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Use of antibiotics in plant agriculture

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Summary
Antibiotics are essential for control of bacterial diseases of plants, especially fire blight of pear and apple and bacterial spot of peach. Streptomycin is used in several countries; the use of oxytetracycline, oxolinic acid and gentamicin is limited to only a few countries. Springtime antibiotic sprays suppress pathogen growth on flowers and leaf surfaces before infection; after infection, antibiotics are ineffective. Antibiotics are applied when disease risk is high, and consequently the majority of orchards are not treated annually. In 2009 in the United States, 16,465 kg (active ingredient) was applied to orchards, which is 0.12% of the total antibiotics used in animal agriculture. Antibiotics are active on plants for less than a week, and significant residues have not been found on harvested fruit. Antibiotics have been indispensable for crop protection in the United States for more than 50 years without reports of adverse effects on human health or persistent impacts on the environment.

Keywords
Antibiotic residue – Bacterial panicle blight of rice – Bacterial spot of peach – *Burkholderia glumae* – *Erwinia amylovora* – Fire blight of pear and apple – Gentamicin – Oxolinic acid – Oxytetracycline – Plant disease control – Streptomycin – *Xanthomonas arboricola* pv. *Pruni.*

Introduction

Bacterial diseases of plants are severe constraints to crop production, and few materials are efficacious or available to mitigate crop loss. Following the discovery of antibiotics, in the 1950s streptomycin was found to be an excellent chemical tool for the control of several bacterial diseases of plants (12, 14, 35, 48). Unfortunately, streptomycin resistance in plant pathogens was detected within five to ten years of commercialisation of the antibiotic (20, 31, 34). Resistance of plant pathogenic bacteria to streptomycin resulted in:

– limiting the use of streptomycin primarily for control of fire blight, a bacterial disease of pear and apple trees caused by *Erwinia amylovora*

– developing disease risk models based on environmental parameters to optimise the timing and reduce the number of antibiotic sprays in orchards

– adding the antibiotics oxytetracycline, oxolinic acid or gentamicin for plant disease control in some countries.

In clinical medicine, the development of antibiotic resistance in human pathogens has been widely publicised and is recognised as a major threat to the control of bacterial diseases and infections worldwide (22). The clinical antibiotic resistance crisis has focused attention on all uses of antibiotics, including their uses in plant agriculture. It has been speculated that spraying antibiotics across several hectares of plants in the environment might increase the frequency of antibiotic resistance genes in bacteria living on plant surfaces and that genes conferring resistance might then be transferred into clinically important bacteria. However, the antibiotics streptomycin and oxytetracycline have been used on pear and apple orchards in the United States (USA) for over 50 years without any reports of adverse effects on humans (61, 62, 63). Nevertheless, the use of antibiotics on plants is a controversial practice (31, 66).

The purpose of this review is to address the global use of antibiotics on plants. The mechanisms of resistance to each of the antibiotics and the putative risk factors for their use on plants will be discussed. Each of the antibiotics registered for use on crops is used primarily to control fire blight of pome fruits, caused by the plant pathogenic bacterium *E. amylovora*, which will be the focus of this paper.

Fire blight: the primary use of antibiotics on plants

Fire blight is the most important bacterial disease of apple, pear and related ornamental plants. Fire blight was first described in New York and has spread throughout North America, New Zealand, the Middle East and Europe (2). The disease is sporadic, and generally it does not occur in orchards every year but can be severe under favourable environmental conditions. In the USA alone, fire blight costs growers over US$100 million annually as a result of disease outbreaks and the expenses incurred in managing the disease (37).

The deployment of antibiotics for the control of fire blight can be understood in the context of the disease cycle (19, 59). The pathogen, *E. amylovora*, overwinters in stem cankers and infected tissues. In the spring, the pathogen multiplies and oozes from the surface of the cankers and is subsequently spread to open flowers by foraging honey bees, insects, wind and rain. The pathogen multiplies rapidly on the nutrient-rich stigmas of flowers to population sizes exceeding 10^6 colony-forming units per flower when temperatures are approximately 15°C and higher. After saturated populations of the pathogen have been established on stigmas, the pathogen migrates to the nectary, which is the infection site on the flower. Successful migration of the pathogen and colonisation of the nectary tissue is aided by moisture from heavy dew or rain. The pathogen infects the flower through natural openings, such as the nectar-secreting cells called nectarthodes. After ingress, *E. amylovora* multiplies within the floral tissues and migrates into branches, causing rapid death and progressive necrosis and wilting. Once the symptoms of the disease are visible, there are no effective chemical treatments to cure the plant of fire blight (41). To save a tree after infection, branches must be removed at least 0.3 m below the visible symptoms. If the infection occurs near the tree trunk, then the entire tree may be killed. Young trees are particularly sensitive to the disease, and pear cultivars are generally more susceptible than apple cultivars (37).

Effective disease control focuses on preventing the growth of the pathogen on flower surfaces before infection (31, 41). Streptomycin has been an effective antibiotic against fire blight because it is bactericidal and kills the pathogen on the flower surface. Oxytetracycline, which inhibits the growth of the pathogen but does not kill it, is less effective than streptomycin (29).

Even though growers may use antibiotics to control fire blight, epidemics of the disease do still occur. Fire blight epidemics in the USA have occurred when weather conditions were favourable to the disease, and in most cases streptomycin-resistant populations of the pathogen were present. A fire blight epidemic in south-western Michigan in 2000 caused losses of US$42 million to apple growers (37). In 1998, apple and pear growers in Washington and northern Oregon suffered an estimated US$68 million in losses due to fire blight. In 2002, the worst epidemic of fire blight in 100 years hit a major pear production region in southern Oregon. In countries where antibiotics are not registered for use in plant agriculture, fire blight has inflicted severe damage on orchards. In an unsuccessful effort to eradicate fire blight in Italy in the late 1990s, approximately 500,000 pear trees were destroyed in the Po Valley, a major pear production area (4). Likewise, nearly one million pear, apple and quince trees were destroyed in Romania and Croatia during the 1990s in an unsuccessful effort to stop the spread of fire blight in those countries (9, 47).

Registered antibiotics, their activity and mechanisms of resistance in *Erwinia amylovora*

Streptomycin

Streptomycin is an aminoglycoside antibiotic, naturally produced by soil actinomycetes (51), which was commercialised for use in plant agriculture in the USA as early as 1955 (10, 15). Currently, 90% of the streptomycin used in plant agriculture in the USA is for control of fire blight (31). Minor uses of streptomycin include control of bacterial diseases in floriculture and on potato tubers, tobacco seedlings and other vegetable seedlings in the field or greenhouse (66). Streptomycin is currently also registered for fire blight control in Israel, New Zealand, Canada and Mexico; it has been permitted on an emergency use basis, subject to annual review and under tightly restricted conditions, in Germany, Austria and Switzerland.

Streptomycin is bactericidal, binding irreversibly to the bacterial ribosome and blocking the synthesis of proteins (7, 20, 38). Two mechanisms for resistance to streptomycin have been observed in samples of the fire blight pathogen from orchards. The most common mechanism is spontaneous mutation of the chromosomal gene *rpsL*, which encodes the production of a ribosomal protein (20). The single-step point mutation in *rpsL* prevents binding of streptomycin to the ribosome, and the bacterium is immune to the antibiotic. This mutation is prevalent among streptomycin-resistant isolates of *E. amylovora* in orchards in the USA, Israel and New Zealand (7, 24, 32, 59).

Acquired resistance to streptomycin has been detected in *E. amylovora* only in orchards in Michigan and once in California (5, 20, 39). The pathogen acquired plasmids that carry the tandem gene pair *strA* and *strB*, which codes for the production of an aminoglycoside phosphotransferase that inactivates streptomycin. Both *strA* and *strB* and an associated transposon, Tn5393, have been detected in several genera of plant-associated bacteria in many environments (5, 6).

Regardless of the mechanism, streptomycin resistance appears to be a stable trait in plant pathogenic bacteria. Streptomycin-resistant isolates of *E. amylovora* were detected in an orchard in California ten years after applications of the antibiotic were stopped (34). The persistence of streptomycin-resistant populations has led to the use of additional antibiotics to protect orchards.

Oxytetracycline

Oxytetracycline (Terramycin) is a naturally produced tetracycline antibiotic of *Streptomyces rimosus*, with a spectrum of activity similar to chlortetracycline and tetracycline and remarkable thermostability (38). In plant agriculture, oxytetracycline is used in the USA primarily on pear and apple for fire blight management. Oxytetracycline is also important for the management of a serious disease of stone fruits (e.g. peach and nectarine) in the USA called bacterial spot, caused by *Xanthomonas arboricola* pv. *pruni* (8, 31). Oxytetracycline is used in Mexico and Central America to control *E. amylovora* on apple and diseases caused by *Pectobacterium* spp., *Pseudomonas* spp. and *Xanthomonas* spp. on several vegetable crops. Oxytetracycline is the only antibiotic that can be used internally in plants: it may be injected into the trunks of palm and elm trees to treat lethal yellowing diseases and other high-value plants to treat other diseases caused by phytoplasmas. However, this is an expensive and labour-intensive treatment and is rarely used (27, 28).

Oxytetracycline is bacteriostatic, inhibiting the multiplication of bacterial cells by binding reversibly to the bacterial ribosome, and blocks the synthesis of proteins only while bound to the ribosome (31). Bacteria have three major strategies for developing tolerance to oxytetracycline: efflux pumps, alteration of the ribosome to block binding of oxytetracycline and production of enzymes that inactivate oxytetracycline. Isolates of *E. amylovora* with resistance to moderate concentrations (>20 parts per million [p.p.m.]) of oxytetracycline have not been detected in orchards in the USA (31).

Gentamicin

Gentamicin is an aminoglycoside antibiotic used in Mexico to control fire blight of apple and pear. Gentamicin is also used in Mexico and Central America to control various bacterial diseases of vegetable crops caused by species of *Pectobacterium*, *Pseudomonas*, *Ralstonia*, and *Xanthomonas* (66). In the 1990s, an attempt to register gentamicin for plant agriculture in the USA was contested, owing to its clinical importance, and the application was withdrawn without prejudice (31).

Gentamicin, like streptomycin, inhibits protein synthesis by binding to the bacterial ribosome. Unlike streptomycin, gentamicin binds to several sites on the ribosome; thus, several mutations in the bacterial chromosome are needed to generate spontaneous mutants resistant to gentamicin (31). Bacterial cells can become resistant to gentamicin by acquisition of genes encoding enzymes that modify the antibiotic. Bacteria harbouring transferable genes for gentamicin resistance have been detected in several environments irrespective of exposure to gentamicin (17). It is unclear whether gentamicin resistance will emerge in plant pathogenic bacteria and decrease the efficacy of the antibiotic against fire blight in pome fruits and the plethora of other diseases caused by other genera on vegetable crops in Mexico and Central America.

Oxolinic acid

Oxolinic acid, a synthetic quinolone antibiotic, is used only in Israel to manage fire blight of pear and related plants, especially in areas where *E. amylovora* is resistant to streptomycin (50). Oxolinic acid also is registered in Japan for management of bacterial panicle blight of rice, caused by *Burkholderia glumae* (26, 36). In rice production, seeds and plants during panicle emergence or flowering are treated with oxolinic acid (26). Oxolinic acid inhibits DNA replication and consequently bacterial growth. The inhibition of DNA replication is due to binding of the quinolone to the target enzymes DNA gyrase and topoisomerase IV (45). Resistance to quinolones is often the result of a single-step spontaneous mutation of the bacterial chromosome that alters the antibiotic binding site on DNA gyrase. Within ten years of the registration of oxolinic acid for use in rice production in Japan, resistant populations of *B. glumae* had been detected (18, 26). Some of the oxolinic acid-resistant isolates of *B. glumae* also exhibited cross-resistance to other quinolones, including ciprofloxacin, a fluoroquinolone important in clinical medicine (18). Just two years after oxolinic acid was introduced for control of fire blight in Israel, oxolinic acid-resistant isolates of *E. amylovora* were detected in several orchards (21). The rapid emergence of resistance to oxolinic acid diminished interest in the use of this antibiotic for sustainable disease control on plants in other countries.

Addressing the risk factors of antibiotic use in plant agriculture: countries where antibiotics are registered and annual use

It is difficult to determine which countries permit the use of antibiotics in plant agriculture. Such information is not widely available on the Internet, and enquiries to governmental agencies are often unsuccessful. Considering antibiotic use on pome fruit trees, data from the statistical division of the Food and Agriculture Organization of the United Nations indicate that the People's Republic of China (hereafter referred to as China) has the greatest acreage of apples and pears in the world. The acreage of apples alone in China is more than 2,000,000 ha (http://usda.mannlib.cornell.edu). The authors have been unable to determine whether antibiotics are used currently in plant agriculture in China. To date, fire blight has not been reported in the country (2). Without pressure from fire blight, and owing to the expense of the materials, it is unlikely that growers in China use antibiotics in pome fruit orchards. Nonetheless, the authors recognise that antibiotics may be used to combat other bacterial diseases on rice or other vegetable crops in China.

Considering the top 15 countries in terms of apple or pear acreage or production, fire blight is present in the USA, Iran, Poland, Turkey, Mexico, Italy, France, Spain, the Netherlands and Egypt (2). Among the top producing countries where fire blight is also present, the authors confirmed that antibiotics are registered for use only in the USA and Mexico (http://usda.mannlib.cornell.edu). In addition to being used in the USA and Mexico (the major pome fruit producers), streptomycin is used for plant disease control in Canada and New Zealand and, on a strictly regulated, emergency-use permit basis, in Germany, Switzerland and Austria.

As mentioned previously, oxolinic acid is registered in Israel for fire blight management (49, 50). Israel has only 1,500 ha of pome fruit orchards, and the antibiotic is applied one to three times per season (50). In Japan, oxolinic acid is used for the management of bacterial panicle blight of rice, with three applications during rice cultivation (26). According to the International Rice Research Institute, Japan has 1.6 million ha in rice production, but the area actually treated with oxolinic acid was not indicated. Oxolinic acid is not registered for plant disease control in the USA (36). The authors do not know if oxolinic acid is used in other countries for the management of plant diseases. Gentamicin and oxytetracycline are registered in Mexico and Central America; however, data on their usage are not available. Oxytetracycline also is registered in the USA, and usage data are discussed below.

Quantitative data on antibiotic use on crops are scarce. Requests for data for some countries were refused on the basis that the permission to use antibiotics on plants and crop usage data is proprietary to the companies that market antibiotics. The USA has major hectarages under apple, pear and peach orchards. Since the 1990s, data on antibiotic use in plant agriculture in the USA have been collected, and searchable databases on the United States Department of Agriculture National Agricultural Statistics Service website (www.nass.usda.gov) are publicly available. Through these databases, we can obtain estimates of the quantities of antibiotics applied to plants.

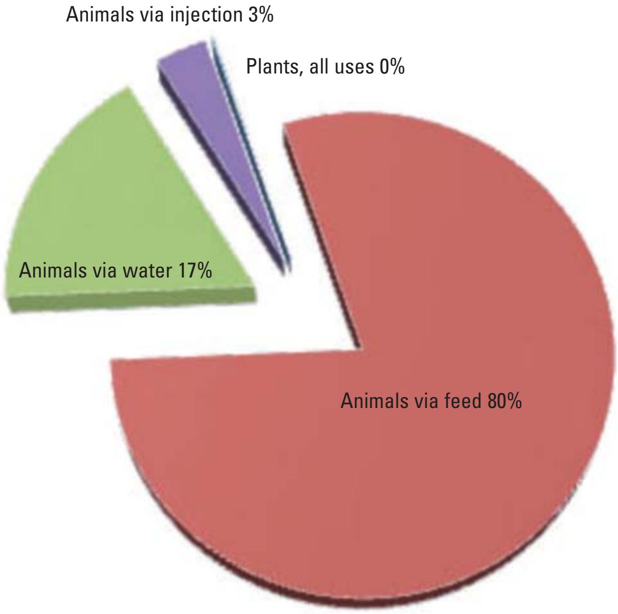
Historically, the antibiotic streptomycin has been sprayed on a calendar basis, beginning when orchards are in early bloom and continuing every five days until about 45 days before fruit harvest, regardless of disease risk (30, 34, 48). Streptomycin and oxytetracycline are active against the pathogen on flowers for less than a week after application (55). The timing of sprays is critical to fire blight control: unnecessary sprays are expensive, but omitting a spray during an infection period can leave an orchard in danger of an epidemic. For example, many antibiotic sprays applied on a strict calendar schedule are unnecessary when temperatures during flowering are too low to support the growth of the pathogen on flowers or subsequent disease (1, 59). The development and use of plant disease risk models, based on environmental parameters and the potential of pathogen populations present in orchards (1), has reduced the number of antibiotic applications. Although none of the models is perfect in predicting when and where fire blight will occur, they are useful management tools and have reduced the number of antibiotic sprays needed for disease control. From usage data, it is readily apparent that growers in the USA use disease risk models and are judicious in their use of antibiotics. In 2009, about 10% of the area planted with peach trees, 15% of the apple hectarage, and 40% of the area under pears were treated with streptomycin and/or oxytetracycline (Table I). Since the 1990s there has been a fairly consistent pattern of not applying antibiotics to the majority of orchards in the USA in a single growing season (30, 31).

Table I
Use of antibiotics on plants in the United States in 2009 according to the United States Department of Agriculture National Agriculture Statistical Services (www.nass.usda.gov)

Crop	Hectares planted	Oxytetracycline: Percentage treated	Oxytetracycline: Average no. of sprays	Oxytetracycline: Total active ingredient per year (kg)	Streptomycin: Percentage treated	Streptomycin: Average no. of sprays	Streptomycin: Total active ingredient per year (kg)	Total amount of antibiotics (kg)
Apple	123,996	12	1.2	3,084	16	1.9	6,169	9,253
Peach	46,458	9	2.2	1,406	–	–	–	1,406
Pear	24,106	41	3.3	3,901	30	2.7	1,905	5,806
Total				**8,391**			**8,074**	**16,465**

In orchards where antibiotics were applied for fire blight control, the compounds were sprayed on average one to three times, mainly during flowering (Table I). For control of bacterial spot, about 10% of the peach hectarage was sprayed with oxytetracycline twice. Streptomycin is applied at concentrations of 50–100 µg/ml and oxytetracycline at 150–200 µg/ml, often with air-blast sprayers at a general rate of 936 l/ha. Considering all applications, about 8,000 kg active ingredient (a.i.) streptomycin and 8,400 kg a.i. oxytetracycline were applied to orchards in the USA in 2009 (Table I).

The quantity of antibiotics applied to plants in the USA is dwarfed by the quantities used in human medicine or animal agriculture. In the USA, the Food and Drug Administration estimates that 3,300,000 tonnes of antibiotics are dispensed to humans annually. The Government Accountability Office in the USA (64) reports that 13,100,000 tonnes a.i. of antibiotics are used in animal agriculture annually in the USA, with 3% of the total injected into animals, 17% added to water and 80% added to feed (Fig. 1). In 2009, a total of 16,465 kg a.i. of antibiotics was applied to tree fruits in the USA (Table I), just 0.12% of the combined amount of antibiotics used clinically and in animal agriculture.



Source: data were obtained from the United States Department of Agriculture National Statistical Service (www.nass.usda.gov) for antibiotic use on plants, and the Government Accountability Office (64)

Fig. 1
Distribution of antibiotic use in plant and animal agriculture in the United States in 2009
In animal agriculture, 13,100,000 tonnes a.i. antibiotics were dispensed, and in plant agriculture 16,465 kg a.i. antibiotics were applied to orchards in the United States in 2009

Plant agriculture-grade antibiotic formulations did not carry resistance genes or 16S rRNA

After alarming reports that low-grade avoparcin formulations added to animal feed for growth promotion were highly contaminated with *Amycolatopsis orientalis*, the producer organism, and its resistance genes for the glycopeptide antibiotic (25, 68), the cleanliness of antibiotic formulations used in plant agriculture was questioned. An array of commercial streptomycin formulations were tested to determine if plant agriculture-grade formulations were contaminated with the producer strain *Streptomycetes griseus* subsp. *griseus* or its resistance genes for streptomycin (43). Quantitative polymerase chain reaction methods were developed to detect streptomycin resistance genes and 16S rRNA for general bacterial DNA in commercial formulations. Rezzonico *et al.* (43) did not detect bacterial DNA or streptomycin resistance genes in commercial formulations of streptomycin for plant agriculture in samples from the

USA, New Zealand or Europe. They concluded that plant agricultural formulations of antibiotics are highly unlikely to introduce resistance genes into the environment.

Regulations reduce direct human exposure to antibiotics used in plant agriculture

The United States Environmental Protection Agency is responsible for setting regulations to minimise exposure to any chemicals applied to crops, including antibiotics (30). In comparison with most pesticides used on plants, antibiotics are relatively non-toxic and were assigned the lowest toxicity rating of pesticides by the agency. Workers are required to wear protective clothing and equipment during handling and application of the antibiotics to crops to mitigate direct exposure to antibiotics. Furthermore, no one is permitted to enter an orchard treated with antibiotics for 12 h after application. The Environmental Protection Agency also regulates the pre-harvest interval, or the minimum time period permitted between last spray and crop harvest. In the USA, the pre-harvest interval for application of oxytetracycline and streptomycin varies from between 21 and 60 days, depending upon the compound and the crop (30). The regulations established in the USA are similar to those established by governmental agencies in other countries.

Governmental regulations also restrict the level of permissible residues on crops. For oxytetracycline, the residue tolerance level on tree fruit crops is 0.35 p.p.m. (61, 63). To date, there are no reports of fruit with residues greater than the permitted tolerances of oxytetracycline. In a risk assessment study (63), the Environmental Protection Agency states that typical pharmaceutical oxytetracycline exposure to humans would be 50,000 to 200,000 times greater than the theoretical dietary exposure (i.e. combined food and potentially contaminated water sources) associated with the use of oxytetracycline in plant agriculture. The agency concluded that the potential dietary exposure of humans to oxytetracycline used in plant agriculture would result in no harm compared with its pharmaceutical usage (63).

For streptomycin, the residue tolerance level on tree fruit crops is 0.25 p.p.m. (62). Shaffer and Goodman (48) published the first evaluation of residues of streptomycin on apple leaves and fruit. They sprayed trees up to ten times from flowering in April to early fruit development in mid-June. They detected residues on leaves during the season (detection limit of 0.1 µg/ml) and on developing fruit, but residues on fruit were below the residue tolerance within a month after the last spray (about 70 days before harvest). At harvest, residues were not detected on apple fruit, even on trees sprayed ten times with streptomycin (16, 48). Subsequent studies by numerous independent investigators have corroborated their results – fruit from trees treated with streptomycin for fire blight management does not have residues near the tolerance levels permitted by governmental agencies (see, for example, reference 13). The Environmental Protection Agency (62) concluded that anticipated dietary residues of streptomycin from plant agriculture were extremely low: even in worst-case scenarios with contaminated water sources and food, the dietary exposure dose would be 3,000 to 21,000 times lower than a typical therapeutic dose. A recent, highly publicised report of streptomycin detected in apples harvested from Austrian orchards treated with just one to three applications during the flowering period (33) has renewed consumer and regulatory concerns in Europe. However, serious questions have been raised (and remain to date unresolved) regarding the reliability, reproducibility, and practical relevance of some of the statements in this report.

Antibiotics are non-persistent on plant surfaces and lose activity rapidly

Even though antibiotics can be detected on plant surfaces, using sensitive analytical chemistry methods, for up to a month after application, their capacity to inhibit bacterial growth is lost within a week after application. In a laboratory experiment, streptomycin no longer prevented fire blight 5 days after spraying apple flowers (65). Stockwell *et al.* (55) treated trees in a screenhouse with streptomycin and/or oxytetracycline. Under conditions where trees were protected from rain and ultraviolet irradiation from sunlight, growth of *E. amylovora* was suppressed for only 4 days after antibiotic treatment (55). The persistence of antibiotics is probably even lower under fully exposed conditions (3). Christiano *et al.* (8) conducted an extensive study of the stability of oxytetracycline (applied at 300 µg/ml a.i.) on peach leaves. At least 50 p.p.m. oxytetracycline (0.06 µg/cm leaf surface) on leaves was required to control bacterial spot of stone fruits (8). Oxytetracycline was thermostable on leaves, but rapidly degraded when exposed to natural sunlight: by 44% within 1 day, 92% within 4 days, and to levels near the detection limit (50 p.p.b.) by a week after application (8). Oxytetracycline was not rainfast on leaves: 2 min of simulated rain (44 mm/h) reduced residual concentrations of oxytetracycline by 67%, and after an hour of simulated rain the material was near the detection limit. The authors concluded that oxytetracycline concentrations on trees in orchards would be insufficient to suppress the pathogen

X. arboricola pv. *pruni* after 2 days under full sunlight, 4 days under overcast skies, or 2 min during a heavy rainstorm (8).

Even though chemical residues may be detected, antibiotics have a short duration of activity on plants in the environment. The low persistence of antibiotic activity on plant surfaces is remarkable given that compounds such as streptomycin can reduce the incidence of fire blight by 90% on pathogen-inoculated trees (53). Before the epidemiology of fire blight was understood (1, 59), growers realised that antibiotic activity on plants was limited, and they sprayed trees every five days for up to 20 applications in season (34). Understanding when the pathogen is predicted to be on plant surfaces (and vulnerable to antibiotics) has reduced the number of sprays to one to three sprays for economic disease control and decreased the potential for residues on fruit.

A direct link between antibiotic sprays on plants and antibiotic resistance in clinical bacteria has not been demonstrated

Models generated by the United States Environmental Protection Agency indicate that the potential for direct exposure of humans and their microflora to antibiotics deployed for crop protection is several thousand-fold less than for the medical use of antibiotics (61, 62, 63). One potential threat is that antibiotic applications on plants may select for antibiotic-resistant bacterial pathogens, resulting in adverse effects on human health (61, 62, 63). Flower tissues of pear and apple are the target of most antibiotics used in plant agriculture. When flowers open, few bacteria are detected (19, 42, 54). Bacteria from environmental sources immigrate and may colonise flowers over time given favourable environmental conditions. As flowers develop and form fruit tissues, detectable populations of bacteria decrease and are restricted to the stem end and the calyx end of the fruit (58). The intact waxy surface of the fruit does not support bacterial growth. The genera of bacteria on flowers that may be treated with antibiotics are common plant-associated bacteria – human pathogens have not been detected in surveys (42, 54). Given this, direct enrichment of antibiotic-resistant human pathogens with antibiotic sprays on plants is unlikely.

It is well established that bacteria harbouring transmissible antibiotic resistance genes are common in the environment, even in environments that have never been exposed to exogenous antibiotics (11, 17, 40, 46, 57). Furthermore, from experience with fire blight, it is clear that antibiotic applications on plants can enrich populations of antibiotic-resistant bacteria present at the time of the application by reducing competition from other microorganisms. The antibiotic-resistant bacteria that are competent phyllosphere colonisers can persist in the environment. Acquisition of antibiotic resistance genes, such as *strA/strB*, which inactivates streptomycin, by *E. amylovora* has been demonstrated in two locations (6, 39), but acquired resistance in this prevalent floral bacterium is uncommon. Given that human pathogens are not common colonisers of pome fruit flowers, the probability of direct acquisition of antibiotic resistance genes from resident phyllosphere bacteria in the tree canopy is reduced.

During the process of spraying trees with antibiotics, undoubtedly a portion of the material lands on the orchard floor and potentially could select for pools of antibiotic resistance genes in the soil, but this supposition has not been supported by recent studies (11, 40, 67). Rodriguez-Sanchez *et al.* (44) repeatedly applied gentamicin and oxytetracycline to coriander plots, and the abundance of antibiotic-resistant bacteria, resistance genes or plasmids was not influenced by antibiotic treatment. This finding is not actually surprising given that many antibiotics do not remain active in soils. Subbiah *et al.* (56) demonstrated that tetracycline was absorbed onto soil particles and rapidly rendered inactive. The fluoroquinolone ciprofloxacin was also found to be inactive in soils (56), and the authors speculate that the chemically related quinolone antibiotic, oxolinic acid, used in rice paddies (26), may also be inactivated in soil. From these studies, the authors speculate that antibiotic residues introduced to soils from foliar applications would have minor effects, if any, in exerting selection pressure for an increase in antibiotic resistance genes in soils.

Conclusion

In plant agriculture, antibiotics are used primarily on perennial plants, specifically pear and apple for the control of fire blight. Growers make substantial investments in orchards, which start producing fruit about five years after planting and may be productive for decades. This investment can be lost in a single spring as a result of an epidemic of fire blight. Antibiotic sprays during flowering in the spring are essential when disease pressure is high and are the most effective intervention tools for the control of fire blight. Antibiotics are effective only when used as a prophylactic; they are not curative when sprayed on infected trees. Antibiotics are expensive, and, coupled with concerns over the development of antibiotic resistance, tree fruit growers limit their use and utilise disease risk models to predict their need and the optimum timing for greatest efficacy.

Currently, only four antibiotics are used for plant disease control, and it is unlikely that additional antibiotics will be registered, especially those that are clinically relevant. Growers are in the precarious position of needing to spray antibiotics to control disease while trying to conserve sprays to minimise the development of antibiotic-resistant plant pathogens. They use other management tools that can be integrated with antibiotics to provide disease control and minimise the development of resistance. Managing bacterial diseases depends mostly on host plant resistance (which may not be available in desirable crop varieties), sanitation (e.g. preventing the introduction of pathogens and removing diseased plants) and cultural practices (e.g. avoiding overhead irrigation and limiting nitrogen fertilisation) (37). In some cases, chemical bactericides (e.g. copper compounds) and biological control agents can be integrated into the disease management programme (19, 37, 41, 52, 55). Disease risk models are used to optimise the timing of antibiotic sprays and reduce the number of unnecessary sprays for disease control. Finally, when available, antibiotics are often used in combination or as alternating sprays. Excellent control of fire blight has been obtained in the USA with sprays containing both streptomycin and oxytetracycline or by using antibiotics in rotation with bacterial biological control agents such as *Pseudomonas fluorescens* strain A506 (19, 23, 55). The prudent use of antibiotics in agriculture contributes to the long-term efficacy of these important tools for the management of bacterial diseases of plants and mitigates potential undesirable effects on the environment.

Utilisation des antibiotiques en agriculture (productions végétales)

V.O. Stockwell & B. Duffy

Résumé

Les antibiotiques jouent un rôle essentiel pour lutter contre les maladies bactériennes des végétaux, en particulier le feu bactérien des poiriers et la tache bactérienne des pêchers. La streptomycine est utilisée dans plusieurs pays ; en revanche, l'utilisation de l'oxytétracycline, de l'acide oxolinique et de la gentamicine est limitée à quelques pays seulement. La pulvérisation d'antibiotiques au printemps empêche la croissance des agents pathogènes sur les fleurs et les feuilles avant qu'une infection ne se produise ; après l'infection, les antibiotiques sont sans effet. Les antibiotiques ne sont utilisés qu'en cas de risque sanitaire élevé, de sorte que le traitement annuel ne concerne qu'une minorité de vergers. Aux États-Unis, le total d'ingrédients actifs administrés dans les vergers en 2009 s'est élevé à 16 465 kg, soit l'équivalent de 0,12 % de la quantité totale d'antibiotiques utilisés en production animale. La durée d'activité des antibiotiques sur les végétaux est inférieure à une semaine et aucune présence significative de résidus n'a été constatée dans les fruits récoltés. Aux États-Unis, les antibiotiques ont été indispensables pour protéger les récoltes pendant plus de 50 ans, sans aucun effet indésirable constaté sur la santé humaine ni d'impact durable sur l'environnement.

Mots-clés

Acide oxolinique – *Burkholderia glumae* – Dépérissement bactérien des panicules de riz – *Erwinia amylovora* – Feu bactérien des pommiers et des poiriers – Gentamicine – Lutte contre les maladies des végétaux – Oxytétracycline – Résidu d'antibiotiques – Streptomycine – Tache bactérienne des pêchers – *Xanthomonas arboricola* pv. *pruni*.

Uso de antibióticos en la agricultura

V.O. Stockwell & B. Duffy

Resumen

Los antibióticos son indispensables para luchar contra enfermedades bacterianas de las plantas, en particular el fuego bacteriano del peral y el manzano y el chancro bacteriano del melocotonero. En varios países se utiliza estreptomicina, mientras que muy contados países se sirven de oxitetraciclina, ácido oxolínico y gentamicina. La fumigación primaveral con antibióticos inhibe el crecimiento de patógenos en la superficie de flores y hojas antes de la infección; una vez iniciada ésta, los antibióticos son ineficaces. Estos fármacos se emplean cuando existe un elevado riesgo de infección, por lo que la mayoría de las plantaciones frutícolas no son tratadas cada año. En 2009, en los Estados Unidos se aplicaron a las plantaciones 16.465 kg de principio activo, lo que representa un 0,12% de la cantidad total de antibióticos utilizados en producción animal. Los antibióticos son activos en la planta durante menos de una semana, y en la fruta cosechada no se han hallado cantidades importantes de residuos de antibióticos. En los Estados Unidos, estos fármacos vienen siendo un elemento indispensable de la protección de los cultivos desde hace más de 50 años, sin que se hayan descrito consecuencias perjudiciales para la salud humana ni efectos persistentes en el medio ambiente.

Palabras clave

Ácido oxolínico – Añublo bacteriano de la panícula del arroz – *Burkholderia glumae* – Chancro bacteriano del melocotonero – *Erwinia amylovora* – Estreptomicina – Fuego bacteriano del peral y el manzano – Gentamicina – Lucha fitosanitaria – Oxitetraciclina – Residuo de antibióticos – *Xanthomonas arboricola* pv. *Pruni.*

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