Commentary

A decision tree for evaluation of exotic plant pathogens for classical biological control of introduced invasive weeds

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Abstract

Plant pathogens for biological control of weeds must satisfy criteria for efficacy, safety, and deployment before they are actually included in the list of candidates for weed management strategies. Decisions are made throughout the development of each candidate agent concerning whether or not further research is justified. These decisions concern pathogen attributes such as collection information, Koch’s postulates, long-term storage, host specificity, risk, and other factors, including deployment. In addition to the scrutiny from researchers, who become advocates at the time a proposal is made to regulators for introduction or utilization of a candidate, the proposed use of exotic pathogens for release receives additional review by regulators in the United States Department of Agriculture (USDA)-Animal and Plant Health Inspection Service (APHIS). In this paper, the processes for deciding whether to continue or abandon research on a candidate exotic pathogen for classical biological control of weeds in the United States are examined. Discussion is based on the experience accumulated at the Foreign Disease-Weed Science Research Unit of the USDA-Agricultural Research Service but has broader application to programs involving evaluation of foreign candidates for biological control of any invasive species.

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1. Introduction

The United States (US) Council for Agricultural Science and Technology currently lists 95 species of invasive weeds that were introduced (i.e., not native) to the US. Many of these have become major threats to native plant ecosystems in the US (Mullin et al., 2000), including rangeland, aquatic, riparian, natural, wild, and recreational areas (Asher and Harmon, 1995). These weeds reduce both productivity (Bridges, 1992) and biodiversity (Kummerow, 1992) of affected ecosystems, and their highly competitive nature leads to displacement and potential endangerment of native species (Cheater, 1992).

In intensive agroecosystems (e.g., row crops, orchards), most of these weeds can be managed effectively and economically by chemical (e.g., herbicides) and cultural (e.g., plowing and mowing) practices. However, in pasture and rangeland agriculture, the cost of conventional weed control is prohibitive. Moreover, these weeds are not restricted to agroecosystems but have invaded riparian and natural wild areas where chemical control, in particular, is neither economically feasible nor desirable because of potential damage to sympatric communities of native flora or to sensitive environments (e.g., bodies of water). In many cases these weeds are simply impossible to control by conventional

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means because they exist in terrain inaccessible to spray equipment, plows, or mowers.

One strategy for effective and economical management of invasive weeds is biological control. This method uses plant pathogens and arthropods that specifically damage target weeds without harm to desirable plants. Although there are several approaches to biological control of weeds (Boyetchko, 1997), the primary focus of this paper concerns the decision-making processes for candidate agents within the classical biological control strategy. The classical approach entails finding agents effective in controlling invasive weeds through systematic surveys, selection of damaging agents, isolation, and testing of natural enemies from areas where the weed species are native and, presumably, under “natural” control. This approach involves surveying and characterizing disease(s) of a selected weed where the target plant is native, identification of the causal agent(s), and evaluating or developing the(se) agent(s) for biological control where the weed is a pest in the intended region of release. These exotic natural enemies need to be efficacious (i.e., damaging to the weed) and safe (i.e., not damaging to other plants of economic or ecological value in the intended region of release). Once an organism is approved for release—following intensive screening for efficacy and specificity, and with regulatory approval—success depends on the ability of the agent to increase naturally and spread through the target weed population. Ideally, the agent will increase greatly on available susceptible plants, thus creating sufficient stress or damage to the target plant that populations will fall below acceptable economic and ecological thresholds.

In the US, scientists of the Foreign Disease-Weed Science Research Unit (FDWSRU) of the United States Department of Agriculture, Agricultural Research Service (USDA, ARS) routinely collect pathogens from selected invasive weeds in their native habitats and evaluate them for potential in biological control in the US. Because the pathogens are exotic to the US all work on these pathogens is done in either a bio-safety level 3 (BSL-3) quarantine facility at FDWSRU (Melching et al., 1983) or in the field in the country of origin (Bruckart et al., 1996). Despite this constraint, FDWSRU has been the source of three exotic pathogens released in the US, *Puccinia carduorum* Jacky for control of musk thistle, *Carduus nutans* L. (Baudoin et al., 1993; Baudoin and Bruckart, 1996), *Puccinia chondrillina* Bubák & Syd. for control of rush skeletonweed, *Chondrilla juncea* L. (Emge, 1977, 1981); and recently *Puccinia jaceae* Ooth for control of yellow starthistle, *Centaurea solstitialis* L. (Federal Register, 2002; Suszkiw, 2004).

However, the process of developing a foreign agent (pathogen or arthropod) is not simple; critical decisions must be made constantly during evaluations to insure the best information is generated concerning potential usefulness and safety of each candidate. For these reasons, most pathogens collected abroad do not advance to the stage of release. Frequently, research on newly collected pathogens is abandoned because the organism either has been reported already in the US or the host range is considered too broad for safe use in weed control. Quick abandonment of a candidate is, by far, preferable to a scenario in which years of research are spent on a pathogen that ultimately has to be abandoned as a candidate for release. Although the latter scenario is rare, it is frequently difficult to judge when to abandon pathogen development, and it is even more difficult to actually abandon a project. In this paper, we attempt to describe a process for decision-making based on our experiences from developing foreign pathogens for weed biological control. We attempt to cast these experiences into a formalized decision-making process. The following, therefore, describes decisions needed during evaluations of candidate biological control agents, including logical processes and caveats for each step. Concepts and philosophy described in this paper should apply for anyone with similar objectives with exotic agents.

2. Steps in development of a candidate biological control agent (the decision tree)

There are three major focal points in developing any candidate agent for biological control of a pest. The organism must be identified and shown to be potentially damaging to the target pest (Phase I), and the candidate must be tested for safety (Phase II), so that it can be used with confidence around species (plants and animals) of value in North America. Finally, there are a number of decisions and processes that relate to satisfying the regulatory processes and deployment or implementation of a selected candidate (Phase III). Phase I, therefore, concerns characterization of the agent, Phase II includes steps pertinent to the risk analysis, and Phase III involves regulation and deployment.

Many of the following research processes and decisions that lead to a proposal are obvious to anyone using good laboratory practices. Nonetheless, there are specific issues and possible exceptions that need to be addressed within each criterion. The basic criteria and caveats are described in the following sections. Data for several of the criteria, in reality, are developed concurrently; they are not necessarily generated in the sequence described. This is a dynamic process and generation of information in each of the steps occurs at different rates. A generalized decision tree, around which the following discussion was developed, is presented in Fig. 1. As mentioned, there are exceptions to the “rules” within the tree, but these exceptions should be pondered at length, evaluating further research versus potential for release, before deciding to proceed with or abandon a pathogen candidate.
Fig. 1. Decision tree for advancing exotic plant pathogens toward release in the US for biological control of introduced invasive weeds. Further research on a pathogen is halted when a “stop sign” is reached in the decision-making process.
3. Phase I: About the candidate biological control agent

3.1. Surveys of pathogens present in the intended area of release

Ideally, the first step in considering pathogens in evaluations for weed biological control is to determine what pathogens are present already on the target weed in the area of intended release. Although this can be accomplished, in part, through reviews of herbaria and databases, the more comprehensive approach is to involve national collaborators to conduct field surveys of the target weed in the intended area of release. Combined with published information, these surveys prevent superfluous introductions of biological control agents, and allow funds to be used in development of other agents. Pathogens of the weed where it is a pest are not, nor would they be likely to, maintain weed population densities at manageable levels.

3.2. Collection information

The steps and information needed for release of exotic plant pathogens for biological control of weeds in the US are presented in Fig. 1. Beginning with the most basic step, complete information about the origin of an acquisition is essential for any scientific work. This may seem simplistic for the first criterion in developing a candidate pathogen for biological control of weeds, since most researchers know precisely the when, the where, and the what, about each candidate accession. However, at FDWSRU we have acquired nearly 5000 organisms in more than 25 years from numerous collectors, and there are many accessions which have incomplete, or in some cases, no descriptors. Acquisitions that lack important information are of very low priority for development and generally not processed at all.

A corollary to this problem is the fact that occasionally a new disease develops during greenhouse tests of other candidates. Pathogens isolated from these diseases have been of interest, since they have caused significant damage, in the greenhouse, on the target weed. In one sense, these also constitute pathogens of unknown origin, and as such, present a dilemma. Considering our objective to develop plant pathogens for biological control of weeds, there may be justification for pursuing these unsolicited pathogens. Further research might be justified for organisms of very important weeds, if the candidate meets the other criteria described in this paper. It is important to note that any proposal would be about the specific organism, and if it is thoroughly tested, then issues of source and other details may be of less importance than the fact that there is a very good, safe candidate for weed control.

There are other considerations that would remove such a candidate from development in containment. If the source is identified to be of North American origin, then research on such an agent technically falls outside of the purview of biological control at FDWSRU. Also, if it is not possible to determine whether the source is “local” (i.e., North American) or foreign, then this poses an interesting dilemma since these pathogens of unknown origin are not supported by any collection data on extent or intensity of the diseases they cause under field conditions, and disease development under greenhouse conditions may be an anomaly that is not reproducible in the field. Certainly, pursuit of a permit to introduce such a pathogen would pose unique and untested challenges to the regulatory community.

3.3. Fulfillment of Koch’s postulates

It is necessary, of course, that the organism isolated is the one responsible for causing the disease. This is satisfied by completion of Koch’s Postulates for proof of pathogenicity (Agrios, 1997), or a corollary thereof: (1) the microorganism is present in all cases of the disease; (2) the microorganism can be isolated from diseased tissue; (3) the isolate can be grown in pure culture; (4) inoculation with the isolate from pure culture produces the disease in healthy, susceptible plants; (5) the microorganism can be re-isolated from the plant inoculated with the pure culture; and (6) the isolate recovered in “5” is the same as that from the pure culture used for the inoculation. For obligate plant parasites, collection of infectious propagules from diseased plants and reproduction of disease after inoculating healthy plants immediately with these propagules are accepted substitutes for steps involving culturing of the organism. Completion of this process may involve considerable time and effort.

3.4. Maintenance of organism in an axenic or distinguishable state

The ability to maintain an isolate in an axenic state is a very important component in its development for biological control. Although axenic isolates may be genetically homogeneous, this latter property is not implied in this discussion. Herein, “axenic” refers to noncontaminated cultures of a single species or subspecific taxon, since permits granted for use of an organism for biological control are made for one species or an isolate within a species. Development of an organism for biological control is a long-term process involving several years of work, at the minimum. This necessitates protocols for maintenance of each organism in an axenic state for extended periods.

Generally, this is not a major concern. Facultative saprophytes can be maintained through several different methods, whereas rust fungi and other obligate parasites can be maintained on living plants or in storage at ultracold temperatures (Dhingra and Sinclair, 1995; Tuite, 1969). Challenges to long-term maintenance of
candidate agents include loss of virulence, contamination by other microorganisms or mites, and the difficulty of maintaining some groups of pathogens such as fastidious bacteria, phytoplasms, and downy mildews among others.

Obligate plant parasites are preferred for classical biological control of weeds because they, as a group, tend to have an higher degree of host specificity. These pathogens can be maintained on living plants in greenhouses, but these pathogens are virtually impossible to maintain in a truly axenic state, since the organism or its propagules on the host plant are frequently (usually) contaminated with other organisms. This contamination is typically by saprophytic organisms that do not cause plant diseases, but in the rare case of contamination with another plant pathogenic organism, the disease caused by the contaminant may be distinguished from the disease caused by the potential biological control agent. Distinguishing from contaminating organisms is usually easier with rust and smut fungi which produce distinct disease symptoms and whose spores are relatively distinct. The requirement becomes more difficult with anamorphs of, for example, powdery mildews that can be mixed with contaminant powdery mildew spores that frequently exist in greenhouse environments. Asexual powdery mildew spores are difficult to distinguish and the diseases produced are virtually the same, making it virtually impossible to separate the contaminant from the potential agent. An overview of procedures for increasing the population of obligate plant parasites can be found in Tuite (1969) and Dhingra and Sinclair (1995). Failure to maintain the organism in an axenic state, or a state that can be distinguished from contaminants, whether based on characteristics of the organism, the propagule, or the disease, is likely to nullify results from other aspects of candidate evaluations. Abandonment of these pathogens is a good decision.

3.5. Identifying the pathogen

There is a lot of information in a name, so, at an early stage of evaluation, some rudimentary identification, at least to the genus level, is desirable. Identification, even to genus, provides much important information about the potential usefulness and safety of a candidate pathogen. Framework for procedures to culture, store, inoculate, and evaluate, the pathogen can be established. Identification facilitates a preliminary determination as to whether development of the pathogen is justified. Proceeding to yield loss, host range, or other tests without identification of a candidate to genus could result in a lot of wasted effort. Ultimately, identification to, or the creation of, a species is necessary for approval to release. Identification to genus may be all that can be achieved for the initial period of evaluation if the pathogen constitutes a new discovery. Work should not be interrupted while waiting for identification at the species level, once a generic name is available for the organism.

Like fulfilling Koch’s postulates, identifying the pathogen can be very involved, particularly for identification to species or subspecific levels. Combining classical morphological approaches with new molecular approaches, such as DNA sequence analysis of ribosomal spacer regions, facilitates this process and helps to clarify issues of taxonomy and naming. Molecular information also is useful in post-release monitoring of an approved pathogen.

3.6. Use of information from pathogen genus and species

3.6.1. Genus information

A candidate pathogen may or may not have representatives of the genus in North America. If the genus is not known in North America, then the pathogen is likely to be unique and therefore of interest on the basis of potential specificity. In either case, the isolate must satisfy other requirements of impact and safety before it is fully considered for release.

Pathogens in some genera are known to infect a large variety of hosts (e.g., Colletotrichum and Fusarium). In such cases, identification to species will be necessary. For other genera, however, the reported host range may be narrow, i.e., each pathogen species includes only one or a few plant species. Examples include pathogens in the genera Septoria and Puccinia. Such information is very useful in the formulation of a test plant list.

3.6.2. Is the pathogen species already present in North America?

If the species of the pathogen is known, then it is necessary to determine whether it has been reported in North America. If reports exist, then further evaluations in quarantine are usually abandoned since the pathogen could be researched elsewhere in the US under natural conditions using a local/indigenous isolate. If the pathogen is particularly promising, information about the candidate is given to colleagues working with indigenous pathogens, who might consider an indigenous isolate of the same species for development outside of containment. Excellent sources of information on hosts and pathogens in North America, particularly for fungi, include Farr et al. (1989); http://nt.ars-grin.gov/SBML-Web (Farr et al., n.d.); Conners (1967); and Ginns (1986).

There are circumstances in which development (in quarantine) of a foreign isolate of a species known in North America might be justified. One example would be if the exotic isolate is much more aggressive and damaging to the target than indigenous isolates, and this difference is judged to be sufficient for successful biological control. The amount of efficacy and host range testing will be about the same as for any other exotic pathogen, given the current regulatory oversight of
foreign pathogens in the US. In such a case, advice for development of the candidate would be sought from the Technical Advisory Group for Biological Control Agents of Weeds (TAG; http://www.aphis.usda.gov/ppq/permits/tag/) before proceeding. Another option in such a case would be to conduct a thorough screen of North American isolates for an aggressive variant of the pathogen.

A second example where development of a foreign pathogen already known in North America is justified involves an organism that has been reported only infrequently and does not appear to be established in its new range. Currently, such a pathogen, Septoria lepidii Desmaz. is in our collection from Lepidium draba L. (hoary cress). This pathogen causes severe epidemics on L. draba in the countries of origin and would seem to have potential as a classical biological control agent for hoary cress in the US. It has been collected only once and only on the target genus in the US (BPI 789059b) in 1897. No known reports or collections have been made since in the US. The apparently rare occurrence of the pathogen in the US warrants surveys of the intended area of release (Montana) to see if this pathogen is present and on which species and subspecies. Clarification on whether distribution remained restricted is of great interest as it may have resulted from geographical isolation or an inherent limitation of the pathogen to disperse.

There are three taxa of the target weed, hoary cress, in the US. These are L. draba L. subsp. draba, L. draba L. subsp. chalepense, and L. appelianum Al-Shehbaz. Simultaneous with the proposed surveys, fulfillment of Koch’s postulates on the target weed present in the intended area of release is necessary to prove that at least one of the taxa of the weed is susceptible to the exotic pathogen. Subsequent to fulfilling Koch’s postulates, tests to document host specificity and protocol to satisfy other requirements of development will be made to support use of this and similar pathogens.

3.6.3. Is species of the pathogen reported from a wide range of host genera?

If the pathogen species has been reported from a wide range of host genera, then the isolate under consideration may parasitize nontarget plants and thus not be permitted for release. However, for many species there are reported differences in specificity among formae speciales, varieties, or pathovars (Weidemann, 1991), and further characterization (e.g., subspecific identification or host specificity tests) might be justified for promising candidates. As an example, subspecific taxa (e.g., formae speciales) uniquely pathogenic to one plant species are well known for both Fusarium oxysporum Schlecht and Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. in Penz. Additional research is justified in these cases.

However, subtaxon identity may pose a problem in getting approval for release. Clear data supporting pathogen taxonomy and specificity must be obtained, if the pathogen is to advance much further. In the case of a formae speciales of F. oxysporum, the host range test required to prove specificity would be very large and likely prohibitive. Two isolates of C. gloeosporioides that are extremely host specific have been approved for introduction into Hawaii (Killgore et al., 1999; Trujillo et al., 1986). One is C. g. f. sp. elidiemiae collected from Panama for biological control of Cidemia hirta (L.) D. Don (Trujillo et al., 1986), and the other is C. g. f. sp. miconiae collected from Brazil for biological control of Miconia calvescens DC. (Killgore et al., 1999).

Prior to conducting these more elaborate taxonomic studies and/or host range tests, it may be worthwhile to proceed through the following sections. If the criteria in these sections are met, then the additional work in identification and characterization of a subspecies variant may be warranted.

3.7. Does the pathogen have special requirements for transmission or infection?

Candidate pathogens that have specific requirements for transmission or infection are likely to be poor candidates for classical weed biological control. Additional research might be justified for such a candidate if it is for control of a major weed pest or exceptional aggressiveness is noted either in field or greenhouse tests. Regardless, it must also meet other criteria described in this paper for approval.

3.7.1. The need for a vector

Some pathogens require a vector for transmission. This is an issue only if a suitable vector is not present already in North America. If both the pathogen and its vector are exotic, then requirements for evaluation essentially double, because both pathogen and vector must satisfy efficacy and safety criteria before use is permitted in North America. Also in such situations, it is necessary to identify both the pathogen and all vectors that might transmit it. For foreign (exotic) vectors, the host range determination expands to include potential hosts for both pathogen and the vector(s), and to determine which, if any, is damaged by the vector outright and which, if any, become diseased by the interaction. To accomplish this in quarantine is extremely difficult, and we believe that, in the case of an exotic agent and exotic vector, efforts could be better spent finding and working with another organism.

3.7.2. Requirements for disease

Candidate pathogens are evaluated under conditions optimal for disease. Usually this includes high concentrations of inoculum, optimal conditions of temperature
and dew, and young host plants; it is unnatural in many ways, but it also provides the most stringent tests for evaluations.

Indications of field efficacy are first obtained from impressions and notes at the collection site, which justified collection of the disease in the first place. However, a pathogen that requires an unnaturally extended dew or leaf-wetness period in order to get “good” infection will be dropped as a candidate, unless a means of achieving a leaf-wetness period in order to get “good” infection will be provided in the field. Making judgment whether disease requirements are extreme for greenhouse evaluations is difficult, and it is well understood that the natural field environment is very different from the artificial conditions in greenhouse tests. If the pathogen advances further, based on satisfying options that follow, field tests in the country of origin may provide insight into potential field expectations and options for deployment of a given candidate. For example, formal collaborations have been established between FDWSRU scientists and colleagues in the countries of pathogen origin.

Occasionally, infection results only after wounding. A candidate requiring a wound, however, would not likely disperse readily enough for use as a classical agent, and the additional materials or protocols needed for adequate field inoculations would greatly reduce its utility.

3.8. Does the pathogen cause significant disease severity in greenhouse tests?

A pathogen that does not readily infect a target plant from the US under greenhouse conditions is probably not a good candidate for biological control. Ideally, observations on damage and epidemic potential at the collection site warranted the collection in the first place, but if a candidate is hard to work with in the greenhouse and, under reasonable conditions, does not infect the target plant, then it is not likely to be sufficiently damaging in the field in the US. The discrepancy between observations at the collection site and greenhouse results might be due to: infraspecific differences in subspecies between US and foreign plants, differences in resistance/susceptibility between plants of different geographic origins, or differences in genotype × environment interactions that affect disease reactions.

Considering the above, it is important to note that a candidate biological control agent does not have to devastate the target in controlled tests. Successful weed management through biological control frequently relies on the presence of several agents that, as individuals, are damaging under specific environmental conditions. When combined with other agents, however, sustained pressure and damage can result from the constant attack of agents operating optimally under varying environmental conditions. It is important to note also that greenhouse and growth-chamber tests only provide crude measures of the field potential of any pathogen. Actual potential is realized only after the pathogen is released into the field. So, in most cases, if the pathogen readily infects and causes measurable damage under optimal greenhouse and growth-chamber tests, development and evaluation go forward.

4. Phase II: Determining risk

4.1. How specific does the pathogen appear under restricted host range tests?

Central to the risk assessment is a host range determination for the candidate organism, since demonstration of host specificity under optimal conditions for disease in controlled studies strongly indicates that nontarget species will not be affected in the field. Host range test plant lists are generally organized on the basis of Wapshere’s centrifugal-phylogenetic testing sequence (Wapshere, 1974). It is based on the phylogenetic relationship of test plants to the target plant, from which the pathogen was collected. It is based also on the assumption that plant species most likely susceptible to a pathogen from a target species are those most closely related. Those plant species are tested first and testing then progresses outwardly to more distantly related plants.

Lists often include safeguard plant species such as important crop plants and native plants that are considered rare, endangered, or threatened, particularly if they are likely subject to exposure by release of an approved candidate. A list developed by scientists evaluating the candidate pathogen is submitted as a preproposal for review by the TAG and other regulatory organizations, particularly the APHIS and the US Department of the Interior, Fish and Wildlife Service (F&WS). Scientists are encouraged to discuss entries on proposed lists with regulatory officials in order to reach consensus of the plants considered important for the risk assessment. Once a list of test plants is developed that satisfies risk analysis and regulatory issues, then test plants become part of the evaluation.

4.2. Risk = Hazard × Exposure

Risk assessment is based on the formula that Risk = Hazard × Exposure (R = H × E). Since exposure of native and beneficial North American plants is assumed following release of a foreign pathogen, the attempt then is to control hazard; and host specificity provides the best approach to reducing hazard, and related risk, to zero. Ideally, a high level of host specificity is desired for foreign candidate pathogens; i.e., the
pathogen parasitizes only the target weed without causing disease on closely related or important North American species. This is determined by inoculating related test plants with the pathogen under optimal conditions for disease. In most cases, test plants will not be infected, and thus would be considered not at risk, even if exposed in the field following release of the candidate pathogen.

However, a more common scenario is that some symptom development or disease occurs on one or a few closely related species under optimal conditions for infection. This constitutes the identification of a potential hazard. In most cases, the infection is limited and not damaging, and further research on specific measurement of the damage is pursued. In some cases, susceptible nontarget plants are considered weeds in the country of intended release, so there are no issues of risk. A recent review by Barton (2004) indicates that, historically, host range tests of this nature have been conservative, and that fungi causing limited infection in pre-release tests have not been recovered from nontarget plants in the field. Evans (2000) reiterates this and states that greenhouse screening can, because of the extremely favorable conditions for disease development, lead to induced susceptibility that is never seen in nature. If disease on a nontarget plant from the test plant list is extreme and damaging, then the pathogen will be abandoned as a candidate for biological control.

In the event of limited nontarget infection and thus identification of a potential hazard, additional research may be conducted to clarify the hazard and enable proper judgment about risk. If the hazard is truly “low” or “limited,” then the conclusion is that risk would be reasonable following release of the organism. One approach used to clarify issues of nontarget infections is to include a pathogen from North America for comparison with the candidate under similar, controlled conditions (Bruckart et al., 1996). For example, minor infections of safflower (Carthamus tinctorius L.) were noted after inoculations with P. jaceae, a candidate for biological control of yellow starthistle (YST) (Bruckart, 1989). Safflower, which grows symptomatically with YST, is infected by a rust fungus, Puccinia carthami Corda, in the US that is readily managed in safflower production areas. Isolates of P. carthami from US safflower production areas were obtained and tested in a containment greenhouse using side-by-side studies with YST and safflower inoculated with either P. jaceae or P. carthami. It was clear in these studies that P. jaceae was much less aggressive and did not damage safflower when, under the same conditions, P. carthami readily infected and damaged safflower (Bruckart, 1999; Bruckart and Eskandari, 2002). The conclusion, which was accepted by regulatory officials, was that P. jaceae would not be detrimental to safflower production in the US if released for biological control of YST.

4.3. Decision to release

Although ultimate determination of whether an organism can be released for biological control depends upon the regulatory process, scientists conducting risk analyses do not send a proposal forward until they, as researchers, are satisfied that the candidate agent is safe; they then become the advocates for release. Judgment of safety is made if issues about risk can be resolved directly (e.g., via demonstrated host specificity) or by some mitigating factor (e.g., the target weed and mildly susceptible nontarget plant do not occur together in nature either geographically or seasonally).

5. Proposal to release

When sufficient data have been accumulated supporting the efficacy and safety of a candidate pathogen for biological control, a proposal is developed for release and utilization of the organism in the US and submitted for review by regulatory officials. During development of any candidate organism, there is considerable communication and interaction with regulators, particularly through the TAG and APHIS, but also with other interested parties, including the F&W, State Departments of Agriculture, and grower groups. Decision to proceed is made only after concerned parties are satisfied about issues concerning the candidate and the release. Foreign organisms for classical biological control are regulated through APHIS with notice given to the US Environmental Protection Agency (EPA).

Once a proposal is submitted to obtain a permit for release of a candidate, scientists follow it through the review and permitting process. The original proposal is submitted to the TAG with copies to other interested agencies and parties (APHIS, F&W, State Departments of Agriculture, and grower groups). The TAG provides recommendation to APHIS for release following a satisfactory review. If APHIS agrees, then an Environmental Assessment is made, and notice of intent is published in the Federal Register with opportunity for comments from the general public. Comments are reviewed and responses developed for each comment. If everything is in order, APHIS issues a “Finding of No Significant Impact” (FONSI). At this point, a permit is issued to the individual or institution that is to receive the pathogen for release, and scientists conducting the original evaluations organize to supply the pathogen for release.

The proposal, then, represents science-based information that enables regulators to make the best decision about safe deployment of a candidate recommended for release. Development of many organisms is terminated long before it is considered a candidate; most decisions to abandon a project relate to issues of virulence or safety, whether perceived or real.
6. Case studies

6.1. Abandonment: Melampsora euphorbiae on Euphorbia esula

Leafy spurge (LS, *Euphorbia esula* L.) is a major pest in the rangelands of the Great Plains in the US. It has been the target of biological control for more than 20 years. One of several rust fungi occurring on LS in Eurasia is *Melampsora euphorbiae* (Schub.) Cast. The fungus, which occurs in the US on *Euphorbia* species other than *E. esula*, is autecious and was considered of potential use in biological control (Step 1). The potential of *M. euphorbiae* was investigated at both FDWSRU and at the Institut fur Phytomedizin, ETH-Zentrum in Switzerland (Bruckart et al., 1986). Plants inoculated included source clones of LS, European collections of LS, and US collections of LS, and collections of the pathogen were well documented (Step 2). It was evident that only the source clone for each of three LS isolates (and two isolates from cypress spurge (*E. cyparissias* L.)) were susceptible. Koch’s postulates were fulfilled (Step 3), the pathogen could be maintained in a distinguishable state in the greenhouse (Step 4), and it was identified to species (Steps 5 and 6). Although *M. euphorbiae* has been reported on three species of *Euphorbia* in the US, it has not been found on leafy spurge (Farr et al., 1989) (Step 7). As it is a rust fungus, the host range was regarded as being narrow (Step 8), and it is not transmitted by vectors (Step 9), nor does it require a wound to cause infection (Step 10). The issue with isolates of *M. euphorbiae* tested at the two laboratories is that each isolate only infected the source clone of LS or cypress spurge. None of the other clones developed significant infections, whether from Europe or the US (Bruckart et al., 1986) (Step 11). For this reason, further development of *M. euphorbiae* for biological control of LS was abandoned.

6.2. Success: Puccinia jaceae on Centaurea solstitialis

Yellow starthistle is an introduced invasive weed of major importance in the Western US, particularly California. It has been (and remains) a target of biological control efforts for at least 30 years. In 1978 an isolate of the rust fungus *P. jaceae var. solstitialis* (Savile, 1970) was collected by R.G. Emge (USDA-ARS, Retired) from YST in Bulgaria. Later several other isolates of this fungus were collected by S. Rosenthal (USDA-ARS, Retired) in 1984 from Turkey, by F. Eskandari in 1995 from Iran, and by R. Sobhian (USDA-ARS, Retired) in 1998 from Uzbekistan. The following are the steps in Fig. 1 that lead to the release of this fungus in the US. The fungus was not present in the area of intended release (Klisiewicz, 1986; Woods and Fogle, 1998), and indigenous pathogens identified in these studies were not effective in reducing stand densities nor were they considered potentially useful for biological control (Step 1). Fungi were collected from YST overseas, and complete collection information was available for each isolate (Step 2). Soon after the initial collection, Koch’s postulates were fulfilled (Step 3). The rust was relatively easily maintained in a distinguishable and collectable state on YST in the greenhouse (Step 4). The pathogen had been identified to species (Step 5), and the species had not been reported in the area of intended release (Steps 7, 7c). At the time, the pathogen had only been reported from YST (Step 8). No vector was involved and wounding of the plant was not required for infection (Steps 9, 10). Inoculation of YST with the rust resulted in consistent significant reductions in root biomass (Bennett et al., 1991; Shishkoff and Bruckart, 1993), and greenhouse effects in that study were similar to those from inoculations of rush skeletonweed with *P. chondrillina*, a pathogen associated with the successful control of its target in the US and Australia (Cullen, 1985; Supkoff et al., 1988) (Step 11). Of 65 plant species from 10 plant families that were inoculated, plants from four genera within the Asteraceae, all from the tribe Carduae, were symptomatic after inoculation (Bruckart, 1989; Shishkoff and Bruckart, 1993). These were a few species of *Centaurea*, *Cirsium*, *Carthamus*, and *Amberboa* (Steps 12, 12b). Reactions on all the hosts were limited and the pathogen could not be maintained on species other than YST, except for *Centaurea cyanus* L., a weedy plant in wheat that was not damaged by the infections (Shishkoff and Bruckart, 1993) (Step 12c).

A proposal was made to use this rust fungus in California on YST (Step 13). Additional tests of native North American *Cirsium* species and modern safflower cultivars were requested by regulators and specific interest groups (Step 14). The additional research was in response to hazards identified in the earlier study (i.e., limited infection on two species of *Cirsium*), changes in safflower cultivars (new varieties with high oleic or linoleic acid content), and concern that *P. jaceae* might cause a seedling disease similar to that resulting from infestation of safflower with teliospores of the safflower rust fungus, *P. carthami*. All tests were conducted in a containment greenhouse. Tests of 19 *Cirsium* species and nine safflower cultivars involved direct inoculation of foliage with urediniospores followed by dew at 18–20 °C for 16 h. None of the *Cirsium* species were infected in the present study and only minor infections, similar to those in earlier studies, occurred on the modern safflower cultivars. *P. jaceae* could not be maintained under optimal greenhouse conditions on the foliage of any of these plants. Quantitative inoculation provided no evidence of infection of safflower hypocotyls inoculated with teliospores of *P. jaceae* (Bruckart and Eskandari, 2002), even though large cankers were observed on plants inoculated with *P. carthami* and clear microscopic evidence of infection was observed in hypocotyls...
following treatment with *P. carthami*. These data suggested that native (including rare, threatened, or endangered) *Cirsium* spp. and modern safflower cultivars were not likely to be adversely affected by the use of *P. jaceae* for biological control of YST (Step 15). Results of these studies substantiated findings from previous tests and were incorporated in a proposal for permission to use *P. jaceae* for YST control in California (Step 16). After extensive review by the TAG and APHIS (with input from the F&WS), an Environmental Assessment was made, and notice of intent was published in the Federal Register (Federal Register, 2002). Comments were reviewed and responses developed, after which APHIS issued a FONSI (Step 17). A permit was issued and the first release of *P. jaceae* for biological control of YST in the US was made in July, 2003 (Suszkiew, 2004).

7. Conclusions

Most exotic pathogens collected for classical biological control of invasive weeds are never released for the reasons outlined in this paper. However, deciding if and when to abandon further work on a pathogen is often a difficult process since discarding pathogens that may provide effective and safe biological control is as undesirable as continuing work on pathogens that will not provide effective or, particularly, safe control. In this paper, we have tried to formalize the decision-making process that we use in evaluating exotic plant pathogens for classical biological control of invasive weeds in the US. Much of the decision-making process is based on our evaluations of the relative agricultural and ecological safety of the exotic pathogens. In turn, most of these evaluations are scientifically objective based on host range tests. However, in the case of pathogens that cause minor disease on nontarget agriculturally important or native plants, the process becomes more dependent upon what the regulatory agencies will accept. This regulatory atmosphere is dynamic and based, in part, on public attitudes toward biological control organizations. As public attitudes and the regulatory atmosphere change, the decisions we make on evaluating pathogens will change as well, but we anticipate that favorable change for pathogen release will be a gradual process. However, negative attitudes could develop much more quickly if we (and others) are not diligent in ensuring releases of safe pathogens.

References


