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Present Status of the Biological Control Programme for the Graminaceous Weed *Rottboellia cochinchinensis*

Carol A. Ellison and Harry C. Evans

CAB International Institute of Biological Control (IIBC), Buckhurst Road, Silwood Park, Ascot, Berks SL5 7TA, UK

Rottboellia cochinchinensis (itch grass) is a pantropical agricultural weed which is increasing its range at an alarming rate. Intensive surveys for fungal pathogens of this weed were conducted in both the Old and the New World. Fifteen different genera were found to be represented in the mycoflora, 2 of which are biotrophs with potential as classical biological control agents. Some 800 isolates of necrotrophic fungi were obtained and >200 of these passed through a primary screen, which included the major graminaceous crop plants, to assess their potential as mycoherbicides. Isolates from 2 genera, *Colletotrichum* and *Sphacelotheca*, were selected for more intensive specificity screening and to determine infection parameters. Both pathogens were found only in the Old World and the former was restricted to South-East Asia. Glasshouse experiments demonstrated that maximum infection of *R. cochinchinensis* with *Colletotrichum* isolate *KP1D1* from Thailand was obtained at 5 X 10^6 spores ml^{-1}, when applied to the 2-leaf growth stage with a minimum dew period of 15 h and a repeated 8-h period of wetting over the next 3 d. A synergistic response was demonstrated when a low dose chemical herbicide was added to the fungal inoculum. A small plot-field trial was carried out in Thailand to test the efficacy of this isolate as a mycoherbicide. *Sphacelotheca ophiuri* is a systemic head smut which has demonstrated intraspecies specificity towards *R. cochinchinensis*. It may have limited potential as a classical biological control agent due to exceptionally high inoculum requirements but, since sporidia are readily produced in culture, it may be possible to apply these to the soil as a form of mycoherbicide.

Introduction

Rottboellia cochinchinensis (Lour.) W.D. Clayton (= *R. exaltata* L.f.) (Gramineae: Andropogoneae) or itch grass, is continuing to expand its range in the tropical world. It occurs as a number of biotypes, the complete documentation of which is still under preparation (Milhollon, R., personal communication, 1991). This variation in biotypic form enables the weed to grow in both annual (maize, upland rice, soybean, sugar cane) and perennial (fruit trees, oil palm) cropping systems. The weed was introduced into the New World, probably at the being of the century, and it is here that infestations are considered to be the most severe (Holm *et al.* 1977). However, there are new records of its invasive nature in North Malaysian sugarcane fields (Chan *et al.* 1990) and maize in the North-East of Thailand (Jahom, S., personal communication, 1990). Indeed, the weed was targeted in the recommendations section of Baker and Terry (1991) as one of the most important tropical grassy weeds that should be selected for increased research effort.

The continued spread of *R. cochinchinensis* and its increase in importance, particularly in graminaceous crops, can be attributed to a number of factors. For example, as farming methods intensify there is a decrease in manual weed control, by which method this weed can be successfully controlled, and an increase in herbicide use. *R. cochinchinensis* is rapidly developing resistance to the limited number of selective herbicides that can be applied in graminaceous crops (Thomas 1972). In addition, the seed is carried in water and mud on farm machinery, thus the increasing proportion of irrigated land, and the intra- and inter-farm movement of machinery, facilitate the dissemination of seed within an infected area.

Due to the inadequate control of this weed using conventional methods and the alarming rate of its spread, *R. cochinchinensis* was selected for an investigation into the potential of fungal pathogens as biological control agents in a joint project between CAB International Institute of Biological Control (IIBC) and Long Ashton Research Station (LARS), funded by the UK Overseas Development Administration (ODA). Both classical and mycoherbicide approaches were investigated. The primary phase of the project (Ellison and Evans 1990) had involved the collecting and assessment of fungal isolates from the New World and Africa. No *R. cochinchinensis*-specific isolates were found. However, isolates from the genus *Curvularia* had demonstrated high levels of virulence, some causing damping-off in seedlings after only a few days, presumably due to toxin production. This paper summarises the progress of the research to date.

Field Surveys

Surveying for fungal pathogens continued in the Old World (India, Pakistan, Nepal, Sri Lanka and Thailand) focusing around India, where *R. cochinchinensis* is thought to be native (Holm *et al.* 1977), and hence, the most likely area to find host specific isolates. Table 1 lists the fungi isolated from and found to be pathogenic to *R. cochinchinensis* from all the surveys completed during this project. The results show a regional variation in the mycoflora; as would be expected, only typically plurivorous fungi were isolated in the introduced range of the weed (New World). A large range of genera are included in the Old World mycoflora, 2 of them being biotrophic fungi.

Evaluation of Necrotrophic Fungi As Potential Mycoherbicides

Screening

Screening was carried-out under quarantine conditions at IIBC, UK. Some 800 isolates of necrotrophic fungi were obtained from diseased *R. cochinchinensis* plants, of these, >200 were examined in primary tests of pathogenicity and specificity. The remaining isolates were rejected on taxonomic and other grounds (e.g., inability to sporulate *in vitro*). The plants used in the primary screen were as follows: *R. cochinchinensis*, biotypes from Thailand, Zimbabwe and Bolivia; maize (*Zea mays* L.); rice (*Oryza sativa* L.); sorghum (*Sorghum bicolor* [L.] Moench); pearl millet (*Pennisetum typhoides* [Burm.f.] Stapf & Hubbard); finger millet (*Eleusine coracana* [L.] Gaertn.) and sugar cane (*Saccharum officinarum* L.). This latter species was only included with the re-screening of pathogenic isolates.

The isolates were grown *in vitro* and spray inoculated following a similar method as described in Ellison and Evans (1990). Plants were assessed for symptoms 2 wks after inoculation and discarded after one month. All isolates that demonstrated pathogenicity to *R. cochinchinensis* were re-isolated onto agar to confirm Koch's Postulates, and then re-screened. Isolates which caused primary lesion development on the crop plants were rejected at this stage, based on both sets of data.

All the isolates screened from the following genera (*Coniothyrium, Leptosphaeria, Myrothecium, Phaeoseptoria, Pyrenochaeta)* were only mildly pathogenic to *R. cochinchinensis*. Some isolates of other genera were highly virulent but demonstrated poor specificity, these were: *Ascochyta, Cercospora, Cochliobolus, Diaporthe, Diplodia, Magnaporthe.* In addition, many of the isolates of *Cercospora* and *Coniothyrium* failed to sporulate adequately *in vitro*, and so were difficult to conclusively reject in the screen. Isolates from the genus *Colletotrichum* showed the most promise as mycoherbicides, since they demonstrated a high level of specificity and acceptable virulence towards *R. cochinchinensis*.

A number of the *Colletotrichum* isolates demonstrated the phenomenon of latent infection, as discussed by Cerkauskas (1988), on some of the crop test plants. This refers to colonisation and sporulation on naturally senescing tissue (i.e., the pathogen is behaving as a saprophyte), rather than physiologically active leaves, as evidenced by the control plants which exhibited a similar senescence of first leaves. Many species of *Colletotrichum* produce pigmented appressoria which can act as survival structures, being resistant to UV light and desiccation (Muirhead and Deverall 1981). Latent colonisation from these appressoria occurs when the host undergoes major physiological change, as for example during senescence, and the plants defence mechanisms are no longer functioning. This phenomenon required careful attention when the results of the screening were interpreted.

Table 1. Fungi collected on *Rottboellia cochinchinensis* (Lour.) W.D. Clayton and their distribution.[1]

Fungus	New World	Africa	S.E. Asia
Ascochyta sp. nov. (Coelomycetes)	–	x	–
Cercospora spp. (Hyphomycetes)	x	x	x
Cochliobolus (*Helminthosporium*) *bicolor* Paul & Parbery (Dothideales)	–	x	–
C. (*Curvularia*) *cymbopogonis* (Dodge) Groves & Skolko (Dothideales)	x	x	x
C. heterostrophus (*Drechsler maydis*) (Drechsler) Drechsler (Dothideales)	x	x	x
Colletotrichum spp. (Coelomycetes)	–	–	x
Coniothyrium sp. (Coelomycetes)	–	x	x
Diaporthe (*Phomopsis*) sp. nov. (Diaporthales)	–	x	x
Diplodia sp. (Coelomycetes)	–	x	–
Leptosphaeria sp. (Dothideales)	–	x	x
Magnaporthe (*Pyricularia*) *grisea* (Herbert) Barr (Polystigmatales)	–	x	–
Phaeoseptoria sp. nov. (Coelomycetes)	–	x	x
Puccinia rottboelliae P. & H. Sydow (Uredinales)	–	x	–[2]
Pyrenochaeta sp. (Coelomycetes)	–	–	x
Sphacelotheca ophiuri (P. Henn.) Ling (Ustilaginales)[3]	–	x	x

[1] Key: x = fungus collected in this region; – = not found.
[2] Recorded from India (Evans 1987).
[3] Now *Sporisorium ophiuri* (P. Hem.) Vánky.

A large number of *Colletotrichum* isolates were screened but none were found to be virulent on all the biotypes of *R. cochinchinensis*. However, all isolates were found to be most virulent on the biotype from which they were originally isolated. Although an arguable concept, it may have been expected that biotypes, isolated in space and time from their co-evolved mycoflora, would lose any genes that confer resistance, rather than obtain them. On the contrary, these results may be evidence for *R. cochinchinensis* having more than one centre of diversity in the Old World; certainly the associated mycoflora in Africa and South-East Asia are significantly different. This biotypic variation in susceptibility to the different *Colletotrichum* isolates could be used to identify the origin of an introduced biotype. For example, all pathogenic isolates of *Colletotrichum* from Sri Lanka were poorly virulent on the Thai-biotype of *R. cochinchinensis*, but highly virulent on most of those tested from the New World. This suggests that these biotypes may have originated from the Indian sub-continent.

The most promising 3 *Colletotrichum* isolates (2 from Thailand and 1 from Sri Lanka) were passed through an expanded screen which included; other graminaceous crop plants, a number of different varieties of all the test crop plants, other monocots and *R. cochinchinensis* biotypes from other countries. Maize in particular was focused on, because of the evolutionary closeness of the genus *Zea* to *Rottboellia*, and approximately 12 varieties, from all over the tropics, were tested (Clayton and Renvoize 1986). Indeed, isolates from a number of the fungal genera screened were found to infect these 2 plant genera alone. From these tests, isolate *KP1D1* (ex Thailand) was selected, based on its virulence, and experiments were carried-out to establish infection requirements, prior to field testing in Thailand.

Investigation of Infection Requirements of Colletotrichum *Isolate* KP1D1 *from Thailand.*

Three experiments were undertaken to investigate the dew period requirements for infection of *R. cochinchinensis* (ex Thailand) by KP1D1, and whether there is a critical plant age at which to inoculate. In each experiment, test plants were spray-inoculated and placed in a dew chamber (Mercia Scientific, UK; Clifford 1973). Plants were left to develop infection for 3 wks and then assessed using some or all of the following parameters:

(a) Disease score.

0 no symptoms;
1 a few scattered lesions, no leaf necrosis;
2 many lesions over inoculated area, some minor leaf necrosis and tip shrivelling;
3 dense lesion formation, extensive leaf necrosis and shrivelling, plant growth rate apparently unaffected;
4 all inoculated area shrivelled, plants visibly stunted;
5 stem necrosis, plants damp-off

(b) Plant height from soil level to the top node.

(c) Total number of live leaves/plant.

In the first experiment, the minimum dew period requirement for *KP1D1* was established. Plants were subjected to dew periods of 15-31 h. The results are represented as a mean disease score for each dew period (Fig. 1). It can be seen that a minimum requirement for free water on the leaf surface, to achieve maximum infection, lies between 23 and 27 h. Less than 19 h results in little or no infection. In the field situation plants rarely sustain free water on the leaf surface for such long periods (except during rain and then, particularly with grass-leaves, most of it will run-off). However, repeated dew periods at night of 8 h or more occur regularly. Thus, the effect of repeated, shorter dew periods, with intermittent periods of complete drying out of the leaf surface, on the ability of *KP1D1* to infect the weed was investigated.

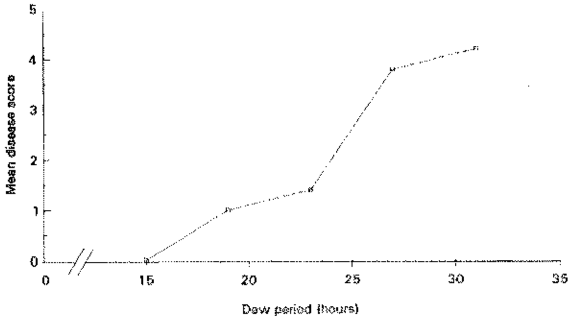

Figure 1. The effect of dew period on disease score of *Rottboellia cochinchinensis* inoculated with *Colletotrichum* isolate *KP1D1*.

Plants were subjected to a first dew period of 8-23 h, and then removed and allowed to dry for at least 12 h during daylight. They were then replaced in the dew chamber for periods of 8 h until each treatment had exceeded 27 h total dew. The results (Fig. 2) are represented as a mean disease score for each treatment. It is clear that the critical first dew period, in order to obtain a high level of infection (>3 disease score), is 15 h, with little increase in disease score after that time. From microscopic anaylsis of the inoculated leaf surface using the Bruzzese and Hasan (1983) staining technique, large scale appressorial formation is achieved by 15 h. Presumably, the appressoria are able to tolerate drying out and can continue the process of infection during the following dew periods.

A final experiment was carried-out to discover the optimum age of *R. cochinchinensis* for inoculation; i.e., the age at which *KP1D1* would be the most debilitating to the weed. Five growth stages were used, defined by the leaf stage of the plants, and ranging between: 0/1 (plants just emerged, some with coleoptile intact and others with expanding first leaf) and 5/6 (five fully expanded leaves and all stages up to 6 fully expanded leaves). Test plants were removed from the dew chamber after 27 h. The recorded data (disease score, plant height and leaf number) for inoculated and control plants within each plant age category were compared using Mann Whitney U-tests. The mean disease score was similar for all ages of inoculated plants (3.00 to 3.25), but only age category 2 contained severely infected plants belonging to 4 and 5. Mean height of inoculated and control plants are given in Figure 3. This shows that height was only significantly reduced by inoculation for age category 2 ($Z = 2.700$, $P<0.01$). In category 5/6, the inoculated plants were significantly taller than the controls but also appeared etiolated; i.e., they appeared to be compensating for leaf area loss by increased growth towards the light. Figure 4 gives mean number of live leaves per plant. All age groups showed a significant reduction in the number of live leaves due to inoculation ($Z = 2.138$ to 3.059, $P<0.05$ to $P<0.01$). However, this leaf kill only appeared to reduce plant vigour when inoculation was carried out at the 2-leaf stage.

It is clear from these experiments that the control of *R. cochinchinensis* using *KP1D1* is not going to be a robust system unless formulation can reduce some of the constraints. The pathogen has a long dew period requirement even when multiple dew periods are used, and complete kill of a high percentage of the seedlings is not achieved at any growth stage. However, the natural dew period can be extended by early application of the spores, i.e. just before dark, and severe debilitation of the seedlings may be sufficient to adequately reduce their effect on crop growth.

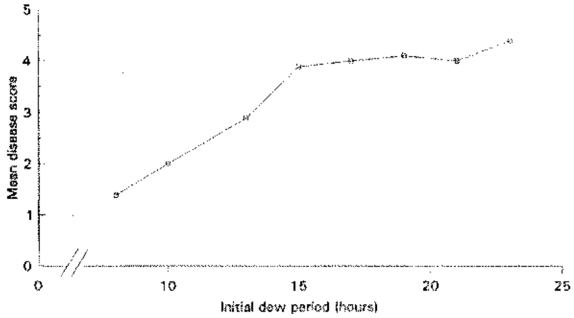

Figure 2. The effect of initial dew period, in combination with successive dew periods, on the disease score of *Rottboellia cochinchinensis* inoculated with *Colletotrichum* isolate *KP1D1*.

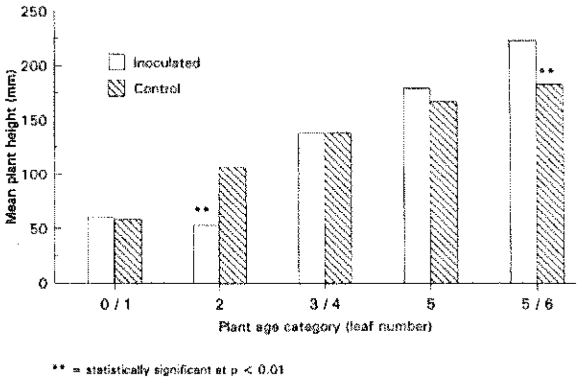

Figure 3. The effect of age of *Rottboellia cochinchinensis* plants at inoculation with *Colletotrichum* isolate *KP1D1* on subsequent plant height.

Field Testing

Two series of small-plot field trials were carried out at the National Corn and Sorghum Research Centre (Suwan Farm), Pak Chong, in the North-East of Thailand, during the rainy season (April to November) of 1990 and 1991. The aims of the trials were primarily to test the efficacy of the selected *Colletotrichum* isolate in the field situation and, where necessary, to manipulate the host-pathogen environment, using different formulations to achieve control of *R. cochinchinensis*.

The pathogen screening documented in the previous section had resulted in the selection of *Colletotrichum* isolate *KP1D1* for field testing. This isolate was highly selective and had demonstrated an acceptable level of virulence towards *R. cochinchinensis* (ex Thailand). However, the selection of this isolate was, to some extent, a trade-off between virulence and selectivity. Those fungal isolates that showed the highest levels of virulence towards the weed were invariably the least selective (i.e., those from the genus *Cochliobolus*). Even those isolates that were screened within the genus *Colletotrichum* showed a similar pattern.

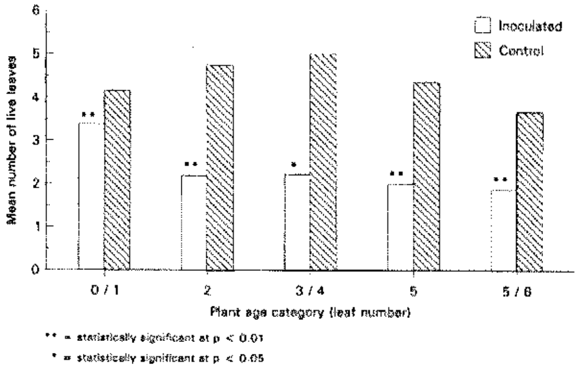

Figure 4. The effect of age of *Rottboellia cochinchinensis* plants at inoculation with *Colletotrichum* isolate *KP1D1* on subsequent number of live leaves.

(a) 1990 trials. Plots were sown with *R. cochinchinensis* seed, to achieve a typical level of weed infestation, and pearl millet at standard planting density. When the weed had reached the 2-leaf stage, inoculation was carried-out using a Herbeflex ULV sprayer (Micron, UK). A basic formulation was used to carry the *Colletotrichum* spores (produced on solidified Richard's V8 agar [TeBeest 1988]), which was composed of the organic sticker gum guar, and the surfactant (sorbitan ester) Tween 85. During the inoculation and infection period, conducive environmental conditions prevailed, and within one week lesions had developed over the entire inoculated leaf area. However, infected plants were still able to out grow the pathogen. Statistical analysis of the data recorded for plant growth 6 wks after inoculation, resulted in no significant difference between inoculated and control plants. In addition, there was no difference in crop yield.

(b) 1991 trials. It was clear from the 1990 trials that *KP1D1* was going to require more specialised formulation to enable it to control *R. cochinchinensis* in the field situation. Wymore and Watson (1989) demonstrated that a synergistic response on the control of a weed could be achieved between a sub-lethal dose of a herbicide and a fungus. This approach to enhancing the effect of *KP1D1* was extensively investigated at LARS. A large number of herbicides (at various concentrations) were screened for effect on spore germination and appressorial formation (Martin, A.D.E., personal communication, 1991). Those showing the least toxicity to the spores were screened on *R. cochinchinensis* plants in a tank mix with isolate *KP1D1*. The most promising were included in the formulations tested in the 1991 trials. These were DPX E9636 (Du Pont) and Fluazifop-P Butyl ("Fusilade" 5, ICI).

The primary dew period requirements of *KP1D1* (15 h) also proved to be limiting. Although such dew periods did regularly occur at the field site, they were not predictable. Quimby *et al.* (1990) showed how the use of invert emulsions can replace the dew period requirement in the biological control of sicklepod. A series of oil emulsions developed by LARS (Martin, A.D.E., personal communication, 1991) from those described in the aforementioned paper, were screened to assess their effect on the pathogen. Those formulations including the non-ionic surfactant Adherbe and soybean oil were tested in the 1991 field trials and found to enhance the pathogenicity of *KP1D1*.

However, due to adverse climatic conditions at the time of inoculation, and technical problems concerning the correlation of herbicide dosage, it was not possible to achieve full control of *R. cochinchinensis* in the field.

The Potential of *Sphacelotheca ophiuri* (Head Smut) as a Classical Biological Control Agent

S. ophiuri was the only non-necrotrophic species found on *R. cochinchinensis* in the Asian areas surveyed. It has not been recorded from the New World and thus potential exists for its introduction as a classical release agent. Detailed studies had previously established that the smut infects germinating seeds via teliospores which had been shed into the soil the preceding season. The infection is systemic, with virtually no seed set from infected plants. *R. cochinchinensis* has a seed bank duration of only 3-4 yrs, and a high percentage of the seeds germinate in the first year, after an 8-month enforced dormancy (Thomas 1972). Hence, a significant reduction in the weed would be observed after the first year. In addition, evidence suggests that smutted plants have reduced vigour (Ellison and Evans 1990) and thus may be less competitive in a cropping system.

Screening

The host-specificity of *S. ophiuri* (ex southern Thailand) to *R. cochinchinensis*, was investigated in this primary screen. Teliospores were shaken from the infected seed heads and dispersed in rain water to give a concentration of approximately 1 x 10^6 spores ml^{-1}. This inoculum was applied to pots of test seeds, as a soil drench. The test plants comprised: *R. cochinchinensis*, biotypes from Thailand, Zimbabwe and Honduras; maize, varieties *SU 1* and *KC 3002* (Thailand), Amarillo Carbonal (Central America); upland rice (local Thai variety); sorghum, *SU 630* and loose panicled variety, *SU 203* (Thailand); pearl millet, *KU 01* (Thailand) and finger millet, *B3* (Thailand). Plants were checked for signs of smut infection at flowering.

No crop plants showed any symptoms of smut infection. Only the biotype of *R. cochinchinensis* from Thailand became infected, with 80% of the plants producing smutted heads only. As with *Colletotrichum*, intraspecies specificity was demonstrated. Further screening is now required to test isolates of smut from Sri Lanka and Kenya against the South American biotypes. Evidence from the *Colletotrichum* screening work suggests that the smut isolates from Sri Lanka should attack the New World biotypes.

Effect of Teliospore Concentration in the Soil on Percentage Infection of R. cochinchinensis

Although 80% infection of *R. cochinchinensis* plants (ex Thailand) was observed from the pot experiments, it was important to establish whether such high percentage infection occurs at lower concentrations of spores in the soil. Three concentrations were tested; 1 x 10^6, 10^5 and 10^4 spores ml^{-1}. Figure 5 indicates that there may be a clear linear relationship between concentration and percentage infection.

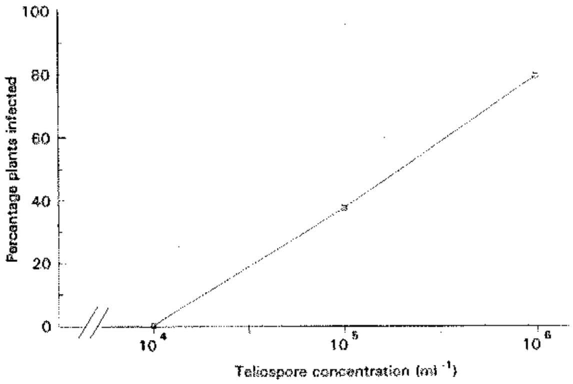

Figure 5. Percentage infection of *Rottboellia cochinchinensis* in relation to spore concentration of *Sphacelotheca ophiuri* applied to the soil.

From these results, it appears that *S. ophiuri* may have limited potential as a classical biological control agent due to exceptionally high inoculum requirements in the soil. The spread of the pathogen from a centre of release would be slow and it would take many seasons to build-up in a population of *R. cochinchinensis*. Indeed, field observations agree with these conclusions; the smut naturally occurs in patches, often with most of the plants in one patch being infected. However, it may be possible to use the smut as a mycoherbicide. Although a hemi-biotroph, the smut forms teliospores which germinate readily on agar to produce sporidia indeterminately. If the sporidia were found to be infective, then it may be possible to mass produce them and apply a formulated inoculum to the soil.

When investigating the smut as a biological control agent, it is also essential to consider the population dynamics of *R. cochinchinensis*. Although an 80% reduction in seed set could be achieved in a population, this may not actually affect the density of the weed population the following year.

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