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Risks to Agriculture and Forestry Arising From the Industrial Use of Phytopathogenic Microorganisms

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Abstract

The main danger in industrial use of plant pathogens is inadvertent failure to respect existing regulations on quarantine organisms and import of cultures. The risk from industrial use of organisms already present in a country is minor, and mostly limited in time and space. However, users should know the pathogenicity, geographical origin, form or pathotype and pesticide resistance of a culture they acquire and should always check whether any of these are new to the country of use. Stricter considerations will apply to genetically manipulated organisms or species imported to be released in biological control programmes.

Keywords: quarantine organisms, phytosanitary regulations

1. Introduction

From the first industrial use of microorganisms on a large scale, risks have had to be analyzed, with dangers to staff and the general public of course in priority. The perception that there might be a risk to agriculture and forestry was very secondary, but it is clear that plant pathogens, for which culture conditions, and enzyme and secondary metabolite production, have often been well studied for the purposes of pathology research, will tend to be among the first candidates as industrial organisms because of the availability of this information. It was accordingly quite essential to pose the question of potential risk, which has been examined already, for example, in the paper

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"Microbial Plant Pathogens: Natural Spread and Possible Risks in their Industrial Use" by Evans, Preece and Sergeant for the Commission of the European Communities, and in discussions of the Working Party on Safety in Biotechnology of the European Federation of Biotechnology.

The risk assessment concerns on the one hand the technical aspects: can aerosols of infective spores or bacteria be released, can contaminated liquids and solid wastes escape, how physically can this dangerous material reach crops or forests, and of course what type of containment and precautions are needed to minimize the risk? On the other hand, the risk can also be assessed purely from the plant pathologist's point of view. In the worst case of escape of infective material, how serious an epidemic, and how serious losses, are liable to arise?

In fact, it appears to this author that the answer to the second question largely makes it unnecessary to discuss the first. For most plant pathogens, there is little risk and only elementary precautions are needed. For a few plant pathogens, there is a very high risk, so high that the only satisfactory recommendation is not to use such an organism at all.

2. Risk from Quarantine Organisms

The real risk comes from the introduction into a country or area of a pathogen not previously present. In the past, the main possible channel of introduction of these has been plants and plant products, and especially imported large commercial consignments of planting material. Plants and plant products for consumption, or carried by travellers, also present a definite risk. Countries have developed phytosanitary (plant health) regulations to counter these risks. Since the list of plant pathogens not present in a country is necessarily an enormous one, one approach (followed by the Working Party on Phytosanitary Regulations of EPPO, and by most EPPO member countries — Smith, 1979) is to select the pests (insects, nematodes, etc. are evidently also covered) which present the greatest risk, in order to focus attention especially on them. EPPO uses the following criteria to select the most dangerous pests from the long list of those not present in Europe or of limited distribution there (Mathys and Smith, 1984).

1. a significant risk to an economic crop (for example, specific pineapple pathogens present a low risk to Europe);
2. possibility of survival, outdoors or on a significant glasshouse crop (thus, *Puccinia polysora* causing tropical maize rust is not a significant risk to Europe);

3. no possibility of natural spread (*Peronospora tabacina* cannot naturally move between continents or, for example, cross the Sahara, but cannot usefully be considered a quarantine pest within Europe, since it is readily dispersed by wind between European countries);
4. real possibility of import by trade (since, as far as the author knows, Japan does not export onions to Europe, no very useful purpose is served by worrying in priority about Japanese onion pathogens. However, this criterion is above all liable to sudden change, and indeed the import of cultures for industrial use introduces a new 'real possibility' where none previously existed).

The selected organisms are now known as quarantine pests* and the international phytosanitary certificate which accompanies consignments of plants and plant products in international trade certifies that the material has been found free from quarantine pests. This freedom is also referred to as a 'zero tolerance', the distinction being made from other pests (quality pests), for which a small tolerance may be allowed or as the certificate states 'consignments must be practically free'. Typically, such quality pests are already present in the importing country and the risk is only to the grower immediately using the material rather than to the agricultural community as a whole. In contrast, quarantine pests may present a high or low risk to the immediate user, but by definition present a high risk to the country at large.

A country defines its list of quarantine pests and then imposes a zero tolerance for them by prohibiting import of such pests in any form, whether on plants (some or all species) or as cultures. In addition, in Europe at least, regulations may specify a series of special requirements for different plants, designed to minimize the risk of the presence of quarantine pests (which simple pre-export inspection cannot always ensure).

Some countries also prohibit all imports of cultures, and then have special licensing systems to authorize import and use of organisms under well defined conditions.

So, although not all countries have necessarily yet covered this risk in regulations, many already have strict rules controlling the import of cultures of plant pathogens, and especially those considered to be quarantine pests. The main problem is that the industrial user is not necessarily aware of them and that international exchange of cultures is so easy to do and so difficult

* According to FAO, a quarantine pest is "a pest of potential national economic importance to the country endangered thereby and not yet present there or present but not widely distributed and being actively controlled".

to check or control. So, inadvertent introduction is easy. The regulations depend on goodwill for their enforcement rather than on any possible policy of policing or sanctioning. Any user of plant pathogens has to take the responsibility of informing himself of current regulations in order to respect them. Indeed, it has to be stressed that plant pathology research workers have themselves in the past been responsible for introducing plant pathogens to new countries or continents, notwithstanding their claim that they should know better than others how to handle them safely. The risk from plant pathologists is probably greater than that from industrial users — but all must face their responsibilities.

3. Examples From the EPPO A Lists

EPPO has compiled two lists of quarantine pests for EPPO member countries (EPPO, 1982) — the A1 list of organisms not present in any member country which all, should join together to exclude, and the A2 list of organisms present in some member countries but not others, which many member countries will still seek to exclude. Here, we need to consider only the bacteria and fungi, and Tables 1–4 give the species concerned. An important point to stress is that, while some are easily cultured, many are 'obligate parasites', not yet cultured and probably very difficult or impossible to culture. In addition, a number are fairly specialized plant pathogens, spending all their life cycle on their host with hardly any saprophytic existence. They can perhaps be cultured, but probably with difficulty, and with slow growth. They are not very likely candidates for industrial use. These distinctions have been brought out in the Tables, and it is probably only worth being seriously concerned about the readily cultured species. The ones of most interest are briefly reviewed below.

A1 bacteria

The pathovar of *Xanthomonas campestris* on citrus is a major hazard to the Mediterranean countries, while those on rice concern a somewhat less important but still very significant crop for Europe. Readily cultured, and potential sources of extracellular polysaccharide gums, these non-European pathovars of a species already used in industry are a major hazard.

A1 fungi

Many of the A1 fungi are not readily cultured, but special attention can be focussed on species such as *Ceratocystis fagacearum*, the cause of oak

Table 1. A1 quarantine bacteria for Europe.

Organism	*Range*	*Disease*	*Culture**
Citrus greening bacterium	Asia Africa	Greening	–
Xanthomonas campestris pv. *citri*	Asia +	Citrus canker	C
Xanthomonas campestris pv. *oryzae*	Asia	Rice leaf blight	C
Xanthomonas campestris pv. *oryzicola*	Asia	Rice leaf streak	C

* In Tables 1–4, C: readily cultured, ?: probably rather difficult to culture, –: obligate parasite.

Table 2. A1 quarantine fungi for Europe.

Organism	*Range*	*Disease*	*Culture*
Angiosorus solani	S. America	Potato smut	?
Apiosporina morbosa	N. America	Prunus black knot	?
Atropellis spp.	N. America	Pine branch canker	?
Ceratocystis fagacearum	N. America	Oak wilt	C
Cercoseptoria pini-densiflorae	Asia	Pine needle blight	?
Chrysomyxa arctostaphyli	N. America	Spruce broomrust	–
Cronartium spp.	N. America Asia	Conifer rusts	–
Guignardia laricina	Asia	Larch shoot blight	?
Gymnosporangium spp.	N. America Asia	Pear and apple rusts	–
Hamaspora longissima	Africa	Rubus rust	–
Inonotus weirii	N. America	Conifer butt rot	C
Melampsora farlowii	N. America	Hemlock rust	–
Monilinia fructicola	N. America Australasia	Fruit rot	C
Mycosphaerella laricileptolepis	Asia	Larch needle cast	C
Mycosphaerella populorum	N. America	Poplar canker	C
Phoma andina	S. America	Potato leaf spot	C
Phyllosticta solitaria	N. America	Apple blotch	?
Puccinia pittieriana	S. America	Potato rust	–
Septoria lycopersici var. *malagutii*	S. America	Potato leaf spot	C
Tilletia indica	Asia	Wheat karnal bunt	?
Trechispora brinkmannii (*Phymatotrichopsis omnivora*)	N. America	Cotton root rot	C

Table 3. A2 quarantine bacteria for Europe.

Organism	*Disease*	*Culture*
Corynebacterium flaccumfaciens	Bean wilt	C
C. insidiosum	Lucerne wilt	C
C. michiganense	Tomato canker	C
C. sepedonicum	Potato ring rot	C
Erwinia amylovora	Fireblight	C
E. chrysanthemi	Wilt	C
E. stewartii	Maize wilt	C
Pseudomonas caryophylli	Carnation wilt	C
P. syringae pv. *persicae*	Peach dieback	C
P. syringae pv. *pisi*	Pear blight	C
P. solanacearum	Tomato wilt	C
Xanthomonas ampelina	Grapevine blight	C
X. campestris pv. *corylina*	Hazel canker	C
X. c. pv. *phaseoli*	Bean common blight	C
X. c. pv. *pruni*	Prunus black spot	C
X. c. pv. *vesicatoria*	Tomato scab	C
X. fragariae	Strawberry blight	C
X. populi	Poplar canker	C

wilt in N. America (since other species of the genus are used in industry), *Monilinia fructicola*, a fruit-rot and canker pathogen not present in Europe and classically used in plant pathology research as an inducer of phytoalexin production, and *Trechispora brinkmannii*, better known as its anamorph *Phymatotrichopsis omnivora*, the notorious cause of Texas cotton root rot, known also as a good cellulase producer.

A2 bacteria

Of the A2 bacteria, we may single out *Erwinia amylovora*, causing fireblight of pear and apple, and possibly the most significant quarantine pest already present in one part of Europe and threatening another part (Italy and Spain especially). Inadvertent introduction of a culture by an industrial user could lead to a disaster, setting to zero massive programs of preparation and protection against the possibility of introduction on planting material. *Corynebacterium sepedonicum*, causing ring rot of potatoes, is perhaps less significant as a direct risk to crops, but most European countries do not have it and quite rightly wish to avoid the losses it would cause. Seed-potato producers have to be able to export ring-rot-free material, and face great costs,

Table 4. A2 quarantine fungi Europe.

Organisms	*Disease*	*Culture*
Ceratocystis fimbriata f. sp. *platani*	Plane canker stain	C
Ceratocystis ulmi	Dutch elm disease	C
Cochliobolus carbonum	Maize leaf spot	C
Didymella chrysanthemi	Chrysanthemum ray blight	?
Cryphonectria parasitica	Chestnut blight	C
Fusarium oxysporum f. sp. *albedinis*	Date palm bayoud disease	C
Glomerella gossypii	Cotton anthracnose	C
Melampsora medusae	Poplar rust	–
Mycosphaerella linicola	Flax pasmo disease	C
Ophiostoma roboris	Oak vascular mycosis	C
Phaeoisariopsis griseola	Bean angular leaf spot	?
Phialophora cinerescens	Carnation wilt	C
Phoma exigua var. *foveata*	Potato gangrene	C
Phytophthora fragariae	Strawberry red core	C
Puccinia horiana	Chrysanthemum white rust	–
P. pelargonii-zonalis	Pelargonium rust	–
Scirrhia acicola	Pine needle blight	?
Stenocarpella macrospora & *S. maydis*	Maize stalk rot	C
Synchytrium endobioticum	Potato wart	–
Tilletia controversa	Wheat dwarf bunt	?
Uromyces transversalis	Gladiolus rust	–
Hop strains of *Verticillium albo-atrum* & *V. dahliae*	Hop wilt	C

Table 5. Plant quarantine organisms producing hydrolases of potential interest (Bertheau et al., 1985).

Ceratocystis ulmi	Pectinase & cellulase
Cryphonectria parasitica	Protease
Trechispora brinkmannii	Cellulase
Verticillium spp.	Pectinases
Xanthomonas campestris pv. *oryzae*	Pectinase

if this disease were introduced, to satisfy their customers on its absence in their exports.

All the other A2 bacteria could be used in culture and present a greater or lesser risk to the countries where they are not yet present. One case does need special mention, however — *Erwinia chrysanthemi*, since it has already been used industrially. It is in fact widespread in European countries, and presents, in general, no more risk than other indigenous species. However, the best way to exclude it from carnation and chrysanthemum glasshouses is rigorous sanitation of the premises and use of absolutely healthy cuttings. In modern practice, a large proportion of cuttings is imported (e.g. from Mediterranean countries to Northern Europe) and this makes phytosanitary control of this planting material international in nature and *E. chrysanthemi* a somewhat special type of quarantine organism. It is clear, however, that industrial culture of the species in Europe presents no special risk to floriculture.

A2 fungi

Ceratocystis ulmi, the cause of Dutch elm disease, is likely to be removed from quarantine lists in Europe fairly soon, since the measures taken against it have not limited its spread. A quarantine pest does not necessarily always remain one but may continue to be one elsewhere (for example, Australia in this case). *C. fimbriata* f. sp. *platani* causes a somewhat slower spreading and less damaging canker stain of plane, now limited to parts of France and Italy, but actively spreading unless strong measures are taken. Plane is the major urban tree in Europe.

Fusarium oxysporum is a well known and diverse pathogen, existing in saprophytic, unspecialized parasitic and host-specific forms (formae speciales). It seems a likely candidate for industrial use and it is then critical to ensure that the form used is not new to the country. At present, f. sp. *albedinis* on date palm (bayoud disease) is listed as the form of concern for EPPO, but new forms are regularly described of this highly variable and damaging fungus, and great care should be exercised.

The other main vascular wilt pathogen (*Verticillium*) has less clearly defined forms — not always recognized as clear taxonomic entities. Nevertheless, certain strains are more damaging to certain hosts, and the 'progressive wilt' strain on hops has caused great concern in United Kingdom and could be as dangerous in other hop-growing countries. *V. albo-atrum* and *V. dahliae* are

again well studied fungi, known to produce many potentially useful polysaccharidases.

Indigenous pathogens

The quarantine organism which escapes from industrial use may cause very little damage at first, but if it spreads and establishes itself, the whole country suffers. The indigenous organism which escapes probably, in just the same way, causes very little damage — and it is simply of no importance what becomes of it in future, since the escape makes a negligible contribution to the existing population. In a very few cases, a special combination of circumstances might allow such an escape to cause substantial local damage. The most reasonable approach to such cases would seem to be 'to make the polluter pay' as he might if his industry were releasing sulphur dioxide and it could legally be proved that this was responsible for economic damage to a farmer's crops.

One point which is potentially more serious arises with soilborne pathogens with long-lived resting structures (chlamydospores, sclerotia, resting sporangia). In such cases, bulk wastes should evidently not be allowed to contaminate soil, but it is doubtful whether one would ever discover that a small amount of inoculum had been released, given the low potential for epidemic spread.

Risks from plant pathogens already in industrial use

Bertheau et al. (1985) list a large number of fungi and bacteria producing 'interesting' hydrolases, and the papers presented at the current conference refer to a long list of plant pathogens already or potentially in industrial use. Most of these are widespread organisms indigenous to European countries, and presenting very little risk (e.g. *Sclerotinia sclerotiorum, Fusarium solani, Agrobacerium tumefaciens, Erwinia carotovora, Glomerella cingulata, Rhizoctonia solani, Phytophthora infestans, Cephalosporium acremonium*).

However, Table 5 picks up a few cases of listed quarantine organisms, some already referred to above, which are of industrial interest. It is useful to stress again that such cases do exist — there are organisms which should not be used or only after very thorough consultation.

The question of strains

Some plant pathogens have high-risk strains, races or forms. These may be more aggressive (*Ceratocystis ulmi*), or carry special virulence with respect to host resistance genes, or be resistant to fungicides. Formae speciales (as with *Fusarium oxysporum*) or pathovars (as with *Xanthomonas campestris* and *Pseudomonas syringae*) present a somewhat similar problem. Such forms may often be absent from an area where the species is present in other forms. For practical plant quarantine, it is often difficult to try to make a distinction, since the measures taken to keep out a special strain will generally apply to all strains. However, the industrial user does not face this problem. He knows, or should know, the exact origin and characterization of a strain he imports. Accordingly, it is possible and indeed essential for him to check whether an imported strain does present a special risk.

Two special cases

The general principles discussed here will not apply in some cases. One is the use of genetically manipulated plant pathogens. These may not, indeed, present any great risk but it is impossible yet to be sure of their potential, which may of course vary greatly from case to case. Special guidelines are needed, and have been under study in OECD. Another case is the culture of fungi or bacteria as biological control agents. The problem is not one of accidental release, since these organisms are intended to be released. Again, special guidelines have to be developed.

4. Conclusions

It would be premature for an article such as this to lay down specific guidelines for these have to be based on international consultations which are already under way. However, the author's conclusions can be summarized as a basis for discussion. The industrial user must first clearly know whether he is using a plant pathogen and whether this occurs naturally or not in the country of intended use. This geographical criterion is important, and could conceivably pose problems for firms operating in several countries (especially in different continents). If the pathogen used does occur locally, one should check whether it has long-lived resting stages liable to contaminate land. One should equally check whether the strain used carries genetic characters (virulence, fungicide resistance) not present locally.

Evidently, any imported culture presents a risk (consider also the possibility that a locally obtained strain was ultimately imported). If the species is a listed quarantine organism, it would in most cases be out of the question to use it. One should substitute a related but safe species. Non-pathogenic strains of the same species are a possibility, but evidently only after very full consultation with plant health authorities, which may well prefer not to authorize such exceptions to a good general rule.

Finally, one should recall that most species not present in the country of use have not been listed as quarantine organisms for they did not meet criteria of immediate danger like those presented above in this article. Such species may or may not be dangerous. Import of cultures provides a short-cut for introduction of species which are very unlikely to enter on plants or plants products with existing trade. In such cases, therefore, it is not for the industrial user to judge the risk. The plant health authorities should be consulted and indeed, in some countries, there is a legal obligation to do so.

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