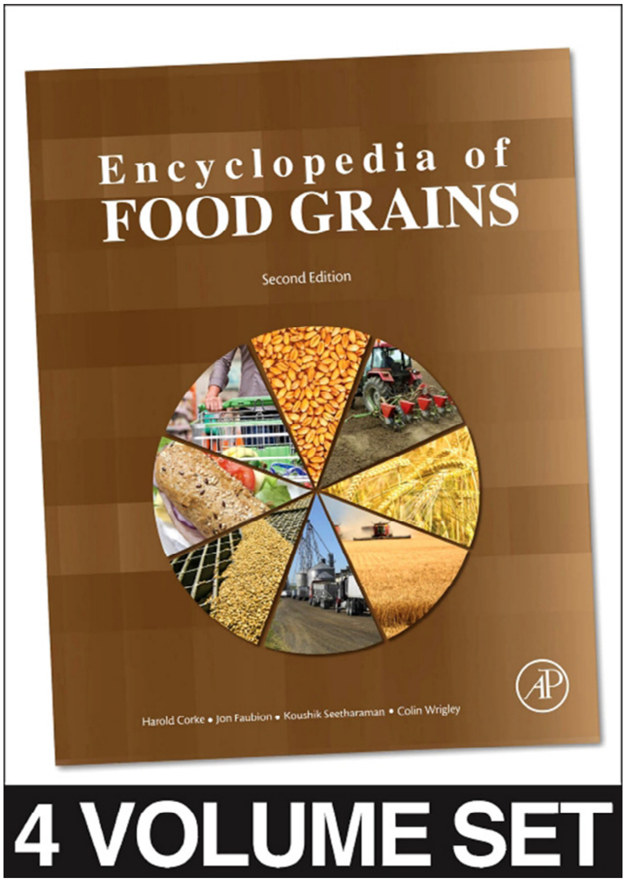

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Natural Disease Control in Cereal Grains

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Topic Highlights

- Microbial diseases cause major losses to grain yields and negatively impact human health and ecosystems.
- Biocontrol is the use of living organisms to suppress crop disease.
- Competition-based biocontrol outcompetes pathogens for nutrients and/or space.
- Antibiosis involves the production of a substance that targets and destroys a pathogen.
- A biocontrol agent may induce natural defense mechanisms in host plants.
- A soil may suppress crop disease due to the specific structure of its microbial community.
- Hyperparasitism is a situation in which there is specific recognition between the antagonist and the pathogen that terminates in pathogen death.
- Transgenic plants can contain introduced genes to combat pathogens as a strategy of biocontrol.

Learning Objective

- To understand different strategies underlying biological control of grain crop disease, with specific examples provided for each strategy.

Introduction

The expected dramatic increase in human population and shortages in food supply make it a necessity to explore new strategies to improve crop yields and effectively manage crop diseases with more natural, environmental-friendly strategies. Given the nutritional and economic importance of grains, microbial diseases are a real danger to global food security. Approximately 10% of global food production is lost due to plant pathogens, contributing to more than 800 million people not having enough food. Plant pathogens may be difficult to identify and pathogens may evolve, which cause further challenges. Phytopathogens may result in a catastrophe as illustrated by the southern corn leaf blight epidemic of 1970–71 in the United States caused by the fungus *Cochliobolus heterostrophus*. Another example was the Great Bengal Famine of 1943 in India, caused by the fungus *Cochliobolus miyabeanus*, which resulted in the deaths of 2 million people due to their dependence on rice as the staple food. Today, the rice blast fungus, *Pyricularia oryzae*, causes 10–30% crop losses annually. Moreover, the host range of this fungus extends to include other cereals such as wheat and finger millet (*Eleusine coracana*) where the infection may result in complete crop loss.

Grain infection caused by toxin-producing fungal species, such as *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, and to a lesser extent *Pithomyces*, *Phomopsis*, and *Acremonium*, is serious, because they produce mycotoxins that may be consumed by animals including humans. For example, the rice and maize (corn) pathogenic fungus *Fusarium moniliforme* secretes the mycotoxin fumonisin B1, which has been linked to esophageal cancer, equine leukoencephalomalacia, and porcine pulmonary edema. *F. graminearum*, the disease-causing agent of head blight in barley and wheat and ear rot in maize, synthesizes cancer-causing trichothecenes such as deoxynivalenol (DON). *Aspergillus flavus*, the causal agent of kernel rot in maize, produces aflatoxin on preharvest maize and during storage. Together, aflatoxin and DON result in $1.5 billion in losses annually across different crops in the United States alone. There is extensive contamination of grains in the United States and Canada with trichothecenes, with a higher incidence reported in maize than wheat. *Fusarium* mycotoxins have been reported worldwide, particularly fumonisins on maize.

Overview of Disease Management Strategies

Crop diseases are managed by chemical, physical, cultural, and/or natural control methods including biological control. Chemical management involves the use of synthetic pesticides (fungicides). However, these chemicals may lack specificity and affect the beneficial soil microbiota; their indiscriminate use may result in negative impacts on soil ecosystems. Moreover, some chemical pesticides may remain in the soil and in crop tissues, potentially causing harmful effects to end users, either animals or humans. Physical methods to control crop disease include burning of infected crops, barriers that prevent insect vectors from gaining access to crops, and segregation of infected seeds from healthy seeds by grain weight (infected grains have less weight). Cultural methods include crop rotation (when a pathogen has a specific crop host), field sanitation (e.g., burning of infected crop residues), disinfecting of field tools and machinery, site selection, and use of seeds certified to be free of pathogens. Natural control strategies are numerous and include the selection and breeding for disease-resistant crop cultivars. For example, naturally resistant Chinese genotypes of wheat have been shown to have lower *Fusarium*-derived mycotoxin levels compared to more susceptible Canadian cultivars. An additional natural control strategy involves the use of intercropping 'companion crops' that attract or repel insect vectors of disease. A final major type of natural control is biological control or biocontrol that is defined by Eilenberg (2006) as "the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be." Biocontrol involves the use of soil- and

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plant-associated beneficial microbes, including bacteria and fungi. The increasing public interest in organic products strongly enhances the idea of biocontrol, since organic agriculture does not allow synthetic chemicals but does permit spraying with beneficial microbes.

Introduction to Soil- and Plant-Associated Microbes

The plant is an attractive host to a variety of beneficial soil microbes (bacteria and fungi). These microbes include those that live in the (1) rhizosphere (area surrounding the root and influenced by root secretions), (2) mycorrhizosphere (area surrounding root-associated mycorrhizal fungi), and (3) endosphere (internal plant tissues; these microbes are termed endophytes). There may be cross talk between the plant host, beneficial microbes, and pathogens. On the plant side, roots produce secretions such as sugars, amino acids, and organic acids that stimulate the microbial population including both antagonists and pathogens. The roots may also produce pathogen-stimulating factors, allelochemicals, and repellents that have their peak effects during vegetative development, with these effects decreasing with increased distance from the root system. On the microbial side, plant growth-promoting rhizobacteria (PGPR), plant growth-promoting fungi, nitrogen-fixing rhizobia, and mycorrhizal fungi play roles in nutrient cycling and also in biocontrol.

Overview of Biocontrol Mechanisms

Mechanistically, beneficial soil- and plant-associated microbes may control pathogens indirectly or directly. Indirect antipathogenesis occurs when microbes enhance plant health by making soil nutrients more available (e.g., solubilize rock phosphate) or fix nitrogen (convert atmospheric N_2 gas into NH_3 fertilizer). Direct antipathogenesis occurs when microbes combat pathogens by competition, antibiosis (production of antipathogen substances), induced systemic resistance (stimulation of plant defenses), soil suppression (enhanced populations of soil microbiota to suppress disease), hyperparasitism (use of microbes as parasites of the pathogen), and the use of microbial-derived genes with antipathogenesis activity inserted into transgenic crops (i.e., genetically modified organisms, GMOs). This article will focus on the direct mechanisms of biocontrol of grain disease.

Mechanisms of Biocontrol

Competition

To colonize plant tissues, pathogens require unblocked access and nutrients for energy during the infection process, such as from the plant surface. Plant surfaces are typically limited for nutrients originating from leachates, exudates, senescing tissues, soil, and waste products from surface insects. Competition-based biocontrol involves the use of fast-growing, antagonist organisms to compete with soilborne pathogens that share the same ecological niche, where nutrients and/or space is limited. The success of the biocontrol agent is determined by its ecological fitness among a soil microbial community that is already in equilibrium. Competition-based biocontrol is also affected by the physical/chemical properties of the soil. For example, the biocontrol fungal agent *Trichoderma* has its activity enhanced in moist soil and diminished in higher-pH soils. The use of nontarget fungicides also diminishes the competition ability of any fungal biocontrol agent.

An example of a biocontrol agent that competes for nutrients is the bacterium *Pseudomonas fluorescens* that secretes siderophores to chelate iron, decreasing its availability for many soilborne pathogens including the bacterium *Erwinia carotovora*. Another example of competition-based biocontrol is the use of nonantagonist soil bacteria including *Pseudomonas* sp., *Brevundimonas* sp., *Pedobacter* sp., and *Luteibacter* sp., which, when combined, were shown to reduce the biomass (ergosterol content) of the fungal pathogens *Rhizoctonia solani* and *F. culmorum* and the saprophytic fungus *Trichoderma harzianum*. The authors argued that the combined biocontrol species competed for nutrients or triggered the production of antimicrobial metabolites. Interestingly, nonpathogenic strains of *F. oxysporum* have the ability to control *F. oxysporum* strains, the causal agents of wilt, a disease that affects many crops including grains; the suppressive mechanism of action involves competition for carbon if the biocontrol inoculum is applied in excess to the pathogen.

Antagonists can also compete for space by occupying infection sites, thus preventing the pathogen from gaining access to the plant host. For example, the fungus *Phialophora graminicola* effectively controls the fungal pathogen *Gaeumannomyces graminis* var. *tritici* by occupying the infection sites of wheat root cortical tissue, resulting in reduced pathogen invasion. The protective *F. oxysporum* strain was isolated from the stems of healthy plants or from wilt disease-suppressive soil (see succeeding text) where it was present at an exceptionally high density. However, when the inoculum is low, *F. oxysporum* also retains some of its biocontrol activity; one possibility is that it may induce host resistance as an avirulent form of the pathogen (explained in the succeeding text). More recently, competition on root hairs and branches has been proposed to hinder the pathogen attachment and penetration.

The addition of animal manures is widely employed particularly in organic agriculture to provide soil with nutrition and also to enhance soil beneficial microbiota, which then compete against pathogens. For example, animal manures can combat many plant pathogens including *Gaeumannomyces graminis*, *Fusarium* sp., *Phymatotrichum omnivorum*, and *Sclerotinia sclerotiorum*.

Antibiosis

In addition to competition for space or nutrients, biocontrol agents may also actively produce destructive metabolites that target a pathogen. Antibiotics are toxins that prevent growth or kill microbes at low concentrations. Antibiosis is a major mechanism of biological control, in which the antagonist produces substance(s) that could be an antibiotic, lytic enzyme (degrades plant cell wall), volatile substance, or toxin that effectively targets and destroys the pathogen. In the succeeding text, examples are provided for each mechanism.

Antibiosis by production of antibiotics

Antibiotics from bacteria

The phenolic polyketide 2,4-diacetylphloroglucinol (DAPG) is produced exclusively by *Pseudomonas* bacterial species (mainly fluorescent *Pseudomonas*) and is one of the primary compounds responsible for the biocontrol activity of *Pseudomonas* spp. against different plant pathogens. For example, *Pseudomonas brassicacearum* was isolated as an endophyte from the roots of *Artemisia* sp. (Jinju area, Korea) and was shown to control the fungal plant pathogens *Phytophthora capsici*, *Colletotrichum gloeosporioides*, and *F. oxysporum* mainly by secretion of DAPG and 2,4,6-trihydroxyacetophenone. *Pseudomonas fluorescens* strain HC1-07, isolated from the wheat phyllosphere, suppresses wheat take-all disease caused by the fungus *Gaeumannomyces graminis* var. *tritici* and root rot of wheat caused by *Rhizoctonia solani*. The mechanism of action involves production of a cyclic lipopeptide (CLP). Extracted CLP inhibited the growth of *Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia solani in vitro*.

The fungus *Chaetomium globosum*, isolated from decaying organic matter and soil, effectively controls seedling blight of corn caused by *F. roseum* and *F. graminearum* through the production of cochliodinol antibiotics.

Ecomycins, which are peptide compounds purified from the bacterium *Pseudomonas viridiflava*, an endophyte that colonizes *Lactuca sativa* (lettuce), showed broad-spectrum antifungal activity against some fungal pathogens of plants, including *Rhizoctonia solani*, *F. oxysporum*, and *Sclerotinia sclerotiorum*.

Munumbicins, peptides obtained from a *Streptomyces* sp., an endophyte of the medicinal plant *Kennedia nigriscans*, Australia, showed activity against some plant pathogenic fungi such as *Pythium ultimum*, *Rhizoctonia solani*, *Phytophthora cinnamomi*, *Geotrichum candidum*, and *Sclerotinia sclerotiorum*.

Fusaricidins, A–D peptides originally purified from the methanolic culture extract of *Paenibacillus polymyxa*, have been isolated from different *Paenibacillus* strains and have been reported to combat several important fungal pathogens of plants including *Aspergillus niger*, *Aspergillus oryzae*, *F. oxysporum*, and *Penicillium thomii*.

Oocydin A, a chlorinated macrocyclic lactone produced by the bacterium *Serratia marcescens*, an epiphyte (plant surface dwelling) living on the aquatic plant species *Rhyncholacis penicillata*, showed antifungal activity against several crop pathogens, including *Phytophthora cinnamomi*, *Phytophthora parasitica*, *Phytophthora citrophora*, and *Pythium ultimum*.

Myxobacteria can efficiently combat the plant fungal pathogens *Phytophthora capsici*, *Pythium ultimum*, *Rhizoctonia* sp., *Sclerotinia minor*, and to a lesser extent *F. oxysporum*, through the production of a group of macrocyclic lactone, lactam rings, and linear cyclic peptide antibiotics.

Antibiotics from fungi

Rice blast disease, caused by *Magnaporthe oryzae*, is one of the most devastating diseases worldwide. Spore germination of *Magnaporthe oryzae* was shown to be strongly inhibited by helvolic acid, a terpenoid compound purified from the yeast *Pichia guilliermondii*, isolated from the medicinal plant *Paris polyphylla*. *Magnaporthe oryzae* is also affected by cryptocin, isolated from the fungal endophyte, *Cryptosporiopsis quercina*, which colonizes the inner stem bark tissue of *Tripterygium wilfordii*. *F. culmorum* and *F. graminearum* were inhibited by pestalachloride A isolated from *Pestalotiopsis adusta*, a fungal endophyte that inhabits the stem tissue of a Chinese tree.

The fungal pathogen *Helminthosporium sativum*, the causal agent of seedling blight and root rot of cereals, was inhibited by colletotric acid, a tridepside compound purified from *Colletotrichum gloeosporioides*, a fungus that colonizes the stems of *Artemisia mongolica*.

Phomenone, an antifungal sesquiterpene (eremophilane class) purified from *Xylaria* sp., a fungal endophyte isolated from *Piper aduncum*, was demonstrated to combat the wheat pathogens *Cladosporium cladosporioides* and *Cladosporium sphaerospermum* (common indoor mold).

Pyrrocidines, a group of polyketide–amino acid-derived antibiotics, were originally cultured from an endophyte of maize kernels, *Acremonium zeae*; this fungus has a protective effect on preharvest kernels against fungal pathogens, perhaps by effectively competing for the same host habitat. Pyrrocidines showed antifungal activity on agar disk experiments against the mycotoxin-producing fungi, *F. verticillioides* and *Aspergillus flavus*. Previous experiments have suggested that pyrrocidine A reduces the growth of several Gram-positive bacteria. It has been claimed that *Acremonium zeae* can cause stalk rot and hence may be selected against by pathologists and breeders, perhaps making commercial hybrid maize more susceptible to fungal pathogens. Pyrrocidine A showed biocontrol activity against stalk and ear rot pathogens of maize, including *F. graminearum*, *Nigrospora oryzae*, *Stenocarpella maydis*, and *Rhizoctonia zeae*. It was proposed that pyrrocidine A may protect disease-susceptible seedlings against pathogens, after colonization of seedlings by the endophyte. Pyrrocidine A also showed antipathogen effects against the seed rot saprophytes *Aspergillus flavus* and *Eupenicillium ochrosalmoneum* and against the biological agent of fungal leaf spot disease *Curvularia lunata* and the bacterium *Clavibacter michiganense*, the disease-causing agent of Goss's wilt.

Antibiosis by production of lytic enzymes and volatiles

The enzyme chitinase was found to antagonize different fungal pathogens by degrading chitin present in the fungal cell wall. Chitinase secretion is correlated with the antipathogenic activities of many bacterial strains including *Pseudomonas*, *Streptomyces*, *Bacillus*, and *Burkholderia*. Chitinases produced from fluorescent *Pseudomonas* strains isolated from the sugarcane rhizosphere showed antifungal activity against the fungus *Colletotrichum falcatum*, the causative agent of red rot disease in sugarcane. *Actinoplanes missouriensis*, a Gram-positive bacterium, was reported to produce high levels of chitinase that was shown to degrade the hyphae of *Plectosporium tabacinum*, the causal agent of lupin root rot in Egypt, through plasmolysis and cell wall lysis. The bacterium *Bacillus cereus*, isolated from a soil that suppresses take-all decline disease, was found to lyse the hyphae of the corresponding fungal pathogen *Gaeumannomyces graminis* var. *tritici*.

Antibiosis by volatile compounds

Muscodor albus, an endophytic fungus obtained from *Cinnamomum zeylanicum* (Sri Lankan cinnamon), produces a mixture of volatile compounds including acids, alcohols, ester, lipids, and ketones. Among these volatiles, 1-butanol, 3-methyl-, acetate ester is the most potent and targets a range of pathogens including the smut fungus *Ustilago hordei* (a pathogen of barley), *F. oxysporum* (pathogen of wheat and maize), *Rhizoctonia solani*, and *Pythium ultimum*.

Induced Host Resistance

Though plants do not have an immune system (e.g., antibodies) as found in humans, they do have genetically programmed strategies to combat pathogens including the production of antibiotic chemicals, and the ability to increase leaf thickness and close vascular tissues to prevent spread of the pathogen. Normally, the host resistance mechanisms can be induced by pathogens. In addition, biocontrol agents may also induce natural defense mechanisms in host plants, a process that is termed priming or induced host resistance. Plant host resistance can be induced either locally or systemically (throughout the plant) by biotic or abiotic inducers resulting in disease control without direct contact between the antagonist and the pathogen. This form of biocontrol usually results in a 20–85% reduction in disease symptoms rather than complete control. The induced host resistance form of biocontrol is also dependent on the genotype of the host and the level of induction in addition to environmental conditions such as crop nutrient health. Induced host resistance involves stimulation of plant signaling hormones that induce plant defense genes (salicylic acid, ethylene, and jasmonic acid). Host disease resistance can be mediated by plant recognition of surface components of some biocontrol species such as the flagella (flagellin protein) and lipopolysaccharides, which mimic the surface features of pathogens.

Arbuscular mycorrhizal fungi (AMF) and other root colonizing fungi are well known as biotic inducers of systemic disease resistance in host plants. Examples of such root colonizers are (1) the AMF *Glomus intraradices*, which induces pathogenic resistance genes in rice upon root colonization to combat the fungal blast pathogen *Magnaporthe oryzae*; (2) the AMF *Glomus mosseae*, which induces systemic resistance in maize against *Rhizoctonia solani*, the causal agent of corn sheath blight; and (3) the endophytic fungus *Piriformospora indica*, which induces host resistance against the basidiomycete fungus *Blumeria graminis*, the disease-causing agent of powdery mildew in barley. With respect to abiotic inducers, the commercial fungicide probenazole was used to induce resistance in maize against the fungus *Bipolaris maydis*, the disease-causing agent of southern leaf blight; the application of this elicitor did not significantly affect the vegetative growth of the plant under greenhouse conditions.

Acetoin (3-hydroxy-2-butanone) is a volatile compound produced by some microbial species such as *Bacillus subtilis* and thought to play a role in plant growth promotion, with potential to induce host resistance. For example, acetoin has been shown to decrease disease severity caused by *Erwinia carotovora* on *Arabidopsis* seedlings when the seedlings were exposed to a mixture of volatiles (including acetoin) produced by *Bacillus subtilis* and *Bacillus amyloliquefaciens* when compared with untreated seeds. Moreover, exposure to a racemic mixture of 2,3-butanediol also activated induced host resistance.

Disease Suppressive Soil

Some soils have been observed to suppress diseases in crops grown upon them. Soils are a rich source of microbes that are thought to help plants suppress pathogens by improving the health of the plant, induce natural plant defense, produce antibiotics, compete against pathogens, or hyperparasitize the pathogen (see succeeding text). Soil that suppresses crop disease due to the specific structure of its microbial community is known as disease-suppressive soil. Suppressive soil is an attractive method of biocontrol, because it has the potential to be sustainable over many seasons under favorable conditions. Suppressive soil is divided into two categories, general and specific.

General soil suppression

General suppressive soils are those that have a high total microbial biomass, resulting in low levels of protection against multiple pathogens. This strategy is dependent on the quality and quantity of soil organic matter (composts, green manures, and cover crops) that provides supplemental nutrients to enhance populations of beneficial microbes intended to antagonize associated crop pathogens primarily by occupying plant infection sites.

Specific soil suppression

In contrast to a general suppressive soil, specific suppressive soil is one that has a high concentration of one or more specific microbial species and results in high levels of protection against specific pathogens. Take-all disease of wheat, caused by *G. graminis* var. *tritici*, is one of the most studied root diseases, and its control by specific soil suppression is used as a model system for biocontrol research. This disease is mainly controlled by biological and cultural strategies as there is no resistant cultivar nor effective fungicide available. The suppressive soil is developed by continuous monocropping up to 5–7 years. The suppression is due to soil enhancement of *Pseudomonas fluorescens* that produces the antibiotics, phenazine carboxylic acid and 2,4-diacetylphloroglucinol.

Another example of specific soil suppression involves control of *Fusarium* wilt, a crop disease caused by *F. oxysporum*. Here, an abiotic factor such as soil pH affects the microbial community and results in enhancement of nonpathogenic *Fusarium* spp., which induce host systemic resistance and outcompete the pathogen for access to nutrients and infection sites. This disease-suppressive soil was also found to contain a high density of *Pseudomonas fluorescens*, which induces host defense and releases siderophores (to decrease iron availability to other organisms). As previously mentioned, both *Fusarium* sp. and *Pseudomonas* act cooperatively rather than individually.

Hyperparasitism (Parasite of the Parasite)

In addition to plants having natural defense mechanisms, in nature, plant pathogens have natural non-plant enemies such as antagonistic predators. Hyperparasitism involves specific recognition between the antagonist and the pathogen that terminates with the death of the pathogen. Hyperparasitism is most common in fungi that form sclerotia, which are dense masses of pathogenic hyphae that remain in crop residues to promote infection in the next crop season. For example, the soil fungus *Coniothyrium minitans* is a potential microparasite of *Sclerotinia sclerotiorum*, a pathogen of legumes (e.g., beans); the biocontrol agent colonizes and penetrates the sclerotia resulting in reduced inoculation of the pathogen in the soil. As another example, *Trichoderma* is a common parasite of *Rhizoctonia solani*, the causative agent of bare patch disease of cereals and sheath blight of rice. *Trichoderma* can specifically recognize and effectively penetrate the hyphal cell wall of the parasite by release of cell wall-degrading enzymes.

Transgenic Plants (GMOs)

As already noted, microbes can attack plant pathogens, for example, through the production of antibiotics. It is possible to isolate the genes encoding these microbial products and insert them into plants, creating GMOs. A GMO that uses a gene from a microbial antagonist is a bio-based method of disease and pest control. This technology is controversial, has varying degrees of public acceptance, and is restricted by government regulations worldwide. A few examples are provided here, focusing on *Fusarium*-derived mycotoxins including fumonisins.

The most common transgene for crop pest control on the market today involves transgenic maize plants that express the *Cry* genes (*CryIA*(*b*), *CryIA*(*c*), or *Cry9C*), isolated from the bacterium *Bacillus thuringiensis*, which encodes the Bt proteins that are toxic to certain insects. This technology is noted here because these transgenic plants were found to contain lower concentrations of the mycotoxin fumonisin than non-transgenic maize, perhaps by reducing the number of entry sites of *Fusarium* pathogen associated with insect damage.

Recently, a patented method was developed to isolate a fumonisin-degrading enzyme by fermentation of the fungi *Exophiala spinifera* or *Rhinocladiella atrovirens* on fumonisin-containing media. The gene encoding this degrading enzyme was isolated and introduced into plants.

Transgenic plants have been reported that are claimed to detoxify the trichothecene mycotoxins of *Fusarium*. These transgenic plants contain TR1101, a gene encoding trichothecene-3-*o*-acetyl transferase cloned from *F. sporotrichioides*, which catalyzes the acetylation of the C3 hydroxyl position of many trichothecenes including DON.

Microbes that Combine Multiple Mechanisms for Biocontrol

Some microbes can contribute to biocontrol by integrating multiple mechanisms. Examples of such microbes include mycorrhizal fungi and endophytes.

Mycorrhizal fungi have the potential to combat many pathogens by different mechanisms: (1) induced host resistance as noted in the preceding text; (2) physical blockage of the inoculation and penetration sites; (3) increased lignification of roots; (4) increased production of antimicrobial isoflavonoids; (5) hyperparasitism; and (6) improved nutrient availability including as a bridge to deliver phosphate solubilized by PGPR, resulting in better plant health.

As noted in the preceding text, endophytes are microbes that live inside plants without causing disease. Endophytes may be able to combine multiple mechanisms to combat plant pathogens including (1) competition for plant nutrients, (2) induced host resistance, and (3) secretion of anti-pathogenic molecules (e.g., secondary metabolites, antifungal peptides, or enzymes such as chitinase). For example, the fungal endophytes of corn, *Trichoderma koningii* and *Alternaria alternata*, were found to possess antifungal activity against *Fusarium* sp., the causal agent of seedling blight and root and stalk rot. *Paenibacillus polymyxa*, an endophytic bacterium that inhabits wheat (Huaibei City, Anhui Province, China), was identified as a strong antagonist against *F. graminearum* by inhibiting mycelium growth and spore germination.

Examples of Commercial Biocontrol Products

The aforementioned strategies are more than theoretical. Though active research continues to identify and validate diverse biocontrol agents, there are many examples around the world where the aforementioned biocontrol strategies have been commercialized for numerous crops. For example, Fravel (2005) provided a list of 25 biopesticides commercially registered by the US Environmental Protection Agency, sold by companies such as Novozymes, Becker Underwood, Gustafson, and Eco-Soil. Among the most famous examples of biocontrol products currently on the market include *Bacillus* sp. and *Pseudomonas* sp., which have received considerable attention, with more than ten registered products of *Bacillus* now available. *Pseudomonas* species exert direct antifungal activity against various phytopathogens by the production of various secondary metabolites including siderophores, hydrogen cyanide, 2,4-DAPG, phenazines, 2,5-dialkylresorcinol, quinolones, and gluconic acid, in addition to lipoproteins. Though biocontrol products hold significant progress, they accounted for only 1% of chemical sales in 2005. Fravel (2005) has described the challenges of commercializing biocontrol products.

Exercises for Revision

- Compare and contrast direct versus indirect methods of biocontrol.
- What are the major mechanisms of biocontrol?
- Explain the difference between general and specific soil suppression.
- Provide examples of plant host-based mechanisms of biocontrol.
- What are the soil-based strategies for biocontrol?
- What are the promising strategies to enhance the ecological fitness of biocontrol agents?

Exercises for Readers to Explore the Topic Further

Biocontrol faces some challenges including government regulation, public acceptance, and the high costs of commercial production (e.g., fermentation), in addition to safety concerns for ecosystems, animals, and humans. In practice, the key challenge is how to enhance the ecological fitness of the biocontrol agent within a complex ecosystem (field) under real-world environmental conditions. The introduction of an antagonist in a well-established soil is usually ineffective because the biocontrol agent may be lost due to competition with preexisting soil microbiota and environmental unpredictability (temperature, moisture, seasonal variation, and synthetic chemicals). Overcoming this challenge will require a better understanding of the complex factors that impact the interactions between host, pathogen, and biocontrol agent under varied environmental conditions. Promising areas of future research are:

- *To target a pathogen at its most susceptible life cycle stage*. For example, a promising direction is to target biocontrol strategies to the saprophytic stage of the pathogen (when a pathogen lives on dead host tissue) and to the dormancy stage of the pathogen (the stage of the pathogen life cycle outside of its host plant when the pathogen has less energy and hence lower resistance). Hyperparasites are most effective at these stages, because the pathogen is most unshielded. In contrast to hyperparasites, competitive antagonists may be most effective during pathogen spore germination when the pathogen has consumed large amounts of stored energy. In situations where the pathogen only survives in a living host (obligate pathogen), then host-based strategies such as induced resistance and transgenic plants and endophytes might be most effective.
- *To target a pathogen with the appropriate biocontrol based on its mechanism, timing, and location of action*. For example, endophytes and antibiosis-secreting biocontrol agents are most appropriate when they share the same host tissue/cell type and developmental stage with the target pathogen. For soil-borne pathogens, soil suppression is an ideal strategy because it can reduce the reservoir of the pathogen through competition for nutrients and/or compete for common host entry sites such as on roots. For fast-growing pathogens, the competitive antagonist should be added prior to the rapid multiplication stage of the pathogen.
- *To take into account the defense strategies of the pathogen*. For example, when considering antibiosis-based biocontrol, it is important to consider whether the target pathogen can degrade the biocontrol-derived antibiotics.
- *To consider environmental stress conditions*. It is important to select biocontrol agents that can either tolerate anticipated unfavorable conditions such as dryness or promote stress resistance in the host. For example, *Trichoderma* biocontrol agents have been well documented to improve host tolerance to low nitrogen and water.

See also: **Agronomy of Grain Growing**: Necrotrophic Pathogens of Wheat; Plants: Diseases and Pests; Wheat: Biotrophic Pathogen Resistance; **Bioactives and Toxins**: Mycotoxins; **Genetics of Grains**: Biotic Stress Resistance Genes in Wheat; Development of Genetically Modified Grains; **Grain Harvest, Storage and Transport**: Chemicals for Grain Production and Protection; Postharvest Operations for Quality Preservation of Stored Grain; Stored Grain: Invertebrate Pests; Stored-Grain Pest Management; The Nature, Causes, and Control of Grain Diseases in the Major Cereal Species.

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