RNRRS PROJECT FINAL TECHNICAL REPORT

The development of an integrated management strategy for *Rottboellia cochinchinensis* (itchgrass) in maize-based cropping systems in selected areas of Latin America

R NUMBER: R6690 (ZA0051, ZA0052, ZA0140)

PROGRAMME MANAGER: Dr. S. Eden-Green, NR International Ltd

SUB-CONTRACTOR: CATIE, Turrialba, Costa Rica; CABI Bioscience, Ascot centre

RNRRS PROGRAMME: Crop Protection

RNRRS PRODUCTION SYSTEM: High Potential

COMMODITY BASE: maize, legumes.

RNRSS PROGRAMME PURPOSE: improved methods for the management of broad-leaved and grass weeds in cereal crops and pasture developed.

	Planned	**Actual**
START DATE:	1st APRIL 1995	1st APRIL 1995
FINISH DATE:	31st MARCH 1999	31st MARCH 1999

Executive Summary

The field component of this project continued validation of sustainable methods for itchgrass (*Rottboellia cochinchinensis*) management at farmer's fields in the seasonally dry Pacific region of Costa Rica. Additional research was conducted to further develop mucuna or velvetbean (*Mucuna deeringiana*) as a cover crop and itchgrass suppresser in maize, aiming to better use resources and the complete or partial elimination of chemical herbicides. The scope of the project was widened by including surveys and limited research into validation plots in Bolivia and Mexico.

Farmers perceived itchgrass as a troublesome species in Campeche, Oaxaca and Veracruz and dedicate part of their resources to management, especially by a combination of manual control (slashing) and herbicides. Based on field visits and information provided by farmers it seems likely that the weed was introduced to maize-producing areas by contaminated rice seed and subsequently spread on tillage equipment. Itchgrass was also frequent in all of 17 locations surveyed in Santa Cruz, Bolivia and was ranked as an aggressive weed in 78% of sampled sites.

Field research in Costa Rica determined that two varieties of indeterminate mucuna similarly suppressed itchgrass density and biomass, with only minor reduction in maize yield. In experiments replicated in two years, mucuna better covered the soil and reduced itchgrass density and biomass when sown simultaneously with maize than when planted later. Lower maize grain yields were obtained in both years if maize was grown in association with mucuna at 50 000

plants/ha, especially, when the cover crop was planted simultaneously with maize. Itchgrass itself decreased maize yield by about 46%. In a separate experiment conducted in 1998, a determine-growth mucuna variety proved useful in suppressing itchgrass.

Information of itchgrass density-dependent mortality and seed production under field conditions was generated to support modelling studies. Additionally, preliminary screening of cover crops for itchgrass management was conducted at El Vallecito Experiment Farm in Santa Cruz, Bolivia. Promising species were *Dolichos lab lab*, *Canavalia ensiformis* and *Mucuna* (*Stizolobium*) *cinereus*.

Three-year validation plots at farmer's fields in Costa Rica evaluated the integration of no-tillage, use of a selective herbicide (pendimethalin) in the first season to lower the initial density of itchgrass, planting of velvetbean (*Mucuna deeringiana*) between maize rows as a cover crop, and prevention of itchgrass seed set in the fallow period. Pendimethalin effectively controlled itchgrass at the onset of validation plots and allowed the establishment of the cover crop during the first corn crop. At all sites itchgrass densities were lower in validation plots than in grower's fields and infestation levels decreased throughout the years with integrated management. In general, yields (corn and dry beans - planted as a rotation crop in one of the sites) were higher in validation plots at all locations and cropping seasons; however soil fertilisation regimes differed between validation and grower's plots and, in some cases, improved corn varieties were used in validation plots. Soil core samples taken each year at the beginning of the cropping season revealed substantial reductions in the itchgrass soil seed bank in validation plots. Partial budget analyses also demonstrated that integrated itchgrass management was economically feasible for smallholders. A validation plot was also established in Oaxaca, Mexico in 1998 but only included minimum tillage and the use of pendimethalin to reduce the initial itchgrass infestation. Nevertheless, this validation plot was always less infested with itchgrass than the commercial one.

The project consolidated an important contribution to the goal of improving yields by demonstrating the feasibility for uptake of improved integrated management strategies for one of the most troublesome weeds in tropical America. Sustainability of these high potential cropping systems was enhanced as a result of development of farmer-accessible technology suitable to recover productive lands from high weed infestations that threaten continued cultivation and to maintain low weed populations thereafter.

During the first two years of research, native pathogens with biocontrol potential were identified and investigations with stress factors to increase the severity of the pathogens performed, under greenhouse conditions. Fifty-one native pathogens of *R.. cochinchinensis*, were evaluated. The best strains were: strain 2 (*Curvularia* sp.), strains 99 and 130 (*Drechslera* sp.) and strains 69 and 127 (*Fusarium* spp.).These pathogens were evaluated on rice, sugar cane, *Brachiaria* spp. and rice cultivars, on which no disease symptoms were observed.

To identify the stress factors which could improve the effectiveness of native pathogens, trials were conducted with shading, flooding and sub-lethal doses of many herbicides including pentimetaline and nikosulfuron. Good results were only observed with haloxifop methyl (Galant). The best stress factor and pathogen combinations were the inoculation of strains 69 or 127 (*Fusarium* spp.) after the application of a sub-lethal dose of the herbicide haloxifop methyl (0.096 a.i. Kg / ha or 2 ml / litre), to seedlings 30 days old or less.

In order to evaluate the native itchgrass pathogens (strain 69, strain 127 and both combined) under field conditions, trials were conducted in five maize plots located in Guanacaste. The

pathogens were tested in water or formulated with cannola oil and tween 40, and inoculated after the application of sub-lethal doses:0.048 , 0.072, and 0.096 a.i. of haloxifop methyl. This research also evaluated the potential of a *Mucuna* cover crop to reduce the itchgrass population. The results showed that both pathogens in combination with the two lowest herbicide doses (1 and 1.5 ml/l) caused the death of 15 - 25 days old seedlings, depending on the environmental conditions. Under field conditions, the best results were observed with lower doses. Individual pathogens were more effective than a mixture. Combining the pathogen formulation with a *Mucuna* cover crop did not improve itchgrass control.

The potential of a head smut S*porisorim ophiuri*, as a classical biocontrol agent, for use in an integrated management strategy against *Rottboellia cochinchinensis* in Costa Rica was investigated. For this, a comprehensive host range screening programme was undertaken using the Madagascan isolate of the smut. The results show that the smut is extremely host specific, as none of the 49 species/varieties of graminaceous test plants became infected.

Electrophoretic techniques were used to characterise the genotypic variation of weed biotypes. The results suggested that the biotypes form a narrow genetic base with greater than 80% similarity. This high degree of similarity can be related to the predominately inbreeding nature of the weed and to the relatively recent expansion of its geographic range. Weed biotypes can be broadly grouped according to their geographic origins. Latin American biotypes form two distinct groups indicating that these populations probably arose from a relatively small number of introductions. Neotropic invasions of the weed appear to have mainly been from Africa, although biotypes in Brazil and Colombia probably originated in Asia. This genetic homogeneity suggests that the smut should infect the majority of Latin American biotypes, some of which would be new associations.

Molecular characterisation of a range of smut genera was also undertaken. The genus *Sporisorium* could be readily distinguished from other smut genera and this should enable the Madagascar strain to be “finger printed” if the need should arise. In addition smut pathotypes from Africa and Asia could be separated.

Infection studies showed that both sporidia and teliospores are capable of initiating infection.

An existing model was adapted specifically to explore itchgrass population dynamics in maize based cropping systems in Costa Rica. The effects of the head smut on its own, and in combination with surpressive cover crops were explored. A simple simulation of smut population dynamics was also incorporated later. The interaction of smut and cover crop depend on how they are modelled and hence on how biocontrol with the smut is implemented. Under a purely natural infestation the cover crop affects smut reproduction as well as the itchgrass, and therefore reduces effectiveness of the smut. However when smut infection was modelled as a constant proportion of itchgrass plants each season (e.g. by augmentation or if applied as a mycoherbicide), then the smut in combination with a cover crop could be a highly effective. Results also suggest that under a natural infestation of smut there can be a trade off between infectivity, reproductive rate, and mortality rate in the soil in selecting effective smut strains. The interaction between the smut and itchgrass is stable over a wide range of parameter values for the smut. The ability to reduce population density to a low stable equilibrium density is one of the requirements for a good biological control agent.

Background

Rottboellia cochinchinensis [Lour.] W.D. Clayton (Gramineae) or itch grass, is a pantropical agricultural weed that is increasing its range at an alarming rate. It is native to the Old World, but was introduced to the New World, probably at the beginning of the century, and it is here, in its exotic range, that infestations are considered to be the most severe (Ellison & Evans, 1992). This could be due to a number of factors, including improved climatic compatibility, mans activity's in disseminating the grass, favourable agronomic practices, and the absence of co-evolved natural enemies. In Central America *R. cochinchinensis* is found infesting both annual and perennial crops and has been reported causing significant yield loss in maize, sugar cane, upland rice, beans and sorghum (Herrera, 1989). Elsewhere, infestations can result in up to 80% crop loss, or even abandonment of agricultural lands (Holm *et al*., 1977).

Previous studies and surveys conducted in Costa Rica documented the importance of the weed and the need to develop suitable and economically feasible control alternatives for small-scale farmers. In Mexico there were indications of localised invasions, especially in the state of Campeche, whereas in Bolivia it was observed as an important weed in maize and rotation crops. Initial research under EMC X0179 demonstrated that effective in-crop control was essential for maintaining low levels of the weed, but that planting in zero tillage and prevention of seed set during the fallow period were also important. It was also shown that little viable seed remained after 12 months in the soil, underlining the importance of prevention of seed set in the weed's management.

Sustainable methods for managing itchgrass in the seasonally dry Pacific and humid tropical Atlantic regions of Costa Rica were further developed and began being validated under R6047 (X0261). The main focus was the integration of cultural and herbicide management systems with the use of suppressive cover legumes for sustainable itchgrass control. Completion of a four-year experiment in heavily infested lands in the seasonally dry Pacific maize growing area demonstrated the usefulness of combining in-crop herbicides, control of residual plants during the off season and zero tillage. Early planting of *Mucuna* and *Canavalia ensiformis* gave good ground cover, suppressed itchgrass and increased maize yields compared to unweeded controls. Under different conditions, both the itchgrass suppressive ability of *Mucuna* and the yields of maize were inversely related to the planting density, indicating that yield penalties might sometimes be expected. In initial validation plots farmers (despite higher initial costs) perceived the technology developed by the project very favourably.

A promising area of research is the use of fungal pathogens to control itchgrass. Both the mycoherbicide and the classical biological approaches have been investigated with funding from ODA-UK (DFID), in Costa Rica (NRI EMC R5344, R6047) and the UK (NRI EMC-R5225, R5883). Since many farmers currently use chemical herbicides, adopting mycoherbicides as an environmentally benign alternative, that is not hazardous to apply, would appear to be feasible. The use of a classical biological control (CBC) agents is considered to be of direct value to the farmers since they will neither cost the farmer in time nor money. They will form a self sustaining 'back ground' inhibition of the weed population, potentially reducing the need for other control methods, or at least enhancing their effectiveness. These agents particularly target areas not typically tended by farmers (e.g. fallow fields, road sides and headlands), and weeds that escape the control measures used by farmers (e.g. those weeds between crop plants within a row), all of which constitute an important source of seed for re-infestation. This component is also considered to be of indirect value to Costa Rica (and other Latin American countries), since it will potentially lay the foundations for the introduction of other fungal biological control agents for the control of weeds.

At CATIE, endemic pathogens (*Fusarium, Curvularia*) have been screened for pathogenicity to the weed and for potential use as mycoherbicides (Jiménez *et al.*, 1990, Lenne 1990). *Fusarium moniliforme* and *Fusarium* spp. are effective endemic biocontrol agents of *R. cochinchinensis* in the wild. Isolates of *Curvularia*, a previously known pathogen, also caused severe damage under screen house conditions. High susceptibility to *F. moniliforme* was also found in 20 and 30 days old weed seedlings; local varieties of maize, sorghum and sugarcane were not affected. The fungus is seed transmitted and there were significant differences in pathogenicity among isolates (Bustamante *et. al.,* 1993; Fuentes & Bustamante, 1996). Itchgrass was most prone to the seed pathogens when planted in soil sterilised with methyl bromide. Development and yield of itchgrass was greater when grown in high fertility soil compared with low fertility, or non-sterilised soils (Fuentes & Bustamante, 1996).

Previous investigations had identified two obligate pathogens of *R. cochinchinensis* with potential as CBC agents for Costa Rica; a head smut *Sporisorium ophiuri* (P.Henn) Vanky (Ustilaginales) and a rust *Puccinia rottboelliae* P&H Sydow (Uredinales). Initial host range screening of these pathogens showed that both were highly host specific. The smut was found to be a soil-borne, systemic pathogen, infecting seedlings before they emerged from the soil. In pot experiments, 80% infection, and hence, significant reductions in seed set was achieved. Observations from the field suggested that, in the endemic range of the weed, natural epiphytotics of the smut were common, often with a high percentage of plants infected within a population. Although isolates of the smut were found to be itchgrass-biotype specific, one isolate from Madagascar was found to infect a wide range of biotypes including a number from Latin American, and hence selected for further host range screening. The number of biotypes in the introduced range of the weed has not been fully documented and, hence, the abiity of the Madagascan isolate to infect all populations of *R. cochinchinensis* in the New World was unknown. The use of isoenzyme analysis of seed proteins and Amplified Fragment Length Polymorphism (AFLP) analysis of total DNA, to distinguish between biotypes of *R. cochinchinensis*, could help in predicting the potential effectiveness of the smut isolate, against New World biotypes. It is important that once a CBC has been released into a new region, that it should be identifiable in the future. The use of molecular characterisation (using Variable Number Tandem Repeats (VNTR) as Polymerase Chain Reaction [PCR] primers) for 'finger printing' of the candidate smut strain should enable this.

The leaf rust has also been observed to cause severe damage to *R. cochinchinensis* in the field, particularly to seedlings, and could complement the effect of the smut fungus by reducing the competitive ability of the weed within a cropping system. Strains capable of causing full symptoms on Latin American biotypes have yet to be identified.

The dynamics of the itchgrass-head smut system were explored in a previous project (A0358, R6003; Smith, 1996; Smith *et al*., 1997) This work suggested that a very high annual infection rate >85% would be required for the smut to be effective as the sole agent of control. The smut cannot be released in Costa Rica until host specificity tests are completed. Therefore, this model can be adapted to investigate the effects of biological control with the head smut in combination with other control practices on itchgrass densities in maize cropping systems.

Project Purpose

The purpose of the project is to improve methods for the management of *Rottboellia cochinchinensis* in maize, by developing an integrated management strategy, and disseminating the results to the target farmers in Latin America (Costa Rica, Mexico and Bolivia), through the training of local extension agents.

The field research (agronomic) component (ZA0052) of the project reported here built upon achievements of a previous project (R6047 Integrated management of itchgrass [*Rottboellia cochinchinensis*] -dominated weed complexes in upland cropping systems) and extended its area of coverage from Costa Rica to include Mexico and Bolivia. The project was to focus on integrating cultural tactics, especially the use of cover crops as weed suppressers and appropriate fallow management, aiming to promote sustainable production schemes that would be substantially less dependent on chemical herbicides. Technologies developed would then be further disseminated in Costa Rica and promoted in both Mexico and Bolivia once the distribution and importance of itchgrass had been assessed in major maize production regions. Research would address in more detail the interaction between maize and the legume cover crop velvet bean (*Mucuna deringiana*) as well as the appropriate planting dates and densities to avoid excessive competition between the legume and maize. During the process of executing the project, additional research needs were identified, especially to support the development of a model (ZA0051) to predict the impact of long-term use of improved weed management practices and their integration with classical biological control (CBC) (ZA0140). Thus additional field experiments were conducted to study density-dependent relationships in itchgrass as well as a growth analysis study. Concerns about costs of labour and negative impact on the crop of cutting the vines of velvetbean that roll around the maize culms prompted the establishment of an additional experiment in the last year to compare a determinate-growth velvetbean variety with the regular indeterminate variety. Interaction with small stakeholders was a major goal of the project and to this purpose validation plots were established at three locations in Costa Rica for the length of the project. Efforts were also made to establish similar validation plots during the last year of the project both in Mexico and Bolivia but weather and operational constraints only allowed limited field demonstrations in Mexico.

The mycoherbicide component of the CATIE subproject (ZA0052) built on the previous projects R5225 and R5344. The purpose was to develop a mycoherbicide against *R. cochinchinensis* and to investigate how such a product could be integrated in to the over-all management strategy for the weed. The further component of this programme (ZA0140) looked at the classical biological control tactic, using the head-smut *Sporisorium ophiuri* and the rust *Puccinia rottboelliae*, and the potential for their release in to the New World. A model was also developed to predict the long-term effect of the different control strategies on the weed populations, and the likelihood of successfully integrating the classical biological control agents into the system. This was based on a model developed under a previous project (A0358).

Overall, the project has contributed important management alternatives for one of the most troublesome weeds in tropical America that should allow its sustainable control with reduced negative impact on the environment. Results and observations in validation plots indicate the financial suitability of integrated management of itchgrass for small stakeholders. Information on appropriate integration of available tactics was distributed to direct and indirect beneficiaries in accordance to the goal of improving yields and enhancing sustainability in high production cropping systems by cost-effective reduction of losses due to pests.

Research Activities

1. Survey of the distribution and importance of *R. cochinchinensis* in Mexico and Bolivia.

Special Facilities and Resources: CATIE Plant Protection Unit weeds laboratory, screenhouses, and spray chamber. CATIE administrative and financial management infrastructure. Project-funded and CATIE core budget personnel. Secure land for long-term experiments at the University of Costa Rica Regional Centre Experiment Station, Santa Cruz, Guanacaste. Collaboration from National Reference Centre for Bird, Rodents and Weeds, Cuernavaca, Mexico and Universidad Autonóma Gabriel René Moreno, Santa Cruz de la Sierra, Bolivia.

Through collaboration with partner institutions in Mexico and Bolivia surveys and field visits were programmed to determine the distribution and importance of itchgrass in their cropping systems. In Mexico, a total of 39 growers were surveyed (24 in Campeche, 3 in Veracruz and 12 in Oaxaca). Survey determined their perceptions about itchgrass, costs of controlling this weed and the preferred control methods through personal interviews (approximately 30 minutes each). Information obtained was analysed by descriptive statistics. Additional information was gathered through field visits by B. Valverde and C. Riches to important maize growing areas in the above mentioned states. In Bolivia, a survey to determine the distribution and aggressiveness of itchgrass in Santa Cruz, Bolivia was conducted during the 1997 summer cropping season. The survey covered 17 locations ranging from 253 to 840 m above sea level, with rainfall varying between 840 to 1800 mm per year. Itchgrass density was determined by counting the number of individuals present in 5 quadrats (50 x 50 cm) randomly selected in each field. Frequency, abundancy-dominancy and aggressiveness indexes were calculated according to conventional methods. Similarly B. Valverde and C. Riches obtained additional information through field visits.

2. Cover crop planting density and timing to avoid competition with maize while maintaining itchgrass suppression.

Interaction between mucuna varieties, itchgrass and maize. Field experiments were established at University of Costa Rica Regional Centre Experiment Station, Santa Cruz, Guanacaste, in 1996 and 1997 to study the interaction between two mucuna varieties, two maize varieties and itchgrass. Trials were planted in May each year (23-05-96 and 15-05-97) after one pass of a disk plough plus two passes of a disk harrow for soil preparation. Plots (5 x 4 m) were established in a factorial arrangement in a complete block design with 4 replications. Factors included a locally adapted "*Criollo*" and an improved "*Diamantes*" maize variety) grown in association with mucuna (two varieties differing in the colour of their seeds, black and grey seeded, respectively) in presence and absence of the natural itchgrass infestation. Itchgrass density (measured in 0.25 m^2 quadrants) and soil cover by *Mucuna* (visual assessment) was determined at 15, 30, 45 and 60 days after planting (DAP). Itchgrass and *Mucuna* biomass was also determined at 45 and 90 DAP. Maize was harvested from 8.40 m^2/plot and yields were converted to kg/ha at 12 % moisture. Data were subjected to analyses of variance and means were separated by Tukey's multiple range test. The angular transformation was applied to percent ground cover by mucuna and itchgrass densities before statistical analysis.

Effect of mucuna density and planting time on itchgrass and maize. Repeated experiments also were established in 1996 and 1997 at University of Costa Rica Regional Centre Experiment

Station, Santa Cruz, Guanacaste, to study the impact of mucuna density and planting time on itchgrass and maize. Trials were planted on 03-06-96 and 14-05-97 with the same soil preparation scheme used in the previous experiments. Plots (5 x 4 m) were established in a factorial arrangement in a complete block design with 4 replications. Factors included two mucuna densities (25 000 or 50 000 plants/ha) and four mucuna planting dates (0, 5, 10 or 15 DAP maize). Two additional treatments (weeded and unweeded controls) were also included. Maize (cv Diamantes) was sown at 40 by 80 cm, with two plants per planting hole. Itchgrass density and soil cover by mucuna were determined as before. Itchgrass and mucuna biomass was also determined at 45 and 90 DAP. Maize was harvested from 9.6 m^2/plot and yields were converted to kg/ha at 12 % moisture. Data were subjected to analyses of variance and means were separated by Tukey's multiple range test.

Comparison of two mucuna varieties with contrasting growth habits as cover crops for itchgrass management in maize. An additional field experiment was established at University of Costa Rica Regional Centre Experiment Station, Santa Cruz, Guanacaste in 1998 to study the suppressive effect of a determinate-growth variety of mucuna compared to the conventional, indeterminate-growth variety already developed for itchgrass management. The experiment was sown on 02 July 1998 with maize (cv. Diamantes) in a factorial arrangement of treatments that included two densities of the determinate mucuna variety (50 000 and 66 667 plants/ha) and three planting dates in relation to maize (0, 4 and 8 DAP). For comparison, the indeterminate mucuna variety (black-seeded) was planted at the recommended date of 8 DAP at the same densities. Two additional treatments were also included (weeded and unweeded controls without mucuna). Unfortunately, excessive rainfall and floods brought by hurricane Mitch precluded completion of the experiment and obtaining maize yield data. Observations could only be taken for the first two months after planting. Itchgrass density and soil cover by mucuna were determined as before at 15, 30, 45, and 60 DAP. Additionally, itchgrass and mucuna biomass was also determined at 55 DAP. Data were subjected to analyses of variance and means were separated by Tukey's multiple range test.

Growth analysis of mucuna, itchgrass and maize growing in interaction. A field experiment was established at the University of Costa Rica experimental farm in Santa Cruz, Guanacaste in 1997. Treatments were arranged in a 2 x 2 x 2 factorial in a randomised complete block design with four replications. Factors evaluated were presence and absence of each of the species in interaction: maize, mucuna and itchgrass. Plot size was 6 m^2 (3.0 x 2.0 m) with a sampling unit of 1.2 m^2 (1.5 x 0.8 m) that included four maize plants, two mucuna plants and the infesting itchgrass plants. Maize (cv Diamantes) was planted at 1.0 x 0.4 m and mucuna at 50,000 plants/ha between the maize rows. Natural itchgrass infestation was allowed in plots where the weed was to be present otherwise it was controlled by pendimetalin 1.5 kg a.e./ha. Sampling for leaf area measurements (Li-Cor Li-3000 leaf area meter) and biomass production (fresh weight) of each species was done at 15, 30, 45, 60 days after maize germination. An additional harvesting was done at maize maturity. Dry weight was obtained after leaving the plants for up to four days in an oven at 70 C. Based on leaf area and dry weight measurements, mean leaf area ratio (LAR), net assimilation rate (NAR) and relative growth rate (RGR) for each species was calculated. Data were subjected to analysis of variance.

3. Validation of integrated weed management tactics for itchgrass control in Costa Rica, Mexico and Bolivia.

Validation plots at farmer fields in Costa Rica. In 1996, field validation plots (about 1000 m^2 each) were established at grower's fields in Corralillo, Arado and Palmira, Guanacaste province, Costa Rica and continued throughout 1998. Validation at these sites had been initiated under

X0261 in 1995. In Arado and Corralillo, corn is grown twice per year; in Palmira the cropping system is based on a corn-dry beans-fallow rotation. The integrated tactics evaluated with farmers co-operation were zero tillage, planting of an improved maize variety (when possible), decreased herbicide use and avoidance of paraquat by substitution with the pre-emergence, selective herbicide pendimethalin at plot establishment, planting mucuna as a cover crop to suppress itchgrass, and fallow management. Most growers rely on a combination of slashing and direct applications of paraquat to control itchgrass; selective herbicides such as atrazine are rarely used. Itchgrass density was determined at regular intervals during each cropping season both on validation and adjacent grower's plots. Cost-benefit balance was assessed based on a partial budget analysis considering grower's technologies and those being validated for itchgrass control. Before the first cropping cycle each year, soil cores were taken at two depths from validation and grower's plots to assess the impact of itchgrass management practices on the size of the soil seed bank.

Validation activities in Mexico and Bolivia. A validation plot was also established at Ejido San Bartolo, Oaxaca, Mexico in 1998 but not in Bolivia. At El Vallecito Experiment Farm in Santa Cruz, Bolivia, initial testing of cover crops for itchgrass control was completed as part of Mr. Jorge Ortiz BS thesis' research. Species included as cover crops were *Mucuna* (*Stizolobium*) *cinereus*, *Dolichos lab lab*, *Crotolaria juncea*, and *Cannavalia ensiformis*. The experiment was conducted over two cropping seasons under a heavy itchgrass infestation but in absence of any crop

4. Detailed characterisation of endemic pathogens for controlling itchgrass (CATIE, mycoherbicide component)

4.1 Selection and evaluation of pathogens

The investigation was performed at CATIE, at a latitude of 9° 53´ N and a longitude of 83° 38´ W, 602 metres above sea level, with an average annual temperature of 21.7°C and a relative humidity of 87.8% (CATIE, 1995).

*Collection and isolation of pathogens***.** Diseased plants were collected from different zones of Costa Rica: Atlantic zone (Turrialba, Siquirres, Limón), North Pacific (Guanacaste, Santa Cruz) and Central Pacific (Quepos). Microorganisms were isolated from the seeds, leaves, stems and roots on artificial media of water agar and PDA. Two surveys were performed; in the first 123 isolates, and in the second 35, were obtained. These are maintained in the pathogen collection of the Plant Protection laboratory of CATIE.

Evaluation of pathogenicity of the isolated organisms under greenhouse conditions. *R. cochinchinensis* plants were planted in 0.5 Kg pots, with soil previously heat sterilised at a temperature of 200 °C for 24 hours. When the plants were 30 days old they were inoculated. The isolates were inoculated in four ecotypes (Cuestas, Silencio, Bagatzi and Esparza) at a concentration of 1 x 10^5 reproductive structures / ml. The pathogenicity and its severity were evaluated according to the following scale: 0 = healthy, 1 = 1 - 15 % of area affected, 2 = 15 - 20 % of area affected, 3 = more than 50 % of area affected and 4 = plant dead.

Evaluation of pathogenicity of selected strains on crop species. To determine if *R. cochinchinensis* pathogens infect phytogenetically related, crop species, *Brachiaria brizantha* (CIAT 16322*), B. brizantha* (CIAT 6780), *B. dictyoneura* (CIAT 6133) and two types of native maize (white and purple cobs), from the zone of Guanacaste, as well as rice varieties CR 5272, CR 1113, Orizica 1, Llano 5 and CR 5272, were inoculated. A dose of 1 x 10^7 reproductive structures /ml was calibrated, applying a total of 5 ml / plant. Every 8 days the severity of the

disease was evaluated, for a total of four evaluations, according to the following scale: 1 = Healthy, 2 = less than 50 % of leaf area affected, 3 = more than 50 % of leaf area affected.

4.2 Evaluation of stress factors as agents to predispose *Rottboellia cochinchinensis* plants to attack by native pathogens in the greenhouse.

Greenhouse experiments were performed at CATIE. Twenty-two days old seedlings of *R. cochinchinensis* were planted in pots with heat sterilised soil. Seed originating from Cañas, Guanacaste was used.

With the aim of predisposing the weed to attack by the selected pathogens, sub-lethal doses of herbicides with distinct modes of action, saturation of soil with water and shade were evaluated. To establish the levels of humidity in the soil, the pots with *R.. cochinchinensis* plants were placed in containers with enough water to maintain the level of water 2-cm above the level of the soil. To obtain the effect of shade, the plants were placed in a greenhouse with 40 % of exterior light.

The herbicides evaluated were pendimetaline (Prowl), fluazifop butyl (Fusilade), propanil (Stam-M48), atrazine (Gesaprin), haloxifop methyl, root mixture 24 % (Galant), glyphosate (Round up), paraquat (Gramoxone) and nikosulfuron. Sub-doses of 6 -40 % of the commercially recommended dose rate were evaluated. A careful calibration was performed to guarantee the exact sub-dose of the herbicide. A spray of constant pressure (CO_2 sprayer) with nozzle 8002 in 30 seconds was used to apply 200 litres of compound per hectare. The herbicide spray was performed 24 hours before the inoculation of the pathogens with the aim of predisposing the plant in order to favour the development of the pathogens.

Strains of pathogens previously selected for their high pathogenicity were evaluated: strains 2 (*Curvularia*), 130, 105, 99 (*Dreschlera*), 127, 69 (*Fusarium*). After herbicide application 15, 25, 30 and 35 days old seedlings, were inoculated with a suspension of 1 x 10^7 reproductive structures. After the inoculation of the pathogens, the plants were left for 12 hours under natural condensation to increase humidity and in this way favour the process of infection. In all of the experiments the severity of the disease was evaluated every 4 days, as a percentage of the foliar area affected using the following scale: 0 = Healthy, 1 = 1 - 15 % of area affected, 2 = 15 - 50 % of area affected, 3 = more than 50 % of area affected and 4 = plant dead. An analysis of variance of the data and regression was performed. The data was grouped using Tukey at a level of 5 %.

4.3 Evaluation of native pathogens and sub-doses of herbicides in the field.

Trials were performed at the experimental station of the University of Costa Rica, situated in Santa Cruz, Guanacaste, with an average temperature of 27.6^{o}C and precipitation of 881.8 mm per year (U.C.R., 1998), to test the results from the greenhouse. Plots were established in an area of high itchgrass infestation, 15- 25 days old seddeling, to study the interaction between a local adapted (criollo) maize cultivar with a Mucuna cover crop, the effect of the native pathogens, strains 69 and 127 (Fusarium), a mixture of these, and in combination with three sub doses of haloxifop methyl. Sowing of maize and Mucuna was begun simultaneously.

The first trial was performed with the highest dose of haloxifop methyl obtained under greenhouse conditions (0.096 a.i. Kg / ha, 2 ml / litre) however this dose was lethal in the absence of the pathogens. For this reason two further trials were performed, evaluating two lower doses 0.072 y 0.048 a.i Kg / ha, 1.5 y 1 ml / litre, respectively, shown in table 1.

Table 1. Sub doses of haloxifop methyl evaluated in the field trials.

Recommended active ingredient Dose	Sub doses evaluated	a.i. gr /litre	c.p. ml /litre	a.i..Kg /ha	c.p.Litre /ha
0.24 gr. / litre	1	0.24	1.0	0.048	0.2
0.24 gr. / litre	2	0.36	1.5	0.072	0.3
0.24 gr. / litre	3	0.48	2.0 *	0.096	0.4

gr.= grams, a.i..= active ingredient, ml= millilitre, c.p.= commercial product, ha= hectare
* Best dose under greenhouse conditions

The pathogens were applied in water at a concentration of 1.0 x 10^5 reproductive structures and in formulation. A formulation of a proportion of 1:9 of sunflower oil and a suspension of spores in water with 0.1 % tween 40 was evaluated. In all of the trials, the severity of the disease was evaluated every 2 weeks, as a percentage of foliar area affected, according to the following scale: 0 = healthy, 1 = 1 - 15% area affected, 2 = 15 -50 % area affected, 3 = more than 50 % of area affected and 4 = plant dead. An analysis of variance of the data and regression was performed. The data was grouped using Tukey at a level of 5 %.

Research Additional to Original PMF

Density-dependant seedling survival seed production in itchgrass. To have a better understanding of density-dependent mortality and seed production of itchgrass under field conditions, an experiment was established at two locations (Santa Cruz, Guanacaste and Parrita, Puntarenas) in Costa Rica. Itchgrass was grown alone at increasing densities and monitored throughout its life cycle. Data obtained from these trials was used to improve the itchgrass model developed under ZA-0051 (see Research Activity/Outputs 8).

5. Full scale screening of the itch grass head smut (*Sporisorium opiuri*) and leaf rust (*Puccinia rottboelliae*)

Seed inoculation. The teliospores of *S. ophiuri* used in these screening tests were from glasshouse-grown *R. cochinchinensis* plants and had been harvested a minimum of 8 months prior to use. The spores were shaken from the infected seed heads and dispersed in Sterile distilled water (SDW). The suspensions were filtered through a 250 µm mesh sieve to remove coarse debris. The teliospore suspension was adjusted to a standard concentration using a haemocytometer (Weber Scientific International Ltd., Teddington , UK). This method of measuring teliospore suspensions proved to be both time consuming and impractical due to the large volumes of suspensions required and the tendency of the spores to form spores balls. Therefore, a reference suspension containing approximately 1 X 10^6 spores ml^{-1} was made using the haemocytometer and all subsequent teliospore suspensions were compared and adjusted by visual estimation.

The test seeds were sown in fine silt loam (Surrey Cricket Loam), contained in 5 cm diameter plastic pots at a depth of 3 cm. The soil was in a dry state, defined as unable to hold a structure upon hand-compression. Small-seeded varieties eg rice were planted at a density of 3 seeds per pot; all other test seeds were planted at a density of 2 seeds per pot. The inoculum was applied to the pots as a soil drench, until maximum soil water-holding capacity had been reached, approximately 60 ml per pot. The plants were grown in an incubator (Mercia Scientific, UK)

under a 12 hour light (26^0C) at and 12 hour dark lighting regime (23.5^0C). After 24 hrs. the pots were covered with 8cm diameter Petri dishes to prevent drying out, the lids were maintained on the pots until the seeds had germinated 3-5 days later. Emerged seedlings were randomly thinned to one per pot and transplanted in a mixture of 3 parts Levington general purpose compost (peat based), 1 part coarse perlite, 1 part fresh water sand. Thirty replicate pots per treatment were arranged at random on benching in a greenhouse chamber (20-25^{o}C) fitted with artificial lights (Metal halide and sodium lights (Sunlighter 500, Sodium 400 Sunlight Systems, Leicester, UK) set at a 16 hr photoperiod (8,000 - 13,000 Lux). Each screen also included seeds of *R. cochinchinensis* from Costa Rica (Biotype 3) as positive controls. Plants were fertilised at regular intervals using a soluble N: P: K fertilizer (20:20:20), and checked for signs of smut infection at flowering.

Inoculation of sugar cane setts. Sugar cane is propagated vegetatively, sections of cane with nodal buds (setts) being used as planting material. Inoculation experiments by various workers (Antoine, 1961) have shown that sugar cane smut (*Ustilago scitaminae* H. Sydow) must reach the inner meristematic regions of sugar cane buds before infection can take place. To ensure consistent levels of infection, sugar cane varieties were inoculated by injecting teliospores of *S. ophiuri* beneath the outer bud scales, using a hypodermic needle (Microlance ® 3). Inoculum placed in this region overcomes any resistance which particular varieties may show under natural conditions (Waller, 1970). After inoculation the plants were placed in an incubator set at 26^{o}C, the optimum for *S. ophiuri* infection (Ellison, 1993) with a photoperiod of 12 hours.

Setts inoculated with sugar cane smut *U. scitaminae* acted as positive controls and these were incubated at 30^{o}C, the optimum for *U. scitamimae* infection (Waller, 1970). After 48 hours the setts were removed from the incubator and the pots placed at random on benching in a greenhouse. The plants were maintained as previously described and periodically checked for signs of smut infection. Plants infected with *U. scitaminae* are distinguished by the production of the characteristic 'whip'. This is a long tapering, cylindrical structure produced from the apex of diseased canes. The period from infection to whip production is about 6 - 8 months, canes that showed no symptoms after this period were recorded as being uninfected.

Inoculation of Rottboellia cochinchinensis *seedlings with the rust* Puccinia rottboelliae. Urediniospores of the rust, *P. rottboelliae,* were harvested from infected plants of *R. cochinchinensis* and suspended in SDW + T80. Eight strains (3 from Kenya, 2 from Ghana, and 3 from Madagascar) were tested for their pathogenicity towards Latin American biotypes of the weed. The spore suspension was passed through a 63 µm sieve, adjusted to approximately 1x 10^6 spores ml^{-1} (using a haemocytometer) and then applied to both surfaces of the test plants to incipient run-off, using a airbrush (Humbrol sprayer). Inoculations were carried out in a reverse laminar flow cupboard. The plants were left for approximately 5 minutes, sprayed with a fine layer of SDW and placed in a dew chamber (Mercia Scientific, UK) for 24 hours at 19^0C. The infected plants were transferred to a quarantine glasshouse equipped with a negative pressure unit and maintained as previously described. The plants were monitored daily for disease development.

6. Characterisation of the itch grass head smut *Sporisorium ophiuri*

Strains studied. Isolates from three different genera of the smut fungi (Ustilaginales) were characterised Table 2. Twelve isolates of *Sporisorium ophiuri,* 2 isolates of *Ustilago maydis,* and single isolates of *Sphacelotheca sorghi, Sphacelotheca destruens, Sphacelotheca reilana* and

Ustilago scitaminea were tested. Sporidial cultures of the smut were maintained throughout on 5cm Petri dishes containing Potato Dextrose Agar (PDA) (Oxoid) at 26°C.

Table 2. Strains of smut fungi used in molecular assessment

Isolate	Host	Country	Location
Sphacalotheca destruens	*Pennisetum typhoides*	India	Andra Pradesh
Sphacalotheca reilana	*Zea mays*	New Zealand	Christchurch
Sphacalotheca sorghi	*Sorghum halepense*	Argentina	Tucaman
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Thailand	Doi Suthep, Chiang Mai
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Ethiopia	Paire, Gojam
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Thailand	Kasetsart, Bangkok
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Thailand	Phimai, Nakhon Ratchasima
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Thailand	Pak Chong, Nakon Ratchisima
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Kenya	Solai, Central Kenya
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Kenya	Kilife, Coastal Kenya
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Philippines	Ifugao, North Luzon
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Philippines	Ifugao, North Luzon
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Madagascar	Mandoto, Antsirabe (East)
Sporisorium ophiuri	*Rottboellia cochinchinensis*	Sri Lanka	Bundala, Southern region
Ustilago maydis	*Zea mays*	Kenya	Nairobi.
Ustilago maydis	*Zea mays*	Thailand	Pak Chong, Nakon Ratchisima
Ustilago scitaminea	*Saccharum officinarum*	Costa Rica	Guanacaste,

DNA extraction. Suspension cultures of sporidia were grown in flasks containing 100ml of Difco Potato Dextrose Broth (PDB) which were autoclaved, allowed to cool and inoculated from the plates. Flasks were placed on a rotary shaker (Gio Gyrotory®) at 26°C and 150 rpm for approximately 3 days, after which the cultures were cloudy with sporidia. DNA was extracted using the rapid extraction method described by Cenis (1992). This method provides small yields of partially purified DNA. suitable for amplification.

Approximately 500 µl of the sporidial suspension was transferred to 1.5 ml Eppendorf tubes and the cells pelleted by centrifugation for 5 mins at 13,000 rpm. The pellet were washed with 500µl of TE buffer (10mM Tris HCl, 1mM EDTA, pH 8 1) and pelleted again by centrifugation for a further 5 minutes at 13,000 rpm. The TE buffer was decanted and 300µl of extraction buffer added. This buffer is the same as described by Raeder and Broda (1985) (200mM Tris HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5% Sodium dodecyl sulfate (SDS)). The cells were disrupted by crushing for 1-2 minutes with a conical grinder, fitting exactly the tube and powered by an electric motor at approx. 200 rpm. To this, 150µl of 3M sodium acetate, pH 5.2 was added and the contents mixed. The tube was incubated at $^{-}20^{o}$C for 10 mins. Then the precipitate pelleted by centrifugation for 10 mins at 14,000 rpm . The supernatant was transferred with a micropipette into a clean microcentrifuge tube and an equal volume of cold isopropanol, added mixed gently, and the tube left to stand for 5 mins at room temperature. The precipitated DNA was pelleted by centrifugation for 5 mins at 14,000 rpm. The isopropanol was decanted and the pellet washed with 70% ethanol. The DNA was pelleted by centrifugation for 10 mins at 14,000 rpm before being dried under vacuum in a dessicator. The pelleted DNA was redissolved in 5-10 µl of TE buffer (pH8.0).

The DNA samples were diluted 1 in 100 with SDW before being used in polymerase chain reactions (PCR) with three different single primers based on simple sequences. The primers were based on known repetitive DNA [(RY, 5'$(CAG)_5$3', GACA, 5'$(GACA)_4$3'; MR 5'(GAGGGTGGCGGTTCT)3] of which two, RY and $(GACA)_4$ were multiple repeats, and one MR, was based on the "universal" tandem repeat from M13 phage.

The amplification reactions were carried out in a total volume of 50µl, containing

C) PCR amplification with VNTR selective primers

Selective VNTR reactions were set up as follows in microtubes

10x Tth buffer	5µl
1.5mM $MgCl_2$	3µl
dNTPs (200mM)	1.25µl
Primer (30pmols)	5µl
Taq polymerase (1 Unit)	0.25µl
dH_2O	31.75µl
DNA (approx. 25ng)	1µl

Each reaction was overlaid with a drop of mineral oil and the microtubes placed in a thermal cycler (PTC-100 MJ Research USA) and amplified with the following profile.

1 cycle	94°C 5 minutes
45 cycles	94°C 1 minute
	45°C 1 minute
	72°C 1 minutes
1 cycle	72°C 5 minutes
	4°C hold temperature

Efficiency of amplification was analysed by adding 2µl of loading buffer (40% glycerol, 0.25% bromophenol blue) to each well and running 15 µl of each reaction in a 1.5 % (w/v) agarose gel (SeaKem LE) prepared with Tris-acetate-ethylenediaminetetraacetic acid (EDTA) buffer (TAE) (40 mM Tris acetate, 1 mM EDTA, pH 7.2) (Sambrook *et al*, 1989). The gels were run at 75V for 500Vh and stained with ethidium bromide (0.5 $\mu g ml^{-1}$) before visulisation under u.v. light.

Data analysis. The VNTR profiles were compared by treating each band as a character and scoring these as present or absent for each isolate. Similarities were determined from the percent similarity (Sorensen's) coefficient and clustering was UPGMA. Calculations were undertaken through the MVSP package (Kovach Computing Services).

7. Characterisation of selected worldwide *Rottboellia cochinchinensis* biotypes

Plant material. Rottboellia cochinchinensis seeds from 20 countries or territories were collected either on surveys or sent to CABI Bioscience UK. The sites for these collections are given in Table 3. The seeds were collected over a 13-year period from 1986 to 1999 and stored in a refrigerator at 10^oC until experiment initiation. Caryopses were extracted by hand from the seed heads

Table 3. Source of itch grass biotypes

Country or state	Nearest city, Province or region
Australia	Queensland
Bolivia	Santa Cruz
Brazil	Rio de Janero
Columbia	Valle de Cauca
Costa Rica–1	Margareita
Costa Rica–2	Jimenez
Costa Rica–3	Quepos
Costa Rica–4	Dominicah
Costa Rica–5	Esparza
Costa Rica–6	Rio Hondo
Costa Rica–7	Barranca
Cuba	La Romero
Ecuador	Quevedo, Los Rios
Honduras	Francisco Morazán
India–1[a]	Tehri, Uttah Pradesh
India–2[b]	Bhubanaswa, Orissa
Kenya-1	Meru, Central Kenya
Kenya-2	Western Province
Kenya-3	Kilifi, Coastal Kenya
Madagascar–1	Tanadava
Madagascar-2	Zazafotsy
Mexico	San Francisco, Sonora
Nicaragua	Santa Rita, Managua Department
Papua New Guinea–1	Ramu Sugar Ltd, Madang Province
Papua New Guinea-2	Ramu Sugar Ltd, Madang Province
Peru	Tingo Maria, Huanuco Department
Philippines–1[a]	Los Banos,Central Luzon
Philippines–2[a]	Bagabag, North Luzon
Philippines-3[b]	Ifugao, North Luzaon
Sera Leone	-
South Africa	KwaZulu-Natal
Sri Lanka–1[a]	Rsdivihara
Sri Lanka-2[b]	Puttalem, NorthWestern Province
Thailand–1[b]	Phimai, Nakhon Ratchisima
Thailand–2[b]	Doi Suthep Chiang Mai
Thailand–3[b]	Kasetsart University, Bangkok
Thailand–4[b]	Pak Chong, Nakhon Ratchisima
Thailand-5[a]	Pak Chong, Nakhon Ratchisima
United States of America	Louisiana ND
United States of America	Louisiana SD
Zimbabwe	Henderson Research Station

- no details available
ND Neutral daylength flowering
SD Short daylength flowering
Plants were of two kinds;
[a] those with small-diameter seeds
[b] those with large -diameter seeds

7.1 AFLP analysis of *Rottboellia cochinchinensis* isolates.

DNA extraction. Approximately 0.1g of freeze-dried *R. cochinchinensis* in a mortar and pestle until a course powder was obtained. The genomic DNA was extracted from each sample using a Phytopure DNA extraction kit from Nucleon Biosciences following the manufacturers instructions with the following modification. The ground seed material was incubated overnight at 37°C in 600µl solution 1 to which was added 20µl Proteinase K at a concentration of 20mg/ml. Genomic DNA was resuspended in 100µl distilled water and the concentration determined by running 5µl on a 1.0% (w/v) agarose gel against standards.

AFLP fingerprinting. The protocol used was adapted from Mueller *et al.* (1996)

A) DNA restriction digestion and adapter ligation

Individual reactions were set up on ice in 0.5ml tubes as follows and incubated at 37°C for 2 hours.

10x enzyme buffer	2.0µl
0.2µg adapter	1.0µl
0.5mM ATP	0.1µl
20 Units *Pst* I	1.33µl
1 Unit T4 DNA ligase	0.17µl
genomic DNA	1µg
dH_2O	to 20µl

The adapter/ligation mixes were precipitated by adding 80µl distilled water, 50µl 7.5M ammonium acetate and 300µl ice cold 100% ethanol. Tubes were mixed and centrifuged at 16,000g for 10 minutes. DNA pellets were washed with 70% ethanol, recentrifuged, air-dried and resuspended at 4°C overnight in 25µl 1xTE (pH8.0). The samples were then diluted 1 in 10 with SDW.

B) Preselective amplification with Adapter primer AD A (see Table 4).

Preselective amplifications were set up in 0.5ml tubes as follows.

10x PCR buffer	2.5µl
50mM $MgCl_2$	1.25µl
dATP (20mM)	0.25µl
dCTP (20mM)	0.25µl
dGTP (20mM)	0.25µl
dTTP (20mM)	0.25µl
Primer AD A	2.5µl
Taq polymerase (1 Unit)	0.2µl
dH_2O	15.05µl
diluted adapter/ligation	2.5µl

Each reaction was overlaid with a drop of mineral oil and placed in pre-heated PCR machine and amplified with the following profile.

1 cycle	94°C 5 minutes
40 cycles	94°C 1 minute
	50°C 1 minute
	72°C 2 minutes 30 seconds
1 cycle	72°C 5 minutes
	4°C hold temperature

After amplification 5µl of each reaction were run on a 1.5% (w/v) agarose gel to ensure amplification. Samples were diluted 1 in 10 with distilled water and stored at 4°C until amplification with selective primers.

C) PCR amplification with AFLP selective primers

Selective AFLP reactions were set up as follows in microtitre plates.

10x PCR buffer	2.5µl
50mM $MgCl_2$	1.25µl
dATP (20mM)	0.25µl
dCTP (20mM)	0.25µl
dGTP (20mM)	0.25µl
dTTP (20mM)	0.25µl
Primer (30pmols)	2.5µl
Taq polymerase (1 Unit)	0.2µl
dH_2O	15.05µl
preselective product	2.5µl

A drop of mineral oil was added to each well and the microtitre plate, placed in pre-heated PCR machine and amplified using the same profile as before. After amplification 2µl loading buffer (40% glycerol, 0.25% bromophenol blue) was added to each well and 15µl run on 1.5% (w/v) agarose gels at 100V for 600Vh.

The primers used in the AFLP analysis are given in Table 4.

Table 4. Primers used for AFLP analysis

Primer	**Nucleotide sequence**
Adapter AD A	5' CTCGTAGACTGCGTACATGCA 3'
A	5' GACTGCGTACATGCAGGT 3'
B	5' GACTGCGTACATGCAGGA 3'
C	5' GACTGCGTACATGCAGGC 3'
D	5' GACTGCGTACATGCAGAC 3'
E	5' GACTGCGTACATGCAGAG 3'
F	5' GACTGCGTACATGCAGCG 3'

Data analysis. The AFLP profiles were analyzed using the GelCompar software package (Applied Maths). Data matrices, calculated using the Dice index for individual primers, were exported to Excel and a matrix of average values calculated. Trees were constructed using PHYLIP.

7.2. Isoenzyme analysis of *Rottboellia cochinchinensis* isolates.

Seed protein extraction. Extracts of endosperm were prepared by grinding approximately 0.1g of freeze-dried *R. cochinchinensis* seeds with a mortar and pestle until a coarse powder was obtained. The powdered seed was placed into a 500µl Eppendorf and 300µl of acetone added. The mixture was mixed for 1minute using a bench mixer before being centrifuged for 5 minutes at 14,000 rpm. The liquid portion was poured off and samples placed in a desiccater for 15-20 minutes to allow the acetone to evaporate. Total soluble whole-cell protein was extracted by resuspending the pellet in 200 µl of Tris-glycine buffer (0.3% Tris, 1.44 % glycine w/v, pH 8.3) to which 1mM Dithiothreitol (DTT) was added at 1/3 v/v DTT/buffer. The resultant slurry was centrifuged for 12000 x G for 40 minutes at 4°C, and the protein containing supernatant collected. Protein concentrations of extracts were determined with Folin reagent according Lowry *et al.* (1951).

Electrophoresis. Discontinuous electrophoresis was carried out in 9 x 8 cm vertical slab electrophoresis cell . The anionic discontinuous system consisted of a 1 mm thick (12% cross linking) bispolyacrylamide resolving gel below a 4% bispolyacrylamide stacking gel. The gels was prepared with Tris citrate gel buffer (1.37 % Tris, 0.525 % citric acid) and Tris-glycine running buffer. Sample volumes were adjusted to give a total loading of 50 µg of protein, and samples loaded onto gels with 2 µl of 0.1 % bromophenol blue in 2M sucrose. Gels were run at 22 mA constant current until the tracking dye was approximately 5 mm from the edge of the gel. The gels were stained stained for α and β naphthal esterase activities according to Bridge & Paterson (1994).

Research Additional to Original PMF

Field survey. Surveys were conducted in the Philippines during April of 1998 for new strains of the smut to test for their pathogenicity towards neotropical biotypes of *R. cochinchinensis*. The survey procedure followed a similar format to that discussed by Ellison (1987). Smutted heads were collected from diseased plants in the field and allowed to air dry for a week. The heads were placed intact into wax packets and stored at 10°C until required for testing. Teliospores stored in this manner have been successfully maintained for several years without significant losses in viability. Mature seeds were also collected from healthy plants at sites throughout the Philippines in order to provide test material for the biotype screening and for subsequent infection studies. Seeds collected from *R. cochinchinensis* plants cannot be germinated for a period of between 6-12 months after being collected due to innate dormancy.

Light microscopy and SEM studies of the modes of infection of the smut *S. ophiuri*

Isolation and culturing of monosporidial strains. The sporidial phase of *S. ophiuri* can be readily cultured *in vitro* on either solid or liquid media. A reference collection of sporidial strains was maintained PDA (Johnston & Booth, 1983). Haploid sporidial lines were obtained by single spore isolations from teliospores collected from Madagascar. The teliospores were obtained by aseptically removing the spore column (sorus) from the surrounding leaf sheath. Short sections of the sorus were agitated in 20ml of SDW plus Tween 80 concentration *circa* (*ca*) 0.01%

volume/volume (v/v). The spore suspension was poured through a 15 µm nylon-mesh screen to remove large particles and coarse debris. Teliospores were applied to 8cm diameter Petri dishes containing PDA by pipetting 0.3ml aliquots of suspension per plate. The teliospores were evenly dispersed over the surface of the agar using a flamed glass rod. The plates were incubated under a lighting regime of 12h light/12h dark, for 2-3 days at 26^0C. Teliospores that germinated and produced primary sporidia were aseptically removed from the plate surface using a flamed loop, and suspended in a small volume of SDW. The spore suspension was then streaked over the surface of a fresh 8cm Petri plate containing PDA to spread the sporidia out singly. Individual sporidia were located microscopically and marked for transfer when the colony had grown visible to the naked eye. Suspension cultures of sporidia were grown in flasks containing 100ml of Difco PDB which were autoclaved, and allowed to cool before being inoculated from the plates. The inoculated flasks were placed on a rotary shaker (Gio Gyrotory®) at 26°C, and 150 rpm, for approximately 3 days, by which time the cultures were cloudy with sporidia.

*Inoculation of plant material before microscopic observation***.** Twenty seeds of *R. cochinchinensis* were dehusked and the embryo surface sterilised by immersion in 20% (w/v) hydrogen peroxide for 8 minutes. The embryos were washed briefly in SDW before being placed onto moistened filter paper inside an 8cm plastic Petri dish. The dish was sealed with Parafilm® before being placed in an incubator at 26°C, under a fixed 12 h light-dark cycle. Germination of the embryo occurred after approximately 3-4 days. The emerging coleoptiles were inoculated with a water suspension of teliospores. Several 5µl drops of teliospore suspension were placed on the surface of each developing coleoptile using a micro-pipette. The plates were re-sealed with Parafilm® and returned to the incubator for a further 3-4 days, to give the teliospores time to infect the coleoptile.

*Clearing and staining of plant tissues for infection studies***.** Studies of penetration of the host tissue were carried out using a whole leaf clearing and staining technique developed by Bruzzese & Hasan (1983). Samples inoculated with suspensions of the spores of *S. ophiuri* were immersed in a clearing-staining solution where they were kept for two days at room temperature. The specimens were then transferred to a chloral hydrate solution (2.5 g ml-1 distilled water), in which they were kept for a minimum of two days to remove excess stain. Before microscopic examination the samples were washed in water, and a few drops of aniline blue in lactophenol were added to facilitate observation. Specimens prepared in this manner can be kept in chloral hydrate solution for long periods before viewing.

Scanning Electron Microscope (SEM). Detailed studies of pathogen penetration were carried out with the aid of a SEM (Hitachi S570, Japan) fitted with a cryopreparation unit (Emscope). Low temperature scanning electron microscopy (LTSEM) was used to view 5 day old coleoptiles of *R. cochinchinensis* that had been previously inoculated with teliospores of *S. ophiuri.* Short (2 cm) sections of coleoptile were mounted and fixed by rapid freezing in subcooled-nitrogen (cryofixation). The surface ice was removed by sublimation, and the specimens were coated in gold for 3 minutes at 25 milliamps in a sputter coater (Polaron). The samples were returned to the SEM (operating at 15 Kv), for viewing at low temperature on a temperature controlled stage. The technique is described in more detail by Beckett & Read (1986).

Water cultures of sporidia. Suspension cultures of monosporidial strains were grown in flasks containing PDB as previously described. The sporidia were separated from the liquid broth medium by centrifugation (MSE Mistral 2000) for 2 minutes at 4500 rpm, and then resuspended in an equal volume of sterile distilled water. For *in vitro* investigations of mating sporidia, 350 µL of sporidial suspension of one strain were placed into 2 cm plastic Petri dishes, and mixed

with an equal volume of suspension of a second strain. The dishes were incubated at 26°C under a fixed 12 h light-dark cycle.

Longevity of teliospores under different soil moisture conditions.

Experiments were conducted with sori of *S. ophiuri* (ex Madagascar) collected from glasshouse infected *R. cochinchinensis* plants. The sori were allowed to air dry for 1 week, then rubbed on the surface of a 250 µm sieve to free the teliospores from the sorus surface.

The soil used was a fine silt loam (Surrey loam) that had been passed through a sieve with 1mm openings before being sterilised by autoclaving twice (ST 19 bench autoclave, Express Equipment). The soil was then dried in an oven at 110°C for 7 days before use.

Teliospores were mixed into the soil at a rate of 347 mg of spores per 10 g of soil. Soils were adjusted to give five moisture contents; dry; 25%; 50%; 75% and 100% saturation. Four replicates per soil moisture were set up. Exact percentages of soil saturation are difficult to secure and maintain therefore, the aim was to obtain a range of soil moistures from dry to a relatively wet. The water holding capacity of the soil was first determined and then its actual moisture content by drying in an oven for 1 week at 110°C. Sufficient water was then added to a given weight of soil to bring it up approximately to a given percentage saturation. Ten grams of soil/teliospore mix was placed in each of 30cm^3 glass universal tubes and the lids tightly sealed and wrapped in Parafilm® to prevent evaporation. The samples were paced in an incubator at 26°C under a fixed 12 h light-dark cycle until being removed for sampling.

At weekly intervals, 0.1 g of soil was removed from each treatment and added to 10 ml of deionized water. The samples were vortex-mixed for 2 mins, and 0.3 ml of solution was spread over a PDA plate. The plates were sealed with Parafilm® and incubated for 3 days at 26 °C, and the percentage of spore germination was determined for four replicates of each treatment. Teliospore germination was recorded when the promycelium had extended by at least the width of the spore. At the end of the experiment the exact water holding content of the soil was determined for each treatment.

8. Modelling of the integrated management strategy for itchgrass

Two models were developed: I to investigate itchgrass population dynamics in a maize cropping system in which the smut was modelled by assuming a constant proportion of smutted itchgrass plants each season; II a model including a simple simulation of smut dynamics to investigate release strategies. It was also intended to develop models of itchgrass dynamics in sugar cane however there was a lack of information with which to do this, beyond the conclusions drawn already in Smith *et al.*, 1997 and model II. Data were derived from Costa Rican literature and reports and discussions with collaborators from CATIE during visits in years 1 and 3. Collaborators also set up experiments to provide data for the model in year 3. The work was presented in Year 3 at the Itchgrass Workshop and at the International Congress of Plant Pathology (Edinburgh, 1998) (both additional to Project Memorandum).

Outputs

1. Survey of the distribution and importance of *R. cochinchinensis* in Mexico and Bolivia.

During field visits to Mexico, itchgrass presence was confirmed at two locations in Campeche (Felipe Carrillo Puerto and Salinas de Gortari) where it had previously been reported. Itchgrass was also observed infesting a few maize fields near to the Edzna field station of the federal agricultural research organisation INIFAP and in a maize growing area south of Champoton, where INIFAP had carried out a herbicide screening study. Use of the pre-emergence herbicide nicosulfuron is frequent in Campeche to control itchgrass. In areas in Campeche where itchgrass is a problem, rice had been planted in the past.

Serious widespread infestation of maize, rice and sugar cane crops were also found in the Tres Valles and Tierra Blanca area (state of Veracruz) and around Tuxtepec, Jalapa de Diaz and Nopaltepec (state of Oaxaca). Itchgrass is a conspicuous component of the road verge flora throughout this area and farmers claimed itchgrass to be found wherever rice has been planted and as early as in the late 1960s. It seems almost certain that itchgrass was introduced as a contaminant of rice seed and subsequently spread on tillage equipment used for sugar cane grown in rotation with rice or when rice is replaced due to increasing weed pressure. It is interesting to point out that in repeated visits to the maize and rice growing area of Veracruz-Oaxaca, itchgrass was seen sporadically along the main highway, including Tres Valles and Ciudad Aleman. Itchgrass was also observed interspersed with *Panicum maximum* in roadsides along highway 150 at km 64, close to intersection to Tinaja, and then in Moralito and Palmarillo, close to Piedras Negras, Veracruz. In the Papaluapan watershed (Oaxaca) most growers plant maize and bananas/plantains, itchgrass being the most important weed along with *Sorghum halepense*. Itchgrass is a particular problem in maize production that is now dependent on herbicide use for its control. Maize, upland rice and sugar cane are mostly produced within the co-operative "ejido" system of social organisation which provides growers with land and through which agricultural activities are planned. Ejido members plant in the region of 8 to 12 ha plots although the situation is complicated by the formation of "Rural Production Societies" which may plant up 300 ha or more. The society allows access to production subsidies available from government programmes. On low lying land maize monoculture itchgrass control commonly depends on the use pre-emergence herbicide applications of an ametryne/2,4-D ester mixture and directed applications of paraquat, supplemented by slashing if required. In the highlands maize is grown with a sesame relay crop and here growers have adopted the use of paraquat, followed by two manual weedings, to avoid complete lost of maize yield. Prior to the introduction of itchgrass only hand weeding was used in the area. Agrochemical retailers in the area provide a comprehensive range of products and in the absence of an effective extension service are probably the major source of advice on weed control. Itchgrass is also spreading in other areas of the state of Veracruz and it is commonly seen invading roadsides. Moving north itchgrass has already reached Martinez de la Torre and its surroundings where it has become a troublesome weed in citrus production. Researcher Valentin Esqueda-Esquivel from Cotaxtla Research Station (Veracruz) indicated that according to his personal observation itchgrass was present in 1991 in banana field

margins and roadsides in Ejido Mentidero, San Rafael, Veracruz. San Rafael is located near Martinez de la Torre/Poza Rica. However, citrus growers interviewed in 1997 indicated that itchgrass was a recently introduced weed.

According to the survey conducted in Champoton municipality, Campeche, most farmers (53%) cultivate maize plots of less than 5 ha, which belong primarily (62%) to the ejido society, the rest being private owners. The vast majority (about 80%) of the growers interviewed recognised itchgrass as a troublesome weed and most of them (63%) indicated having the weed in their fields for 5-10 years. The most commonly cited reasons for considering itchgrass were its competitive ability with maize (58%) and making it difficult to harvest the crop (38%). Two third of the interviewed growers use herbicides to control itchgrass either alone (54%) or in combination with slashing (21%); the remaining 25% rely exclusively on manual (slashing) control. Ninety percent of the growers stated not receiving technical assistance and most commonly used herbicides are nicosulfuron, paraquat and glyphosate.

The three growers from Veracruz own their farms and have different views about the importance of itchgrass, none considering it the most important weed but two citing itchgrass as troublesome. They were uncertain about the origin of the infestation but indicated that itchgrass was quite common on roadsides. All growers rely on herbicides for weed control, especially paraquat, atrazine and nicosulfuron.

In the Tuxtepec area (Oaxaca) 80% of the 15 growers interviewed belong to ejidos, the same proportion considered itchgrass to be a troublesome weed, citing competition effects resulting in lowered yields and increased production costs. The weed was said to be present in their fields for 4-30 years, most growers believe it was introduced with contaminated seed. All growers us both herbicides and manual control to eliminate weeds and most of them (11) rely on paraquat for itchgrass control; two of them use pendimethalin.

The Bolivian survey recorded 46 genera belonging to 20 families, the most important being Poaceae, Asteraceae and Euphorbiaceae. Itchgrass was very frequent (80-100%) in all locations, with densities ranging from 12 to 138 plants/m^2. The highest densities were recorded in Pailón Norte (138 plants/m^2), El Vallecito Experiment Station (111 plants/m^2), and Okinawa II (44 plants/m^2). Itchgrass was ranked as an aggressive weed in 78% of all sites sampled.

Large areas of soybeans, upland rice and, to a less extent maize and sorghum are grown under a high input system by the Japanese settlements at San Juan de Yapacani and Okinawa. During field visits, itchgrass was noted on field margins and scattered in sorghum and maize crops throughout Japanese settlements. According to high-input growers at San Juan de Yapacani, itchgrass was a problem 10 years ago but is now readily controlled by herbicides used in soybeans. It is sometimes still seen as a problem in maize.

A population was also seen by the roadside near Abapo, about 100 km south of Santa Cruz. This was in an area where forest has been felled recently to provide land for maize and extensive pasture. In San Julián, Chiquitos province, in the so-called *Expansion Zone*, an area being deforested for agricultural purposes, the main crops being planted include cotton, soybeans, maize and rice. Weeds typically associated with those crops in other areas are infesting these

recently opened fields, also suggesting the possibility of long-distance dispersal of weed seeds by contaminated crop seed and agricultural or road construction equipment. In several farms visited in this field itchgrass is becoming an important weed. Based on interviews with growers, common control practices include the use of paraquat and slashing, similar to what is regularly practised in Central America and Mexico. A grower at Nueva Vida, San Julián (16º 48.93 S and 62º 45.58 W) indicated that his fields severely infested with itchgrass which he finds very difficult to control, especially when it emerges simultaneously with maize or before. For successful control, he relies on crop rotation: three successive cropping seasons with soybeans substantially reduced itchgrass infestation, especially as a result of herbicide applications (fomesafen and fluazifop-buthyl).

2. Cover crop planting density and timing to avoid competition with maize while maintaining itchgrass suppression.

Interaction between mucuna varieties, itchgrass and maize. Initial itchgrass densities at the experimental site (15 DAP) were low in both years, ranging between 3 and 7 plants/m^2. (Table 5). In 1996, maize variety did not affect itchgrass density or fresh weight throughout its life cycle (Table 6). Presence of mucuna resulted in decreased itchgrass density, especially at or after 60 DAP, regardless of its variety. The suppressive effect of mucuna on itchgrass was evident in the fresh weight evaluations made at 45 and 90 DAP; both mucuna varieties suppressed itchgrass biomass by 80-90% compared to the control treatment without the cover crop. Because itchgrass density was only slightly affected by the cover crop the suppressive effect of mucuna must have been on itchgrass size, plants being smaller in plots with mucuna than in plots without the legume. The black-seeded mucuna covered the ground faster than the grey seeded variety and produced more biomass up to 45 DAP; after this period, both varieties were similar in soil coverage and biomass production at 90 DAP. Mucuna also grew better in absence of itchgrass. The improved variety (Diamantes) yielded more than the local (Criollo) variety. The presence of either mucuna variety did not decrease maize yield.

In the second year, both black-seeded and grey-seeded varieties of mucuna suppressed itchgrass density by about 25% and biomass by 60% at 45 DAP. In contrast to 1996, both mucuna varieties were similar in soil cover and biomass production. Both mucuna and itchgrass slightly reduced maize yield. Under the 1997 conditions the criollo variety was more competitive with itchgrass and yielded about 70% more grain (1554 kg/ha) than Diamantes (903 kg/ha). This could be associated with a shorter cycle of the local variety that decreased the negative impact of severe water stress late in the cropping season. In general, yields were lower than normal because of drought.

Table 5. Effect of maize and mucuna varieties on itchgrass density at different intervals in the maize cropping cycle. Guanacaste, Costa Rica, 1996-1997.

Treatment		Itchgrass density (plants/m^2) at days after planting (DAP)					
		15	30	45	60	75	90
1996							
Maize variety	Criollo	6.48 a[1]	9.48 a	9.95 a	10.18 a	10.88 a	7.87 a
	Diamantes	4.93 a	6.17 a	10.44 a	9.26 a	8.33 a	6.02 a
Mucuna variety	Grey-seeded	5.54 a	6.94 a	10.07 a	8.68 a	5.56 b	5.56 ab
	Black-seeded	6.49 a	6.01 a	9.72 a	6.60 a	5.90 b	3.47 b
	Without mucuna	5.10 a	10.18 a	10.76 a	13.90 a	17.36 a	11.81 a
1997							
Maize variety	Criollo	3.47 a	3.93 a	17.13 a	4.17 a	4.17 a	7.87 a
	Diamantes	5.57 a	8.80 b	21.99 a	12.96 b	11.34 b	21.99 b
Mucuna variety	Grey-seeded	3.47 a	5.56 a	16.67 a	7.90 a	8.33 a	4.20 a
	Black-seeded	3.47 a	4.86 a	18.06 a	5.90 a	5.21 a	10.74 a
	Without mucuna	6.60 a	8.68 a	23.96 a	11.81 a	9.72 a	29.86 b

[1] Means followed by the same letter within main effect (maize or mucuna variety) within year are not significantly different according to Tukey's multiple range test at 5%.

Effect of mucuna density and planting time on itchgrass and maize. Mucuna was more effective in reducing itchgrass density at 50 000 plants/ha than at 25 000 plants/ha throughout the experiment in both years (Table 7). Better soil cover by mucuna was obtained when it was sown simultaneously with maize than when planting the legume was delayed in relation to the crop (Table 8), probably because of the competition imposed by maize on the cover crop. As a result of the better soil cover with the highest mucuna density and with earlier sowing, the cover crop reduced both itchgrass density and biomass more effectively under such conditions. At 45 DAP, which is a good estimate of the critical period of itchgrass competition with maize, mucuna (planted simultaneously with the crop at 50 000 plants/ha) reduced itchgrass density to 23 and 46% of that recorded in the unweeded controls in 1996 and 1997, respectively (data not shown). Concomitantly, itchgrass biomass decreased between 10 and 15% when mucuna density increased from 25 000 to 50 000 plants/ha, although these differences were not statistically significant (Table 9). The same tendency was observed as the mucuna planting date was closer to that of maize. Lower maize grain yields were obtained in both experiments when maize was grown in association with mucuna at its highest density and, especially, when the cover crop was planted simultaneously with maize (Table 10). Itchgrass itself had a substantial negative impact on maize grain yield (comparison of control treatments), decreasing it by about 46% in both years. Maize yields in 1997 were significantly lower than in 1996 because of drought.

Table 6. Effect of maize and mucuna varieties on their percent groundcover and fresh weight and on maize grain yield. Guanacaste, Costa Rica, 1996-1997.

		Ground cover by mucuna (%)		Mucuna fresh weight (kg/m^2)		Itchgrass fresh weight (kg/m^2)		Maize yield
		30 DAP	45 DAP	45 DAP	90 DAP	45 DAP	90 DAP	(kg/ha)
1996								
Maize variety	Criollo	68.7 a	60.3 a	0.815 a	0.142 a	0.170 a	0.095 a	2194 b
	Diamantes	69.4 a	65.0 a	0.880 a	0.182 a	0.129 a	0.084 a	3560 a
Mucuna variety	Grey-seeded	65.6 b	57.5 b	0.905 a	0.163 a	0.069 b	0.038 ab	2796 a
	Black-seeded	72.5 a	67.8 a	0.790 a	0.162 a	0.063 b	0.022 a	2910 a
	Without mucuna	-	-	-	-	0.316 a	0.209 b	2926 a
Itchgrass	With	64.1 b	53.4 b	0.653 b	0.099 b	-	-	3210 a
	Without	74.1 a	71.9 a	1.043 a	0.233 a	-	-	2544 b
1997								
Maize variety	Criollo	13.9 a	75.6 a	0.380 a	0.407 b	0.269 a	0.227 a	1554 a
	Diamantes	12.8 a	75.6 a	0.375 a	0.547 a	0.310 a	0.418 a	903 b
Mucuna variety	Grey-seeded	13.9 a	75.0 a	0.383 a	0.481 a	0.179 a	0.090 a	1131 a
	Black-seeded	12.8 a	76.3 a	0.372 a	0.473 a	0.207 a	0.193 a	1148 a
	Without mucuna	-	-	-	-	0.481 a	0.684 a	1394 a
Itchgrass	With	13.9 a	74.1 a	0.325 b	0.415 b	-	-	1115 a
	Without	12.9 a	77.2 a	0.430 a	0.540 a	-	-	1317 a

[1] Means followed by the same letter within main effect (maize or mucuna variety) within year are not significantly different according to Tukey's multiple range test at 5%.

Table 7. Effect of mucuna density and planting date on itchgrass density. Guanacaste, Costa Rica, 1996-97.

Main treatment or Factor		Itchgrass density (plants/m^2)									
		15 DAP		30 DAP		45 DAP		60 DAP		90 DAP	
		1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
Density (plants/ha)	25,000	16.7 a[1]	69.6 a	18.2 a	66.5 a	20.0 a	71.2 a	17.2 a	57.5 a	16.0 a	50.8 a
	50,000	15.2 a	60.9 b	13.0 a	67.4 a	12.2 b	77.6 a	9.5 b	46.7 a	11.5 a	46.9 a
Planting date (DAP)	0	11.0 a	62.5 ab	14.0 a	50.0 a	10.0 b	53.8 a	6.5 c	43.1 a	6.5 c	32.3 b
	5	12.5 a	89.2 a	13.0 a	65.3 a	14.5 b	82.3 a	10.5 bc	61.4 a	12.5 bc	54.5 ab
	10	17.8 a	71.2 ab	14.5 a	97.9 a	14.5 b	86.8 a	16.5 ab	62.5 a	21.0 ab	57.6 a
	15	18.0 a	38.2 b	20.8 a	54.5 a	25.5 a	74.6 a	20.0 a	41.3 a	15.0 a	50.7 ab
Unweeded control	-	14.0	131.2	18.0	127.8	26.0	111.1	44.0	70.83	38.0	75.0

[1] Means followed by the same letter within main effect (density or planting date) and year are not significantly different according to Tukey's multiple range test at 5%.

Table 8. Percent mucuna ground cover in relation to its density and planting date. Guanacaste, Costa Rica, 1996-97.

Main treatment or Factor		Mucuna ground cover (%)									
		15 DAP		30 DAP		45 DAP		60 DAP		90 DAP	
		1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
Density (plants/ha)	25,000	2.25 a	3.69 a	19.25 a	10.94 a	32.81 a	26.25 a	13.12 a	15.50 a	7.44 a	7.94 a
	50,000	3.50 a	7.60 b	30.31 b	18.07 b	44.37 b	42.00 b	16.12 a	19.67 a	11.56 b	14.20 b
Planting date (DAP)	0	8.12 a	14.50 a	53.75 a	27.12 a	79.37 a	54.37 a	21.87 a	31.25 a	15.00 a	20.62 a
	5	3.37 b	6.37 b	24.00 b	15.87 b	33.75 b	35.62 b	20.00 a	17.50 b	10.00 b	11.00 b
	10	0.00 c	0.86 c	11.75 c	8.14 c	21.25 c	23.57 c	8.75 b	13.50 b	6.75 b	5.86 b
	15	0.00 c	0.00 d	9.63 c	5.62 c	20.00 c	20.62 c	7.87 b	7.25 c	6.25 b	5.75 b

[1] Means followed by the same letter within main effect (density or planting date) and year are not significantly different according to Tukey's multiple range test at 5%.

Table 9. Mucuna and itchgrass fresh weight in relation to mucuna density and planting date. Guanacaste, Costa Rica, 1996-97.

Main Factor or Treatment		Fresh weight at 45 DAP (kg/m^2)			
		Mucuna		Itchgrass	
		1996	1997	1996	1997
Density (plants/ha)					
	25000	0.650 a[1]	0.293 a	0.497 a	0.655 a
	50000	0.781 a	0.392 a	0.450 a	0.576 a
Planting date (DAP)	0	1.071 a	0.642 a	0.402 a	0.477 a
	5	0.740 ab	0.397 b	0.495 a	0.693 a
	10	0.590 b	0.162 c	0.450 a	0.737 a
	15	0.541 b	0.170 c	0.545 a	0.558 a
Unweeded control		-	-	1.076	0.997

[1] Means followed by the same letter within main effect (density or planting date) and year are not significantly different according to Tukey's multiple range test at 5%.

Comparison of two mucuna varieties with contrasting growth habits as cover crops for itchgrass management in maize. Since indeterminate mucuna varieties impose extra labour for cutting the vines that climb and twist around maize plants, it was important to evaluate the possibility of using a determinate variety recently made available. When planted 8 DAP the determinate variety cover the soil slightly less than the traditional variety, with better coverage at the higher density (Table 11). As seen in previous experiments with the indeterminate mucuna, the new variety better covered the soil when planted at the highest density and earliest in relation to maize planting time. As it would be expected, more biomass accumulated at 55 DAP when mucuna was planted simultaneously with maize. On average, the determinate variety produced about 50% more biomass than the traditional variety at 55 DAP (Table 12). However, the indeterminate variety better suppressed itchgrass density and biomass accumulation than the determinate legume (Table 12), probably in relation to its better soil covering ability. Itchgrass density and biomass suppression also was similar regardless planting date of the determinate variety, with only minor improvement with increased sowing density.

Table 10. Effect of mucuna density and planting date on maize yield. Guanacaste, Costa Rica, 1996-97.

Planting date (DAP)	1996 Mucuna density (plants/ha)			1997 Mucuna density (plants/ha)		
	25000	50000	Average	25000	50000	Average
	Maize yield (kg/ha)					
0	3050.59 bc**	3720.24 abc	3385.48 B	286.46 c	273.18 c	278.81 B
5	3968.21 abc	3675.59 abc	3801.07 B	446.35 bc	280.21 c	363.28 AB
10	4880.95 ab	4032.74 abc	4456.90 AB	441.41 bc	590.28 ab	505.21 A
15	5401.79 a	4503.93 abc	5017.02 A	409.89 bc	350.52 c	380.21 AB
Average	4349.79 A	3948.45 A		396.03 A	359.09 A	
Weeded control	4568.45 abc			650.00 a		
Unweeded control	2455.45 abc			349.48 c		

[1] Means followed by the same capital letter within rows or columns in the sasme year are not significantly different according to the Tukey's multiple range test at 5%. Means followed by the same small letter within year do not differ among themselves or with the respective control based on the Tukey's multiple range test at 5%.

Table 11. Percent ground cover by an determinate variety of mucuna according to density and planting time compared to the conventional (indeterminate) variety. Guanacaste, Costa Rica, 1998.

Main treatment or Factor[1]		Mucuna ground cover (%)			
		15 DAP	30 DAP	45 DAP	60 DAP
Density (plants/ha)	50,000	3.00 b	19.33 a	42.08 b	74.58 a
	66,667	4.08 a	20.50 a	55.00 a	78.75 a
Planting date (DAP)	0	6.62 a	24.25 a	57.50 a	84.37 a
	4	3.00 b	20.25 b	47.50 b	74.37 b
	8	1.00 c	15.25 c	40.62 b	71.25 b
Indeterminate mucuna (plants/ha)	50,000	1.00 d	25.00 a	61.25 abc	75.00 ab
	66,667	1.00 d	25.50 a	72.50 a	86.25 a

[1] Indeterminate mucuna was planted 8 DAP.

Table 12. Effect of density and planting date of a determinate variety of mucuna on itchgrass density and fresh weight compared to the conventional (indeterminate) variety and control treatments. Guanacaste, Costa Rica, 1998.

Main treatment or Factor[1]		Itchgrass density (plants/m^2)				Fresh weight at 55 DAP (kg/m^2)	
		15 DAP	30 DAP	45 DAP	60 DAP	Mucuna	Itchgrass
Density (plants/ha)	50,000	6.71 a	22.68 a	15.42 a	12.17 a	1.015 a	0.364 a
	66,667	6.71 a	16.90 a	15.05 a	11.83 a	0.934 a	0.333 a
Planting date (DAP)	0	6.60 a	14.93 a	10.76 a	9.25 a	1.195 a	0.306 a
	4	7.30 a	21.90 a	17.01 a	12.75 a	0.967 ab	0.336 a
	8	6.25 a	22.60 a	17.71 a	14.00 a	0.763 b	0.406 a
Indeterminate mucuna (plants/ha)	50,000	7.64 a	15.28 b	11.11 b	12.00 b	0.437 d	0.280 a
	66,667	9.72 a	7.64 ab	10.42 b	11.50 b	0.569 cd	0.297 a
Weeded control	-	-	-	-	-	-	-
Unweeded control	-	9.03 a	13.19 ab	34.72 a	32.00 a	-	0.618 a

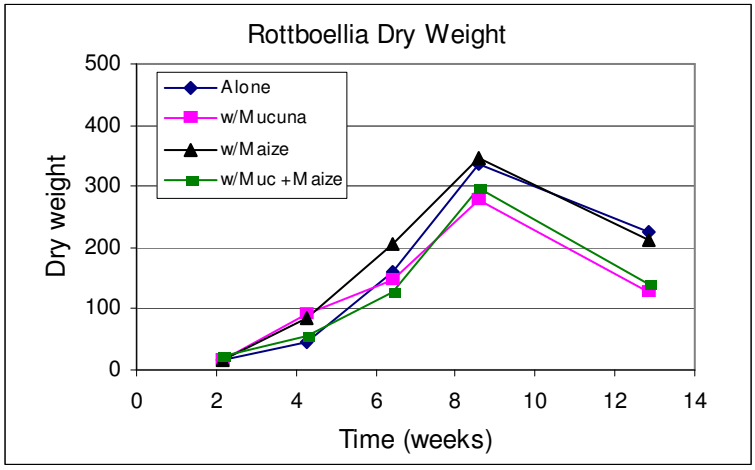
[1] Indeterminate mucuna was planted 8 DAP. Mucuna was not sown in additional control treatments.

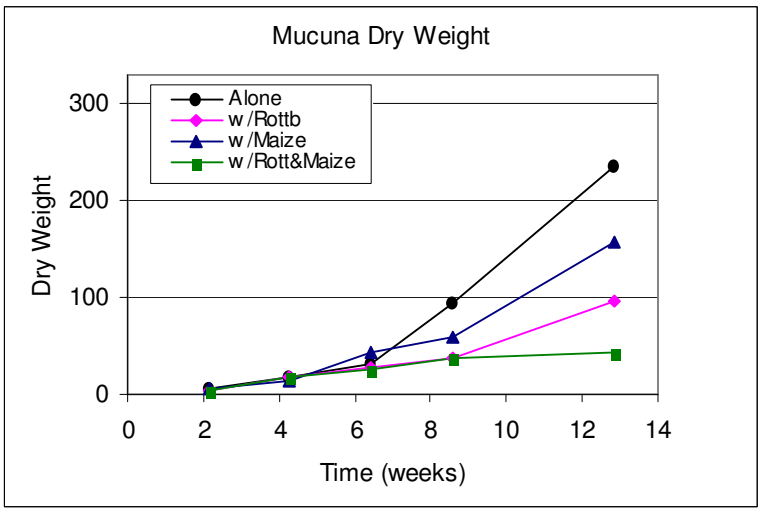
Growth analysis of mucuna, itchgrass and maize growing in interaction. Maize grown in absence on interspecific competition increased in vegetative biomass (fresh weight) up to 8 weeks after germination reaching almost 1.8 kg/plot (about 450 g/plant) and drastically decreased at plant maturity (Figure 1). Presence of mucuna alone had no impact on maize fresh weight but itchgrass alone or in association with mucuna reduced maize biomass, especially during the critical period of weed competition, which we have determined to be between 45 and 60 days after planting under similar conditions. On average, itchgrass decreased maize fresh weight by 34%. A similar relationship was obtained with dry weights (data not shown). Maize leaf area was also reduced if the crop grew in association with itchgrass alone or with both mucuna and itchgrass (Figure 2). Mucuna by itself had little ($p = 0.15$) impact on maize leaf area.

It is important to distinguish between absolute biomass measurements and biomass accumulation over time. The mean relative growth rate (RGR) describes the increase in plant material per unit time and it is considered to be highly responsive to environmental conditions and to initial plant size. There was no difference in relative growth rate for maize growing alone or in interaction with mucuna, itchgrass or both (Figure 3). The leaf area ratio (LAR), calculated as the amount of leaf area per unit of total plant biomass, is a measurement of the leafiness of the plant whereas the net assimilation rate (NAR) is a physiological index closely related to the photosynthetic activity of the leaves that represents the net gain in weight per unit leaf area. Mucuna decreased the LAR of maize ($p = 0.006$), especially when the crop became mature (Figure 3), and itchgrass slightly increased it ($p = 0.034$). The maize NAR was not differentially affected by the species in interaction with the crop.

Because senescence did not occur during the evaluation period, biomass accumulation in mucuna did not decrease in the last harvest (Figure 1). At the end of the evaluation period, maximum fresh weight was about 522 g/plant when grown in absence of competition. There was a significant effect of itchgrass on all mucuna variables except NAR. Maize affected biomass accumulation as well as leaf area and the derived leaf area index (ratio of plant leaf area over ground area). The effect of these species was also evident over time (significant interaction of itchgrass x time and maize x time) for all direct measurements but not for calculated growth parameters (LAR, NAR and RGR). On average, itchgrass was more suppressive of mucuna biomass accumulation and leaf area development than maize. Overall dry weight was reduced 28% and 53% by maize and itchgrass, respectively. A similar effect was observed on mucuna leaf area: 20% and 40% reductions were caused by maize and itchgrass, respectively. On average, mucuna RGR was reduced about 14% by itchgrass; maize had no effect on mucuna RGR (Figure 4). When itchgrass was present, mucuna plants responded by slightly increasing their LAR. Mucuna NAR was not affected by the species in association.

There was no effect of maize or mucuna on the fresh weight of itchgrass; however, mucuna slightly decreased itchgrass dry weight ($p = 0.05$, data not shown) and leaf area ($p = 0.09$). Although statistically not significant, there was a trend towards increased weight and leaf area in itchgrass when grown in association with maize. Neither maize nor mucuna affected the calculated growth parameter of itchgrass (Figure 5).





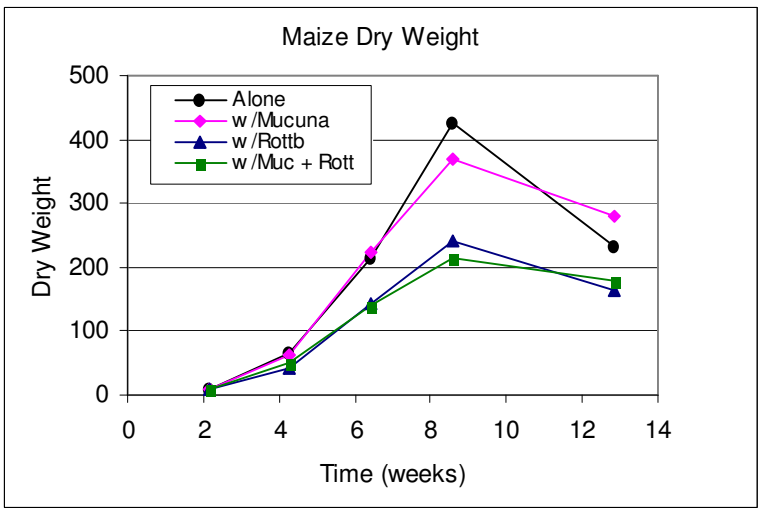
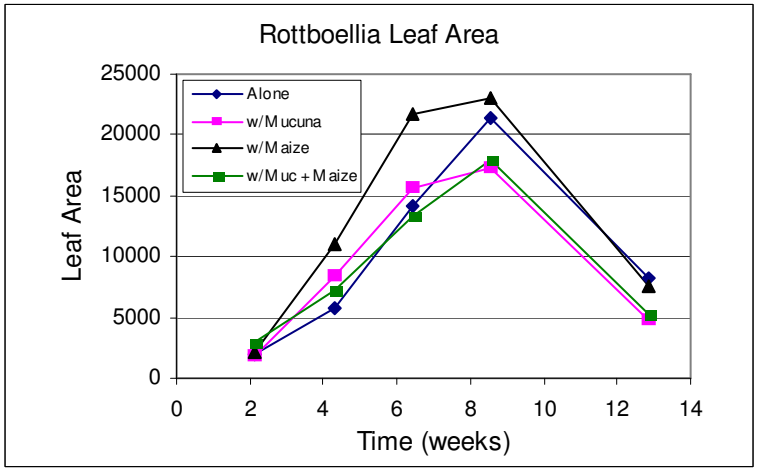
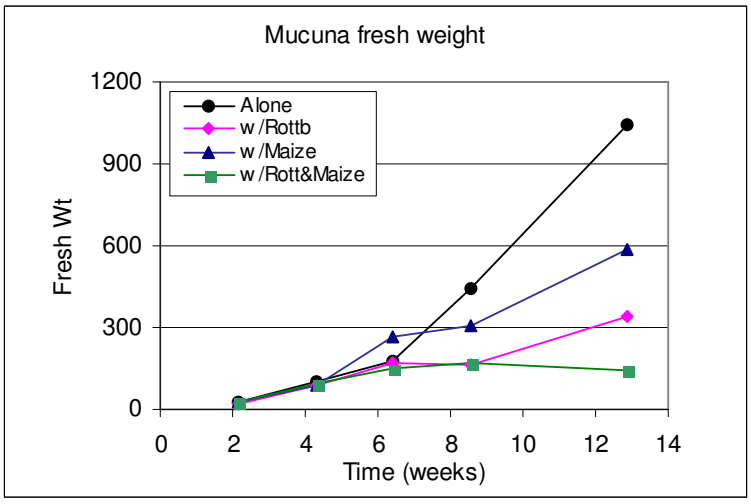


Figure 1. Dry weight response of itchgrass, mucuna and maize growing in interaction. Field study, Guanacaste, Costa Rica, 1998.





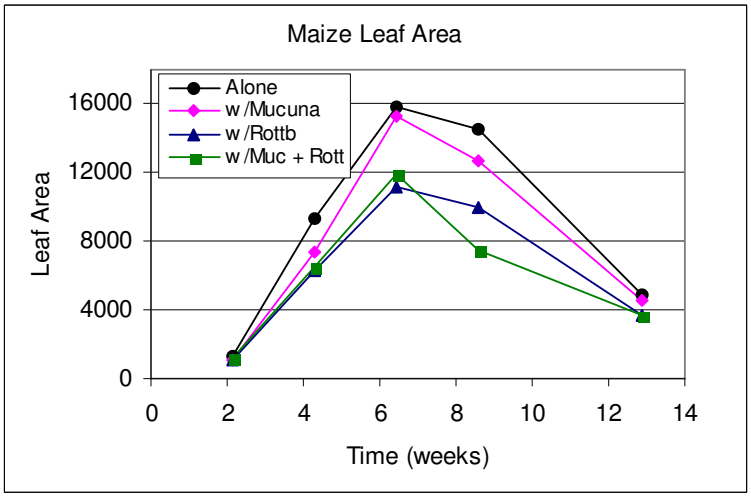
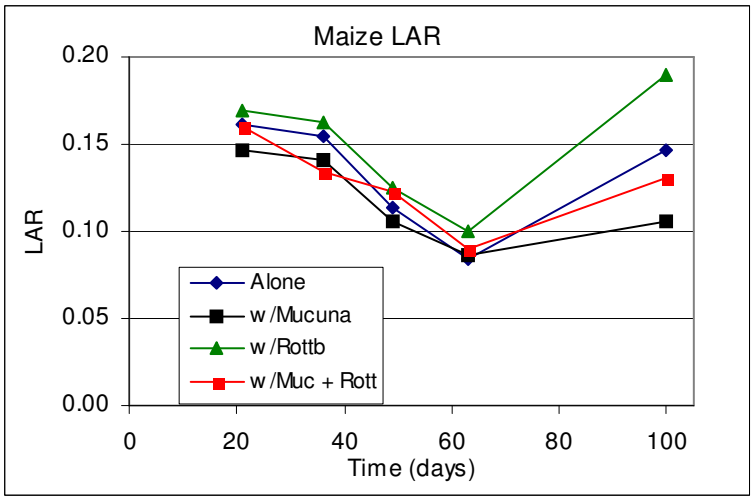


Figure 2. Leaf area response of itchgrass, mucuna and maize growing in interaction. Field study, Guanacaste, Costa Rica, 1998.



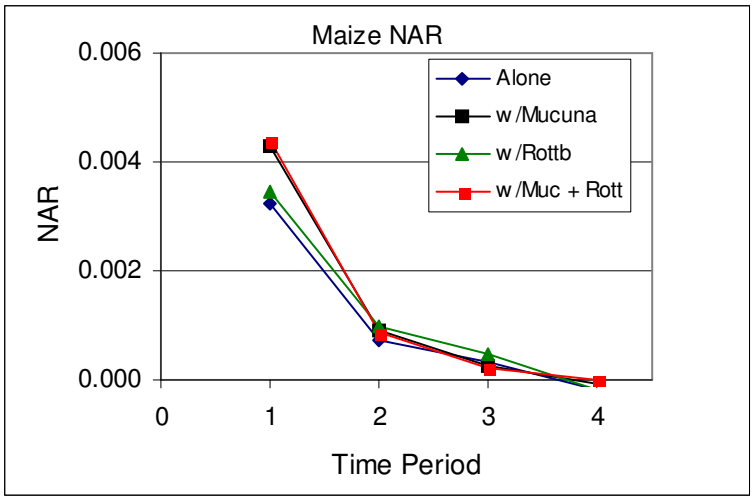
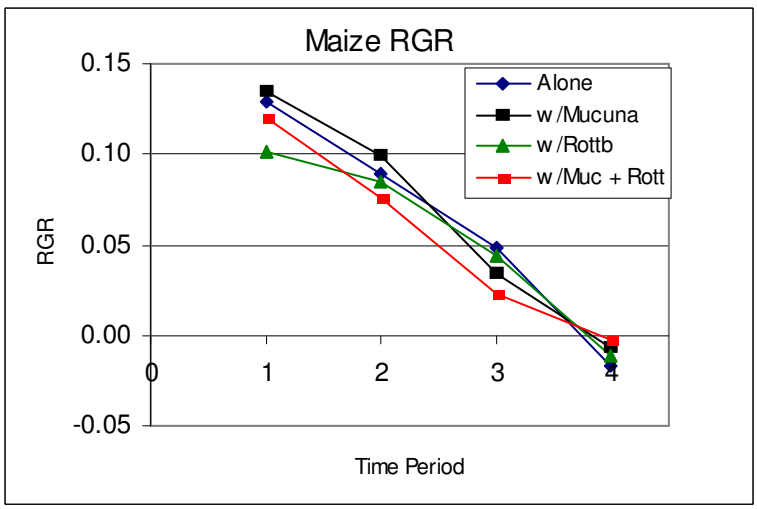


Figure 3. Growth parameters for maize planted alone and in interaction with mucuna and itchgrass. Field study, Guanacaste, Costa Rica, 1998.



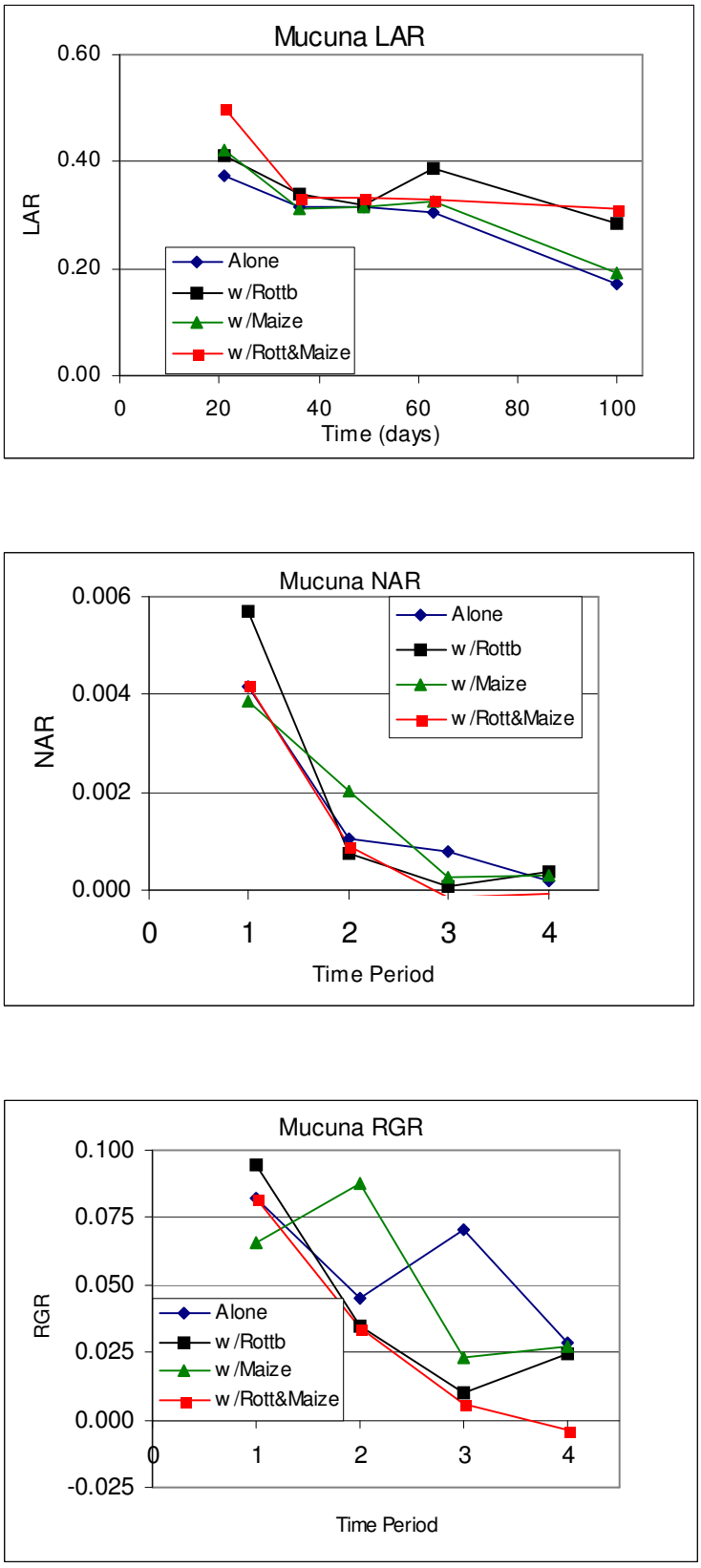
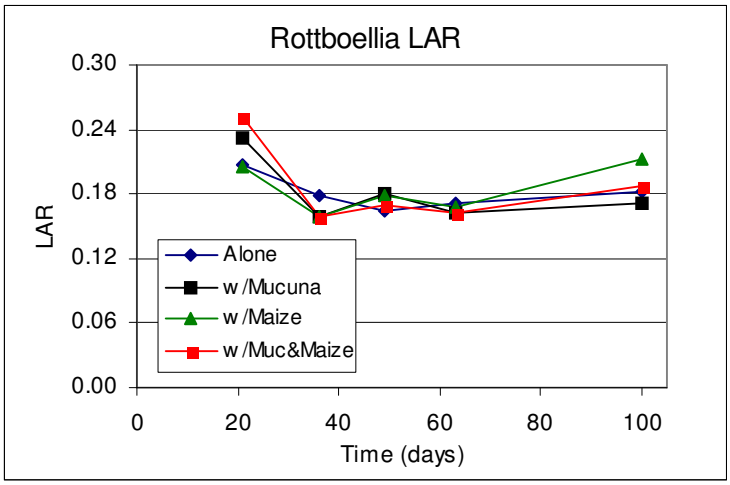
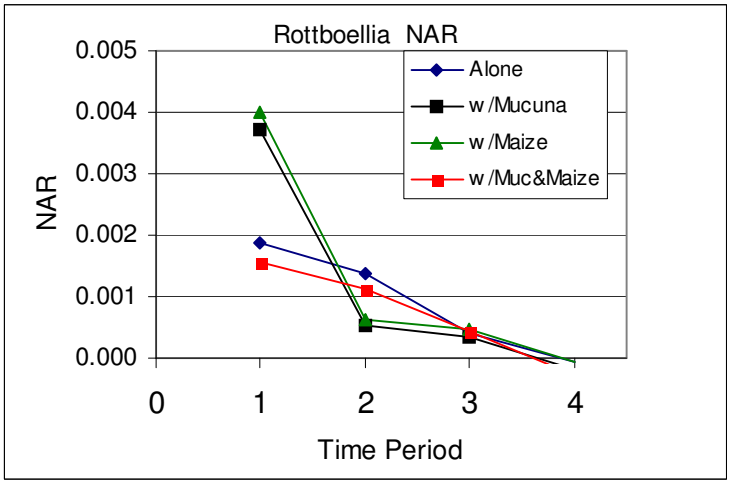


Figure 4. Growth parameters for mucuna planted alone and in interaction with maize and itchgrass. Field study, Guanacaste, Costa Rica, 1998.





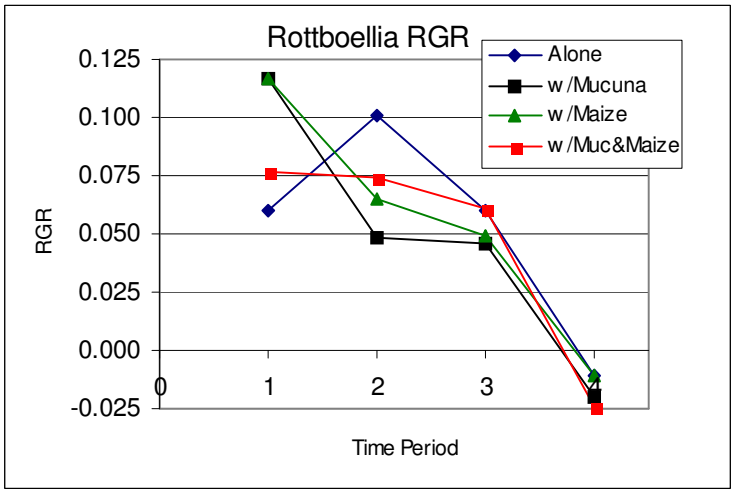


Figure 5. Growth parameters for itchgrass growing alone and in interaction with mucuna and maize. Field study, Guanacaste, Costa Rica, 1998.

3. Validation of integrated weed management tactics for itchgrass control in Costa Rica, Mexico and Bolivia.

Validation plots at farmer fields in Costa Rica. Pendimethalin effectively controlled itchgrass at the onset of validation plots and allowed the establishment of the cover crop during the first corn crop. In the first cropping cycle of 1996, itchgrass density at 45 DAP was between 28% and 95% lower and maize grain yield was higher (1.2 to 1.7 times) in validation plots than in the commercial ones (Table 13), although the cover crop growth was less vigorous than expected based on experimental plots and initial validation plots established in the second cropping season of 1995. The second cropping cycle was initiated in September 1996 by planting maize in Corralillo and Arado and dry beans as a rotation crop (following collaborator grower's practice) in Palmira. Itchgrass density at 45 DAP was reduced between 65% and 94% in validation plots at the first two locations. Maize grain yields were also higher at validation plots than commercial ones (between 1.2 and 1.6 times). In Palmira, where dry beans were planted as a rotation crop, itchgrass density at 45 DAP was 70% lower in the validation plot than in the grower's plot. Partial economic analyses gave an early indication that improved itchgrass management practices were profitable (Table 14).

Validation plots were planted at the same locations in 1997 but were severely affected by drought. In Palmira maize was planted twice but both plantings were lost; mucuna survived but only 35% ground cover was obtained 45 DAP. In Arado mucuna grew slowly, reaching only 15% ground cover by 45 DAP, but still reduced itchgrass populations from 22 to 7 plants/m^2 compared with the grower's plot (Table 13). In Corralillo, development was normal, reaching 80% ground cover at 45 DAP, and itchgrass was only 2 plants/m^2 compared to 32 plants/m^2 found in the grower's plot. The effect of adverse weather conditions also reflected on maize grain yields. In Palmira maize was lost. In Arado, grain yield in the validation plot was higher (2782 kg/ha) than in the grower's plot (1864 kg/ha). In Corralillo where Diamantes variety was planted yields were similar (1198 kg/ha in validation plot, 1293 kg/ha in grower's plot). In September, the second cropping season began and maize in association with mucuna was planted in Corralillo and Arado and beans, in Palmira, following the same scheme as the year before. The farmer at Corralillo could not plant maize because of late maize harvesting in the previous season. In Palmira, the rotation crop (beans) yielded about 50% more in the validation plot than in the grower's plot. Partial economic analyses in both cropping seasons demonstrated the profitability of improved itchgrass management practices (Table 14).

A final set of validation plots was established in 1998. The plot established in Corralillo was lost to severe drought at crop establishment but surviving mucuna was allowed to colonise the site to suppress itchgrass development and seed production. Maize yields in Arado and Palmira were substantially (29% and 93%) higher in validation plots than those obtained by the grower proving financial success of the improved management tactics (data not shown). Second-cropping season validation plots were established in Corralillo (maize) and Palmira (beans) but because floods and excessive rain one of them (Palmira) was lost and the other (Corralillo) was severely damaged. The validation plot in Arado could not be established because the collaborating grower decided to rotate his field and planted sugar cane.

The effect of improved itchgrass management on the soil seed reservoir was more pronounced at 0-10 cm than at 10-20 cm depth (Table 15). On average, 1.1 seeds/kg germinated and emerged from soil samples taken from validation plots at 0-10 cm whereas in the grower's plots germinating seeds amounted 5.22, 17.23 and 17.00 per kilogram in 1996, 1997 and 1998, respectively. Germinating seeds at 10-20 cm depth ranged between 0.12 and 0.48 seeds/kg and 0.23 to 2.12 seeds/kg in grower's plots. The sustained depletion of the soil seed bank corroborates the biological suitability of integrated itchgrass management.

Table 13. Itchgrass density at 45 days after planting, percent ground cover by mucuna and crop yield obtained at validation plots in two cropping seasons per year in Guanacaste, Costa Rica, 1996-97.

Location/	Itchgrass density at 45 DAP (Plants/m^2)				Mucuna ground cover at 45 DAP (%)		Maize yield (kg/ha)			
Cropping season	First		Second		First	Second	First		Second	
1996	V.P.[1]	G.P.	V.P.	G.P.	V.P.	V.P.	V.P.	G.P.	V.P.	G.P.
Arado	11.8	16.3	7.4	21.5	55	50	3541	2040	1462	1202
Corralillo	2.2	15.6	2.9	48.0	50	30	3015	1933	1622	1025
Palmira	2.2	42.2	4.4	14.8	45	NCC	3010	2452	1301*	1236*
1997										
Arado	6.67	21.48	6.67	21.50	15	40	2782	1864	1833	1656
Corralillo	0.00	32.89	0.00	----	80	35	1198	1293	2178	Not planted
Palmira	2.22	32.00	1.50	11.00	35	NCC	Lost[2]	1477	887*	725*

[1] Abbreviations: DAP, days after planting maize; V.P., validation plot; G.P., growers plot; NCC, cover crop not planted. An asterisk (*) indicates dry-beans yield.
[2] In 1997, validation plot at Palmira was lost because of severe drought. Grower at Corralillo did not planted maize in the second cropping cycle of 1997

Table 14. Partial budgets of weed management validation and farmer maize plots (in $US) in Guanacaste, Costa Rica, 1996-97.

	Arado		Corralillo		Palmira	
Cropping cycle/year	Validation	Farmer	Validation	Farmer	Validation	Farmer
First/1996						
1. Gross income	1,343	858	1,498	1,087	1,265	1,309
2. Management costs	139	126	176	183	146	163
3. Other costs	265	66	260	93	228	79
1-(2+3)	938	666	1063	811	892	1066
Validation-farmer		273		252		(174)
Second/1996						
1. Gross income	615	506	635	431	1,015	964
2. Management costs	94	129	211	218	86	72
3. Other costs	195	275	190	0	101	60
1-(2+3)	325	101	234	49	827	828
Validation-farmer		224		185		(5)
First/1997						
1. Gross income	1,043	699	449	485	-	554
2. Management costs	160	158	149	76	149	115
3. Other costs	243	160	150	93	84	88
1-(2+3)	640	352	151	316	-233	351
Validation-farmer		259		(165)		(584)
Second/1997						
1. Gross income	1,113	1,006	1,323	-	934	763
2. Management costs	172	290	149	-	77	115
3. Other costs	227	477	197	-	495	448
1-(2+3)	714	238	976	-	361	201
Validation-farmer		476		976*		161

Table 15. Number of itchgrass seedling germinating from soil cores taken at validation and farmer plots at three locations in Guanacaste, Costa Rica. 1996-1998

Year/Soil depth	May 1996				May 1997				May 1998			
	0-10 cm		10-20 cm		0-10 cm		10-20 cm		0-10 cm		10-20 cm	
Location	V.P.[1]	G.P	V.P.	G.P	V.P.	G.P	V.P.	G.P	V.P.	G.P	V.P.	G.P
Arado	1.0 a	8.2 b	0.0 a	0.3 a	0.88 a	6.01 b	0.29 a	0.44 a	2.82 a	18.38 b	0.00 a	0.00 a
Corralillo	0.6 a	1.5 a	0.0 a	0.2 a	0.29 a	2.93 b	0.00 a	0.00 a	0.29 a	28.89 b	0.73 a	3.84 b
Palmira	0.4 a	2.2 b	0.4 a	0.4 a	0.44 a	4.84 b	0.00 a	0.59 a	0.36 a	4.44 b	0.73 a	2.51 b

[1] Abbreviations: V.P., validation plot; G.P., growers plot.

Validation activities in Mexico and Bolivia. The validation plot at Ejido San Bartolo, Oaxaca, Mexico, could not include a cover crop because local seed was not made available on time. Instead, the only tactics that could be included in the validation plot were minimum tillage and the application of the selective herbicide pendimethalin to reduce the initial itchgrass infestation. The validation plot was always less infested with itchgrass than the commercial one; glyphosate was used twice by the grower to control itchgrass. According to visual estimates (by B. Valverde during a field visit), itchgrass infestation was about 60% and 25% in the commercial and validation plots, respectively.

In Bolivia validation plots were not established but cover crops were experimentally tested as itchgrass suppressers. In the first experiment (planted 28 January 1997), itchgrass infestation was very high (408 plants/m^2 in control plots). Cover crops slightly reduced itchgrass density (*C. juncea* by 37%) but had a greater impact on itchgrass biomass, the most suppressive being *D. lab lab* (71%) and *C. ensiformis* (67%). In the second experiment (planted 4 May 1998) the itchgrass infestation was reduced from 91 plants/m^2 in control plots to 53 and 83 plants/m^2 by *C. ensiformis* and *M. cinereus*, respectively. Biomass measurements better reflected the suppressive effect of the cover crops. In the presence of these two cover crops, itchgrass biomass was reduced by 32% and 30%, respectively. Six months after planting, *M. cinereus* was still providing excellent control of itchgrass. A final (thesis) report is yet to be prepared by the student, Mr. Ortiz.

4. Detailed characterisation of endemic pathogens for controlling itchgrass (CATIE, mycoherbicide component)

4.1 Selection and evaluation of pathogens

Collection and isolation of pathogens. As is shown in Figure 6, the greatest number of pathogens were isolated from the zone of Quepos (Central Pacific), with a total of 51 isolates, followed by Siquirres (Atlantic zone) with 44. The smallest number of pathogens, a total of 23, was isolated from Turrialba and Guanacaste. Such results suggest that there are zones in Costa Rica where there is greater attack of *R. cochinchinensis* by pathogens, although the weed originates from Asia.

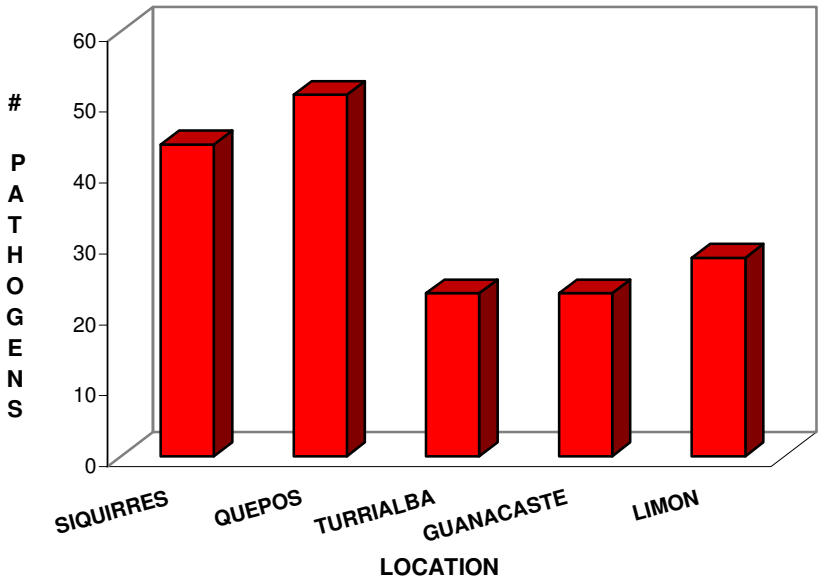


Figure 6. Pathogens isolated in different location in Costa Rica .

It was also found that 26 of the pathogens isolated from Siquirres correspond to isolations coming from the inflorescence, whilst in the case of Quepos most correspond to isolations from the root (30), followed by the inflorescence (18). In Turrialba, Guanacaste and Limón most isolations were from the leaves (Figure 7).

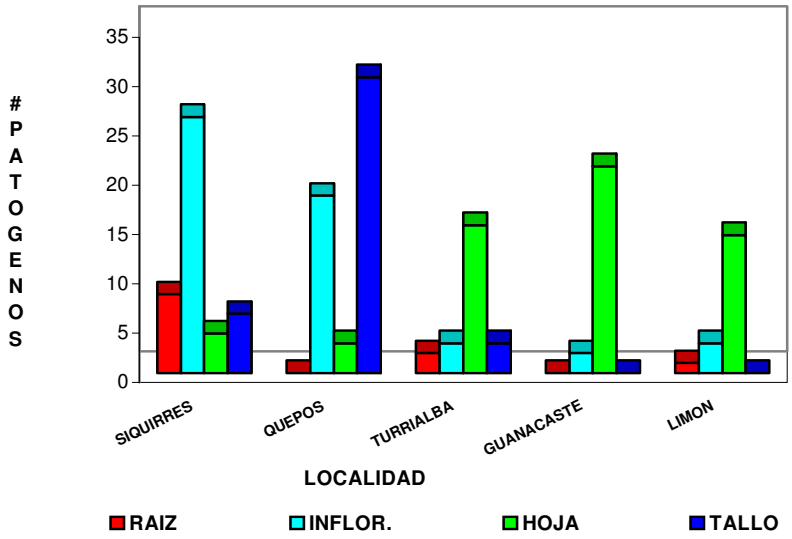


Figure 7. Pathogens isolated from each part of the plant of *R. Cochinchinensis*(root (raíz), inflorecencia (inflor), leaf (hoja) and stem (tallo).

It was observed that 30 days old or less seedlings were more susceptible to the isolated pathogens. These results indicate the importance of clearly recognising the phenological stages of the weed in order to determine which of these is most susceptible to attack by pathogens and to which a strategy of biological control can be adapted.

Evaluation of pathogenicity of the isolated organisms under greenhouse conditions. Figure 8 shows significant differences ($p<=0.05$), in the pathogenicity of strains 69, 105, 2 and 99 with respect to the absolute control, however the levels of pathogenicity shown by the pathogens were never greater than the value of two on the scale adopted and the weed was able to recover from attack by the pathogens. The analysis of pathogenicity also shows that there were no significant differences in the pathogens according to their ecotypes. Nevertheless those which showed greatest pathogenicity against the weed were: strain 2 identified as a Curvularia sp., strains 99 y 130 as Drechslera sp. and strains 69 y 127 as Fusarium sp. Other strains such as 105, 126, 130, 134, 140 163 and 169 are distinct from the control, although their pathogenicity was low, and so they should be considered for application in combination with stress factors. Also initial observations indicate that some of the selected strains may lose their virulence after manipulation for 6 months *in vitro.*

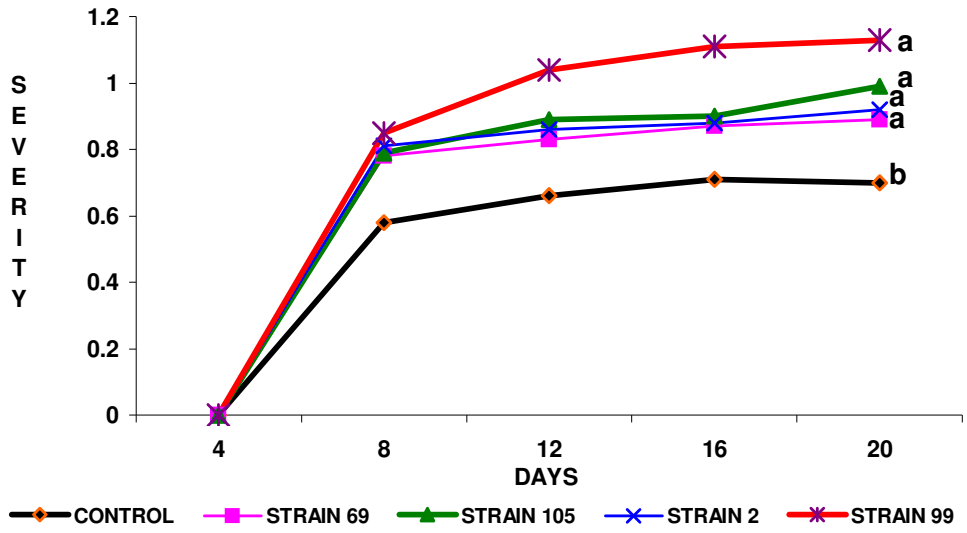


Figure 8. Comparison of the progress of disease caused by different strains,related to the control, under field conditions

Evaluation of pathogenicity of selected strains on crop species. No disease symptoms of selected strains pathogenic to *R. cochinchinensis* were observed on any of the evaluated crop species (Table 16).

Table 16. Pathogenicity of itchgrass native pathogens on crops of economic importance.

CULTIVAR/CROP	**Strain 2**	Strain 69	Strain 99	**Strain 127**	Strain 130
CR 5272 CERTIFICADA/RAICE	-*	-	-	-	-
CR 1113/RICE	-	-	-	-	-
ORIZICA 1/RICE	-	-	-	-	-
LLANO 5/RICE	-	-	-	-	-
CR5272 SIN CERTIFICAR/RICE	-	-	-	-	-
CRIOLLO TUZA BLANCA/MAIZE	-	-	-	-	-
CRIOLLO TUZA MORADA/MAIZE	-	-	-	-	-
CIAT 6780/Brachiaria sp	-	-	-	-	-
CIAT 6133/ Brachiaria sp	-	-	-	-	-
CIAT 16322/ Brachiaria sp	-	-	-	-	-

* No disease symptoms observed.

4.2 Evaluation of stress factors as agents to predispose *Rottboellia cochinchinensis* plants to attack by native pathogens in the greenhouse.

*Sub-doses of herbicides***.** With the exception of haloxifop methyl, none of the evaluated herbicides predisposed the plants to attack. For example, in the experiments with paraquat the effect on pathogenicity was principally due to the action of the herbicide, and so even at sub-doses the effect of the herbicide was enough to kill the seedling, due to its mode of action. (Figure 9).

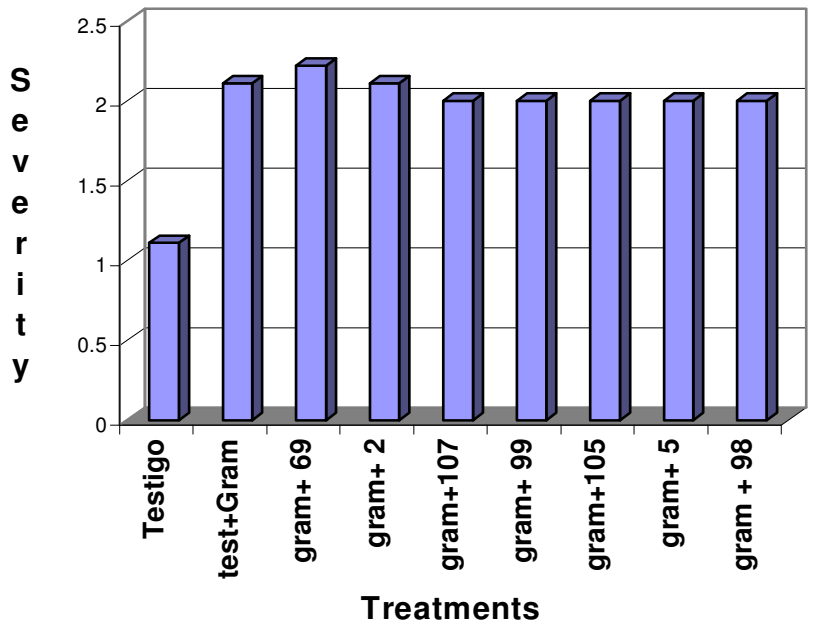


Figure 9. Strains (2, 5, 69, 98, 99, 105 and 107) evalued in combination with paraquat (gram) 0.37 ml commercial product / litre.

*Effect of haloxifop methyl***.** With this herbicide the severity of the disease was greater with four of the pathogens evaluated: strains 105 and 99 (*Dreschlera)*, 69 and 127 (*Fusarium*). Also the strains of *Fusarium* showed signs of pathogenicity principally in the zones of active growth of the weed. The best dose observed in the greenhouse was 0.48 g, a.i./L or 2ml Pc/L, which combined with strains 69 and 127 (*Fusarium*), gave the greatest levels of pathogenicity of the weed 30 days old or less (Figure 10).

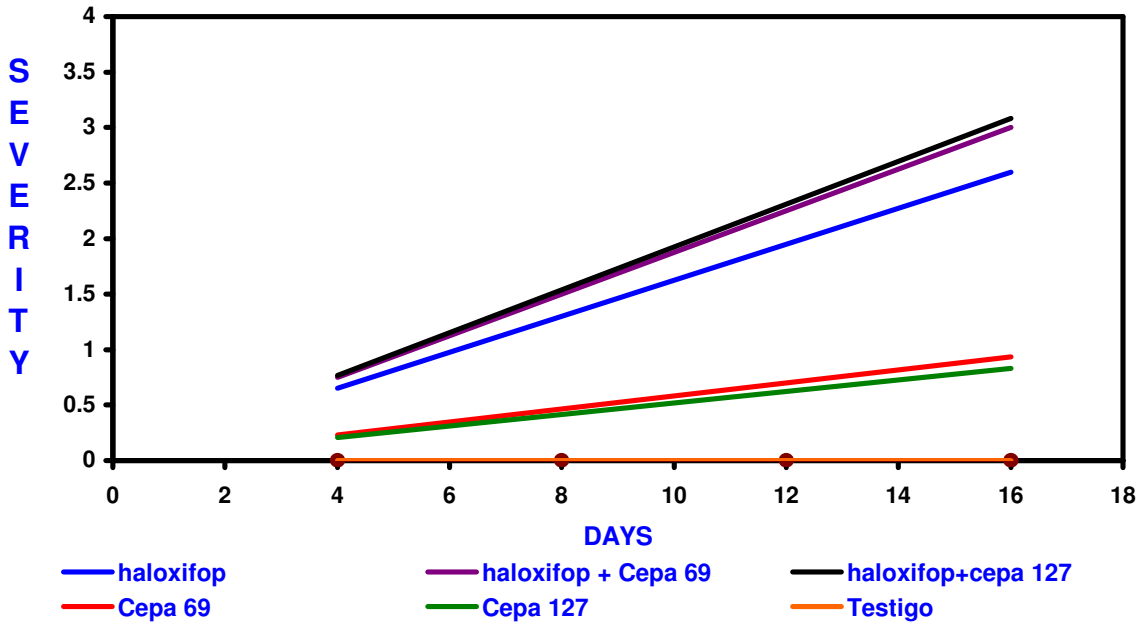


Figure 10. Effect. of haloxifop methyl (0.096 kg a.i./ ha or 2ml c.p./litre) and the strains (cepas) 69 and 127 (*Fusarium*), under greenhouse conditions.

Effect of saturation with water and shade. The results show that neither shade nor saturation of the soil favoured the effect of the pathogens. However a clear increase in severity was observed when shade was combined with a sub-dose haloxifop methyl. (Figure 11).

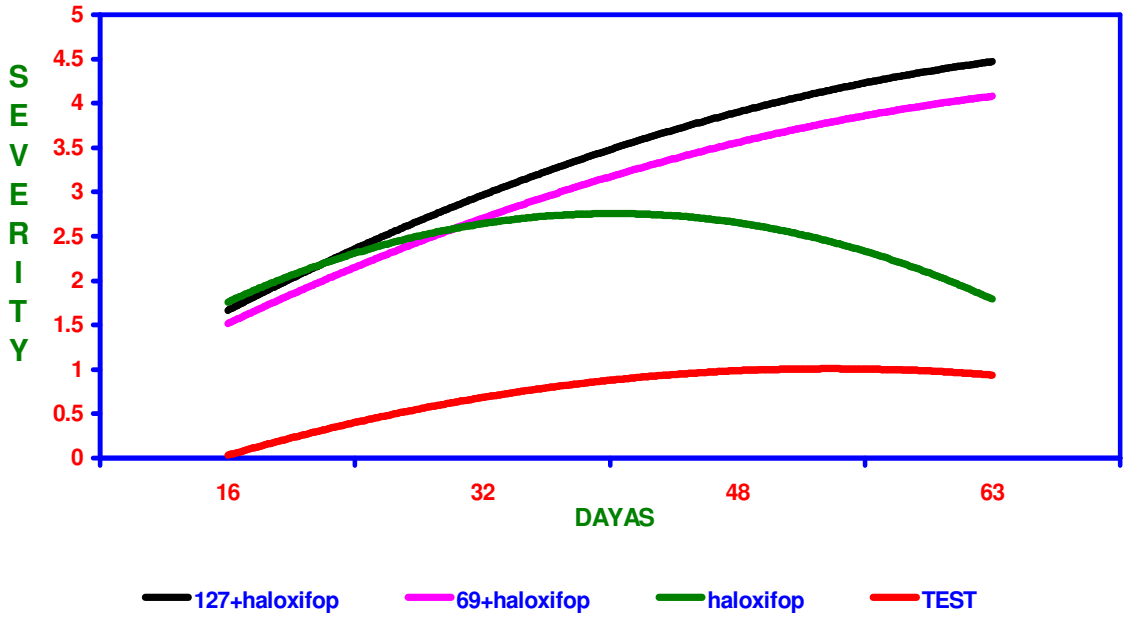


Figure 11. Effect of shade and haloxifop methyl (0.096 kg a.i./ ha or 2ml c.p./litre) on the severity of strains 69 and 127, under field conditions.

4.3 Evaluation of native pathogens and sub-doses of herbicides in the field.

Effect of native pathogens and formulation. Both pathogens, strains 69 and 127, caused a maximum pathogenicity of 4 on the scale used, when they were combined with the herbicide, and the itchgrass plants treated died. Isolate 127 was slightly different to isolate 69. The mixture of the two strains resulted in a lower pathogenicity (3), which could be due to antagonism between them. No differences were observed when the pathogens were applied in a formulation with canola oil. Other formulations need to be tested. (Figure 12).

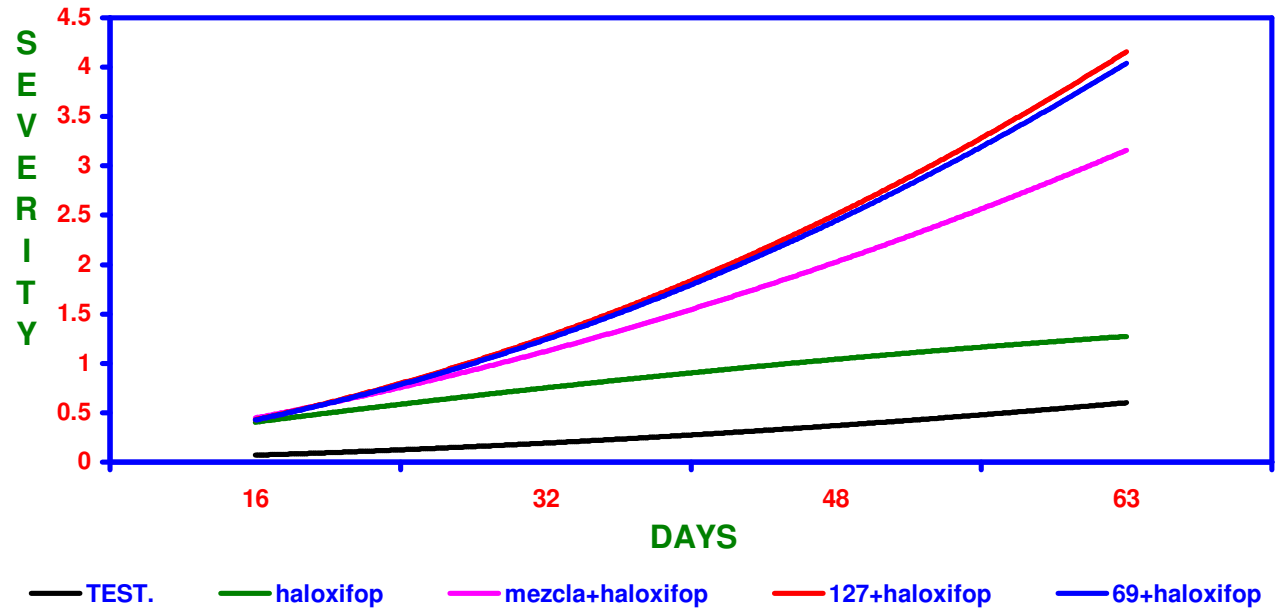


Figure 12. Effect of strains 69 and127 and their mixture (mezcla), in combination with haloxifop metil (0.048 kg a.i. / ha or1 ml c.p. / litre), under field conditions.

Effect of haloxifop methyl. The first field trial was performed with the highest dose of haloxifop methyl obtained under greenhouse conditions (0.096 a.i. Kg / ha, 2 ml/Litre), however in the field this dose was lethal in the control without pathogens. This was probably due to conditions of high luminosity and temperature at the site. Under such conditions the metabolism of the plant is much more active favouring the translocation and action of the herbicide. For this reason two further trials were performed to evaluate two lower doses of 0.072 and 0.048 a.i. Kg / ha, 1.5 and 1 ml/Litr, repectively. There were significant differences in the effects of the two lowest subdoses of haloxifop methyl and the pathogens over time. However the best dose was the lowest evaluated (0.048 Kg a.i./ ha, 1ml/L). Figure 12 shows that the herbicide and the pathogens could only kill the plants when they were applied in combination.

*Effect of Mucuna cover***.** Populations of *R. cochinchinensis* were not affected by a cover crop of Mucuna, however the period of evaluation was probably too short to observe the effect of the cover. It is probable that if the cover is sown well before the crop it has a greater chance of establishing and covering the soil before the weed and an interaction could be obtained between the pathogens and the herbicide.

Research Additional to Original PMF

Density-dependant seedling survival seed production in itchgrass. There was no density-dependent mortality when initial population was less than 10 plants/m^2. Mortality increased over time, especially when the initial density was equal or more than 40 plants/m^2. Biomass per unit area followed the law of constant final yield with a maximum of about 600 g/m^2. On a per plant basis, biomass followed the reciprocal yield law until reaching about 15 g/plant. Similar relationships were obtained at both locations for seed production. Maximum seed production was between 7400 and 8900 seeds/m^2. A single itchgrass plant growing in isolation produced between 700 and 820 seeds. See section on ZA-0051 for additional details.

5 Full scale screening of the itch grass head smut (*Sporisorium opiuri*) and leaf rust (*Puccinia rottboelliae*)

Both the smut *Sporisorium ophiuri* and rust *Puccinia rottboelliae* were found to be intra-species specific, whereby isolates of these pathogens would only infect a limited number of biotypes of the weed, presumably from those countries in which the strain had co-evolved with its host. The exception to this was a smut isolate collected from Madagascar that was capable of infecting Latin American biotypes (ex Africa) and hence selected for further host range screening. None of the rust strains screened proved sufficiently virulent towards any of the South American biotypes that were challenged, therefore, further host range screening was suspended.

A pathogen that shows biotype selectivity within its host species, and comprises co-evolved pathotypes, by definition, therefore, would not infect plants from any other species, even those closely related. However, for quarantine purposes, it was necessary to undertake a comprehensive host range testing programme. The genus *Sporisorium* Vanky (*Ustilaginaceae*) is only recorded from species of the *Gramineae*. For this reason it was decided to restrict testing to the *Gramineae* concentrating particularly on those species and varieties of agronomic importance.

The results of the screening (Table 17) clearly showed that the smut is a highly host-specific pathogen, since the inoculated plants of all test species remained healthy and produced only fertile flowers. In all cases, the *R. cochinchinensis* plants included as positive controls consistently became infected, demonstrating that conditions for infection were favourable.

Table 17. Graminaceous plant species screened for susceptibility to *Sporisorium ophiuri:* Head Smut of *Rottboellia cochinchinensis*

Plant species	*Variety*	**Number tested**
Andropogon gayanus (Pasture grass)	Andropogon gayanus, Central America	15
Brachiaria brizantha (Palisade grass)	CIAT No. 664 Atenos, Costa Rica	17
Brachiaria brizantha (Palisade grass)	CIAT No. 26110 Atenos, Costa Rica	21
Brachiaria decumbens (Surinam grass)	CIAT No.16497 Atenos, Costa Rica	18
Eleusine coracana (Finger millet)	Wild type, Thailand	8
Ischaemum rugosum (Saramacca grass)	ex Colombia	20
Oryza sativa (Upland rice)	Local variety, Thailand	16
Oryza sativa (Upland rice)	IR64, Philippines	26
Oryza sativa (Upland rice)	1113, Costa Rica	27
Oryza sativa (Upland rice)	Llano, 5 Costa Rica	21
Oryza sativa (Upland rice)	Orizica, Costa Rica	24
Oryza sativa (Upland rice)	CR 5272, Costa Rica	30
Panicum maximum (Guinea grass)	CIAT No.6177 Atenos, Costa Rica	10
Panicum maximum*(*Guinea grass*)*	CIAT No.6177 Atenos, Costa Rica	12
Panicum maximum*(*Guinea grass*)*	CIAT No.16028 Atenos, Costa Rica	14
Pennisetum typhoides (Pearl Millet)	BJ104, India	21
Pennisetum typhoides (Pearl Millet)	KU 01, Thailand	40
Rottboellia spp.	Unidentified, Thailand	15
Saccharum officinarum (Sugar cane)	B6 409, Barbados	11
Saccharum officinarum (Sugar cane)	BJ 75-04, Barbados & Jamaica -	9
Saccharum officinarum (Sugar cane)	CP 73-1312, USA	9
Saccharum officinarum (Sugar cane)	CP80-1953, USA	14
Saccharum officinarum (Sugar cane)	Ja 605, Jamaica	10
Saccharum officinarum (Sugar cane)	Na 5642 ®, Central America	10
Saccharum officinarum (Sugar cane)	Q 96 ®, Australia	13
Saccharum officinarum (Sugar cane)	SP70-1284 ®, Brazil	12
Saccharum officinarum (Sugar cane)	SP71-5574 ®, Brazil	10
Saccharum officinarum (Sugar cane)	SP71-1680, Brazil	13
Saccharum officinarum (Sugar cane)	LAICIA 82-2436, Costa Rica	4
Saccharum officinarum (Sugar cane)	LAICIA 82-2020, Costa Rica	4
Saccharum officinarum (Sugar cane)	Noble cane, Tanzania	3
Saccharum officinarum (Sugar cane)	NC310,	5
Setaria italica (Foxtail millet)	Wild type, Thailand	12
Sorghum bicolor (Sorghum)	SU 630, Thailand	32
Sorghum bicolor (Sorghum)	SU 203, Thailand	29
Zea (Euchlaena) mexicana (teosinte)	B 73-75 (balsas); Mexico	
Zea mays (Maize)	SU 1, Thailand	30
Zea mays (Maize)	KC 3002, Thailand	25
Zea mays (Maize)	Amarillo Carbonal, Central America	25
Zea mays (Maize)	Pioneer H3078 Brunca, Central America	17
Zea mays (Maize)	Maicena amarillo, Central America	11
Zea mays (Maize)	Enano amarillo , Central America	17
Zea mays (Maize)	Maicena Blanca, Central America	10
Zea mays (Maize)	Rosani , Central America	10
Zea mays (Maize)	Maicena Enano, Central America	8
Zea mays (Maize)	Tico var. 7, Central America	3
Zea mays (Maize)	Guarare, Central America	13
Zea mays (Maize)	Var. Diamantes, Central America	6
Zea mays (Maize)	Criollo, Central America	10

® Known variety resistant to *Ustilago scitaminae*

6. Characterisation of the itchgrass head smut *Sporisorium ophiuri*

Amplification products were obtained from all isolates of each species with at least two of the primers tested. However, no amplification was achieved with the MR primer previously used as a finger-printing probe for a number of fungal genera (Bridge *et al.*, 1997). The reason for this is unclear, as it is likely that the sequence occurs within the isolates and failure to detect banding patterns may be due to amplification conditions. The $(GACA)_4$ primer was found to be most useful for distinguishing between the *Sporisorium* isolates (Fig. 13) and the results from the UPGMA cluster analysis generated with this primer are represented as a dendrogram (Fig. 14).

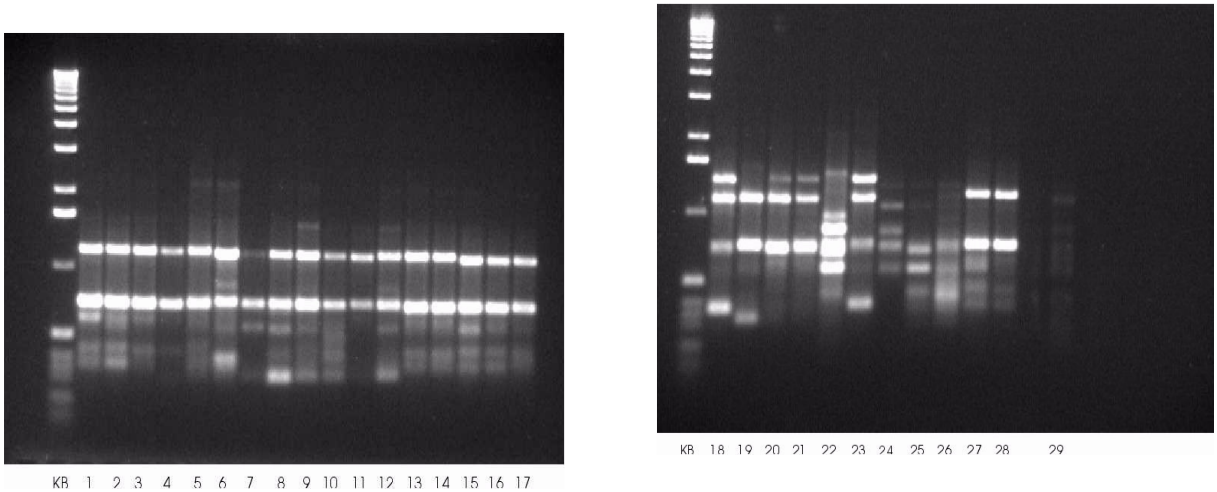


Figure 13. Agrose gel of amplification products of Ustilaginaceae DNA using the $(GACA)_4$ Primer

(1) *Sporisorium ophiuri* (Thailand–4[b]), (2) *Sporisorium ophiuri* (Thailand–1[b]), (3) *Sporisorium ophiuri* (Thailand–2[b]), (4) *Sporisorium ophiuri* (Thailand–3[b]), (5) *Sporisorium ophiuri* (Sri Lanka-2[b]), (6) *Sporisorium ophiuri* (Kenya-3), (7) *Sporisorium ophiuri* (Kenya-1), (8) *Sporisorium ophiuri* (Madagascar-2), (9) *Sporisorium ophiuri* (Madagascar-2), (10) *Sporisorium ophiuri* (Ethiopia), (11) *Sporisorium ophiuri* (Zimbabwe), (12) *Sporisorium ophiuri* (Philippines –2[a]), (13) *Sporisorium ophiuri* (Philippines –2[a]), (14) *Sporisorium ophiuri* (Philippines –3[b]), (15) *Sporisorium ophiuri* (Philippines –3[b]), (16) *Sporisorium ophiuri* (Philippines –3[b]), (17) *Sphacelotheca reilina* (New Zealand), (18) *Ustilago maydis* (Thailand), (19) *Sphacelotheca destruens* (India), (20) *Sphacelotheca destruens* (India), (21) *Sphacelotheca sorgi* (Argentina), (22) *Ustilago maydis* (Kenya), (23) *Ustilago scitaminea* (Costa Rica), (24) *Ustilago scitaminea* (Costa Rica), (25) *Ustilago scitaminea* (Costa Rica), (26) *Sporisorium ophiuri* (South Thailand), (27) *Sporisorium ophiuri* (North Thailand), (28) Water control.

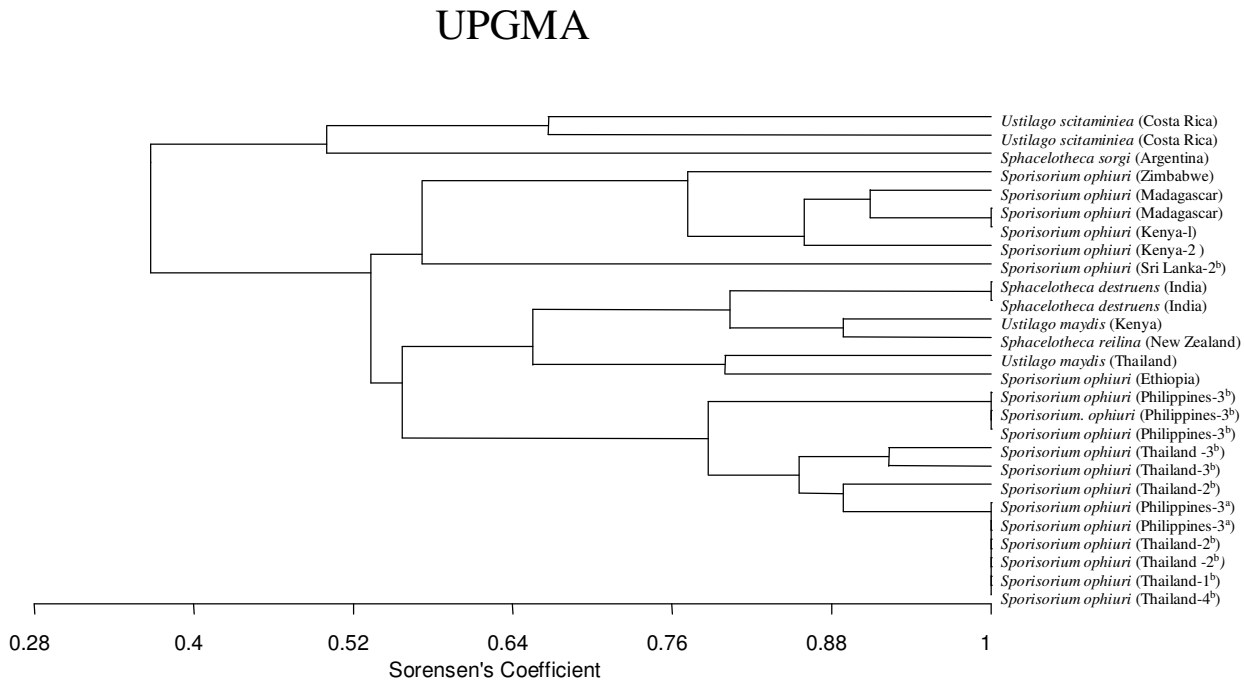


Figure 14. Dendrogram obtained from Sorensen's coefficient after UPGMA clustering of band data by PCR amplification of Ustilaginaceae DNA by the $(GACA)_4$ primer.

The cluster analysis revealed that the six smut species form only four groups. The majority of the *Sporisorium* isolates were split into two main sub clusters of either African or Asian origin. The two exceptions to this grouping were isolates from Ethiopia and Sri Lanka. The Ethiopian isolate was placed within a separate sub-cluster along with species of *Ustilago* and *Sphacelotheca* and appeared more closely related to *Ustilago maydis* from Kenya than to the other African *Sporisorium* isolates. This anomaly may be explained by the comparatively poor amplification of the Ethiopian isolate which meant that only the largest bands common to the bulk of the smut isolates were detected. In other PCR reactions, where the Ethiopian isolate underwent stronger amplification, the banding patterns appeared very similar to the African isolates of *Sporisorium*. The isolate from Sri Lanka had more bands in common with the African group of *Sporisorium* isolates, however, this isolate was clearly different from the African isolates and formed only a relatively weak linkage (56 %) to the group.

The African group with the exception of the Sri Lankan isolate all had greater than 76% of their bands in common. Within this group, the Madagascan isolate appeared very similar to Kenya, but the isolate from Zimbabwe showed a greater degree of polymorphism. The Asian group also formed a discrete cluster with greater than 76% similarity between isolates. The Philippines RLB isolate was almost identical to the isolates from Thailand. The largest sub division within this group was formed by the Philippines RC isolate, although the degree of separation was not especially wide.

The other two groups identified by the cluster analysis contained smut species from genera other than *Sporisorium*. The sugar cane smut *Ustilago scitaminea* from Costa Rica was grouped with the sorghum smut *Sphacelotheca sorghi* from Argentina, although the final linkage to this group was relatively weak (49%). Within the grass tribe of Andropogonae, *Sorghum* is very closely related to *Saccharum* (Clayton & Renvoise, 1986). Therefore, the grouping of these pathogens, although distant, might be related to the taxonomic similarities

of the host plants. The last group identified by the cluster analysis was a rather heterogeneous group that contained smuts species belonging to *Ustilago*, *Sphacelotheca* and *Sporisorium*. The cluster analysis indicated that the *Ustilago maydis* isolate from Kenya was very close to *Sphacelotheca reilina* from New Zealand. This degree of similarity is difficult to explain, however, both smuts are pathogenic to maize, which may possibly account for some, but not all of their similarities as the *Ustilago maydis* isolate from Thailand appeared quite different from these isolates. Also forming a separate cluster within this rather diverse group was the millet smut *Sphacelotheca destruens* from India.

Sporisorium ophiui can be readily distinguished from the other smut species included in the screen. This "finger-printing" of isolates should enable any *S. ophiuri* isolate released into the New World to be identified, should the need arise. The Philippines robust creeping biotype of *R. cochinchinensis* was both morphologically and genetically quite different from the other *R. cochinchinensis* biotypes. Therefore, it is perhaps not surprising that the smut isolated from this biotype appeared different from the rest of the Asian smuts although this difference was not wide. The inclusion of the Sri Lankan pathotype within the African group was an unexpected result based on the clear divisions between the other members of these groups shown by the cluster analysis. Nevertheless, there was some pathological evidence from the host range studies of this isolate to suggest a tentative link between this isolate and at least one pathotype within the African group.

The Madagascan isolate has demonstrated an ability to infect a number of Latin American biotypes of the weed. This ability is shared at least in part by the Sri Lankan biotype, which also infected a limited number of New World biotypes of the weed. However, the genetic relatedness of the smut pathotypes cannot always be taken as a definitive guide to their pathogenicity. For example, the central Kenyan isolate was found to be very closely related to the Madagascan isolate. The genetic similarity between the smut pathotypes was also paralleled in the biotypic relatedness of the Kenyan and Madagascan biotypes of the weed, identified by both the AFLP and isoenzyme analyses. However, despite these genetic similarities, in glasshouse infection studies the Kenyan smut isolate proved to be biotype specific and unable to infect Latin American biotypes of the weed.

The mechanisms of host resistance that allow only certain pathotypes of *S. ophiuri* to parasitise a particular biotype of *R. cochinchinensis* are unknown. However, it is suspected that each isolate of *S. ophiur*i is capable of gaining entry into all of the biotypes that it challenges (C. A. Ellison, unpublished data). The extreme level of host specificity exhibited may be due to small biochemical differences within the plant, which, in the case of incompatible associations, prevent the smut from invading the meristematic regions of the plant. Although the Madagascan isolate of the smut was consistently capable of infecting Latin American biotypes of the weed, the percentage of infected plants achieved was less than that obtained with the fully compatible Madagascan biotype. Hence, the Madagascan isolate might represent a highly virulent but less specific form of the smut capable of infecting a broader range of biotypes within its host species.

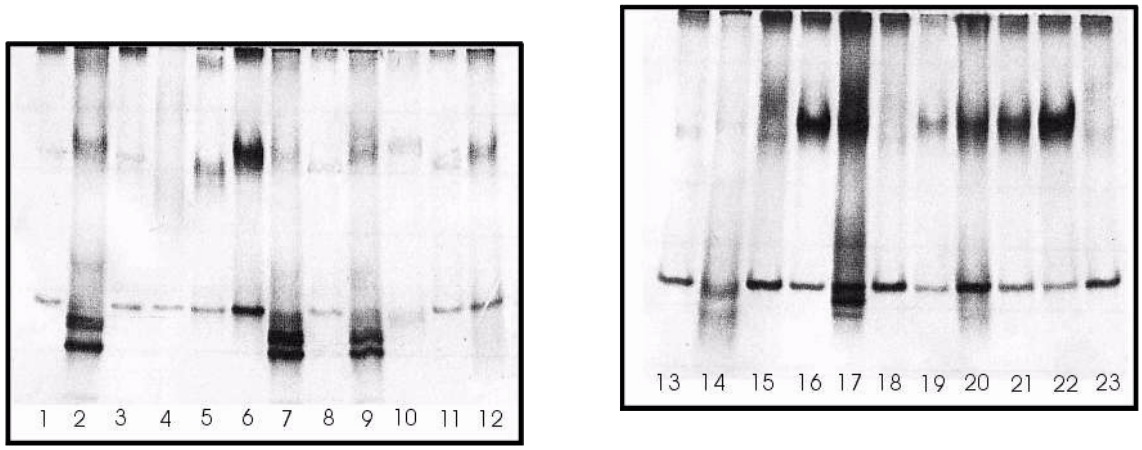
The Madagascan biotype and its co-evolved smut pathotype have been geographically separated from the rest of the African weed population, possibly for many thousands of years. This period of isolation may have led to the evolution of a more virulent strain of the pathogen. Alternatively, the ability of this pathotype to infect New World biotypes of the weed may be due the increased pathogenicity that has often been ascribed to new encounters by pathogens, especially as it appears that the Latin American biotypes may have originated

from Africa. The argument put forward for the enhanced pathogenicity of new encounters is based on a genetic feedback mechanism proposed by Pimentel (1961). Host species that have co-evolved with their natural enemies within the centre of origin of the host, must have evolved a mutual balance or interspecific homeostasis. Within this balance the pressures from natural enemies have reduced the population of the host by reducing the numbers of highly susceptible individuals within the population. The genetic feedback mechanism suggests that host resistance represents a genetic compromise and that plants do not become completely host resistant due to the costs of maintaining these resistance mechanisms (the costs of maintaining physical and chemical resistance in the population when the natural enemies are not present) as the resistant genotypes must still compete with non-resistant genotypes. Once the host is moved to a new ecosystem without the natural enemies present, then the more resistant individuals are at a selective disadvantage in competition with other members of their own species, because they must divert resources away from growth and reproduction in order to maintain their resistance. This allows the previously more susceptible members of the population to dominate often leading to an 'explosion' in the population of the weed. This situation may have occurred in the New World and the Madagascan pathotype is capable of infecting the now dominant but more susceptible members of the population.

7. Characterisation of selected worldwide *Rottboellia cochinchinensis* biotypes

Species with a greater genetic diversity tend to be better able to respond to different environmental conditions. It is particularly important to be able to assess diversity in weedy species, both in general terms, and in relation to specific traits, in order to develop effective control measures. The electrophoretic techniques used in this study looked at 38 biotypes selected from 20 counties in an attempt to map the genetic diversity of the weed on a global basis.

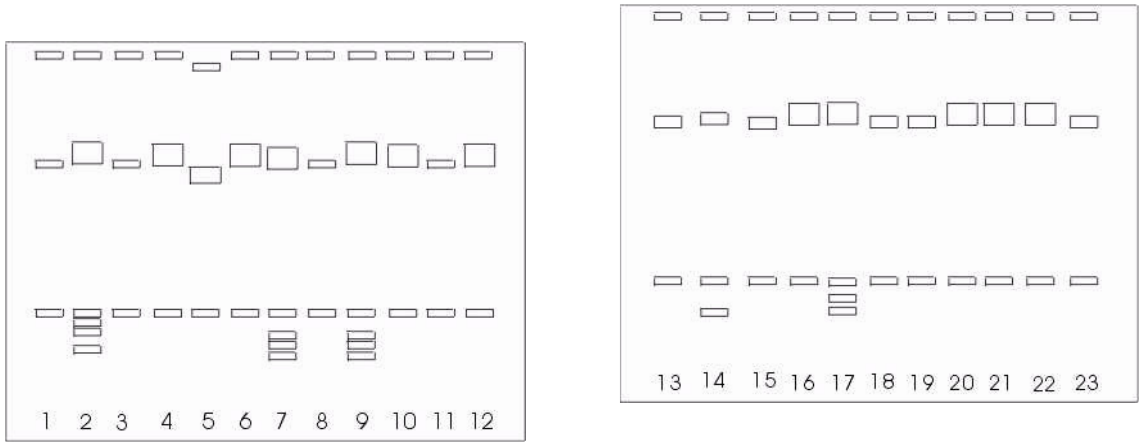
The stain for esterase activity permitted a clearer distinction between biotypes, and was selected in preference to the other isozyme stains, which did not yield easily perceptible differences (Fig. 15). This is similar to Fisher *et al* (1987) who also found that esterase isozymic staining was the most useful in distinguishing between two *R. cochinchinensis* biotypes and their F_2 progeny.



a

Figure 15. a Polyacrylamde gel for the isoenzyme banding patterns obtained for the for α naphthal esterase stain; **b** interpretation of banding patterns.

(1) Thailand-5[a], (2) Thailand 2[b], (3) Bolivia, (4) Madagascar-1, (5) India–1[a], (6) Australia, (7) Thailand –3[b], (8) Costa Rica-3, (9) Thailand–4[b], (10) Sri Lanka–2[b], (11) Peru, (12) Kenya-1, (13) Papua New Guinea-1, (14) Colombia, (15) Costa Rica-5, (16) Ecuador, (17) India-2[b], (18) Cuba, (19) Honduras, (20) South Africa, (21) Louisiana LD, (22) Louisiana SD, (23) Solomon Isles



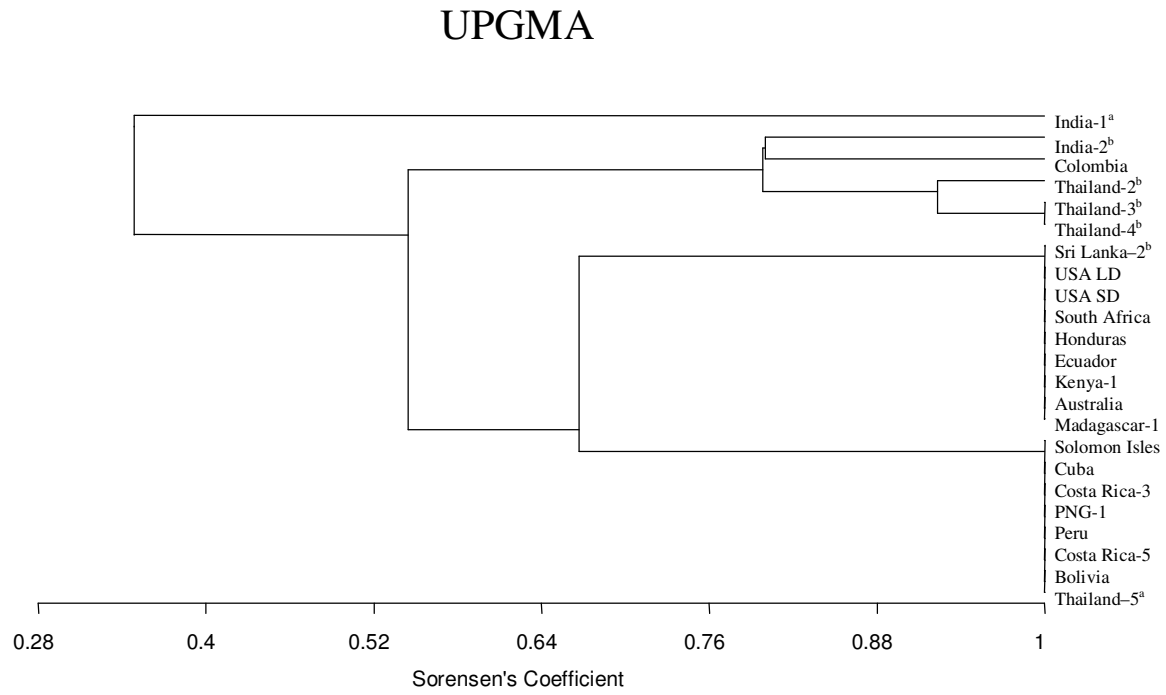


Figure 16. UPGMA cluster analysis of the banding patterns produced from the stain for α naphthal esterase for *Rottboellia cochinchinensis* biotypes

The UPGMA cluster analysis of the banding patterns produced from the stain for α naphthal esterase (Fig. 16) revealed that the *R. cochinchinensis* biotypes could be divided into three broad groups based on the similarity of their isozymic banding patterns (Table 18). These groups were largely associated with the geographical origins of the biotypes and represent weed populations from America, Africa and Asia, although there was some overlap between these groups. The remaining biotype from India –1[a] was placed into a separate group (group 4) being distinctly different from the other biotypes.

Table 18. Characterisation of itchgrass biotypes based on Isoenzyme analysis

Group 1	Group 2	Group 3	Group4
Thailand-5[a]	Australia	Thailand-1[b]	India-1[a]
Costa Rica-3	Kenya-1	Thailand-2[b]	
Costa Rica-5	Madagascar-1	Thailand-3[b]	
Peru	Ecuador	India-2[b]	
Cuba	USA (Louisiana LD)	Colombia	
Solomon isles	USA (Louisiana ND)		
Papua New Guinea	Honduras*		
Bolivia	South Africa*		
	Sri Lanka–2[b]		

The groupings are based on the isozyme banding patterns for the enzyme α naphthal esterase

*Grouping based on rather unclear (diffuse) banding patterns needs to be re-confirmed.

Results from the cluster analysis of the AFLP data (Fig. 17) showed that *R. cochinchinensis* biotypes formed an extremely narrow genetic base, there being greater than 80% similarity between all of the biotypes. It was not until the 91% level that biotypes could be separated out into five broad groups, again related to their geographical origins (Table 19).

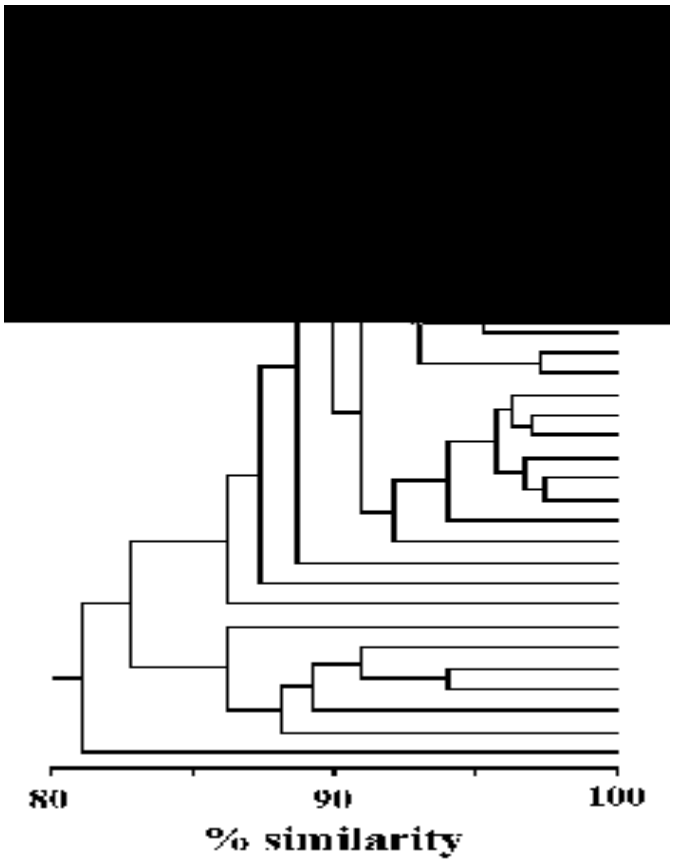


Figure 17. UPGMA tree calculated from the average similarity matrix produced by AFLP analysis of 35 *Rottboellia cochinchinensis* isolates with all 6 primers

Table 19. Groups identified by AFLP cluster analysis based on the 92% level of similarity

Group 1	Group2	Group 3	Group 4	Group 5	Group 6
Kenya– Kenya-2 Madagascar -1	Costa Rica-3 Bolivia USA ND Mexico Peru	Australia South Africa Zimbabwe USA SD PNG-1 Costa Rica-2 Philippines–1[a] Costa Rica-4	Honduras PNG-2 Ecuador Costa Rica-6 Costa Rica-5 Nicaragua Cuba Kenya–3	Colombia Sri Lanka–2[a] India–2[b]	Costa Rica-7

Table 19. Continued. Groups identified by AFLP cluster analysis based on the 92% level of similarity

Group 7	Group 8	Group 9	Group 10	Group 11	Group12
Thailand–4 [a]	India–1[a]	Philippines–2[a]	Brazil	Sri Lanka–1[b]	Phillippines–3[b]

Although, the techniques used to differentiate between the biotypes relied on quite different characteristics, there are many similarities between the groupings obtained using either technique.

Group 1 of the isoenzyme analysis and group 2 and 4 of the AFLP analysis were comprised of biotypes predominately of Latin American origin. Four out of the seven biotypes from group 1 of the isoenzyme analysis and ten out of the twelve biotypes from groups 2 and 4 of the AFLP analyses were from North, Central or South America. The similarity of these biotypes identified in both studies suggests that the majority of Latin American biotypes might have arisen from just a small number of introductions. The weed has most likely been transported to one or more of these Latin American countries in contaminated grain shipments and has since spread to neighbouring countries, by further seed exchanges, washed into water courses or transported on the wheels of vehicles. Also identified in both studies as belonging to this Latin American group was a biotype from Papua New Guinea (PNG). This and the geographically related biotype from the Solomon Isles identified in the isoenzyme analysis may represent a New World introduction of the weed from Latin America. Similarly the isoenzyme analysis also placed the exotic introduced Thai biotype (Thai–5[a]) within this Latin American group. This biotype shares many morphological and physiological similarities with the Latin American biotypes, being comparatively rapid to flower and producing seed of a relatively small size. Pathological evidence also links this biotype with those of Latin America, which are susceptible to the Madagascan pathotype of the smut *S. ophiuri*. This evidence indicates that the introduction into Thailand was via Latin America possibly in contaminated seed used in a CIMMYT breeding trial conducted at the farm. However, the AFLP analysis does not appear to support this hypothesis placing the Thai-5 [a] biotype in a separate group (group 7) although, it should be noted that the genetic separation between the biotypes identified by the cluster analysis was not large. The isoenzyme analysis does not include a biotype of Old World origin from which these Latin American biotypes might have originated. However, group 4 of AFLP analysis contains a biotype from coastal Kenya perhaps linking Africa with the centre of diversification for this group.

Group 2 of the isoenzyme analysis contained biotypes from a broader range of geographical areas, including all of those from Africa (Madagascar, Kenya and South Africa), four from the America's (Louisiana LD and ND, Ecuador and Honduras), Asia (Sri Lanka) and a biotype from Australia. The African biotypes within this group suggest this might be the origin for at least some of the Latin American introductions and also for the Australian biotype. The inclusion of the Sri Lankan biotype (Sri Lanka-2[b]) within this group was unexpected based on its morphological and phyisiological similarities to the other Asian biotypes (group 3), which were characterised as having a robust growth habit, purple banding at the ligules, large seed size and a relatively long period between germination and flowering. Group 2 of the isoenzyme has many biotypes in common with those found within groups 1 and 3 of the AFLP analysis. Group 1 contained only African biotypes (Madagascar and two biotypes from Kenya), whereas group 3 incorporated biotypes from Africa, the Americas, PNG, Australia and the Philippines. The AFLP analysis again linked the Australian biotype with biotypes

from South Africa and Zimbabwe to which it appears almost identical. This supports an African origin for the introduction of this biotype into Australia. Additionally, this group comprised several biotypes from the Americas including three from Costa Rica and one from Louisiana, again implicating Africa with the Latin American introductions. The PNG biotype (PNG –1) also appeared in this group, as did a biotype from the Philippines (Philippines-1[a]). The Philippine biotype was a particularly aggressive biotype collected from trial plots located at the International Rice Research Institute (IRRI). This biotype was separate from all of the other Asian biotypes in the cluster analysis and therefore may represent an introduction into the Philippines. The weed was most likely to have entered the Philippines as part of a contaminated rice seed shipment, and there is recent evidence to support this claim (Hulma *et al*, 1996).

Group 3 of the isoenzyme analysis contained biotypes from Thailand, Colombia and India. This indicates that the introduction to Colombia may have been via Asia. The AFLP data concurs with this result as Group 5 which is predominantly Asian, also included a Colombian biotype. The last group of the isoenzyme cluster analysis (group 4) contained a single biotype from India (India 1[a]). This Indian biotype was morphologically quite distinct from the rest of the Asian biotypes having relatively small seed and no purple banding at the ligules. This biotype was also identified by the AFLP cluster analysis (group 8) as being different from the other Asian biotypes having a greater number of polymorphism.

The AFLP analysis also had six biotypes predominately of Asian origin, which fell outside the 92% similarity cut off point, used to distinguish between the major groups. However, the biotypes from the Philippines (Philippines–2[a]), Brazil and Sri Lanka (Sri Lanka–1[b]), groups 9, 10 and 11 respectively clearly formed part of the main Asian group and had 87% of their bands in common. The inclusion of Brazil with this group points to an Asian introduction along with the Colombian biotype. Indeed, the Colombian biotype was reportedly introduced into Brazil in 1961 in a shipment of rice (Millhollon & Burner, 1993). The introduction was further supported based on the similarity between the Brazilian and Colombian biotypes in terms of chromosome number (2n = 60), response to day length and purple banding at the ligules (Millhollon & Burner, 1993.)

The Costa Rican biotype(Costa Rica–7) of group 6 was sufficiently different to be placed in a group of its own and may be the result of a separate introduction or an example of rare outcrossing event. The Philippines (Philippines-3[b]) biotype of group 12 was the most genetically distant of all the biotypes having 82 % similarity with the other biotypes. This was perhaps not unexpected since this biotype was morphologically quite different to the other biotypes found in the Philippines and elsewhere. This biotype was typically found above (900m) and had a prostrate creeping habit, frequently forming spreading colonies on steep banks.

The characterisation of *R. cochinchinensis* biotypes has historically concentrated on those selected from within individual countries. For example, in the Philippines, at least five biotypes have been reported (Pamplona, & Mercardo, 1981a; Pamplona & Mercardo, 1981b; Pamplona. & Mercardo, 1982), while in Louisiana two geographically separate biotypes have been described. (Millhollon, 1982). In both cases the biotypes were differentiated according to their flowering and tillering responses to daylength, and on the morphological and physiological characteristics of each biotype. The two Louisiana biotypes have additionally been shown to differ using isoenymic techniques (Fisher *et al*, 1987). Similarly seven

biotypes have been described from Costa Rica based on their isoenzymic differences. (Le Paz, 1995).

A more global perspective was taken by Millhollon & Burner (1993) who looked at biotypes gathered from 34 countries or territories. The biotypes were divided into five broad groups based primarily on the effect of day length on flowering, but also on general morphology and pattern of growth (Table 20).

Table 20. Characterisation of itchgrass biotypes based on flowering under two controlled day lengths in the greenhouse**.**

Group 1:	Group 2:	Group 3:	Group 4:	Group 5:
Argentina	Australia	Brazil	Cuba-2[c]	Indonesia[e]
Bolivia	Kenya-1	Colombia	Dominican Republic	Kenya-2
Costa Rica	Reunion	Jamaica	Eduador-2[c]	Liberia
Cuba-1	South Africa	Philippines-2	Honduras-2[c]	Nigeria
Ecuador- 1	Zambia		India	Sudan
USA (Florida)	Zimbabwe		USA (Louisiana-2)	Tanzania
Guadeloupe			Malaysia[d]	Trinidad
Honduras-1			Papua New Guinea-2[c]	
USA (Louisiana-1)			Puerto Rico	
USA (North Carolina)			Taiwan	
Papua New Guinea-1			Thailand[d]	
Panama				
Peru				
Philippines				
Venezuela				

Biotypes were grouped according to day-length response and morphology (adapted from Millhollon & Burner, 1993).

[c]Seed of biotype 2 occurred in a mixture with seed of biotype 1.
[d]Plants were of two kinds: those with or without purple bands at the ligules.
[e]Plants were of two kinds: those with or without brown bands at the ligules.

Direct comparisons between the groupings proposed by Millhollon & Burner and those made using electrophoretic techniques were complicated by the fact that the seeds used in each study were not from a common source. This presents problems in interpreting the biotypic differences, as a number of the countries used in the studies are reported as having more than one biotype of the weed. Moreover, the characteristics used to separate the biotypes of *R. cochinchinensis* have frequently been based on subjective morphological differences, which are influenced to a greater or lesser extent by the environment. *R. cochinchinensis* is plastic in its response to environmental changes, therefore, many of the differences observed may be due to phenotypic plasticity modifying the mode of growth and energy allocation of the plant, rather than to genetic variations. Indeed plasticity of development is considered to be an important adaptive mechanism in many weedy plants (Oka & Morishima, 1982). Nevertheless, there was considerable agreement between the results of the studies where geographically similar biotypes were used.

Thirteen out of the fifteen biotypes belonging to Group 1 of the day length study were also from North, Central or South America. These biotypes were not affected by day lengths of 12 and 14 h (day length neutral) and flowered rapidly (35 to 57 days after germination). The

similarity of this Latin American group to those identified by either AFLP or isoenzyme techniques gives credence to the hypothesis that the Latin American biotypes may have arisen from a limited number of introductions. Further evidence for the similarity of these biotypes is provided by the fact that these countries have only diploid biotypes with 2n =20 chromosomes (Millhollon & Burner, 1993). Also included in group 1 of the day length analysis is a biotype from the Philippines perhaps linking Asia with the origins for this group. However, this evidence is contradictory to the AFLP cluster analysis, which strongly links Africa with this group.

Group 2 of the day length analysis was almost entirely composed of biotypes from Africa with the inclusion of a single biotype from Australia. According to Millhollon & Burner (1993) these biotypes were somewhat different to those from the other areas sampled in that they lacked the relatively short, early flowering, day length neutral (ND) biotypes that were prevalent in North and Central America and also lacked polyploids. Again, similarities can be drawn with group 2 of the isoenzyme analysis and groups 2 and 4 of the AFLP. All three studies placed biotypes from South Africa and Zimbabwe with Australia, adding more weight to the argument that the Australian biotype was introduced from Africa.

Group 3 of the day length analysis contained biotypes from Brazil Colombia, Jamaica and the Philippines. This group had some biotypes in common with Group 3 of the isoenzyme analysis, which contained biotypes from Thailand, India and Colombia and also with group 5 of the AFLP cluster analysis that likewise contained biotypes from Colombia and India, but also a biotype from Sri Lanka. In all of the studies the Colombian biotype was consistently associated with Asian biotypes of the weed pointing to this being the centre of origin of this biotype. Indeed, Millhollon & Burner (1993) also considered that the Colombian biotype probably arose as a introduction from Asia, since the 2n = 60 chromosome number seems prevalent there.

Group 4 of the day length study was divided into biotypes from Central and South America and those from Asia. Biotypes in this group were short day plants that did not flower until 130 to 163 days after germination. This group had some similarities with group 4 of the AFLP analysis and Group 3 of the isoenzyme although neither of these studies linked Asia with these biotypes.

Group 5 of the day length study contained strict short day plants mainly from Africa. Most biotypes were diploid with 2n = 20 but some were polyploids 2n = 40, 2n = 60. The majority of these biotypes were not included in the either the isoenzyme or AFLP study but point to the diversity of chromosome number found within Africa, but lacking in the New World.

Overall, the analysis by various methods points to there being little variation within the species. The low levels of genetic diversity observed could be due to a high level of self-fertilisation taking place or, due to very recent global distribution of the plant. The results suggest that Africa is the most likely centre of origin of the Latin American biotypes. The exception being biotypes found in Colombia and Brazil that appear to have originated from Asia. The Asian biotypes appear to form a discreet group being genetically distinct from the African and Latin American biotypes of the weed indicating a greater degree of genetic diversity.

According to Millhollon & Burner (1993), most biotypes are diploid 2n = 20, including all those found in North America the majority from Central and South America and Africa. The

six Indian selections were tetraploid (2n = 40), while those from Brazil, Colombia, Thailand, Indonesia Taiwan and Philippines were hexaploid (2n = 60). In typical polyploid complexes in grasses, the diploids represent only a small proportion of the complex and are greatly outnumbered by more vigorous and better adapted polyploids (Stebbins, 1956). Such does not seem the case with *R. cochinchinensis,* indicating that the complex may be of recent origin.

Rottboellia cochinchinensis is predominately an inbreeder and rarely recombines genes with other individuals. Despite these restraints, considerable variation may occur within a natural population of a strongly inbreeding or apomictic species (Allard *et al,* 1968; Harper, 1977). In some circumstances, however, such plant populations may show an extremely depauperate genetic structure (Burdon *et al*, 1980). This is particularly true of weed species that have colonised new habitats through accidental or deliberate movement of a few seeds by man. Populations which arise from such small inputs, almost invariably show considerably less genetic variation than that found within the naturally occurring populations of the same species (Harper, 1977), because of so called 'genetic bottleneck' or 'founder' effects (Mayr, 1963).

The importance of genetic diversity in protecting plant species against parasite attack has been widely recognised and recently summarised by (Burdon *et al*, 1980). This suggests that where introduced weeds successfully establish large-scale monocultures that are genetically uniform, these plants might be easier to control biologically than species that exhibit a wider genetic base. Indirect evidence for this was provided by Burdon *et al* (1980) who compared the average degree of control achieved using natural enemies with the predominant means of reproduction of the target species. The analysis showed that plants which reproduced primarily asexually (assumed to have a narrower genetic base) have been controlled more often than those that reproduce sexually. Burdon concluded that the greater effectiveness of biological control agents against asexually reproducing species might be due to their greater genetic homogeneity particularly after dispersal over long distances, and/or their limited capacity to evolve resistant strains when heavily attacked by natural enemies.

The AFLP analysis points to the weed having a rather narrow genetic base, particularly within Latin America, and suggests that the smut should be capable of infecting the majority of the biotypes. Costa Rica has been proposed as the country for the New World release of the smut. The AFLP analysis placed the Costa Rican bitotypes within 4 groups, however, the majority (71%) of the biotypes formed just two groups. This is in contrast to that reported by Rojas *et al* (1992) who distinguished at least 7 ecotypes in Costa Rica based on their morphology, and by La Paz (1995), who also identified 7 biotypes of the weed using isoenzymic techniques. It is unclear why there should be such a large number of biotypes of the weed reported from Costa Rica, especially as the founder populations would most likely have originated from a relatively small number of introductions as part of contaminated seed shipments, thereby limiting the genetic diversity. It is likely, therefore, that much of the diversity reported is due to phenotypic expression of environmental conditions rather than to true genotypic differences.

Rottboellia cochinchinensis is well established in Central America, Islands of the Caribbean sea, and the northern and north-western coast of South America. These infestations are potential reservoirs for introductions into other regions particularly Mexico, the United States, and South America, particularly Brazil.

One of the most important factors determining the weediness of the biotype is the time taken from germination to flowering. The Latin American biotypes have a relatively short time to flower and are usually more aggressive in annual cropping systems since the weed typically flowers before the crop is harvested and therefore can shed considerable numbers of seed. Polyploid biotypes such as those in India and Thailand, which take longer to flower and are not usually reported as pests of annual cropping systems, are more suited to perennial crops.

It is the close links that *Rottboellia* shares with other economically important crop species within the *Andropogoneae* that make it such a difficult weed to control using chemical herbicides. Herbicides tend to target a single enzyme or step within a biological pathway in order to achieve control. However, these chemicals often lack the specificity needed to differentiate between species or even genera within the same family. A classical biocontrol agent, such as the smut, does offer the necessary host specificity needed to target *Rottboellia.* The smut does not rely on a single enzyme or pathway for its pathogenicity, but instead utilises a broad spectrum of mechanisms of action to overcome the hosts defences, such subtle host-pathogen interactions have developed over thousands of years of co-evolution.

Research additional to original PMF

Light microscopy and SEM studies of the modes of infection of the smut *S. ophiuri.*

Microscopic investigation of plant material inoculated with a teliospore suspension of S. ophiuri. Since the overwhelming majority of smuts have been shown to be heterothalic, requiring fusion between haplonts of opposite mating types, fusion, and the establishment of the dikaryophase is essential for the subsequent infection of the host (Holton, *et al*., 1968). Infection is by means of the dikaryotic mycelium which can be initiated by either fusion between sporidia; mycelium; branches of the promycelium; or nuclei.

Germination of teliospores on the coleoptile of *R. cochinchinensis* was rarely observed, and when it was, it did not usually result in penetration of the host. The promycelium emerged from the teliospore in a similar manner to that observed on solid agar, but relatively few sporidia were produced compared to the proliferation observed on nutrient agar.
The paucity of sporidia formed by teliospores germinating on the host tissues has been noted for many other smut fungi (Fischer & Holton, (1957); Falloon, (1979); Mills & Kotze, (1981). The behaviour of *S. ophiuri* teliospores germinating on *R. cochinchinensis* was similar to that observed in water cultures, where the promycelium cells germinated and gave rise to long unbranched hyphae with retraction septa in the old, exhausted regions. This response might be an abnormality due to the germination conditions, or may be a prelude to the fusion and plasmogamy of promycelium cells. The trigger for the germination of the promycelium cell may be low levels of nutrients or host exudates.

Growth of the promycelium on the coleoptile epidermis was frequently occurred across the grooves formed by the epidermal cells. Direct teliospore penetration of the host was seen in one instance. The teliospore germinated and produced a short promycelium, close to the junction of an epidermal cell. Penetration of the host's cells appeared to be via the apical cell of the promycelium, which directly penetrated the wall of the epidermal cell without the formation of any specialised penetration structure. A halo of host material was seen around the penetration peg, that was swollen and creased at the point of entry. The halo of host material is analogous to the collar-like structure seen during the penetration of the host's cells viewed under the SEM. No intracellular mycelium was observed in this instance. However,

in other samples viewed 7 days after inoculation, intracellular mycelium were observed in the outermost layers of the stained coleoptiles.

Because infection appears to be initiated without sporidial fusion, it is possible that extensive sporidial growth only occurs under optimum conditions and as a response to relatively high nutrient conditions. The nutritional requirements for the prolific multiplication of sporidia, therefore, are presumably better fulfilled in culture than on the host. (Falloon, 1979).

Good evidence of the direct penetration of the host via a germ tube was provided using the SEM. Penetration of the host occurred after the production of a relatively short germ tube. At the tip of the germ tube, a protuberance was produced that appeared to be two lobed, this structure, although somewhat distorted, could represent an appresorium. Directly beneath this lobed swelling, there was evidence of the host cell wall being disturbed, possibly by a penetration peg. The hole formed by this peg appeared to be bordered by a lip or collar of host material closely adhering to the fungal structure. The penetration hypha was of a smaller diameter (1.5 µm) to that of the germ tube (3µm). It was collapsed, except for the last third, indicating that the cytoplasmic contents had been pushed out into the penetrating tip, which suggests that penetration may be by mechanical as well as chemical means.

These results agree broadly with other studies in which teliospore penetration has been closely followed, although some disagreement exists whether penetration occurred by mechanical means , or through small wounds. Mills (1996), using light microscopy, described a similar penetration event in the loose smut (*U. avenae*) of oats. Penetration of the epidermal cell wall was also from the apical cell of a promycelium. The penetration hyphae were reported as being sheathed by material derived from the cell wall of the host, which was probably invaginated. The penetration hole was 4µm in diameter, and bounded by a prominent lip. Likewise Walter (1934) reported that teliospores of *U. maydis* form short germ tubes which, in their growth along the leaf surface, favour the troughs between epidermal cells. In these depressions the germ tubes bulge, flatten, and press closely to the epidermis, and infect the maize leaf through direct mechanical penetration. In contrast, Mills & Kotzé (1981) demonstrated in an SEM study, that germinating teliospores of *U. maydis* infected maize leaves through the stomata and wounds, and not by direct mechanical penetration.

Direct penetration of the host via teliospores is a relatively unusual mode of infection, because the majority of smuts undergo a haploid sporidial phase, with fusion and dikaryotisation before infection can occur. Perhaps in the case of *S. ophiuri*, meiosis and dikaryotisation takes place within the teliospore or germtube prior to infection. This occurs in *Ustacystis walsteiniae* (Peck) Zunde, the diploid nucleus undergoes meiosis in the spore at the time of germination, although no fusion takes place between sporidia or promycelial cells, all vegetative development is dikaryotic.(Hanson & Atkinson, 1938). Similarly, for many Ustilaginaceous smuts which germinate directly, there probably is no haplophase, and as no promycelia develop in these species, the dikaryon is probably formed in the first vegetative hypha which develops at the time of spore germination.

The smut fungi *Ustilago nuda*, the cause of loose smut of wheat and barley, has another variation in the way in which it penetrates the host's cells. This species produces no sporidia, but on germination a promycelium develops and becomes septate, the dikaryophase is established by the fusion of germ tubes derived from individual uninucleate cells of the promycelium (Malik, 1974). *Sporisorium ophiuri* also appears capable of establishing the dikaryon through the fusion of promycelium cells. Falloon (1979) also noted that sporidia

may not be essential for successful infection of the host. He observed that penetration of the host coleoptile of *Bromus catharticus* Vahl by *U. bullata* was sometimes achieved by dikaryotic hyphae. These hyphae were formed as a result of plasmogamy between promycelium cells, and in some cases, growth of dikaryotic hyphae began without any apparent external connection between cells.

Microscopic investigation of sporidial fusion from water cultures. For smut genera that are capable of producing the haplophase, sporidia are considered to be the main infective propagule. However, for many smut fungi, even those of agronomic importance, the sequence of microscopically observable events that occur during mating of haploid sporidia have often not been well-characterised or illustrated

Sporidial mating in *S. ophiuri* appears similar to that described for *U. violacea* (Pers.) Rouss. (Day & Jones, 1968), *U. maydis* (DC.) Cda., (Snetsellar & Mims, 1992), and for many other fungi (Fisher & Holton, 1957), but was difficult to document. Pairs of fused sporidia were scarce in all cultures. The best results were obtained using the method described by Snetsellar & Mims (1992), where shallow layers of liquid were incubated in closed dishes. Perhaps these conditions more closely resemble the thin liquid films found around soil particles where presumably mating and infection normally take place.

When two compatible lines of haploid sporidia were mixed in water, mating was seen after 16 hours. Each pair of sporidia was joined by a conjugation tube that was frequently coiled. However, mating of sporidia was sometimes seen between three, or occasionally four sporidia. The cytoplasmic contents of the fused sporidia moved out from the paired sporidia into the conjugation tube, and then into the developing infection hyphae, which emerged from either the conjugation tube or one of the sporidia. The sporidia in control cultures, inoculated with single haploid isolates, did not fuse in pairs, as they did in the mated haploid cultures, instead their growth remained yeast-like, although some abnormal morphologies were observed after extended incubation.

The majority of sporidia viewed using the SEM continued to bud from the polar ends of each cell. However, on occasions, fused sporidia were observed. Each pair of sporidia was joined by a short conjugation tube. The infection hyphae were produced from either the conjugation tube or one of the sporidia. These observations confirmed those made when using the light microscope.

Confirmation of mating was obtained from mating tests conducted on Holiday's Complete Medium with the addition of 1% activated charcoal (HCM+C) (Holiday, 1974). Crosses of strains with opposite mating type resulted in the formation of white aerial mycelium. The number of nuclei formed in sporidial, and hyphal cells, obtained from the surface of (HCM+C), confirmed that all sporidia contained one nucleus while the hyphae were found to be dikaryotic.

In conditions where nutrients are limited, *S. ophiuri* switches from sporidial production to the formation of swollen thick walled hyphae or chlamydospores. The chlamydospores are formed from terminal or intercalary segments of the mycelium which become packed with food reserves, and develop thick walls. Many groups of fungi form chlamydospores which act as important organs in the asexual survival of fungi, especially soil fungi (Webster, 1980) Chlamydospores formed by *S. ophiuri* also offer a survival mechanism as the fungus would die if it did not encounter its host or sufficient nutrients to support the proliferation of

sporidia. Generally there is no mechanism for the detachment and dispersal of chlamydospores, but they can become separated from each other by the disintegration of the intervening hyphae. The chlamydospores, once transferred to nutrient-rich agar, germinated in a manner similar to that of the teliospores, producing septate hyphae on which sporidia were borne.

Variations abound within the smut fungi, in both their morphology and physiology. Physiological variation involves all phases of the smut life cycle, including nutritional requirements, spore germination, compatibility relationships, nuclear behaviour, and pathogenicity. S. *ophiuri* may be capable of infecting its host by either teliospores or sporidia. The relative importance of each method of infection within the life history of the smut has not been fully elucidated and may be dependent upon the environmental conditions..

Longevity of teliospores under different soil moisture conditions.

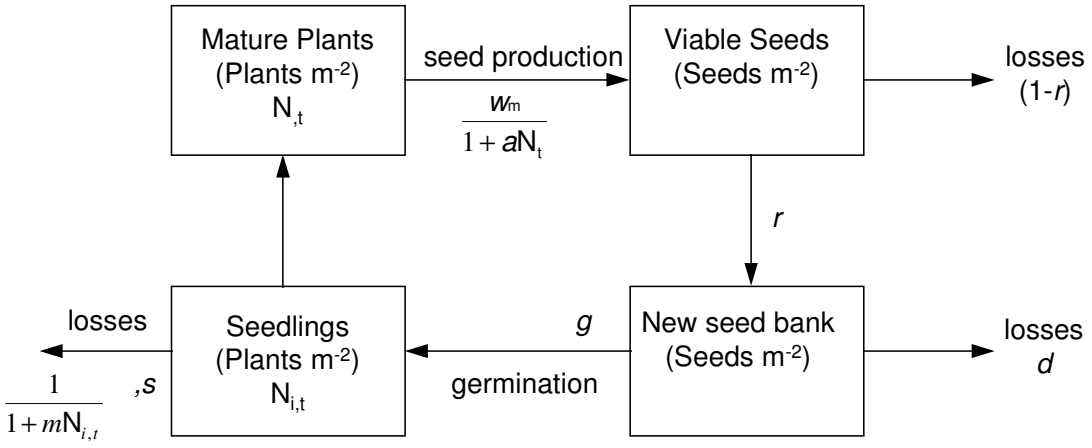
This work is still ongoing and will in the future be published in international scientific journals.

8. A model to predict the long term effect on the weed populations, of the control strategies recommended, and the likelihood of successfully integrating the classical biological control agents into the system developed.

Model I: A model of itchgrass population dynamics in a maize cropping system in Guanacaste, Costa Rica

The model is deterministic with transition probabilities defining the proportion of itchgrass plants surviving from one life stage to the next (Fig. 18). The proportion of plants surviving from seedlings to the flowering stage, and seeds set per plant were density dependant i.e. decreased with density. The model was essentially similar to that in Smith *et al*. (1997), but with some adaptation to allow for the annual crop/crop/fallow cycle. A seasonal (four monthly) time interval was used instead of the annual one in Smith *et al.* (1997). There are two generations of itchgrass each year; but in the third season each year, which is dry, there is no germination of itchgrass seed. Itchgrass plants did not survive between seasons in the model. Life cycle characteristics in the two cropping seasons were considered to be similar to each other in this model. Smut infection was simulated as a constant proportion of itchgrass plants. The effect of a leguminous cover crop sown at two different densities was simulated as a reduction in itchgrass seed germination, seedling survival, and seed set per plant (derived from Valverde *et al.*, 1995). A pre-emergence herbicide application was modelled by assuming a second flush of itchgrass of lower competitive ability with the maize (potential competitive interactions were considerably simplified for the model).

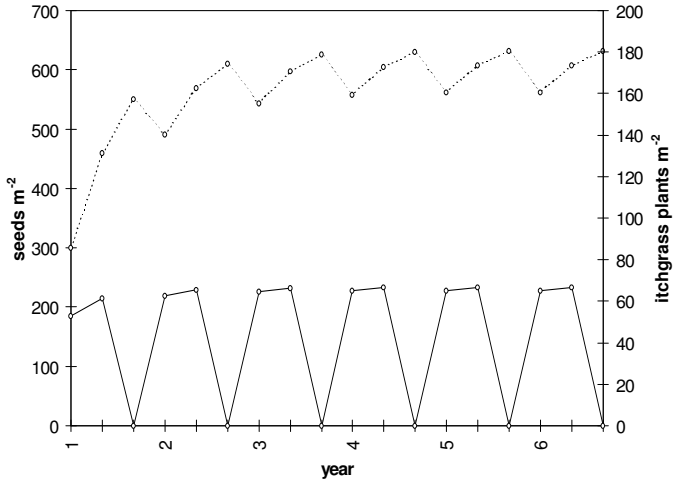
Figure 18. Structure of the model of the itchgrass life cycle showing flows between the state variables [seedbank; itchgrass seedlings (m^{-2}) ($N_{i,t}$); itchgrass plants (m^{-2}) (N_t); seed production (m^{-2})] and parameters [proportion of seed germinating from the seed bank each season (g); proportion of seed lost per season (d); density independent seedling mortality rate (s); degree of density dependence in seedling survival (m); seeds produced per plant in the absence of competition (w_m); degree of density dependence in seed production (a); proportion of seed rain incorporated into the new seed bank (r)].



Where possible parameter estimates were taken from data collected on itchgrass in Costa Rica (Appendix I). Some data on density dependence in seedling mortality and seed set was kindly collected by colleagues at CATIE at a late stage in the project and hence itchgrass densities given here, though not conclusions, may differ from those presented in the PCSS. No yield loss curves have been calculated for itchgrass in maize, although there are a number of yield

loss estimates in various countries. Percentage yield loss in maize, was assumed to follow a rectangular hyperbola with increasing weed density (Cousens, 1985). The curve was parametrised to fit the combined data from the literature. Several different parametrisations of the yield loss curve due to itchgrass and the cover crop were used (Appendix I). Scenario I is identical to that with no cover crop. In scenarios II-IV yield loss due to the cover crop and yield loss per itchgrass plant were varied. Itchgrass plant and seed bank densities approached levels which were constant for a given season after only 5 or 6 years (Fig. 19). Average itchgrass densities and associated yield losses were obtained from model runs for a range of control measures (Table 21).

Figure 19. **Final itchgrass count (transformed to natural logarithms) against initial seedling count. Guanacaste data: data points open circles, solid line fitted curve. Parrita data: solid symbols and dashed line.**



.

Table 21. Estimates of densities of mature itchgrass plants and yield losses under various combinations of control measures. Itchgrass densities are the average over the 10 cropping seasons in years 10-14. The same control measures were applied each season except for the last two scenarios where it was assumed that the cover crop was used in the first season of each year only. A range of yield losses were calculated for control combinations involving cover crops under four yield loss scenarios

Combination of controls used				**% yield losses under scenarios I-IV**			
cover crop (low or high density, see Table 21)	**% smut**	**pre-emergence herbicide ("y" if used)**	**itchgrass density (plants m^{-2})**	**I**	**II**	**III**	**IV**
no itchgrass control			65.9	52.3%	___	___	___
Low			14.1	19.2%	33.5%	22.7%	33.6%
High			2.6	4.2%	27.5%	16.5%	19.2%
	25%		60.2	50.1%	___	___	___
	50%		50.9	45.9%	___	___	___
	75%		32.7	35.5%	___	___	___
		Y	60.8	45.6%	___	___	___
Low	50%		2.1	3.4%	27.2%	16.2%	18.4%
High	50%		0.1	0.2%	26.1%	15.1%	15.2%
Low		Y	0.0	0.0%	26.0%	15.0%	15.0%
High		Y	0.0	0.0%	26.0%	15.0%	15.0%
	50%	Y	35.6	32.7%	___	___	___
low (first crop each year only)			48.5	44.8%	45.7%	40.6%	50.5%
high (first crop each year only)			43.0	41.9%	43.6%	38.5%	47.9%

The effects of periodic complete failure of the cover crop, or of periodic 100% maize yield loss due to smothering by the cover crop were investigated for the higher density cover crop. Results suggest (Table 22) that one failure in ten seasons resulted in only a slight increase in % maize yield lost. Under scenarios II and III the effect of one failures in five seasons also resulted in quite a small loss in yield relative to successful cover crop control in all seasons. However under scenarios I and IV the resultant decreases in yield were quite large. The average yield losses when the cover crop smothered the maize one season in ten or one season in five, were higher under all scenarios than when cover crop control failed in the equivalent number of seasons.

Table 22. Estimated average maize yield losses under scenarios I-IV, under situations where **a.** the cover crop fails completely, or **b.** where the cover crop overruns the maize crop. For **b.** the cover crop was assumed to control itchgrass to the usual density but cause 100% yield loss in years when it overruns the crop. Parametrisation is for the high density cover crop in both cases. Predicted yield losses for the high density cover crop applied each season were 4.2%, 27.5%, 16.5% and 19.2% under scenarios I-IV respectively.

high density cover crop	**I**	**II**	**III**	**IV**
successful in all seasons	*4.2%*	*27.5%*	*16.5%*	*19.2%*
a. **frequency of cover crop failure**				
1 year in 10	10.1%	27.7%	17.9%	23.3%
1 year in 5	20.1%	31.9%	23.2%	31.5%
1 year in 2	41.9%	43.6%	38.5%	47.9%
b. **frequency of cover crop overrunning maize crop**				
1 year in 10	13.8%	34.8%	24.9%	27.2%
1 year in 5	23.4%	42.0%	33.2%	35.3%
1 year in 2	52.1%	63.8%	58.3%	59.6%

The value of the increased maize yield to the farmer over no control , was predicted (Table 23) on the basis of a grain yield of 4000 kg ha^{-1} in an itchgrass free plot selling at 0.4$\$kg^{-1}$, giving 1600 $\$ha^{-1}$. These gains were compared with estimated financial costs and human inputs required for the control measures (Table 24). Except under yield-loss scenario I, financial returns on a second control method on top of a high density cover crop were very low, too low for a pre-emergence herbicide to be a financially viable control method. For herbicide on top of a low density cover crop yield benefits were ~$100 ha^{-1} which would make it a financially viable addition.

Table 23. Estimated financial gains to the farmer in years 10-14 of various combinations of control measures. Gains are those over no control assuming maize grain yields of 4000 kg ha^{-1} from itchgrass free plots selling for 0.4$ kg^{-1}.

combination of controls used			**financial gain ($ ha^{-1}) over no control**			
cover crop (low or high density, see Table 21)	**% smut**	**Pre-emergence herbicide ("y" if used)**	**I**	**II**	**III**	**IV**
low			529	300	474	299
high			769	396	572	530
	25%		35	___	___	___
	50%		101	___	___	___
	75%		268	___	___	___
		Y	106	___	___	___
low	50%		782	401	577	542
high	50%		833	419	595	593
low		Y	836	420	596	596
high		Y	836	420	596	596
	50%	Y	313	___	___	___
low (first crop each year only)			120	105	186	28
high (first crop each year only)			166	138	221	69

Table 24. Costs and labour time associated with some itchgrass control measures: a Mucuna cover crop and a pre emergence herbicide application (Francisco Fonseca pers. comm.).

	Cost ($ ha^{-1})	**Labour (hours ha^{-1})**
cover crop		
seed*	35	
Sowing		50
cutting		7
total**	**3.5**	**71**
biological control	unknown but small to the farmer if applied as a classical biological control agent	unknown but probably low for the farmer if applied as a classical biological control agent
Herbicide		
Chemical	44	
Tractor	3	
Application		11
Total	**47**	**11**

* Farmers should be able to harvest their own *Mucuna* seed at the end of the second cropping cycle so this investment should only need to be made once.
**Total includes time and money invested in cutting back the cover crop three times per season; 1/10th of cost of purchase of cover crop seed since only one purchase is required over the 10 cropping seasons simulated.

Conclusions from Model I

a) When uncontrolled, itchgrass densities were estimated to reach 66 plants m^{-2} , averaged over the first and second crops each year.
b) The lower density of cover crop brought itchgrass densities down to 14 plants m^{-2}; and the higher level brought them down to 3 plants m^{-2}. Hence the cover crop appears to be a highly efficient means of itchgrass control.
c) Simulation of a single pre-emergence herbicide application suggested this control alone would be ineffective (as expected). The head smut was only effective at high levels of infection: 75% infection rate was required to reduce the itchgrass density to 33 plants m^{-2}.
d) However simulations suggest that one of these methods in combination with a cover crop could be highly effective. A low density cover crop plus 50% smut infection rate resulted in 2 plants m^{-2} in each crop. Simulations of a low density cover crop plus herbicide, or a high density crop plus smut or herbicide all resulted in ≤0.1 itchgrass plant m^{-2}.
e) Under the various yield loss scenarios (Appendix I) no control of itchgrass resulted in 52% yield loss reduced to 19-34% by a low density cover crop or 4-27% by a high density cover crop. These results suggest that even a cover crop which induces a considerable maize yield loss could be of benefit over no control, if it is effective at surpressing itchgrass.
f) The yield gain from a second control measure on top of the cover crop was in most cases relatively modest, an argument for a relatively cheap secondary method of control. In this model the value of the yield increase resulting from a pre-emergence herbicide in addition to a high density cover crop was not enough to cover the cost of the herbicide.

g) Comparing yield losses resulting from high and low cover crop densities, and the different yield loss scenarios, suggest that in the long term the best return may be from a lower density cover crop giving an intermediate level of itchgrass control, plus a cheap additional control. This could potentially be the smut biological control agent. This scenario is likely if the higher density cover crops result in relatively high maize yield penalties.
h) Using the cover crop in the first crop only each year was considerably less effective. For the low density cover crop itchgrass densities were still 48 plants m^{-2} and 43 plants m^{-2} for the high density cover crop. The above results also simulate the effect of a cover crop planted, but failing every other season, suggesting that it is worth taking some risk of yield loss (but not being over run by the cover crop) due directly to the cover crop than to risk frequent failure of the cover crop and an itchgrass epidemic resulting.
i) Timing of planting of the cover crop is important so that it surpresses itchgrass but does not over compete with the maize crop. Simulations of complete failure of the cover crop in one or two seasons out of ten still resulted in a considerably improved average maize yield over no control. Likewise when 100% maize yield loss was assumed one year in ten, due to smothering of the maize by the cover crop. More frequent mis-timings of cover crop sowing resulted in considerable average maize yield losses.

Model II

The model was modified from that described in that for a previous itchgrass modelling project under the CPP (A0358,R6003). The approach, and assumptions regarding the itchgrass life history are the same as the "mycoherbicide" model (Model I) but the smut population dynamics were modelled in a very simple way by varying the amount of smut infection depending on smut spore density. The probability of smut infection is random i.e. follows a Poisson distribution. A constant proportion of these spores die annually, and a number are added depending on Itchgrass population density, and smut infection rate. This is a very simplified view of the smut life cycle. In fact germinated teliospores produce sporidia, these fuse in pairs, and it is the dikaryon that infects Itchgrass seedlings. It is possible that the saprophytic life stages may be able to survive and multiply in the soil. However, in view of the lack of knowledge regarding these parts of the life cycle, it seems best not to include them in detail at this stage. Transition probabilities were annual and itchgrass parametrisation was as Smith (1997) with the exception of relative competitiveness of smutted and unsmutted plants which was assumed to be equal. Parametrisation of the smut component was from data collected by CABI under previous projects (Ellison, 1993 and Mwijage, MSc project 1994).

Conclusions from Model II

a) Under the default parameter values the model came to equilibrium without oscillations and percent survival (that is the ratio of itchgrass densities at equilibrium under biocontrol v uncontrolled) was about 50%. Changing the parameter values for the smut showed that the smut could control the itchgrass to fairly low densities without inducing undamped oscillatory behaviour in the populations. The minimum % survival without undamped oscillatory behaviour was about 6.8% for parameters except when the smut component was parametrised with very high spore longevity. The latter could be increased until extinction of the weed without inducing undamped oscillations. The ability to reduce population density to a low stable equilibrium density is one of the requirements for a successful biological control system.
b) The effects of the same relative changes in the smut parameters spore production rate , infectivity and spore longevity was found to be identical . The greater these parameters the lower the resultant equilibrium itchgrass density. Hence in selecting a smut strain there

could be a direct trade off between infectivity, reproductive rate, and mortality rate in the soil.

c) The model showed equilibrium behaviour over a wider range of values of spore longevity than the other parameters and so might be the better characteristic to select for. Since the smut has a saprophytic phase in the soil the phase in the soil is not however as simple as represented here. The possibility of a positive growth rate in the soil was not considered.
d) The sensitivity anlayses also showed that the parameters defining the effects of competition on propagule production have a considerable effect on the predicted effectiveness of the smut. Assuming that competition is symmetrical it seems essential to the success of control that smutted plants are as competitive as unsmutted ones or nearly so. However a lower intensity of intraspecific competition could result in more effective control since both seed and spore production are affected.
e) Of the parameters defining the itchgrass life cycle(germination rate, seedling survival and seed production rate) all appeared to have similar equilibria for the same proportionate change from the default. The behaviour of the model was very stable, coming to equilibrium over a wide range of these parameter values. Decreasing these parameter values below the default itchgrass parameterisation resulted in the smut becoming less effective in terms of itchgrass percent survival (that is the ratio of itchgrass densities at equilibrium under biocontrol v uncontrolled). All of these parameters are reduced when itchgrass is controlled by a *Mucuna* cover crop. Therefore although the smut could give some additional control on top of a cover crop, the more effective the cover crop control, the less effective the smut. This suggests that the idea of initial releases of the smut in irrigation ditches and areas where itchgrass control is otherwise very poor is probably a good one.
f) The above results contradict model I, and therefore the manner of employing the smut (as a classical biological control agent or where smut levels are supplemented by some means) will affect the success of the interaction with other control methods.
g) The model suggests that equilibrium is most rapidly achieved when the smut is applied to an itchgrass infestation which has already reached a high density, rather than early on in an infestation. Crude results suggest that there is an optimum initial dose which is large enough to have an immediate effect on itchgrass densities but not so large that both organisms are temporarily reduced to levels close to extinction.

References

References for mycoherbicide work

Bustamante, E.; Sanchez, V.; Gamboa, A. 1993. Reconocimiento de patgenos de la caminadora (Rottboellia cochinchinensis) en Costa Rica. In Congreso Nacional de Fitopatologia (2, 1993, San Jos, C.R.) Memoria. San Jos, C.R. Asociaci n Costarricense de Fitopatologia. p 26.

Fuentes, G.J.; Bustamante, E. 1996. Severidad de pat genos de semilla de Rottboellia cochinchinensis en dos suelos de differente fertilidad y desinfecci n. Manejo Integrado de Plagas (Costa Rica) 39.

Jiménez, J.M., E. Bustamante, R. Gómez & M. Pareja (1990) La pudrición de la espiga de la caminadora, *R. cochinchinensis*, su etiologia y posible uso como agente de combate biológico. *Manejo Integrado de Plagas (Costa Rica)* **15:** 13-23.

Lenne, J. (1990) A world list of fungal diseases of tropical pasture species. Plant Pathologist Tropical Pastures Program. CIAT, Cali, Colombia. P.18

References for classical biological control work

Allard R. W., Jain S. K., & Workman, P. L. (1968). The genetics of inbreeding populations. *Adv. Genet*. **14**: 55-131

Antoine, R.(1961). Smut. In *Sugarcane Diseases of the World*. Martin, J. P., Abbott, E. V. Hughes, C. G. (eds.). Elsevier Publishing Co. Amsterdam, Netherlands **I**: 327-345.

Barret, S. C. H. (1982). Genetic variation in weeds. In*: Biological Control of Weeds with Plant Pathogens.* Charudattan, R. & H. L. Walker (eds.). J. Wiley & Sons, New York: 73-79.

Beckett, A. & Read N. D. (1986). Low-temperature scanning electron microscopy. In *Ultrastructural Techniques for Microorganisms*. (Eds) H. C. Aldrich & W. J. Todd, Plenum Press, New York, U.S.A.: 45-86

Bridge, P.D., Pearce, D. A., Rivera A., & Rutherford, M. A. (1997). VNTR derived oligonucleotides as PCR primers for population studies in filamentous fungi. *Lett. Appl Microbiol* **24**: 426-430.

Bruzzese, E & Hasan, S. (1983). A whole leaf clearing technique for host specificity studies of rust fungi. *Plant Pathology* **32**: 335-8.

Burdon ,J. J.,Marshall, D. R., & Groves R. H., (1980). Aspects of weed biology important to biological control. *Proc. V. Symp. Biol. Control. Weeds. Brisbane. Australia.*: 21-29.

Cenis, J.L. (1992). Rapid extraction of fungal DNA for PCR amplification. *Nucleic Acids Research* **20**: 2380

Clayton, W. D. & Renvoize, S. A. (1996). In: *Genera Graminum Grasses of the World*.. Her Majesty's Stationery Office. Royal Botanic Gardens Kew. Kew Bulletin. Additional series XIII. p 321

Day, A. W. and Jones J. K. (1968). The production and characterisation of diploids in *Ustilago violacea. Genet Res.* **11**: 63-81.

Ellison C. A. (1987). Preliminary Studies to Assess the Potential of Fungal Pathogens for Biological Control agents of the Graminaceous weed *Rottboellia cochinchinensis* (Lour) W. D. Clayton. M.Sc. Thesis, Imperial College, University of London: 90pp.

Ellison, C. A. (1993) An evaluation of fungal pathogens for biological control of the tropical graminaceous weed *Rottboellia cochinchinensis.* Ph.D. Thesis, University London: 254pp.

Ellison, C.A. & H.C. Evans (1992). Present status of the biological control programme for the graminaceous weed *Rottboellia cochinchinensis*. *Proceedings of the Eighth International Symposium on Biological Control of Weeds*, 2-7 February 1992, Lincoln University, Canterbury, New Zealand (eds. Delfosse, E.S. & R.R. Scott). DSIR/CSIRO: Melbourne.

Falloon, R. E. (1979). Seedling and shoot infection of *Bromus catharticus* by *Ustilago bullata*. *Trans. Br. Mycol. Soc*. **73** (1): 49-56

Fischer, G. W. & Holton C. S. (1957). *Biology and Control of the Smut Fungi.* Ronald Press Co., New York: 622pp.

Fisher, H. H., Lopez, F., Margate, L., Elliot, P. & Burrill, L. (1985). Problems in control of *Rottboellia exaltata* L.f. in maize in Bukidnon Province, Mindanao, Philippines. *Weed Research*. **25**: 93-102.

Gómez Flores, M. A. (1985). *Rottboellia exaltata* L.f. Gramineae: Una Nueva Maleza en el Cultivo de Azucar en la Region del Papaloapan. Instituto para el Mejoramiento de la Produccion de Azucar, Centro Nacional de Investigaciones Azucareras. Cordoba Veracruz, Mexico: 11pp.

Hanson, E. W., Atkinson, R. E. (1938) Preliminary studies on the cytology of *Urocystis waldsteiniae*. *Phytopahology,* **28**, 8.

Harper, J. L. (1977). '*Population Biology of plants*.'(Academic Press: London)

Herrera, F. (1989). Situación de *Rottboellia cochinchinensis* en Costa Rica. In: *Seminario tallersobre* Rottboellia cochinchinensis y Cyperus rotundus. *Distribución, problemas y impacto ecónomico en Centroamérica y Panamá.* Proyecto MIP-CATIE, Honduras:pp.14.

Holliday, R. (1974). *Ustilago maydis.* In: *Handbook of Genetics*, Vol. **1**. R. C., King (ed.). Plenum Press, London.: 575-595

Holm, L.G. Plucknett, D.L., Pancho, J.V. & Herberger, J.P. (1977). *The World's Worst Weeds, Distribution and Biology*. University Press of Hawaii, Honolulu.:139-145

Holton, C. S., Hoffman; J. A. & Duran R. (1968). Variation in the smut fungi. *Annual Review of Phytopathology* **6**: 213-242.

Huelma, C. C., Moody, K. & Mew T. W. (1996) Weed seeds in rice shipment: a case study. *International Journal of Pest Management*. **42**: 147-150

Johnston, A., & Booth C., (eds) (1983). *Plant PathologistsPocketbook*. Commonwealth Mycological Institute, Kew, U.K.: 439pp.

Le Paz, M. G. (1995). Carcterizacion isoenzimatica de *Rottboellia cochinchinensis* en 11, poblaciones de diferentes de Costa Rica. Unpublished CATIE Report, Turrialba, Costa Rica

Lowry, O. H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**: 265-275.

Malik, M. M. S. (1974) Nuclear behavior during teliospore germination in *Ustilago tritici* and *U. nuda* . *Pakistan journal of botany*, **6**: 59-63.

Mayr, E. (1963). '*Animal species evolution*.' (Harvard Univ Press: Cambridge.)

Millhollon, R. W. & Burner, D. M. (1993). Itch grass (*Rottboellia cochinchinensis*) biotypes in world populations. *Weed Science*. **41**:379-387.

Millhollon, R. W.(1982). Reproduction variability in biotypes of Johnsongrass (*Sorghum halepense* (L.) Pers). and itch grass (*Rottboellia cochinchinensis*) interference on growth and yield of sugarcane (*Saccharum* spp. hybrids). *Weed Science*. **40**: 48-53.

Mills, L. J. and Kotzé J. M. (1981). Scanning electron microscopy of the germination, growth and infection of *Ustilago maydis* on maize. *Phytopathol. Z.* **102**: 21-27.

Mills, J. T. (1966). The development of loose smut (*Ustilago avenae*) in the oat plant with observations on spore formation. *Transactions of the British Mycological Society*. **49**: 651-663.

Mueller, U.G., Lipari, S.E. & Milgroom, M.G. (1996) Amplified fragment length polymorphism (AFLP) fingerprinting of the symbiotic fungi cultured by the fungus-growing ant *Cyphomyrmex minutus*. *Molecular Ecology*, **5**: 119-122.

Oka H., I. & Morishima, H. (1982). Ecological genetics and the evolution of weed. In: Holzner, W. and N. Numata, (eds.) *Biology and ecology of weeds*. Dr W. Junk Publishers, The Hague.: 73-89

Pamplona, P. P., &. Mercado, B.L (1981a). Ecotypes of *Rottboellia exaltata* L.f. in the Philippines I. Characteristics and dormancy of seeds. *Philippine Agriculturist*. **64**:59-66.

Pamplona, P. P., &. Mercado, B.L (1981b). Ecotypes of *Rottboellia exaltata* L.f. in the Philippines II. Response to day length and nitrogen application. *Philippine Agriculturist*. **64**: 371-378.

Pamplona, P.P. & Mercado, B.L. (1982). Ecotypes of *Rottboellia exaltata* L.f. in the Philippines. III. Competitive relationship with corn (*Zea mays* L.). *Philippine Agriculturist*. **65**: 395-402.

Paterson R. R. M. & Bridge, P. D. (1994). *Biochemical Methods for filamentous fungi*. IMI Technical Handbooks no.1. Wallingford: CAB international

Pimentel, D. (1961). Animal population regulation by the genetic feedback mechanism. *Am. Nat*., 95: 785-792.

Raeder, U. & Broda, P. (1985). Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1**: 17-20.

Rojas, C.E., A.Merayo & R. de la Cruz (1992) Determinación de posibles ecotipos de *Rottboellia cochinchinensis* (Lour.) W.D. Clayton) en varias zonas ecológicas de Costa Rica. *Manejo Integrado de Plagas* **24/25:** 22-25.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edition. New York: Cold Spring Harbor Laboratory Press

Smith, M.C. & Holt, J. (1995). Assessing the potential of head smut as a biological control agent of *Rottboellia cochinchinensis* flush using a simple model. *Proceedings of the Brighton Crop Protection Conference. – Weeds*: 219-24.

Smith, M.C., and Holt. J. (1996). Theoretical models for weed biocontrol using sterilising fungi. *Proceedings of the IX International Symposium on Biological Control of Weeds*, 19-26 January 1996, University of Cape Town, South Africa (eds. Moran V. C. & Hoffman J. H.): 319-325.

Smith, M.C., Reeder, R. H. & Thomas, M. (1997). A model of the biological control of *Rottboellia cochinchinensis* with the head smut *Sporisorium ophiuri*. *Journal of Applied Ecology*. **34**: 388-398.

Snetselaar, K. M. & Mims, C. W. (1992). Sporidial fusion and infection of maize seedlings by the smut fungus *Ustilago maydis. Mycologia*. **84**: 193-203.

Stebbins, G. L. (1956). Cytogenetics and the evolution of the grass family. *Am J. Bot*. **43**: 890-905.

Waller, J. M. (1970). Smut (*Ustilago scitaminea*) in Kenya II infection and resistance. *Transactions of the British Mycological Society*. **54**: 405-414.

Walter, J, M. (1934). The mode of entrance of *Ustilago zeae* into corn. *Phytopathology* **24**: 1012-1020.

Webster, J. (1980). *Introduction to fungi second edition* Cambridge University Press.: 669 pp.

References and data sources for modelling work

Cousens R (1985) A simple model relating yield loss to weed density. Annals of Applied Biology 107, 239-152.

Ellison CA (1993) *An evaluation of fungal pathogens for biological control of the tropical graminaceous weed* Rottboellia cochinchinensis. Ph.D Thesis, University Of London

Mercado BL (1978) Biology, problems and control of *Rottboellia exaltata L.f.*. A monograph. Biotrop Bulletin 14. 39 pp.

Rojas CE, de la Cruz R and Merayo A (1993b) Effecto competivo de la caminadora (*Rottboellia cochinchinensis* (Lour.) W.D. Clayton) en el cultivo del maiz (*Zea mays* L.) Manejo Integrado de Plagas (Costa Rica), 27, 42-45.

Rojas E, Merayo A and Calvo G (1994) La profundidad duracion en el suelo de la semilla de caminadora (*Rottboellia cochinchinensis* (Lour) W.D. Clayton) y su effecto sobre la viabilidad y persistencia en el tropico seco. Manejo Integrado de Plagas (Costa Rica) 32, 25-29.

Sharma D and Zelaya O (1986) Competition and control of itch grass (*Rottboellia exaltata*) in maize (*Zea mays*). Tropical Pest Management 32, 101-104.

Smith MC (1996) Final technical report :A model of biological control of *Rottboellia* (Itch Grass) with the head smut *Sporisorium ophiuri* (A0358, R6003)

Smith MC, Reeder RH and Thomas MB (1997) A model to determine the potential for biological control of *Rottboellia cochinchinensis* with the head smut *Sporisorium ophiuri*. Journal of Applied Ecology 34, 388-398.

Valverde BE, Merayo A, Rojas CE and Alvarez T (1995) Interaction between a cover crop (*Mucuna* sp.), a weed (*Rottboellia cochinchinensis*) and a crop (maize). Proceedings of the Brighton Crop Protection conference (Weeds), 1995. p197-200.

Appendix I

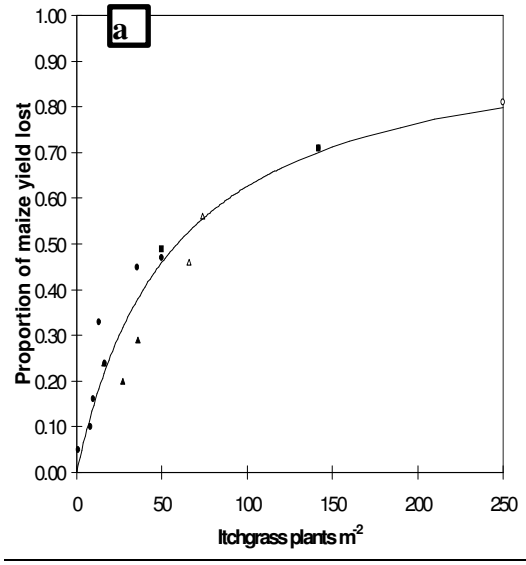
Parameter values and data sources for itchgrass population dynamics models

Parameter values for the itchgrass population dynamics model I and their data sources

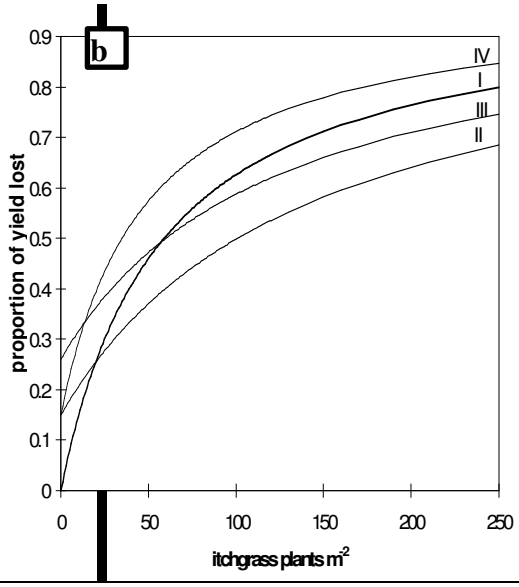
Symbol	Description	value	source
g	seasonal germination rate	0.43	reanalysis of seed burial experiment data described in Rojas *et al.,* 1994
M	degree of density dependence in seedling survival	0.01123	density dependence experiment conducted by Costa Rican collaborators under this project
s	density independent seedling mortality	00	density dependence experiment conducted by Costa Rican collaborators under this project
a	degree of density dependence in seed production	0.0557	density dependence experiment conducted by Costa Rican collaborators under this project
W_m	seed production per isolated plant	730	density dependence experiment conducted by Costa Rican collaborators under this project
r	proportion of seed rain incorporated into seed bank	0. 03	quadrat counts of seeds on soil surface by Costa Rican collaborators under this project
d	seasonal seed mortality rate	0.11	reanalysis of seed burial experiment data described in Rojas *et al.,* 1994

a Yield loss estimates in maize due to itchgrass. Estimates were taken directly from these sources or derived from maize yield and itchgrass densities in different treatments. Data sources: ●Richards and Thomas (1970),○ Sharma and Zelaya (1986), solid triangle Valverde et al (1995), unfilled triangle Rojas et al. (1993b), ■ Mercado (1978), solid line fitted rectangular hyperbola. **b** Alternative yield loss scenarios for models of itchgrass control involving a cover crop.

000000



000300



Contribution of Outputs

The original and additional outputs of the project have been consistently achieved. The contribution of the project to the goal of improving sustainability in high potential cropping systems by cost-effective reduction in losses due to pests, can be summarised as follows: Identification of suitable integrated itchgrass management tactics that substantially reduce the negative impact of the weed on the crop and that result in decreasing infestations over the longer term. Integration has been made possible by specific research aimed to improve our knowledge of the biology and importance of itchgrass and addressing the best combinations of planting density and timing of mucuna as a cover crop to allow for effective weed suppression while maintaining profitable grain yields. Interactions between the three key species of the production system (maize, itchgrass and mucuna) have been well characterised and demonstrated to leader collaborating growers in the seasonally dry Pacific region of Costa Rica. In addition, a mycoherbicide has been developed that may have potential for integrating in the overall control strategy. Importance and distribution of itchgrass has been determined in the areas where infestations were known or suspected to occur in the states of Campeche, Veracruz and Oaxaca, Mexico and, with less detail, in the maize producing areas of Santa Cruz, Bolivia. Information related to the biology and population dynamics of itchgrass under field conditions has been generated to improve existing models that address the long-term impact of management practices. Best management practices have been validated for seven cropping seasons (3 years) at farmers' fields where financial feasibility was fully demonstrated. Extension materials have been widely distributed in Mexico, Bolivia and Costa Rica and several publications in Spanish contribute to further project results dissemination throughout tropical America. Technical results have been disseminated according to proposed pathways to farmers, extension agents, and the scientific community.

The main aim of the CBC component of the research was to carry-out host specificity screening of the smut pathogen *Sporisorium ophiuri;* which unequivocally showed the smut to be specific to *Rottboellia cochinchinesis.* However, integral to this was the characterisation of both the biotypic variation of the weed, and the pathotypic variation of the smut. Both of these outputs are important in helping to establish the potential effectiveness of the smut, and in enabling the smut to be identified at a later date, i.e. once released in Costa Rica. The results of all this work are to be submitted as part of a dossier on the classical biological control (CBC) potential of the agent, to the Costa Rican plant health authorities, so they can assess the risk of introducing the smut into the New World. The introduction and successful establishment of the smut will potentially help in the management of *R. cochinchinensis* and thus lead to a reduction in the infestation and spread of this weed. Direct initial beneficiaries of the introduction of this pathogen are the maize farmers and their families in Costa Rica, who will benefit from reduced crop losses, reduced expenditure on herbicides, and more sustainable production. Indirectly, the same benefits can be expected throughout tropical America as a result of the dissemination and application of the results.

The modelling results confirm (in the absence of an ability to release the smut and test this directly) that a biocontrol agent which prevents seed production in itchgrass could have a significant impact on itchgrass populations. They also suggest that under a natural infestation of smut there can be a trade off between infectivity, reproductive rate, and mortality rate in the soil. The interaction of smut and cover crop depend on how they are modelled and hence on how biocontrol with the smut is implemented. Under a purely natural infestation, the cover crop affects smut reproduction as well as the itchgrass, and therefore reduces effectiveness of the smut. Models of natural infestations suggest that the interaction between the smut and

itchgrass is stable over a wide range of parameter values for the smut. The ability to reduce population density to a low stable equilibrium density is one of the requirements for a good biological control agent.

Publications:

ELLISON, C.A. & EVANS, H. C (1998). Classical biological control of weeds with pathogens in Latin America. Paper presented at the *'Taller International de Manejo Intergrado y control Biológico de Rottboellia cochinchinensis'* (*International Workshop on the Integrated Management and Biological Control of Rottboellia cochinchinenis*), held at CATIE, Costa Rica 11-13 May. [In: Control biológico de *Rottboellia cochinchinensis*. (Ed V. Sánchez-Garita). In Press].

EVANS, H. C. & ELLISON, C.A. (1998). Biological Control of Weeds with Pathogens: Potential and Risks. Paper presented at the *'Taller International de Manejo Intergrado y control Biológico de Rottboellia cochinchinensis'* (*International Workshop on the Integrated Management and Biological Control of Rottboellia cochinchinenis*), held at CATIE, Costa Rica 11-13 May. [In: Control biológico de *Rottboellia cochinchinensis*. (Ed V. Sánchez-Garita). In Press].

FONSECA, J.F., MERAYO, A., VALVERDE, B.E. & ALVAREZ, T. (1998) Interacción entre dos tipos de *Mucuna deeringianum*, dos variedades de maíz y la caminadora (*Rottboellia cochinchinensis*). Memoria. VII Congreso Internacional de Manejo Integrado de Plagas, Nicaragua, p. 55.

MERAYO, A., FONSECA, J.F., VALVERDE, B.E. & ALVAREZ, T. (1998) Efecto de la densidad y momento de siembra de Mucuna sobre la supresión de *Rottboellia cochinchinensis* y el rendimiento del maíz. Memoria. VII Congreso Internacional de Manejo Integrado de Plagas, Nicaragua, p. 77.

MERAYO, A., ROJAS, C.E., VALVERDE, B.E. & UMÃNA, E. (1998) Leguminosas de cobertura para el manejo de *Rottboellia cochinchinensis* en el asocio yuca/maíz. *Manejo Integrado de Plagas* 48:49-53.

MERAYO, A., VALVERDE, B.E., ROJAS, C.E., CALVO, G., FONSECA J.F. & ALVAREZ, T. (1996). Avances en el manejo de *Rottboellia cochinchinensis* en maíz. Memorias VI Congreso Internacional de Manejo Integrado de Plagas, Mexico, p. 114.

MERAYO-MILLER, A. & FONSECA-RAMIREZ, J.F (1998) Control de la *Rottboellia cochinchinensis* en el maíz. CATIE, Turrialba, 9 p.

MERAYO-MILLER, A. & FONSECA-RAMIREZ, J.F. (1998) Usos y beneficios de la Mucuna. CATIE, Turrialba, 11 p.

REEDER, R.H. & ELLISON C. A. (1998). Current status of research into the classical biological control of *Rottboellia cochinchinensis* with the smut *Sporisorium ophiuri*: potential and risks. Paper presented at the *'Taller International de Manejo Intergrado y control Biológico de Rottboellia cochinchinensis'* (*International Workshop on the Integrated Management and Biological Control of Rottboellia cochinchinenis*), held at CATIE, Costa

Rica 11-13 May. [In: Control biológico de *Rottboellia cochinchinensis*. (Ed. V. Sánchez-Garita). In Press].

SÁNCHEZ -GARITA, V. (1999). Control biológico de *Rottboellia cochinchinensis* (Biological Control of *Rottboellia cochinchinensis)*. (Ed V. Sánchez-Garita). (In Press).

SANCHEZ-GARITA V & ZÚÑIGA C. (1998). Patógenos Nativos de América Latina con potencial como agentes de control biológico de malezas. Native pathogens with potential as biological control agents of weed. IN Control biológico de *Rottboellia cochinchinensis* y Resumenes del Taller Internacional De Manejo Integrado y Control Biológico de *Rottboellia cochinchinensis.* [In: Control biológico de *Rottboellia cochinchinensis*. (Ed V. Sánchez-Garita). Abstract p.185. In Press].

SANCHEZ-GARITA V., ZÚÑIGA C.; & CERVANTES, M. (1998). Control Biológico de *Rottboellia cochinchinensis*. (Biological Control of *Rottboellia cochinchinensis)*. IN 12 th ATACORI Congress (Association of Sugar Cane Technicians of Costa Rica), 2-3 September, 1998, Guanacaste, Costa Rica. Abstract p. 25.

*SÁNCHEZ-GARITA, V.; & ZÚÑIGA, C. (1998). Patógenos Nativos de América Latina con potencial como agentes de control biológico de malezas (Native pathogens with potential as biological control agents of weed). [In: Control biológico de *Rottboellia cochinchinensis*. (Ed V. Sánchez-Garita). Pp 151-181. In Press.].

SMITH M C & HOLT J (1997) Analytical models of weed biocontrol with sterilizing fungi: the consequences of differences in weed and pathogen life-histories. *Plant Pathology* 46, 306-319.

VALVERDE BE, MERAYO A, FONSECA JF, ALVAREZ T & RICHES CR, (1999). Validation of integrated methods to control itchgrass (*Rottboellia cochinchinensis*) in corn with subsistence growers in Costa Rica. WSSA Abstracts, 1999 Meeting of theWeed Science Society of America, volume 39:155.

* VALVERDE, B.E., MERAYO, A., FONSECA, J.F., ALVAREZ, T. & RICHES, C.R. (1999) Validation of integrated methods to control itchgrass (*Rottboellia cochinchinensis*) in corn with subsistence growers in Costa Rica. WSSA Abstracts 39: 308.

ZÚÑIGA C.; SANCHEZ-GARITA V.; & CERVANTES, M. (1998). Control Biológico de *Rottboellia cochinchinensis* con patógenos nativos. (Biological Control of *Rottboellia cochinchinensis* with native pathogens*)*. IN Memoria del VII Congreso Internacional de Manejo Integrado de plagas. VII Taller Latinoamericano y del Caribe de Mosca Blanca y Geminivirus y XXXVIII Reunión Anual de la Sociedad Americana de Fitopatología, División Caribe. Abstract, 60 p.

ZÚÑIGA, C.; SÁNCHEZ -GARITA, V.; BUSTAMANTE, E.; & CERVANTES, M (1999). Uso de herbicidas como agentes de predisposición de *Rottboellia cochinchinensis* al ataque de patógenos nativos (Herbicides as predisposition agents of of *Rottboellia cochinchinensis* to the native pathogens damage). *Revista Manejo Integrado de Plagas*. (In Press).

ZÚÑIGA, C.; SÁNCHEZ -GARITA, V.; BUSTAMANTE, E.; & CERVANTES, M. (1999) Selección de patógenos nativos con potencial de micoherbicida para el control biológico de

Rottboellia cochinchinensis (Selection of native pathogens with potential as mycoherbicides to control *Rottboellia cochinchinensis*). *Revista Manejo Integrado de Plagas*, (In Press).

* ZÚÑIGA, C.; SÁNCHEZ GARITA, V.; & BUSTAMANTE. (1999). Evaluación del efecto de patógenos nativos y factores de estrés en el control de *Rottboellia cochinchinensis.* (Evaluation of native pathogens and stress factors to control of *Rottboellia cochinchinensis).* Poster for CATIE Scientific Week, 6-9 April 1999. (In Press).

Internal Reports:

*CATIE Annual Report 1998.

* MERAYO, A., FONSECA, J.F., VALVERDE, B.E. & ALVAREZ, T (1997) Validación y transferencia de técnicas mejoradas para el manejo de la maleza *Rottboellia cochinchinensis* en maíz. *In* Actas III Semana Científica, 3-5 de febrero 1997. CATIE, Turrialba, Costa Rica, p.95.

Quarterly Technical Reports (ZA0140, ZA0051, ZA0052)

01 April to 30 June 1996	Undated
01 July to 30 September 1996	Undated
01 October to 31 December 1996	Undated
01 April to 30 June 1997	Undated
01 July to 30 September 1997	Undated
01 October to 31 December 1997	15 January 1998
01 April to 30 June 1998	10 June 1998
01 July to 31 August 1998	10 September 1998
01 September to 31 December 1998	15 December 1998

Annual Technical Reports

01 April 1996 to 13 March 1997	Undated
01 April 1997 to 13 March 1998	Undated

Final Technical Report

01 April 1996 to 31 March 1999	In preparation

RICHES C R. Back To Office Report VS: 5298 Visit to CATIE, Costa Rica. 16 - 24th February, 1998

ELLISON C.A., R. H. REEDER CABI Bioscience (Ascot site). P.J. SHANNON AND M.C. SMITH NRI, Chatham. BTO Report on a Visit to Costa Rica, 8-18 May 1998.

Other Dissemination of Results:

BAÑOS-MEJÍA, E.I. (1998) Field day with 6 leader growers on itchgrass management and demonstration of validation plot. Cuenca del Papaluapan Plant Health Committee, Oaxaca, Mexico, 01 August 1998.

ELLISON, C.A. (1998). A proposal for the potential introduction of *Sporisorium ophiuri* into Costa Rica for the classical biological control of

Rottboellia cochinchinensis. Talk presented at the *'Taller International de Manejo Intergrado y control Biológico de Rottboellia cochinchinensis'* (*International Workshop on the Integrated Management and Biological Control of Rottboellia cochinchinenis*), held at CATIE, Costa Rica 11-13 May.

International Workshop of Integrated Management and Biological control of *Rottboellia cochinchinensis* (11 to 13, May 1998), was carry out successfully with 36 participants, including specialist researches and farmers, their summaries and conclusions are included in the book: Control Biológico de *Rottboellia cochinchinensis* (Biological control of *Rottboellia cochinchinensis*).

MERAYO, A. & FONSECA, J.F. (1998) Integrated Management of itchgrass in Maize: Informal Lectures to two groups of undergraduate students (total 48) from EARTH University, Costa Rica, 23 and 26 November, 1998.

MERAYO, A. & FONSECA, J.F. Two field days were organised in Corralillo and Palmira (19 and 25 November 1997, respectively), Guanacaste, Costa Rica with participation of 8 and 12 growers, respectively.

MERAYO, A. (1998) Lectured on integrated management of itchgrass at Organic Agriculture graduate course at CATIE's Graduate Programme, CATIE, Turrialba.

MERAYO, A., FONSECA, J.F. & VALVERDE, B.E. (1998) Distribution of pamphlets (see Publications) on the Use of mucuna as a cover crop and on Management of itchgrass in maize. Total number of copies distributed: 410.

MERAYO, A., FONSECA, J.F. & VALVERDE, B.E. (1998) Field days on integrated management of itchgrass in maize, held in Santa Cruz and Filadelfia, Guanacaste province, Costa Rica, 12 and 13 August 1998, with a total attendance of 28 extension agents and farmers.

MERAYO, A., FONSECA, J.F. & VALVERDE, B.E. (1998) Avances en el manejo de *Rottboellia cochinchinensis* bajo condiciones de campo [Advances in *Rottboellia cochinchinensis* management]. Paper presented at an International Workshop on Management and Biocontrol of *Rottboellia cochinchinensis.* 11-13 May, 1998, CATIE, Costa Rica.

*REEDER, R. H. & ELLISON C. A. (1998). Prospects for the biological control of *Rottboellia cochinchinensis* in Latin America using the head smut *Sporiosorium ophiuri.* Poster presented at the *British Mycological Society International Symposium The Future of Fungi in the Control of Pests, Weeds & Diseases*. University of Southampton 5-9th April 1998

*REEDER, R. H. & ELLISON C.A. (1998) Prospects for the biological control of *Rottboellia cochinchinensis* in Latin America using the head smut *Sporiosorium ophiuri.* Paper presented at Asia-Pacific Mycological Conference on Biodiversity and Biotechnology, Hua Hin, Thailand, 6-9 July 1998.

SHANNON P. Introduction and discussion of the importance of *Rottboellia* (Talk) *Rotboellia* WorkShop 11-13 May, 1998, CATIE, Costa Rica.

SMITH M C (1998) Models of a head smut epidemic in itchgrass: the potential for biocontrol. Poster presentation at ICPP98, Edinburgh 9-16 August 1998. Abstracts of offered papers to ICPP98, Edinburgh, 9-16 August 1998. Abstract number 2.1.2, Volume 2.

SMITH M. C. (1998). A modelling approach to assessing the potential of the head smut in controlling *Rottboellia*. (Talk) *Rotboellia* WorkShop 11-13 May, 1998, CATIE, Costa Rica. A written version should be published in the Proceedings of the Workshop

VALVERDE B. & GARITA I. presented research results to collaborators and crop protection industry representatives in Bolivia. Santa Cruz, Bolivia, 03-05 June 1997.

VALVERDE, B.E. (1998) Informal meetings with Bolivian and Mexican collaborators A. Claros and G. Torres while attending *Rottboellia* Workshop, to discuss current / planned project activities

VALVERDE, B.E. 1996-1999. Informal weekly to biweekly meetings were held with collaborating maize growers involved in validation plots to discuss field observations on itchgrass management practices

VALVERDE, B.E. (1998). Informal meetings with Bolivian and Mexican collaborators A. Claros and G. Torres while attending Herbicide Resistance Workshop, to discuss current / final project activities

VALVERDE, B.E. (1998). Importancia de la biología y la ecología de malezas para el control biológico [Importance of weed biology and ecology for their biocontrol]. Paper presented at an International Workshop on Management and Biocontrol of *Rottboellia cochinchinensis*. 11-13 May, 1998, CATIE, Costa Rica.

VALVERDE, B.E. (1998) Integrated Management of itchgrass in Maize: Lecture at University Grabriel René Moreno, Bolivia (17 students, 3 representatives from local crop protection industry, 1 grower, and 2 university/government researchers). 16 November 1998.

VALVERDE, B.E. (1998). Meeting with Mr. Valentin Esqueda-Esquivel from Cotaxtla Research Station (Veracruz) to discuss about itchgrass distribution in Veracruz. Morelos, Mexico, 19 September 1998.

Follow-up action/research

Publications drafted or in preparation will be submitted to appropriate journals. A review paper on Integrated Management of Itchgrass will be presented at the 1999 Brighton Crop Protection Conference –Weeds. CATIE will continue distributing pamphlets already published.

The mycoherbicide component requires the implementation of a validation stage, in different areas and over time, for a minimum of two years. Virulent isolates are available for which other formulations need to be tested. The stability of pathogen virulence should be evaluated over time. For further investigation new sources of financial support will be looked for.

An application has been submitted to NRI to obtain funding for a mini-project to enable the CBC component of the programme (ZA0140) to enter an adaptive phase. The aim is to release the smut, *Sporisorium ophiuri,* into the field in Costa Rica as a CBC agent of *R. cochinchinensis* (itch grass). Meetings held with the Costa Rican Sanidad Vegetal at the May 1998 Workshop (CATIE) suggested no opposition to the concept of introducing the smut. However, they were keen to introduce the smut into quarantine in Costa Rica, and to undertake selective host specificity screening prior to field releases. The screening is to be conducted at CATIE, in a specially converted screen-house. Bulking-up of inoculum prior to field release will also be undertaken in this facility. This mini-project will allow the potential impact of the smut on the weed populations to be realised.

Although further development of the modelling work is not anticipated, the conclusions of models I and II provide ideas for further trials of itchgrass control options, and for selection and deployment of the smut should release take place. Such explorations of the potential of the smut as a classical biocontrol agent are the best that can be provided until it is released. A joint publication is planned with the Costa Rican collaborators on the project, to describe the results of analysis of Model I and the experimental data collected for it. (Authors to be decided: Exploring control options for Itchgrass in a maize based cropping system in Costa Rica: a modelling approach)

Name and signature of author of this report:

Bernal E. Valverde (with information and help from A. Merayo), Elkin Bustamante (with information and help from Vera Sánchez Garita and Cristhian Zuñiga), Margaret C. Smith, Robert H. Reeder and C. A. Ellison

(Compiled by: C.A. Ellison & R.H. Reeder 13.5.99)