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Uses of Antimicrobials in Plant Agriculture

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Bacterial diseases of plants are less prevalent than diseases caused by fungi and viruses. Antimicrobials for prophylactic treatment of bacterial diseases of plants are limited in availability, use, and efficacy, and therapeutic use is largely ineffective. Most applications are by spray treatments in orchards. Monitoring and surveillance for drug resistance are not routinely done. In the United States, data on use of antimicrobials for treatment of bacterial diseases of plants are limited to streptomycin and oxytetracycline. Resistance to streptomycin has become widespread among bacterial phytopathogens; no resistance among these bacteria has yet been reported for oxytetracycline. No human health effects have been documented since inception of use of antimicrobials in plants in the 1950s. Transfer of antimicrobial resistance from marker genes in transgenic plants to bacteria has not been documented under natural conditions in field-grown plants. However, antimicrobial-resistance genes are being eliminated from use as marker genes because of concerns about possible transfer from plant genomes back to bacteria, with further horizontal transfer to the bacteria in the environment, or from plant genomes to animals by plant consumption. No new antimicrobials are expected to be used in plant agriculture because of high costs of development, regulatory constraints, and environmental and human health concerns. Alternatives to antimicrobials, such as biocontrol agents, transgenic plants, and novel chemicals, are being developed and marketed, although their efficacy remains to be determined.

CURRENT USE OF ANTIMICROBIALS IN AGRICULTURE

Antimicrobials originated from microorganisms isolated from the environment [1]. Although there are some studies of phenotypic antimicrobial resistance and a few studies of genetic determinants associated with resistance in natural isolates of commensal and phytopathogenic bacteria, as Salyers has pointed out, there are no systematic studies of microbes in an ecosystem (A. Salyers, personal communication). This lack of data is the case even for environments in which antimicrobials are used for managing bacterial plant diseases of fruit trees, for which antimicrobial use in the United States has proven to be economical [2]. The extent of naturally occurring antimicrobial resistance is not well known because, except for monitoring the target pathogen treated with antimicrobials, even fewer studies have monitored the resistance of nontreated, wild-type pathogens [3, 4] and commensal bacteria [5].

An estimated 40 million pounds of antimicrobials are used in the United States each year, of which ~0.1% is used in plant agriculture [6]. Antimicrobial use in US plant agriculture is limited in type and quantity used as a result of economics, lack of antimicrobial efficacy for a number of diseases, and environmental concerns. The US Environmental Protection Agency (EPA) has regulatory responsibility for antimicrobial use in plants, whereas the Food and Drug Administration regulates all other antimicrobial use. Eventually, the Food Quality Protection Act of 1996 may eliminate the use of antimicrobials in plant agriculture because the required reregistration and compliance with the higher standards involved may not be cost-effective.

Only 2 antimicrobials, streptomycin and oxytetracycline, are currently registered by the EPA for use in plant agriculture. Streptomycin and oxytetracycline are often grouped with fungicides in data reports, and both are used primarily as prophylactic treatments—that is,

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Table 1. Antibiotics registered for use in plant agriculture in the United States.

Crop use, crop	Disease	Disease agent	Registered treatment: Streptomycin[a,b]	Registered treatment: Oxytetracycline[b,c]
Terrestrial food and/or feed crop use				
Apple	Fire blight	*Erwinia amylovora*	F	F
Bean	Halo blight	*Pseudomonas syringae* pv. *phaseolicola*	S	—
Celery	Bacterial blight	*Pseudomonas cichorii*	F*	—
Crabapple	Fire blight	*E. amylovora*	F	—
Nectarine	Bacterial leaf and fruit spot	*Xanthomonas campestris* pv. *pruni*	—	F
Peach	Bacterial leaf and fruit spot	*X. campestris* pv. *pruni*	—	F
Pear	Fire blight	*E. amylovora*	F	F
Pepper	Bacterial spot	*X. campestris* pv. *vesicatoria*	F*	—
Potato	Bacterial soft rot	*E. chrysanthemi, E. carotovora* subspecies *carotovora*	S	—
	Blackleg	*E. carotovora* subspecies *atroseptica*	S	—
Quince	Fire blight	*E. amylovora*	F	—
Tomato	Bacterial spot	*X. campestris* pv. *vesicatoria*	F* and S	—
Nonfood crops				
Sugar beets (grown for seed)	Bacterial rot/blight	*Erwinia* species	S	S
Tobacco	Wildfire	*Pseudomonas syringae* pv. *tabaci*	F* and S	—
Ornamental herbaceous plants, shrubs, and vines, and greenhouse ornamentals				
Anthurium	Bacterial blight	*X. campestris* pv. *dieffenbachiae*	F	—
Cotoneaster	Fire blight	*E. amylovora*	F	F
Chrysanthemum	Bacterial wilt	*E. chrysanthemi, E. carotovora* subspecies *carotovora*	F	C
Crabapple, flowering	Fire blight	*E. amylovora*	F	—
Elm	Lethal yellows	*Phytoplasma*	—	I
Dieffenbachia	Bacterial stem rot	*Erwinia* species	F	—
Hawthorn	Fire blight	*E. amylovora*	F	—
Palm	Lethal yellows	*Phytoplasma*	—	I
Philodendron	Bacterial leaf spot	*X. campestris* pv. *dieffenbachiae*	F	F
Pyracantha	Fire blight	*E. amylovora*	F	—
Quince, flowering	Fire blight	*E. amylovora*	F	—
Roses	Crown gall	*Agrobacterium tumefaciens*	F	—

NOTE. F, foliar; F*, foliar, seedling stage only; S, seed, seed piece, or bed treatment; C, cutting; I, internal injection.

[a] Adapted from 1992 US Environmental Protection Agency (EPA) Reregistration Eligibility document [7].

[b] Data from [8, 9].

[c] Adapted from 1993 US EPA Reregistration Eligibility document [10].

when disease is expected on the basis of previous experience, predictive systems, or recommendations of local agricultural advisors. Streptomycin is registered for use on 12 fruit, vegetable, and ornamental fruit crops, and oxytetracycline is registered for use on 4 fruit crops (table 1). Some data on minor uses for other crops and seed treatment are not available.

A major plant disease, fire blight, is caused by *Erwinia amylovora*, a relative of *Escherichia coli* and other enteric bacteria. Spray treatments may be used every 3–4 days (streptomycin) or 4–6 days (oxytetracycline) as prophylactic treatment to limit fire blight damage during blossom time, when fire blight damage is the most devastating [11]. Approximately 53,000 hectares (∼131,000 acres) are sprayed annually with antimicrobials [12]. Blossom time may extend 6 weeks or more and differs among species and varieties. Residue studies described in the public literature are limited to streptomycin. These studies showed that fruit had no detectable streptomycin residue at the time of harvest, but streptomycin activity was still detectable in leaves [13]. The 1992 EPA fact sheet on streptomycin [7, p. 5] indicates that "all ecological effects data requirements are satisfied" and that streptomycin is nontoxic to birds, freshwater invertebrates, and honeybees and is slightly toxic to fish (both cold-water and warm-water species). Interestingly, streptomycin is reported to be "toxic to algae." The 1993 EPA fact sheet addressing oxytetracycline usage [10, p. 5] states, "oxytetracycline is practically non-toxic to birds, fish, aquatic inverte-

Table 2. Use of the antibiotic agent gentamicin in food crops by country.

Country, crop	Disease	Disease agent
Chile		
Tomato	Bacterial canker	*Clavibacter michiganensis* subspecies *michiganensis*
Pear	Fire blight	*Erwinia amylovora*
Central America (Costa Rica, Honduras, Guatemala, El Salvador)		
Potato	Blackleg	*Erwinia carotovora* subspecies *atroseptica*
	Bacterial wilt	*Ralstonia solanacearum*
Tomato	Bacterial speck	*Pseudomonas syringae* pv. *tomato*
Chili	Bacterial spot	*Xanthomonas campestris* pv. *vesicatoria*
Cauliflower and broccoli	Bacterial soft rot	*Erwinia* species
Cabbage	Bacterial black rot	*X. campestris* pv. *campestris*
Mexico		
Potato	Black leg	*E. carotovora* subspecies *atroseptica*
Apple, pear, and ornamentals	Fire blight	*E. amylovora*
Tomato and chili	Bacterial spot	*X. campestris* pv. *vesicatoria*
Agave	Bland rottenness of the heart of agave	*Erwinia* species
Watermelon	Bacterial spot	*Xanthomonas* species

NOTE. The gentamicin used is Agry-gent (Quimica Agronomica de Mexico, Rhode Island No. 4908, Residencial Campestre, C.P. 31238, Chihuahua, Chihuahua, Mexico).

brates and non-target insects such as honey bees." On the basis of limited public-domain data and on limited patterns of oxytetracycline use, the EPA waived all environmental data requirements. However, its open application in the environment remains a concern.

Recommended concentrations for streptomycin range from 50–200 ppm (50–200 μg/mL), depending on treatment objective and crop. For use in fire blight on apples and pears, an application rate of 24–48 ounces per acre (~2–4 L/hectare) is recommended. Oxytetracycline is used at concentrations of 150–200 ppm (150–200 μg/mL). For treatment of peaches and nectarines, the application rate at 150 ppm is 3 gallons per tree or 240 gallons per acre, which may be increased for large trees, not to exceed 500 gallons per acre per application. For treatment of pears, the application rate at 200 ppm is 50–100 gallons of solution per acre.

In 1999, the latest year for which data are available through the US Department of Agriculture [14], 30% of the pear acreage received a total of 6000 pounds of streptomycin and 40% of the acreage received a total of 12,000 pounds of oxytetracycline. Apples received >15,000 pounds of streptomycin on ~20% of the acreage, or 3000 pounds of oxytetracycline on 5% of the acreage. In 1997, 39,800 pounds of streptomycin and 26,800 pounds of oxytetracycline were used, mostly on pears and apples. Streptomycin use has decreased over the decade, but oxytetracycline use has increased, except in 1999. One reason for the increased use of oxytetracycline is the increasing prevalence of streptomycin resistance in the target bacterium, *E. amylovora* [6, 15, 16].

Most worrisome is the use of gentamicin for plant agriculture in Latin America (table 2). The extent and quantity of antimicrobial use in this region are not known, and the degree of human exposure is unclear. The American Society for Microbiology and others persuaded the EPA that fruits and vegetables treated with gentamicin should not be imported, and a tolerance level for gentamicin in food should not be considered because of the importance of gentamicin in human medicine. The concern was that any unnecessary residues on food could compromise use of this antimicrobial, which is the last economically feasible drug for some human bacterial infections. No data are available on gentamicin use in agriculture in Latin America or on the occurrence of antimicrobial resistance of bacteria on fruits and vegetables from Latin America.

ANTIMICROBIAL RESISTANCE IN PLANT PATHOGENS

Antimicrobial resistance in plant pathogenic target bacteria began to appear as early as the 1960s, a few years after introduction of use of streptomycin [15, 17]. Resistance has also been found to be linked with copper resistance [16, 18]. Genetically, resistance genes may be chromosomal or carried on plasmids or transposons; all genetic forms are found in environmental, human, and plant pathogenic strains [19, 20]. Tetracycline resistance has not been reported in target bacteria—that is, the pathogen—but it has been found in plant surface–associated (phylloplane) bacteria [5].

Although there is at present no evidence for a correlation between the agricultural use of azoles as fungicides and fungal resistance in humans, such concerns have been expressed [21], and research on this issue is merited. In principle, the same concerns that apply to development of resistance with the use of bacterial antimicrobials are applicable to antifungals. The reverse concern may apply to antiviral agents, which have not yet been used in plants.

Antimicrobial-resistance genes have been used as selectable markers in producing transgenic plants. Under optimized laboratory conditions, the *nptII* gene (conferring resistance to kanamycin) could be transferred from transgenic sugar beets to the soil bacterium *Acinetobacter* sp. BD413 at a frequency of 10^{-9} to 10^{-10} [22]. This gene can also be transferred from transgenic potatoes to *Acinetobacter* BD413 and *Pseudomonas stutzeri* ATCC 17587, both of which harbor plasmids carrying the *nptII* gene with a small deletion [23]. In these experiments, detectable marker rescue was dependent on sequence homology in the recipient cells. Even if such transfer were to occur, Gebhard and Smalla [22] point out that the promoter sequences used in the transgenic constructions are not active in most bacteria, so that the recipients would not express a kanamycin resistance phenotype. Also, most of the antimicrobial-resistance genes used as marker genes are widely disseminated in environmental bacteria. Nevertheless, such use is being phased out because of concerns about potential transfer of these bacterial antimicrobial resistance genes from plant chromosomes back to bacteria, with subsequent horizontal transfer among bacteria in the environment [12].

At the genetic level, little information exists on the extent of antimicrobial susceptibility and resistance occurring naturally in environmental bacteria. Consequently, implications for human health from resistance arising from these sources remain problematic. Alternatives to antimicrobials under investigation include biocontrol agents [24, 25], transgenic plants, and novel chemicals. Some of these agents or compounds have been recently marketed, but efficacy and safety over time still remain to be determined.

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