Mycoherbicidal control of *Rottboellia cochinchinensis*: a viable alternative?

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

Summary

Itch grass (*Rottboellia cochinchinensis*) is an Old World weed now with a pantropical distribution. Because of its increasing economic importance, this weed was selected as the candidate in a project to develop a mycoherbicide that could be used throughout the tropics. The original terms of reference and choice of target weed, placed a number of constraints on the project, which are discussed in the context of the research findings. The hope of obtaining a single fungal isolate that could be used in any *R. cochinchinensis* infested area, proved to be naive, as the weed shows high biotype variation between countries, with varying levels of susceptibility to the different isolates. The results from the screening also demonstrated a clear correlation between high pathogen virulence and low specificity to *R. cochinchinensis*. However, isolates of the genus *Colletotrichum* from Thailand proved to be the most promising (high selectivity and adequate virulence), and a series of small-plot field trials were conducted in Thailand to test two of the best isolates. The results from the field trials were variable, due in part to adverse environmental factors, the presence of an exotic biotype at the field site and an exceptionally vigorous weed. A synergistic response, between the *Colletotrichum* isolates and a sub-lethal dose of a chemical herbicide, was demonstrated in the glasshouse. This appears to be the most promising method of using pathogens to control the weed.

Introduction

Rottboellia cochinchinensis (Lour.) W.D. Clayton (= *R. exaltata* L.f.) (Gramineae: Andropogoneae) or itch grass, is an Old World weed, with increasing importance throughout the tropics (Terry 1991). It infests both annual and perennial cropping systems but in subsistence agriculture can be relatively successfully controlled by hand-hoeing (Sharma and Zelaya 1986). However, as agricultural practices have intensified, with an increased reliance on herbicides, *R. cochinchinensis* has become more important, particularly in graminaceous crops, where it is demonstrating an increasing tolerance to the limited number of herbicides that can be used (Thomas 1972).

Due to the increasing threat of this weed to both small- and large-scale tropical agriculture, *R. cochinchinensis* was selected as the target weed for a U.K.-Overseas Development Administration-funded project to investigate the potential of mycoherbicides as an alternative strategy for control of tropical weeds. Investigations in this area of weed control have tended to target dicotyledonous weeds (Templeton 1982), despite the fact that eight out of the ten world's worst weeds are grasses (Holm *et al.* 1977). This reflects, perhaps, the innate difficulty in controlling graminaceous weeds (Evans 1991). The original terms of reference of the project were to develop a mycoherbicide against *R. cochinchinensis*, that could be used throughout the tropics, and be produced locally at the cottage industry level.

This is a collaborative project between the International Institute of Biological Control (IIBC) and Long Ashton Research Station (LARS), Bristol. The role of the latter organization was to develop the formulation in which to apply the selected fungal isolate and to investigate the mass production of inoculum. The preliminary investigations carried-out by IIBC are summarized in Ellison and Evans (1990). This paper discusses the IIBC input into the project, in the light of the problems encountered, and the feasibility of using mycoherbicides as a method of control for *R. cochinchinesis*.

Field surveys and screening

Interspecies selectivity within the Gramineae

During the course of this project extensive surveys for fungal pathogens were carried-out in both the Old and New World. More than 200 necrotrophic isolates, from 13 different genera, were passed through a primary screen, which consisted of major graminaceous crop plants and a range of biotypes of *R. cochinchinensis*.

The screening for fungal isolates specific to a graminaceous weed (*R. cochinchinensis*) in graminaceous crops, proved to be equally as difficult as the screening of chemicals for selective herbicidal compounds in such cropping systems. However, unlike chemical herbicides (that can be recommended for use in a limited number of crops), and in contrast to all previous mycoherbicide programmes (TeBeest and Templeton 1985), the selected fungal isolate against *R. cochinchinensis* had to be rigidly host specific, since the product is intended for use outside of its natural range. Hence, in this programme, a necrotrophic fungus would have to be treated as a classical release agent (which traditionally are biotrophs, such as rusts), with equally stringent screening requirements. These are not necessary for mycoherbicides registered for use in their area of discovery. This whole concept of considering mycoherbicide pathogens for exotic introduction has yet to be fully addressed by regulatory authorities. Indeed, movement of mycoherbicides outside of the region of endemic occurrence of the pathogen, is generally considered as one of the risks involved in the development of such products, rather than a possible progression of the programme (Leonard 1982, Weidemann 1991).

Maize (*Zea mays* L.) proved to be the most problematic species within the screening phase of this project. Many fungal isolates were found that infected only *R. cochinchinensis* and maize out of the species tested. This is perhaps not surprising due to the evolutionary closeness of the two genera involved (Clayton and Renvoize 1986).

The results of the screening were sometimes difficult to interpret. *Sorghum* spp., for example, are very sensitive to any foreign body placed on their leaves, often giving a massive hypersensitive response, that can be mistaken for infection. To help interpret these ambiguous cases, the Bruzzese and Hasan (1983) staining technique was used to investigate the plant-pathogen response at the microscopic level. Maize, as previously discussed, was the key screen species. Microscopic examination of the leaf surface of some maize varieties, inoculated with certain isolates of *Colletotrichum* from *R. cochinchinesis*, revealed that the spores were actually penetrating the leaf surface, but the infection hyphae were prevented from further development by the formation of an encircling callous. Macroscopically, this would be seen as a yellowing of the leaf and tip die-back, when a high concentration of spores was applied.

An added complication was the phenomenon of latent infection (Cerkauskas 1988). In some test species, sporulation of the test pathogen was observed on dead leaves. However, this was not considered or recorded as a positive result, since no primary infection had occurred. The spores had survived on the leaf surface until senescence, probably as melanised appressoria, after which saprophytic invasion of the necrotic tissues occurred.

Isolate virulence

Although rigid specificity to *R. cochinchinensis* was required for any candidate fungal isolate, virulence was of equal importance; the mycoherbicide had to be capable of severely debilitating the weed. Unfortunately, the results of the screening demonstrated a clear correlation between high pathogen virulence and low specificity. However, a number of isolates from the genus *Colletotrichum* were found to be sufficiently virulent as well as possessing high levels of specificity to *R. cochinchinensis*. This genus was only collected in South-East Asia, the purported centre of origin of the weed (Holm *et al.* 1977), and thus predictably where the most highly co-evolved (and thus selective) isolates would be found. Fungal isolates from the New World, although capable of consistently killing seedlings of the weed (particularly species from the genus *Curvularia*), were extremely unselective. Presumably, pathogens on the weed outside of its native range would have "jumped" from other graminaceous hosts.

Thus, in cases where mycoherbicide projects target exotic weeds using indigenous fungi, there is less chance that a host-specific fungal isolate will be found, as the mycoflora will have originated mainly from the resident flora. For example, with the control of johnsongrass (*Sorghum halepense* [L.] Pers.) (Chaing *et al.* 1989) and goosegrass (*Eleusine indica* [L.] Gaertn.) (Figliola *et al.* 1988) in the USA, no totally selective fungal isolates were found.

Intraspecies selectivity within R. cochinchinesis

R. cochinchinesis occurs over its tropical range as a number of morphologically and/or physiologically different biotypes (Fisher *et al.* 1987, Millhollon, R.W., personal communication, 1991). These biotypes demonstrated variable susceptibility towards *Colletotrichum*; different isolates of the fungus were capable of infecting different combinations of biotypes. Hence, the original project aim of trying to find one fungal isolate that could be used in any *R. cochinchinensis*-infested area, proved to be naive.

Field trials

A series of small-plot field trials were conducted at the National Corn and Sorghum Research Centre (NCSRC), Pak Chong, in the North-East of Thailand, during the rainy season (April to November) of 1990 and 1991. These trials were carried out to test the efficacy of the two most promising *Colletotrichum* isolates (KP1D1 and WM4), both of which had originally been collected in Thailand. The initial trials, conducted using natural infestations of the weed and isolate KP1D1, were unsuccessful, only a hypersensitive response had been elicited on the seedlings. It was later discovered that the biotype of the weed infesting the field plots was not the same as the native biotype from which the test isolate had been collected. It was, in fact, an exotic that appeared to be very close in type to the New World biotypes. The origin of the introduction is unknown, but, since the NCSRC is used for new variety testing by a Central American based international plant breeding institute, it could have entered in contaminated seed lots.

Subsequent trials were more successful. These used either sown seed of the native biotype, sprayed with the KP1D1; or natural infestations of the exotic biotype, inoculated with WM4, which was selected for virulence on this biotype. When conducive environmental conditions prevailed, good field infection was achieved, with all the inoculated leaves dying prematurely. Unfortunately, the protected meristem escaped infection and this enabled the majority of the seedlings to outgrow the pathogen; a feature of grasses that make them particularly difficult targets for herbicides.

Formulation

Because the field plots were located on an exposed site, test plants were subjected to periods of drought, hot temperatures (>40°C) and strong winds, cycling with heavy monsoon rain storms, which in 1990/91 were the driest on record. Thus, the timing of the mycoherbicide application was critical. It had to coincide with an adequate dew period (most reliable when the soil was wet), but not immediately prior to a rain storm, as the spores may be washed-off the leaves before they had time to adhere. To some extent the LARS formulations in which the fungal isolates were applied, were able to reduce the dependency on climatic conditions. The most successful formulation tested was based on an oil-in-water emulsion (Quimby *et al.* 1988), mixed with an organic sticker (gum guar). This helped reduce run-off from the leaves and length of dew period requirements.

Synergism

From these initial trials, the fungus was found to be insufficiently virulent in the field to provide an acceptable level of weed control. Hence, a synergistic approach was investigated (Wymore and Watson 1989), whereby a sub-lethal dose of a chemical herbicide was included with the fungal formulation. Glasshouse investigations gave promising results; complete kill of seedlings was attained, which neither fungus nor low-dose chemical herbicide alone could achieve.

Discussion and conclusions

The prospects of controlling *R. cochinchinesis*, on a pantropical scale, with a single mycoherbicide product, does not appear to be a viable proposition. The biotypic variation of the weed makes the use of a single product invalid, regardless of the quarantine restrictions of using an exotic, necrotrophic fungus. However, on a regional scale, this approach to control of the weed appears promising. Local fungal isolates would have to be used, and screened, but the specificity requirements of the candidate fungi would not be so stringent. The commercially produced mycoherbicide Collego® (Upjohn Company, Kalamazoo, MI), for example, is known to infect certain varieties of English pea (*Pisum sativum* L.), lupin (*Lupinus densiflorus* Benth.), broad bean (*Vicia faba* L.) and perennial sweet pea (*Lathyrus* sp.) (TeBeest 1988), but is not used in areas where these species are grown. Jiménez *et al* (1990) have also been looking at the potential of mycoherbicidal control of *R. cochinchinensis* in Central America, using local isolates of *Fusarium moniliforme* Sheld.

During the screening of pathogens from *R. cochinchinensis*, a number of near host specific, highly virulent isolates were rejected. For example, a promising, undescribed *Diaporthe* sp. (*Phomopsis* anamorph), found in Kenya, was rejected due to its ability to infect some varieties of maize but no other test plant species. It may be possible to use this isolate in Kenya, specifying which maize varieties it should not be used in. In addition, an isolate of *Curvularia* from Somalia, was highly virulent to *R. cochinchinensis*, killing the plants in a matter of days, presumably due to the production of a toxin. It was also able to infect maize, but the plants readily recovered from the damage. It may be possible to isolate the toxin and use this as a conventional chemical herbicide.

The concluding message is that the isolates of *Colletotrichum*, which were field tested in Thailand, cannot, on their own, offer a viable control strategy for *R. cochinchinensis*. There has been a definite "trade-off" during their selection between specificity and virulence towards this highly vigorous weed. However, their potential when applied to seedlings of the weed together with a chemical herbicide, at a sub-lethal dose, appears promising, and merits further investigation.

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