Extensive efficacy tests of non-antibiotic fire blight control agents

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Abstract

Application of control agents to prevent new infections is an integral part of disease management for fire blight on pome trees caused by *Erwinia amylovora* (Burrill) Winslow et al.. Due to resistance development and human health concerns when using antibiotics, non-antibiotic control agents with high efficacy are urgently needed. In this study extensive efficacy tests with some classical antibiotic and relatively new non-antibiotic control agents for the prevention of bloom and shoot blight as well as phytotoxicity tests based on fruit russetting were carried out using young apple trees. Furthermore an experimental design for testing control agents preventing fire blight following hail injury was developed. The high efficacy of streptomycin (> 90%) and streptomycin containing spray strategies (> 80%) was confirmed. Non-antibiotic agents with high efficacy are LMA (85%), a potassium aluminum sulfate, and Juglon (80%). LMA is already in use in practice in Austria and Germany with a temporal and restricted permission and the administrative process to gain the legal status of a pesticide is ongoing whilst already admitted in Switzerland. Juglon, an organic compound from walnut, did show a high efficacy, but no consistent results (80.0 and 69.5%). Myco-Sin showed good results to prevent infection after artificial hail simulation. Antinfek showed high efficacy (90%) in preventing bloom blight, but could be tested only once so further research is needed to confirm this result. The design of the presented study enabled an extensive evaluation of the potential control agents and the aggregation and comparison of results and can be advised for other evaluations.

Keywords *Erwinia amylovora* · Control measures · Chemical control · Bacterial disease · Apple

Introduction

Erwinia amylovora (Burrill 1882) (Winslow et al. 1920), a Gram-negative, flagellated and rod shaped bacterium with a width of 0.3 µm and a length of 1 to 3 µm and an extracellular polysaccharide capsule, is the causative agent of fire blight, the most devastating bacterial disease of rosaceous plants, especially on apple and pears (Eastgate 2000). Implications are high economic losses in commercial pome fruit

production, namely on apple and pear (Bonn and van der Zwet 2000). First observed in North America in the nineteenth century, it successively spread around the world and today is present in most fruit growing areas in North America, Europe, the Mediterranean, the Near East and in New Zealand (Bonn and van der Zwet 2000). Disease symptoms are necrosis of twigs, flowers, and leaves and the typical “shepherd’s crook” of infected shoots (Eastgate 2000). Infestation mainly leads to die back of branches or whole plants. Flowers are the main point of entry for *E. amylovora*, but also wounds on leaves and shoots as a result of wind, rain, hail and insects are possible entry points (Thomson 2000). Bacterial ooze from infected tissue or fruit mummies is the primary source of inoculum. Dispersion between plants occurs by natural occasional vectors like insects, wind and rain, or through human activities (Thomson 2000).

Fire blight control in an integrated way includes a combination of different measures such as the application of chemical and/or biological control agents to prevent new infestations, planting tolerant cultivars and application of proper cultivation techniques such as careful pruning, proper fertilization and other phytosanitary measures to reduce vulnerability

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of plants (Steiner 2000; Joos et al. 2014). One of the main management tools however still is the prevention of new infections by applying control agents during critical stages particularly during bloom or after hail storms (Psallidas 2000). Several prediction models have been developed over the years and indicate days with high infection risk based on weather data and developmental stages of the plants (Steiner 2000; Pusey and Smith 2008). Agents used in practice are antibiotics (mainly streptomycin), yeasts or bacterial antagonists, copper containing pesticides or fertilizers, products with metal compounds, and resistance inducers (Psallidas 2000). According to their mode of action these agents are sprayed before favorable infection conditions occur to establish defense reactions through resistance inducers or through the action of antagonistic microorganisms. Otherwise agents with direct effects on epiphytic populations of *E. amylovora* are sprayed directly onto vulnerable plant parts during infection conditions.

Streptomycin was the first agent widely used to control fire blight under field conditions with a high and reliable efficacy and no phytotoxic effects (van der Zwet and Beer 1995). Due to the frequent development of resistance of *E. amylovora* strains against streptomycin caused by intense application, its use was reduced and substituted by antibiotics with lower efficacy (Adaskaveg et al. 2008; Rezzonico et al. 2009). Additionally, governmental restrains for the application of antibiotics or their ban in agricultural production in some countries led to a decline of their use in the control of fire blight. This decision was mainly based on fears that the use of clinical antibiotics in orchard environments may select for transmissible antibiotic resistance genes (Stockwell and Duffy 2012). Due to these developments the search for alternative control agents and strategies without antibiotics has become a hot topic for researchers worldwide.

Specific characteristics of future control agents are a high efficacy, “non-antibiotic” properties in terms of not used clinically to protect human or animal health, security for the environment, consumers and producers, no phytotoxic effects (plant and fruit), and potential use after hail storm damage. Despite the efforts put into this search for alternatives, no agent with the same efficacy and characteristics as streptomycin could be found until now (Tsiantos et al. 2003; Adaskaveg et al. 2008). Experimental designs were not uniform in these studies - e. g. designs with natural (Adaskaveg et al. 2008) or artificial (Tsiantos et al. 2003) inoculation exist and their statistical analyses vary greatly. A direct comparison of results has therefore to be viewed critically. A standardized experimental design under almost natural conditions using artificial inoculation for primary infection and evaluation of trees with secondary infections (Moltmann et al. 2002) was developed into a standard for efficacy evaluation of plant protection products by the European and Mediterranean Plant Protection Organization (EPPO 2002).

Apart from the efficacy of an agent, safety of plants and fruits are critical as phytotoxic effects, or the enhancement of fruit russetting have been described (Psallidas 2000; Spotts and Cervantes 2002). Tests for effects on plants and fruits should therefore be an integral part of efficacy testing. Infection after injury by hail is well known as a critical factor in fire blight epidemiology and described as trauma blight (Thomson 2000). Several studies have confirmed the possibility of infection and disease development after wounding of leaves and shoots and describe the efficacy of streptomycin to prevent fire blight symptoms in these cases (Babini and Mazzucchi 2000; Ockey and Thomson 2006). However, up to now to our knowledge no study on the efficacy of prevention of fire blight infection after hail injury with a standardized experimental design has been performed.

Here we describe a multilevel test design for novel agents that considers not only the efficacy of prevention of new infections, but also possible phytotoxic side effects on fruits. We wanted to know its applicability for prevention of fire blight infection after hail injuries as well.

Our multilevel test used an approved experimental design for field trials on young potted apple trees with artificial spray inoculation (EPPO 2002). Potential fire blight control agents were tested over a period of five years in different locations in Germany and an enhanced statistical analysis was applied. In addition, potential fire blight control agents were tested for their phytotoxic effects by evaluation of fruit russetting on harvested apple fruits. These extensive efficacy tests were complemented by a unique inoculation experiment with simulated hail injury to recognize the need of a control agent not only during bloom but also after hail damage.

Materials and methods

Control agents A total of 29 agents were tested in different experimental settings from 2009 to 2013. Table 1 shows all agents used grouped according to their mode of action and with their effective ingredients and applied concentrations. Table 1 also indicates the experimental setup for which the agents were used. Plots with artificial inoculation but no application of agents were used to calculate infestation and efficacy rates.

Bacterial strains and culture Bacterial strains are listed in Table 2. All strains used were virulent and streptomycin sensitive strains isolated from infected plants in Southwest Germany. All strains were provided by Dr. Esther Moltmann (Landwirtschaftliches Technologiezentrum Augustenberg, Außenstelle Stuttgart, Germany). Strains were cultivated in King’s B medium for 12 h at 27 °C under agitation at 250 rpm. Cell number was determined photometrically at 600 nm and calculated by the formula

Table 1 List of tested agents with common name, concentration and number and type of experiment

Group	Name	Agent	Concentration [%]*	No. of tests / Type of trial		
				Field	Phyto-toxicity	Hail
A. Antibiotic	Strepto	streptomycin	0.06	3	3	2
B. Metal compounds	Br-612	metal salt	0.03	1	–	–
	Lime sulfur	lime sulfur	1.5	–	2	–
	LMA	potassium aluminum sulfate	1/2/3 (resp.)	2	3	2
	Myco-Sin	aluminum sulfate	1	–	–	2
	VP 20	aluminum salt	2	1	2	–
C. Antagonists	Abi 09	<i>Bacillus amyloliquefaciens</i>	5	1	–	–
	Bloomtime	<i>Pantoea agglomerans</i>	0.002	1	1	–
	Blossom Protect	<i>Aureobasidium pullulans</i>	1.05 + 0.15**	1	4	–
	<i>Erwinia tasmaniensis</i>	<i>Erwinia tasmaniensis</i>	0.015	1	–	–
	Serenade Max/ASO	<i>Bacillus subtilis</i>	0.5/1	1	2	–
D. Copper containing	CuCaps	copper capsulated	2.8	1	1	–
	DM 31/32	copper	1	–	1	–
	Selecta Disperss	copper	0.15	–	–	1
	Sergomil	copper	0.15	–	1	1
	Vittafos, Curamin	phosphate, copper	0.2	2	1	1
E. Resistance inducers	Chitoplant	chitosan	0.1	1	1	1
	Vacciplant	laminarin	0.075	–	1	–
F. Fertilizers	LX 4630 + additive	calcium formiate	1.5	1	2	–
	Xedaphos	phosphate, potassium	1	–	–	2
	3DS	3-deoxy-flavonoide	10	1	–	1
G. Others	AFB	polyhexamethylene biguanide	2	1	1	1
	Akasoil	polymeric guanidine	0.2	1	2	–
	Antinfek	polyhexamethylene biguanide	1	1	1	–
	Juglon	5-hydroxy-1,4-naphtalindion	0.087	2	1	2
	NPA 25	organic acid	2	1	1	–
	Planta 4+	disinfectant	1	1	1	–
	Regulex	gibberellins a4 + a7	0.00044	–	2	–
	Test agent WS 2	not known	not known	–	1	–
H. Spray strategies	Strategy 1: Serenade Max (1X)/ Strepto (1X)/ LX4622 (2X)	<i>B. subtilis</i> / Strepto/ calcium formiate	0.5 / 0.06 / 2	1	–	–
	Strategy 2: Blossom Protect (1X)/ Strepto (1X)/ LX4622 (2X)	<i>A. pullulans</i> / Strepto / calcium formiate	1.05 + 0.15** / 0.06 / 2	1	–	–
	Strategy 3: Blossom Protect (1X)/ Strepto (3X)	<i>A. pullulans</i> / Strepto	1.05 + 0.15** / 0.06	1	–	–

*Concentration of formulated product in spray mixture

**Two different components

Application of agents according to manufacturer's data and fire blight forecast model

cell number [cfu/ml] = $OD_{600} \times 3.3 \times 10^9$. In the field trails a mixture of strains isolated in the previous year in Southwest Germany with a tested high virulence were used to reflect the actual threat for apple producers in the region.

For the mixtures the three strains were separately cultivated and cell densities photometrically determined. Then they were mixed in a 1:1:1 (v:v:v) ratio to the final concentration of the inoculation suspension (Table 2).

Table 2 *Erwinia amylovora* strains used and concentration in inoculation suspension. Only trials that yielded results were taken into account

Strain, strain mixture	Concentration [cfu/ml]	Experiment	Number/timing of inoculation events
Ea 385	10^6	hail simulation	4 h after leave injury
Ea 763, Ea 839, Ea 840	1.2×10^8	field trial 2009	05.05.; 09.05.
Ea 797, Ea 839, Ea 851	1.2×10^8	field trial 2010	17.05.; 21.05.
Ea 782, Ea 797, Ea 914	1.2×10^8	field trial 2012	02.05.; 08.05.; 11.05.

Strain names follow the denominations of the Julius Kühn-Institut (JKI), Quedlinburg, Germany. Strain mixtures were in a ratio of 1:1:1 (v:v:v)

Field trials with artificial spray inoculation Field trials were conducted with three year old 2.0 m high potted ‘Gala’ apple trees on rootstock M9 in a randomized complete block design with four replicates per treatment. Each plot comprised ten surveyed trees in a row, distance between rows was 2.0 m, and distance between plots in row was 1.5 m. One extra tree was directly spray inoculated with a hand sprayer until run off with a suspension of *E. amylovora* (1.2×10^8 cfu/ml) in the mixtures listed in Table 2. The directly spray inoculated tree was in the middle of the plot having 5 trees on both sides with a distance of 1.2 m to the next tree. Distance between the evaluated trees was 0.7 m.

Tests were carried out at two different locations in Southwest Germany. The locations “Mühlingen” and “Vogt” are on the boundary of the Lake Constance fruit production area in Baden-Württemberg in a remote quarter to prevent spreading of the bacterium to production sites. Dispersal of bacteria within the plots occurred by natural vectors, wind, and rain. To improve the spread of inoculum natural vectors of the genera *Apis*, *Bombus*, and *Osmia* were released in every experiment. The directly inoculated trees were sprayed with the *E. amylovora* suspension at the beginning of bloom and 2 to 3 times during bloom when the forecasting model, a Maryblyte model adapted to the regional characteristics of Southwest Germany (Moltmann and Herr 2011), showed a high infection risk to keep disease pressure in the plots high. Application of the control agents (concentrations indicated in Table 1) with a water volume of 1000 l per ha (0.3 l per tree) took place directly after initial inoculation and again during bloom when the forecasting model showed a high infection risk (3 to 4 applications per location and year). According to the forecasting model, infection risk varied over years between high (2009, 2011, and 2012) and medium (2010, and 2013).

At the location “Mühlingen” application was carried out with a parcel fan sprayer (SZA 32, Wanner, Wangen, Germany; flat spray nozzle TeeJet TP80015, Spraying Systems Co., Wheaton, USA), at a pressure of 9.0 bar and a speed of 4.0 km per hour. At the location “Vogt” application was conducted with a handheld backpack sprayer (SHR 170SI, Echo Motorgeräte, Metzingen, Germany; flat spray nozzle TeeJet TP8004, Spraying Systems Co., Wheaton, USA) at 4.0 bar.

Twenty-four days after the first inoculation each tree was evaluated by the number of flower clusters with visible fire blight symptoms compared to the total number of flower clusters at that time. Total number of flower clusters per tree was around 60 resulting in *ca.* 2400 flower clusters per treatment over all plots per location.

To gain homogeneity of variance data were transformed with an arcsine angular transformation for statistical analyses and re-transformed for graphic display. An ANOVA was carried out with the statistical models shown in Table 3. Whereas in all trials the same design elements were used as model effects, in 2012 an additional design element (distance = space between sampled tree and inoculated tree) was integrated to improve the accuracy of the model. The efficiencies of the tested agents were calculated in each block as real repetition according to Abbott: efficacy = (no. of infected flower clusters in control plot - no. of infected flower clusters in treated plot) / no. of infected flower clusters in control plot (Abbott 1925). The experimental design of the field trials with artificial spray inoculation is according to the EPPO standard PP 1/166 (3) (EPPO 2002).

Phytotoxicity tests for fruit russetting Phytotoxicity tests were conducted with 2.5 m high ‘Golden Delicious’ apple trees planted 2000 and 2001 in a randomized block design with four replicates. This cultivar is known for its sensitivity towards fruit russetting. Five trials, one per year, were conducted from 2009 to 2013 in Bavendorf, Baden-Württemberg, Germany. Agents were applied by a parcel fan sprayer (SZA 32, Wanner, Wangen, Germany; flat spray nozzle TeeJet TP80015, Spraying Systems Co., Wheaton, USA) at 7.0 bar at a speed of 5.5 km per hour three times during bloom with concentrations provided in Table 1 and a water volume of 500 l per ha.

Per treatment, 4 * 250 fruits were harvested and scored for fruit russetting (% fruit surface with russetting) and grouped in four categories (category 1: no russetting; category 2: up to 10% russetting; category 3: 10% to 30% russetting; category 4: more than 30% russetting). Categories 1 and 2 are generally marketable fruits. A fruit russetting index (FRI) was calculated using the formula: (no. fruits in category 1 * 1 + no. fruits in category 2 * 2 + no. fruits in category 3 * 3 + no. fruits in category 4 * 4) / no. of total fruits. The FRI shows

Table 3 Statistical models and procedures applied in the different experiments. Only trials that yielded results were taken into account

Experiment	Year	Model	SAS procedure
1 Field trials with artificial spray inoculation	2009, 2010	infection = treatment + place: place • row + place • column	Proc MIXED
	2010	infection = treatment + place + distance: place • block + place • block • treatment + place • block • distance	Proc MIXED
2 Phytotoxicology tests for fruit russetting	2010, 2012	russetting = treatment: replication	Proc MIXED
3 Simulated hail injury inoculation experiment	Trial no. 1	infection = treatment + column + new leaves: replication necrosis = treatment + column: replication	Proc GLIMMIX Proc MIXED
	Trial no. 2	infection = treatment + column: replication necrosis = treatment + column + shoot length: replication	Proc GLIMMIX Proc MIXED

Notation according to Piepho et al. (2003): “+” addition of effects; “•” crossing of effects; “:” separates fixed from random effects, listing fixed effects first
 Model elements: place = location of trail; row, column = site within experimental plot; distance = space between sampled tree and inoculation tree; new leaves = number of new leaves per sampled sapling; shoot length: length of sampled shoot

marketability of fruits by grade. Statistical analyses were done by ANOVA using the statistical model shown in Table 3. A mean comparison test with a letter-based representation of all pairwise comparisons was performed for years with significant treatment effect (Piepho 2004).

Simulated hail injury inoculation experiments Potted apple saplings (‘Gala’ on M9) with a height of 50 cm and 10 plants per treatment, were cultivated in a climate chamber with 12 h, 24 °C day and 12 h, 18 °C night cycle; RH > 80%. Leaves were injured using a portable flower thinning machine with rotating plastic strings (Electro’flor, INFACO, Cahuzac-sur-Vère, France). Plants were subsequently spray inoculated with an *E. amylovora* suspension (10^6 cfu/ml). This procedure aimed to simulate the natural conditions after a hail storm event with irregular wounds on leaves, high temperature and moisture (24 °C, RH > 80%). To analyze temperature effects pre-tests were conducted with colder (20 °C) and warmer (27 °C) conditions during the 4 h after injury. Application of control agents (concentrations listed in Table 1) took place 4 h after injury with a handheld backpack sprayer (SHR 170SI, Echo Motorgeräte, Metzingen, Germany; flat spray nozzle TeeJet TP8004, Spraying Systems Co., Wheaton, USA) at 4.0 bar. Plants were set up in a randomized complete block design afterwards under the same conditions as during the cultivation period. After three weeks plants were scored on incidence (yes/no) and severity of fire blight infection (visible length of necrosis [cm]). Statistical analyses were done by ANOVA using the statistical models shown in Table 3. The models used include uniform spatial design elements. In order to enhance the accuracy of the model vegetative characteristics of the plants (number of new leaves, shoot length: length) were integrated as effects in the model if a significance level of $p < 0.05$ was achieved for their influence on the results. For the infestation rate (percentage of infected plants per treatment) a generalized linear model was used to account for the binomial distribution of the data. It must be taken into account that due to the characteristics of a binomial model confidence

intervals are relatively wide. The results of two simulated hail injury inoculation experiments are presented in this paper.

Data analyses Statistical analyses were carried out with the software SAS 9.3. (SAS Institute Inc., Cary, USA). Models used for investigations are listed in Table 3. The achieved significance level for statistical tests was $p < 0.05$. The effective value lies with a probability of 95% within the borders of the displayed confidence interval based on the model used.

Results

Field trials with artificial spray inoculation Field trials were conducted in five subsequent years (2009 to 2013). In three years untreated plots showed severe infestation rates (2009: 8.2%; 2011: 8.5%; 2012: 8.6%). In 2010 (3.8%) and in 2013 (2.3%) the infestation rate in untreated control plots did not reach the required 5% level (EPPO 2002) due to unfavorable cold weather conditions. This prevented vigorous disease development. Therefore, no conclusion on the efficiency of a treatment can be drawn and these data were excluded from further analyses. Statistical analyses showed a significant treatment effect in all three years ($p < 0.0001$). The effect of location was never significant (2009: $p = 0.3083$; 2011: $p = 0.1815$; 2012: $p = 0.1513$). Results were therefore grouped according to year. Results for the analyzable years 2009, 2011, and 2012 are shown in Figs. 1, 2 and 3.

Streptomycin showed high efficacies in all trials with 93.3, 95.0, and 99.5% in the respective years. Therefore, streptomycin can be used as a reference standard in all experiments conducted in this study. Likewise, spray strategies involving streptomycin also proved to be effective (77.0, 83.9, and 88.3%). The different spray strategies that consisted of applications of streptomycin in rotation with non-antibiotic agents with lower efficacy (Table 1) did not significantly differ from the single application. The number of streptomycin applications in the spray strategies in total is reduced from 3 to 4 times

Fig. 1 Results of the field trial with artificial spray inoculation in 2009. Median of infected flower clusters (bars) with confidence interval and efficacy of treatment (circles). Strategy 1: Serenade Max, Strepto, LX4622. Strategy 2: Blossom Protect, Strepto, LX4622

to 1 application in combination with agents of lower efficacy. Apart from the high efficacy in preventing bloom or shoot blight, streptomycin caused no phytotoxic reactions on plants and fruits.

Other agents with comparable high efficacy were Antinfek (92.8%), LMA in the concentrations in the tank of 1%, 2% and twice 3% (88.4, 87.6, 91.9, and 85.6% respectively), and VP 20 (91.9%). Juglon (80.0 and 69.5%) did show a high efficacy, but no consistent results. None of the applied antagonists showed such high efficacies. Blossom Protect (56.0%) and Bloomtime (45.6%) resulted in the highest score within the antagonists. The only copper containing agent that reached high efficacy (CuCaps, 98.3%) caused severe phytotoxic effects on leaves of treated plants. The resistance inductor Chitoplant showed insufficient efficacy, as well as all other agents tested in the field trial. Apart from streptomycin, only the agents Antinfek, Juglon, LMA, and VP 20 resulted in an

acceptable high efficacy but further research is necessary to finally define the potential of the agents under other climatic conditions and with other cultivars and fruits.

Phytotoxicity tests for fruit russetting Over years 32 agents were tested for phytotoxic effects on fruits (agents shown in Table 1). The fruit russetting index (FRI) in untreated plots varied over the years reflecting different natural conditions that favor or prevent russetting (2009: 2.5; 2010: 2.0; 2011: 1.8; 2012: 1.9; 2013: 1.6). In three years (2009, 2011 and 2013) no treatment resulted in a significant higher FRI than in untreated plots (data not shown). In 2010 and 2012 (Figs. 4 and 5) significant increased fruit russetting resulted only from the application of copper agents (DM 31, DM 32, CuCaps). In the pairwise comparison with the control, a slightly positive statistical effect was observed for Regulex ($p < 0.048$), but with unlikely relevance for practical use. Moreover, this effect

Fig. 2 Results of the field trial with artificial spray inoculation in 2011. Median of infected flower clusters (bars) with confidence interval and efficacy of treatment (circles). Strategy 3: Blossom Protect, Strepto

Fig. 3 Results of the field trial with artificial spray inoculation in 2012. Median of infected flower clusters (bars) with confidence interval and efficacy of treatment (circles)

could not be confirmed in the following year when there was no significant treatment effect. Regarding the FRI, none of the agents with high efficacy against *E. amylovora* in the field trials showed an effect on fruit russetting.

Simulated hail injury inoculation experiment In the hail simulation experiments pre-tests showed clear effects of temperature of the plant on shoot blight incidence. Lower temperature (20 °C) during the first 4 h after inoculation led to only few fire blight symptoms in all plots with only 5 to 20% infected shoots (data not shown). By contrast, disease incidence was high (all shoots infected) and no reducing effects of agents were observed when temperatures after inoculation were high (27 °C).

Fire blight incidence in untreated plots differed in the two main experiments presented (72.6% and 84.3%) which can be taken to reflect differences in infection pressure as temperature

conditions were the same (24 °C day, 18 °C night). Streptomycin showed a very high efficacy in reducing disease in the simulated hail damage experiment with a low absolute number (0% and 6.7% in the two experiments respectively) and a low lesion length of affected shoots as a parameter for disease dynamics (0 cm; 8.1 cm). Under lower disease pressure (experiment 1, Fig. 6) LMA, Sergomil FB, and Xedaphos showed some reduction in affected shoot number and in necrotic shoot length, while this reduction proved not to be significant in the statistical model. Under higher disease pressure (experiment 2, Fig. 7) Juglon, LMA and Myco-Sin showed a reduction, while Sergomil FB and Xedaphos did not perform as well as in the first experiment. Treatment effect from the applied agents on infected shoots was significant ($p = 0.0431$). In none of the tests treatment effect from the agents showed significance on length of necrosis. In experiment 2 the total shoot length [cm] had a significant effect ($p = 0.0122$) on the

Fig. 4 Effects of treatments on the russetting of harvested apple fruits 2010. Numbers with the same letter are not significantly different

Fig. 5 Effects of treatments on the russetting of harvested apple fruits 2012. Numbers with the same letter are not significantly different

length of necrosis. Combining results of both experiments, lowest number of affected shoots after simulated hail injury showed streptomycin (0% and 6.7%), followed by LMA (8.7% and 30.8%), Juglon (26.4%; 27.6% and 39.9%), and Myco-Sin (27.8% and 27.9%) in the two experiments respectively. All other agents tested gave no reduction and showed a high number of affected shoots and extensive necrotic lesions on the affected shoots. Due to the low sample size per treatment confidence intervals were relatively wide and differences between treatments therefore need to be interpreted with caution. But this possibly can be overcome by planning bigger sample sizes in future experiments.

Discussion

In this study extensive efficacy tests of non-antibiotic fire blight control agents were carried out. These included field

trials over three years (two years with lower infection pressure excluded) with artificial spray inoculation, tests on phytotoxic effects in the form of fruit russetting, and tests with simulated hail injury. By this test scheme the identification of an agent with high efficacy for preventing bloom and shoot blight with no phytotoxic effects as a follow-up agent for streptomycin proved to be possible. The control compound streptomycin showed an efficacy of over 90% in preventing fire blight infections during bloom or after simulated hail injuries. This result confirms the findings of other researches (van der Zwet and Beer 1995; Psallidas 2000) and is well-known from practical use. Application of streptomycin in rotation with non-antibiotic agents with lower efficacy resulted in efficacies from 77.0 to 88.3%. This is likely to be still acceptable for practical use.

In the group of control agents with metal compounds an agent with potassium aluminum sulfate (LMA) resulted in a high efficacy (> 85%) in preventing bloom blight and hail-

Fig. 6 Results of the first simulated hail injury inoculation experiment with lower disease pressure by infected shoots (dark grey) and length of necrosis (light grey) with confidence intervals. Juglon A/B: with/without exposition to light after application

Fig. 7 Results of the second simulated hail injury inoculation experiment with higher disease pressure by infected shoots (dark grey) and length of necrosis (light grey) with confidence intervals

simulated trauma blight in several tests, while no phytotoxic effects were observed. Therefore, this agent might be suited to substitute streptomycin. Experiments under field conditions performed by other research groups using LMA showed similar results (Fried et al. 2014; Kunz and Donat 2014). A mixed application of LMA together with several other agents commonly used in apple production is also possible (Scheer et al. 2014). The agent LMA has a low solubility in water. This problem can be solved in practice by vigorous mixing of a stock solution (max 6%) in warm water. LMA (2%) is already in use in practice in Austria and Germany with a temporal and restricted permission and the administrative process to gain the legal status of a pesticide is in process. In Switzerland the permission was granted in 2019.

Another agent with a metal component we tested, Myco-Sin (aluminum sulfate), which is approved for organic production, showed sufficient efficacy in preventing infection after hail injury. It was not tested in the experiments for prevention of bloom blight but is known to have medium efficacy (Kunz et al. 2012). VP 20, a test agent with an aluminum salt, showed a sufficient efficacy in one year but necessary further research is not possible as the producer did not continue to develop the agent.

Out of the tested products containing antagonists, Blossom Protect and Bloomtime showed medium efficacy while the others could not reduce fire blight incidence sufficiently. In other studies clearly higher efficacies were reported for Blossom Protect (Kunz et al. 2012) and Bloomtime (Sundin et al. 2009). Establishing a sufficient colonization of open flowers by antagonists is necessary to achieve prevention of bloom blight (Kunz et al. 2012). This implies that application of antagonists must occur before the infection risk is high and under favorable conditions for propagation of the antagonist. These requirements might not have been fulfilled in our study and could therefore explain the relatively low efficacies. An aggravation of fruit russetting due to the application of

Blossom Protect is described in literature (Spotts and Cervantes 2002), but could not be observed in our experiments. The treatment with Blossom Protect did not lead to significant difference with the untreated plots in four phytotoxicity tests when applied three times.

One agent with copper (CuCaps) showed a high efficacy in field trial but severe phytotoxic effects on fruits. Other tested copper containing agents showed no phytotoxic effects but only exhibited medium or low efficacy, which confirms other findings (van der Zwet and Beer 1995; Psallidas 2000). All agents that significantly increased the FRI in phytotoxicity tests with application during bloom contained copper. Copper agents can therefore offer only a basal protection and may be used on young trees with no fruiting (van der Zwet and Beer 1995; Psallidas 2000). Furthermore it is reported that application until the phenological stage “green tip” does not lead to russetting, even not on the fruits of russet sensitive pear cultivars (Elkins et al. 2015).

A resistance inducer tested (Chitoplant) showed only medium efficacy but was tested only in one test. This might result from the low potential of chitosan towards *E. amylovora* (El Hadrami et al. 2010), or it might be due to difficulties in application that are not known yet. In order to induce resistance, respective defense mechanisms must be activated prior to contact with the pathogen (Zeller 2006). This might not have happened in our trial due to wrongly timed applications. However, the potential of resistance inducers in preventing fire blight infections is proven for Acibenzolar-s-methyl (Baysal and Zeller 2004) and prohexadione-calcium (Norelli and Miller 2004) and further research might focus on their correct mode of application. The resistance inducers tested (Chitoplant, Vacciplant) did not influence fruit russetting.

Among the tested fertilizers with possible control on fire blight as side effect, calcium formiate showed medium efficacy to prevent bloom blight when applied alone and exhibited

sufficient efficacy when integrated in a spray strategy in combination with streptomycin. This result confirms results from other tests (Scheer 2012). Xedaphos showed a high efficacy in the hail experiment with lower disease pressure. However, this could not be confirmed in the hail experiment with higher disease pressure. None of these tested agents influenced fruit russetting.

Other tested agents contained various effective compounds. Antinfek, containing a polyhexanide, showed a high efficiency in preventing fire blight - comparable to streptomycin. But the number of tests in this study containing this agent was too low to have clear evidence (only one test). Unfortunately, this test agent was no longer available and therefore could not be further tested. Other agents with the same (AFB) or similar (Akasoil) active ingredient did not reach the same efficacy level. Further research is necessary to clarify the potential of these agents.

The control agent Juglon, containing juglone, an ingredient from walnut (*Juglans regia* L.) (Cosmulescu et al. 2011), yielded medium to high efficacy in flower and hail experiments. As the tested agent is a simple spray mixture of the compound in water with no additives, further enhancement by developing it as a pesticide seems possible. None of the agents with various effective compounds, but Regulex, had an influence on russetting. This agent with gibberellins slightly reduced the incidence of russetting but with no significant effect for the marketability of the fruits based on the FRI. Results from materials tested only in one year in a successful trial should be considered preliminary, as the efficacy varies greatly under different environmental conditions as shown in our experiments. The existence of two controls per experiment, untreated plots and streptomycin as a reference standard, still makes it possible to interpret the results.

The field trials presented here originate from the urgent need of the producers for an alternative to streptomycin. Therefore the emphasis was on screening as many potential agents as possible under natural conditions. This led to an inhomogeneous experimental design regarding the tested agents over the years and locations whilst fundamental experimental design was consistent (replications, measures etc.). This leads to the question how reliable the results for a single agent can be. Taking into account the untreated control in every single experiment to reflect the disease pressure and the application of streptomycin in every single experiment as a well known reference, interpretation of the effectiveness of a single agent can be drawn in comparison to these two levels.

Our statistical analysis of the field trials enables an aggregation of site specific results and the comparison of several years with different weather conditions, agents, application techniques, and inoculation suspension. These statistical analyses using adequate linear models combined with a standardized experimental design is a possibility to overcome the heterogeneity reported for worldwide tests (Psallidas 2000).

Efficacy tests that follow our scheme can possibly be used to benchmark potential fire blight control agents that are effective under diverse environment conditions.

The experimental design of our field trials with artificial spray inoculation of flowers, dissemination within the plots by natural vectors and evaluation of secondarily infected trees represents almost natural conditions (Moltmann et al. 2002). In trials with natural infection (Laux et al. 2003; Adaskaveg et al. 2008) there is always the risk that disease pressure can be too low or even absent. In experiments with direct inoculation of flowers, followed by treatment and evaluation of the same flowers (Sundin et al. 2009), disease pressure can be much higher than in naturally occurring infection and therefore the efficacy of the tested agents might be underestimated. Our experimental design, closely following Moltmann et al. (2002), proved to be a possible good compromise between the two experimental designs mentioned above and hence may lead to results more relevant for practice. Still, due to unfavorable weather condition during bloom, our experimental design can lead to insufficient infestation rate in untreated control plots (<5%) and results can therefore not be interpreted and used what happened in two of the five test years.

The simulated hail injury inoculation experiment proved to be suitable to evaluate the efficacy of the tested agents on damaged leaves. This overcomes the lack of an adequate experimental design with simulating natural conditions after a hail storm event with irregular wounds on leaves, high temperature and moisture under controlled environment. The statistical analysis enables to describe the infestation rate by percentage of infected plants per treatment and the severity of fire blight infection by the visible length of necrosis. To gain more statistical accuracy, further experiments with bigger sample sizes are necessary. Application of effective agents could also accompany pruning activities during days with high infection risk to prevent subsequent occurrence and/or intensification of shoot blight. This could be evaluated in detail in future research.

In this study various experiments to evaluate the efficacy of fire blight control agents were conducted in a successive screening process. Additionally, tests to assess phytotoxic effects of the agents were performed by evaluating impact on the fruit russetting. Testing was completed by simulated hail injury inoculation experiments to gain knowledge upon the effectiveness of the agents studied when applied after plant injuries.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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