44 Take-all Decline: Model System in the Science of Biological Control and Clue to the Success of Intensive Cropping

R. JAMES COOK

Washington State University, Pullman, Washington 99164-6430, USA, rjcook@wsu.edu

Overview: Take-all is a root disease of wheat and barley caused by the soil-borne ascomycete, *Gaeumannomyces graminis* var. *tritici.* The fungus causes serious limitations to grain yield when wheat is grown for 2 consecutive years in the same field. Crop rotation, preferably breaks of 2 or more years away from wheat or barley, was the only method recommended for reliable control. The discovery that this disease declines in severity and can all but disappear where wheat is grown in the same field over many years provided an exemplary model system for studying soil ecosystems that become 'disease suppressive'. This chapter summarizes the results of 40 years of research that led to the understanding of the key biological and biochemical components responsible for creating take-all suppressive soils and the resultant take-all decline. The article provides approaches to unravelling the complex microbial ecosystems in the rhizosphere and gives directions for management of root diseases in intensive cropping systems heretofore considered to be only controllable by broad-spectrum biocides.

Introduction

It was with both a sense of amazement and a tinge of pride that I looked over the audience of some 200 in attendance at the symposium 'The Nature and Application of Biocontrol Microbes III. *Pseudomonas* spp.' at the 2005 annual meeting of the American Phytopathological Society in Austin, Texas; amazed that so many plant pathologists were still interested in this group of biocontrol microorganisms and proud that four of the eight invited speakers were alumni or current leaders of the Pullman team working to understand and exploit take-all decline. Take-all is a disease caused by a soil-borne ascomycete, *Gaeumannomyces graminis* var. *tritici*, that develops on the roots and stem bases of wheat and on the roots but rarely stem bases of barley. It stunts early plant development, accelerates plant maturity later in the season and can seriously limit yields, sometimes to the extent that it 'takes all' the crop. 'Take-all decline' is the spontaneous remission of disease following continuous monoculture of wheat and barley. This phenomenon

©CAB International 2007. *Biological Control: a Global Perspective* (eds C. Vincent, M.S. Goettel and G. Lazarovits)

has become a model system in the science of biological control of soil-borne plant pathogens. In fact, I will suggest that as a scientific milepost in rhizosphere microbiology the story of take-all decline today is approaching the sophistication and scientific interest of another beneficial plant–microbe interaction, namely that of *Rhizobium* biology and associated nitrogen fixation.

One year earlier, I was an invited speaker at the 4th International Crop Science Congress in Brisbane, Australia, with the assigned topic: 'In Defence of Continuous Crop Monoculture'. There is almost no end to the list of successful crop monocultures globally, including the decades-long establishment of managed turf and other perennials. However, very little has been done to reveal why they are successful production systems. Arguably, research into take-all decline is opening an entirely new way of understanding the success of, and opportunities for, continuous crop monocultures specifically and intensive cropping more generally.

One could say that my interest in biological control was by default, that I had no other options to control root diseases of wheat, but this would not be correct. It is true that options for root disease control in modern wheat-based cropping systems were and remain very limited. The traditional 3-year (and longer) crop rotations promoted in cereal-producing areas during the 20th century as the best means to control root diseases (Cook and Veseth, 1991) are now being replaced increasingly with intensive cereals, typically wheat 2 years in 3 or every year. The amount of land under clean tillage continues to decline and 'no-till' (direct-seed) systems, where the crop residue is left on the soil surface as a trashy seedbed, continue to increase. Some growers in the Inland Pacific Northwest made their transition to direct seeding less risky by burning the wheat straw prior to seeding wheat, but public pressure has greatly limited the use of this practice. Soil fumigation provides the most spectacular increases in crop growth response for wheat and barley, especially for fields most frequently cropped to cereals (Cook and Veseth, 1991), but is not affordable for wheat. The most ideal control would be to have cultivars with resistance to take-all, but genes for resistance to root diseases remain essentially unknown in the pool of germplasm available to wheat breeders.

My job, starting in the mid-1960s, was to provide the science and technology needed to limit or eliminate the yield-depressing effects of root diseases on wheat (and later barley) while depending little or not at all on traditional crop rotations, tillage, stubble burning, chemical pesticides or host-plant resistance. Indeed, disease suppression by soil microorganisms antagonistic to the pathogens was about the only option left.

My days as a graduate student at the University of California, Berkeley, from 1961–1964 with W.C. Snyder, K.F. Baker, S. Wilhelm, M.N. Schroth, T.A. Toussoun and A.R. Weinhold, all world leaders in research on ecology and control of soil-borne pathogens, prepared me to consider biological control by disease-suppressing soil microorganisms as the first line of defence against soil-borne plant pathogens. This philosophy was the foundation for the *International Symposium on Factors Determining the Behavior of Plant Pathogens in Soil*, held on the Berkeley campus in April 1963, and published in 1965 as *Ecology of Soil-borne Plant Pathogens: Prelude to Biological Control* (Baker and Snyder, 1965). I attended that symposium as a graduate student, and the proceedings, published the year I was hired by the USDA, Agricultural Research Service (ARS) and stationed at

Washington State University, Pullman, became the foundation for my work on ecology and biological control of soil-borne plant pathogens of cereals in the US Pacific Northwest.

My decision to focus on biological control in soil and the wheat rhizosphere was sealed when, in 1969, the late Kenneth F. Baker invited me to join him as co-author of the book, *Biological Control of Plant Pathogens* (Baker and Cook, 1974). It took 5 years to write that first book, which also meant 5 years of thinking deeply about biological control of plant pathogens with Ken Baker, an awesome opportunity for someone in their early thirties. I also made a conscious decision during that period of my career that I was not going to only write about biological control of plant pathogens, I was going to *do* biological control – of wheat and barley root pathogens.

Initiation of Take-all Decline with 'Starter' Soil

By the time I began my work in 1965, take-all was already well known for its severity on 'back-to-back' wheat in the US Pacific Northwest. Crop rotation, preferably breaks of 2 or more years, was the only method recommended for its control, and incidentally, is still the best method of control (Cook and Veseth, 1991). Indeed, conventional wisdom among plant pathologists in the region at that time was that, because crop rotation is so effective, no further work was needed on take-all. My question was: if crop rotation is so effective, why is there so much take-all? There were, and still are, many economic, environmental and ecological reasons why farmers around the world not only risk second and third consecutive wheat crops and even continuous cropping with wheat or wheat/barley sequences but now specialize in intensive wheat-based cropping systems if not continuous wheat monoculture. For me, there was the personal challenge: we know the effectiveness of rotations in managing soil-borne plant pathogens, but managing them in crop monoculture, while taboo to those that espouse a certain philosophy of 'sustainable agriculture', to me was the scientific frontier.

Early experimental evidence that take-all could be managed in continuous wheat monoculture was published in two PhD theses in Europe. One was the report of M. Gerlach in The Netherlands on the early development of take-all in new polders and its decline (Gerlach, 1968), and the other the work of Peter Shipton at Reading, UK, on soil assays to predict fields undergoing, or already into, take-all decline (Shipton, 1972). I met Shipton at the First International Congress of Plant Pathology held in London in 1968 and discussed with him the possibility that the virgin arid lands brought into wheat production with irrigation in eastern Washington might offer special circumstances to better understand take-all decline, similar to the virgin polder soils studied by Gerlach (1968) in The Netherlands. Shipton received a NATO Postdoctoral Fellowship for 2 years and joined me in Pullman in 1969.

We immediately began testing the applicability of Shipton's soil assay for take-all suppression associated with putative take-all decline. This assay measured the severity of take-all on seedlings grown in pots filled with test soil (from wheat-monoculture fields) amended with a standardized amount of inoculum of

G. graminis var. *tritici* that we produced on an artificial food base. After nearly a year of ambiguous and inconsistent results, we decided to see whether it would be possible to transfer take-all suppression from a field in long-term wheat monoculture to a field with no known history of wheat or take-all. This idea did not arise in a vacuum. First, Menzies (1959), working with common scab of potato in the arid irrigated lands of eastern Washington, had reported suppression of common scab of potato with the addition of 10% scab-suppressive soil mixed into a scab-conducive soil. Second, we realized that with Shipton's soil assay the results were greatly influenced, if not dominated, by variations in soil type. We needed a common soil type as our rooting medium and to this we could add a standard amount of test soil (e.g. 1 or 10% w/w) as well as inoculum of the pathogen. Third, Baker, with all his experience in biological control, convinced me that our hypothesis was worth testing.

We selected six soils from eastern Washington fields, three with a history of wheat and three corresponding non-cropped sites near the respective three wheat fields (later described by Shipton *et al*., 1973). The three fields cropped to wheat were: (i) (near Pullman) in a traditional wheat/pea rotation, with wheat every second or third year; (ii) (near Lind) in a traditional fallow/winter wheat rotation, with wheat every other year; and (iii) (near Quincy) in its 12th year of continuous wheat monoculture. The corresponding non-cropped sites were: (i) undisturbed grassland (near the Pullman field); and (ii) undisturbed native sagebrush-dominated vegetation (near the Lind and Quincy fields). Soil from the top 15 cm at each site was hauled over the Cascade Mountains to the Washington State University Research and Extension Center at Puyallup, Washington, where each was tested for its ability to initiate take-all decline. As a precaution against naturally occurring *Gaeumannomyces graminis* var. *avenae*, a related fungus affecting turf, the experimental site was fumigated with methyl bromide under clear plastic tarp at about 4.4 kg/m^2. Two days later, the tarp was removed and the six soils sprinkled uniformly and respectively over the surface of plots approximately 1.3 m $\times$ 3.0 m, with each soil treatment replicated four times. The two control plots included an equivalent rate of native soil and no soil (fumigated only). The entire site was then rotovated in the long direction of the plots and to a depth of 15 cm, starting with the no-soil control, followed by the native-soil control, then the non-crop (virgin) soil treatments, and finally the cropped soil treatments. The site was then planted to high-quality seed of winter wheat mixed in the drill box with oat grains colonized by *G. graminis* var. *tritici* (to assure the occurrence of take-all in the first season).

Arbitrarily deciding how much oat-grain inoculum to add and taking into account that the soil was fumigated, we overdosed and take-all was uniformly devastating that first year (1969/70 crop year). The site was planted a second time (1970/71 crop year) with wheat only, depending entirely on inoculum from the diseased crop from the 1969/70 crop year. In that second year, the effect of 'starter' soil from the 12-year wheat monoculture field near Quincy was spectacular. To the very border of each of the four replicate plots amended with this one soil, at the tillering stage the roots were still white, whereas in all the other treatments they were classic black from take-all (Baker and Cook, 1974; Fig. 44.1). The amount of 'starter' soil mixed to 15 -cm depth amounted to only about 0.5% by weight. With the third sowing (1971/72 crop year), again depending entirely on inoculum

Fig. 44.1. Representative wheat plants from a field trial in the second year of wheat monoculture showing severe take-all (left and centre) or no apparent disease because of take-all decline (right) in response to the introduction of 'starter' soil (0.5% w/w, rotovated to 15 cm depth) 2 years earlier from a field in the 12th year of continuous wheat monoculture near Quincy, WA (right). Plots not yet into take-all decline (represented by plants on left and centre) were amended with the same amount of soil from a non-cropped (virgin) site adjacent to the12-year wheat monoculture field (centre) or no soil (control, left). Take-all was uniformly severe in the first wheat crop and take-all decline occurred uniformly in the third wheat crop, regardless of the one-time initial soil amendment (Baker and Cook, 1974).

from diseased roots and stem bases of the previous crop(s), the wheat was uniformly healthy regardless of the treatment. Take-all decline had occurred throughout the test site.

I reached four conclusions from this one experiment.

- Something in soil from the field in continuous wheat monoculture was suppressive to take-all, it was transferable, and it could multiply.
- The results of this experiment justified a full-scale project to explain them.
- There was no need to repeat this 3-year field experiment, since even if the performance of the soil from the Quincy field could not be confirmed that would not negate the results of this one experiment.
- Never again would I doubt whether take-all decline is real.

From Field Plots to Glasshouse Pots

With the Puyallup field tests underway, we began to refine Shipton's assay for specific suppression to take-all. We diluted test soils with a fumigated soil (our

standardized rooting medium) and amended this mixture with a standardized amount of ground oat grains colonized by the pathogen. This procedure was patterned after that described by Menzies (1959) and our field test for transfer of suppressiveness (Shipton *et al*., 1973). Working with one part test soil blended with 99 parts stock fumigated soil, we confirmed in the glasshouse that the disease-suppressive factor was transferable. We also showed that the transferable factor was eliminated by steam-air pasteurization at 55°C, confirming the findings of Gerlach (1968) for the factor he associated with take-all decline in the Dutch polders.

However, it was not until 1973 when I was in Adelaide, Australia, working with A.D. Rovira that this pot assay system was perfected. The end of the 3-week incubation period of my first attempt with the assay in Adelaide coincided with the time set for me to give a seminar to the CSIRO Division of Soils faculty. I lined up pots representative of the treatments on a table next to the podium and hid them behind a large sheet of brown paper. After discussing the results of the Puyallup field experiment, and how this field test led to development of a pot test as a measure of suppressiveness, I ceremoniously removed the brown paper to reveal the row of pots, showing the tall plants in response to soil from the Quincy long-term wheat monoculture field and short (stunted) plants in response to the non-cropped virgin Quincy soil (Cook and Rovira, 1976; Fig. 44.2). I explained that each pot contained only 1% of the test soil, about a tablespoon, and the rest was the same

Fig. 44.2. Pot test showing the suppressiveness to *Gaeumannomyces graminis* var. *tritici* (Gg) of one part (by wt) soil from the continuous wheat-monoculture field near Quincy, WA (QF) mixed with 99 parts of a stock soil fumigated with methyl bromide (MB) (MB/Gg/QF), and the absence of suppressiveness in the stock fumigated soil amended with the pathogen only (MB/Gg), amended with one part soil from a non-cropped (virgin) site adjacent to the Quincy monoculture wheat field soil plus the pathogen (MB/Gg/QV), or amended with one part QF soil treated with methyl bromide plus the pathogen (MB/Gg/QF MB). Controls include fumigated soil only (MB), natural soil with the pathogen only (CK Gg), fumigated soil amended with 1% of the QV soil (MB/QV) and the fumigated soil amended with 1% of the QF soil (MB/QF) (Cook and Rovira, 1976).

locally available stock fumigated soil used as the rooting medium. I then announced that I had come to work with the world's leading rhizosphere microbiologist to answer the question: what is in that tablespoon of soil?

Fast Forward to 2005

After more than 30 years of research, multiple PhD theses and postdoctoral projects and base support provided by ARS plus multiple grants from the USDA's National Research Initiative Competitive Grants Program starting in 1978, all evidence now indicates that take-all decline is a natural biological control caused by a specific genotype or select few genotypes of rhizosphere-inhabiting bacteria (rhizobacteria), taxonomically classified as *Pseudomonas fluorescens.* These bacteria are inhibitory to the take-all pathogen through production of the antibiotic 2,4-diacetylphoroglucinol (DAPG) (Weller *et al*., 2002). While offering a milestone in rhizosphere microbiology that is arguably second only to the story of *Rhizobium* biology associated with nitrogen fixation, it is doubtful that the investment in research that has produced this remarkable story could or would have been justified without the unequivocal results of that early field trial laid out at Puyallup. Equally noteworthy, an estimated 2 million acres of crop land in the Inland Northwest is now cropped continuously or nearly continuously to cereals, using combinations of spring wheat, spring barley and winter wheat, with only minimal damage from take-all, presumably because of take-all decline. One can only imagine the millions of acres of wheat worldwide that benefited both health- and yield-wise because of the activity of this remarkable subpopulation or sub-subpopulation of DAPG-producing rhizobacteria.

A Brief Review of the Steps Leading to Today's Picture of Take-all Decline and Its Implications for Intensive Agriculture

By the time I left Australia in 1974, there was strong evidence to suggest that the factor suppressive to take-all in our pot test was: (i) operating in the wheat rhizosphere and not the bulk soil; and (ii) involved strains of fluorescent *Pseudomonas* species putatively inhibitory to the take-all pathogen (Cook and Rovira, 1976). One line of evidence came from the work of Smiley (1979), done prior to our work but published later, indicating that the disease suppression associated with ammonium nitrogen involved rhizoplane-inhabiting fluorescent pseudomonads inhibitory to the wheat take-all pathogen. D.M. Weller joined me in Pullman in January 1979, and isolated the now well-studied *P. fluorescens* strain 2-79 (Weller and Cook, 1983; Weller, 1988) from the rhizosphere of wheat in a pot test of soil from a plot on the Washington State University Research Unit, Lind, Washington, in the 10th year of continuous monoculture wheat. We thought at the time that this strain represented the population responsible for take-all decline as it provided significant suppression of take-all in the field plots (Weller and Cook, 1983; Fig. 44.3).

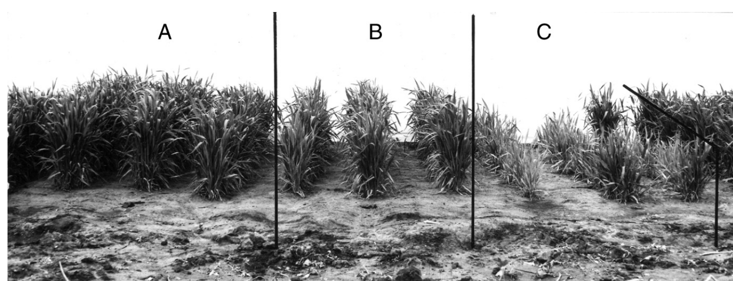

Fig. 44.3. Field test showing (B) the suppressiveness of *Pseudomonas fluorescens* strains 2-79 and 13-79 to take-all caused by *Gaeumannomyces graminis* var. *tritici* when applied as a mixture to the seeds of wheat with the pathogen introduced into the seed furrow, compared with (A) no seed-applied bacteria and no soil-applied pathogen (healthy control) and (C) pathogen added to the seed furrow but no seed-applied bacteria (diseased control) (Weller and Cook, 1983).

Weller was joined in 1984 by Linda Thomashow, and thus began their now-classic studies that led to the identification of the antibiotic phenazine-1-carboxylate (PCA) as the mechanisms of *in vitro* and *in vivo* inhibition of the take-all pathogen by strain 2-19 (reviewed in Weller, 1988; Weller *et al*., 2002). Using Tn-5 mutagenesis, a plasmid that inserts itself randomly into genomic DNA, they generated mutant strains of 2-19 that lost ability to produce PCA and also became ineffective in disease suppression. Genetic complementation of the mutant restored PCA production as well as disease suppression in the rhizosphere. These results, together with quantitative documentation of PCA production in the rhizosphere of wheat inoculated with the wild type and complemented mutant, provided the first evidence, after decades of debate, that antibiotics are produced in soil and they play a role in the ecology of the producing microorganism.

Like so many culturable soil bacteria, the antibiotics produced by fluorescent *Pseudomonas* species had been described and structures worked out by microbiologists and organic chemists years earlier. They include pyroleuteorin, pyrolnitrin, 2,4 diacetylphloroglucinol (DAPG) and the family of phenazines, each highly conserved worldwide within subpopulations of this large and diverse genus *Pseudomonas* (Weller *et al*., 2002; Hass and Defago, 2005). With the basic chemistry worked out, an international effort by investigators interested in exploitation of these bacteria for biological control took this area of science to the next level by cloning and sequencing the genes involved in the biosynthesis of each of these antibiotics (Hass and Defago, 2005). Linda Thomashow led the effort that identified and characterized, respectively, a seven-gene locus for biosynthesis of PCA by strain 2-79 and a five-gene cluster for biosynthesis of DAPG in a strain of *P. fluorescens* Q2-87 isolated from the rhizosphere of wheat grown in the Quincy long-term wheat monoculture soil (Weller *et al*., 2002). The use of genetic probes and primers specific for genes in the PCA and DAPG biosynthetic loci, along with colony hybridization and PCR, allowed quantification of PCA- and DAPG-producers in the rhizosphere of wheat grown, respectively, in suppressive and conducive soils and thus the testing of the hypothesis that one or both kinds

of antibiotics contribute to suppressiveness. These approaches showed that it is the subpopulation of *P. fluorescens* with ability to produce DAPG, not the phenazine-producing subpopulation as previously thought, that accounts for take-all decline under continuous wheat monoculture in Washington State and in The Netherlands (Weller *et al*., 2002; De Souza *et al*., 2003).

The threshold populations of DAPG-producing strains of *P. fluorescens* required for take-all suppression is log 5 CFU/g root, and this amount was shown to occur naturally in the rhizosphere of wheat growing in soils that had undergone take-all decline (Weller *et al*., 2002). Elimination of the DAPG producers eliminates disease suppression, whereas restoration of these bacteria to log 5 CFU/g root by mixing a small amount of take-all decline soil into conducive soil and planting wheat restores suppression. Further, the amount of DAPG produced in the rhizosphere of wheat is a constant 0.62 ng/10^5CFU at populations of the DAPG-producing strain ranging between log 6 and log 7.

Polymorphisms in the *phlD* gene from the five-gene DAPG-biosynthetic operon, together with DNA fingerprinting, is now used worldwide to detect, quantify and characterize the distinct genotypes of DAPG-producing *P. fluorescens* in the rhizosphere of economically important crops (Weller *et al*., 2002). At last count, 22 distinct genotypes of DAPG-producing *P. fluorescens* have been identified among the thousands of isolates obtained from rhizospheres (Landa *et al*., 2005).

Of particular significance is the evidence that the genotype(s) dominating the population of DAPG producers in any give rhizosphere is modulated, in part, by the crop grown, length of monoculture and the geographic location of the field (Picard *et al*., 2000; Landa *et al*., 2005; McSpadden Gardner *et al*., 2005). Among the DAPG-producing genotypes associated with continuous wheat monoculture and take-all decline, the D genotype has been the dominant strain in Washington take-all decline fields, whereas genotypes F and M were dominant in Dutch take-all decline fields (Weller *et al*., 2002). Strain Q8r1- 96 is of the D genotype (Weller *et al*., 2002) and, like all genotype D isolates, is a highly aggressive colonist of the wheat rhizosphere, which no doubt contributes to its ability to efficiently suppress take-all in TAD fields under continuous wheat monoculture.

On the campus of North Dakota State University, Fargo, where wheat and flax were grown as monocultures in side-by-side plots for more than 100 years, populations of DAPG-producing pseudomonads exceeded the threshold log 5.0 CFU/g root in the rhizospheres of both wheat and flax grown in the soils. However, the genotypes that made up these two populations were very different. About 80% were of equal frequencies of genotypes F and J in soil where flax had been grown in monoculture, and 77% were genotype D in soil where wheat had been grown in monoculture. DAGP-producers were below the level of detection (10^4 CFU/g root) on roots grown in soil from a third adjacent plot that had been in crop rotation (i.e. bean, maize, oat, soybean, sugar beet, sunflower etc, or left fallow) for over a century (Landa *et al*., 2005).

Similarly in a plot on the Washington State University, Northwest Research and Extension Center at Mount Vernon, Washington, DAPG-producers exceeded log 5.0 CFU/g root in the rhizosphere where peas had been grown in monoculture for the past 30 years and the soil was suppressive to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *pisi*. Among the six DAPG-producing genotypes

identified in the rhizosphere of pea growing in this soil, D and P were dominant. Greenhouse studies showed further that D and P colonized the rhizospheres of wheat and pea, respectively, better than four other of DAPG-producing genotypes (A, L, O and Q) also isolated from this pea-monoculture plot (Weller *et al*., 2002). In Ohio, McSpadden Gardner *et al*. (2005) found the D genotype in the rhizosphere of maize, soybeans, or both crops in all 15 counties sampled, and it represented the most abundant of seven genotypes identified in total. On average, the D genotype was detected at populations exceeding log 3.4 CFU/g root on 77, 84 and 81% of maize plants sampled in years 2001, 2002 and 2003, respectively, and 78, 67 and 52% of soybean plants sampled during those three years, respectively.

In re-examining the early experiment on initiation of take-all decline in the Puyallup field plot with 'starter' soil transferred from a long-term wheat monoculture field (Shipton *et al*., 1973; Baker and Cook, 1974), and the demonstration of take-all suppression with one part wheat monoculture soil mixed with 99 parts stock fumigated soil as the rooting medium (Cook and Rovira, 1976), it would seem nearly certain that the tiny amounts of soil that we used to transfer suppressiveness to the conducive soil contained genotype D isolates of DAPG- producing *P. fluorescens.*

A Brief Review of Concurrent Research with Biocontrol Pseudomonads

As in any advancement in science, the work on take-all decline is but part of a much larger and international effort in research on biological control with fluorescent pseudomonads over the past 30+ years. Here, and for a more proper perspective, I provide a very brief review of some of the concurrent, collaborative and supporting research with these ubiquitous and ecologically and agriculturally important rhizobacteria. The definitive and unambiguous studies continue to point to direct inhibition of soil-borne pathogens (antibiosis) as the primary reason for the obvious plant-growth enhancement have come to be known collectively as 'plant growth promoting rhizobacteria' (PGPR) (Kloepper *et al*.,1980). However, the scope of this area of science continues to expand, with recent evidence that rhizobacteria can also suppress disease development through induction of systemic resistance (ISR) to plant pathogens, described as enhanced basal resistance in plants to their pathogens (Hass and Defago, 2005). Interestingly, the ISR depends on the jasmonic acid signalling pathway first shown to trigger a defence response in plants to herbivorous insects (Farmer *et al*., 1992). In addition to these multiple mechanisms of plant growth promotion through biological control of root pathogens, genetic evidence is now also forthcoming for a direct role of rhizobacteria in promotion of root growth, including under gnotobiotic conditions (Wang *et al*., 2006). For more comprehensive reviews, see Bakker (1989), Cook and Baker (1983), Hass and Defago (2005) and Weller *et al*. (2002).

One of the first lines of evidence that fluorescent *Pseudomonas* species had potential for biological control of soil-borne plant pathogens in the rhizosphere was from work done at the University of California, Berkeley, led by M.N. Schroth.

The seminal work from this group was their discovery that the enhanced growth of potatoes was associated with the production of siderophores proposed to inhibit pathogen growth through iron starvation (Kloepper *et al*., 1980). Under Kloepper's leadership, an international PGPR workshop has been held somewhere in the world every 4 years since 1982. The 7th International Workshop on Plant Growth Promoting Rhizobacteria was held from 28 May to 2 June in The Netherlands.

Equally comprehensive work on the effectiveness of PGPR strains in biological control of pathogens in the potato rhizosphere has been done in The Netherlands. This work began with the report of increased growth of potatoes in response to treatment of potato seed pieces with fluorescent pseudomonads, with the greatest growth responses occurring in fields cropped every 3 years to potatoes (short rotation) compared with lesser growth responses in fields cropped every 6 years to potatoes (long rotations) (Schippers *et al*., 1987). These results pointed clearly to a role of pathogen displacement or suppression in the rhizosphere since pathogen pressure (and hence response to pathogen control) would be highest in fields in short rotations (but not quite continuous potato monoculture). With no evidence that plant pathogens well known to be favoured by short rotations were inhibited by the introduced rhizobacteria, e.g. *Verticillium dahliae*, the hypothesis was advanced that the relatively poor performance of potatoes grown in short rotations is due to deleterious rhizobacteria enriched by frequent cropping to potatoes and that, in turn, are displaced or suppressed through iron starvation by the introduced PGPR (Shippers *et al*., 1987; Bakker, 1989).

Parallel to the Berkeley and Dutch studies on growth promotion of potato, and about the time that D.M. Weller isolated strain 2-79, investigators working on cotton in Texas reported control of seedling blight caused by *Rhizoctonia solani* using one strain of *P. fluorescens* or its antibiotic, pyrolnitrin, and seedling blight caused by *Pythium ultimum* by a different strain of *P. fluorescens* or its antibiotic, pyroluteorin (reviewed in Cook and Baker, 1983). This research provides some of the first evidence, albeit circumstantial, for a role of antibiotic production in biological control of soil-borne plant pathogens by these bacteria. Whether the seed-applied bacteria colonized the rhizosphere of cotton or served only to protect the germinating seed against infection was not determined.

Similar to the take-all decline story, researchers in Switzerland led by G. Defago demonstrated a primary role of fluorescent pseudomonads in the suppression of black root rot of tobacco caused by *Thielaviopsis basicola* in a soil cropped 24 years to monoculture tobacco (Stutz *et al*., 1986). Early work indicated that this example of disease suppression with monoculture of the host crop involved multiple mechanisms of iron starvation by production of siderophores and inhibition of the pathogen by production of hydrogen cyanide and antibiotics (reviewed in Hass and Defago, 2005). Subsequent work with *P. fluorescens* strain CHAO, obtained from the suppressive soil and now one of the premiere model strains for fundamental research, pointed to the importance of DAPG in this natural suppression (Keel *et al*., 1992). *P. fluorescens* CHAO is member of the A genotype of DAPG-producing *P. fluorescens*.

Interestingly, the pyrolnitrin-producing strain *P fluorescens* Pf-5, active against *R. solani*, also produces DAPG, is a member of the A genotype, and has become another model strain for fundamental studies. As another milestone, and an effort

led by Joyce Loper with ARS at Corvallis, Oregon and Linda Thomshow with ARS at Pullman, the genome of Pf-5 has been completely sequenced (Paulson *et al*., 2005), revealing six secondary-metabolite gene clusters.

Still another in the growing list of DAPG-producing pseudomonads, and apparently the only known member to date of the K genotype (Weller *et al*., 2002), has been shown in Ireland to suppress damping-off of sugarbeet and pea seedlings caused by *Pythium ultimum* (Fenton *et al*., 1992), soft rot of potato caused by *Erwinia carotovora* (Cronin *et al*., 1997a), and the potato cyst nematode, *Globodera rostochiensis* (Cronin *et al*., 1997b).

Finally, and an interesting twist on the role of rhizobacteria in crop monoculture, soils not previously planted to apples are naturally suppressive to a fungal/oomycete complex of apple root pathogens but become conducive to these pathogens by the 3rd or 4th year after apples are grown in the soil, owing to a displacement of populations of microorganisms antagonistic to these pathogens by pseudomonads not inhibitory to these pathogens. This shift in microbial communities involving the concurrent enrichment in the inoculum density of root pathogens and populations of rhizobacteria that have no inhibitory activity to these pathogens accounts for the long-studied apple replant problem. However, planting wheat in old apple-orchard soil prior to replanting apples restores the population-inhibitory pseudomonads, in particular *Pseudomonas putida*, and coordinately the soil reverts from its disease-conducive to a disease-suppressive state and controls the apple replant disease (reviewed in Weller *et al*., 2002).

What is Biological Control?

In his review of Baker's and my first book, Hirst (1974) wrote: 'The dilemma faced by the authors was how far to extend beyond the difficulty of defining biological control into the wider problems of managing microbial ecology to lessen plant diseases.' The definition put forward by Baker and Cook (1974) was 'Biological control is the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists.' Hirst (1974) considered this definition as 'so broad that it embraces all of microbial ecology', and preferred himself to restrict the term to control achieved by 'the manipulation of "third organisms" such as hyperparasites, antagonists, and competitors'.

Hirst's preference to exclude 'managing of microbial ecology to lessen plant diseases' from the concept of biological control of plant pathogens was incomprehensible to Baker and me at that time, and it is even more incomprehensible to me and probably most plant pathologists today. Restricting the concept in this way implies that unless the biological control agent is introduced or until one knows the resident agent, i.e. antagonist, responsible for the control, as in the case of take-all decline, it is not biological control. Yet Hirst's views were more nearly in line with 'conventional wisdom' at that time, possibly because of the many successes in biological control of insects and weeds with introduced agents. The late Ken Hagen, well known for his outstanding work and scientific leadership on

biological control of insects, tried to resolve what Hirst referred to as our 'dilemma' by suggesting to me in a personal conversation that most examples from plant pathology should be categorized as 'biological *methods* of control', as distinct from what entomologists considered 'biological control'. This struck me as splitting hairs and presumably would be as unnecessary as trying to distinguish between chemical control and chemical methods of control.

The definition offered in our first book and made more explicit in our second book (Cook and Baker, 1983) also included host plant resistance as biological control. Excluding host plant resistance from the concept of biological control always struck me as artificial if not political. Going back to Hirst's preference to restrict the concept to control achieved by 'the manipulation of "third organisms"', resistance induced in an otherwise susceptible genotype of the host by an avirulent or weakly virulent strain of the pathogen or PGPR strain would be included in the concept, but the same or similar mechanism(s) of resistance expressed in a resistant genotype of the host without the aid of the *third organism* would not be biological control. Whether resistance is manipulated through deployment of an organism or a gene, the control achieved is *biological* in nature and therefore logically should be included within the concept of biological control.

In a report released nearly 20 years ago, the US National Academy of Sciences defined biological control as 'The use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms (pests), and to favor desirable organisms such as crops, trees, animals, and beneficial insects and microorganisms' (NAS, 1987). This broad definition included the use of the crystalline protein of *Bacillus thuringiensis* as biological control whether delivered by the insect pathogen itself, a plant-associated microbe, e.g. endophyte or rhizobacterium genetically transformed to express the *Bt* gene, or as a gene expressed as transgenic resistance in the plant to the insect pest. Similarly, this NAS definition included as biological control of plant viruses both cross protection achieved by pre-emptive inoculation of the host with a weakly virulent strain of the virus or resistance achieved by expression of the coat-protein (or other gene) of the virus as a transgene in the host.

As scientists, we logically view problems and opportunities in science based on our own experiences and usually also by what we were taught by others within our own discipline. One can understand why those working on biological control of insect pests and weeds would focus entirely on regulating the population of the pest. By the classic definition of DeBach (1964), 'The use of pathogens, parasites and predators to regulate the population of a pest at a level lower that it would occur in the absence of these natural enemies', lowering the weed or insect pest population is the only acceptable outcome. Likewise, one can understand why those of us working on biological control of plant pathogens would focus on disease suppression, which, like the endophytic fungi antagonistic to herbivorous insects, may or may not involve reducing the population of the pathogen or pest. The focus in biological control of plant pathogens has been on protecting the health of the host, often with no reliable information on what happens to the population of the pathogen.

Whether or not one agrees with Hirst's preference to limit the concept to 'the manipulation of "third organisms"', scientists and policy makers today can justifiably ask: 'After 30+ years of research and investments of millions in research

dollars, where are the commercial products?' Indeed, the standard for documentation that a strain fits the definition of PGPR (Kloepper *et al*., 1980), and now applied by investigators worldwide, is to document an improved stand, increased growth and/or higher yield in response to the strain or strains introduced on or with the seed or other planting material at planting. Our work on take-all at Pullman, and similar work in Australia and China, has involved extensive and intensive field testing of these strains over a 20-year period, starting with the PCA-producing 2- 79 (Weller and Cook, 1983) and then with the DAPG-producing D-genotype Q8r1-96 (Cook *et al*., 2002). However, with few if any exceptions, the increased growth and yield responses have been too variable or inconsistent to meet the standards set for commercialization (Weller, 1988; Bakker, 1989). I have argued unsuccessfully that the standards are unrealistically high (Cook, 1993), and that development and use of microbial biocontrol products should be modelled after the development and release of plant varieties rather modelled after pesticides. Obviously no company is going to invest in the cost of obtaining regulatory approval under standards set for pesticides, especially if the microbial agent will be used for biological control of only one disease on one crop. Yet experiment stations and seed companies have released thousands of crop varieties based on a single superior trait or slightly improved agronomic performance.

PGPR strains introduced with the planting material, or by other means, should eventually expand from their current minor use to become part of mainstream agriculture, including as strains genetically engineered to express more than one antibiotic (Fenton *et al*., 1992; Blouin Bankhead *et al*., 2004). However, for now we are left with the approach that Hirst (1974) excluded from the concept of biological control, i.e. 'managing of microbial ecology to lessen plant disease', and what Baker and Cook (1974) included in the concept, which is '. . .reduction of . . . disease-producing activities of a pathogen . . . by one or more organisms, accomplished naturally . . . through manipulation of the . . . host. . .' Even more intriguing than the millions of acres of intensive cereals that benefit health- and yield-wise year after year because of the activity of DAPG- and other antibiotic-producing rhizobacteria, it would be interesting to know the area of crops, turf or plants in natural ecosystems more generally that are healthier because of the antibiotic-producing rhizobacteria that team up with roots of their host to provide biological control of the soil-borne pathogens of that host.

Acknowledgement

My thanks to David Weller for critically reviewing this manuscript and for his many helpful suggestions.

References

Baker, K.F. and Cook, R.J. (1974) *Biological Control of Plant Pathogens*. W.H. Freeman and Co., San Francisco, California, 433 pp. (Book, reprinted in 1982, American Phytopathological Society, St. Paul, Minnesota.)

Baker, K.F and Snyder, W.C. (eds) (1965) *Ecology of Soil-borne Plant Pathogens: Prelude to Biological Control*. University of California Press, Berkeley, California, 535 pp.

Bakker, P.A.H.M. (1989) Siderophore-mediated plant growth promotion and colonization of roots by strains of *Pseudomonas* spp. Ph.D thesis, Willie Commelin Scholten Phytopathological Laboratory, Department of Plant Pathology, State University Utrecht, Javalaan 20, 3742 Baarn, The Netherlands, 100 pp.

Blouin Bankhead, S., Landa, B.B., Lutton, E., Weller, D.M. and McSpadden Gardner, B.B. (2004) Minimal changes in rhizobacterial population structure following root colonization by wild type and transgenic biocontrol strains. *FEMS Microbiology Ecology* 49, 307–318.

Cook, R.J. (1993) Making greater use of introduced microorganisms for biological control of plant pathogens. *Annual Review of Phytopathology* 31, 53–80.

Cook, R.J. and Baker, K.F. (1983) *The Nature and Practice of Biological Control of Plant Pathogens.* American Phytopathological Society, St. Paul, Minnesota, 539 pp.

Cook, R.J. and Rovira, A.D. (1976) The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. *Soil Biology and Biochemistry* 8, 69–273.

Cook, R.J. and Veseth, R.J. (1991) *Wheat Health Management.* APS Press, St. Paul, Minnesota, 151 pp.

Cook, R.J., Weller, D.M., El-Banna, A.Y., Vakoch, D. and Zhang, H. (2002) Yield responses of direct-seeded wheat to fungicide and rhizobacteria seed-treatments. *Plant Disease* 86, 780–784.

Cronin, D., Moenne-Loccoa, Y., Fenton, A., Dunne, C., Dowling, D.N. and O'Gara, F. (1997a) Ecological interaction of a biocontrol strain of *Pseudomonas fluorescens* producing 2,4-diacetylphloroglucinol with the soft rot potato pathogen *Erwinia carotovora* subsp. *atroseptica*. *FEMS Microbiology Ecology* 23, 95–106.

Cronin, D., Moenne-Loccoa, Y., Fenton, A., Dunne, C., Dowling, D.N. and O'Gara, F. (1997b) Role of 2,4-diacetylphloroglucinol in the interaction of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. *Applied Environmental Microbiology* 63, 1357–1361.

DeBach, P. (ed.) (1964) *Biological Control of Insect Pests and Weeds.* Reinhold, New York, 844 pp.

De Souza, J.T., Weller, D.M. and Raaijmakers, J.M. (2003) Frequency, diversity, and activity of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in Dutch take-all decline soils. *Phytopathology* 93, 54–63.

Farmer, E.E., Johnson, R.R. and Ryan, C.A. (1992) Regulation of expression of proteinase-inhibitor genes by methyl jasmonate and jasmonic acid. *Plant Physiology* 98, 995–1002.

Fenton, A.M., Stephens, P.M., Crowley, J., O'Callaghan, M. and O'Gara, F. (1992) Exploitation of gene(s) involved in 2,4-diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a *Pseudomonas* strain. *Applied Environmental Microbiology* 58, 3873–3878.

Gerlach, M. (1968) Introduction of *Ophiobolus graminis* into new polders and its decline. *Netherlands Journal of Plant Pathology* 74 (Supplement 2), 1–97.

Hass, D. and Defago, G. (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews. Microbiology* 3, 307–319.

Hirst, J.M. (1974) What is biological control? *Nature* 252, 147.

Keel, C., Schnider, U., Maurhofer, M., Voisard, C. and Burger, D. (1992) Suppression of root diseases by *Pseudomonas fluorescens* CHAO: importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Molecular Plant–Microbe Interactions* 5, 4–13.

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. (1980) Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature* 286, 885–886.

Landa, B.B., Mavrodi, O., Schroeder, K., Allende-Molar, R. and Weller, D.M. (2005) Enrichment and genotypic diversity of phl-D containing fluorescent *Pseudomonas* spp. in two soils after a century of wheat and flax monoculture. *FEMS Microbiology Ecology* 55, 351–368.

McSpadden Gardner, B.B., Gutierrez, L.J., Raghavendra, J., Edema, R. and Lutton, E. (2005) Distribution and biocontrol potential of *phlD+* pseudomonads in corn and soybean fields. *Phytopathology* 95, 15–724.

Menzies, J.D. (1959) Occurrence and transfer of a biological factor in soil that suppresses potato scab. *Phytopathology* 49, 648–652.

National Academy of Sciences (1987) *Biological Control in Managed Ecosystems.* National Academy Press, Washington DC, 55 pp.

Paulson, I.T. and 27 coauthors (2005) Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. *Nature Biotechnology* 23, 873–878.

Picard, C., Di Cello, F., Ventura, M., Fani, R. and Guckert, A. (2000) Frequency and biodiversity of 2,4-diacetylphloroglucinol-producing bacteria isolated from the maize rhizosphere at different stages of plant growth. *Applied Environmental Microbiology* 66, 948–955.

Schippers, B., Bakker, A.W. and Bakker, P.A.H.M. (1987) Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annual Review Phytopathology* 25, 339–258.

Shipton, P.J. (1972) Take-all in spring sown cereals under continuous cultivation: disease progress and decline in relation to crop succession and nitrogen. *Annals Applied Biology* 71, 33–46.

Shipton, P.J., Cook, R.J. and Sitton, J.W. (1973) Occurrence and transfer of a biological factor in soil that suppresses takeall in wheat in eastern Washington. *Phytopathology* 63, 511–517.

Smiley, R.W. (1979) Wheat-rhizoplane pseudomonads as antagonists of *Gaeumannomyces graminis*. *Soil Biology and Biochemistry* 11, 371–376.

Stutz, E.W., Defago, G. and Kern, H. (1986) Naturally occurring fluorescent pseudomonads involved in suppression of black root rot of tobacco. *Phytopathology* 76, 181–185.

Wang, K., Conn, K. and Lazarovits, G. (2006) Involvement of quinolinate phosphoribosyl transferase in promotion of potato growth by a *Burkholderia* strain. *Applied and Environmental Microbiology* 72, 760–768.

Weller, D.M. (1988) Biological control of soilborne plant pathogens in the rhizosphere by bacteria. *Annual Review of Phytopathology* 26, 379–407.

Weller, D.M. and Cook, R.J. (1983) Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* 78, 1094–1100.

Weller, D.M., Raaijmakers, J.M., McSpadden Gardener, B.B. and Thomashow, L.S. (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* 40, 309–348.