

Population dynamic aspects of the interaction between the weed *Rottboellia cochinchinensis* (itch grass) and the potential biological control agent *Sporisorium ophiuri* (head smut)

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Abstract. Pot- and field-based experiments were undertaken in Thailand on the population dynamics of *Rottboellia cochinchinensis*, an aggressive tropical grassy weed, and the systemic head smut fungus, *Sporisorium ophiuri*. This work formed part of an on-going study to determine whether the smut can significantly affect populations of the weed in the long term, and as such, whether it would be suitable as a classical biological control agent in the New World. Pot-based experiments using plants grown separately, revealed no significant difference in tiller number between infected and healthy plants. However, when plants were grown eight to a pot, the smut-infected plants produced significantly fewer tillers. This suggests that under severe competition, infected plants may be less vigorous than non-infected plants and that reductions in seed production due to infection may be compensated for at the population level. To investigate this further, a small-plot field trial was conducted to examine the effect on fecundity of varying the smut-infected to healthy-plant ratio, whilst maintaining the overall density of plants in the plots. The results indicated a negative linear relationship between the percentage of plants infected and the total number of seeds produced by all plants. This suggests that healthy plants do not compensate for loss of seed production by infected plants, under the imposed field conditions. The results are discussed in the context of an integrated approach to the control of the weed.

Introduction

Rottboellia cochinchinensis (Lour.) W.D. Clayton (Gramineae) or itch grass, is an aggressive, C4, annual grass, and an important weed in many areas of the tropics. It has an Old World centre of origin but was introduced into the New World, probably at the beginning of the century, where it is increasing its range at an alarming rate (Ellison and Evans 1990). In parts of the New World, infestations can result in up to 80% crop loss, or even the abandonment of agricultural land (Holm, *et al.* 1977). It is a difficult weed to control by conventional methods as it is resistant to some herbicides and because seedlings emerge intermittently in the field, especially after soil disturbance such as hoeing or ploughing. It is a prolific producer of seed with some biotypes beginning to shed seed only seven weeks after germination. Hundreds of seeds can be produced by a single plant in a growing season and

densities of up to 500 plants m⁻² have been recorded (Pamplona and Mercado 1982).

The increasing threat of *R. cochinchinensis* to small- and large-scale tropical agriculture and the inadequate or expensive control methods available, make it a suitable target for biological control. Ellison (1993) found a number of pathogens of *R. cochinchinensis* which could have potential as biological control agents. One of these was the highly host-specific head smut, *Sporisorium* (*Sphacelotheca*) *ophiuri* (P. Henn) Vanky (Ustilaginales). This fungus is absent from the New World and thus has potential for introduction as a classical biocontrol agent. The smut is soilborne, infecting seedlings before they emerge from the soil. The resultant systemic infection is only manifested at flowering when the seed head is converted to a mass of black, powdery teliospores which are shed into the soil to infect the next population. The fact that *R. cochinchinensis* is an

annual weed, with a relatively short-lived seed-bank (3-4 years *vide* Thomas and Allison 1975), and because seed is the plants only means of propagation, indicate that such a sterility-inducing fungus could have potential as a biological control agent.

The efficacy of the smut in reducing population levels of the weed will depend on many factors related to the population dynamics of the weed-smut pathosystem, and the weed control practices employed. The work presented here is part of an on-going two-year population dynamics study on *R. cochinchinensis* in Thailand (within the endemic range of the weed and smut), to investigate the levels of smut-infection needed to reduce significantly the density of the weed, and the suitability of integrating the smut with other control measures. As part of this investigation the data from these studies are being used to improve a model of the weed-pathogen system, constructed initially using parameter estimates from the literature. This model will be used to make predictions regarding the potential value of introducing the smut, thus aiding the decision making of the quarantine authorities in the countries of introduction.

Materials and methods

Experiment 1. The effect of Sporisorium ophiuri infection on the growth characteristics of Rottboellia cochinchinensis

Seeds of *R. cochinchinensis* originating from Thailand (native biotype), were sown in local, dry, humus-rich, lateritic soil, in 27-cm diameter clay pots (each containing approximately 0.01 m³ of soil), at a depth of 3 cm. Six pots were used, each containing 12 seeds; three pots were inoculated with teliospores of *S. ophiuri* (ex *R. cochinchinensis*, Thai native biotype) that had been harvested eight months previously, and three had only rain water applied. The inoculum was prepared by shaking spores from the infected seed-heads into rain water and adjusting the spore suspension to give a concentration of 1x10⁶ spores ml⁻¹. The inoculum was applied to the pots as a soil drench using a watering can, until maximum soil water-holding capacity was reached. Sacking was placed over the pots, and the soil surface lightly sprayed with water on a daily basis to prevent drying out, until the seeds had germinated. Upon emergence the seedlings were randomly thinned to eight plants per pot. The following characteristics of each plant were recorded: (i) time of seedling emergence from soil; (ii) height from top

strut-root to uppermost node of each tiller, every 2/3 weeks from germination to emergence of first raceme; (iii) number of living leaves per plant (including tillers), until week five; (iv) number of tillers per plant, from start of tillering until the emergence of first raceme; and (v) the time of first raceme emergence.

Those plants in the inoculated pots that were subsequently found to be uninfected were excluded from the results (mean of 80% infected plants). Means were calculated for each replicate pot and the data analysed using a Mann-Whitney U-test, since there were only three replicates.

Experiment 2. The effect of Sporisorium ophiuri infection on the tiller formation of Rottboellia cochinchinensis

Experiment 1 was repeated, except that plants were grown individually in separate pots rather than eight to a pot, to allow for a more rigorous statistical analysis. Thirty-two pots were set-up as before, with 16 inoculated treatments and 16 uninoculated healthy plants. Tiller number was recorded over time, and the treatments compared using an analysis of variance.

Experiment 3. The effects of infection by Sporisorium ophiuri on the fecundity and seed biomass of Rottboellia cochinchinensis

Seeds of *R. cochinchinensis* were planted in plastic trays (40x30 cm and 12 cm deep) and the soil in half of these was inoculated as described above. The soil in the remainder of the trays was untreated. After emergence from the soil (3-4 days), the seedlings were maintained in the gauzehouse for a further week until they had reached the 3-4 leaf stage before transplanting into the trial plots. The plots were 0.7x0.6 m (0.42 m²), arranged according to a randomized complete block design with four replicates per treatment. The plots were planted out on a 5x7 grid (83 plants m⁻²) with varying numbers of inoculated and healthy control plants, with the aim of achieving a gradation of infected plants between treatments. Within each plot the seedlings were 0, 30, 60 and 100% inoculated. Plants that did not survive transplanting were replaced with plants of the same age and inoculation status. After maturation, all seed heads which had fully emerged from the leaf sheath, along with smut-infected heads, were collected from each plot, at seven-day intervals throughout the period of seed set (10 weeks). The total number of infected and uninfected seed heads collected from each plot was recorded. On each

collection date, 30 intact seed heads were selected at random from each plot and the number of fully formed seeds was recorded. Analyses of variance were computed for total number of seed heads, total number of seeds and total number of smutted heads collected at each harvest. Duncan's multiple range test was used to determine significant differences among means at the 5% level.

Results and discussion

Experiment 1. The effect of Sporisorium ophiuri infection on the growth characteristics of Rottboellia cochinchinensis

The first set of pot experiments gave the following results:

- (1) There was no significant difference in the time taken for infected and uninfected plants to emerge from the soil. ($U > 0$, $P > 0.05$). Both sets of plants emerged on average at 3.5 (± 0.5) days after sowing.
- (2) There was a significant difference in combined tiller height from day 39 onwards; the height of smut-infected plants was significantly less than that of healthy plants ($U = 0$, $P = 0.05$). At the last measurement (day 77), mean combined tiller height for the control plants was 4.20 m and for infected plants was 3.55 m; representing a 15% reduction in total height. However, if the main tiller height is considered alone, there was no significant difference between treatments (control = 1.15 m, infected = 1.2 m).
- (3) There was no significant difference in the number of leaves per plant (all tillers combined) up to day 25 after inoculation, but infected plants had produced significantly fewer leaves at day 32 and day 39 ($U = 0$, $P = 0.05$ at both times). At the last measurement (day 39), the mean number of leaves per plant for the controls was 22.5 leaves and for infected plants was 16.6. There was a trend towards divergence in leaf number between treatments as the plants matured.
- (4) Figure 1 shows that from day 32 onwards, the treatment had a significant effect on the number of tillers a plant produced ($U = 0$, $P = 0.05$). The healthy (control) plants had significantly more tillers than infected plants. For example, at day 39, the control plants had a mean of 6.8 tillers per plant, and the infected plants 4.6. This difference was maintained until the end of the experiment.

- (5) There was a significant difference between the dates of first raceme emergence ($U = 0$, $P = 0.05$). On average, the smutted heads of infected plants emerged 74 days after planting, while healthy plants flowered three days later.

The results show that *R. cochinchinensis* plants infected with *S. ophiuri* have an altered development when compared with healthy plants. This is manifested in smutted plants by an earlier flowering date, and a significant reduction in the number of tillers, and leaves per plant. The combined tiller length of smut-infected plants was significantly shorter than for healthy plants. This, coupled with the fact that infected plants produce fewer leaves, suggests that infected plants do not compensate for reduced tiller number by increasing their biomass. This could be due to the parasite reducing the vigour of infected plants through competition for nutrients within the plant. Therefore, infected plants, besides setting no seed, may exert less competitive pressure within a cropping system. No significant difference was found between the maximum height attained by the main tiller of infected and healthy plants. This does not agree with field investigations undertaken by Ellison and Evans (1990), the results of which showed a significant reduction in the overall height of smut-infected plants. The tiller number of control plants in Fig. 1 declined towards the end of the experiment because of the death of some of the youngest tillers prior to flowering.

Experiment 2. The effect of Sporisorium ophiuri infection on the tiller formation of Rottboellia cochinchinensis

The results from experiment 1 indicated that the most important difference (from a population dynamic perspective) between infected and uninfected plants was related to tiller number. Hence, only this parameter was measured in experiment 2. An analysis of average tiller production over the total measurement period revealed that smutted plants grown individually in pots did not produce significantly fewer tillers than healthy plants. The average number of tillers produced by smutted plants was 3.48, compared to 3.79 tillers produced per healthy plant ($P = 0.457$, $F = 0.561$, d.f. = 52). This suggests that the growth of *R. cochinchinensis* is not affected by the parasite when plants are grown individually under optimum growth conditions (density in this experiment was the equivalent of approximately 17 plants m^{-2}). This is in contrast to when smut-infected plants were grown at

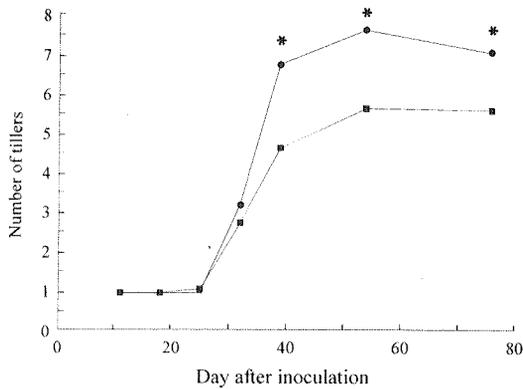


Fig. 1. Effect of infection by *Sporisorium ophiuri* smut on the mean number of tillers produced per *Rottboellia cochinchinensis* plant (see experiment 1). Closed circles - control plants; closed squares - infected plants. Asterisks show significant differences at the 5% level between treatments for individual dates.

densities of eight plants to a pot (the equivalent of 140 plants m^{-2}), where they did not appear to compete as well as healthy plants.

Experiment 3. The effects of infection by Sporisorium ophiuri on the fecundity and seed biomass of Rottboellia cochinchinensis

Until *R. cochinchinensis* plants flower there is no reliable, non-destructive way of telling if the plant has become infected by the smut. In this experiment, considerable variation in the numbers of inoculated plants that became infected in each plot was revealed at flowering (due to variable rates of infection in the inoculation trays). The total number of smut-inoculated plants used in the experiment was 268 but of these only 139 subsequently proved to have been infected, an overall infection rate of 51.8%. The actual infection rates from the four different treatments fell into three main groups; a control group of five plots with an average of 2% (0-6%), six plots with an average of 20% (14-29%), and five plots with an average of 50% (46-60%) infected plants, respectively (see Fig. 2).

The results in Table 1 show that there was a significant difference ($P = 0.002$, d.f. = 173, $F = 8.85$) in the average number of seeds produced by plants between the infection categories. Duncan's multiple range test revealed this difference to be between the control, 20%, and high (50%) infected group.

From experiments 1 and 2 it is unclear whether infected and healthy plants compete equally or

whether, because of greater tillering, healthy plants may be able to compensate at the population level for loss of seed production in the infected plants. In experiment 3, if infected and healthy plants compete equally and infected plants produce no viable seed, there should be a linear relationship between the reduction in seed number and the level of smut infection. To investigate this hypothesis, a simple linear model was fitted to the data which showed that there was no significant relationship between the level of total seed production per plot and smut infection ($R^2 = 21.10\%$, $P = 0.073$). However the data in Fig. 2 show that there is a point from a 20% infected plot which can be considered as an outlier. This plot had an unexpectedly high value for total seed production, especially when a 20% infection rate would be expected to lower the total seed-production relative to the control. If this value is excluded from the analysis, a significant negative linear relationship between smut infection and total seed-production is obtained ($P = 0.045$), although it still has a relatively low R^2 value of 27.40% due to quite large variations in seed production between plots with similar, and even identical, percentage infection.

There could be two possible reasons for the high-yielding plot in the 20% smut-infected treatment group. (1) This plot bordered onto an maize trial where standard fertilizer treatments were being used, therefore the soil may have been richer in nutrients. An increase in nutrients, particularly nitrate ions, will improve the growth and seed production of *R. cochinchinensis*. Thomas (1972) reported a 16% increase in seed production from fertilized plots. (2)

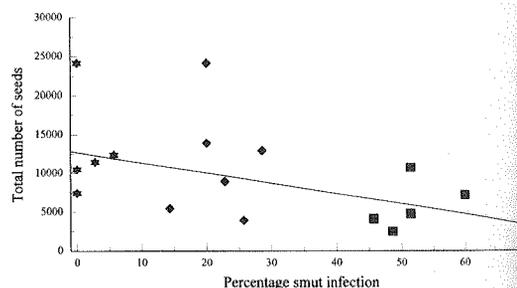


Fig. 2. Relationship between total seed production by *Rottboellia cochinchinensis* plants, and percentage infection by *Sporisorium ophiuri* smut. Infected groups: closed stars - 2%; closed diamonds - 20%; closed squares - 50%. The line shows a significant negative relationship between seed production and smut infection ($y = 12680 - 132x$; $P = 0.045$; $R^2 = 27.40$)

Table 1. Average number of seeds produced by *R. cochinchinensis* per plot per week over a 10-week period of seed set. * - LSD at 0.05 =324.91. ** - LSD at 0.05 =311.08.

% infection by smut	Average number of seeds produced	% difference from control	Average number of seeds per uninfected plant	% difference from control
Control	1185.0*	0.0	372.5	0.0
20	1050.0**	-11.4	422.5	+13.4
50	533.0*	-55.0	344.8	-7.4

Occasionally, infected plants may produce healthy tillers, the meristems of these tillers having escaped early mycelial invasion due to growing conditions that favour the plant more than the fungus. Some of the first tillers produced by the infected plants in this high-yielding plot escaped infection allowing some seed-set, however seed heads of subsequent tillers were infected and destroyed by the fungus.

Figure 3 shows a steady, weekly increase in the numbers of seeds produced by *R. cochinchinensis* plants, reaching a peak after six weeks and then declining over the next four weeks. The peak in smutted-head production, by infected plants, occurred one week earlier (between week 4-5). This is in agreement with the results of the pot experiments where infected plants flowered earlier. There is also a steady decrease in the average number of seeds per seed head, with time. Seed heads at the beginning of seed-set had about 15 seeds per head, this steadily declined throughout the sampling period to about nine seeds per head at the end of the collections. This trend was followed in all three groups, and is presumably in response to the plants declining resources.

Previous work in Thailand has shown that the number of seeds produced by *R. cochinchinensis* plants, is density dependent (Authors' unpublished data). At low densities the plants produce significantly more seed due to compensatory growth through increased tiller production. Plant density becomes a limiting factor at densities of 80 plants m⁻². At this density, seed production per unit area approaches its maximum because of the increased competition for resources, especially light. This is a similar result to Thomas (1972) who observed that over a range of plant densities, from 43-172 m⁻², seed production was not significantly different. In the present study, seedlings were planted at a density of 83 plants m⁻², where significant inter-plant competition occurs. If smut-infected plants are less competitive than healthy plants they might be expected to be either excluded from the

plots, or to exert little influence on the seed production of neighbouring healthy plants. However, there was no significant difference in the average seed production per plant between healthy plants growing in smut-infected and uninfected (control) plots, (the values ranging from 344.8 to 422.5 seeds per plant). This suggests that there was little compensatory growth in healthy plants through increased seed production. Infected plants must still be capable of competing for resources and affecting the seed production of neighbouring plants, under the field conditions of experiment 3. This is in contrast to experiment 1 which implied that infected plants are less competitive than healthy plants.

Conclusions

In studies based on artificial inoculations of itch grass seedlings in pots, Ellison (1993) achieved a maximum infection rate of 80% with the smut fungus, when the soil was inoculated with 2x10⁵ spores per cm⁻³. The results of experiment 3 in the current study, indicated that such an infection rate would result in an 80% (or more) reduction in seed production in the field. The effect of this reduction in fecundity on the *R. cochinchinensis* population over time is unclear. A model of *R. cochinchinensis* population dynamics developed by Smith *et al.* (1996), suggested that with the smut as the sole control agent acting on an uncontrolled equilibrium density of 145 plants m⁻², an annual infection rate of about 85 % would take 20 seasons to reduce the plant population by 90% (i.e. to a density of 14 plants m⁻²). One of the assumptions in obtaining this result was that smutted and healthy plants were equally competitive (with reduced competitiveness of smutted plants, even greater levels of infection are likely to be required for equivalent control). From experiment 3 this does appear to be the case as a linear relationship between seed production and smut infection was observed (any reduced competition in smutted plants would lead to non-

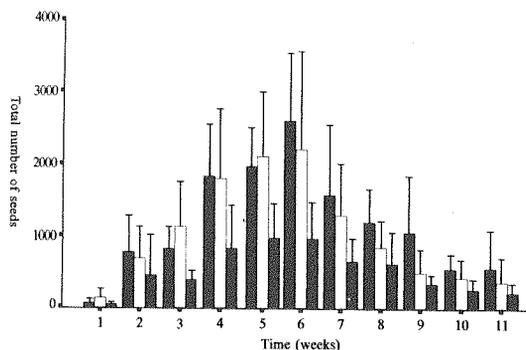


Fig. 3. Effect of infection by *Sporisorium ophiuri* smut on the average (+SE) seed production of *Rottboellia cochinchinensis* plants from each infection group in experiment 3. Closed histogram bars - 2%; open bars - 20%; shaded bars - 50% infection group.

linearity in this relationship). Such consistently high levels of infection are likely to be difficult to achieve in the field. However, the use of the smut in an integrated approach to the control of the weed, incorporating both biological and cultural methods of control, may prove more effective.

Control strategies that reduce the amount of *R. cochinchinensis* seed in the soil can be effective in the management of this weed. Fisher *et al.* (1985) observed that in several small (>1 ha) fields of maize and upland rice at Bukidnon in the Philippines, *R. cochinchinensis* was almost eliminated by repeated cultivation and hand-weeding over several years. Although mechanical practices such as ploughing can control *R. cochinchinensis* in the inter-row region, the weeds within the crop rows often survive and can contribute considerably to the seed-bank. Further problems arise from the inadequate control of the weed in fallow fields, headlands and areas of waste ground which all act as seed reservoirs for further infestation. Seeds from these areas are washed into the fields after heavy rain, or are transported on the wheels of farm vehicles. The effectiveness of cultural methods therefore could be enhanced by the use of the smut fungus which would help to control *R. cochinchinensis* plants in these situations.

Since *S. ophiuri* is soil borne, it may be limited, as a classical biological control agent, by the narrow window of opportunity for infection. In addition, *S. ophiuri* only has one disease cycle a year, and consequently it will have a slow intrinsic rate of spread within a population of *R. cochinchinensis*. From field

observations of natural populations of the weed-smut pathosystem, the percentage of smutted plants in a population can be very high, but such population densities may have built up over many years (C. Ellison personal observations). However, at the local level, farmers may be able to augment the natural spread of the pathogen by ploughing smutted plants back into the soil as a form of green manure, helping to spread the spores and adding organic matter to the soil. Farmers aiding the natural spread of a biotrophic pathogen is not a new concept. For example, Phatak *et al.* (1983) report the augmentative control of *Cyperus esculentus* L. (Cyperaceae) with the rust *Puccinia canaliculata* (Schw.) Lagerh. (Uredinales). In this system, rust culture is maintained on the host plant in the glasshouse during the winter and then released into the weed population at the beginning of the growing season. This gives significant control of the weed, that is not achieved if the pathogen is allowed to build up naturally in the population. For control of itch grass, it may also be possible to exploit the smut as a type of mycoherbicide. Although it is an obligate pathogen, teliospores of the smut germinate readily on agar to produce sporidia indeterminately. If the sporidia are found to be infective, then it may be possible to mass-produce them and apply a formulated inoculum to *R. cochinchinensis*-infested soils. This would help to overcome some of the constraints due to the slow spread of the smut.

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