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Management of the Environment for the Control of Pathogens [and Discussion]

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Management of the environment for the control of pathogens

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Pathogens can be controlled by management of the environment of (1) the host plant, to maximize resistance, (2) nonpathogens associated with the pathogen, to enhance antagonisms, and (3) the pathogen itself, to limit its activity or longevity directly. Each can be illustrated with controls developed for root diseases of Pacific Northwest wheat. For example: management of plant water potentials to minimize water stress in the host controls *Fusarium* foot rot; root-colonizing fluorescent pseudomonads, with ability to inhibit *Gaeumannomyces graminis* by antibiosis, achieve highest populations on roots and provide best control of take-all at rhizosphere matric water potentials of −0.3 to −0.7 bar (1 bar = 10^5 Pa) and rhizosphere pH values below 7.0; and infection of wheat seedlings by *Pythium* spp., being directly limited by soil matric water potentials drier than −0.4 to −0.5 bar, is controlled by sowing in early autumn while the seed zone is still well-drained or by burning surface residues to expose the soil to drying, and possibly also killing propagules by heat-treatment of the soil. Depending on the host–pathogen–nonpathogen interaction, tillage can maximize host plant resistance, intensify antagonism of pathogens by nonpathogens, and directly limit inoculum potential of the pathogen in soil.

1. Plant disease: an outcome of host–pathogen–nonpathogen interactions

The development of a plant disease is the outcome of a succession of biological interactions involving host, pathogen, and nearly always one or more nonpathogens coexisting on, or in, the host with the pathogen. The nonpathogens may be weakly parasitic to the plant or strictly saprophytic, and they may be related (e.g. a strain or a species of the same genus) or unrelated (e.g. nonpathogenic bacteria with a pathogenic fungus) to the pathogen. These nonpathogens occur in diverse and commonly dense populations on plant surfaces and in the courts of infection and may well provide the initial defence of plants against pathogens. Nonpathogens also become secondary colonists of lesions, where they compete with or parasitize (in special cases) the pathogen; these effects may retard lesion development and production of inoculum by the pathogen. Some nonpathogens, as cohabitants with pathogens, may increase disease severity, but examples of this outcome are rare compared with the converse. Of course, the living host has its own succession of defence mechanisms against ingress by the pathogen, and some resistance responses may be induced by the presence of a nonpathogen (Kuć 1982).

In considering the influence of environment on plant disease development (and ultimately the use of environment to control plant pathogens) we must keep all three of these interacting biological systems in mind and not just the host and pathogen as done so often in the past.

2. Definitions of cultural and biological controls

'Cultural control' is used broadly in this paper to include any control of a pathogen achieved by a cultural practice. Cultural controls generally work by bringing about a change in the environment unfavourable to the pathogen, favourable to the host, favourable to antagonists, or some combination of these three possibilities. The controls discussed in this paper for soil-borne pathogens of wheat in the Pacific Northwest U.S.A. are of these types, designed to change the environment so as to favour the host, nonpathogens, or both relatively more than the pathogen and thereby minimize disease severity. Some cultural practices control pathogens entirely through direct effects of the modified environment on the pathogen, for example soil that is too dry for pathogen growth at the time of sowing. In most cases, however, the practice works through effects on the outcome of biological interactions involving host and nonpathogens as well as the pathogen.

'Biological control' is also used broadly in this paper to include any pathogen control accomplished through one or more organisms (antagonists, host, or pathogen used against itself) other than man (Cook & Baker 1983). Any adjustment in the environment that decreases inoculum density or suppresses disease-producing activities of a pathogen but is achieved through greater host-plant resistance or increased antagonistic effects of the nonpathogens is an example of biological control.

An important point not commonly recognized is that often only the slightest change in the environment will bring about a major change in disease severity. For example, soft rot of potatoes, caused by *Erwinia carotovora*, develops in tubers at −6.5 bar† matric water potential but not in tubers at −7.5 to −8.0 bar (Pérombelon & Kelman 1980). The difference in severity of soft rot over this narrow range of water potentials seems too dramatic to be explained simply on the basis of a higher and hence more favourable turgor potential for growth of the bacterial cells at −6.5 than at −7.5 to −8.0 bar. Host-susceptibility factors almost certainly are involved. A slight change in pH or temperature can have equally dramatic effects on the interactions between a pathogen and nonpathogens on the phylloplane or rhizoplane (Cook & Baker 1983). In the case of host factors, the different reactions (e.g. resistant or susceptible) under the different environments may be near instantaneous, requiring only that the host tissues or cells achieve the water potential, temperature, oxygen status, or other environmental conditions required for maximal expression of resistance. In the case of interactions between a pathogen and associated nonpathogens, the outcome is usually decided over a longer time, more typical of interacting populations in an ecosystem.

3. Rationale for use of the environment to bring about biological control of plant pathogens

It is sometimes stated that plant pathologists have been relatively unsuccessful in developing biological controls for plant pathogens. This statement can be made only with reference to the narrowest possible definition of biological control, namely the introduction or application of an antagonist, probably a single antagonist, to control the pathogen. Baker (1987) refers to this approach as the 'one-on-one' syndrome that seems to dominate the thinking in biological

† 1 bar = 10^5 Pa.

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control and to parallel our use of single genes and single chemicals for pathogen control. Obviously, many successes can be cited for the single-agent approach to pathogen control. The use of *Agrobacterium radiobacter* var. *radiobacter* strain K84 to control crown gall (Kerr 1980) and *Phlebia gigantea* to control *Heterobasidion* root rot of pine (Rishbeth 1979) are excellent examples of single, introduced antagonists effective in the field. However, most of the biological controls in plant pathology have involved manipulations of the environment to manage host-plant susceptibility together with a myriad of resident, nonpathogenic and potentially antagonistic microorganisms on the host and in host residues and thereby to relegate the pathogen to a lesser position in its ecological niche. In this approach, our science has been quite successful.

Biological control achieved through management of the environment brings to bear the combined effects of many antagonists or potential antagonists, all adapted to the niche or replaced by those that are adapted. Suppressive soils, defined as those soils where the pathogen does not establish, establishes but causes little or no disease, or causes disease at first but then declines (Baker & Cook 1974), are mostly examples of environments relatively more favourable to a community of resident antagonists than to some pathogen that otherwise would cause severe disease. Detailed studies of a suppressive soil (or of other pathogen-suppressive environments) may, on occasion, produce a single antagonist or group of related antagonists responsible for the effect. This antagonist can then be isolated and reintroduced to enhance the effect. One example is *Trichoderma harzianum* obtained from a Colombian soil suppressive to *Rhizoctonia solani* (Chet & Baker 1981), and another is the fluorescent *Pseudomonas* species obtained from wheat-field soils that have undergone take-all decline, and used to control take-all (Weller & Cook 1983). In some cases, the quality of the microbial community is the key, in other cases the quantity (total numbers or mass of microorganisms active at a time critical to the pathogen) is more important. Generally, however, the quality and quantity of nonpathogenic microorganisms are both important. 'The greater the complexity of the biological community, the greater is its stability.' At the very least, cultural practices should be selected that do not environmentally upset the suppressive communities of microorganisms that help defend plants against pathogens.

4. Control of *Fusarium* foot rot of wheat in the low- to intermediate-rainfall areas of the Pacific Northwest U.S.A.

Fusarium foot rot of wheat, caused by *Fusarium culmorum*, is a problem in the Pacific Northwest, U.S.A., mainly on winter wheat subjected to severe plant water stress (Papendick & Cook 1974; Cook 1980). The pathogen had been noted on wheat for decades (Sprague 1950), but acute foot rot typified by crown rot, premature plant blight, and a chocolate-brown discolouration extending one to two internodes up the stem, did not become important until the 1960s (Cook 1968). The appearance of the severe form of the disease coincided with the use of the high-yielding, semi-dwarf cultivars of winter wheat. Moreover, those who noted the first outbreaks of this disease also noted that it was most serious for the 'best' farmers in the low- to intermediate-rainfall areas (25–40 cm annual precipitation) of Washington State. Papendick & Cook (1974) related the occurrence of severe plant water stress to excessive nitrogen fertilization. The so-called better farmers were fertilizing for yields greater than possible for the available water. Wheat plants are remarkably resistant to foot rot caused by

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F. culmorum, provided their mid-day plant water potentials go no lower than −28 to −32 bar, but they become extremely susceptible to the disease if their mid-day plant water potentials go below −32 to −35 bar (Cook 1973). Some plants developed water potentials as low as −40 to −50 bar.

The control, now widely practised in the dryland Pacific Northwest of the U.S.A., is to fertilize for a yield no greater than can be expected for available water (Cook 1986). This exemplifies the use of cultural practices to manage the environment of a pathogen. The environment, in this case, is the water potential of the host tissue occupied by the pathogen. Nonpathogenic (strictly saprophytic) fusaria related to *F. culmorum* are commonly present as secondary colonists of wheat roots and basal stem tissues occupied by *F. culmorum*, but whether they play any role in this disease control on non-stressed plants is unknown.

Field surveys done over a ten year period, approximately 1965–75, revealed that populations of *F. culmorum* sufficient to produce disease (greater than 100 propagules per gram dry mass of soil) occurred almost exclusively in the low- and intermediate-rainfall areas and not in the high-rainfall areas (greater than 45–50 cm precipitation annually) such as near Pullman. Large populations of *F. culmorum* established naturally or experimentally in the Pullman area were observed to disappear from the soil in only 1–2 years. This raised the possibility that soils around Pullman were suppressive, of the type that did not allow the pathogen to establish (Baker & Cook 1974). However, Inglis & Cook (1986) showed that chlamydospores of this pathogen die faster in the dry, harsh soil environment in the low- than in the high-rainfall areas. They proposed that the higher frequency of infested fields in the low- than in the high-rainfall areas was the result of conditions more favourable to disease, and hence replenishment of inoculum of the pathogen, and not of differences in conditions required for survival of the pathogen between susceptible crops. The presence of *F. culmorum* in wheat-field soils of the Pacific Northwest is therefore thought to be a function of its parasitic (pathogenic) activities and not of its ability as a soil saprophyte.

Cook & Bruehl (1968) similarly concluded that the presence of *F. culmorum* in fragments of wheat stubble after harvest was the result of parasitism, i.e. the establishment of the fungus in the lower internodes as a parasite while the plant was still alive, rather than of saprophytic colonization of the straw after harvest. Those portions of standing stubble not occupied by *F. culmorum* as a parasite become quickly colonized by airborne saprophytes, and these pre-empt *F. culmorum* as a colonist once the stubble is mixed with soil infested with this pathogen (Cook 1970).

By managing plant water potentials, the parasitic activities of *F. culmorum* are virtually prevented. By leaving the stubble standing to become mouldy after harvest, the saprophytic activities of this fungus are virtually prevented. Because chlamydospores gradually die out in soils of the region (Inglis & Cook 1986), the pathogen without recourse to either parasitism or saprophytism has ceased to be a factor in wheat production in the region.

5. Control of *Pythium* root rot of wheat in the intermediate- to high-rainfall areas

Pythium root rot of wheat is caused by several *Pythium* species (Chamswarng & Cook 1985), with *P. ultimum* and *P. irregulare* being the most widespread and thought to be the most important. These fungi existed in association with roots and residues of the prairie grasses

(Sprague 1950) long before wheat farming began in the region in the late 19th century. The disease can be divided into two phases: a generally non-lethal infection of the embryos of seeds during the first 1–2 d after sowing that results in stunted, twisted leaves, and spindly appearance (in severe cases) of seedlings (Hering *et al.* 1987); and infection of root hairs and the cortical tissues of roots and rootlets that may continue during the life of the plant (Cook & Haglund 1982; Cook *et al.* 1987). Adult wheat plants affected by *Pythium* root rot are generally 3–5 cm and sometimes up to 10 cm shorter, have 15–25 % fewer tillers, and head 3–5 days later than plants of the same age but grown in soil freed of *Pythium* spp. by either heat treatment (e.g. solarization) or fumigation (Cook *et al.* 1987).

Although *Pythium* spp. pathogenic to wheat are ubiquitious, and *Pythium* root rot occurs across all major wheat-management systems in the region, control is needed only in the intermediate- to high-rainfall areas (35–40 cm of available water or more, annually). The disease occurs in the drier areas, probably during October–March when most rainfall occurs, and elimination of inoculum of *Pythium* spp. from soil by fumigation invariably produces an early increased-growth response for wheat in the drier areas. However, available water and not *Pythium* root rot is almost always the yield-limiting factor in areas of the region with less than 35–40 cm (Cook 1986; Cook *et al.* 1987). For this reason, all efforts to date aimed at control of *Pythium* root rot have been focused in the higher rainfall areas of the far-eastern edge of Washington State, northeastern Oregon, and northern Idaho.

The most effective controls at present for damage to wheat caused by *Pythium* spp. are those combinations of practices that: (1) minimize the amount of wheat straw (especially the chaff) at the soil surface or mixed with the top 10–15 cm of soil (Chamswarng 1984); (2) keep the soil surface exposed to drying winds and the sun, especially during emergence and early seedling growth (R. J. Cook, unpublished results); and (3) keep the soil matric potentials in the top 10–15 cm of soil drier than −0.4 to −0.5 bar (Hering *et al.* 1987). These three factors determine whether the soil environment will be suitable to activity of *Pythium* spp. and are not mutually exclusive.

The benefits of a well-drained seedbed and removal of potential foodbases for control of *Pythium* spp. are perhaps best illustrated by noting that *Pythium* root rot is most severe for winter wheat when direct-drilled through a layer of straw into wet soil late in the autumn (e.g. mid-October or later). Average daily temperatures are dropping during this period and the chance is very low that the soil will dry during establishment of the stand. In this situation, final grain yields are commonly only 50–75 % of what they could be with the moisture available to the crop (Cook 1986). Infection of germinating wheat seeds is maximal at −0.1 bar matric water potential but is virtually prevented in soils at −0.4 to −0.5 bar or drier (Hering *et al.* 1987). Most wheat seedlings emerge in spite of embryo infections, but fewer seedlings emerge and those that do are distinctly spindly in straw- (or chaff-) amended soils, and the addition of nitrogen with the straw does not correct the problem (Ingram 1987). All evidence indicates that the chaff and other straw fragments are used by *Pythium* spp. as a foodbase, unless the straw is already colonized by other fungi (Rush *et al.* 1986). The straw can be eliminated (and damage from *Pythium* spp. is almost always reduced accordingly) by burning, deep burial with a plough (i.e. deeper than 10–15 cm), or rotation with a crop such as dry peas or lentils (which leaves the soil surface relatively bare).

The benefits of straw removal are apparent from the results of a field experiment conducted in eastern Washington during the 1985–86 crop year. Winter wheat direct-drilled in the autumn of 1985 into standing stubble of a 1985 spring wheat crop averaged (five replicates)

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4.5 t ha^{-1}†. Injection of chloropicrin 10–12 cm below the soil surface (about 10 l ha^{-1} 5–7 cm below and 5–7 cm to one side of the seed) with the fertilizer at the time of sowing reduced the population of *Pythium* spp. by nearly 50 %, produced a typical increased-growth response of the wheat, and resulted in an average yield of 5.2 t ha^{-1}. However, winter wheat that was direct-drilled the same day at the same site but with stubble removed by burning averaged 5.8 t ha^{-1}, and injection of chloropicrin into these plots resulted in about the same yield (5.9 t ha^{-1}). The adult plants in the burned, non-fumigated plots showed no evidence of *Pythium* root rot and their yield probably was the maximum possible for available water at that site (Cook 1986) and therefore could not be increased by fumigation. Differences in initial inoculum density of *Pythium* spp. cannot account for the growth and yield differences of the wheat in the stubble-standing and stubble-burned sites. Complete removal of the straw to produce a black soil surface was obviously of great benefit, possibly because the fungus was denied access to a foodbase, or possibly because of a greater opportunity for the soil to dry below the critical (−0.4 to −0.5 bar) matric water potential. The response of wheat to fumigation in the standing stubble supports our findings in glasshouse studies that, although surface crop residues are a contributing factor to the stunting of wheat associated with direct drilling, it is the soil and not the straw that must be fumigated to eliminate the microorganisms responsible for the effect.

Burning wheat straw on the soil surface may also reduce the population of *Pythium* spp. by a heat or possibly also by a smoke effect. At Pullman, wheat straw placed as a layer on bare fallow (surface already black and the soil drained) at 0, 3, 6 and 12 t ha^{-1} and burned resulted in average yields of 5.1, 5.8, 5.9 and 6.2 t ha^{-1} for winter wheat grown in the replicated plots (Cook *et al.* 1987). In another experiment, burning a layer of straw (12–15 t ha^{-1}) on the soil surface was nearly as effective as solar heating of the soil in reducing the *Pythium* population in the top 5–10 cm of soil, and the wheat yields were 15–20 % greater than where no treatment or where straw ash only was applied (Cook *et al.* 1987). The temperature of moist soil needs to be elevated to only 42–43 °C for 10 min virtually to eliminate the population of *Pythium* in these soils (Cook *et al.* 1987; W.-h. Tang, unpublished results). Moreover, a 10 min exposure of *Pythium*-infested soil to smoke-filled air, produced by burning wheat straw, was sufficient to render the population of *Pythium* spp. undetectable when the soil was returned to clean air and dilution-plate counted (W.-h. Tang, unpublished results).

Pythium root rot of winter wheat is also limited by seeding early in the autumn, while the soils are still well drained or are not likely to be wet for a prolonged period. The chances for adequate drainage and improved control of *Pythium* root rot are greatly increased if the soil has no tillage pan (plough-sole layer) formed by prior tillage operations (R. R. Allmaras and J. M. Kraft, unpublished results). Even a slight difference in the soil matric potential during times critical to the saprophytic or parasitic activities of *Pythium* spp. in the spermosphere and rhizosphere of wheat can greatly affect the amount of damage caused by this fungus.

6. Control of take-all in consecutive crops of wheat in the high-rainfall and irrigated areas

Take-all, caused by *Gaeumannomyces graminis* var. *tritici*, is easily controlled in most areas of the world by not growing wheat or barley in the same field more than every other year (minimum of a two year rotation). The pathogen survives in fragments of root and basal stem

† 1 hectare = 10^4 m^2.

tissues colonized through parasitism, while the fragments were still part of the living host. Rotation to non-host crops is effective because the pathogen either depletes its nutrient supply in these fragments or is displaced by more competitive microorganisms within a few months in most biologically active soils. The disease can also be managed with consecutive crops of wheat or barley. Biological control is also involved in this approach, and is thought to include the combined effects of competition from fungi and bacteria in the foodbase (inoculum source), which reduce the inoculum potential of the pathogen, and antagonistic microorganisms in the rhizoplane and in young lesions, which prevent infection or limit the secondary spread of the pathogen by runner hyphae (Cook & Weller 1987).

The competitive effects of fungi and bacteria with *G. graminis* var. *tritici* in fragments of infested crop residue is a continuous process that probably begins as the lesions age but intensifies once the host dies. Garrett (1976, 1985) has done extensive studies of the factors affecting longevity of *G. graminis* var. *tritici* in tiller bases as a means of saprophytic survival and as a foodbase for this pathogen. The importance of competition from fungi, in particular, was demonstrated by W.-h. Tang (unpublished results) using a benomyl-resistant strain of the pathogen; the infection efficiency (number of root lesions produced per unit mass of inoculum (Wilkinson *et al.* 1985)) was 25–50 % greater when benomyl was infused (5 parts in 10^6 by mass) into the pathogen-infested (inoculum) fragments. The increase was equivalent to that achieved by moist heat treatment of the soil at 60 °C for 30 min (Wilkinson *et al.* 1985; Cook *et al.* 1986). Experiments with the pasteurized soil revealed only that soil microorganisms have a suppressive effect on the infection efficiency of this pathogen, but experiments with benomyl infused into the particles indicated that the suppressive agent(s) is (are) benomyl-sensitive. Subsequent experiments indicated that nonpathogenic (saprophytic) *Fusarium* spp. are partly or largely responsible for the suppression; a strain of *F. oxysporum* reduced the infection efficiency for the take-all pathogen when added with inoculum of the benomyl-resistant pathogen to pasteurized soil, but not if benomyl was present in the fragments of inoculum.

From a practical standpoint, the competitive effects of microorganisms on *Gaeumannomyces graminis* var. *tritici* in its foodbase can be maximized by tillage (Moore & Cook 1984) together with a delayed seeding date (Taylor *et al.* 1983). Tillage is well known to promote temporary bursts in microbial activity and to accelerate breakdown of organic matter in soil. The response is thought to result from a combination of the fresh surfaces on fragments of residue made available for microbial colonization and the temporary improvement in soil aeration. In Washington State, some tillage is essential for take-all control in consecutive crops of wheat (Moore & Cook 1984). Delayed seeding is also beneficial, possibly because the extra 1–2 weeks between harvest of one crop and sowing the next crop allows more time for the nonpathogens to displace the pathogen.

The suppression of take-all by antagonistic bacteria on the roots of wheat is also a common phenomenon but intensifies or becomes more effective with any of the following practices: (1) the use of ammonium rather than nitrate fertilizer (Smiley 1978); (2) growing consecutive crops of wheat (Cook & Rovira 1976; Weller 1985); and (3) introducing the bacteria with the wheat seed (Weller & Cook 1983).

Workers in Idaho (Huber *et al.* 1968), Oregon (Christensen *et al.* 1981), and Washington (Smiley & Cook 1973) all have reported that the use of ammonium nitrogen results in less take-all on wheat than occurs with the use of nitrate nitrogen. The ammonium effect is apparently the result of a lower pH in soil around roots taking up this form of nitrogen (Smiley & Cook

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1973). However, the lower rhizosphere pH cannot, by itself, account for the disease suppression because the effect did not occur in soil fumigated with methyl bromide. Smiley (1978) showed that the frequency of fluorescent pseudomonads inhibitory to *G. graminis* var. *tritici* is significantly greater in the rhizosphere of wheat treated with ammonium compared with nitrate fertilizers. Howie (1985) presents evidence that this effect is also the result of a lower rhizosphere pH; both the indigenous population of fluorescent pseudomonads and the population of *P. fluorescens* strain 2-79 introduced with the seed tended to be higher at rhizosphere pH values of 6.0 to 6.5 than at 7.0. Rouatt & Katznelson (1961) noted that pseudomonads are commonly favoured in the rhizosphere by slightly acidic conditions.

Manipulation of the rhizosphere pH with form of applied nitrogen illustrates the potential practical value of managing environmental factors in specific courts of infection, namely microsites such as the root–soil interface, rather than more generally in the bulk of the soil. Manipulation of rhizosphere pH may not be sufficient itself, but when combined with tillage and the presence of certain nonpathogens, e.g. fluorescent pseudomonads inhibitory to the pathogen, the additional degree of disease control can be significant.

Populations of fluorescent pseudomonads inhibitory to *G. graminis* var. *tritici* also tended to increase spontaneously with consecutive crops of wheat and in the presence of the take-all fungus. The evidence of Weller (1985, in Cook & Baker 1983) points to a qualitative as well as quantitative change towards higher populations of fluorescent *Pseudomonas* sp. inhibitory to the take-all pathogen. Indeed, this phenomenon is thought to contribute to or be responsible for the well-known take-all decline phenomenon (Cook & Weller 1987). Certain strains of the bacteria are also now providing modestly effective control against take-all under field conditions when introduced as a living seed treatment; the average yield compared with appropriate checks was 7.6% (400 kg ha^{-1}) greater for ten experiments done in naturally-infested commercial fields over a 5 year period (R. J. Cook, D. M. Weller & E. N. Bassett, unpublished results).

Both the indigenous population of fluorescent pseudomonads and the population of *P. fluorescens* strain 2-79 introduced with the seed were consistently 1–2 $\log_{10}$ units larger on roots of wheat seedlings that were 28 days in the presence than in the absence of the take-all fungus (Howie 1985). This response of fluorescent pseudomonads to the take-all fungus was also observed by Weller (1984), with a marked strain monitored in the field, and is thought to result from response of the bacteria to root lesions caused by the pathogen. Several workers have noted the proliferation of Gram-negative bacteria in root lesions caused by *G. graminis* var. *tritici* (Vojinović 1973; Cook & Rovira 1976). By establishing in root lesions, the bacteria are then ideally positioned both to inhibit further spread of the pathogen on the roots and to carry over as cohabitants with the pathogen in host-plant fragments after the crop is harvested (Cook *et al.* 1986). Thus, as the incidence and severity of take-all increases, the populations of fluorescent pseudomonads (and possibly other bacteria) increase in response to the amount of infected root tissue. Cook & Weller (1987) have proposed that after one or two severe outbreaks of take-all, an equilibrium may develop between populations of bacteria, which is sufficient to limit future outbreaks of severe take-all, but which also allows enough lesions to maintain populations of the bacteria. This hypothesis can account for why, after take-all decline, the pathogen can still be readily isolated from wheat roots and is fully virulent in a conducive rooting medium but is prevented from causing severe disease in the field (Asher 1980; Cook & Naiki 1982).

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In addition to the presence of the take-all pathogen, and pH of the rhizosphere, any source of inorganic nitrogen (either ammonium or nitrate) added to a nitrogen-impoverished soil resulted in 1–2 $\log_{10}$ unit larger populations of *P. fluorescens* strain 2-79 than occurred on roots of plants in the soil with no added nitrogen. Nitrogen deficiencies are well-known to favour take-all and many reasons have been proposed for the effect. This list of proposed reasons can now be extended to include larger populations of potentially antagonistic bacteria in the rhizosphere, possibly brought about by greater root exudation from the better-nourished plants.

Another major factor is rhizosphere water potential. Populations of *P. fluorescens* strain 2-79 introduced on wheat seeds achieved largest populations when the water potential of the rhizosphere was -0.5 to -0.7 bar, and oxygen entry into the soil and turgor potential of the bacterial cells both presumably are maximal (Howie *et al.* 1987). The optimal water potential for the take-all pathogen is also in this range of soil water potentials (Cook *et al.* 1972); as such, the pathogen is maximally exposed to fluorescent pseudomonads and probably other Gram-negative bacteria. This may explain why the environmental factors that affect activity of the bacteria have such an effect on the activity of this pathogen.

The suppression of take-all on wheat in soils fertilized with ammonium nitrogen, and after wheat monoculture and one or two outbreaks of the disease, is associated not only with larger populations of fluorescent pseudomonads but also with a greater frequency of types inhibitory to the pathogen (Weller, in Cook & Baker 1983). *P. fluorescens* strain 2-79 produces a phenazine-type antibiotic inhibitory to *G. graminis* var. *tritici* at only 1 $\mu g\ ml^{-1}$ of medium (Gurusiddaiah *et al.* 1986). Antibiotic-negative mutants, produced by inactivating single genes by *Tn5* mutagenesis, were significantly less suppressive to take-all than the parent when introduced with the seed (Thomashow *et al.* 1986). Restoration of phenazine-producing ability, by complementation with a fragment of wild-type DNA introduced using a cosmid vector, also restored ability of the mutant to suppress take-all (L. S. Thomashow and D. M. Weller, unpublished results). This evidence makes clear that antibiotic-producing ability is important for biocontrol with this strain of *P. fluorescens* and justifies seeking this trait in either natural or engineered strains. However, the stage is also now set for studies of ways to enhance antibiotic production through manipulation of the rhizosphere environment. Rhizosphere pH, oxygen supply, redox potential and nutrient availability are among the many factors that could influence either production or activity of the antibiotic, and can also be studied experimentally in finding ways to control this pathogen by management of the environment.

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Discussion

G. Défago (*Swiss Federal Institute of Technology, Zürich, Switzerland*). Can I ask Dr Cook for more information on the location of his rhizobacteria? Are they on the root surface or inside the roots? We have some evidence that in the case we are studying it is necessary that the rhizobacteria are inside the roots to suppress disease.

R. J. Cook. According to results of my associate, Dr D. M. Weller, about 90% of the bacteria are in the rhizosphere and 10% are on the rhizoplane. This is the distribution found on and around wheat roots after introduction of an antibiotic-resistant strain on the seed. However, I have seen results from my own earlier experiments that indicate a portion of the bacteria on roots are either attached very tightly (cannot be washed off by standard techniques) or they are inside root tissues. A portion of the cells are immersed in or beneath the mucilage of roots.

R. R. M. Paterson (*C.A.B. International Mycological Institute, Kew, U.K.*). A number of years ago there were reports about the use of fungal spores (e.g. *Penicillium*) to coat seeds in a manner that sounds similar to that described for the bacterium that was mentioned. Does Dr Cook know what has happened to the work?

R. J. Cook. This work has been, and continues to be, done by Dr T. Kommedahl and his students at the University of Minnesota. No patent was ever obtained on the effective organisms (or on their use as seed treatments) and without the prospects of an exclusive licence, private companies generally are not interested in developing these agents for commercial use. However, Dr Kommedahl's best strains are as good as standard chemical seed treatments.

J. M. Lynch (*Glasshouse Crops Research Institute, Littlehampton, U.K.*). About the effects of *Pythium* and *Fusarium*, Dr Cook also recognized that phytotoxins produced in the microbial degradation of straw could contribute to the total reduction in yield. Growth-inhibitory pseudomonads can also contribute to the yield reduction (Elliott & Lynch 1985). These effects could be synergistic

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in reducing yield. Does Dr Cook envisage that a single biological control agent could be applied to counteract these various effects or does he see more prospect in the use of a microbial 'cocktail' consisting of two or more antagonists?

Reference

Elliott, L. F. & Lynch, J. M. 1985 Plant growth-inhibitory pseudomonads colonizing winter wheat (*Triticum aestivum* L.) roots. *Pl. Soil* **84**, 57–65.

R. J. Cook. We know from results with selective treatments applied in both greenhouse and field experiments that the poor-growth syndrome for wheat sown into a trashy seed bed (e.g. direct-drill or conservation tillage) is associated mainly, if not entirely, with soil-inhabiting microorganisms and not the microorganisms present as a natural component of the straw microbiota. *Pythium* spp. are a major component of this deleterious microbiota, along with possibly *Rhizoctonia* spp., inhibitory pseudomonads, and other pathogens. Knowing this leads me to believe that a good seed and root protectant would go far to solve the problem. However, I am not so encouraged to believe that a single antagonist around the seeds and roots would be sufficient. We may need a mixture of antagonists or seed-treatment chemicals plus antagonists.

D. Hornby (*Rothamsted Experimental Station, Harpenden, U.K.*). Would Dr Cook say in what proportion of tests in commercial fields he has obtained biological control of natural epidemics of take-all? How was this assessed: by yield increases, or by actual measurements of the disease?

R. J. Cook. As of 1986, we have completed ten replicated trails in cooperation with growers in fields with natural infestations of the take-all fungus. These fields were all seeded with the grower's own drill. Each field was second- or third-year wheat and in each case, take-all turned out to be the main yield-limiting factor. Root ratings, plant heights (a highly sensitive indicator of take-all) and yields were measured in each trial. Evidence of efficacy was obtained by one or more of these three measurements in at least five of the nine trials. Root ratings were nearly always lower with than without the treatment, but owing to variation, these differences were significant in only two or three cases. Differences were easiest to demonstrate statistically by measurement of plant heights. Yields were greater by only 1.5–5% in five trials (not significant) and 9.6–22.0% in four trials (significant at p equal to 0.05 or 0.01). The average for the nine trials was 7.6% greater yield (0.4 t ha^{-1}). Where the treatment worked, it was obvious. However, independent assessments in these trails indicated that complete control of take-all would have elevated yields by 20–25%, on the average. Thus we have some way to go. Nevertheless, we are encouraged by the results obtained thus far. We think of our strains as prototypes of strains still to come.

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