1978) found that *F. oxysporum* f.sp. *cannabis* formed the largest number of chlamydospores in diffusates prepared from soybean meal. In the same study barley straw and oat straw gave low yields of chlamydospores. *Fusarium* isolates differ in requirements for growth and sporulation, consequently no one set of conditions is optimum for all (Chi and Hanson 1964). Maize meal agar is used to produce large quantities of *F. solani* f. sp. *pisi* for field inoculation (Kraft and Berry 1972), and so maize was expected to be a good medium for mass production of *F. solani*. *F. solani* isolate 2a3 responded favourably in this medium, but not isolate 5aex25, which produced relatively few conidia. Composition of the medium and the environmental requirements vary considerably from one organism to another in the same genus and even within the same species (Abbasher 1994). Differences in availability of nutrients, moisture content, surface area, vitamins and other growth factors may be responsible for the variable sporulation response of the *Fusarium* spp.

on the solid media tested.

Conidia production on straw was low, with the exception of straw obtained from the host, waterhyacinth. Perhaps the nutritional requirements of the fungi were not met in these media. Hilderbrand and McCain (1978) found that specific amino acids were important, and concluded that these nutritional effects probably explained why various natural plant products affected spore formation differently as the plant products would all have a different nutritional composition. Good conidia production by *Fusarium* spp. on grain and the host straw was reported in Sudan (Abbasher 1994). Sorghum grain and *Striga* straw were used as inoculum substrates for the fungus *Fusarium nygamai* and they gave better control of *Striga hermonthica* compared to sorghum straw (Abbasher 1994).

When comparing the liquid media, both isolates of *F. solani* produced the highest number of conidia in modified Richard's medium. This was in contrast to the result obtained by Chi and Hanson (1964) who evaluated several media for growth and sporulation of *F. solani*, and found that it sporulated best on potato-glucose medium and Richard's medium, while modified Richard's medium was inferior for growth and sporulation. A delicate balance between nutrition (carbon, nitrogen and minerals) and environment (temperature, pH and aeration) control sporulation of filamentous fungi in liquid media (Vezina, Singh and Sehgal 1965). Since all of these parameters were not monitored in this experiment, it is not possible to account for this difference.

Conidia production by *F. solani* isolate 5aex25, *F. moniliforme* and *F. pallidoroseum* in PDB was better than conidia production in PSB while the reverse

was true for *F. solani* isolate 2a3. Carbon compounds are used by fungi as a source of energy and of the chief structural element (Lilly and Barnett 1951). Chi and Hanson (1964) evaluated different carbon sources (in Richard's medium less carbon as the basic medium) and found that starch, mannitol, xylose and glucose were the best sources of carbon for sporulation of *F. solani*. Lilly and Barnett (1951) reported dextrose to be the best carbohydrate source for most fungi. Peptone, a complex nitrogen source, did not favour production of *Fusarium* spp. in liquid culture.

Production of conidia on solid substances is time consuming, labour intensive, prone to contamination, may be uneconomical and submerged production techniques are favoured in the West since the expertise and technology are available, and because scale up of the process is relatively easy (Churchill 1982, TeBeest 1985). The solid substrate system however, may be appropriate in developing countries where agricultural wastes are available, elaborate facilities limited, and labour is abundant.

All the fungal isolates produced on the different media germinated within 24 hours on water agar. Macroconidium germination by *Fusaria* can be a rapid process, completed after four to seven hours of incubation in some instances (Griffin 1981). Although spore viability should be determined before application, a high germination reading does not necessarily indicate a high infectivity potential (Dhingra and Sinclair 1995).

Good weed control indicated by low weed weights, was obtained when wet soybean inoculum was used. The medium used to increase fungus inoculum can influence its infectivity potential. Generally fungi grown on a rich medium are more

vigorous than those grown on a nutritionally poor one (Dhingra and Sinclair 1995). Soil infestation with the *Dreschlera* state of *Cochliobolus sativus* produced on agar, liquid medium, or autoclaved seeds caused little or no infection on barley; however, when soil was infested with inoculum grown on maize meal, high levels of disease occurred (Ludwig, Clark, Julien and Robinson 1956).

Use of crushed soybeans helped in disseminating the pathogen and created more infection sites, especially for *F. solani* whose main effect on the stolons is on the top surface of the waterhyacinth plant. Plants treated with *F. pallidoroseum* had necrotic lesions on the underside of the petiole and hence infested wet whole soybean seed which tended to sink in the tray, allowing the inoculum around the seed to dissolve in the water, was effective in causing damage to waterhyacinth plants. Both the whole and the crushed soybean seed were difficult to handle when wet, as the particles tended to stick together. Drying the media was therefore intended to improve handling. Desiccation of the fungi during drying of the media may have affected the performance of the fungi, and hence the dry soybean seed did not give results similar to those obtained when wet seed was used.

The choice of media tested was based mainly on the availability on farms. Although soybeans are produced by many farmers in Zimbabwe (both commercial and communal) it is produced as a cash crop and is therefore of high value. Although the straws are used in communal areas as a mulch as well as feed for cattle, they are considered to be of lower value, and would have been the media of choice. It may be necessary therefore, to examine the performance of waterhyacinth treated with

Fusarium as this would be a cheaper medium, readily available in the communities where it is a problem weed. However, there is legislation against the use of waterhyacinth in Zimbabwe, and unless this is relaxed, it might not be possible to use waterhyacinth as a substrate to mass produce fungal inoculum.

4. HOST RANGE STUDIES

4.1 Introduction

4.1.2. Determination of host range

A critical consideration in the development of a biological control agent is the determination of host range (Weidemann 1991). Careful study of the host specificity of a pathogen serves to provide some assurance that crops and valuable species would be safe from disease produced by the pathogen when it is used as a bioherbicide. Although various schemes have been proposed to systematically identify susceptible species, the centrifugal-phylogenetic test proposed by Wapshere (1973, 1974,1975) has been most widely accepted. A small group of taxonomically related plants with morphological and biochemical similarities to the target weed is first tested, gradually expanding the number of tested species to include more distantly related plants in order to delimit the extent of the biocontrol agent's host range. Cultivated plants that are related to the weed, poorly characterized for associated pests, evolved apart from the agent, attacked by related pests, and previously recorded as possible hosts are also tested. Despite thorough testing, it is possible to fail to determine host range adequately with organisms that attack plants irregularly distributed in several plant families, organisms specific to two alternate hosts in different taxa, and organisms attacking several phylogenetically separated plant groups (Wapshere 1974).

Plant pathogens range from highly host specific obligate parasites to facultative necrotrophs with a wide host range (Brian 1976). Phylogenetic testing is most precise with highly host-specific pathogens that are well characterized in the literature. The

precise delimitation of host range is more questionable with pests that are less host-specific. Most fungi that are being evaluated as potential bioherbicides are facultative saprophytes with relatively wide host ranges, including some host-limited strains (restricted to one or a few species) (Watson 1985). However even pathogen species considered to have a wide host range may consist of subspecies populations with more limited host preferences (Caten 1987).

Charudattan (1989) proposed modifying the test requirements based on the level of specificity of plant pathogens. A centrifugal phylogenetic test would be used with highly host specific pathogens, whereas pathogen taxa known to be less specific would also include plants ecologically and economically important at the release site and known or reported to be suscepts of the pathogen.

Differences exist in the level of specificity considered acceptable between pathogens imported for classical biological control and endemic pathogens used as bioherbicides (Weidemann 1991). It is generally accepted that imported pathogens present a greater potential threat to non-target plants (Leonard 1982, Wapshere 1982). Detailed host range information is still required, however, to avoid potential conflicts of interest, to avoid exerting increased disease pressure on cultivated plants, to avoid potential hazards associated with introductions into an area where the pathogen did not occur previously, or to accommodate changes in cropping practices (Leonard 1982, Weidemann 1991). In some respects, the host range evaluations of candidate bioherbicides are analogous to crop safety and efficacy testing for chemical herbicides (Watson 1985). There must be a margin of safety required for desirable plants

occurring in close proximity to the target weed, and this safety margin is less important for plants far removed from the target weed.

4.1.2. Classification of waterhyacinth

Waterhyacinth belongs to the order Pontederiales (Dahlgren, Clifford and Yeo 1985). There is only one family Pontederiaceae, in this order which consists of nine genera. *Pontederia, Reussia, Zosterella, Hydrothrix* and *Eurystemon* are confined to the Americas, *Eichhornia* and *Heteranthera* occur in both the New and the Old World, *Monochoria* is found in the old world tropics, and *Schlleropsis* is eastern Asiatic (Dahlgren et al. 1985).

There is one species of *Eichhornia* native to Southern Africa, *E. natans* (Beauv.) Solms Laubach (Dyer 1976). It occurs in the northern parts of South West Africa and in Botswana. There are two other plants of the family Pontederiaceae native to Southern Africa, *Monochoria africana* (Solms) N.N. Br. is found in the low altitude area of the Transvaal and *Heteranthera callifolia* Reichb. ex Kunth occurs from South West Africa to the Northern Transvaal, including Botswana and Zimbabwe (Dyer 1976).

4.1.3. Objective

The purpose of the experiment reported here was to delimit the host specificity of *F. moniliforme, F. pallidoroseum*, as well as isolates 5aex25 and 2a3 of *F.solani*, and therefore to determine their suitability as biological control agents for

waterhyacinth in Zimbabwe. Host specificity of the fungi to selected varieties of plants was studied in pots, as well as in the field.

4.2. Materials and methods

4.2.1. Selection of Plants

Test species were selected by using both centrifugal (related plants), and varietal (economic plants) strategies (Wapshere 1974, 1975). Crop plants for varietal studies were chosen from recommended cultivars for Zimbabwe. A total of 64 different types of plants (some with several varieties tested, representing 30 families), were used in this experiment (Appendix C).

Commelina benghalensis L. (Commelinaceae), order Commelinales, *Allium cepa* L. (Liliaceae) order Liliales, and *Musa cavendishii* Lam. (Musaceae) order Zingiberales were selected for testing as these orders were the ones most closely related to waterhyacinth in Zimbabwe. Plants were also selected from Poaceae which contains numerous important agricultural crops grown, on a commercial as well as on a subsistence scale in Zimbabwe. Seeds of the long season maize hybrid SR52, four medium season hybrids, and one open pollinated variety, Kalahari were provided by the Maize Agronomist, at Harare Research Institute. Sorghum, pearl millet, and finger millet seed was provided by the Sorghum and Millet Breeder of the Crop Breeding Institute. Sugarcane, wheat, barley and oats, important commercial irrigated crops were included in the experiment. Seed of pasture grasses which included *Paspalum urvillei* Steud, grown in wetland areas where waterhyacinth is likely to be found, was

provided by the Pastures Section of Henderson Research Station, while grass weed seeds were provided by the Weed Research Team of the Department of Research and Specialist Services.

Since the Zimbabwean economy is based on agriculture, food crops, fruit trees and flowers, which are mainly grown for export in Zimbabwe, were included in the experiment. The flowers selected were *Rosa alba* L., *Campanula cinerea* L.f., *Ageratum houstonianum* Mill., *Tagetes erecta* L. and *Zinnia peruviana* L.. Aquatic plants tested were *Azolla filiculoides* Lam. and *Hydrocotyle ranuculoides* L.f., as these were the plants found growing alongside waterhyacinth in the water bodies visited during the survey.

Plants from Cucurbitaceae, Fabaceae and Solanaceae that are known hosts of the *Fusarium* species under study as well as other species of *Fusarium* were tested.

4.2.2. Pot Experiment:

4.2.2.1. Establishment of plants

Seeds of test plants were planted in asbestos flower pots 25cm diameter and 24cm deep on the 23rd of November 1994 at Henderson Research Station (17° 35' S, 30° 58' E). The soil used was a dazomet (granular soil fumigant) treated sandy loam, and the fertilizer used was a commercial formulation with NPK ratios (5%N 18%P 10%K) which was applied in pots at a rate of 250kg/ha and mixed with the soil. Each pot contained four plants and each planting was replicated 15 times. All plants were

derived from seed, except for fruit trees, roses (*Rosa alba*), grape vines (*Vitis vinifera* L.) and strawberries (*Fragaria virginiana* L.) which were bought as established seedlings from Golden Stairs Nurseries, Harare. Sugarcane (*Saccharum officinarum* L.) was planted using planting setts with three segments each, and aquatic plants were generated from ramets, and grown in asbestos trays 60cm long, 10.5cm wide and 20cm deep, to which was added a nutrient solution of 2ml groesia per litre of water. Pots were watered when necessary using tap water.

4.2.3. Inoculation of plants

Every fungal treatment was applied to three pots, and three pots were used as controls. The treatments were arranged in a randomised complete block design. The fungi were grown in PDB, in 2L glass jars on a rotary shaker (250rpm), in continuous fluorescent light for ten days. Conidia were harvested by passing through a soil sieve onto which two layers of cheesecloth were placed, to avoid mycelia in the spray mixture. The fungal material remaining on the cheesecloth and inside the glass jar was rinsed with 50ml of water. Conidia counts were determined with the aid of a haemocytometer. The conidia concentration was adjusted using water, to a conidia suspension of 10^7 conidia/ml, which was used for all the fungi.

Application of pathogens was done using a knapsack sprayer calibrated to deliver 242 L/ha of the spray mixture. All the plants were sprayed to runoff on the 22nd of December 1994. A Hessian screen was used to prevent drift to adjacent plants during spraying. In order to permit the testing of plants and plant parts of

different ages, the plants were inoculated again on the 17th of January 1995, and for a third time on the 8th of February. The plants were left in an open fenced area for the duration of the experiment. Plants were visually assessed first on the 16th of January 1995, and the second and final assessments were on the 7th and 29th of February 1995 respectively, using a disease rating in which 'I' denoted immune, 'HR'-highly resistant (slight flaking),'R'-resistant (small pin-point lesion), 'MS'-moderately susceptible (distinct lesion which does not expand) and 'S'-susceptible (collapse/death).

4.2.4. Field Experiment

4.2.4.1. Plant Establishment

Plots were established on 15 December, 1994 at Henderson Research Station (17°35'S 30°58'E) to ascertain the host specificity of the *Fusarium* spp. under natural conditions. The block of land used had been planted to a commercial crop of sunflower (*Helianthus annus* L.) during the 1993/94 season. The soils are medium grain sandy clay loams with a pH of 6.1.

The land was fertilized with 350 kg/ha of a commercial fertilizer (8N 14P 7K). The fertilizer was applied after disc ploughing and incorporated using a tractor disc harrow. The plots were separated from each other by a distance of 3m. Seeds were sown in 20 plots, each measuring 15.5 m long and 5m wide. The spacing used was 0.5m between rows and 20cm within the row. Twenty asbestos trays (60cm long, 20.5cm wide and 20cm deep) each with 20 healthy plants of waterhyacinth were placed in these plots. A supplementary irrigation of 22mm was applied after planting,

to facilitate germination of the plants, three days after planting. The design of the experiment was a randomized complete block design with four replicates.

4.2.5. Inoculation of plants

Plants were inoculated first on the 17th of January and then again on the 8th of February 1995. The procedure used to inoculate plants was the same as described for the pot experiment. Two millimetres of rain fell a day after the first spraying, and 0.3mm four hours after the second spraying.

4.3. Results

4.3.1. Host specificity- Pots: Twenty one days after the first application of inoculum the laminae of inoculated waterhyacinth showed some chlorosis and a few spots. However, there were no indications of infection on any of the other inoculated plants. Three weeks after the second application of inoculum small lesions on the stems and leaves of *Setaria verticilata* were noticed on plants that had been inoculated with *F. solani* isolate 2a3. However, the lesions did not expand. Inoculation with *F. moniliforme* resulted in yellow patches which later turned grey on the leaves of kale (*Brassica rapa* L.), as well as chlorosis on sunhemp (*Crotalaria juncea* L.) and waterhyacinth leaves in pots. Both isolates of *F. solani* caused dark brown lesions on waterhyacinth leaves and petioles, as well as leaf burning on the margins of the leaves. Waterhyacinth leaves inoculated with *F. pallidoroseum* turned brown starting from the margins, while groundnuts (*Arachis hypogea* L.) variety Flamingo, developed spots on

the leaves. The final assessment did not reveal any further disease development in any of the plants, except for waterhyacinth which continued to deteriorate.

4.3.2. Host specificity - Field:

Evidence of *Fusarium* spp. infection was not found on any of the plants tested, including *Setaria verticilata* three weeks after inoculation. Both isolates of *F. solani* caused yellowing of the older leaves of *Commelina benghalensis* L., three weeks after the second application of inoculum. Typical symptoms of *Fusarium* spp. infection were noted on waterhyacinth in the inoculated plots within three weeks.

4.4. Discussion

Setaria verticilata, a common grass weed of arable lands was the only plant moderately susceptible to *F. solani* isolate 2a3 in pots, and damage was only on older, senescent leaves. Infection occurred only after two applications of the fungus and only after the plants had been confined to pots for ten weeks. When grown in the field, *S. verticilata* was not susceptible to *F. solani*. Conditions in nature would not approach those that existed in pots during this experiment. Host range studies of plant pathogens conducted under controlled conditions have often resulted in broader host ranges than reported or previously known and this may extend to pot grown plants (Watson 1985). Predisposition (the tendency of nongenetic condition, acting before infection to affect the susceptibility of plants) under controlled environmental conditions, could play an important role. The principal predisposing features may not

be known precisely, but the fact that plants grown in pots are usually more liberally watered and fertilized may predispose the plants to disease. Since *S. verticilata* is a member of Poaceae, the result of the pot trial suggests that further studies with *F. solani* isolate 2a3 on predisposed or weakened economically important grass species may be warranted.

Groundnut plants treated with *F. pallidoroseum* developed brown spots on the leaves, but there was no further development of symptoms on the groundnuts, in the pot experiment. *F. pallidoroseum* is not listed as a pathogen of groundnuts in Zimbabwe (Rothwell 1983). Since there were no symptoms on groundnuts (cv Flamingo) when grown in the field, this slight disease reaction might also have been due to predisposition of the pot grown plants.

Both isolates of *F. solani* only caused symptoms on the older leaves of *C. benghalensis* which is also a common weed of arable lands in Zimbabwe, in the field experiment. These plants did not exhibit any other symptoms, and thus appear to be resistant when growing vigorously. However *C. benghalensis* is a close relative of waterhyacinth, and this result may indicate that *F. solani* isolate 5aex25 is restricted in its host range to relatives of waterhyacinth. Although (Dyer 1976) reported the presence of *H. callifolia* in Zimbabwe, the National Herbarium was not able to supply information on where this plant could be collected, and all their records were old, with references to the plant growing in shallow and non-perennial ponds and streams. It was absent in all the water bodies visited during the survey (before the host range testing experiment), and so it was not included in this experiment.

Although species of *Fusarium* have been reported as parasites on virtually all cultivated crop species, many Fusaria are host specific and are classified as *formae specialis* according to their specificity (Jones and Hancock 1990). For example *F. solani* f. sp. *cucurbitae*, applied for control of Texas gourd (*Cucurbita texana*), is limited to infection of cucurbits. Isolations of *F. solani* from red clover (*Trifolium repens* L.) in USA infected only legumes , and the pathogen on pea was distinct from that on bean (Booth and Waterston 1964). This specialization provides a predictable host range, thus reducing the risk of infecting a plant species absent from host range screening (Jones and Hancock 1990).

Kale and sunhemp were moderately susceptible to *F. moniliforme* in addition to waterhyacinth. Kale and sunhemp are not close relatives of waterhyacinth, and this gave the impression that, this fungal isolate was not host restricted. It is known to have a broad host range (Abbas, Tanaka and Duke 1995) and some *F. moniliforme* isolates are highly toxic to mammals (Rabie, Marasas, Lubben and Vleggaar 1982). These moniliformin producing strains were isolated from sorghum, sorghum malt, millet and maize obtained from Southern African countries (Namibia, Mozambique and the Republic of South Africa). *Fusarium moniliforme*-contaminated maize has been linked to human oesophageal cancer, pulmonary edema syndrome in swine, cancer-promoting activity in rats, and a variety of other animal toxicoses (Rheeder, Marasas, Thiel, Sydenham, Shephard and Van Schalkwyk 1992, Richardson and Bacon 1995). It is also known to produce a range of phytotoxic compounds that are chemically diverse and possess a broad range of biological activities and metabolic effects.

Although some of the secondary products of *F. moniliforme* are potent phytotoxins, many of these products also exhibit mammalian toxicity (Abbas, Boyette and Hoagland 1995). The possible production of toxins affecting human health are concerns that prompt very stringent assessment of bioherbicide candidates (Charudattan 1982). This consideration and the fact that *F. moniliforme* is an important pathogen of maize during and after harvest (Abbas and Boyette 1992) led to the dropping of *F. moniliforme* from further evaluation. Because *F. solani* isolate 2a3 appeared to have a broader host range than isolate 5aex25, it was also dropped from further testing. Limited resources also contributed to the dropping of *F. solani* isolate 2a3 and *F. moniliforme* from further evaluation.

F. solani isolate 5aex25 and *F. pallidoroseum* were retained for further evaluation. Based on these host specificity tests, their use for biological control of waterhyacinth would not be expected to create problems either for plants grown commercially or for plants considered to be of ecological importance in Zimbabwe.

5. INTEGRATED CONTROL OF WATERHYACINTH

5.1. Introduction

Waterhyacinth weevils provide substantial control of waterhyacinth, but consistent reliable reductions at all sites where they have been released has not occurred (Center et al. 1990). This variability in the performance of biological control agents may be due to variation in plant quality (Center and Dray 1992). In general weevil population growth is superior on high quality plants. However, high quality plants are often associated with eutrophic conditions and exhibit rapid growth rates. Even though weevil populations fare well under these circumstances their impact may be lessened by profuse plant growth. Size of weevil populations and degree of biological control are not necessarily correlated, rather the severity of the impact depends upon complicated interactions amongst aquatic nutrient loads, proximate composition of the plant tissue, and the physiology of the biological control agents (Haag and Habeck 1991).

Waterhyacinth treated with 2,4-D showed a decrease in lamina hardness for the youngest leaves and an increase in nitrogen (Wright and Bourne 1990). These changes in plant quality may account for improved waterhyacinth control after 2,4-D treatment because larvae of *N. eichhorniae*, and *N. bruchi* could enter leaves and grow effectively (Messersmith and Adkins 1995). *Neochetina* spp. weevils successfully controlled waterhyacinth in ponds when half the area was sprayed with glyphosate in a pattern that left a short boundary along which daughter plants could colonize open

water (Haag et al. 1988). However when glyphosate was applied in a pattern that left a long boundary, daughter plant growth surpassed the weevil population increase and waterhyacinth filled the open water areas.

N. eichhorniae weevils controlled waterhyacinth more effectively when combined with the experimental growth retardant EL-509 [α-(4-chlorophenyl-α-(1-methylethyl)-5-pyrimidine-methanol] than when used alone (Center et al. 1982). EL-509 was ineffective without weevils. Similarly, *N. eichhorniae* plus the growth retardant paclobutrazol [1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol] provided 95% reduction of waterhyacinth growth, which was higher than either method used alone (Van 1988).

The Abbott formulation of *Cercospora rodmanii* was tested together with *Neochetina* spp. (Charudattan 1984). The combination of *C. rodmanii* and the arthropods was capable of eliminating waterhyacinth from the test frames while plants treated with the fungus alone or with the arthropods alone were not adequately controlled (Charudattan et al. 1984).

5.2. Objective

The objective of this study was to examine plant growth of waterhyacinth subjected to waterhyacinth weevils as well as *F. solani* and *F. pallidoroseum*.

5.3. Materials and Methods

Waterhyacinth was grown outdoors in 18 concrete lined ponds with clay bases

which were rectangular (surface area 5.0m^2 and 0.65m deep), and which received pump circulated water from Lake Chivero, at the Fisheries Research Center within Lake McIlwaine National Park. Waterhyacinth plants were collected from various locations in the Hunyani River system. Healthy plants from the different locations were mixed among ponds to provide uniform colonies. Thirty waterhyacinth weevils collected from weeds growing next to Skyline bridge in Hunyani River were placed on waterhyacinth in each of nine ponds on 10 March 1995. Each pond was covered with a mosquito gauze cage which was 30cm high.

On 10 April some waterhyacinth was removed from the ponds to make infestations uniform in area, and to leave every pond covered by an area of 60cm x 2.0m of waterhyacinth. Conidia suspensions of *F. solani* isolate 5aex25 and *F. pallidoroseum* were each used to spray a third of the weevil treatments as well as a third of the ponds without weevils, to give a randomized complete block design with three blocks. The density of the suspensions was 1 x 10^7 conidia/ml (in water), and it was applied over the leaf canopy using a knapsack sprayer.

Infestations of *A. filiculoides* and algae were removed from the ponds fortnightly with small fine nets. Plant coverage was monitored in each tank fortnightly from 10 May, until 29 August, 1995. Plants were gently pushed to one end of each pond before plant coverage measurements were taken. Changes in plant coverage at the end of 18 weeks were used as estimates of weed control in the various treatments. Canopy height was also determined. The heights of five plants selected at random from each pond, were measured from the base of the petiole to the tip of the longest

leaf and the mean of those values was taken as the canopy height. Changes in plant volume (coverage x canopy height) over time were used as nondestructive estimates of plant growth in the various treatments.

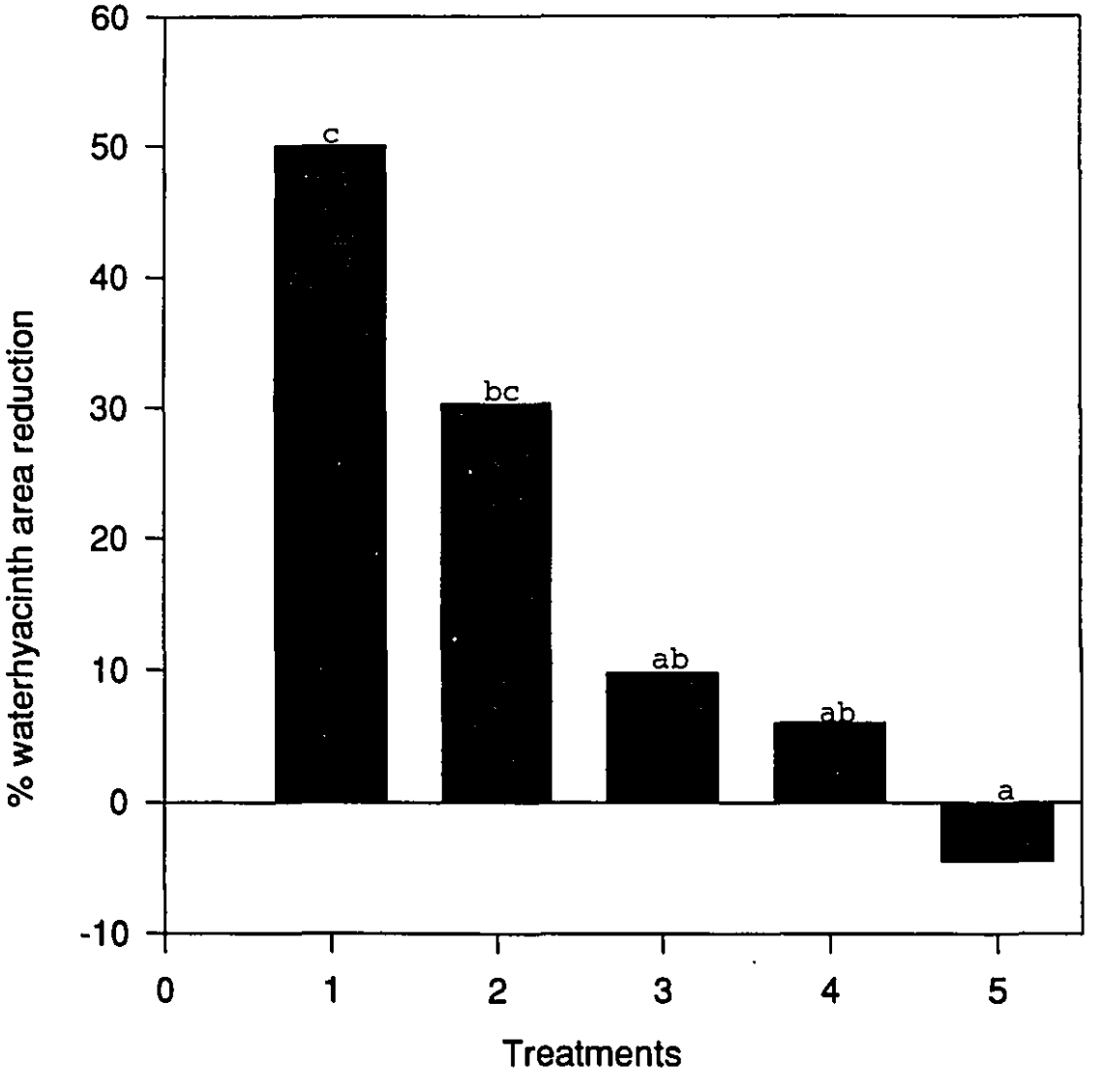
Four plants were randomly selected from each pond, and a plastic tag was attached to the third nodal position leaf (usually the youngest mature leaf) on each of four ramets in each pond. Numbers of live and dead leaves on each ramet were counted. On 29 August, the number of new leaves produced by each ramet was determined by the change in the nodal position of the tagged leaf. Live and dead leaves were again counted, and the number that had died was derived by comparison with previous counts. Count data were transformed using logarithmic transformation (Steel and Torrie 1980) prior to analysis of variance (ANOVA).

The minimum and maximum temperatures at the Research Centre were monitored daily at the weather centre, and the mean monthly minimum and maximum temperature readings were obtained for the duration of the experiment. Plant coverage data were subjected to ANOVA. Plant volume data were subjected to repeated measures analysis and multivariate analysis of variance (MANOVA).

5.4. Results

Treatment with *F. solani* and *Neochetina* weevils resulted in a 50% decrease in the area covered by waterhyacinth relative to the control (Figure 6). This was followed by treatment with *F. pallidoroseum* and *Neochetina* weevils which caused a 30.3% reduction in the area covered by waterhyacinth.

Figure 6. Effect of *Neochetina* weevils, *Fusarium solani* and *F.pallidoroseum* on area covered by waterhyacinth after 18 weeks, in winter. Thirty *Neochetina* weevils were used to infest ponds and the fungi were applied in water after one month at a rate of 1×10^7 conidia /ml. 1. *F. solani* + *Neochetina* weevils, 2. *F. pallidoroseum* + *Neochetina* weevils, 3. *Neochetina* weevils only, 4. *F. solani* only and 5. *F. pallidoroseum* only. Bars with the same letter are not significantly different at P=0.05, according to Duncan's multiple range test.


60
50
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% waterhyacinth area reduction
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Treatments

Plants in ponds treated with *F. solani* and weevils were very thin and spindly, and showed extensive damage from both weevil feeding and the pathogen, especially on the petioles (Figure 7). Petiole bases were necrotic and waterlogged from larval tunnelling. Submerged water-logged plant material pulled the shoot apices below the surface, and spaces opened in the waterhyacinth mat (Figure 8). The spaces were quickly covered by *A. filliculoides* and algae.

Feeding marks were evident on weeds growing in the ponds with the weevils only treatment, and there was a 9.8% reduction in the area covered by waterhyacinth, relative to the control. Typical symptoms of damage by *F. solani* and *F. pallidoroseum* and damage to older petioles were evident in the ponds treated with these fungi alone. There was a slight decrease of 6% (relative to the control) in the area covered by waterhyacinth in ponds treated with *F. solani*, while the area increased in ponds treated with *F. pallidoroseum*. Although the control ponds were not inoculated with pathogens, with time disease symptoms also appeared on the older leaves.

There was a significant time x treatment effect in the waterhyacinth volume. The greatest reduction in waterhyacinth volume was in ponds treated with *F. solani* and *Neochetina* weevils (Figure 9). This was followed by the *F. pallidoroseum* and *Neochetina* weevils treatment. There were no differences in waterhyacinth volumes, between the ponds in which weevils only and fungi only were used, and the control.

Figure 7. Effect of *Fusarium solani* on the petioles of *Neochetina* damaged waterhyacinth plants. Thirty *Neochetina* weevils were used to inoculate each pond, and *F. solani* was applied in water at the rate of 10^7 conidia/ml four weeks later. a) Symptoms of *F. solani* infection six weeks after inoculation, b) collapse of waterhyacinth plants inoculated with weevils and *F. solani* 16 weeks after inoculation with the fungus.


a
b

Figure 8. Effect of *Neochetina* weevils, *Fusarium solani* and *F. pallidoroseum* on waterhyacinth growing in ponds, 16 weeks after inoculating with the fungi. Thirty *Neochetina* weevils were used, and the fungi were applied in water at the rate of $1x10^7$ conidia/ml four weeks later. 1) *F. solani* + weevils, 2) *F. pallidoroseum* + weevils, 3) weevils, 4) *F. solani*, 5) *F. pallidoroseum*, 6) control.

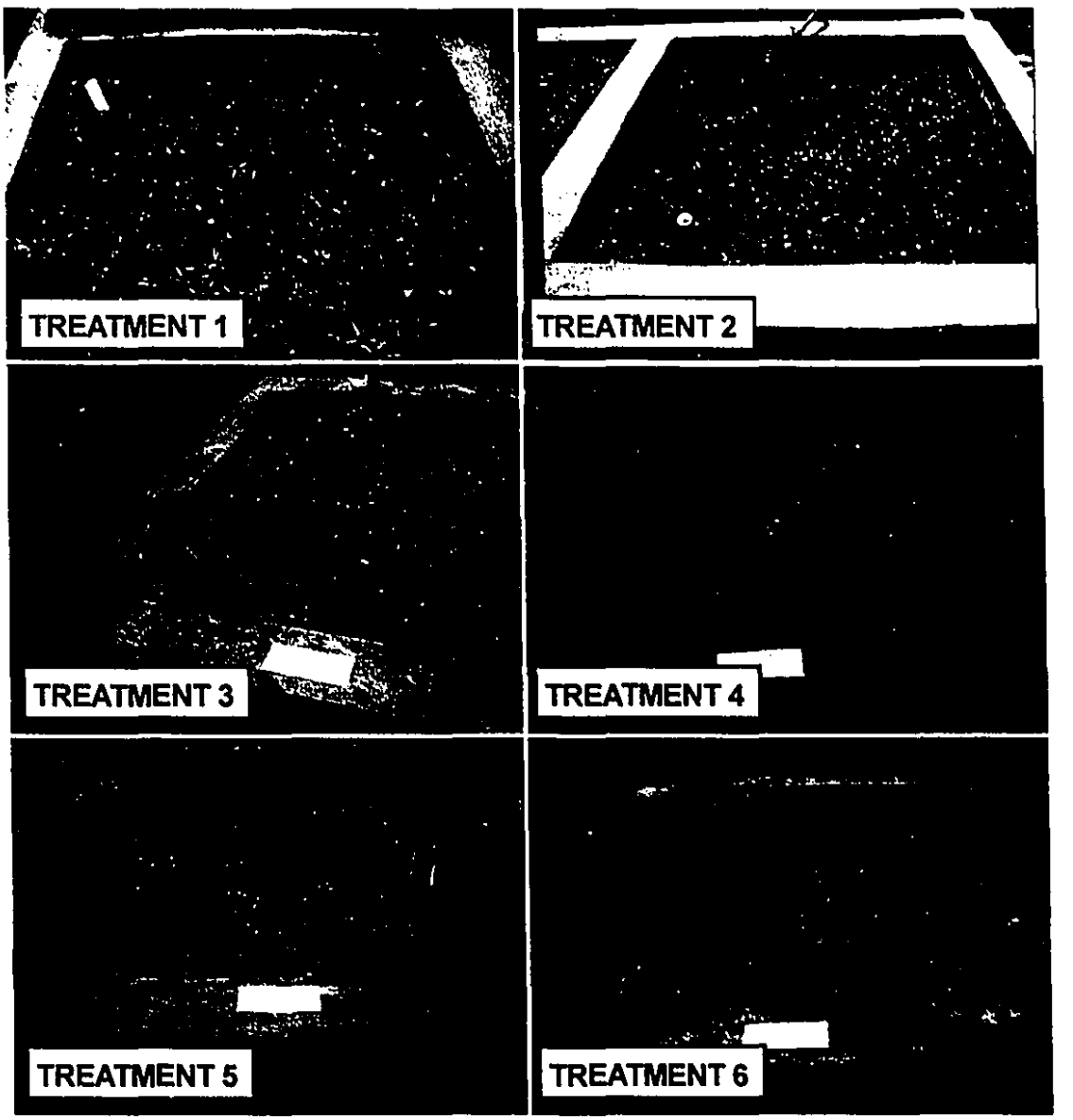
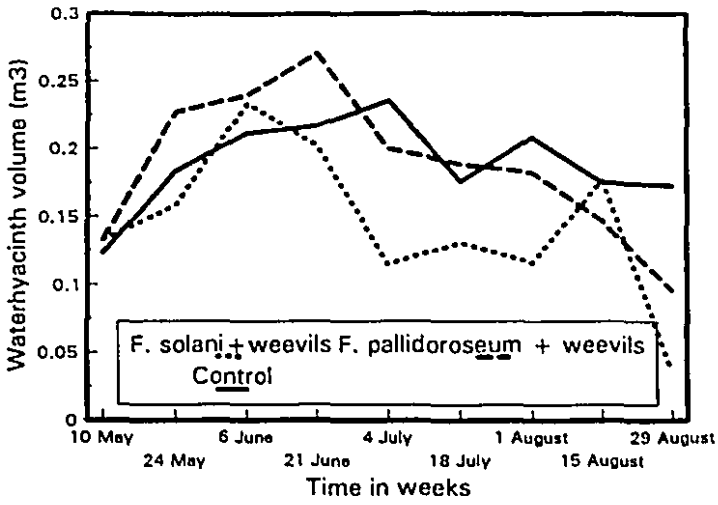
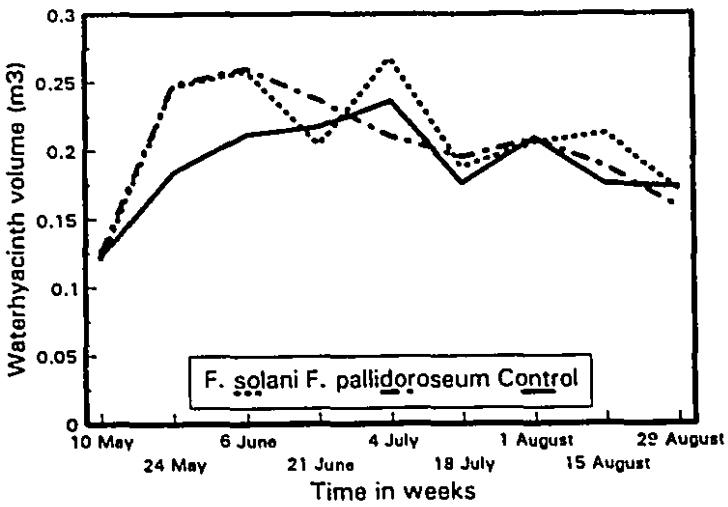

TREATMENT 1
TREATMENT 2
TREATMENT 3
TREATMENT 4
TREATMENT 5
TREATMENT 6

Figure 9. Effect of *Neochetina* weevils, *Fusarium solani* and *F. pallidoroseum* on waterhyacinth volume over 18 weeks in winter. Thirty *Neochetina* weevils were applied into each pond and a month later the fungi were applied at the rate of 1 x 10^7conidia/ml. Waterhyacinth volume was measured fortnightly. a) *F. solani* + weevils, *F. pallidoroseum* + weevils and control; b) *F. solani*, *F. pallidoroseum* and control; and c) *Neochetina* weevils and control.

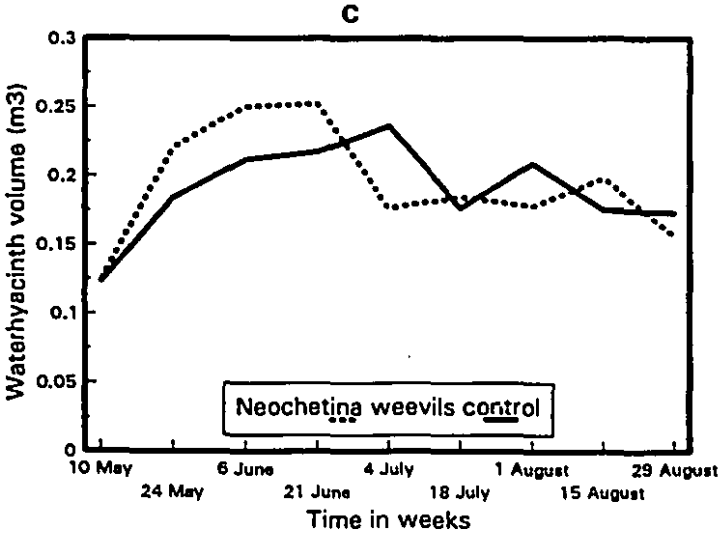
a


Waterhyacinth volume (m3)
0.3
0.25
0.2
0.15
0.1
0.05
0
F. solani + weevils
F. pallidoroseum + weevils
Control
10 May
24 May
6 June
21 June
4 July
18 July
1 August
15 August
29 August
Time in weeks

b


Waterhyacinth volume (m3)
0.3
0.25
0.2
0.15
0.1
0.05
0
F. solani
F. pallidoroseum
Control
10 May
24 May
6 June
21 June
4 July
18 July
1 August
15 August
29 August
Time in weeks

c


Waterhyacinth volume (m3)
0.3
0.25
0.2
0.15
0.1
0.05
0
Neochetina weevils
control
10 May
24 May
6 June
21 June
4 July
18 July
1 August
15 August
29 August
Time in weeks

The mean monthly maximum temperature declined at the Fisheries Research Centre from April to July, and started to increase again in August 1995 (Table 11). There was a reduction in the mean monthly minimum temperature from April to June. The lowest temperature recorded for the duration of the experiment was 9C on the night of 20 June 1995, and the waterhyacinth growing in the experimental ponds was affected. The uppermost parts of the waterhyacinth plants were damaged, and they turned brown. There was an increase in the mean monthly minimum temperature in July and August, 1995.

There were no significant differences between treatments in number of new leaves formed per ramet and in the number of dead leaves per ramet (Table 12). The leaves that were formed on waterhyacinth onto which combination treatments were applied were very small (Figure 7).

Table 11. Mean monthly maximum and minimum temperatures at Lake Chivero from April to August 1995.

Month	Mean maximum temperature (C.)	Mean minimum temperature (C.)
April	22.1	17.0
May	21.3	14.2
June	17.0	11.5
July	15.8	11.8
August	16.8	15.0

Table 12. Number of live and dead leaves on waterhyacinth treated with different biocontrol agents for 18 weeks.

Treatment	Number of new leaves/ramet	Number of dead leaves/ramet
Fusarium solani + weevils	3.2	6.5
Fusarium pallidoroseum + weevils	2.8	4.7
Weevils only	5.2	7.0
Fusarium solani	3.3	4.0
Fusarium pallidoroseum	4.3	4.5
Control	4.2	4.5

The number of live leaves per ramet and the number of dead leaves per ramet are not significantly different at P=0.05.

5.5. Discussion

In terms of vegetative growth waterhyacinth is one of the most productive plants and its growth is directly related to the level of available nutrients in the water in which the plant is growing (Chadwick and Obeid 1966, Wahlquist 1972, Mitchell 1974, Pieterse 1978). The biocontrol efficacy of the weevils as well as the pathogens was related to the growth rate of the weed host. Because waterhyacinth can outgrow disease pressure and insect damage through increased growth, when the biological control agents were used individually, the area covered by waterhyacinth continued to increase. However, when a combination of the fungi and the weevils were used, more pressure was exerted on the waterhyacinth and there was a reduction in the area covered by waterhyacinth.

The main effect of the combinations of biocontrol agents on waterhyacinth appears to be in a reduction in the size of waterhyacinth leaves produced. Thus although there were no significant differences in both the number of new leaves formed and the number of dead leaves in the different treatments, there was still a significant reduction in the area covered by waterhyacinth in ponds where a combination of biocontrol agents were used. Although the experiment was conducted in winter, when waterhyacinth was not expected to be very actively growing, new leaves were formed by the plants. It was difficult to accurately count the number of dead leaves in the combination treatment at the end of the experiment, as many plants and leaves had sunk in the ponds, and therefore some dead leaves might have been missed.

The stocking rate of waterhyacinth weevils was low. Damage by waterhyacinth weevils at low densities does not kill plants but reduces their growth rate and fecundity (Cilliers 1991). In Australia the weevils caused a gradual deterioration in waterhyacinth vigour, with decreased flower and seed production. However, relatively low numbers of adult weevils can kill a waterhyacinth plant under the proper conditions (Perkins 1978). In laboratory studies using as few as five 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. Large, healthy field plants have been found with more than 20 adult weevils, indicating the importance of proper conditions in affecting the weed (Perkins 1978). Conditions at Lake Chivero are not optimum for waterhyacinth weevils and they would be expected to feed and reproduce more slowly in a cool climate, compared to a tropical climate (Harley 1990). The mean annual temperature range in Lake Chivero is from a minimum of 14C in mid-winter (July) to a maximum of 25C in mid-summer (January), although extreme temperatures have been recorded (Thornton and Nduku 1982). The experiment was conducted in winter (April to August), because the ponds which were borrowed from Fisheries Research Centre were only available for use at this time. The low temperatures may have contributed to the poor control of waterhyacinth in ponds with weevils only.

There was no significant difference between the area covered by waterhyacinth in the control ponds and in the ponds where fungi were used individually. It is not possible to maintain biocontrol pressure on an exponentially growing weed population,

unless the agent is capable of killing or substantially damaging the meristematic tissues of the plant (Charudattan et al. 1985). Vigorous waterhyacinth plants with sufficient nutrient supplies can outgrow infection (Charudattan, DeValerio and Prange 1990). The ponds were receiving water directly from Lake Chivero, which is highly eutrophicated. *C. rodmanii* was also not effective in controlling waterhyacinth growing in eutrophicated Lake Alice and this was thought to be a result of the increased nutrition, which may impart a higher degree of immunity to the plants either through an increased growth rate on an altered metabolism (Conway and Freeman 1978).

F. solani and *F. pallidoroseum* appear to behave like facultative parasites (fungi which usually grow on dead or decaying matter, but have at the same time the faculty of attacking living tissues under certain conditions) (Butler and Jones 1949). Some facultative parasites are weak parasites, as they become parasitic only when the host plant has been weakened in its vitality by some harmful agency e.g. insect damage. In many cases, particular tissues of a plant are normally of low vitality and are readily available as food for weak parasites for example, in this case, old leaves.

Following damage by cold temperature, there was a significant reduction in waterhyacinth volume in all the ponds that contained weevils. This suggests that this was a vulnerable period in the life cycle of the waterhyacinth. Interactions between waterhyacinth and biological control agents are different when plants are in a phase of growth compared to a phase of decline (Harley 1990). However, there was an increase in the volume of waterhyacinth treated with fungi only. The environment

may affect both the growth and resistance of the host plant and also the rate of growth or multiplication and degree of virulence of the pathogen (Agrios 1988). At temperatures much below the optimum for the pathogen, disease development is slower. In August, there was an increase in the temperatures at Fisheries Research Centre, and this appeared to favour growth of waterhyacinth in the first fortnight. Waterhyacinth volume declined further to different degrees in all the treatments in the second fortnight in August, indicating that the increased temperatures had further stimulated the activity of the biocontrol agents.

Waterhyacinth treated with a combination of the weevils and the pathogens declined, and at the end of four months, most of the weeds were rotting. In a study assessing the effects of waterhyacinth weevils and *C. rodmanii*, seven months elapsed following initial treatments before the combined stress due to the insects and the pathogen caused a 99% weed eradication (Charudattan 1986). This suggests that for small water bodies, it may be possible to control waterhyacinth infestations using a combination of weevils and *Fusarium* within a shorter period of time rather than three to six years that is generally required where use of *Neochetina* weevils alone has been successful (Harley 1990).

There are a number of ways in which insects and plant disease are linked (Agrios 1980). Insects can be vectors of fungal spores. *Neochetina* weevils feed specifically on waterhyacinth, so the weevils could be important in spreading spores within and between populations of waterhyacinth (Galbraith and Hayward 1984). Injuries caused by the weevils weaken the adjoining tissues, rendering them more

subject to fungal attack. Feeding scars have often been reported as the means of entry to the leaf, particularly for weak pathogens without effective means of penetration (Carter 1973). Pathogens can be distributed as larvae move through the plant tissue (Galbraith and Hayward 1984). The corn stalk rots develop in this way (Christensen and Schneider 1950). Many of the fungi associated with the rot have been found in and outside of the larvae of the corn borer, *Pyrausta nubilasis* Hbn. Frass deposited in the tunnels is an excellent medium for the rapid growth of saprophytes and pathogens such as *Fusarium spp.*, which then invade living tissue (Christensen and Schneider 1950, 1966). Necrosis of waterhyacinth plants might well progress in a similar way as the larvae of *Neochetina spp.* tunnel through the plants and hasten the spread of the *Fusarium* spp.

Injury by insects can predispose a plant to infection (Carter 1973). Increase in water stress and a drop in the rate of respiration can increase susceptibility of the host to the pathogen (Cook and Baker 1983). Insect salivary secretions include enzymes and plant growth regulatory compounds (Anders 1958, Kloft 1960). Waterhyacinth leaves have specialized phenol-storing cells which contain phenols implicated in plant resistance to microbial attacks (Martyn et al. 1983, Charudattan et al. 1990). This plant resistance which is dependant on phenolic compounds may be interfered with at the site of a feeding scar (Miles 1968, Galbraith and Hayward 1984).

In general *F. solani* produced more conidia than *F. pallidoroseum*. The greater the number of conidia, the more inoculum reaches the host, greatly increasing the chances of an epidemic (Agrios 1988). This might partly explain why the *F. solani*

and weevils treatment was more effective than the *F. pallidoroseum* and weevils treatment. Furthermore, *F. solani* effectively damaged waterhyacinth petioles, while most of the damage caused by *F. pallidoroseum* was on waterhyacinth leaves.

F. solani and *F. pallidoroseum* do not appear to be very effective pathogens of waterhyacinth. However, when they are combined with *Neochetina* weevils, they enhance control of waterhyacinth by the weevils. In the present situation they must be contributing to the overall control initiated by the weevils, and there are instances of using weak pathogens in biological control e.g. as saprophytic antagonists to preclude colonisation by facultative parasites (Skidmore and Dickinson 1976). Since the fungi are already present in many waterhyacinth infestations in Zimbabwe, they could be augmented onto waterhyacinth infestations onto which waterhyacinth weevils have been released, and they would hasten control of waterhyacinth. This would be expected to boost the prospects of biological control in Zimbabwe, where decision makers have generally been sceptical of classical biological control with *Neochetina* weevils which is expected to take at least four years under the cool conditions at Lake Chivero, before the waterhyacinth is brought under control. Although the cool temperatures during winter in Zimbabwe, are not conducive to *Neochetina* weevil activity, they also inhibit, excessive weed growth. Applying the fungi at this vulnerable stage in the weevil infested weed, would increase biocontrol pressure and would be expected to reduce the area under waterhyacinth cover.

6. GENERAL DISCUSSION

Waterhyacinth is now found in seven out of eight provinces in Zimbabwe, absent only in the arid province of Matebeleland South. This is of great concern in a country with a severe dry season, and few streams or rivers which continue to flow throughout the year (Mheen 1995). Action should be taken to prevent spread to uninfested areas. An important aspect of preventing further spread of waterhyacinth, is to raise the awareness of local people by a public awareness campaign highlighting the problems caused by waterhyacinth in Zimbabwe. The campaign should stress the importance of not spreading waterhyacinth, not polluting water, and of reporting new waterhyacinth infestations to an appropriate authority (Mitchell 1985).

There is no centralised decision making body assigned to deal with waterhyacinth infestations in Zimbabwe, as responsibility to control the weed is assigned to several government departments. Waterhyacinth outbreaks are dealt with on an ad hoc basis, with no anticipation of the problem. The responsibility for monitoring waterhyacinth spread and implementing control should be vested in a central government agency with expertise, authority and funding to act.

The proliferation of waterhyacinth in its exotic range is determined largely by nutrient supply and the absence of natural enemies of the weeds. To be fully effective control strategies in Zimbabwe must address both watershed management and direct weed control. Because of water pollution, even if effective control measures are applied on the waterhyacinth, it is expected that the niche it vacates will be filled by other aquatic weeds.

A survey conducted by the FAO showed that little work has been done on biological control in Africa (Labrada 1994). As a result policy makers may be somewhat sceptical about the importance of biological control in Zimbabwe. The frequent use of 2,4-D on waterhyacinth infestations affected the establishment of waterhyacinth weevils, because it resulted in extensive loss of habitat for the weevils. Although the adult weevils were expected to be able to fly to unsprayed waterhyacinth, the immature stages perished in the dying weed. As weevil populations have a much slower rate of increase than waterhyacinth, the ratio of weevils to waterhyacinth plants is expected to continue to decline. Furthermore, because of the cool temperatures experienced in Harare, weevil populations in the Hunyani River System may increase at a slower rate, as compared to warmer climates.

Because of the importance of Lake Chivero and the Hunyani River system and the extent of their waterhyacinth infestations, it is unlikely that spraying with 2,4-D will be terminated in the near future. It is therefore necessary to plan and implement biological control efforts judiciously. Further releases of biological control agents should be made in rivers and dams that are less intensively managed. Alternatively, releases should be coordinated together with personnel responsible for spraying waterhyacinth in the Department of National Parks and Wildlife Management. Initial observations showed that the weevils were able to establish and spread in Zimbabwe, even in the relatively cold temperatures experienced in the Hunyani River system. One of the major advantages of classical biological control is that it provides a permanent, self-perpetuating solution to a weed problem (Cook 1994).

Most of the fungi isolated from diseased waterhyacinth were not pathogenic. *Fusarium solani*, *F. pallidoroseum* and *F. moniliforme* are weak pathogens of waterhyacinth. When *F. solani* and *F. pallidoroseum* were used in combination with waterhyacinth weevils, the pathogen contributed to the overall control exerted on the weed, by accelerating decay of the weevil infested plants. The weevil feeding marks as well as the tunnels produced by *Neochetina* larvae provided the fungi with entry points, into the otherwise water-repellant foliage of waterhyacinth. Both pathogens were easily produced in shake-flask fermentation in liquid media as well as on solid substrates. Pathogen infested soybean caused phytotoxicity on waterhyacinth. The narrow host ranges of both pathogens suggests that it would be feasible to conduct further field trials for the control of waterhyacinth. However it is necessary to evaluate both pathogens for production of mycotoxins before further work is implemented.

7. CONCLUSION

On the basis of the results obtained during the survey and in the experiments carried out in this study, the following conclusions can be drawn:

1) Waterhyacinth infestations are now widespread in seven out of the eight provinces in Zimbabwe, and adequate measures are not being applied to control these infestations.

2) Waterhyacinth weevils *Neochetina eichhorniae* and *N. bruchi* are present in Zimbabwe at ever decreasing populations and there is need to rationalize the herbicide spraying programs to allow the waterhyacinth weevils to further establish and spread. Alternatively waterhyacinth weevils should be released in other water bodies which are not being sprayed with 2,4-D, especially in the warmer environments of Mutoko and Masvingo where they are likely to have a better chance of establishing.

3) *Fusarium solani* isolate 5aex25 and *F. pallidoroseum* isolated from diseased waterhyacinth leaves have potential to be used as biocontrol agents on waterhyacinth in combination with waterhyacinth weevils.

4) *F. solani* isolate 5aex 25 and *F. pallidoroseum* can be easily produced in liquid and solid substrate culture. This success in producing large numbers of conidia on various crude agricultural products and in liquid media offers several alternatives to develop effective large-scale or cottage-scale conidia production systems.

5) *Fusarium solani* and *F. pallidoroseum* appeared to be restricted to plants closely related to waterhyacinth. From a total of 64 plant species from 30 families tested, only one species in addition to waterhyacinth was moderately susceptible to either of

the isolates.

6) Further field scale experiments combining the fungi with waterhyacinth weevils are recommended as the next step in order to determine the feasibility of using the *Fusarium* isolates to enhance biological control of waterhyacinth with *Neochetina* weevils.

8. CLAIMS OF ORIGINAL CONTRIBUTION TO KNOWLEDGE

To the best of the author's knowledge, the following are considered to be original contributions to knowledge:

1) This is the first documented report on the status of waterhyacinth weevils in Zimbabwe.

2) This is the first documented record of the pathogens of waterhyacinth in Zimbabwe.

3) This is the first report on extensive testing of *F. solani*, *F. pallidoroseum* and *F. moniliforme* as pathogens of waterhyacinth.

4) This is the first documented report of testing waterhyacinth weevils with *Fusarium spp.* on waterhyacinth.

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10. APPENDICES

Appendix A. Questionnaire

Province_____

Name of Officer_____

1. In your province which dams and/or rivers are infested with water hyacinth? (Please list in space provided).

2. What is the extent of the infestation?

a) 50 - 100 water cover.

b) 20 - 50% water cover.

c) In floating mats covering less than 20% of the water.

d) Covering less than 20% water and growing mainly along the banks.

List **Dams**	**Extent of infestation**
a)	
b)	
c)	
d)	
e)	

List **Rivers**	**Extent of infestation**
a)	
b)	
c)	
d)	
e)	

3. What measures if any have been taken to control the weed?

a) none

b) herbicides

c) mechanical clearing

d) biological control

Structure of the questionnaire that was sent to the different Extension Officers.

Appendix B. Dates and sites at which diseased waterhyacinth plants were collected

	LOCATION	Date of Collection
Insect "Free" Sites		
Chisamvi Dam	16° 56'S 32° 25'E	02/04/93
Kudzwe Dam	16° 56'S 32° 35'E	03/03/94
Lake Mutirikwi	20° 04'S 30° 52'E	11/03/93
Matova River	20° 10'S 30° 45'E	29/03/93
Mucheke River	20° 00'S 30° 45'E	15/03/93
Mushagashe River	19° 50'S 30° 47'E	22/03/93
Nyadiri River	17° 25'S 32° 13'E	24/03/93
Sites with *Neochetina* spp.		
Lake Chivero	17° 54'S 30° 47'E	22/01/93
Darwendale Dam	17° 50'S 30° 30'E	26/01/93
Hunyani River	17° 58'S 31° 00'E	29/01/93
Manyame dam	17° 55'S 31° 08'E	23/03/93
Mukuvisi River	17° 58'S 30° 55'E	02/03/93
Nyatsime River	18° 00'S 31° 00'E	16/02/93
Seke Dam	17° 55'S 31° 08'E	24/02/93

Appendix C. Plants tested in the host range experiment

FAMILY	Pot	Field
Taxonomically Related to Waterhyacinth		
Poaceae		
Eleusine coracana		
Eleusine indica (L) Gaertn		
Hodeum vulgare L.		
Nata		
Pote		
Oryza sativa L.		
Panicum maximum Jacq.		
Paspalum urvillei Steud.	NT	
Pennisetum americanum (L.) V. Schum		
PMV1		
PMV2		
Rottboellia cochinchinensis (Lour) W.D. Clayton		
Saccharum officinarum L.		NT
Setaria verticillata (L.) P. Beauv		
Sorghum bicolor (L.) Moench		
SV1		
SV2		
Triticum aestivum L.		
Kairo		
Sengwa		
Urochloa panicoides Beauv		
Zea mays L.		
R215		
SR52		
SC601		
ZS233		
ZS225		
Kalahari		
Liliaceae		
Allium cepa L.		NT
Musaceae		
Musa cavendishii Lam	NT	

Appendix C. (continued)

	Pot	Field
Economically important		
Anacardiacae		
Mangifera indica L.		NT
Annonaceae		
Annona squamosa L.		NT
Amaranthaceae		
Amaranthus hybridus L.		
Azollaceae		
Azolla filiculoides Lam		NT
Campanulaceae		
Campanula cinarea L.f.		NT
Capparaceae		
Cleome monophylla L.		NT
Chenopodiaceae		
Spinacia oleracea		NT
Commelinaceae		
Commelina benghalensis L.		
Compositae		
Ageratum houstonianum Mill		NT
Bidens pilosa L.		
Helianthus annus L.		
Mopane		
Lactuca sativa L.		NT
Tagetes erecta L.		NT
Tagetes minuta L.	NT	
Zinnia peruviana (L.) L.		
Convolulaceae		
Ipomoea batatas L.		NT
Cruciferae		
Brassica oleracea L.	NT	
Brassica rapa L.		NT
Euphobiaceae		
Ricinus comunis L.		
Fabaceae		
Arachis hypogaea L.		
Falcon		NT
Flamingo		
Heron	NT	
Makulu Red		
Plover		
Valencia		
Crotalaria juncea L.		

Appendix C (continued)

	Pot	Field
Glycine max (L.) Merrill		
Gazele		
Nyala		
Macroptilium atropurpureum (Dc) Urb.		
Phaseolus vulgaris L.		
Ex-rico		
Broad beans		
Natal sugar		
Pisum sativum L.		
Stylosanthes guianensis (Aubl) Sw		NT
Vigna anguiculata (L.) Walp		
Vigna subperranea (L.) Verdic		
Labiatae		
Leucas martinicensis (Jacq) Ait.f.	NT	
Lauraceae		
Persea americana Mill.		NT
Malvaceae		
Gossypium hirsutum L.		
HA2	NT	
var 72		NT
var 75		NT
Myrtaceae		
Psidium guajava L.		NT
Passifloraceae		
Passiflora edulis Sims	NT	
Portulacaceae		
Portulaca grandiflora Hook		NT
Ranunculaceae		
Aquilegia vulgaris L.		NT
Rosaceae		
Fragaria virginiana L.		NT
Rosa alba (L.)		NT
Rubiaceae		
Richardia scabra L.		NT
Rutaceae		
Citrus aurantium L.		NT
Citrus limon (L.) N. Burman		NT
Umbelliferae		
Daucus carota L.		NT
Hydrocotyle ranuculoides L.f.		NT
Vitaceae		
Vitis vinifera L.		NT

Appendix C (continued)

	Pot	Field
Known Hosts		
Cucurbitaceae		
Citrullus vulgaris Schrad.		
Cucumis sativus L.		
Cucurbita maxima Dutch. ex Lam.		
Cucurbita pepo L.	NT	
Solanaceae		
Caspicum annum L.		
Lycopersicon esculentum. Mill.		
Maglobe		
Roma		NT
Rossol		
Nicandra physalodes Scop.		NT
Nicotiana tabacum L.		
Physalis angulata L.	NT	
Solanum tuberosum L.		NT

NT = not tested