Biological Control of Plant Pathogens, with Special Reference to the Take-all Fungus in Suppressive Soils

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摘 要

Cook, R. J. (1985) **抑病土對小麥全蝕病菌之生物防治** （美國農部根部病害及生物防治研究室）

廣義的生物防治包括拮抗微生物，寄主植物，病原菌之變異菌系或以上任何因子之配合應用，達到抑制病原菌之接種源，感染過程或病害發展的目的。許多傳統的生物防治方法，例如輪作，耕犂，淹水及添加有機物等已不適合現代的耕作制度，故必需尋求其他的防治對策。小麥全蝕病菌（*Gaeumannomyces graminis* var. *tritici*）之生物防治即是研究抑病土作用機制發展出來的方法之一。研究結果發現，有兩類型抑病土存在，亦即針對「全蝕衰敗」（take-all decline）之專一性抑制型（specific suppression）及不論耕作歷史，任何土壤皆具有之一般性抑制型（general suppression）。對全蝕病菌而言，一般性抑制型乃是病原行腐生生存期間，拮抗微生物在病斑上或植株殘體中對病原菌的競爭作用，它影響接種源單位容積質量（植株殘體）所能引起的病斑數目，換句話說，倘若去除殘體中之拮抗微生物，則全蝕病菌之感染效率爲之顯著提高。專一性抑制型則被認爲是某類拮抗菌對病原菌之抑制作用，尤其是當這些拮抗微生物屬於侵略性的次生菌，具有抗生作用，並且和全蝕病菌，同時棲息於植株殘體時，更能顯出抑制力。能產生抗生物質之根棲螢光性細菌（*Pseudomonas* spp）是造成「全蝕衰敗」的主要拮抗菌，將它們粉衣於小麥種子上，則可阻擋全蝕病之侵襲。本文亦討論其他可能之生物防治機制，例如 dsRNA mycovirus agent 能使全蝕病菌無毒化，並且自我抑制，以及相關的 *Phialophora graminicola* 對全蝕病菌之交叉保護等。

ABSTRACT

Biological control, broadly defined, includes the use of antagonistic microorganisms, the host plant, modified strains of the pathogen, or any combination of these agents to eliminate or suppress inoculum of the pathogen, prevent infection, or limit disease development. Many traditional biological controls

(e. g. those accomplished by crop rotation, tillage to accelerate biological destruction of inoculum, and flooding, and organic amendments) no longer fit with modern farming practices. Approaches to biological control of take-all illustrate some alternatives developed for one disease where traditional controls have become impractical or obsolete. The controls under development were first recognized for their potential through studies of the mechanisms of suppression of the pathogen, *Gaeumannomyces graminis* var. *tritici* in certain soils. At *least* two kinds of suppressive soils exist; so-called "specific suppression," responsible for take-all decline; and "general suppression," operative to one degree or another in all soils regardless of cropping history. It is suggested that general suppression results from competitive effects of the succession of secondary colonists in the lesion and continuing in the infested crop residue during saprophytic survival of the fungus. General suppression affects the number of root infections per unit mass of infested crop residue (infection efficiency), which increases significantly upon elimination of competitors for the foodbase. Specific suppression acts against a background of general suppression, and is thought to also result from inhibitory effects of secondary colonists. However, the antagonists responsible for the specific suppression are thought to be more potent in their effect, being especially aggressive as secondary colonists of lesions, able to inhibit the pathogen by antibiotic action, and more likely to carryover as cohabitants with the pathogen in the crop residue. Antibiotic-producing root-colonizing fluorescent pseudomonads are implicated as the important agents in the specific suppression responsible for take-all decline. These antagonists have been shown to provide significant protection of wheat when applied as a living seed treatment. Other potential mechanisms of biological control discussed are dsRNA mycovirus-like agents in the take-all fungus, the tendency of the fungus to convert to avirulence and to inhibit itself, and cross protection by the related *Phialophora graminicola*.

INTRODUCTION

Biological control is used in this paper in the broad sense to include pathogen control by microorganisms, the host plant, or even by the pathogen used against itself[13]. A microorganism may be inhibitory to the pathogen by its ability to produce one or more antibiotics, it may be a hyperparasite (or hyperpathogen) with ability to weaken or destroy cells of the pathogen, or it may compete with the pathogen and thereby retard the growth and/or reproduction of the pathogen on or within the host. The host may play a passive role in biological control, by providing the sites for interaction between the antagonist(s) and pathogen; or it may play an active role by resisting the pathogen. Traditionally, the resistance of plants used against pathogens has been obtained by breeding, but resistance can also be induced by inoculation of the plant with certain nonpathogens; either way biological control come into play. The use of a

pathogen for its own biological control includes mainly modified or avirulent strains as carriers of infectious agents (e. g. a dsRNA mycovirus), as competitors in the infection court of the pathogen, or to induce resistance. Ultimately, we may find that modified pathogens will be the best agents of biological control.

While a concept of biological control that includes the use of antagonists, the host, or the pathogen itself may seem too broad[17] there is no logical reason to limit the concept to a more narrow definition[12]. Some would exclude the host as an agent of biological control, but as F. G. H. Lupton[35] states "accelerating or diverting evolutionary processes in order to obtain genotypes adapted to [man's] needs are a most important example of the application of biological control to agricultural and horticultural crops." On the other hand, a concept so broad can be unwieldy, and to avoid this Cook and Baker[13] proposed three subdivisions: biological control of pathogen inoculum; biological control of plant infection; and biological control of disease development after infection. These three approaches fall generally, but with some overlap, into biological control remote from the host, on the host, and inside the host.

The first approach includes those biological controls aimed at reduction of inoculum density in soil, e. g. by crop rotation, tillage to accelerate biological breakdown of crop residue infested with pathogens, or the introduction of hyperparasites to destroy pathogen spores and sclerotia in soil or in host tissue. This approach may also include the use of agents to lower the virulence of pathogen inoculum, e. g. dsRNA mycovirus-like agents to convert virulent *Endothia parasitica* in chestnut cankers to a hypovirulent state, whereupon the canker heals.[2] The second category—control of plant infection—includes any use of nonpathogenic microorganisms in the rhizosphere or on any phylloplane to preempt, exclude, or inhibit pathogens and thereby protect plants. More will be said later about this potentially very useful approach. The third proach—limit disease development after infection or attempted infection—includes the use of cross protection and induced resistance. The third approach can also include genetic improvement of the plant's resistance to pathogens, any cultural practice that helps the plant avoid or tolerate stresses and thereby maintain its resistance to weak parasites, and the use of mycorrhizae that somehow help the plant resist pathogens.

Accepting the broad concept, it is apparent that biological control is not a new way to control pathogens. Already in the 3rd century B.C. farmers in China were advised to fallow their soil if the field gave a poor yield.[13] Crop rotation allows time between the plantings of any given crop for microorganisms in soil to destroy the inoculum of pathogens that otherwise would damage that crop. Rotating crops is thus a very old as well as contemporary method of biological control. Another biological control of long standing and still used today is flooding the soil.[49] Microorganisms, probably anaerobes, are somehow involved in destruction of pathogen inoculum in flooded soil.[51] The incorporation of large quantities of organic material, e. g. barnyard manure or green manure, is an

ancient farming practice. It was shown scientifically 50-60 years ago to induce biological control of common scab of potato[42], take-all of wheat[43], and phymatotrichum root rot of cotton[30]. Organic soil amendments initiate germination followed by lysis of the pathogen propagules in soil,[40] and they increase the microbial suppressiveness of soils to pathogens.[13] Each of these familiar methods are aimed at biological destruction or suppression of pathogen inoculum in soil and therefore fit in the first category given above. Other familiar examples could be cited. It is important to realize that plant plathologists have been doing biological control research all along[12].

Unfortunately, not all pathogens can be controlled by the the traditional crop rotations, flooding, organic manural treatments, or host resistance; or these approaches no longer fit with modern farming practices or are too labor-intensive. Take-all of wheat, caused by *Gaeumannomyces graminis* var. *tritici*, fits this situation. This disease has been studied longer and more intensively than possibly any other root disease, yet it continues to be the most important root disease of wheat worldwide. It occurs in northern Europe, South Africa, Brazil, Chile, Argentina, Australia, China, Hokkaido Island of Japan, and most wheat growing areas of North America.[5] The disease can be controlled by organic amendments to the soil, but organic manures are no longer available in sufficient quantities and the practice is too labor-intensive. The disease is easily controlled by a single year of no wheat, barley, or other grass suscepts in the rotation, but the trend in cereal production, as for many other important food crops, is toward monoculture. The disease can also be controlled by tillage, but the trend in North America and elsewhere is toward less or no tillage. There is no known source of genetic resistance in wheat or barley, and the only effective chemical treatment thus far is soil fumigation, which is too expensive for wheat. Thus, for nearly the past twenty years, our USDA, ARS program at pullman has been seeking biological approaches to the control the wheat take-all fungus that will permit less or no crop rotation and hopefully less or no tillage. Our work is focused mainly on the biological mechanisms responsible for suppressive soils and take-all decline with wheat monoculture.

A mechanism of bioloical control of *G. graminis* var. *tritici* by one or some combination of each of the three approaches given above has now been identified and one method involving the introduction of antagonists of the pathgen as a seed treatment is under consideration for commercial application. The remainder of this paper will therefore be on biological control of the take-all fungus on wheat, as a means to briefly summarize the current state of this work, but also because so many principles of biological control of plant pathogens can be illustrated by the biocontrols or potential biocontrols of this fungus. The main emphasis will be on the mechanisms identified through studies of pathogen suppression in certain soils.

IMPORTANCE OF CROP RESIDUE AS A FOODBASE TO LONGEVITY AND INOCULUM POTENTIAL OF

THE PATHOGEN IN SOIL

Understanding the importance of a foodbase to the survival and inoculum potential of *G. graminis* var. *tritici* in soil is a key to understanding the mechanisms of suppression of this fungus in certain soils and how it can be controlled biologically. Like many root pathogens, *G. graminis* var *tritici* is dependent for survival between host crops on its saprophytic existence in the roots and stem bases of host plants colonized earlier through parasitism.[20] The fungus cannot effectively colonize dead substrates in competition with other microorganisms in the soil, but it overcomes this limitation by colonizing the substrates while still parts of living plants. Thereafter, the challenge to *G. graminis* var. *tritici* is one of "hanging on" to the substrates in an environment where countless saprophytes exist with ability to displace the fungus. The longer the time the fungus exists in the crop residue in soil the less the energy available for infection when a new host root finally comes along.

Wilkinson, et al[56] developed a model system to quantify the inoculum potential of *G. graminis* var. *tritici* in fragments of plant residue. Their system determined both the minimum (threshold) size of infested particles necessary for an infection to occur and also the number of infections per unit mass (infection efficiency) of different size particles. The threshold size was 250-500 μm for both infested stem bases (natural inoculum) and axenially colonized oat grains (artificial inoculum). This range compares favorably with the 420 μm estimated by Hornby[26] as the minimum size. At the threshold size and larger, the number of infections per unit weight of inoculum (infection efficiency) was the same for all particle sizes, i. e. 10 mg of particles 1-2 mm in diameter produced the same number of lesions as did 10 mg of particles either 250-500 μm or 500 μm to 1.0 m in diameter. However, the infection efficiency of artificial (infested oat-grain) inoculum was always greater than that of naturally infected stem bases.

Under field conditions, the size and total amount of infested particles at any given time after harvest will depend on the length of time since the last wheat crop was grown in the field, the frequency of wheat cropping (length of the rotation), and the frequency and kind of tillage. This is revealed by the particle size distribution of infested debris recovered in the fall after harvest and tillage of three irrigated wheat fields in Washington state. In a field cropped to wheat for the first time after rotation for several years to nonsusceptible crops, all infested particles collected 2-3 months after harvest were 12.5 mm in diameter and larger. In a second field cropped two consecutive years to wheat, most of the infested debris was again as particles 12.5 mm in diameter and larger, but in addition, an infested fraction 6.4 to 12.5 mm in diameter was recovered. In a third field, cropped three consecutive years to wheat, a large portion (most) of the infested debris again was 12.5 mm in diameter, but infested particles of many sizes down to only 0.5-1.0 mm in diameter also were recovered. The smaller size particles in the field cropped 3 years undoubtedly represented remnants of plants infected in the second and even first years that wheat was grown in the

field.

On the other hand, if the field is not tilled (seeded directly, with no prior seedbed preparation, a method gaining in popularity to prevent soil erosion), the fragments of infested crop residue are mostly as intact wheat crowns 1-2 cm in diameter[37]. Take-all in consecutive crops of wheat is therefore more severe in the Pacific Northwest of the USA if the fields are direct drilled than if intensive tillage is used. The more intensive the tillage, the smaller the particles of infested residue and the greater the portion of infested residue as particles smaller than the minimum threshold size. The greater surface area of the fragmented inoculum may also favor greater competition for the take-all fungus in the residue by microorganisms.

Wilkinson et al[58] also estimated the distance *G. graminis* var. *tritici* can grow from an inoculum particle to infect a root in different soils. The average distances for infection were estimated 1-2 mm for particles 0.25-0.5 mm in diameter, 3-4 mm for particles 0.5 to 1.0 mm in diameter and 11-12 mm for particles 1-2 mm in diameter. As expected, the estimated distances for infection increased, when the soil was pasteurized or fumigated to over 3 mm for particles 0.25 to 0.5 mm and to 5-7 mm for particles 0.5 to 1.0 mm in diameter. Particles smaller than the threshold size (90 μm to 150 μm in diameter) for natural soil were infectious in pasturized soil, but the 11-12 mm estimated as the distance for infection from particles 1-2 mm in diameter was the same whether or not the soil was treated to eliminate competing microorganisms. Wilkinson, et al[58] concluded that with the larger particles (1-2 mm in diameter), the food base was the main source of nutrients for the fungus and hence, elimination of soil organisms as competitors in the rhizosphere did not result in a significantly distance the fungus could grow to reach the root. In the smaller particles, on the other hand, nutrients in the form of root exudates were thought to be more influential which could explain why any reduction in the intensity of competition from the associated microbiota was reflected in a greater distance the fungus could grow from the smaller particles to reach a root.

CONVERSION OF SOILS FROM CONDUCIVE TO SUPPRESSIVE FOLLOWING ONE OR MORE OUTBREAKS OF TAKE-ALL

Like most root diseases, take-all increases in severity with the first two, three, or possibly four consecutive host crops. Howevre, whereas most diseases caused by soilborne pathogens eventually stabilize at a consistently severe level with monoculture of the host, or fluctuate in severity depending on conditions, take-all eventually goes into remission following one or two severe outbreaks of the disease. "Take-all decline" was first observed about fifty years ago by Fellows and Ficke[18] in Kansas in the USA and independently by Glynn[22] in England. It was not until the work of Gerlagh[21] in Holland, however, that take-all decline was demonstrated experimentally. Gerlagh[21] showed that soils conducive to the pathogen became suppressive with wheat monoculture and the presence of the take-all fungus. This pattern fits one of three categories proposed by Baker and

Cook[6] for pathogen suppressive soils, namely the pathogen establishes in soil and causes disease for a while but then severity of the disease declines. The background information and evidence for take-all decline has been reviewed by Sipton[45]. To avoid confusion with other suppressive soils the suppression responsible for take-all decline will hereafter be referred to as *specific suppression.*[21]

CHARACTERISTICS OF SPECIFIC SUPPRESSION

Specific suppression has been studied in more detail than possibly any other kind of suppressive soil. Several important features of suppressive soils and experimental approaches useful to their study are illustrated by this work and are enumerated below.

a. A bioassay for the suppressiveness is usually needed to study the suppression and, most important, to make certain that any suppressiveness demonstrated experimentally is the same suppressiveness operative in nature. Gerlagh[21] and Shipton[44] each developed bioassays, but probably the best and simplest is the pot assay of Shipton et al[46], described more fully and illustrated by Cook and Rovira [15]. In this bioassay, the field soils are diluted 1:10 or 1:100 with a standardized fumigated soil and the blend then infested with axenically colonized oat-grain inoculum of *G. graminis* var. *tritici*, potted, and sown to wheat. The plants are scored for severity of disease 4 weeks later. Soil from fields where take-all decline had occurred were the only soil suppressive to take-all in this test. D. Weller (unpublished) found it necessary to remove the first crop of seedlings and to sow again, since suppressiveness was best revealed in the second crop of seedlings. The enhanced with a second sowing and the perfect correlation between suppressiveness in the pot test with soils from fields where take-all decline had occurred is good evidence that the suppressiveness measured in the pot test is, indeed, the suppressiveness associated with take-all decline. This bioassay has since been reduced to an even easier "tube test" involving only 10 g of test soil amended with inoculum and sandwiched between layers of vermiculite in a narrow plastic tube, with one or two wheat plants per tube[56].

b. Evidence that the suppressiveness is biological usually can be obtained by the use of heat or chemical treatments to eliminate groups of organisms. In the case of the specific suppression, Gerlagh [21] with his test, and Shipton et al[46] with their pot bioassay, both showed that suppressiveness was eliminated by moist heat-treatment at 60°C/30 min. Cook and Rovira[15] showed that methylbromide and chloropicrin also eliminated the factor(s) responsible for specific suppression. The sensitivity of suppressiveness to 60°C/30 min rules out the heat-resistent, spore-forming bacteria and probably also actinomycetes[6], but leaves in contention all fungi and nonsporing bacteria as candidates to explain the effect.

c. Transferability of suppression can provide useful information on the biological nature of the phenomonon and also help reveal whether conducive soils can be made suppressive on a practical scale. The first demonstration of the transferability of specific suppression was by Shipton et al[46,6] and was done in the field. Soil from six fields in eastern

Washington was moved to a site in western Washington where it was mixed about 15 cm deep into replicate plots. The actual amount of soil incorporated was estimated at only about 0.5% by weight. Wheat seed mixed 50:50 with oat-grain inoculum of *G. graminis* var. *tritici* was then drilled into the entire plot site. Take-all was uniformly severe in all plots the first year, but was almost totally controlled by one introduced soil in the second sowing. The one effective soil was from a field near Quincy, Washington, that had been in irrigated wheat for 12 consecutive years. None of the other five soils initiated suppressiveness by the second sowing but with the third sowing, take-all decline had occurred over the entire site. This is possibly the first successful transfer of a principle responsible for a suppressive soil. The factor(s) transfered with the soil from Quincy obviously multiplied in response to the host and pathogen.

d. The site of action of suppressiveness, if known, can reveal useful information on the mechanism of action. Wilkinson et al[57] found that neither the minimum (threshold) size of infested particles nor the number of infections per unit mass of particles was affected by the microbial factor(s) responsible for specific suppression. This finding held for both natural (stembases) and artifical (axenically colonized oat grains) sources of inoculum. They showed further[58] that the estimated distance the fungus could grow from particles of infested oat grains to infect the root was the same for suppressive (specific) and conducive soils, and that the values increased about the same amount when the respective soils were pasteurized. Estimated distances were affected by microorganisms but apparently not by *the microorganisms* responsible for take-all decline. These data support an earlier demonstration in the field[11] that specific suppression operates to suppress disease progress after infection, but does not affect prepenetration activities of the pathogen. The only opportunity for the specific suppression to affect the pathogen during its saprophytic existence or prepenetration activities is when the responsible microorganisms carry over in the inoculum particles with *G. graminis* var. *tritici*[55]. This point is discussed in more detail below.

e. Persistence of the pathogen in a virulent form on the host in suppressive soil is evidence that the factor (s) in suppressive soil must be external to and act on the pathogen rather than the result of some genetic or physiological change within the fungus itself. Asher[4], in a survey of wheat fields in England, and Cook and Naiki[14], in a survey of wheat fields in the Pacific Northwest, USA, found no evidence that virulence in the pathogen population was significantly different before than after take-all decilne. In the work of Cook and Naiki[14], the pathogen was recovered from 50-100% of random plants in fields after take-all decline and 90-95% of the isolates were virulent, but still disease was mild or nonexistent.

Our evidence continues to indicate that take-all decline does not occur until take-all has occurred. Thereafter, the pathogen persists in a virulent form but is suppressed. At the western Washington plot site, take-all decline occurred in all plots by the third year, which was after

two outbreaks of severe disease. The advantage of the introduced from Quincy soil was to initiate decline already in the second crop. All of this points to a fairly specific microorganism or group of microorganisms responsible for a natural biological control of the wheat take-all fungus.

ALTERNATIVE EXPLAINATIONS FOR TAKE-ALL DECLINE

There are many forms of antagonism of the wheat take-all fungus in soil, the rhizosphere, and within roots, including destruction of hyphae by amoebae[9,25] cross protection by the avirulent or weakly virulent *G. graminis* var. *graminis* and *phialophora graminicola*[16,60] and the inhibitory effects of root-colonizing fluorescent pseudomonads[15,47,48,59]. In addition, *G. graminis* var. *tritici* is commonly infected with dsRNA mycovirus-like agents[19] and workers in France have proposed that transmission and spread of mycoviruses within the population of the pathogen causes a progressive loss in virulence within the population and hence, take-all decline.[33] Probably each of these microbiological phenomonon, and others not yet identified, affect the take-all fungus to one degree or another, but the question remaining is: which, if any, cause take-all decline[27]?

The factor(s) responsible for take-all decline must fit the following characteristics based on information presented in the previous section.

a. Multiply when introduced as a small quantity of soil into a large volume of soil (e. g. 1:100 or 1:200 dilution in soil) and wheat then grown with the take-all fungus present.
b. Operate on the root or in the lesion to suppress disease progress after infection.
c. Act with sufficient speed to suppress symptom expression on seedlings already during the first 4 weeks after planting if in a greenhouse pot [15], or retard disease spread into the crown and from seminal to coronal roots if in the field[11].
d. Be sensitive to moist heat-treatment at 60°C/30 min.
e. Permit the pathogen to remain virulent and to complete its life cycle on roots of wheat without causing serious damage to the plant.

Amoebae are too slow and inefficient to intercept the pathogen on roots and in lesions and to significantly retard disease development already within the first 2-3 weeks after sowning in soil heavily infested with the pathogen. The giant vampyrellid amoebae are active only if the soil is drained (drier than −0.01 to −0.05 matric potential) but not drier than −0.2 to −0.25 bars.[24] Even soil at "field capacity" (about −0.3 bars) is too dry for amoeboid activity. Finally, amoebae are most active in soil at pH 6.5 to 7.5[24], yet this pH range is where take-all is most severe. If amoebae are responsible for take-all suppression, then expectedly there should be less disease where amoeboid activity is greatest. The major effect of amoebae is probably as a predator on hyphae during saprophytic survival of the fungus[9], rather than an interceptor of the fungus during its parasitic activity.

Cross protection by *P. graminicola* cannot explain take-all decline on the basis that populations of this antagonist tend to decline rather than increase

during wheat monoculture.[16] This fungus is widely distributed in England in grass swards, where it exists as an inhabitant and weak parasite of grass roots under cool conditions. *P. graminicola* colonizes the roots of the first wheat crop sown after a grass sward, but with successive crops of wheat, its population declines while that of *G. graminis* var. *tritici* increases. The take-all suppression by *P. graminicola* is therefore less effective with continuing monoculture, a pattern very different from take-all decline.

Hypovirulence caused by dsRNA mycovirus infections cannot explain take-all decline on the basis of two studies, both which showed that the pathogen population is still 90-100% as virulent isolates after take-all decline[4,14]. This does not mean that hypovirulence and dsRNA mycovirus infections cannot co-ntrol take-all if properly understood and exploited. Besides hypovirulence, the fungus may also convert from virulent to avirulent[38], possibly because of a self inhibitor[39] described by Romanos et al [41]. These genetic and physiological mechanisms deserve more study as a means to biocontrol of the take-all fungus, but they are seemingly unrelated to the microbiological factor(s) that act on the fungus to bring about take-all decline.

The only form of antagonism that can explain take-all decline is the inhibition of the wheat take-all fungus on roots and in lesions by the fluorescent pseudomonads. These ubiquitious bacteria are sensitive to 60°C/30 min and are common colonists of plant roots and soil surrounding plant roots. They also produce many antibiotics as well as siderophores[31] and have been shown to colonize roots and inhibit plant pathogens [32,50]. In addition to this circumstantial evidence, there is now direct evidence for their role in take-all decline.

The first evidence that certain strains of *Pseudomonas fluorescens* were involved in take-all decline was obtained by Cook and Rovira[15]. They used the pot assay of Shipton et al[46] but substituted soil cultures of individual strains of bacteria for the field soil added to the fumigated soil. Two hundred strains of bacteria were tested; 100 came from a collection maintained by A. D. Rovira and 100 came from roots of wheat from earlier pot assays with conducive and suppressive soils. Only eight of the 200 soil cultures diluted 1:100 with fumigated soil gave suppression of *G. graminis* var. *tritici* that matched the characteristics of specific suppression as revealed in the pot assay, and of the eight, all were from the group isolated originally from roots of wheat growing in a 1:100 dilution of suppressive soil. Of these eight, seven were fluorescent *Pseudomonas* sp.

Weller[13] isolated bacteria from roots of a second sowing of 4-week-old wheat seedlings grown in conducive and suppressive soils diluted 1:10 with fumigated soil and amended with oat-grain inoculum of *G. graminis* var. *tritici*. Different strains of bacteria seemed to dominate different roots, but the predominant and commonly only bacterial type at the 10^{-4} and 10^{-5} dilutions of macerated roots was fluorescent *Pseudomonas* sp. The total number of fluorescent *Pseudomonas* sp. were about 1 log greater (e. g. 10^5 vs 10^4) on roots of seedlings growing in suppressive compared to those on roots of wheat growing in conducive soils.

Equally significant, 30-55% of those from roots growing in suppressive soil were inhibitory compared with only 10-20% of those from roots growing in conducive soils[13]. Since the total number of fluorescent pseudmonads was about 10 times greater and the number inhibitory was at least two times greater, Weller's data indicated that the total number of inhibitory bacteria could be as much as 20 times greater on the roots in suppressive than on roots in conducive soil.

In another study, random isolates of *P. fluorescens* from wheat roots were tested both in vitro, for antibiotic activity against the wheat take-all fungus on agar media, and in vivo, for ability to suppress take-all in the tube test when applied as a seed treatment[54]. Isolates most inhibitory in vitro were the most suppressive to take-all in vivo, and those least inhibitory or noninhibitory in vivo were the least suppressive or nonsuppressive in vivo. Again, a significantly higher ($P=0.05$) frequency of inhibitory (and take-all suppressive) isolates of *P. fluorescens* were obtained from roots growning in the suppressive soil compared to conducive soil[54]. All of these data indicate a qualitative as well as quantitative shift in the population of fluorescent pseudomonads toward both a higher total number and especially a higher number antibiotic to *G. graminis* var. *tritici*.

Wong and Baker[59] concluded from studies with isolates of pseudomonads added to soil at about 10^7 cfu/g that inhibition by antiboitic production in vitro was not a reliable indicator of ability to suppress take-all in soil. We have also found isolates of fluorescent pseudomonads that suppress take-all in vivo but not in vitro, and vice versa. However, it cannot be concluded on the basis of such results that antibiotic production is not an important trait to strains inhibitory both in vitro and in vivo; our results continue to indicate that of strains with antibiotic-producing ability and also effective in vivo are dependent for their effectiveness in vivo on this antibiotic-producing ability.

A MODEL TO EXPLAIN TAKE-ALL DECLINE

Wilkinson et al[55] performed a highly significant experiment that makes it possible to propose more precisely how take-all decline comes about. Inhibitory-positive (wild-type) and -negative (mutant) strains of *P. fluorescens* were applied as a cell suspension in each of three ways: to natural soil at abouut 10^6 cfu/g soil, as a seed treatment at about 10^7 cfu/seed, and infiltrated into particles of the oat grain inoculum used to infest the soil. The bacteria added as cells to the soil were virtually without effect on take-all. In contrast, significant suppression occurred with the bacteria as a seed treatment and greatest suppression occurred with the antagonist infiltrated into the inoculum particles. In all cases, however, no disease suppression occurred with the inhibitory-negative isolates, even if infiltrated into the inoculum particles. These results confirm the importance of antibiotic-producing ability of the bacteria, but suggest further that the greatest inhibitory effect of these bacteria occurs when they exist as cohabitants with the pathogen in the inoculum fragments.

Following a break of one or more years with no wheat or barley, most

inoculum of *G. graminis* var. *tritici* will have displaced in the crop residue fragments by saprophytic microorganisms. However, a few fragments with viable hyphae of the pathogen will still be present and available to infect roots of the next crop. Since isolates that are highly virulent also survive the longest time[10], the inoculum available after a break is likely to be the most virulent of the original population carried over from the last wheat crop[38]. The most successful infections are also likely to originate from inoculum in crop residue lacking inhibitory isolates of *P. fluorescens*. Since inhibitory fluorescent pseudomonads scattered in soil are also unlikely to protect roots against the fungus, even if at 10^6 cfu/g[55], infections of the first crop after a break, while rare, are likely to be severe. These infected plants will then serve as a larger inoculum source for the next crop.

With the second sowing, more plants will be infected, and infections are again likely to be severe. However, fluorescent pseudomonads are also likely to be establishing in higher populations with the second and subsequent sowings, as secondary colonists of the "leaky" lesions caused by the take-all fungus. Of all secondary colonists, fluorescent pseudomonads seem especially attracted to the lesions. A shift toward a higher frequency of antibiotic types might be explained on the basis that inter and intra species competition among microorganisms in the nutrient-rich lesion may be so intense that gradually the most inhibitory types win out. These types then become cohabitants with the take-all fungus in the crop residue where they are especially effective and also then poised to re-establish and multiply in the next lesion caused when hyphae of *G. graminis* var. *tritici* emerge from the reside in response to roots of the next crop. This cycle is then repeated until an equilibrium is reached between inhibition of *G. graminis* var. *tritici* on the one hand, but enough-infected tissue to support the inhibitory bacteria, on the other hand.

This model can account for the quanitative as well as qualitative shift in population of root-colonizing fluorescent pseudomonads and also why virulent *G. graminis* var *tritici* can be recovered from roots of up to 100% of wheat plants in fields after take-all decline. It can also account for the observation that severe take-all usually must occur before take-all decline will occur; the more severe the infection. the more abundant the sites and nutrients for multiplication of bacteria and the greater the selection pressure in favor of inhibitory bacteria. However, the best direct evidence to support this model is the finding of Weller[13,54] and also W. Howie, (unpublished), that marked strains of *Pseudomonas fluorescens* inhibitory to *G. graminis* var. *tritici*, when monitored as root colonists following their introduction on seeds, achieve the highest populations and persist at the higher populations longer if *G. graminis* var. *tritici* is also on the roots. The bacteria are highly favored by the presence of the wheat take-all fungus on wheat roots, possibly because they respond to nutrients liberated from lesions. Work is now progressing to establish more conclusively the validity of this model.

APPLICATION OF ROOT-COLONIZING

FLUORESCENT PSEUDOMONADS FOR TAKE-ALL SUPPRESSION

The transfer of whole untreated soil from field to field is obviously impractical on a commercial basis. Even a seemingly small amount of soil, e. g. 0.5% by weight, would amount to over 800 mt of soil/ha. The logistical problems of mass rearing antagonistic fluorescent pseudomonads in soil culture and applying this would be even more insurmountable, even if the soil inoculum was applied in the seed row only. The weight of soil would be several times the weight of any dry fertilizer now applied with and beneath the seed by commercial drills. The most practical approach would be a seed treatment.

When the strains of fluorescent pseudomonads inhibitory to *G. graminis* var. *tritici* in vitro were applied to wheat seed and the seed planted in plots together with oat-grain inoculum of the pathogen, the degree of take-all suppression was highly significant[53]. The introduced strains colonize the roots and compete well with native rhizosphere colonists[52]. In some trials, a mixture of strains was more suppressive than a single strain. The treatment is now under test in the field for the seventh consecutive year, including in commercial fields seeded by growers. Field tests are conducted only where take-all is the main yield-limiting factor. Yield increases of 10-25% (significant at $P=0.05$, sometimes at $P=0.01$) have been obtained in approximately two trials out of every three. Unfortunately, the treatment has failed about 1/3 of the time which indicates that either soil conditions are not always satisfactory for root colonization by the introduced strain(s) or that improved strains are needed. Nevertheless, the trials are encouraging and the treatment is now under consideration for commercial application.

The potential for seed bacterization as an approach to biological control of root pathogens is also indicated by recent accomplishments of other investigators with other antagonistic strains of bacteria. A significant breakthrough was by Broadbent et al[7], who's *Bacillus subtilus* strain A13, antagonistic in vitro to all nine test fungi, was shown to increase tillering, accelerate maturity, and increase yields of wheat, barley, and oats when applied as a seed treatment[36]. This same strain as a seed treatment also increased yields of sweet corn and carrots[36]. More recently, Burr et al[6] and Kloepper et al[31] showed that strains of fluorescent pseudomonads isolated originally from roots or tubers of potatoes and inhibitory in vitro to a wide spectrum of bacterial and fungal pathogens increased growth and yield of potatoes in the field in California when applied on the potato seed pieces. The same or similar strains also increased yield and sugar content of sugar beets when applied as a beet-seed treatment[50]. Biological seed treatments have the potential to become a practical method by which plants are protected from root pathogens. All evidence indicates that the bacteria grow or are carried downward with the roots and that they protect roots against pathogens by their competitive and inhibitory ability.

A unique feature of our method is that we isolate the antagonists from roots of wheat growing in soil suppressive

to *G. graminis* var. *tritici*. Effective antagonists can be isolated from roots growing in conducive soil, but the odds of obtaining effective strains is much better (possibly several times better) if the soil is from a field that has undergone take-all decline. In addition, we target our selection process for strains with ability to inhibit the take-all pathogen. Any increased growth response of wheat to the presence of one or a mixture of our strains is the result of suppression of the take-all fungus and not a stimulation of the plant[53].

SUPPRESSIVENESS OF SOILS TO G. GRAMINIS VAR TRITICI NOT RESPONSIBLE FOR TAKE-ALL DECLINE

Gerlagh[21] was the first to distinguish between the suppressiveness of soils that developed in response to severe take-all and the suppressiveness to this fungus characteristic of all soils regardless of their cropping history. He referred to the latter type as *general suppression*. All soils are suppressive to one degree or another to all soilborne pathogens[13] and *G. graminis* var. *tritici* is no exception. The fact that soils become progressively more suppressive with increasing soil temperatures up to 25-30°C[23] may be an example of an enhancement by the higher temperatures of Gerlagh's general suppression[15].

Alabouvette et al[1] and Hwang et al[28] demonstrated that the suppressiveness of certain soils to Fusarium wilt pathogens relates, in part, to greater competition for nutrients between the *Fusarium* and soil microorganisms. More nutrients as glucose was needed for chlamydospore germination in the wilt-suppressive than in the wilt-conducive soil. The possibility that general suppression of soils to the wheat take-all fungus is related to more intense competition for nutrients during prepenetration growth[13] was therefore tested (Alabouvette and Cook, unpublished). The amound of microbial biomass in the soil and available to respond to an introduced source of carbon and energy (such as contained in root exudates) was estimated by both the Jenkinson[29] and Anderson and Domsch[3] methods. Each soil was infested, respectively, with five different sizes of oat-grain particles between 0.25 μm (threshhold) and 1.0 mm in diameter and at concentrations of 1-10 mg/g soil. The number of lesions per plant was counted on 4-week-old seedlings and the number of lesions per unit mass of inoculum (infection efficiency) was then calculated. It was expected that the greater the total microbial biomass available to compete with *G. graminis* var. *tritici* for nutrients (exudates in the rhizosphere), the fewer the number of infections per unit mass of inoculum.

As found by Wilkinson et al[57], the infection efficiency of any given particle size varied greatly among soils but was not be related to whether or not the soil came from fields where take-all had declined. On the other hand, without exception, the infection efficiency was significantly greater in pasteurized than in the same soil used without treatment, suggesting that microorganisms suppressive to *G. graminis* var. *tritici* occurred in all soils. However, there was also little or no relationship between infection efficiency of inoculum in any given soil and the total microbial biomass of the soil as measured by either of the Jenkinson[29] or Anderson and Domsch[3]

methods. For example, the values for infection efficiency were lowest in a noncropped (virgin) Ritzville silt loam having only about 1% organic matter content and registering a very low total microbial biomass, and were highest in an organic garden soil with a very high total microbial biomass, Other soils registering a relatively low microbial biomass supported a relatively high infection efficiency, and vice versa. The addition of increasing rates of inorganic N up to 100μg N/g soil had no significant effect on the values in any soil. Obviously, some mechanisms other than competition for exogenous nutrients from total microbial biomass active during prepenetration growth of *G. graminis* var. *tritici* must be operative.

A MODEL TO EXPLAIN GENERAL SUPPRESSION

Soilborne plant pathogens depend for their carbon and energy supply during prepenetration in the rhizosphere on both their endogenous reserves (within the propagules) and exogenous nutrients (supplied as root exudates). If their main carbon and energy supply is exogenous, then competition from the total microbial biomass active in the rhizosphere should be most important. On the other hand, if the main carbon and energy supply is endogenous, then microbial activity in and around the propagule itself should be most important source of competition. Wilkinson et al[58] concluded that for *G. graminis* var. *tritici*, the larger the inoculum particle the less dependent the fungus on exogenous nutrient supplies and the more it operates on nutrients available in its foodbase. This raises the possibility that general suppression of *G. graminis* var. *tritici* is the result of colonists of the foodbase of *G. graminis* var. *tritici* rather than competitors for the root exudates. Lin and Cook[34] have already demonstrated this as the mechanism of suppression of *Fusarium avenaceum*. *Trichoderma*, *Mucor*, and *Penicillium* species were shown to colonize the foodbase of this pathogen and thereby keep it from infecting lentils. These and other fungal colonists of dead plant remains are cosmopolitan in soils, which can account for the general suppression of all soils to *G. graminis* var. *tritici*. Moreover, these colonists would be eliminated by 60°C moist heat treatment of soil, which can account for why the infection efficiency of inoculum particles is greater in all soils when pasteurized compared to when tested nontreated. The different degrees of general suppression in the different soils may be the result of differing amounts of activity of these fungi in the soils, determined by many biotic and abiotic factors.

Chlamydospores of *F. oxysporum* arc only about 10 μm in diameter and probably supply only a small portion of the total nutrient used by the fungus for germination and growth prior to infection of the root. This may explain why the suppressivenss of soils to pathotypes of this fungus can be overcome by adding more nutrients to the soil[1,28]. The soil microbiota acts as a sink for carbon and energy released as root exudates and the larger and more responsive microbiota, the greater the sink and hence the greater the suppressiveness of the soil to *F. oxysporum*. In contrast, a pathogen such as *Sclerotium rolfsii*, having propagules 1-2 mm in diameter, presumably

would be more vulnerable to competition for its endogenous reserves by colonists of its sclerotia than to the magnitude of the sink for nutrients released a exudates. Substances released from roots may serve to initiate sclerotial germination and may help determine the direction of hyphal growth, e. g. toward the root, but would not be a significant component of the total nutrient supply used by the fungus to reach and infect the root. Probably the inoculum particles of *G. graminis* var. *tritici* are more like the sclerotia of *S. rolfsii* than the chlamydospores of *F. oxysporum*. The nutrient supply in the fragments of crop residue occupied by the fungus is in essence, an endogenous supply for this fungus. It is therefore not surprising that Alabouvette and Cook (unpublished) found no relationship between total microbial biomass of the soil and suppressiveness of the soil to this fungus.

SECONDARY COLONISTS: THE COMMON FACTOR IN THE EXPLAINATIONS OFFERED FOR SPECIFIC AND GENERAL SUPPRESSION

G. graminis var. *tritici* is vulnerable during both its parasitic and saprophytic existence to the competitive and inhibitory effects of secondary colonists. Many bacteria and fungi can be readily demonstrated to occur in lesions caused by this pathogen on wheat and barley. Indeed, they interfere significantly with isolation of the pathogen from infected tissues onto agar media. The older the lesion, the more difficult it is to recover the pathogen, even from infected tissues that are surface sterilized. Secondary colonists continue to pose a problem as competitors of the take-all fungus during its saprophytic survival.

Specific and general suppression both are thought to result from inhibition of *G. graminis* var. *tritici* secondary colonists of the substrate occupied by the pathogen. The main difference between the two types is as follows: General suppression is thought to result from competition from many microorganisms for nutrients in the foodbase, including from saprophytic colonists of the crop residue occupied by the pathogen, but probably also from competitors established already as secondary colonists of the lesion. General suppression could even result from a succession of colonists of the inoculum particles beginning during the parasitic phase and continuing during the saprophytic existence of the fungus. Specific suppression functions against a background of general suppression, i. e. in addition to the competitive effects of a succession of colonists of the substrate of *G. graminis* var. *tritici*, antibiotic-producing fluorescent pseudomonads in particular are thought to become increasingly more dominant, in the lesion first but then carried over as cohabitants with the pathogen in the foodbase. Rather than suppression by competition, specific suppression is thought to result from suppression by antibiosis, and to result from bacterial antagonists rather than fungi.

Obviously, much more work remains to be done to validate or dismiss these explanations for the two kinds of suppression of the wheat take-all fungus in natural soils. Whether we know the mechanisms or not, work can proceed on the use of pseudomonads as a seed treatment to protect wheat against take-

all, and also to find ways besides tillage to encourage secondary colonists of the foodbase used by this fungus for survival and infection. Work should also proceed on the genetic mechanisms of avirulence, hypovirulence, and self inhibition, on the chance that these also can be exploited for biocontrol purposes. Cross protection by *P. graminicola* is still another possibility; it is already working in certain rotations in England and might work elsewhere if the proper strains and methods for their introduction could be found[60]. Successful biological control of the wheat take-all fungus will ultimately depend on several approaches and not just a single approach.

LITERATURE CITED

1. Alabouvette, C., F. Rouxel, and J. Louvet. 1980. Recherches sur la résistance des sols aux maladies VII. Etude comparative de la germination des chlamydospores de *Fusarium oxysporum* et *Fusarium solani* au contact de sols résistant et sensible aux fusarioses vasculaires. Ann. Phytopathol. 12:21-30.
2. Anagnostakis, S. L. 1982. Biological control of chestnut blight. Science 215:466-471.
3. Anderson, J. P. E. and K. H. Domsch. 1973. Quantification of bacterial and fungal contributions to soil respiration. Arch. Mikrobiol. 93:113-127.
4. Asher, M. J. C. 1980. Variation in pathogenicity and cultural characteristics in *Gaeumannomyces graminis* var. *tritici*. Trans. Brit. Mycol. Soc. 75:213-220.
5. Asher, M. J. C. and P. J. Shipton, eds. 1981. Biology and Control of Take-All. Academic Press, New York. 538 pp.
6. Baker, K. F. and R. J. Cook. 1974 (original ed.). Biological Control of Plant Pathogens. W. H. Freeman, San Francisco. Reprinted ed., 1982. Amer. Phytopathol. Soc., St. Paul, MN. 433 pp.
7. Broadbent, P., K. F. Baker, and Y. Waterworth. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. Austral. Jour. Biol. Sci. 24:925-944.
8. Burr, T. J., M. N. Schroth, and T. Suslow. 1978. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68:1377-1383.
9. Chakraborty, S. and J. H. Warcup. 1983. Soil amoebae and saprophytic survival of *Gaeumannomyces graminis tritci* in a suppressive pasture soil. Soil Biol. Biochem. 15:181-185.
10. Chambers, S. C. and N. T. Flentje. 1967. Studies on variation with *Ophiobolus graminis*. Aust. J. Biol. Sci. 20:941-951.
11. Cook, R. J. 1981. The influence of rotation crops on take-all decline phenomenon. Phytopathology 71:189-192.
12. Cook, R. J. 1985. Biological control of plant pathogens: theory to application. Pytopathology 75: 25-29.
13. Cook, R. J. and K. F. Baker. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN. 539 pp.
14. Cook, R. J. and T. Naiki. 1982. Virulence of *Gaeumannomyces gra-*

minis var. *tritici* from fields under short-term and long-term wheat cultivation in the Pacific Northwest, USA. Plant Pathology 31:201-207.

15. Cook, R. J. and A. D. Rovira. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. Soil Biol. Biochem. 8:267-273.
16. Deacon, J. W. 1976. Biological control of the take-all fungus, *Gaeumannomyces graminis* by *Phialophora radicicola* and similar fungi. Soil Biol. Biochem. 8:275-283.
17. Deacon, J.W. 1984. Managing disease, a book review of The Nature and Practice of Biological Control of Plant Pathogens, R. James Cook and K. F. Baker. Nature 309:732.
18. Fellows, H. and C. H. Ficke. 1934. Wheat take-all. Kans. Agr. Exp. Sta. Annu. Rep. 1932-34:95-96.
19. Frick, L. J. and R. M. Lister. 1978. Serotype variability in virus-like particles from *Gaeumannomyces graminis*. Virology 85:504-517.
20. Garrett, S. D. 1956. Biology of Root-infecting Fungi. Cambridge Univ. Press, London 294 pp.
21. Gerlagh, M. 1968. Introduction of *Ophiobolus graminis* into new polders and its decline. Neth. Jour. Plant Pathol. 74:(Suppl. 2):1-97.
22. Glynne, M. D. 1935. Incidence of take-all on wheat and barley on experimental plots at Woburn. Ann. Appl Biol. 22:225-235.
23. Henry, A. W. 1932. Influence of soil temperature and soil sterilization on the reaction of wheat seedlings to *Ophiobolus graminis* Sacc. Can. Jour. Res. 7:198-203.
24. Homma, Y. and R. J. Cook. 1985. Influence of matric and osmotic water potentials and soil pH on the activity of giant vampyrellid amoebae. Phytopathology 75: 243-246.
25. Homma, Y., J. W. Sitton, R. J. Cook, and K. M. Old. 1979. Perforation and destruction of pigmented hyphae of *Gaeumannomyces graminis* by vampyrellid amoebae from Pacific Northwest wheat field soils. Phytopathology 69:1118-1122.
26. Hornby, D. 1969. Quantitative estimation of soil-borne inoculum of the take-all fungus. [*Ophiobolus graminis* (Sacc.)]. Proc. 5th Br. Insectic Fungus Conf. Brighton 1:65-70.
27. Hornby, D. 1979. Take-all decline: A theorist's paradise. Pages 133-156 *in* B. Schippers and W. Gams, eds. Soil-borne Plant Pathogens. Academic Press, London. 686 pp.
28. Hwang, S. F.. R. J. Cook, and W. A. Haglund. 1982. Mechanisms of suppression of chlamydospore germination of *Fusarium oxysporum* f. sp. *pisi* in soils. Phytopathology 72:948.
29. Jenkinson, D. S. 1966. Studies on the decomposition of plant material in soil. II. Partial sterilization of soil and the soil biomass. Jour. Soil Sci. 17:280-302.
30. King, C. J., C. Hope, and E.D. Eaton. 1934. Some microbiological activities affected in manurial control of cotton root rot. Jour. Agric. Res. 49:1093-1107.
31. Kloepper, J.W., J. Leong, M. Teintze, and M. N. Schroth. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885-886.

32. Kloepper, J. W., M. N. Schroth, and T. D. Miller. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078-1082.
33. Lemaire, J. M., H. Lapierre, B. Jouan, and G. Bertrand. 1970. Découverte de particules virales chez certaines souches d'*Ophiobolus graminis*, agent du piétin-échaudage des céréales: Consequences agronomizue prévisibles. C. R. Hebd. Séanc. Acad. Agric. Fr. 56:1134-1138.
34. Lin, Y. S. and R. J. Cook. 1979. Suppression of *Fusarium roseum* 'Avenaceum' by soil microorganisms. Phytopathology 69:384-388.
35. Lupton, F. G. H. 1984. Biological control: the plant breeder's objective. Ann. Appl. Biol. 104:1-16.
36. Merriman, P. R., R. D. Price, K. F. Baker, J. F. Kollmorgen, T. Piggott, and E. H. Ridge. 1975. Effect of *Bacillus* and *Streptomyces* spp. applied to seed. Pages 130-133 *in* G. W. Bruehl, ed. Biology and Control of Soil-borne Plant Pathogens. Am. Phytopathol. Soc., St. Paul. 216 pp.
37. Moore, K. J. and R. J. Cook 1984. Increased take-all of wheat with direct drilling in the Pacific Northwest. Phytopathology 74:1044-1049.
38. Naiki, T., and R. J. Cook. 1983. Factors in loss of pathogenicity in *Gaeumannomyces graminis* var. *tritici*. Phytopathology 73:1652-1656.
39. Naiki, T., and R. J. Cook. 1983. Relationship between self-inhibition and inability of isolates of *Gaeumannomyces graminis* var. *tritici* to cause disease. Phytopathology 73:1657-1660.
40. Papavizas, G. C., and R. D. Lumsden. 1980. Biological control of soilborne fungal propagules. Annu. Rev. Phytopathol. 18:389-413.
41. Romanos, M. A., C. J. Rawlinson, M. R. Almond, and K. W. Buck. 1980. Production of fungal growth inhibitors by isolates of *Gaeumannomyces graminis* var. *tritici*. Trans. Brit. Mycol. Soc. 74:79-88.
42. Sanford, G. B. 1926. Some factors affecting the pathogenicity of *Actinomyces scabies*. Phytopathology 16:525-547.
43. Sanford, G. B. and W. C. Braodfoot. 1931. A note on the biological control of root rots of cereals. Studies of the effects of other soil-inhabiting microorganisms on the virulence of *Ophiobolus graminis* Sacc. Sci. Agric. 11:460,512-528.
44. Shipton, P. J. 1972. Take-all in spring sown cereals under continuous cultivation; Disease progress and decline in relation to crop succession and nitrogen. Ann. Appl. Biol. 71:33-46.
45. Shipton, P. J. 1975. Take-all decline during cereal monoculture. Pages 137-144 *in* G.W. Bruehl, ed. Biology and Control of Soil-borne Plant Pathogens. Am. Phytopathol. Soc., St. Paul, MN. 216 pp.
46. Shipton, P. J., R. J. Cook, and J. W. Sitton. 1973. Occurrence and transfer of a biological factor in soil that suppresses take-all of wheat in eastern Washington. Phytopathology 63:511-517.
47. Smiley, R. W. 1978. Antagonists of *Gaeumannomyces graminis* from the rhizoplane of wheat in soils fertilized with ammonium- or nitrate-nitrogen.

Soil Biol. Biochem. 10:169-174.

48. Smiley, R. W. 1978. Colonization of wheat roots by *Gaeumannomyces graminis* inhibited by specific soils, microorganisms and ammonium-nitrogen. Soil. Biol. Biochem. 10:175-179.
49. Stover, R.H. 1979. Chapter 3. Flooding of soil for disease control. Pages 19-28 *in* D. Mulder, ed. Soil Disinfestation. Elsevier Scientific Publishing Company. Amsterdam. 368 pp.
50. Suslow, T. V. and M. N. Schroth. 1982. Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathology 72:111-115.
51. Taylor, J. B. and E. M. Guy. 1981. Biological control of root-infecting basidiomycetes by species of *Bacillus* and *Clostridium*. New Phytol. 87:729-732.
52. Weller, D. M. 1984. Distribution of a take-all suppressive strain of *Pseudomonas fluorescens* on seminal roots of winter wheat. Appl. Environ. Microbiol. 48:897-899.
53. Weller, D. M. and R. J. Cook. 1983. Suppression of take-all of wheat by seed-treatment with fluorescent pseudomonads. Phytopathology 73:463-469.
54. Weller, D. M., B. X. Zhang, and R. J. Cook. 1985. The application of a rapid screening test for selection of take-all suppressive bacteria. Plant Disease 69: In Press.
55. Wilkinson, H. T., D. M. Weller, and J. R. Alldredge. 1982. Enhanced biocontrol of wheat take-all when inhibitory *Pseudomonas* strains are introduced in inoculum or seed as opposed to directly into soil. Phytopathology 72:948-949.
56. Wilkinson, H. T., R. J. Cook, and J. R. Alldredge. 1985a. Relation of inoculum size and concentration to infection of wheat roots by *Gaeumannomyces graminis* var. *tritici*. Phytopathology 75:98-103.
57. Wilkinson, H. T., R. J. Cook, and J. R. Alldredge. 1985. Evidence that microorganisms in suppressive soil associated with wheat take-all decline do not limit the number of lesions produced by *Gaeumannomyces graminis* var. *tritici*. Phytopathology 75: In Press.
58. Wilkinson, H. T., J. R. Alldredge, and R. J. Cook. 1985. Estimated distances for infection of wheat roots by *Gaeumannomyces graminis* var. *tritici*. Phytopathology 75:557-559.
59. Wong, P. T. W. and R. Baker. 1984. Suppression of wheat take-all and Ophiobolus patch by fluorescent pseudomonads from a Fusarium-suppressive soil. Soil Biol. Biochem. 16:397-403.
60. Wong, P. T. W. and R. J. Southwell. 1979. Biological control of take-all in the field using *Gaeumannomyces graminis* var. *graminis* and related fungi. Pages 597-602 *in* B. Schippers and W. Gams, eds. Soil-borne Plant Pathogens. Academic Press. London. 686 pp.