European Journal of Plant Pathology **103:** 775–784, 1997.

Selection of biological control agents for controlling soil and seed-borne diseases in the field

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Accepted 1 August 1997

Key words: antagonists, biological control, comparative screening, field screening, rhizosphere competence, field performance

Abstract

Different screening methods for selection of biological control agents (BCAs), for controlling soil and seed-borne diseases, are discussed. The shortcomings of laboratory methods focused on mechanism of action are discussed and we conclude that these methods should be used with caution if candidates with multifactorial or plant mediated mechanisms of control are to be obtained. *In vitro* screens may be useful for specific groups of microorganisms, thus, screens for antibiotics may be relevant for *Streptomyces* spp., and promising results have been obtained using soil plating or precolonized agar methods to screen for mycoparasitism and competitive saprophytic ability. Experience with screening in the Nordic programme 'Biological control of seed borne diseases in cereals' is summarized. Research in the four participating countries – Finland, Sweden, Norway and Denmark – followed the same paradigm: that of obtaining antagonists, well adapted to different Nordic environments, and developing them as effective BCAs. Potential antagonists were isolated from different sources and *in planta* screening methods were developed in order to optimize selection of antagonists effective against a range of seed borne pathogens. Screens in the laboratory or greenhouse were followed by screening in the field. The different screening procedures are compared and evaluated.

Introduction

The relevance of much of the research in biological disease control for practical farming has been questioned (Deacon, 1988; Gerhardson and Larsson, 1991; Campbell, 1994). Although the reasons for the often highly variable results obtained in biocontrol experiments carried out under practical growing conditions are difficult to analyse, one of the explanations offered is the *ad hoc* nature of the screening procedures with undue emphasis on *in vitro* methods (Merriman and Russel 1990; Gerhardsson and Larsson, 1991).

Although a number of authors have stressed the importance of appropriate screening procedures (Campbell, 1986; Whipps and Magan, 1987; Lumsden and Lewis, 1989; Merriman and Russell, 1990; Deacon, 1991; Campbell, 1994; Jensen et al., 1996; Whipps, 1997), only a few studies have been carried out, comparing results of different screening methods (e.g. Kommedahl and Windels, 1978; Renwick et al., 1991; Duczek, 1994; Teperi et al., subm.). The aim of this paper is to discuss some of the approaches to screening with special reference to our own results which were reached in an Internordic research pro-

gramme carried out in Denmark, Finland, Sweden and Norway. From screenings within this programme four BCA products based on antagonistic bacteria and fungi are presently being developed and patented (Tahvonen and Teperi, 1995; Jensen et al., 1996; Hökeberg et al., 1997).

The background for screening methods

Any screening method is selective, therefore it is to be expected that only a part of the antagonistic microflora will be detected. The important question is however: Will the methods used select the organisms most suitable for disease control in the environment, in which they are meant to work? (Deacon, 1991).

Biocontrol strategies may be divided into two broad categories. One strategy follows a fundamentally ecological approach. This strategy has been termed classical biocontrol (Hokkanen and Lynch, 1995) or biocontrol with one-time introduction (Cook, 1993). The other strategy uses microorganisms as biopesticides and resembles in some important respects the approach known from chemical pesticide treatment which aims at control for a limited period of time. This strategy has also been referred to as augmentative biocontrol (Hokkanen and Lynch, 1995). These differences in control strategy ought to influence the choice of isolation as well as screening method.

In the ecological approach, selected organisms should be able to function in the same environmental niche as that of the pathogen they are to control. Thus, suitable places for isolation of such ecologically adapted antagonists against seed and soil borne pathogens would be the surface of seeds and roots of the plants susceptible to the pathogen in question (Renwick et al., 1991; Knudsen, 1994). In order to obtain antagonists against *Fusarium culmorum*, Knudsen (1994) isolated fungi from the seed coat and rhizosphere of *F. culmorum* infected barley, from *F. culmorum* infested straw and from bulk soils. A comparison of all tested sources showed that the highest frequency of antagonistic isolates was obtained from the rhizosphere of barley roots (53%) as compared to the other sources which yielded similar but lower numbers of antagonists (about 20% each). Hökeberg et al. (1997), on the other hand, concluded that host plant or climatic zone were not critical factors in finding effective antagonists against seed-borne *Microdochium nivale*, *Drechslera teres* and *Tilletia caries*.

An often stated recommendation is to look for healthy plants in a field with diseased plants of the same species (e.g. Linderman et al., 1983). When screening for antagonists against *Gaumannomyces graminis*, Renwick et al. (1991) found that although 72% of the isolates originated from fields cropped continously with wheat, only 23% of the effective strains came from such fields. Thus, their initial hypothesis, that biocontrol agents of take-all would be most easily found in fields naturally suppressive to the disease, was not confirmed. Others, however, have found very effective isolates in soils suppressive to take-all (Weller and Cook, 1985), to *Fusarium*-wilt (Alabouvette, 1990) and in *Sphagnum* peat suppressive to *Pythium* diseases (Tahvonen, 1982; Wolffhechel, 1989).

At least in the case of the augmentative control strategy, it might seem that less attention need be paid to the source of the antagonist. Even so, knowledge about the origin of an antagonist will always be relevant in connection with risk evaluation and wild type candidates, isolated from natural locations, can be expected to give less complications in the registration process (Lumsden and Lewis, 1989).

A valuable background for any screening approach is a thorough knowledge of the etiology and life cycle of the causal agent to be controlled: particularly, it is important to have knowledge about inoculum transfer, survival, critical inoculum threshold level, the infection process, climatic conditions favorable for disease outbreak and development etc. These aspects need to be mirrored in the biological control strategy, time and place of application etc. and thus, initially also the choice of an appropriate screening method. More often, however, investigators have focused on the mode of action of the antagonist in rather artificial environments far removed from the field situation. Thus, often the search for isolates or strains of microorganisms with antagonistic properties has been carried out using purely laboratory methods. As most of these methods have important drawbacks and shortcomings as discussed below, they should be used with great caution.

Types of screening protocols

Screening methods can be arranged after the level of complexity they represent. Methods with low numbers of components (e.g. an antagonist and a pathogen) mainly give information about mechanisms of antagonism. More complex methods, with higher numbers af components – including, for example, antagonist,

pathogen, host and environmental factors – may give less exact information about the mechanisms of action, but mimic more closely the field situation.

In the following we will discuss several types of screening protocols and some of the problems and shortcomings in using them.

Methods based on hydrolytic enzyme activity of the antagonists

Finding organisms with specific enzyme activity or toxin production is the object of some procedures. This type of approach is most often used to differentiate amongst candidates of a species already known to possess antagonistic potential. Thus, it is focused on some facet of the mechanism of antagonism itself.

Screening based on assays for enzymatic activities has been used to differentiate isolates of *Gliocladium virens* and *Trichoderma longibrachiatum* (Sreenivasaprasad and Manibhushanrao, 1990), and others have similarly found correlations between different hydrolytic enzyme activities and biocontrol efficiency within the genera *Trichoderma* and *Gliocladium* (Elad et al., 1982; Lorito et al., 1993). Nevertheless, even when the presence of lytic enzymes can be demonstrated, there may be lack of good evidence of causation between this factor and biological control. Thus Ordentlich et al. (1991), found lack of correspondence between biocontrol efficiency and *in vitro* activity, in dual culture, of three *Trichoderma* isolates against *Fusarium oxysporum*. This was further emphasized in enzymatic assays, where 1,3-β-glucanase and chitinase activity of the three antagonists reached a maximum at the same time but the crude filtrates of the three isolates degraded mycelium of *F. oxysporum* differently. It was obvious from this study, that there was no correlation between biocontrol *in vivo* and the results of the enzyme assays (Ordentlich et al., 1991).

Ridout et al. (1988) proposed that a screening method based on 1,3-β-glucanase and chitinase activity was a possible method for selecting antagonistic strains of *Trichoderma* spp. against *Rhizoctonia solani*. However, following purification by chromatofocusing, these enzymes were shown to represent only a small fraction of the total extracellular proteins induced by cell walls. They concluded that the breakdown of *R. solani* cell walls appears to be a complex process involving many different enzymes, and that this should be considered when developing a screen for biocontrol activity.

Methods based upon interaction studies

Dual culture on agar

Two-component screening (e.g. dual cultures of a candidate antagonist and a pathogen on agar) is exclusively related to interaction studies and potential antagonists are typically ranked according to their ability to inhibit the growth of the pathogen expressed by an inhibition-zone. Some *Streptomyces* species isolated from sphagnum peat produce polyene antibiotics, which are known to be the substances active against fungi *in vivo*. The production of these antibiotics correlate very well with the biocontrol ability of *Streptomyces* isolates (Raatikainen et al., 1993) and the dual culture method has performed reasonably well for screening *Streptomyces griseoviridis* (R. Tahvonen, unpubl.). However, others have found that production of antibiotics *in vitro* does not correlate with their production *in vivo* (Fravel, 1988; Renwick et al., 1991). One reason could be that antibiotics may be induced by specific nutrients. For example Whipps (1987), and Whipps and Magan (1987) studied interactions between pathogens and antagonists in dual cultures on different media, by measuring the percentage inhibition of growth. In the majority of cases very different results were obtained on the different test media. No single medium can, it seems, promote the expression of the full range of antibiotic production of an organism. Studying the antifungal compounds produced by *Epicoccum purpurascens*, Brown et al. (1987) found that nutrients played a role for the amount of antibiotic produced and the authors suggested that caution should be taken when attempting to equate *in vitro* and *in vivo* production of these compounds. They further showed that amino acids act as precursors for the production of some antibiotics (Brown et al., 1987). Stack et al. (1987) found, that the source of carbon and nitrogen used in the carrier substrate and the C:N ratio significantly affected the growth of antibiotic producing antagonists subsequent to the placement of the carrier granules in nonsterile soil. Temperature, pH and water potential can also be expected to influence antibiotic production but references are scarce.

Antibiotics in soil

Another reason for the often poor correlation between *in vitro* and *in vivo* results may be related to inactivation of antibiotics which can occur rapidly in natural soil (e.g. after adsorption to colloids (Williams, 1982)). For example, in sterile soil chloramphenicol concentration

remained constant for 14 days, while in non sterile soil it decreased rapidly within 3 days and only a trace remained after 14 days (Gottlieb and Simonoff, 1952). On the other hand, other studies have demonstrated biological control due to fungal secondary metabolites in soil. *Talaromyces flavus*, although known to produce four antibiotics on agar, is suggested to control *Verticillium dahliae* in the soil by releasing glucose oxidase, an enzyme that generates hydrogen peroxide from glucose to the detriment of the pathogen (Kim et al., 1988). Strains of fluorescent pseudomonads are thought to suppress *Thielaviopsis basicola* (black root rot of tobacco) by producing cyanic acid in the rhizosphere (Défago et al., 1990) and take-all is likewise suppressed by fluorescent pseudmonads producing the antibiotic phenazin-1-carboxylic acid in the rhizosphere of wheat (Thomashow et al., 1990). So far antibiotic production on the surface of plant roots or the seedcoat where interactions take place has been difficult to analyze under natural conditions due to inadequate methodology (J. Friesvad pers. comm). For example, *Streptomyces griseoviridis* produces an heptaene antibiotic in soil in relatively small amounts compared to the amount needed for chemical analysis (Raatikainen et al., 1994). However, in the future, new techniques may be helpful in elucidating production of specific antibiotics in soil e.g. by immunological methods as shown by Lumsden et al. (1992).

Competitive saprophytic ability (soil plate method)

In vitro, the mycoparasitic ability of *Trichoderma* isolates correlated well with their ß(1-3)glucanase and chitinase activity (Elad et al., 1982). However, as demonstrated by Davet (1986, 1987), for a *Trichoderma* isolate to be effective in non-sterile soil, a strongly developed saprophytic competitive ability was found to be essential. Good correlation was found between the competitive saprophytic ability of a range of *Trichoderma* isolates and their ability to destroy sclerotia in non sterile soil and a simple soil plate method to measure the competitive saprophytic ability of *Trichoderma* isolates has been described and documented (Davet, 1986).

Precolonized agar method

As pointed out by Deacon and Berry (1992), competition for substrates already occupied by other fungi is an ecological feature shared by many mycoparasites. Competitive possession of substrates is utilized in the precolonized plate method to selectively isolate mycoparasites from natural habitats. In this method an agar plate is first colonized by a host fungus after which particles of soils or plant material are placed on the surface. Only fungi that are able to derive nutrients from the precolonizing fungus and to tolerate its waste products will be able to grow. As there is no universally susceptible host fungus, and all mycoparasitic fungi have restricted host ranges, the choice of host fungus used to precolonize the plate will greatly influence the type of mycoparasite that will be detected (Deacon and Berry, 1992; Mulligan and Deacon, 1992). Mycoparasites isolated by this method include *Gliocladium roseum* and related species (Foley and Deacon, 1985), *Papulaspora* sp. (Mulligan and Deacon, 1992), *Pythium oligandrum* and related species (Deacon and Henry, 1978; Foley and Deacon, 1985; Mulligan et al., 1995), *Pythium mycoparasiticum* (Foley and Deacon, 1985; Mulligan et al., 1995) and *Verticillium biguttatum* (van den Boogert and Gams, 1988).

The method can also be adapted to rank isolates for aggressiveness and several easy-to-use criteria are available including linear growth across the precolonized plate (Deacon, 1976; Laing and Deacon, 1990), reduction in sporulation of the host alone or together with an increase in sporulation by the antagonist (Davanlou and Hockenhull, 1996; Hockenhull et al., 1995), and microscopy of mycoparasitizing of host conidia (Davanlou and Hockenhull, 1996) or of hyphae (Bradshaw-Smith and Whalley, 1991; Laing and Deacon, 1991; Berry et al., 1993; Ribeiro and Butler, 1995). Little work has yet been published correlating aggressiveness scored by such laboratory methods with biocontrol performance under field conditions.

Studies based on approaches related to the plants

By extending the screening program to include plants growing in natural substrates, other mechanisms such as induced resistance and plant growth promotion, and edaphic or nutritional factors such as root exudates and plant residues will be included in the system.

In recent years, several reports have revealed that not only one but several mechanisms may be responsible for a biological control effect. The involvement of various mechanisms, and especially the role of competition in biological control has been reviewed by Faull (1988). Thus, even though antibiosis or mycoparasitism has been shown to occur, it is often competition for nutrients and the ability to compete against other organisms, in the rhizosphere, spermosphere etc., that are the essential attributes of successful biocon-

trol organisms (Deacon, 1991). Clearly, in the absence of plants, selection for antagonism alone will not say anything about an organism's ability to colonize and protect roots and seeds. It is well established that seed and root-infecting pathogens are often highly dependent on exudates to initiate plant infections (Nelson, 1991) and the ability of the antagonist to metabolize these exudate molecules may be an important step in biocontrol processes (Nelson, 1991; Green and Jensen, 1995). Indeed, rhizosphere competence has in some cases been seen as an important prerequsite for obtaining successful biocontrol and specific tests have been devised to select for this characteristic (e.g. Kloepper, 1991). Inoculation with BCAs based on single clonal strains may result in poor or short-lived colonization of the rhizosphere. To overcome this problem, Deacon (1994) advocates the use of mixtures of ecotypes of a BCA organism or combinations of different BCAs in order to achieve synergism and more persistent control.

Induced resistance

An area of increasing attention is the role of induced resistance in biocontrol. Induced resistance has been intensively studied in various plant pathosystems using biotic or abiotic inducing agents and is the subject of a recent monograph (Hammerschmidt and Kuć, 1995). Such studies have indicated that multiple defence mechanisms in plants can be activated by, and may be effective against diseases caused by a broad range of fungi, bacteria and viruses. Recently, several reports have been concerned with the detection of induced resistance in connection with the use of BCAs (Schippers et al., 1993; Benhamou et al., 1997). Naturally, only screening methods employing plants can take advantage of the phenomenon of induced resistance in the evaluation of putative biological control agents.

Field performance

Screening methods involving plants in which the results are measured as disease severity or disease incidence do not reveal the mechanisms involved. But, from a practical point of view – at least at this stage in the selection process – knowledge about mechanisms is not critically important. *In planta* screening is carried out in defined media such as sand (Knudsen et al., 1992; Knudsen et al., 1995), peat soil (Teperi et al., subm.) or in natural soils, possibly containing amendments such as sand.

An ability to protect infection sites *in situ* may be one of the key attributes of a biocontrol agent. This is the case in two classical examples of biocontrol in routine use; *Phlebiopsis gigantea* (against *Heterobasidion annosum*) in pine forests (Rishbeth, 1975) and *Agrobacterium radiobacter* for control of crown gall (New and Kerr, 1972). Although the period of protection may be more prolonged, a similar attribute is required of antagonists used of protecting seeds and roots from infection by seed borne and soil pathogens. For example, the possibility of protecting roots of flax from attack by the pathogen *Fusarium oxysporum* f.sp. *lini*, by saprophytic *Fusarium oxysporum* has been convincingly demonstrated (Eparvier and Alabouvette, 1994).

Deacon (1991) claims that 'Ecological attributes relating to the microenvironments of pathogens are the obvious base of screens for antagonists, and this is true irrespective of mode of action because the overriding requirement is that the antagonists must grow where the pathogen is found.' While a much wider acceptance of this concept is to be encouraged, the importance of field screening has already been demonstrated in the results of a number of studies. Lumsden and Lewis (1989), for example, recommend screening *in vivo* in non-sterile natural soil, and prefer field screening for organisms to be used in field crops. However, screening in the field may be difficult to perform due to inconsistent abiotic and biotic parameters and is time and space consuming and thus expensive. Because the requirements of a primary screen is often that it be simple, rapid and repeatable, we are often forced to make compromises. One compromise is to attempt to simulate field conditions in pot tests. However, here the physical, chemical and biological characteristics of the soil will not be identical with those in the field. Moreover, sterilized field soil or artificial substrates often are used in order to avoid compaction and similar cultural problems or to minimize labour. All such simplifications may cause problems in selecting the fittest antagonists for use under field conditions.

Comparison between simple and complex protocols: some case studies

With the aim of selecting the most useful antagonist from among a group of isolates a tiered or hierarchic approach to screening has frequently been adopted. In such an approach the first, preliminary, screening will typically be the most artificial and least laborious e.g. dual cultures, while the final screening will be the most natural as well as the most laborious e.g.

screening in the field. Additionally, one or even two intermediate level screenings may be carried out e.g. screening in a 3-component system (potential antagonist + pathogen + host plant) in a simple substrate in the growth chamber followed by screening in natural soil in the greenhouse. While an overestimation of numbers of potential candidates found in the initial and intermediate screenings may be acceptable, a more serious danger with the hierarchic approach is if the best candidates for biocontrol under field conditions are overlooked. Therefore, studies comparing different screening methods are needed to elucidate the extend of this problem. Only occasionally has this been done, and investigations carried out as part of a programme initiated by the Nordic Joint Committee for Agricultural Research entitled 'Biological control of seed borne diseases in cereals' included this type of work. In the programme, the projects in the four participating countries – Finland, Sweden, Norway and Denmark – followed the same paradigm: obtain antagonists, well adapted to different Nordic environments, and develop them as effective BCAs. Potential antagonists were isolated from different sources and different *in planta* screening methods were developed in order to optimize selection of the best antagonists against a range of seed borne diseases. Screening in the laboratory or greenhouse was followed by screening in the field. The different screening procedures could thus be evaluated and compared.

Screening against Drechslera teres

Fungi were isolated from soils collected in different regions in Norway and the isolates were screened in sand using a barley seedlot with 96% natural infection of *Drechslera teres*. Antagonists from this test were further screened in two different types of non-sterile field soils in selfwatering pots. The general efficacy of the antagonist was lower and the ranking different in the second test compared to the first screening. The most promising isolates were further tested in two barley field trials. The results from the field trials were more comparable with the non-sterile field soil test than with the sand test, and gave reason to believe that the non-sterile field soil test gave the most reliable result (Henriksen and Sundheim, 1994).

Screening against Fusarium culmorum *and* Bipolaris sorokiniana

In the screening programme carried out in Denmark, 340 fungal isolates from organic and conventional farming systems (Knudsen et al., 1994) were screened to select antagonists against *Fusarium culmorum*. The initial screening was carried out in small pots filled with moistened sand in which seeds were inoculated with spore suspensions of the presumed antagonist and the pathogen (Knudsen et al., 1992, Teperi et al., subm.). After 19 days at 15 °C, disease severity was evaluated. Isolates giving more than 50% control were thereafter tested on seeds naturally infected with *F. culmorum* or *Bipolaris sorokiniana*. Further selection was performed in pots containing field soil. Using this hierarchic screening system, a few very effective isolates were selected (Knudsen et al., 1995) and three of these isolates were further tested in field experiments. Two of the isolates gave significant control of *F. culmorum* measured as reduced disease incidence one month after sowing, and one isolate gave significant control when measured as grain yield. Similarly, the two isolates gave significant control of *B. sorokiniana* measured as increased dry weight after one month and yield and 1000-grain weight at harvest. Furthermore, the effect of the best isolate, identified as *Gliocladium roseum*, was as least as good as the fungicide treatment. It is interesting to note, that none of the best 7 isolates selected in this hierarchic screening process showed inhibition of *F. culmorum* or *B. sorokiniana* in dual cultures on potato dextrose agar (I.M.B. Knudsen, unpubl.).

In the experiments of Teperi et al. (subm.), 1700 isolates obtained from soil from different Finnish climatic regions were initially screened in sand against *F. culmorum*. Further screening was done in pots containing sphagnum peat or field soil. The best isolates were later tested in the field under different climatic conditions and also here, very effective isolates were found (Tahvonen and Teperi, 1995). In order to evaluate this hierarchic screening system a comparative screening experiment was carried out in the field (Teperi et al., subm.) using spring wheat c.v. 'Luja', naturally infected with *F. culmorum*. Two hundred and ten of the original 1700 isolates were included in this experiment. They comprised isolates previously found to be antagonistic as well as non antagonistic isolates. All were non pathogenic to wheat seedlings and belonged to genera of which at least one isolate had been found

to be antagonistic in one or more of the preliminary screenings, in sand, sphagnum or soil.

Generally, a high percentage of the isolates found to be antagonistic in the field experiment had also been found to be antagonistic in the earlier screenings carried out in pots. Furthermore, a comparison between the results from this field experiment and the initial screenings in sand, peat and soil showed that only one group of fungi were high performers in all systems: *Gliocladium* spp. Therefore, the preliminary screening in sand selected most of the antagonistic *Gliocladium* isolates. However, for this genus, the experiments run under controlled environmental conditions did not accurately rank for the biocontrol efficiency in the field.

It should be mentioned that the success of initial screening in sand in the Danish and Finnish experiments may, at least in part, have been due to favorable conditions for the development of *Fusarium*-foot rot in this medium. It has been demonstrated that *F. culmorum* is sensitive to competition from saprophytic microorganisms (Nyvall and Kommedahl, 1973) and that warm and dry soil is conducive to disease development (Cook, 1980; Knudsen, 1994). Although the screening system in sand was carried out at about 15 °C (a relatively high soil temperature in Nordic countries) this temperature appeared to be suitable. However, the outcome of the test was a relatively high number of potential antagonists and further selection was needed.

Screenings for BCAs against Drechslera *spp. and other seed-borne pathogens in cereals*

In the Swedish screening programme (Hökeberg et al., 1997), about 400 bacterial strains, isolated from the roots of wild and cultivated plants, were screened in the greenhouse against diseases caused by *Drechslera teres* alone or together with *Microdochium nivale* and in field screenings against smut, caused by *Tilletia caries.* Isolates showing more than 70% control in these screenings were then tested in large scale field experiments. The procedure can thus be regarded as a two-step hierarchial screening programme. In this programme efforts were made to minimize, as much as possible, *in vitro* cultivation of the bacterial isolates. They were applied as suspensions directly to pathogen-infected cereal seeds and the seeds were then dried under a fan before being sown either in unsterile soil in pots in the greenhouse or, in the case of *T. caries*, in small plots in the field. In this way screenings for antagonistic effects as well as for the ability to survive the drying procedure on seeds were performed. Biocontrol efficacy of the isolates was evaluated from disease incidence readings in the greenhouse after about three weeks and, in the case of *T. caries*, in the field as soon as possible after booting when the symptoms could be seen.

The results showed that the adopted procedure worked well for screening bacterial isolates against *D. teres* and *T. caries* and disease suppression obtained in the initial screening correlated well with the results obtained in the large scale field test (Gerhardson et al., 1994; Hökeberg et al., 1997). However, most of the isolates tested in the initial screening were effective against *M. nivale* which indicates that selection was not rigorous enough. Moreover, some of the high ranking isolates from the *M. nivale* screening in the greenhouse did not perform well in the large scale field experiments. Obviously the screening procedure was, in this case, too 'artificial' to reliably select isolates with good field performance. In both the screenings against *D. teres* and *T. caries* isolates showed a range of effects, from strong disease-suppression to strong disease-promotion, but with a majority of neutral isolates. Probably this distribution mirrors the actual situation in the field where a few bacteria are effective disease antagonists, a few are strong disease synergists – although this is poorly investigated (Gerhardson and Larsson, 1991) – and most are more or less disease neutral.

In conclusion

How then, should we go about selecting the best antagonists suitable for use under field conditions? Tests on agar will identify organisms functioning by antibiosis or hyperparasitism but, with some possible exceptions discussed above, such tests overlook organisms which act by competition or induced host plant resistance. When used alone or as the first method of screening, Petri dish screenings will thus probably function well for only a few special groups of microorganisms. Screening putative antagonists in pots containing test plants and the pathogen *viz* the disease to be controlled, in a more soil-like substrate than agar probably increases the chances of selecting the better agents. In comparison to Petri dish tests, pot tests require, however, more labour, space and equipment.

Also pot tests, at least for some diseases, seem too artificial to effectively select for field relevant antagonists and one general problem we faced in the Nordic programme was that too many promising iso-

lates emerged from the primary pot screenings. In the greenhouse, the temperature of the growing substrate (e.g. soil, peat and sand) is often high compared to the field and the substrate is often too well watered and fertilized to accurately mimic the field situation. Also, the rather uniform environmental conditions in which the pot tests are performed, compared to most field situations, will probably lead to an overestimation of 'good' antagonists. Lastly, some very suitable biocontrol agents may not show up because of the artificial biotic or abiotic surroundings.

In the light of this, screening directly in the field is an obvious option. Although the time period from sowing to scoring often is longer in field testing than in pot tests and the possibilities for retesting often may be restricted because of climatic conditions, this testing method need not be very laborious. However, our experience from using various screening methods in the Nordic projects indicates that *in planta* screening in pots, under favourable conditions for disease development can be a useful approach when it is followed up by small scale field experiments, especially when carried out in different soils, and under different climatic conditions.

Independent of the way *in planta* screening is carried out, the outcome will depend very much on the methods used to apply the antagonist (for example seed treatment, soil drenching, spraying) and on its physiological state. The physiological state is affected for example by culture age, growing medium, temperature etc. Furthermore, when preparing the putative antagonists, different concentrations and dosages of the microorganisms can be used, starter nutrient can be included, different adhering and protective agents can be added, etc. All these factors may affect the screening result. As it would be unrealistic to test and optimize all these factors for a large set of putative antagonists the choice is often governed by earlier experience and intuition. Thus, screening natural microorganisms in order to find utilizable biocontrol agents is becoming an important scientific topic with its own concepts.

Acknowledgements

The Nordic research programme was initiated by The Nordic Joint Committee for Agricultural Research and supported by grants from The Danish Agricultural and Veterinary Research Council, The Forestry and Agricultural Research Council (Sweden), The Research Council of Norway, and The Ministry of Agriculture and Forestry, Finland.

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