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Pathogen–Weed Relationships: The Practice and Problems of Host Range Screening

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From a comparison of host range screening involving potential agents for the biological control of weeds, evidence is presented to show that the test requirements for fungal pathogens are significantly stricter than for insects. As a direct consequence, pathology projects are much more expensive both in time and resources. As a positive spin-off, however, such intensive screening is producing a wealth of new information on some aspects of pathogen-plant relationships which have been little investigated in conventional plant pathology. Weed pathology projects in contrast to crop pathology, offer the opportunity to explore the factors determining host-specificity in a disparate range of plant species. A critical examination of pathogen-plant interactions at the cellular level has identified at least 5 mechanisms conferring resistance or immunity to infection: lysis of spores on plant epidermis (no germination); distortion of germ-tubes (abnormal germination); plasmolysis of infection structures (appressoria); isolation of penetrant hyphae or intercellular mycelium by polyphenol or callose production; and plasmolysis or isolation of feeding structures (haustoria). These mechanisms are discussed using, by way of example, pathosystems involving the rusts *Puccinia abrupta* var. *partheniicola* and *Maravalia cryptostegiae* , potential biocontrol agents of *Parthenium hysterophorus* and *Cryptostegia grandiflora* respectively. Problems relating to the interpretation of results from host range tests, carried out under controlled environmental conditions, and their relevance to pathogen behaviour in the field are also discussed, with particular reference to the tropical weeds: itch grass (*Rottboellia cochinchinensis*), rubber-vine (*Cryptostegia grandiflora*) and Parthenium weed (*Parthenium hysterophorus*).

Introduction

"The critical phase of biological control work against weeds is the selection of species that will not harm other plants, or at least not useful plants. All other considerations are subordinate ..." (Williams 1954). This has since been quoted by Leonard (1982), in addressing the problems and potential hazards involved with using pathogens for weed control, and sets the scene for the central role that host range screening plays in any biological control project. Nevertheless, it was not until more recent times in the history of biological control that a script or protocol for selecting test plants was made available and a more scientific approach to host range screening was adopted (Wapshere 1974 1975). This so-called centrifugal phylogenetic method was later re-assessed, based on the experiences of the intervening 15 yrs, and following numerous examples of insect control agents expanding their host ranges under artificial conditions (Wapshere 1989). Modifications in the testing sequence were proposed in order to reduce the chances of rejecting potentially useful biological control agents. Wapshere *et al.* (1989) also reiterated the Orwellian-like adage close to heart, if one views it as the Ancient Mariner's albatross, or the brain, in its Damoclean content, of all biological control workers: "... agents must not attack any cultivated or socially (aesthetically) important plant in the region of introduction." Haunted by such spectres, those involved with

host range screening are subjected to more than their fair share of self-doubts centering around the inglorious consequences if a released agent should change its spots. Apart from a much-publicised but relatively minor problem with an insect agent in East Africa, imported for control of *Lantana camara* L. (Verbenaceae) which would have been averted using the centrifugal phylogenetic testing strategy, there have been no disasters to hinder the progress of biological control of weeds and to provide extra ammunition for the opposition.

Host range screening has figured prominently in previous *Symposia on Biological Control of Weeds* but, all too often, those involved with drawing-up legislation have been viewed in a "them and us" situation (Harris 1985, Freeman and Charudattan 1985). This could be interpreted as paranoia on the part of the biological control faction, but there is evidence that more legislative obstacles are being placed in the way of biological control projects, particularly those involving weed pathogens, despite their clean and often spectacularly successful track record (Evans and Ellison 1990). Harris (1985) reviewed these difficulties and concluded that the problems were increasing due to ill-founded skepticism about biological control on the part of pressure groups and the authorities responsible for approving the release of agents. Whilst Freeman and Charudattan (1985), at the same *Symposium*, specifically highlighted the prejudice against pathogens on the part of quarantine authorities: "Despite the lack of documented serious conflicts, there is an air of pathophobia that has brought to a virtual standstill the application of the classical approach in the use of plant pathogens for weed control". There is no doubt that these *Symposia* offer an ideal forum to try to resolve the controversial issues surrounding host range screening and to establish guidelines which would satisfy all parties concerned and hence direct more efforts (and funds) at the real enemy: invasive weeds.

At the last *Symposium*, several papers dealt exclusively with problems relating to host-specificity testing, logistical as well as scientific, but only in the context of insect agents (Cullen 1990, Shepherd 1990). However, more recent events give cause for optimism and it would seem that critical problems are being approached and analysed from a scientific rather than an emotive standpoint. The present paper aims to review the actual and potential problems encountered by pathologists practising host range screening for the biological control of weeds, and to illustrate these with examples from on-going projects.

Historical Problems (or Insects *versus* Pathogens)

Since the concept of using pathogens to control weeds is a novel approach, compared to using insects, the practices and priorities have been established by entomologists. Pathologists have built on this framework but the structure is still evolving. The master plan has not been finalised because pathogens are viewed in a much more suspicious light by legislative authorities, and by the public at large, than are insect biocontrol agents. This is rooted in fears, often out-dated and misdirected, about mutation, biological warfare and, of course, the spectacular and historically important plant disease epiphytotics which have affected the socio-economic infrastructure of many countries (Large 1940). The safety requirements and, therefore, the time frames, and hence costs, of weed pathology projects are often considerably more than for equivalent insect projects (Evans and Ellison 1990). By way of example, for Australian weed projects involving insects and classical biological control strategies, the bulk of the host range screening work is carried out under quarantine in Australia, with only minimal testing in the country of origin. In contrast, all host range screening with pathogens is performed in the indigenous area or in a "third party" country. Are pathogens better at escaping from quarantine facilities than insects?

The purported "safer" option offered by insect agents means that weed projects are entomologically-biased from the start and that pathogens are turned to as a last resort when the insect control agents have been fully evaluated. In reality, therefore, the financial inputs into any weed pathology programme are secondary to the main project budget and frequently exhausted before the true potential of

pathogens has been realised: in short, agents are cut. Moreover, the pathologist is faced with an increased number of test plants to be screened, compared with the equivalent list for insect agents, in order to satisfy the official pathophobia identified by Freeman and Charudattan (1985). For example, screening of insect agents for control of Parthenium weed (*Parthenium hysterophorus* L.; Heliantheae) involved the testing of 19 plant species in Mexico (McClay 1985) and a further 50 species in Australia (McFadyen 1985). Conversely, more than 90 plant species have been screened in the UK against Parthenium rust, including 17 cultivars of sunflower (Tomley 1990). Similarly, in the host-specificity tests involving seed-feeding bruchid species (*Acanthoscelides* spp.; Coleoptera: Bruchidae) for control of *Mimosa pigra* L. (Mimosoideae) the number of test plants was 73 species (Kassulke *et al.* 1990). The list for 2 fungal pathogens is now approaching 92 species (Forno, I.W., personal communication, 1991). I rest my case, but would add a final plea that future projects should include in the initial proposal, a pathology as well as an entomology component. In this way, the logistics of the host range tests, in addition to the initial natural enemy surveys, can be shared, the costs reduced and a much more balanced approach adopted to the biological control of weeds.

The Pathogens Involved

Before detailing the types of fungi which have or are being evaluated in host range screening, it is worthwhile to dispel some of the myths surrounding plant pathogens and their host ranges. Most plants are resistant (or more accurately, immune) to most pathogens and only a fraction of micro-organisms have achieved a pathogenic relationship with a plant host. In this compatible interaction, the pathogen has evolved a complex series of mechanisms to enable successful invasion and colonisation of its host. "It is therefore not likely for a pathogen to gain access to new plant hosts through a few mutations, since basic compatibility is thought to be controlled by many genes encoding pathogenicity factors" (Scholtens-Toma *et al.* 1991).

Classical Biological Control

"All agents used in classical biological control show a degree of specialization to their host, whether to genus, species or variety, produced by coevolution of host and natural enemy over a long period of time" (Wapshere *et al.* 1989).

1. Rust fungi: (Uredinales). "The rust fungi are potentially dangerous plant disease organisms" (Gold and Mendgen 1991). However, since the rusts are the most highly evolved of the plant pathogenic fungi they tend to have a highly specialised parasitic life style concomitant with a restricted host range. This character, together with their ability to cause significant damage to their host plants and the possession of efficient spore dispersal mechanisms, make the rust fungi ideal classical biological control agents. Leonard (1982) observed that natural mutations occur regularly in rusts and serve to extend the host range to other cultivars within the host species but concluded that there is no danger of rusts adapting to new hosts, quoting by way of example the skeleton weed (*Chondrilla juncea* L.; Asteraceae) rust (*Puccinia chondrillina* Bubak & Sydenham): "... it is unlikely that simple mutation would be sufficient to extend the host range of *Puccinia chondrillina* Bubak & Sydenham to species other than skeleton weed." The success of this rust has, of course, been spectacular; the rust demonstrating rigid host-specificity during host range testing (Hasan 1972), which was more than realised under field conditions (Cullen and Hasan 1988). Paradoxically, the rust has been almost too specific.

Faced with this pioneering and, to date, the only well-monitored example of classical biological control with rust fungi, why has their potential not been more fully exploited? Part of the reason lies with often conflicting information on disparate and expanding host ranges, especially during screening for host-specificity under artificial conditions. Watson (1985) listed 4 rust species which, during greenhouse screening, infected plant species outside of their normal field range. This has been termed induced susceptibility, the "non-hosts" being predisposed by a combination of optimum conditions for the pathogen and massive

(unrealistic) inoculum levels. In essence, such results can be considered to represent artifacts of the screening programme, although the possibility exists that this represents a new encounter, the pathogen and host having an allopatric distribution.

Bruckart *et al.* (1985) reported the susceptibility of artichoke (*Cynara scolymus* L.; Asteraceae) to the musk thistle rust (*Puccinia carduorum* Jacky) under greenhouse conditions. It was concluded, however, that the low inoculum production (almost 50 times more pustules and 70% larger pustules on its natural host), and the low efficiency of infection, minimises the chances that an artichoke strain will be selected in the field. Indeed, the musk thistle rust has never been found on artichoke in Eurasia where the plant species are sympatric. Similarly, Mortensen (1985*a*) demonstrated an extended host range within the Asteraceae for *Puccinia jaceae* Otth., a potential control agent of diffuse knapweed (*Centaurea diffusa* Lam.). The rust attacked 4 cultivars of safflower (*Carthamus tinctorius* L.) in the host range tests, with fully mature pustules developing only on the cotyledons. However, it was considered that the effect of *P. jaceae* on the development of safflowers was minimal and, moreover, that the rust had little chance of surviving on this plant in the field. Later, Hasan *et al.* (1990) found that bachelor's button (*Cyanus segetum* [L.] Hill) was highly susceptible to this rust species in field trials in Europe. The release of the pathogen in North America was not recommended. This proved to be premature since the rust was discovered subsequently in Canada (Mortensen *et al.* 1989). These authors concluded, however, that the rust presented no problem to crop or garden plants and should be artificially disseminated for biological control of diffuse knapweed instead of attempting to eradicate it. The progress and natural host range of this rust under North American conditions will be interesting to monitor.

Such results from host range screening tests led Adams (1988) to state: "It could thus be argued that the potential benefits of biological control using such agents are not being realised because of the unrealistic findings on host range obtained under controlled environmental conditions." However, a more unusual example, not normally associated with classical biological control is that of groundsel rust, *Puccinia lagenophorae* Cooke. This distinctive rust was unknown in the UK before 1961 and, following its sudden appearance and rapid spread on groundsel (*Senecio vulgaris* L.; Asteraceae), it was later identified as an Australian native, occurring on a range of Asteraceae in Australia (Wilson *et al.* 1965). Daisy, marigold and cinerarias were experimentally infected in the greenhouse and in small-scale field plots in the UK. Concern was expressed about the possible dangers to garden plants posed by the exotic pathogen and, in particular, it was concluded that "... it could be troublesome on greenhouse cinerarias unless measures are taken to eliminate the groundsel carrier" (Wilson *et al.* 1965). Ironically, nearly 30 yrs since its arrival, few records of the rust have been made on anything by *S. vulgaris* (Henderson and Bennell 1979, IMI Herbarium) and, *P. lagenophorae* has proven to be an exceptionally efficient biological control agent of this weed in the UK. Experiments have shown that weed competitiveness is significantly reduced when the rust is present, lettuce yields in rusted plots being 2-3 times greater than in plots with healthy groundsel (Paul and Ayres 1987). Based on the initial host range screening results, particularly the infection of "socially (aesthetically) important plants," *P. lagenophorae* would never have been approved as a biological control agent.

2. Other Fungi. Pathogens as classical biological control agents have rarely been sought for or investigated outside of the rust fungi (see Watson 1991, for lists of fungi released or under consideration). However, one successful example is that of the white smut, *Entyloma ageratinae* Barreto & Evans (Ustilaginales), used for control of mistflower (*Ageratina riparia* [Regel] K. & R.; Eupatorieae) in Hawaii (Trujillo 1985). Like the rusts, this is an obligate biotroph, which, in consequence, has many of the characteristics which embody the ideal classical biological control agent. Ironically, *E. ageratinae*, was initially described as a *Cercosporella* sp. (Deuteromycetes), and, presumably, considered to be a relatively unspecialised facultative pathogen (Barreto and Evans 1988). It was introduced into Hawaii after a relatively short period of host range screening

against 40 plant species in 29 families (Trujillo 1985). This is a somewhat short and disparate test list, compared with the requirements quoted earlier for other pathogens. Indeed, the number of test species would have to be increased significantly if permission were sought to import the smut into Australia (Tomley, A.J., personal communication, 1991). This, of course, is despite the fact that the pathogen has not been reported on any other plant apart from the target weed in Hawaii after nearly 20 yrs in the field.

Facultative pathogens belonging to Coelomycete genera, such as *Septoria* and *Phomopsis*, are being evaluated as classical biological control agents (Morris, M.J.; Shivas, R.G., personal communication, 1991). But, because of their non-selective nutritional status and relatively inefficient spore dispersal mechanisms, questions may well be asked about their suitability, in terms of specificity (genetic stability) and effectiveness in the field. IIBC is currently investigating the potential of a new Coelomycete species for control of *M. pigra*. The test plant list has been modified, and considerably lengthened compared with that for insect agents (see earlier), according to the taxonomic position of this anamorph. However, on the basis of conidiogenesis–often an inexact character–it could also be accommodated in the genus *Phloeosporella*. Based on the morphology of the teleomorph, however, it must be assigned to the genus *Phloeospora*. Thus, without the discovery of the teleomorph, the generic placement, and hence the host range test list, would be significantly different. Doubts have already been raised in Australia concerning the use of a facultative pathogen as a classical biological control agent. Nevertheless, this pathogen shows exceptional promise and, nutritionally, it can now be firmly categorised as a hemibiotroph, since it demonstrates a long period (up to 4 wks) of biotrophic (symptomless) association with the host plant before switching to the necrotrophic phase. This indicates a long association, in evolutionary terms, with its host and a highly advanced (specific) nutritional state. Thus, in order to avoid rejection of a selected pathogen as a classical biological control agent, and to satisfy the legislative authorities in the target country, complex and often new aspects of plant-pathogen relationships have to be investigated and clarified.

Mycoherbicides

Most fungi being used or considered for mycoherbicide development are facultative saprophytes with relatively wide host ranges. The term "sufficiently safe" has been used to describe the host-specificity requirements of these pathogens (Wapshere 1982). Leonard (1982) reasoned that the potential problems of using plant pathogens as biocontrol agents are by-passed in the case of mycoherbicides by selecting endemic species. These can be further reduced by employing pathogens with limited ability for long distance dispersal. Indeed, those genera currently being exploited as mycoherbicides (e.g., *Phomopsis, Colletotrichum*) have poorly-disseminated slime spores which pose little contamination threat to neighbouring crops. Many criticisms have still been voiced about the wisdom of using fungi closely related to dangerous crop pathogens. It has been suggested, for example, that the application of enormous quantities of inoculum would result in increased mutation rates and the appearance of new pathotypes attacking beneficial plants within the ecosystem. However, Leonard (1982), from a plant disease geneticists viewpoint, has dispassionately analysed the hazards: "During their long coexistence with the endemic pathogens, the native plant species have evolved to cope with the genetic variability of the pathogen. Mutations that may occur in the inoculum of a microbial herbicide are not likely to differ from those that have occurred naturally in the pathogen population." Moreover, the poor survival ability of the currently selected fungal genera and the fact that the mycoherbicide inoculum originates each year from the same strain, rather than from a naturally-selected population, completely dispels the mutation argument. Leonard (1982) did emphasise the point that adaptation tests should also be included in host range screening, citing the example of *Phytophthora infestans* (Mont.) de Bary (Peronosporales). Massive amounts of inoculum of the potato race of this pathogen after passaging (5-6 times) through tomato

leaves resulted in highly virulent tomato races. If host range screening of mycoherbicides is to be broadened by such riders then the testing programme becomes even more daunting. Balanced decisions need to be taken and, in fact, it could sensibly be argued that only minimal host range testing is necessary for "endemic" mycoherbicides. As long as the pathogen does not attack the crop plant in which weed control is being exercised, then the dangers are minimal, even if other crop plants were potential hosts. For example, the product DeVine, based on the pantropical and notorious crop pathogen, *Phytophthora palmivora* (Butl.) Butl., (Peronosporales) has been used successfully to control stranglervine (*Morrenia odorata* [H. & A.] Lindl.; Asclepediaceae) in the southern USA (Ridings 1986). The only safety recommendation being that the product is not used within 30 m of a susceptible crop. The so-called *forma specialis* status of the Collego fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. has now been thoroughly investigated and shown to be erroneous and, inevitably, the safety of mycoherbicide has been questioned (Cerkauskas 1988). During the original host range screening, 30 plant species (including 46 crop cultivars) were tested, and the selected strain of *C. gloeosporioides* attacked only species of the weed genus *Aeschynomene* (Daniel *et al.* 1973). On the basis of these results, the strain was designated as *forma specialis aeschynomene.* Later, the list was expanded to 77 species (TeBeest 1988) and species belonging to 4 genera within the sub-family Papilionoideae proved to be susceptible. Further studies extended this to 9 genera, although only the weed host was killed (Weidemann *et al.* 1988). These results induced Weidemann and TeBeest (1990) to conclude that other factors, apart from taxonomic ones, must be considered when evaluating facultative pathogens. But does it really matter, as long as the crop plants in the target area are not put at risk? The DeVine experience would suggest that it does not.

However, the statement by Leonard (1982) that: "The greatest hazard in the use of microbial herbicides is probably not that of genetic variability, but rather the danger that the pathogen may be deliberately disseminated widely as a microbial herbicide and, consequently, introduced into regions where it did not previously occur," presents very real problems for a current IIBC mycoherbicide project. Traditionally, only endemic pathogens have been considered for mycoherbicide development but the latter project has been assessing the mycoherbicide potential of pathogens for control of itch grass, *Rottboellia cochinchinensis* (Lour.) Clayton (Andropogoneae, Gramineae), in the purported Old World centres of origin of the weed (Evans 1991). The terms of reference of the project specifically flagged developing countries in the New World, where *R. cochinchinensis* is becoming more problematical, as recipients of any product. In this case, an exotic facultative pathogen would be introduced into a region and applied as a mycoherbicide. Logic dictates that the host range screening should be as severe as that for any classical agent. Clearly, the host range of the pathogen would need to be considerably narrower than that of the Collego fungus. An apparently *Rottboellia*–specific *Colletotrichum* sp. has already been successfully screened in the country of origin (Thailand), but this may well prove to be a difficult test case if permission is ever sought to introduce the pathogen into the New World.

The Techniques Involved

In artificial screening conditions, the environment, at least during the inoculation phase, should be optimum for infection and based on pre-determined temperature and humidity parameters. The critical dew period can be simulated by a variety of methods, depending on the pathogen under investigation. At IIBC, 3 humidity simulators are currently employed:

1) A custom-built dew chamber (Clifford 1973), which is essential for screening plants, such as grasses, where conventional water droplets rarely stay on the leaves or run-off to non-lethal penetration sites, or where a particularly narrow temperature window may be required.

2) A fan-assisted, misting humidifier (Defensor).

3) A "steam-pot." This is the simplest (maintenance free) and cheapest apparatus available but, due to heat generation, tends to favour those pathogens with relatively high temperature requirements (>20°C) for infection.

With rust fungi, these conditions are necessary only during the initial infection phase (1-2 d). Subsequent growth during the incubation and sporulation period is almost independent of ambient conditions. However, with necrotrophic pathogens a period of high humidity is necessary for sporulation, or symptom expression. In the case of the *Phloeospora* fungus, mentioned earlier for *M. pigra* control, this remains in a symptomless (non-sporulating) phase unless the inoculated, susceptible plants are placed in conditions of high humidity. Thus, after an incubation period, all test plants have to be periodically transferred from the quarantine holding greenhouse (*ca.* 60-70% RH) into an inoculation chamber at 95-100% RH.

Next the unanswerable question: How many test plants are needed for each species and how many replications should be undertaken? This is left to common sense and practicality: a minimum of 4 plants, with inoculum being applied to all stages of growth, and 2 repetitions. The inoculated plants are monitored externally, with the aid of a stereoscopic microscope, and, after the appearance of symptoms and sporulation on the control weed, plants are examined internally using the now classic Bruzzese and Hasan (1983) technique. With the aid of this method, it is possible to follow pathogen behaviour within the test plants and to more accurately determine the reasons for non-host resistance or immunity. This is discussed more fully below.

Shepherd (1990) analysed the practical problems involved with host-specificity testing of insects and divided these into problems with the agent and those with the test plants. The latter are essentially similar, whichever agents are being screened. The former are more specific and, although other organisms could affect the culturing and maintenance of pathogens, hyperparasites can easily be excluded, despite the fact that they may be exerting considerable influence on the agent within the native range. There remains the possibility that a pathogen could be carrying a deleterious virus or mycoplasma and this aspect should not be overlooked. Perhaps the most severe constraint, particularly with obligate pathogens, is obtaining sufficient inoculum under artificial conditions in order to carry out meaningful tests.

One final thought concerns the feasibility or validity of repeating the host range screening in the field, as suggested by Freeman and Charudattan (1985); Wapshere (1989). More emphatically, perhaps, Watson (1985) recommended that host-specificity testing should be two-phased with the second phase being a field evaluation, presumably in the endemic range of the pathogen. This may not be possible for many classical biological control agents since a certain proportion of the test species are invariably restricted to the target area or country. Obviously, these plants can only be screened in containment facilities.

Interpretation of Results

As Heath (1982) has pointed out for crop pathology, the boundary between resistance and susceptibility to a disease is drawn at an arbitrary point along a continuous gradient of disease expression. This author also regards plants which show any infection type differing from maximum susceptibility as expressing signs of resistance. Thus, there are already grey areas, open to misinterpretation, not least of which is differentiating immunity from resistance. In both, defence mechanisms are in operation, from non-specific physical features to induced chemical processes on or within the potential host, which retard or inhibit fungal development: even if the end result is merely the production of less inoculum. In the case of obligate pathogens, and rust fungi in particular, Heath (1982) concluded that: "If multiple potential defence mechanisms, different in each plant, have to be specifically overcome before basic compatibility can be established, it is not surprising that a given rust fungus can successfully attack only a small number of plant species, or that its host range does not change significantly with time." This is the theory behind using pathogens for biological control. Host range screening is the vehicle for testing it, whilst interpretation of the results advances or

retards its acceptance by quarantine authorities and their advisers. Centrepoint to the argument against employing exotic pathogens for weed control, is the fear of host jumps. The information on this phenomenon is often conflicting but there are several well-documented examples of host jumps (or new encounters), most prominent of which, perhaps, is the guava rust, *Puccinia psidii* Wint. This rust, a native of the American tropics, quickly proved to be an important pathogen in plantations of *Eucalyptus* spp. (Myrtaceae) newly established in southern Brazil (Dianese *et al.* 1984). The genus *Eucalyptus* is, of course, uniquely Australasian in distribution. Without speculating on the antecedents of *Eucalyptus*, it can only be concluded that this is a new encounter (rather than a mutation or jump), but that this problem would have been readily identified using the centrifugal phylogenetic testing sequence. *P. psidii*, in fact, has a wide natural host range within the Myrtaceae, infecting plants in 12 indigenous genera, other than the exotic *Eucalyptus* (Marlatt and Kimbrough 1979).

Host range screening offers a unique opportunity in plant pathology in which to investigate pathogen-host relationships in a disparate range of plant species. Only Heath (1974, 1977, 1982, Elmhirst and Heath 1987) has previously researched this area of host and "non-host", the latter being defined as a plant species on which a given pathogen has never been recorded, and this author concluded that: "... amongst obligately biotrophic organisms, the study of non-host resistance has been singularly overlooked" (Heath 1974). Obviously, there are resistance factors present in the actual host as well as in non-host plants and Heath (1982) suggests that different mechanisms are operating in these 2 types of resistance. Incorrect pre-penetration behaviour. for example, is one of the most common types of reaction of a pathogen on non-hosts (Heath 1974, 1977).

Mortensen (1985b) made the first attempts to standardise reaction types (*RT*) for the host range screening of pathogens, delimiting 10 categories based on degree of damage to the test plant, and ranging from immune (0%, *RT* = O) to highly susceptible (>71%, *RT* = 9). Interpretation of *RT* on non-target plants formed the decision-making basis for release of an agent. Thus, guidelines were established enabling rational rather than emotive decisions to be reached. For example, it was argued that, even at a high *RT* (5-9), release of a pathogen would be acceptable if the susceptible plants were allopatric, scattered or rare wild plants or uncommon ornamentals, because the probability of the pathogen reaching them would be small. Even if individual plants were attacked and damaged, the pathogen would have little chance of reaching another susceptible host. Such a reasoned analysis has not been entertained by quarantine authorities until recently when the rust, *Uromyces heliotropii* Sredinski, was introduced into Australia for control of common heliotrope, *Heliotropium europeaum* L. (Boraginaceae) (Hasan, S. personal communication, 1991), despite the fact that it attacks an endemic *Heliotropium*. Bruzzese and Hasan (1986) and Hasan *et al.* (1990) used slightly modified *RT* ratings to assess the reaction of test plants to several rust species, based on a microscopic rather than a purely macroscopic examination. This approach is now being employed routinely at IIBC in host range screening tests in order to put them on a more scientific footing and to provide qualitative evidence as to why a given pathogen cannot infect or "mature" on species of agricultural or social importance.

The first project involves the rust *Puccinia abrupta* Diet. & Holw. var. *partheniicola* (Jackson) Parmelee and the composite weed *P. hysterophorus*. Fourteen *RT* categories have been identified, based entirely on microscopic examination (Tomley 1990), and pathogen development was evaluated according to 5 levels (A-E) and infection types (0-9) used by Bruzzese and Hasan (1986). Within leaves of most of the plants tested, the fungus produced internal hyphae whose further progress was halted by plant cell necrosis and/or callose deposition. In the 17 varieties of sunflower screened, this reaction resulted in leaf necrosis of varying degrees, which has been interpreted as hypersensitivity and the species rated as immune. In other plant species, however, abundant mycelium (up to 55% of leaf tissue) with accompanying haustoria occurred, but the subsequent collapse or isolation of haustoria

following callose formation prevented sorus development and sporulation. These results were presented in a pre-proposal for importation of the pathogen and the consultant committee recommended that those plant species exhibiting significant internal development (level C and D) should be re-screened under extremes of environmental regimes, with varying combinations of temperature, humidity and light, designed to stress the plants (Holden *et al.* 1992). Generally, rust development was slower in the non-host plants following stress. A final series of screens was demanded to test the possibility that sunflower varieties infected with *Puccinia helianthi* Schw. may be predisposed to infection by *P. abrupta* var. *partheniicola*. There was no evidence to support this hypothesis and a rapid hypersensitive response was observed in all test plants. The rust was released in Queensland in April 1991.

A further project for Queensland Department of Lands involves the rust, *Maravalia cryptostegiae* (Cummins) Ono, and its rubbervine host, *Cryptostegia grandiflora* R. Br. (Asclepiadaceae). Pathogen and host are endemic to Madagascar. Surveys for insect agents in Madagascar yielded no host-specific species, although a leaf-feeding lepidopteran with a relatively narrow host range was later released: "In view of the serious threat posed by *C. grandiflora* to the natural ecosystems, the enormous economic losses to the cattle industry and the lack of alternative biological control agents ..." (McFadyen and Marohasy 1990). The field performance of this insect agent has been poor and the main hope for biological control now lies with the rust. Over 60 plant species have undergone host range screening and a variety of external and internal symptoms has been observed. Additional *RT* categories have been defined to accommodate some of the unique responses of the various non-hosts to the pathogen, and at least 5 resistance mechanisms have been identified:

1. Inhibition (total) of spore germination. This was seen only in the fleshy Asclepiadaceae genera (*Hoya, Stapelia, Cynanchum*) and must be associated with powerful fungitoxic substances on or within the plant cuticle. The mechanism has not been reported previously.

2. Abnormal germination, including grossly swollen and/or distorted germ tubes, probably due to the release of phytoalexins in response to pre-penetration activity.

3. Plasmolysis of appressoria over stomata, or failure of appressoria to locate stomata due to topography of host leaf surface.

4. Inhibition or isolation of infection hypha and haustorial mother cell by callose or polyphenol release.

5. Haustorial death due to host cell necrosis–plasmolysis or isolation of haustoria by callose.

The latter reaction has only been observed in the sub-family Periplocoideae, however, in 2 species of the genera *Gonocrypta* and *Cryptolepis*, the rust has achieved maximum *RT* rating (=12, sporulation) under greenhouse conditions. The Madagascan endemic, *Gonocrypta grevei* H. Bn. is a natural host of the rust but field evidence was gathered to indicate that generic-specific pathotypes do occur in Madagascar: heavily-rusted rubbervine, for example, growing amongst disease-free *G. grevei*. Moreover, the majority of plants of this host when inoculated under greenhouse conditions, fail to develop sori or form only a few, slow growing sori compared with the rubbervine host; although several plants have shown a relatively high density of pustule production. Unfortunately, a newly described and rare, endemic Australian member of the Periplocoideae, *Cryptolepis grayi* Forster (1990), has also developed fertile sori in the host range screening. Pustule formation is slow and at a very low density and internal examination shows that in this host, as well as in *G. grevei*, haustorial density is high but haustorial activity is low due to host cell death or rapid callose build-up. It is concluded that these 2 species are not natural hosts of the rust strain (pathotype) occurring on rubbervine, but become susceptible (lose resistance) in host range screening tests due to a combination of favourable infection conditions and unrealistic inoculum levels (1.6×10^6 spores M^{-1}). Evidence will be presented to this effect in support of the proposal to import *M. cryptostegiae* into Australia. The same arguments discussed earlier, and originally put forward by Mortensen (1985*b*), and later used successfully to obtain

release of the heliotrope rust, would apply equally well here.

This section would be incomplete without reference to the problems of host range screening with facultative pathogens for mycoherbicide development. Screening of *Colletotrichum* and *Phomopsis* spp. on itch-grass weed and graminaceous crops has revealed that these fungi will sporulate consistently on maize plants (Ellison and Evans 1992). On this basis, promising but "non-specific" isolates were rejected and not included in the later screens. However, on careful analysis, it was shown that sporulation was restricted to the lower, naturally-senescing leaves and that this was not a case of primary infection but of latent infection. The spores germinated on the young leaves but were unable to infect, remaining as resistant appressoria, and opportunistically invaded the non-functional or senescing leaves. Therefore, far from being a natural host, the maize was succumbing to secondary invasion, and that this constitutes a perfect and, in hindsight, regrettable example of how potentially-useful isolates may fall by the wayside. Cerkauskas (1988) has adopted the opposite stance and believes that latent colonisation is a potential hazard in using mycoherbicides, arguing that there is the possibility of selecting isolates more virulent to the crop by repeated applications of high concentrations of the product. There is little evidence to support this scenario (Leonard 1982).

General Thoughts

Host range screening of pathogens for the biological control of weeds is still evolving. The practices are being modified and refined and the problems scientifically addressed. There is cause for optimism amongst weed pathologists as the objections to or suspicions surroundings the use of pathogens for weed control are gradually being eroded. There should be no complacency, however, because, as Adams (1988) warned "... one error resulting in crop damage could jeopardise the future of all biological control programmes".

Watson (1991) has noted the slow progress in the exploitation of pathogens for control of weeds, particularly from the classical point of view, but predicted that future prospects were bright since the alternatives are few in an evermore environmentally-conscious world. Watson also challenged biological control workers to understand the mechanisms involved in host-agent relationships; at the very centre must be those relating to specificity and safety: host versus non-host. However, a survey of the plant pathology literature soon reveals that resistance mechanisms still remain to be elucidated for many crop-disease relationships.

Importation of exotic pathogens for weed control using the classical biological control strategy has been singularly successful in the few implemented cases. Scientific guidelines have been followed and, as a consequence, the safety record is unblemished. Host range screening forms the backbone of any proposal to release exotic agents and the requirements will differ with each given weed-pathogen relationship. However, if these are too demanding, such as testing rarely-produced aeciospore inoculum to assess genetic variation (Tomley 1990), then classical weed pathology projects will become unmanageable and too costly, in terms of both time and money. Sponsors will need to recognise the constraints, especially the problems involved with host range screening, and allow sufficient funding to overcome them. Cautionary tales, such as those of Wiberg and Walker (1990), may reduce enthusiasm for, and hence investment in, weed pathology by governmental and potential sponsoring agencies. These authors reported the recent occurrence in Australia of the Northern Hemisphere rust, *Uromyces minor* Schroter, on garden peas (*Pisum sativum* L.; Leguminosae). This rust species had earlier been identified on the same host in New Zealand (Savile 1979). The natural hosts of *U. minor* are *Trifolium* spp. and the rust has never been recorded on any other plant genus in the Northern Hemisphere. In this environment, peas would have been exposed to the rust for a considerable period of time, hence, the 2 Southern Hemisphere records are all the more puzzling. The only explanation is that of a host jump through mutation, not just in an isolated instance but in 2 distinct geographic areas. Obviously, there is cause for concern,

particularly as the authors involved are much-respected mycologists and, as such, a powerful anti-biological control lobby. Clearly, much more work will be necessary to solve this conundrum but some comfort can be gained from the fact that this "unpredictable jump" (Wiberg and Walker 1990) would almost certainly have been predicted using the centrifugal phylogenetic sequence of host range screening.

References

Adams, E.B. 1988. Fungi in classical biocontrol of weeds. *Fungi in Biological Control Systems*. Burge, M.N. (ed.). Manchester University Press, Manchester, pp. 111-124.

Barreto, R.W. and H.C. Evans. 1988. Taxonomy of a fungus introduced into Hawaii for biological control of *Ageratina riparia* (Eupatorieae: Compositae), with observations on related weed pathogens. *Transactions of the British Mycological Society* **91**:81-97.

Bruckart, W.L., D.J. Politis and E.M. Sutker. 1985. Susceptibility of *Cynara scolymus* L. (artichoke) to *Puccinia carduorum* Jacq. observed under greenhouse conditions. *Proceedings of the VI International Symposium on Biological Control of Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agriculture Canada, Ottawa, pp. 603-7.

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

Bruzzese, E. and S. Hasan. 1986. Host specificity of the rust *Phragmidium violaceum*, a potential biological control agent of European blackberry. *Annals of Applied Biology* **108**:585-96.

Cerkauskas, R.F. 1988. Latent colonization by *Colletotrichum* spp.: epidemiological considerations and implications for mycoherbicides. *Canadian Journal of Plant Pathology* **10**:297-310.

Clifford, B.C. 1973. The construction and operation of a dew-simulation chamber. *New Phytologist* **72**:619-23.

Cullen, J.M. 1990. Current problems in host specificity screening. *Proceedings of the VII International Symposium on Biological Control of Weeds*, 6-11 March 1988, Rome, Italy. Delfosse, E.S. (ed.). Ministero dell'Agricoltura e delle Foreste, Rome/CSIRO, Melbourne, pp. 27-36.

Cullen, J.M. and S. Hasan. 1988. Pathogens for the control of weeds. *Philosophical Transactions of the Royal Society of London* **318**:213-24.

Daniel, J.T., G.E. Templeton, R.J. Smith and W.T. Fox. 1973. Biological control of northern jointvetch in rice with an endemic fungal disease. *Weed Science* **21**:303-7.

Dianese, J.C., T.S.A. de Moraes and A.R. Silva. 1984. Response of *Eucalyptus* species to field infection by *Puccinia psidii*. *Plant Disease* **68**:314-6.

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

Elmhirst, J.F. and M.C, Heath. 1987. Interactions of the bean rust and cowpea rust fungi with species of the *Phaseolus-Vigna* plant complex. I. Fungal growth and development. *Canadian Journal of Botany* **65**:1096-1107.

Evans, H.C. 1991. Biological control of tropical grassy weeds. *Tropical Grassy Weeds*, Baker, F.W.G. and P.J. Terry (eds). CAB International, Wallingford, Oxon, UK, pp. 52-72.

Evans, H.C. and C.A. Ellison. 1990. Classical biological control of weeds with microorganisms: past, present prospects. *Aspects of Applied Biology* **24**:39-49.

Forster, P.I. 1990. Notes on Asclepiadaceae 2. *Austrobaileya* **3**:273-89.

Freeman, T.E. and R. Charudattan. 1985. Conflicts in the use of plant pathogens as biocontrol agents for weeds. *Proceedings of the VI International Symposium on Biological Control of Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agriculture Canada, Ottawa, pp. 351-7.

Gold, R.E. and K. Mendgen. 1991. Rust basidiospore germlings and disease initiation. *The Fungal Spore and Disease Initiation in Plants and Animals*. Cole, G.T. and H.C. Hoch (eds). Plenum Press, New York and London, pp. 67-99.

Harris, P. 1985. Biocontrol of weeds: bureaucrats, botanists, beekeepers and other bottlenecks. *Proceedings of the VI International Symposium on Biological Control of Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agriculture Canada, Ottawa, pp. 3-12.

Hasan, S. 1972. Specificity and host specialization of *Puccinia chondrillina*. *Annals of Applied Biology* **72**:257-63.

Hasan, S., P. Chaboudez and K. Mortensen. 1990. Field experiments with the European knapweed rust (*Puccinia jaceae*) on safflower, sweet sultan and bachelor's button. *Proceedings of the VII International Symposium on Biological Control of Weeds*, 6-11 March 1988, Rome, Italy. Delfosse, E.S. (ed.). Ministero dell'Agricoltura e delle Foreste, Rome/CSIRO, Melbourne, pp. 499-509.

Heath, M.C. 1974. Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust–*Uromyces phaseoli* var. *vignae*. *Physiological Plant Pathology* **4**:403-14.

Heath, M.C. 1977. A comparative study of non-host interactions with rust fungi. *Physiological Plant Pathology* **10**:73-88.

Heath, M.C. 1982. Host defense mechanisms against infection by rust fungi. *The Rust Fungi*. Scott, K.J. and A.K. Chakravorty (eds.). Academic Press, London and New York, pp. 223-45.

Henderson, D.M. and A.P. Bennell. 1979. British rust fungi: additions and corrections. *Notes from the Royal Botanic Garden, Edinburgh* **37**:475-501.

Holden, A.N.G., A. Parker and A.J. Tomley. 1992. Host range screening of *Puccinia abrupta* var. *partheniicola* for the biological control of *Parthenium hysterophorus* in Queensland. *Proceedings of the VIII International Symposium on Biological Control of Weeds*, 27 February 1992, Canterbury, New Zealand. Delfosse, E.S. and R.R. Scott (eds). DSIR/CSIRO, Melbourne, Australia (*in press*).

Kassulke, R.C., K.L.S. Harley and G.V. Maynard. 1990. Host specificity of *Acanthoscelides quadridentatus* and *A. puniceus* [Col.: Bruchidae] for biological control of *Mimosa pigra* (with preliminary data on their biology, *Entomophaga* **35**:85-96.

Large, E.C. 1940. *The Advance of the Fungi.* Jonathan Cape, London.

Leonard, K.J. 1982. The benefits and potential hazards of genetic heterogeneity in plant pathogens. *Biological Control of Weeds with Plant Pathogens.* Charudattan, R. and H.L. Walker. J. Wiley & Sons, New York, pp. 99-112.

Marlatt, R.B. and J.W. Kimbrough. 1979. *Puccinia psidii* on *Pimenta dioica* in south Florida. *Plant Disease Reporter* **63**:510-1.

McClay, A.S. 1985. Biocontrol agents for *Parthenium hysterophorus* from Mexico. *Proceedings of the VI International Symposium on Biological Control of Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agriculture Canada, Ottawa, pp. 771-8.

McFadyen, R.E. 1985. The biological control programme against *Parthenium hysterophorus* in Queensland. *Proceedings of the VI International Symposium on Biological Control of Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agriculture Canada, Ottawa, pp. 789-96.

McFadyen, R.E. and J.J. Marohasy. 1990. A leaf feeding moth, *Euclasta whalleyi* [Lep.: Pyralidae] for the biological control of *Cryptostegia grandiflora* [Asclepiadaceae] in Queensland, Australia. *Entomophaga* **35**:431-5.

Mortensen, K. 1985*a*. Reactions of safflower cultivars to *Puccinia jaceae*, a potential biocontrol agent for diffuse knapweed. *Proceedings of the VI International Symposium on Biological Control of Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agriculture Canada, Ottawa, pp. 447-52.

Mortensen, K. 1985*b*. A proposal for a standardized scale of attack and its application to biocontrol agents of weeds in laboratory screening tests. *Proceedings of the VI International Symposium on Biological Control of Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agriculture Canada, Ottawa, pp. 643-50.

Mortensen, K., P. Harris and R.M.D. Makowski. 1989. First occurrence of *Puccinia jaceae* var. *diffusae* in North America on diffuse knapweed (*Centaurea diffusa*). *Canadian Journal of Plant Pathology* **11**:322-4.

Paul, N.D. and P.G. Ayres. 1987. Effects of rust infection of *Senecio vulgaris* on competition with lettuce. *Weed Research* **27**:431-41.

Ridings, W.H. 1986. Biological control of stranglervine in citrus–a researcher's view. *Weed Science* **34**:31-2.

Savile, D.B.O. 1979. Fungi as aids in higher plant classification. *Botanical Review* **45**:377-503.

Scholtens-Toma, I.M.J., M.H.A.J. Joosten and P.J.G.M. de Wit. 1991. Appearance of pathogen-related proteins in plant hosts. *The Fungal Spore and Disease Initiation in Plant and Animals.* Cole, G.T. and H.C. Hoch (eds). Plenum Press, New York and London, pp. 247-65.

Shepherd, R.C.H. 1990. Problems which arise with host-specificity testing of insects. *Proceedings of the VIII International Symposium on Biological Control of Weeds*, 2-7 February 1992, Canterbury, New Zealand. Delfosse, E.S. and R.R. Scott (eds). DSIR/CSIRO, Melbourne, Australia, pp. 85-92.

TeBeest, D.O. 1988. Additions to the host range of *Colletotrichum gloeosporioides* f.sp. *aeschynomene*. *Plant Disease* **72**:16-8.

Tomley, A.J. 1990. Proposal for the field release of the Parthenium rust fungus, *Puccinia abrupta* var. *partheniicola* in Australia. Alan Fletcher Research Station, Queensland Department of Lands, 36 p.

Trujillo, E.E. 1985. Biological control of hamakua pamakani with *Cercosporella* sp. in Hawaii. *Proceedings of the VI International Symposium on Biological Control of Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agriculture Canada, Ottawa, pp. 661-71.

Wapshere, A.J. 1974. A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* **77**:201-11.

Wapshere, A.J. 1975. A protocol for programmes for biological control of weeds. *PANS* **21**:295-303.

Wapshere, A.J. 1982. Biological control of weeds. *Biology and Ecology of Weeds.* Holzner, W. and N. Numata (eds.). W. Junk Publishers, The Hague, pp. 47-56.

Wapshere, A.J. 1989. A testing sequence for reducing rejection of potential biological control agents for weeds. *Annals of Applied Biology* **114**:515-26.

Wapshere, A.J., E.S. Delfosse and J.M. Cullen. 1989. Recent developments in biological control of weeds. *Crop Protection* **8**:227-50.

Watson, A.K. 1985. Host specificity of plant pathogens in biological weed control. *Proceedings of the VI International Symposium on Biological Control of Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agriculture Canada, Ottawa, pp. 577-86.

Watson, A.K. 1991. The classical approach with plant pathogens. *Microbial Control of Weeds.* TeBeest, D.O. (ed.). Chapman and Hall, New York and London, pp. 3-23.

Weidemann, G.J. and D.O. TeBeest. 1990. Biology and host range testing for biocontrol of weeds. *Weed Technology* **4**:465-70.

Weidemann, G.J., D.O. TeBeest and R.D. Cartwright. 1988. Host specificity of *Colletotrichum gloeosporioides*

f.sp. *aeschynomene* and *C. truncatum* in the Leguminosae. *Phytopathology* **78**:986-90.

Wiberg, L. and J. Walker. 1990. *Uromyces minor* on peas in Australia, with notes on other rusts of *Pisum*. *Australasian Plant Pathology* **19**:42-5.

Williams, J.R. 1954. The biological control of weeds. *Report of the Sixth Commonwealth Entomological Conference*, CIE, London, pp. 95-8.

Wilson, I.M., D.F. Walshaw and J. Walker. 1965. The new groundsel rust in Britain and its relationship to certain Australian rusts. *Transactions of the British Mycological Society* **48**:501-11.