

Schlussbericht Soul-Bio Projekt: Module ‚Sorten X Unterlagen- kombinationen‘ und ‚Agronomische Eignung für den Bioanbau



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1. Einleitung

Alle vorgesehenen Arbeiten im Projekt Soul-Bio wurden termingerecht abgeschlossen. Die Berichterstattung an das BLW erfolgte jeweils gemeinsam mit Agroscope. In diesem Schlussbericht wird über die abschliessenden Arbeiten in der letzten Projektphase in Form eines ‚Executive Summary‘ berichtet. In Kapitel 4 wird zusammenfassend und ausführlich über das Modul ‚Effekt verschiedener Sorten x Unterlagen-kombinationen auf die Feuerbrand-Anfälligkeit bei Apfel und Birne‘ berichtet.

2. Modul ‚Effekt verschiedener Sorten x Unterlagen-kombinationen auf die Feuerbrand-Anfälligkeit bei Apfel und Birne‘

Die letzten Prüfungen unter Gewächshausbedingungen fanden im Jahr 2013 statt. Am int. ISHS-Feuerbrandtagung an der ETH Zürich im Juli 2013 wurde zu den Resultaten ein Posterbeitrag präsentiert. Das Thema wurde von den mitdiskutierenden SymposiumsteilnehmerInnen als sehr wichtig erachtet. Hingegen war es die einzige Studie, die dort Daten zu dieser Fragestellung präsentiert hat.

Die Jahre 2014 und 2015 wurden in diesem Projektmodul genutzt für profunde Auswertungen über alle 4 Jahre der Versuche mit Gewächshausinfektionen (2010-2013) mittels der Masterarbeit von Matthias Schlucher von der Universität Hohenheim. Betreuender Professor war Prof. Dr. R.T. Vögele (Abteilung Phytopathologie) und betreuender Wissenschaftler war Dr. F. Weibel vom FiBL (Dept. crop science). Die Methodik und Resultate sind in der beigelegten Masterthesis (pdf-Dokument) ausführlich beschrieben. Bei FB-teil-toleranten Edelsorten konnte regelmässig festgestellt werden, dass wenn sie auf FB-toleranten Unterlagen veredelt sind, die Infektionsstärke (gemessen als äusserlich sichtbare Läsionslänge) nochmals um mehr als die Hälfte reduziert werden konnte verglichen mit Veredelung auf einer FB-anfälligen Unterlage (z.B. M.9). M. Schlucher und F. Weibel arbeiten zurzeit am Manuskript zur Publikation der Resultate in einer wissenschaftlichen Zeitschrift (Abstract siehe Kasten).

Die Studien mit den künstlichen Infektionen an frisch getopften und erstmals austreibenden Winterhandveredelungen im Gewächshaus haben uns auch diverse methodische Limiten dieser Methodik gezeigt: besonders bei Birnen ist der Austreiberfolg und die Variabilität der Wuchsstärke sehr variabel. Die Wuchsstärke des Austriebs steht jedoch mit der Symptomausprägung in Zusammenhang, weshalb wir zu schwach austreibende Pflanzen nicht in der Datenauswertung berücksichtigt haben, und so teilweise auch statistische Power verloren haben. Eine Übertragung der Erkenntnisse auf das Verhalten der geprüften Sorten x Unterlagenkombinationen auch unter Feld- bzw. unter Praxisbedingungen ist deshalb mit erheblichen Unsicherheiten behaftet. Für weitere Versuchsaktivitäten wurden deshalb Bäume für künftige Freilandversuche vorbereitet (siehe unten).

Die im Projekt und in den Nachfolgeaktivitäten stets sehr gute Zusammenarbeit mit agroscope erlaubte uns, diverse interessante neue Kernobst-Selektionen, wo uns der Züchter aber keine Angaben zur Feuerbrandresistenz machen konnte, auch nach dem wichtigen Kriterium der FB-toleranz zu beurteilen. Auch alte Sorten im Rahmen einer Zusammenarbeit mit Pro-Spezie-Rara konnten und können so geprüft werden.

Ausgehend von den interessanten Resultaten, die in der Tendenz und in verschiedenen Fällen auch statistisch sicherbar die Hypothese bestätigen, dass Bäume die aus Kombinationen von FB-toleranten Unterlagen mit teil-toleranten Sorten bestehen die FB-Toleranz der Sorte zusätzlich steigern, haben wir zusammen mit den KollegInnen von agroscope auch die Folgeaktivitäten nach Projektabschluss geplant: so wurden zur Überprüfung der Gewächshausresultate unter Freilandbedingungen 2jährige Bäume ausgewählter Unterlagen x Sortenkombinationen angezogen für Freiland-Versuche in der Feuerbrand-Testanlage auf dem Breitenhof in Wintersingen. Diese Versuche werden in den Jahren 2017 in engster Zusammenarbeit mit agroscope durchgeführt.

Wir erwarten mit älteren Bäumen unter Freilandbedingungen eine noch deutlichere Akzentuierung des Unterlageneffekts zur Feuerbrandunterdrückung, da die Unterlage absolut und auch relativ zum Spross nun wesentlich mehr aktive Biomasse besitzt als es bei den jungen Handveredelungen wie sie im Gewächshausversuch verwendet werden der Fall ist. Die nun grössere Wurzelbiomasse sollte in der Lage sein, rascher und/oder mehr Feuerbrand-unterdrückende Sekundärmetaboliten zu produzieren.

Es war im Rahmen dieses Projekts bzw. über den Zeitraum dieses Projekts hinweg nicht möglich, eigene Abklärungen zu machen oder andere Forschungsarbeiten zu finden, die hinreichend den Wirkungsmechanismus der Feuerbrand-unterdrückenden Wirkung bestimmter Unterlagen x Sortenkombinationen erklären können. Wären diese bekannt, könnte man einerseits gezielter geeignete Kombinationen voraussagen, und andererseits könnten in der Züchtung, oder in Genressourcenbanken von Sorten und Unterlagen viel gezielter nach auch in dieser Hinsicht vorteilhaften Genotypen selektioniert werden. Aus diesen Gründen kann diese Thematik als wichtige künftige Forschungsfrage bezeichnet werden (... further reseach is needed ...).

Abstract (Manuskript M. Schluchter und F. Weibel für eine wissenschaftliche Zeitschrift)

Fire blight disease caused by the enterobacterium *Erwinia amylovora* (BURILL) WINSLOW ET AL. is the most devastating and difficult to manage bacterial disease in economic apple (*Malus domestica* BORKH.) and pear (*Pyrus communis* L.) production. There is scientific consensus that utilizing tolerant cultivars and rootstocks is the most sustainable approach to control fire blight. Over a period of four years (2010-2013), this study examined rootstock induced effects on the fire blight tolerance of the cultivar scion after artificial inoculation under greenhouse conditions. For the tests, cultivars and rootstocks were chosen over a range from highly fire blight susceptible to highly tolerant. All possible full-factorial combinations were grafted and tested. Significant cultivar x rootstock interactions on fire blight tolerance were found in all years for apple, and in two years for pear. Cvs. Ladina and Galiwa revealed high rootstock sensitivity. Both, Ladina and Galiwa grafted on Geneva rootstocks G.11 and G.41 were significantly more fire blight tolerant compared to M.9. With pear, however, such a tendency could not be found. Our study reveals and confirms the remarkable, in commercial fruit growing yet unexploited potential of cultivar x rootstock interaction effects as a tool to prevent fire blight damage in apple production. Whereas, our findings with apple under greenhouse conditions should be confirmed in field experiments. More efforts should be carried out to exploit better the fire blight control potential of rootstock x cultivar combinations also with pear. In addition to that more research is needed to better understand the active fire-blight suppressing principle in the different rootstock x scion combinations. This would possibly allow both: i) more specific rootstock and cultivar breeding; and ii) to predict tolerant rootstock x scion combinations without the necessity of conducting extensive inoculation tests.

3. Modul ‚Agronomische Eignung verschiedener feuerbrand-toleranter Apfel- und Birnen Sorten bzw. Apfelunterlagen für biologischen Erwerbsanbau‘

Laufend wurden die aus den Gewächshausversuchen gewonnenen Erkenntnisse auch in die Konzeption der Sorten- und Unterlagenprüfung am FiBL bzw. Bio-Aussenstandorten sowie in die jährliche Sortenbewertung und Anbauempfehlungen für den Bio-Kernobstau einbezogen. Dies betrifft auch die sogenannten „Sorten-Team Versuche“ wo die als für die Bio-Produktion und den Bio-Markt besonders interessanten Apfelsorten auf mindestens 3 Bio-Praxisbetrieben mit Baumzahlen von 400 bis 1000 pro Sorte und Betrieb in Testung stehen. Birnensorten sind aus verschiedenen Gründen wie z.B. Nicht-Erhältlichkeit der für Bio interessantesten Selektionen bzw. sehr konservatives Verhalten bei der Sortenwahl bei Produzenten und im Handel leider noch nicht auf dem Level für Sortenteamversuche mit Apfel. Ebenso hat das gleichzeitige Aufgleisen von Vergleichen verschiedener Unterlagen auch in den Sorten-Team Versuchen bisher leider nur teilweise geklappt, da es schlussendlich in den meisten Fällen schon äusserst schwierig war, überhaupt so hohe Baum-Stückzahlen von ganz jungen Sorten – teilweise sogar erst Selektionen - zu organisieren.

Bezüglich der Unterlagen-Leistungsprüfung lässt sich zusammenfassen, dass sich die Unterlagen der Cornell University am Campus Geneva NY (CG unterlagen) Nr. 41 und 11 als über alle Versuchsjahre sehr FB-tolerant erweisen. Agronomisch überzeugt vor allem CG.11. Diese Unterlage wird nun endlich auch in Europa von holländischen Baumschulen vermehrt und die Praktiker können entsprechende Sorten x Unterlagenkombinationen bestellen. Dem entsprechend empfiehlt das FiBL auch bei den heutigen erhältlichen schorf- und feuerbrand(teil)toleranten Sorten Ariane, Ladina, Natyra diese auf CG.11 veredeln zu lassen.

Bei den Birnen scheint es sich bei der heute zur Verfügung stehenden Wahl an Unterlagen nicht zu lohnen, die FB-anfälligkeit der Unterlagen mitzuberücksichtigen: OH-11 und OH-89 haben die diesbezüglichen Erwartungen nicht erfüllt. Andere FB-toleranten Birnen-Unterlagen sind uns nicht bekannt.

Momentan stehen in den Sorten-Teamversuchen folgende Apfelsorten: die sehr FB-robuste Ariane (in Gewächshausversuchen ähnlich robust wie Rewena), die teilrobusten Sorten Ladina, Natyra und Galiwa sowie die eher anfällige Galant und Rustica. Letztere wurde, trotz den negativen Resultaten aus den Gewächshausversuchen bezüglich Feuerbrandtoleranz einbezogen, da sie a) nicht FB-anfälliger ist als Gala, b) eine im Bio-Anbau sehr wichtige Marktlücke schliesst (sehr lange lagerfähig, grosskalibrig, gesuchte Geschmacksgruppe), c) eine sehr gute Schorf- und Mehlttauresistenz zeigt, d) gute Erträge und einfach zu erziehende Bäume aufweist sowie e) partiell selbstausdünnend ist, was wiederum für den Bio-Anbau, wo Alternanz eines der Hauptprobleme darstellt, sehr vorteilhaft ist. Die Sorte Galant erhält ihre Berechtigung in den Sorten-Team Versuchen dadurch, dass der Biobereich seit Jahren dringend eine Alternative zur leider sehr schorfanfälligen Hauptsorte Gala sucht.

4. Schlussfolgerung und Ausblick

Dank dem Soul-Bio Projekt konnte die für den Bio-Kernobstanbau zentral wichtige Sorten- und Unterlagenprüfung unter Bio-Bedingungen mit wichtigen wissenschaftlichen Detailuntersuchungen begleitet und vertieft werden. Es konnten für die Praxis, aber auch für die Methodik der Sorten- und Unterlagenleistungsprüfung sowie für Gewächshaus Inkubationsversuche sehr wichtige Resultate, Erkenntnisse und Erfahrungen gewonnen werden.

Auch eine stärkere internationale Vernetzung zum Thema Sorten/Unterlagen/Feuerbrand wurde dank dem Soul-Bio Projekt ermöglicht (z.B. Zusammenarbeit mit KOB Bavendorf, Uni Hohenheim, Teilnahme an int. Workshops etc.). Die angesprochenen Erkenntnisse wurden in den Folgeaktivitäten (seit 2015) grösstmöglich und wiederum in enger Zusammenarbeit mit agroscope einbezogen und genutzt.

Dr. F. P. Weibel

Frick, den 17. Juni 2016

5. Annex: Detailbericht , Four year assessment of the cultivar × rootstock interaction on fire blight tolerance'

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Four year assessment of the cultivar × rootstock interaction on fire blight tolerance

Master thesis

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Degree program: Organic Agriculture and Food Systems

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List of Abbreviations

ACW	Agroscope Changins–Wädenswil
B.9	Budagovsky 9
cfu	colony forming units
crc/s	cultivar × rootstock combination/s
cri	cultivar × rootstock interaction
DNA	Deoxyribonucleic acid
FiBL	Forschungsinstitut für biologischen Landbau
Gala	Gala Galaxy
G.11	Geneva 11
G.41	Geneva 41
M.9	Malling 9
OHF 11	Old Home × Farmingdale 11
OHF 87	Old Home × Farmingdale 87
PR	pathogen related (proteins)
QBA 29	Provence Quince
QC	Quince C
QE	Quince Eline
qg	quarantine greenhouse
ROS	reactive oxygen species
sdh	sorbitol dehydrogenase
wpi	weeks post inoculation

1. Abstract/Summary

Fire blight disease caused by the gram negative enterobacterium *Erwinia amylovora* (BURILL) WINSLOW ET AL. is the most important bacterial disease in economic apple (*Malus domestica* BORKH.) and pear (*Pyrus communis* L.) production. Under certain conditions, fire blight can be highly devastating and advance rapidly, possibly leading to complete orchard destruction. Several authors hypothesized that utilizing a tolerant cultivar grafted to a tolerant rootstock is the most promising approach to combat fire blight. Although separate fire blight tolerance assessments of either cultivars or rootstocks are available, there is a lack of combined assessments of the cultivar \times rootstock interaction on fire blight tolerance.

During 4 successive years, between 2010 and 2013, cultivar \times rootstock combinations have been grafted, and the trees were subsequently kept under controlled greenhouse conditions. The concept was to carry out the tests with cultivars and rootstocks that represent a gradient from highly susceptible to highly tolerant plant material: e.g. from cv. Gala to Rewena, and from rootstock M9 to G.41. Shoots were artificially needle inoculated with 10^9 cfu ml⁻¹ of the Swiss *E. amylovora* strain ACW 610. The necrotized shoot length was measured for 3 times in weekly intervals, starting 1 week after inoculation. The reference date for statistical analysis was set to 3 weeks after inoculation since the disease pattern was most distinct at that point. All combinations have been statistically assessed on an annual basis. Additionally, 2-year intervals have been assessed in 3 periods (2010–2011, 2011–2012, and 2012–2013). In order to identify only distinct significant differences that enable a practice-relevant discrimination between trees which can be restored through pruning and severely infected trees that need to be cleared, a Tukey's HSD test was performed as post-hoc analysis. Combining all years, 6 scab resistant cultivars (Ariane, Galiwa, Ladina, Natyra, Rewena, and Rustica) as well as Gala, and 6 rootstocks (AR 295-6, B.9, G.11, G.41, M.9, and Supporter 2), amounting to 34 different cultivar \times rootstock combinations have been included in the tests with apple. For the tests with pear, 7 cultivars (ACW 3764, ACW 3851, Conférence, Elliot, Hortensia, Roksolana, Uta) and 5 rootstocks (OHF 11, OHF 87, QBA 29, Quince C, and Quince Eline), resulting in 20 different cultivar \times rootstock combinations, have been statistically assessed.

In the four single year assessments, the cultivar \times rootstock interaction was significant in all years for apple and in two years for pear. The least relative shoot necrosis in the apple assessment was achieved by Ladina \times G.11 in 2012 (13.4%) and 2013 (3.2%). Moreover, the summarized value of Ladina \times G.11 in 2012–2013 was significantly different to Ladina \times M.9 and even 21% below the relative shoot necrosis of the tolerant reference Rewena \times M.9. Over the same period and independent of the rootstock, the cultivar Ariane showed stable values of relative shoot necrosis, that were below the tolerant reference Rewena \times M.9. Furthermore, Galiwa \times G.41 achieved a significantly lower relative shoot length than Galiwa \times M.9 in 2010 (- 60%). However, in contrast to apple, no specific pear cultivar \times rootstock combination

constantly revealed a low relative shoot necrosis. Nevertheless, the tolerant reference cultivar Elliot grafted on OHF rootstocks (87 and 11) proofed the lowest values in 2011 and 2013. Moreover, the assessment of two-year periods showed a significant annual effect for apples and pears.

The frequently significant cultivar \times rootstock interactions confirm the fire blight tolerance screening of cultivar \times rootstock combinations as useful procedure to identify *E. amylovora* tolerant trees. The results give evidence how important it is to consider fire blight tolerance of the entire combination rather than solely the tolerance of cultivar or rootstock. This is essential to both, growers and scientists. However, to select likely fire blight tolerant cultivar \times rootstock combinations, the separate screening of cultivars and rootstocks remains of major importance. Furthermore, the results imply that a robust rootstock may on the one hand increase the tolerance of a tolerant cultivar, but on the other hand, it cannot be the basis for a tolerant tree in connection to a susceptible cultivar. Moreover, in order to consider the observed annual variability, a repeated testing of combinations are recommended. Since no specific fire blight tolerant pear combination could be identified, further screening experiments with promising combinations must be conducted. For upcoming apple and pear screenings, a sufficient amount of plant material and a perennially stable composition of combinations is recommended. Furthermore, concentration measurements of compounds which are considered to be indicators for fire blight tolerance should be included in future experiments.

2. Introduction

2.1. History, distribution and economic importance of fire blight

Affecting members of the family *Rosaceae*, fire blight nowadays is the most devastating bacterial disease in economic apple and pear production (Vanneste 2000). Under certain conditions, fire blight can advance rapidly and possibly lead to complete orchard destruction. The fire blight disease is caused by the gram negative enterobacterium *Erwinia amylovora* (BURILL) WINSLOW ET AL. (Winslow et al. 1920) and was first observed in 1780 in the State of New York. By movement of humans and goods by horse, rail and car, fire blight spread to every region of the USA within the first 135 years after its detection (Bonn and van der Zwet 2000). Outside of North-America, fire blight was first recorded in Japan in 1903 followed by New Zealand in 1919. The introduction of fire blight to Europe most probably resulted from infested bud wood or trees coming from North America, which have been transported to the United Kingdom. Affecting susceptible pear cultivars, first disease outbreaks in the United Kingdom have been reported in 1958, whereas on the European mainland fire blight was not recorded until 1966 in The Netherlands and 1967 in Poland. The disease reached Germany in 1971 and spread further to Switzerland in 1989. Within Switzerland and Germany, especially around the warm and humid fruit growing region of Lake Constance and in central Switzerland, fire blight has become a severe threat to economic apple and pear production (Bonn and van der Zwet 2000; Hasler et al. 2002). Nowadays fire blight has spread globally, and particularly within the past 30 years, the number of countries in which *E. amylovora* has been detected has rapidly increased from 15 in 1977 to 47 in 2012 (van der Zwet and Beer 1991; van der Zwet et al. 2012).

Economic costs of fire blight incidents are difficult to assess, since small damages like a few blighted flower buds are not recorded and reported. However, Norelli et al (2003) reported that a 10% infection with rootstock blight and subsequent tree loss in a 4 year old high density planting may cause an economic loss of 8.400 US \$ ha⁻¹. They considered tree replacement, investment loss in tree establishment and maintenance as well as yield loss over several years. In 2007, a very severe fire blight year in Central Europe, economic fruit growers in the Austrian region Vorarlberg suffered an average military damage due to fire blight that amounted to 9.500 € ha⁻¹ (Schwärzler et al. 2011). Within Switzerland only, estimated total costs for control of fire blight between the years of 1989 and 2000 amounted to 9 million US \$ (Hasler et al. 2002).



Figure 1: Young apple orchard devastated by fire blight. Source: (Norelli 2015).

Due to several reasons the global economic losses caused by the fire blight disease are likely to increase. First of all, fire blight is still spreading geographically into new apple and pear production areas (Vanneste 2000). Furthermore, high density orchards consisting of susceptible cultivar \times rootstock combinations (crcs) that bear early, pose an increased risk for rapid disease spread and high economic losses (Vanneste 2000; van der Zwet and Beer 1991). Moreover, most economically used apple cultivars have a very low degree of fire blight tolerance (Steiner 2000). The cultivar composition in Lower Saxony, which produces most of the apples in Germany, is highly susceptible, for example. The top 5 produced apple cultivars in 2014, accounting for a share of 74% of all apples produced are Elstar, Jonagored, Jonaprince, Braeburn, and Jonagold, which are all susceptible to fire blight (Statistisches Bundesamt 2015). Also in the organic sector the 4 most-produced apple cultivars that accounted for almost 2 thirds of the whole production in Western Europe in 2011/2012 are susceptible to fire blight: Gala (28%), Elstar (16%), Topaz (11%), and Braeburn (9%) (Weibel 2013). In economic apple production, the susceptible cultivars are mostly combined with the rootstock M.9, which is also highly susceptible to fire blight (Steiner 2000; van der Zwet et al. 2012).

2.2. Disease cycle and plant pathosystem

2.2.1. Disease cycle

Primary infection occurs in spring when bacteria, originated primarily from last year's cankers, but also resident bacteria, invade the host's blossoms or shoots (Figure 2). Bacteria from cankers are transmitted by wind, rain and insects and are able to enter the host plant via natural openings in flower parts – mainly the nectarthodes –, or via wounds e.g. as a result of damage from hail, strong wind or thunderstorms (Thomson 2000). Subsequently, bacteria multiply and move into the intercellular space. At this stage, bacteria are already abundant in sufficient

amounts to be transferred by wind, rain or pollinating insects to blossoms and openings of other plants. Within a few days, death of plant cells sets in, necrosis is evident and small drops of bacterial ooze are visible. However, latent bacterial infestation in asymptomatic tissue has equally been reported (Joos et al. 2012). Subsequent to the primary infection and throughout the growing season, secondary infection may occur, based on the bacterial ooze from primary infection. Particularly the infection of immature fruit results in a fast development of inoculum for repeated secondary infection. Towards the end of the growing season, bacterial multiplication slows down, eventually resulting in canker formation. These cankers serve the bacteria as shelter for overwintering. At the beginning of the next growing season, some of the cankers become active, again providing bacterial inoculum for primary infection (Johnson and Stockwell 1998; Thomson 2000; van der Zwet and Beer 1991). The severity of fire blight depends on the quantity and quality of the pathogen, the susceptibility of the host, and environmental conditions (van der Zwet and Beer 1991). Fire blight symptoms at different stages of infection throughout the disease cycle are displayed in Figure 3.

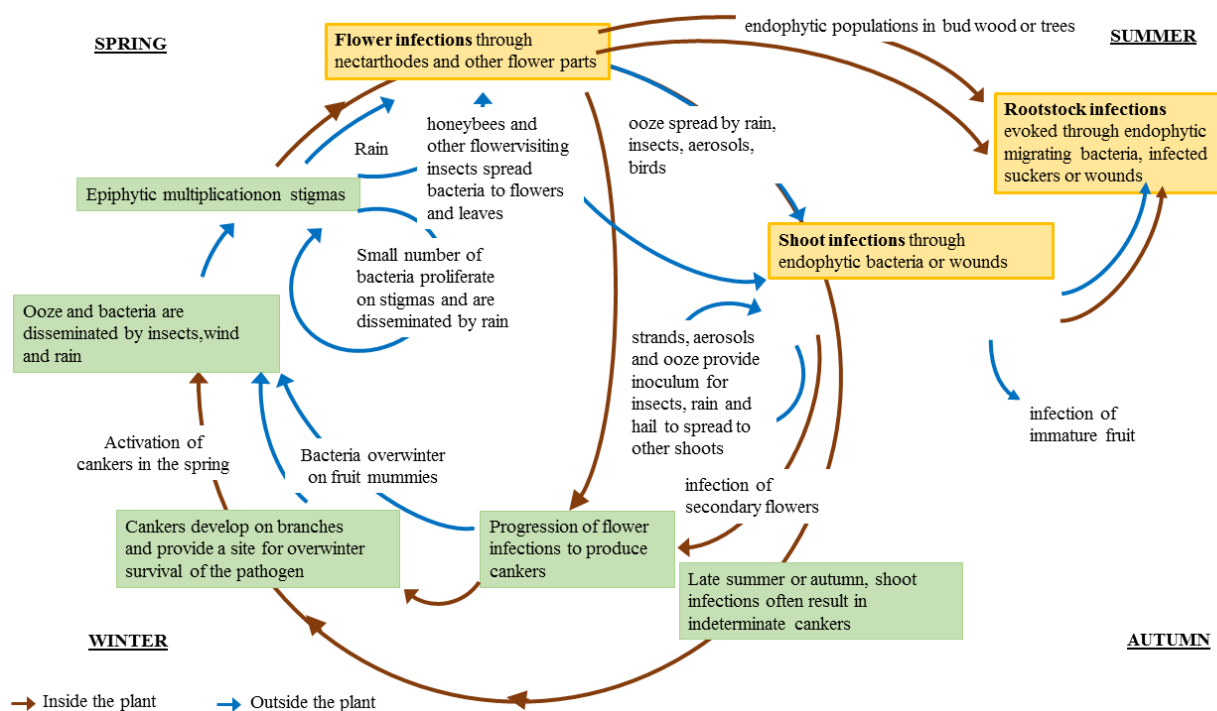


Figure 2: Fire blight disease cycle (modified after Thomson (2000) and Olbrecht (2008)).



Figure 3: Fire blight symptoms.

Top left: Fire blight canker with cracked margins and bacterial ooze. Top right: Infected blossom.
 Bottom left: Infected shoot with characteristic shepherds' crook at the tip and bacterial ooze. Bottom right: Infected Fruit.
 Sources: Top left, top right and bottom right: (Ministry of Agriculture Food and Rural Affairs Ontario 2011) Bottom left:
 (Schöneberg (13.04.2015), Personal Communication)

2.2.2. Pathogenicity of *E. amylovora*

The plant-pathogen interaction of *E. amylovora* and its host plants is well described by Vrancken et al. (2013). Once inside the plant, *E. amylovora* bacteria proliferate and start to produce exopolysaccharides (e.g. amylovoran, levan), leading to a physical pressure that enables bacteria to migrate through the intercellular space. *E. amylovora* bacteria insert effector proteins through their type III secretion system into the cytosol of plants, resulting in a hypersensitive reaction of the plant and programmed cell death (Bantleon 2012; Vrancken et al. 2013). There are currently 12 effector proteins known that are secreted via the type III secretion system of *E. amylovora*, which suppress host defense and promote further infection (Nissinen et al. 2007).

2.2.3. Plant defense

There are several plant defense mechanisms that are induced by an infection with *E. amylovora* (Vrancken et al. 2013). The first observable reaction of a fire blight infected plant is a rapid increase of reactive oxygen species (ROS), elicited by proteins that entered the plant via the type III secretion system (Venisse et al. 2001). Furthermore, a change in levels of compounds that are derived from the phenylpropanoid-flavonoid pathway, such as flavonoids, phenolamines and lignin is identifiable. Moreover, levels of plant hormones such as salicylic acid

and jasmonic acid may change (Vrancken et al. 2013). Another defense mechanism is the production of pathogenesis-related proteins (PR proteins). PR proteins exhibit an antimicrobial activity and are only synthesized as a reaction to pathogen attack. Furthermore, the abundance of phytoalexins was recorded in infected apple and pear trees. These are secondary metabolites with antimicrobial activity that are induced following biotic and abiotic stress (Vrancken et al. 2013).

2.2.4. Cultivar × rootstock interaction

Based on practical field observations, Jackson (2003) hypothesized that “*apple rootstocks also influence the incidence of diseases of the scion through mechanisms distinct from those of rootstock resistance*”. Although rootstock effects on the fire blight tolerance of the cultivar have been observed, only little is known about the mechanisms behind this interaction (Cline et al. 2001; Jackson 2003; Jensen et al. 2012).

As far as rootstock mediated effects to the scion are known, they can be separated into external and internal effects. External effects relate to tree morphology and the relationship between tree and way of production, whereas internal effects relate to plant metabolism. External effects are the development of early bearing and production in high density plantations. Early bearing of the scion, induced by dwarfing rootstocks, increases the susceptibility of young trees, since particularly young plant tissue is highly susceptible to fire blight (Jackson 2003). Utilizing dwarfing rootstocks enables fruit growers to enhance the productivity by increasing the tree density within an orchard. At the same time high density plantings increase the density of host plants and facilitate bacterial dispersion within the orchard.

By applying an apple DNA microarray, several internal cultivar × rootstock interaction (cri) mechanisms have been identified by Jensen et al. (2012). They reported that different rootstocks had a significant effect on the susceptibility of the cultivar Gala. They also examined gene expression levels in Gala scions and associated these with a rootstock-induced decrease in fire blight susceptibility, and identified the expression of the phenylpropanoid pathway as good predictor for fire blight tolerance. Furthermore, sorbitol dehydrogenase (sdh) was found at increased levels in more tolerant trees. Probably sdh impedes the availability of the sugar alcohol sorbitol to *E. amylovora*. In addition, in more tolerant trees, a higher gene expression was generally recorded for genes involved in response to biotic and abiotic stress. Particularly a heat shock protein, calnexin and a Sec61 homolog were associated with reduced fire blight susceptibility. Calnexin is important for protein processing in the endoplasmic reticulum in the context of the secretory pathway. Moreover, the processes endocytosis and peroxisomal pathways were found to be more active in less susceptible trees. These findings allow plant breeders to perform a marker assisted selection of plant material (Jensen et al. 2012).

2.3. Management options

In order to control fire blight, it is generally recommended to set up a strategy combining a range of measures rather than single measure approaches (Häseli 2013; Steiner 2000; van der Zwet et al. 2012). Thus, an application of bactericide or a microbial antagonist should be integrated into a program including the selection of tolerant plant material, cultivation methods such as pruning, proper irrigation and fertilization, and a monitoring system (Steiner 2000).

2.3.1. Preventive measures and adaptation of crop cultivation methods

There are several preventive measures that can be implemented to minimize the risk of a fire blight infection in an orchard. For example, the introduction of infected plant material in an orchard can be prevented or hail nets can be put up in order to avoid tree wounds, which may serve as portal for entry for bacteria.

Crop cultivation methods aim for a reduction of bacterial inoculum in the orchard. Sanitary measures, like pruning of infected plant parts in the growing and the dormant season or clearing of infested trees are important methods to avoid further disease dispersion in the orchard (Steiner 2000; Voegelé et al. 2010). Due to a high susceptibility of young and vigorously growing trees, there are cultural practices recommended – such as proper irrigation and fertilization – that promote the growth of a balanced, slowly growing tree (Steiner 2000). Furthermore, the spread of bacteria within an orchard can be reduced by a proper control of phytophagous and sap-sucking insects (Bantleon 2012).

2.3.2. Application of active agents

Spray treatments are applied to avoid blossom infections. To achieve a good efficacy, they need to be timely harmonized with a fire blight prediction program, such as Maryblyt™. Biological control of fire blight can be achieved by the application of antagonistic microorganisms, specifically *Aureobasidium pullulans* (DE BARY) G. ARNAUD, *Pseudomonas fluorescens* MIGULA, *Lactobacillus plantarum* BERGEY ET AL, *Bacillus subtilis* COHN, or *Pantoea agglomerans* GAVINI ET AL. While Kunz and Haug (2006) reported a fire blight control efficacy of 72% for the preparation ‘Blossom Protect’ that contains the yeast-like fungus *A. pullulans*, a meta-analysis has revealed a comparatively low efficacy of single biocontrol products – namely *B. subtilis*, *P. agglomerans*, and *P. fluorescens* – for fire blight control (Ngugi et al. 2011).

Application of copper formulations reduces the primary *E. amylovora* inoculum. However, copper compounds are phytotoxic (Psallidas and Tsiantos 2000). For direct fire blight control in organic agriculture there are currently two products recommended. The first is based on *A. pullulans* and sold as ‘Blossom protect’. The second product is an aluminium sulfate named

‘Myco-Sin’. To achieve a good efficacy against *E. amylovora*, both products need to be embedded in a multi-level strategy (Häseli 2013).

Antibiotics that are applied in fire blight control are mainly streptomycin and oxytetracycline. Limitations to the use of antibiotics are the occurrence of *E. amylovora* strains that are resistant to streptomycin and regulations either on a national scale or on the production level, particularly in organic agriculture (McManus et al. 2002).

2.3.3. Selection of a cultivar × rootstock combination

Among all management options, the most important measure to sustainably manage fire blight and to reduce financial losses in the long-term is the use of a combination of tolerant cultivars and rootstocks (Bantleon 2012; Ferree et al. 2002; Korba et al. 2008; Sobiczewski et al. 2014; van der Zwet et al. 2012). This is the basis of an integrated strategy, including some of the above mentioned measures. Although there is evidence that the interaction of cultivar and rootstock has an effect on the tolerance of the cultivar, the particular mechanisms behind this tolerance interaction are unknown (Jackson 2003; van der Zwet et al. 2012). This might be one reason why most research in testing fire blight tolerance is conducted by either testing the tolerance of cultivars (Korba et al. 2008; Nybom et al. 2012; Perren et al. 2013; Sobiczewski et al. 2014) or rootstocks (Aichholz 2012; Ferree et al. 2002; Norelli et al. 2003b). However, a significant rootstock effect to the cultivar was reported by Jensen et al. (2012), indicating that the selection of a rootstock significantly affects the tolerance of the cultivar. Contrasting this scientific information, the actual market development in apple and pear production is heading towards a specialization in the cultivation of a low number of crcs, which are mostly susceptible to fire blight (Steiner 2000).

2.4. Scope of this study

This study aims to analyze the cultivar × rootstock interaction of apples and pears regarding its fire blight tolerance. Based on this analysis, significant differences among the fire blight tolerance of cultivar × rootstock combinations can be obtained, and tolerant combinations identified. Together with the knowledge on the agronomic and market properties of the cultivars and rootstocks, this allows to recommend promising *E. amylovora* tolerant cultivar × rootstock combinations for the fruit growers. Furthermore, an extensive literature review shall provide a discussion of the methods and results gained in this study. It is of particular importance for reliable practical recommendation to investigate results of studies with the same plant material. To reassure a reproducibility of the experiment and the subsequent statistical analysis, the obtained methods will be thoroughly documented.

Finally, conclusions addressing separately the scientific methodology on the one hand and practical fruit growing on the other hand will be given.

2.5. Hypotheses

This thesis investigates the following hypotheses:

- (1) Apple and pear rootstocks can have a significant effect on the *E. amylovora* tolerance of the scion (cultivar).
- (2) With highly *E. amylovora* susceptible cultivars, rootstocks cannot prevent severe fire blight damage.
- (3) The results are consistent for several years.

3. Materials & Methods

The fire blight tolerance of crcs was tested during 4 succeeding years from 2010 to 2013. The trials were operated in facilities of Agroscope in Wädenswil, Switzerland.

3.1. Graftage and pregrowing

Rootstocks and cultivar scions were acquired in a period from September until January each year, prior to the experiment. The scions originated from the compound of Agroscope, Wädenswil and were cut by technical staff from Agroscope. At the same time, rootstocks were obtained from different sources (annex, Table 25 and Table 26). In the time between reception of the plant material and graftage, scions and rootstocks were kept in a cooling chamber at 2 °C and 96-97% relative humidity. The graftage was annually conducted in January by members of the technical staff of Agroscope. Depending on the diameter of rootstock and scion, two different graftage methods were applied. If the diameter of rootstock and scion was similar, a whip graft was conducted. In case the rootstock diameter was bigger than that of the scion, a side veneer-grafting (spliced side grafting) was performed (Bärtels 1996; Schöneberg (13.04.2015), Personal Communication). After graftage, the plants were put in moist sawdust and again kept in a cooling chamber at 2 °C and 96 – 97% relative humidity until the start of pre-growing. In May, the plants were potted in single pots filled with potting soil (Kübel- und Dachgartenerde, Floragard, N= 260 mg l⁻¹, P₂O₅= 250 mg l⁻¹, K₂O= 720 mg l⁻¹). Subsequently, the potted trees were kept in the growing station for 4 to 5 weeks. The daytime temperature inside the growing station ranged from 21 to 25 °C and was allowed to decline to a minimum of 18 °C at night. The relative humidity averaged 70% and to balance potentially occurring darkening times (below 250 W/m²), an illumination program was running daily from 7 am until 7 pm. In order to avoid plant infestation with aphids, a 0,75 l mixture of the insecticide PLENUM WG 50 (0,1%) in combination with an impact enhancing formulation, BREAK-THRU S240 (0,1%), was uniquely applied. Furthermore, Hauert Plantaktiv Typ Hydro (15+7+22), a nitrogen-phosphorus-potassium (NPK) fertilizer was applied 3 times at a concentration of 0.15%. The first application took place two weeks after potting the plants, followed by two more applications in weekly intervals. After 4 to 5 weeks of growing and prior to transferring the plants to the greenhouse, the plants were reduced to a single, most vital shoot, which had a length of ≥10 cm. Finally, the ten most promising individuals of each crc were selected and transferred to a quarantine greenhouse (qg). In some cases, due to insufficient growing, problems of affinity between rootstock and scion, or loss because of aphids, less than ten individual plants per crc could be utilized. Inside the qg, relative humidity was around 70% with dehumidification starting at 73%, and the temperature ranged from 18 to 23 °C with a medium temperature of 21 °C. Against the background of sharply rising temperatures and high radiation inside the qg, homogenous shading conditions were created by drawing curtains on the internal side of the windows. In order to avoid bacterial dispersion to the environment,

several security measures, such as restricted entrance for a particular group of scientists, and disinfection when entering and leaving the qg, were taken. An annual chronology of the experiment is given in Table 1.

Table 1: Annual chronology of the experiment

Process	Time
Reception	September-January
Storage until graftage	September-January
Graftage	January
Storage until growing	January-May
Growing	May
Transportation to security greenhouse	Beginning of June
Inoculation	1 week after introduction to qg
Measurement of Lesion Length	3 times in weekly intervals, starting 1 wpi

3.2.E. *amylovora* strain, inoculation and rating of lesion length

Prior to inoculation, shoot length was measured with a ruler. Inoculation of the plants with *E. amylovora* was conducted one week after transferring the plants to the qg. Actively growing shoots of apple and pear trees allow a more rapid progression of *E. amylovora* infection than slowly growing shoots (Hepaksoy et al. 1999; Ozrenk et al. 2012; Sobiczewski et al. 2014). Due to this, solely vigorously growing shoots were inoculated. Single colonies of the *E. amylovora* strain ACW 610 were stored in a freezer in 1-1.5ml tubes filled with Glycerol (40%) at -86 °C. Prior to the inoculation, bacteria were removed from the freezer and placed on nutrient agar plates. An inoculum of 10^9 cfu ml⁻¹ of the *E. amylovora* strain ACW 610 was used for injection with a 0.46-mm-diameter (26-gauge) hypodermic needle 0.5 cm below the shoot apex (Rezzonico and Duffy 2007). As shown in Figure 4, the needle was inserted through the shoot and the inoculum was injected in a way that a droplet at each side of the penetration leakage was visible.

Starting 1 week after inoculation and for 3 times in weekly intervals, visible lesion length was measured with a ruler. The relative lesion length was calculated as the length of the necrotized section of an inoculated shoot as percentage of the total shoot length. Subsequently to the last lesion length measurement, plants were properly disposed due to the high risk of unintentional bacterial dispersion.



Figure 4: Inoculation of a shoot with *E. amylovora* bacteria. Source: Schöneberg (13.04.2015), Personal Communication.

3.3. Experimental design

The experiment was designed for an annual testing of 40 different crcs (S1–S40) and a number of 10 individual plants per crc. This amounts to an overall number of 400 individual plants per year. As shown in Figure 5, the potted plants were split in two types of randomized racks, with each rack containing 20 plants. To store 400 plants, 20 racks were in use. The racks were split on four moveable tables (Figure 6, Tables 1, 2, 4, 5), and one bigger, stationary table (Figure 6, Table 3), with each table containing 3 to 18 racks. The racks were randomly aligned in different directions on the tables. For watering and tending the plants, the smaller tables (Tables 1, 2, 4, and 5) had to be moved.

S1	S2	S3	S4	S21	S22	S23	S24
S5	S6	S7	S8	S25	S26	S27	S28
S9	S10	S11	S12	S29	S30	S31	S32
S13	S14	S15	S16	S33	S34	S35	S36
S17	S18	S19	S20	S37	S38	S39	S40

Figure 5: Setup of randomized rack 1 and rack 2.

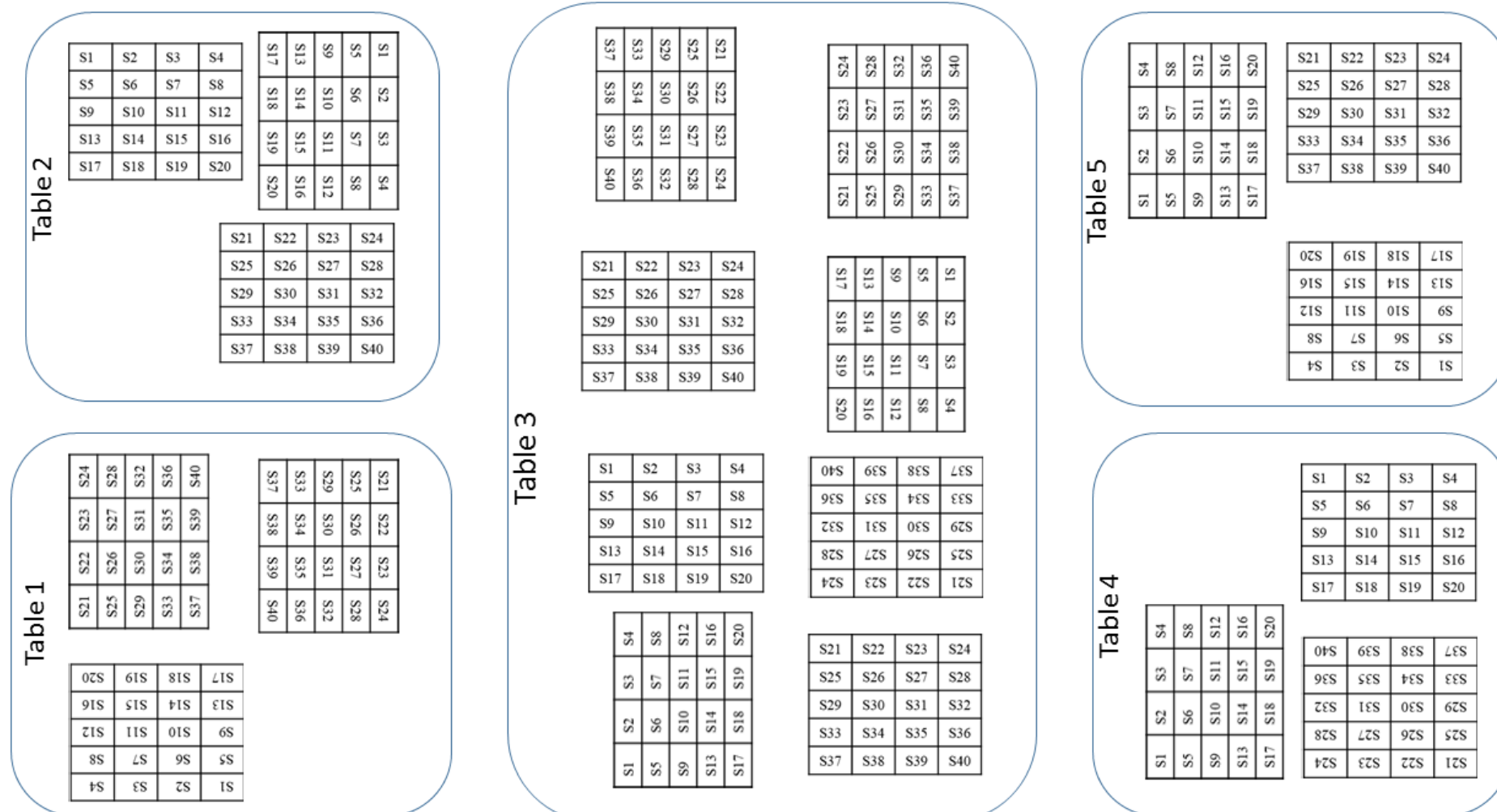


Figure 6: Experimental setup in the greenhouse.

3.4. Utilized plant material

Summing up, 68 different apple and pear combinations were used in the four years of the experiment. 30 combinations were tested in 2010, 33 combinations in 2011, and 40 combinations in 2012 and 2013. Besides fire blight characteristics, there are several other important features of cultivars and rootstocks regarding their horticultural performance. An overview of these features for the utilized cultivars and rootstocks is given in the following subsections separately for apple (Table 2, Table 3) and pear (Table 6, Table 7).

3.4.1. Apple

3.4.1.1. *Cultivars and rootstocks*

Table 2: Selected features of utilized apple rootstocks.

	AR 295-6	B.9	G.11	G.41	M.9	Supporter2
Origin	Robusta 5 × Ottawa 3	M.8 × Red Standard	M.26 × Robusta 5	M.27 × Robusta 5	Chance seedling	M.9 × Malus micromalus
Fire Blight						
---- Direct Inoculation	?	S ²	T ^{1,3}	Very T ¹	S ²	?
---- Scion inoculation	?	T ⁴ S ¹³	T ⁴	T ⁴	S ⁴	?
Phytophthora (Crown and Root Rot)	?	Very T ² I ¹⁰	T ¹	T ¹	T ²	T ¹⁰
Scab	?	I ²	?	?	I ²	T ⁹
Replant disease	?		T ¹ ; I ¹⁰	T ^{1, 10}		
Woolly Apple Aphid Resistance	?	S ²	High ¹	High ¹	S ¹²	High ⁹
Cold Hardiness	?	Yes ²	Yes ¹	Yes ¹	No ¹²	Yes ⁹
Yield efficiency compared to M.9	?	Similar ^{3, 6}	Better ^{1, 3}	Better ¹ Similar ³	-	Better ⁸ Similar ⁷
Suckering	?	Low ¹⁰	Low ¹⁰	Low ¹⁰	Low ¹²	Low ¹⁰
Vigorousness compared to M.9	Similar ¹¹ Lower ⁵	Similar ^{3, 6}	Higher ³	Higher ³	-	Similar ⁷ 15% lower ¹²
Propagation / Market Supply	?	Less productive than M.9 ¹⁴ / Available ¹²	Available e.g. Huber- Brugger, IT	Available e.g. Gebr. Janssen, NL	2.6 per stool ¹⁴ / Available ¹²	Increasingly available ¹²

Letters: T= Tolerant, I= Intermediate, S= Susceptible

Sources: ¹(Fazio et al. 2014); ²(Jackson 2003); ³(Kockerols et al. 2009); ⁴(Russo et al. 2007); ⁵(Johnson et al. 2007);

⁶(Kviklys et al. 2012); ⁷(Autio et al. 2006); ⁸(Rühmer 2014b); ⁹(Höfer et al. 2009); ¹⁰(Monney and Kockerols 2009);

¹¹(Webster et al. 2000); ¹²(Webster and Wertheim 2003); ¹³(Jensen et al. 2012); ¹⁴(Wertheim and Webster 2003)

Table 3: Selected features of utilized apple cultivars.

	Ariane	Gala	Galiwa	Ladina	Natyra	Rewena	Rustica
Origin	Multiple cross-breeding	Kidds Orange × Golden Delicious	Gala × K1R20A44	Topaz × Fuji	Elise × ?	(Cox Orange × Oldenburg) × BX 44,14	Mairac × ?
Fire Blight (<i>Erwinia amylovora</i>)							
----Shoot inoculation	T ^{3, 4, 7}	Highly S ⁷	I ²	T ¹	I ⁷	T ⁷	S ⁶
----Blossom inoculation	S ⁷	Highly S ⁷		T ¹	S ⁷	T ⁷	
Scab (<i>Venturia inaequalis</i>)	R (Vf) ^{3, 4}	S ⁸	R (Vf) ²	R (Vf) ¹	R (Vf) ⁹	R (Vf) ³	R (Vf) ⁶
Sooty Blotch						S ³	
Canker (<i>Necktria galligena</i>)	T ³	S ⁸	S ²		S ⁹		
Powdery Mildew (<i>Podosphaera leucotricha</i>)	Low ^{3,4}	S	S ²	Low ¹	Low ⁹	Low ³	Low ⁶
Biennial Bearing		Low ⁸	I ²	No ⁵	Low ⁹		No ⁶
Vigorousness	I ³	I ⁸	I ²	I ¹	Weak	I ³	
Ripeness/Harvest Time	End of September–Beginning of October ³	End of August–Mid of September ⁸	Mid–End of September ²	Mid of September ¹	Beginning of October ¹⁰	End of September–Beginning of October ³	
Fruit size	Small–I; 60–70 mm wide, 55–65 mm high ³		I ²	I ¹		I–Big; 65–75 mm wide, 70–80 mm high ³	
Storage in cooling chamber/ CA-storage	End of March ³	-/End of August ⁸	March ² /July ²	March ¹ ; January ⁶ /July ¹	Long storeable ¹⁰	Mid of March ³ /May ³	Long storeable ⁶
Market Supply			Available ⁶	Available ⁶			Available ⁶

Letters: T= Tolerant, I= Intermediate, S= Susceptible

Sources: ¹(Leumann et al. 2013); ²(Franck and Kellerhals 2010); ³(Kellerhals et al. 2003); ⁴(Laurens et al. 2005); ⁵(Rühmer 2014a);

⁶(Weibel and Häseli 2015); ⁷(Schöneberg et al. 2015); ⁸(Egger 2007); ⁹(Egger et al. 2013); ¹⁰(Brugger et al. 2013)

3.4.1.2. *Cultivar × rootstock combinations*

Based on the 7 cultivars and 6 rootstocks, 35 apple crcs have been tested. The combinations were tested for varying periods. 9 crcs have been tested for one year (26%), 17 combinations were considered for two years (48%), 2 for three years (6%), and 7 for four years (20%) (Table 4).

Table 4: List of apple cultivar × rootstock combinations intended for the experiment. An orange highlighted box indicates a test of the combination in the respective year.

Cultivar × rootstock	2010	2011	2012	2013
Ariane × AR 295-6				
Ariane × B.9				
Ariane × G.11				
Ariane × M.9				
Gala × AR 295-6				
Gala × B.9				
Gala × G.11				
Gala × G.41				
Gala × M.9				
Gala × Supporter 2				
Galiwa × AR 295-6				
Galiwa × B.9				
Galiwa × G.11				
Galiwa × G.41				
Galiwa × M.9				
Galiwa × Supporter 2				
Ladina × AR 295-6				
Ladina × B.9				
Ladina × G.11				
Ladina × M.9				
Natyra × AR 295-6				
Natyra × B.9				
Natyra × G.11				
Natyra × M.9				
Rewena AR 295-6				
Rewena × B.9				
Rewena × G.11				
Rewena × G.41				
Rewena × M.9				
Rewena × Supporter 2				
Rustica × AR 295-6				
Rustica × B.9				
Rustica × G.11				
Rustica × M.9				
Rustica × Supporter 2				
SUM	15	20	21	21

In addition to the apple cultivars, the annually tested apple rootstocks were grafted on M.9 and subsequently shoot inoculated, to receive an insight about the direct fire blight tolerance of the rootstocks (Table 5).

Table 5: List of apple rootstocks considered for shoot inoculation (all grafted on M.9).
An orange highlighted box indicates a test of the rootstock in the respective year.

Rootstock grafted on M.9	2010	2011	2012	2013
AR 295-6				
B.9				
G.11				
G.41				
M.9				
Supporter 2				
SUM	5	5	4	4

3.4.2. Pear

3.4.2.1. Cultivars and rootstocks

Table 6: Selected features of the utilized pear rootstocks.

	OHF 11	OHF 87	QBA 29	QC	QE
Origin	Old Home × Farmingdale	Old Home × Farmingdale	French selection	Malling selection	Dutch selection
Fire Blight		Very T ²	S ²	S ²	
Pear decline (<i>Candidatus</i> <i>Phytoplasma pyri</i>)	T ¹	T ¹	T ¹	T ¹	T ¹
Bacteria canker (<i>Pseudomonas syringae</i>)		T ²	I ²	I ²	
Collar Rot (<i>Phytophthora cactorum</i>)		I ²	T ²		
Woolly Pear Aphid (<i>Eriosoma pyricola</i>)		S ²	T ²	T ²	
Cold Hardiness		T ²	S ²	S ²	T ³
Iron chlorosis	T ¹	T ¹	T ¹	S ¹	S ¹
Productivity	I ¹	I ¹	I ¹	High ¹	High ¹
Suckering		Low ²	I ²	I ²	
Vigorousness	Very strong ¹	Very strong ¹	Strong ¹	Weak ¹	Weak ¹

Letters: T= Tolerant, I=Intermediate, S= Susceptible

Sources: ¹(Monney and Egger 2013); ²(Lombart and Westwood 1987); ³(Baumschule Fleuren 2015)

Table 7: Selected features of the utilized pear cultivars.

	ACW 3764	ACW 3851	Conférence	Elliot	Hortensia	Roksolana	Uta
Origin	H. Sweet × Verdi	H. Sweet × Verdi	English selection		Nordhäuser Winterforelle × Clapps Liebling		Madame Verte × Boscs Flaschenbirne
Fire Blight (<i>Erwinia amylovora</i>)			S ⁷	T ^{4,7}	S ³		T ¹ , I ³
Scab (<i>Venturia pirina</i>)			T ⁶		Low ³		Low ¹
Incompability to Q rootstocks							Yes ⁴
Biennial Bearing			I ⁶		Yes ²	I ⁵	No ²
Vigorousness		I ⁸	I ⁶		High ²	High ⁵	Weak ¹
Ripeness/ Harvest Time	Mid of September ⁸	Mid of September ⁸	Mid of September ²		Beginning of September ²	Beginning of October ⁵	Beginning–Mid of October ¹
Fruit size		I ⁸	I–Big ⁶		Small ²	Big ⁵	I ²
Storage in cooling chamber			February ⁶		January ²	February ⁵	January ¹

Letters: T= Tolerant, I=Intermediate, S= Susceptible

Sources: ¹(Verband der Bediensteten für Obstbau Garten und Landschaft e.V. 2015); ²(Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau Weinsberg 2015); ³(Höfer et al. 2009);

⁴(Schöneberg (13.04.2015), Personal Communication); ⁵(International Fruit Obtention 2015b); ⁶(International Fruit Obtention 2015a); ⁷(Perren et al. 2013);⁸(Kellerhals 2013)

3.4.2.2. Cultivar × rootstock combinations

Throughout the experiment, 20 different pear crcs compiled out of 7 cultivars and 5 rootstocks have been tested (Table 8). The combinations have been tested for different periods: Nine crcs have been tested for one year (45%), 6 combinations for two years (30%), 3 combinations for three years (15%), and 2 combinations for four years (10%).

Table 8: List of pear cultivar × rootstock combinations intended for the experiment. An orange highlighted box indicates a test of the combination in the respective year.

Cultivar × rootstock	2010	2011	2012	2013
ACW 3764 × OHF 11				
ACW 3764 × OHF 87				
ACW 3764 × QC				
ACW 3764 × QE				
ACW 3851 × OHF 11				
ACW 3851 × OHF 87				
ACW 3851 × QC				
ACW 3851 × QE				
Conférence × OHF 11				
Conférence × OHF 87				
Conférence × QC				
Conférence × QE				
Elliot × OHF 11				
Elliot × OHF 87				
Elliot × QC				
Elliot × QE				
Hortensia × QC				
Roksolana × OHF 87				
Roksolana × QC				
Uta × QBA 29				
SUM	8	6	12	12

Furthermore, the utilized pear rootstocks have been grafted on QC, and tested in the respective experimental years (Table 9).

Table 9: List of pear rootstocks. An orange highlighted box indicates a test of the rootstock in the respective year.

Rootstock on QC	2010	2011	2012	2013
OHF 11				
OHF 87				
QBA 29				
QC				
QE				
SUM	3	2	3	3

3.5. Statistical analysis

3.5.1. Program and Data Grouping

Statistical analysis was conducted using SAS software (SAS® version 9.4, The SAS institute). Data of the conducted experiments and the appendant documentation was provided by supervisor Dr. Franco Weibel (Forschungsinstitut für biologischen Landbau (FiBL)). Apple and pear combinations were analyzed separately. Furthermore, the crcs were separated from the rootstock \times rootstock combinations in both apples and pears. Thus, over the experimental period of four years, annually two groups of apples and two groups of pears (cultivar \times rootstock, and rootstock \times rootstock) were statistically analyzed, amounting to 16 annually analyzed data sets in total.

Furthermore, three two-year periods (2010–2011, 2011–2012, and 2012–2013) of identical crcs have been compiled and were also analysed for the year effect.

In addition to that, the rootstock sensitivity of each cultivar was separately analyzed.

The third lesion length measurement was determined as basis for all statistical assessments in this study, since the disease pattern was most distinct at that point. Nevertheless, also the dynamics of the lesion development was examined whether it might reveal important information.

3.5.2. Data Exclusion and handling of outliers

Based on the provided documentation of the experiments, single plants were excluded from statistical analysis due to the following reasons:

- plants with a shoot length of less than 10 cm
- plants showing a bad grafting unit
- plants accidentally omitted during inoculation
- badly growing plants expressing disease or pest symptoms other than fire blight
- plants with cracked or injured shoots

To identify possible outliers, data were examined for extreme values of the residuals at normal distribution. On basis of this, documentation of dubious data has been reviewed. Data was only retrieved from the analysis if the respective plants were suspected to fulfill at least one of the criteria mentioned above. Crcls which had less than 3 plants, were excluded from the analysis.

3.5.3. Testing of homogeneity of variance and normal distribution

To carry out ANOVA and F-Test procedures, the residuals of the data have to fulfill the requirements of homogeneity of variance and normal distribution. Residuals were gained using PROC MIXED. The residuals were then visually assessed for their homogeneity of variance by a comparison of predicted versus observed values (PROC GPLOT), and for normal distribution

with a Q-Q plot (PROC UNIVARIATE). If both or 1 of the requirements were not fulfilled, transformations of the original data were applied. Root transformations (square root, third root and fourth root) as well as transformations with the natural logarithm and logarithm to the base 10 were considered. The most appropriate transformation was again assessed by visual evaluation. If transformations led to a similar appearance regarding its normal distribution and homogeneity of variance of the residuals, the most suitable transformation was chosen by considering the Shapiro Wilk value for normal distribution of the transformed residuals.

3.5.4. Testing of combinations in single years

On an annual basis, F-tests to assess the cri, have been performed using PROC GLIMMIX ($\alpha=0.05$). The applied model was:

$$Y = \text{Cultivar} + \text{Rootstock} + \text{Cultivar} \times \text{Rootstock}$$

In case the F-value of the interaction indicated significant differences among combinations, crcs were compared with a post ANOVA Tukey test and the single factors were not further investigated. Given the F-value of cri was not significant, the cri was excluded from the model and the single factors ‘cultivar’ and ‘rootstock’ were separately analyzed. To receive least square mean values and back transformed standard errors, the ‘link/ilink’ option was used.

3.5.5. Testing of combinations in two-year periods

Just as in the annual analysis, an F-test to assess significant effects was performed with PROC GLIMMIX ($\alpha=0.05$). The applied model was:

$$Y = \text{Cultivar} + \text{Rootstock} + \text{Year} + \text{Cultivar} \times \text{Rootstock} + \text{Cultivar} \times \text{Year} + \text{Rootstock} \times \text{Year}$$

In case any of the twofold interaction was not significant, it was excluded from the model. Then the test was performed again. Post-hoc analysis was executed with a Tukey test. Significance letters were generated with the ‘lines’ statement. To receive least square mean values and back transformed standard errors, the ‘link/ilink’ option was used.

3.5.6. Testing of rootstock sensitivity

To assess significant rootstock effects, an F-test was conducted for each apple and pear cultivar group separately. If the F-test expressed significant different results among the rootstocks, a post-hoc Dunnett test was performed. Thereby, all crcs of the respective cultivar were pairwise compared to a susceptible reference: the respective cultivar grafted onto M.9 (apples) or QC (pears).

3.5.7. Graphs and plots

3.5.7.1. Bar charts for lesion length development

To visualize the dynamic character of the lesion length development from 1 to 3 wpi, bar charts that show the measured values have been created. Therefore, ls-mean values for relative shoot necrosis were generated by using PROC GLIMMIX. By utilization of PROC SGPLOT, the ls mean values for the 3 measurements of each combination have been compiled in a bar chart.

3.5.7.2. Interaction curves

Least Square-Means (cultivar \times rootstock) were compiled using PROC GLIMMIX. Subsequently, the curves were generated with PROC GPLOT, plotting the estimated value on the y-axis and the rootstocks on the x-axis (plot estimate*rootstock= cultivar). To visually assess the lesion length of apple cultivars, a reference line was drawn at the value of the robust reference Rewena \times M.9 for apples, and Elliot \times QC for pears, by using the 'vref' option.

3.5.7.3. Box Plots

Box Plots were created using PROC BOXPLOT (plot (percent lesion length)*cultivar=rootstock). Additionally, N, minimum, maximum and mean values together with standard deviation were generated by the 'insetgroup' option.

4. Results

4.1. Lesion length development

The general trends of the shoot necrosis development of apples and pears are illustrated in the lesion length measurements 1, 2 and 3 wpi (Figure 7 and Figure 8). The bar charts display variable patterns of necrosis development of different combinations. The last record of lesion length (3 wpi) depicts the peak of this development. Crcs with Gala for example, usually showed a rapid increase of shoot necrosis already in the first and second wpi, whereas at a high lesion length level, the necrosis development slows down at the third wpi. A similar development pattern – but generally with lower values – was recorded for Galiwa. Crcs with Natyra demonstrate a similar rise of necrosis as crcs with Galiwa in the first wpi. However, the development after 1 wpi is either sharply increasing (Natyra \times B.9), steadily increasing (Natyra \times M.9, Natyra \times G.11), or out-leveling between 2 and 3 wpi (Natyra \times AR 295-6). In contrast to that, crcs with Ladina and Ariane emerge on comparatively low value levels, but with a relatively consistent increase over the 3 weeks recorded. Exceptions to this are the combinations Ladina \times G.11 and Ariane \times G.11, which both show almost no further increase of necrosis between 2 and 3 wpi.

A variable pattern of lesion development is also apparent in necrosis dynamics of pear crcs (Figure 8). The sharp necrosis increase of crcs with ACW (ACW 3764 \times QC, ACW 3851 \times QC) between 2 and 3 wpi is particularly striking. This is in contrast to crcs with Conférence and Elliot (Conférence \times QC, Conférence \times QE, Elliot \times QC, Elliot \times QE), which show a comparatively small lesion length increase between 2 and 3 wpi.

Since the pathology of shoot necrosis is most pronounced at the latest measurement, the following statistical analysis is based on the values recorded at 3 wpi.

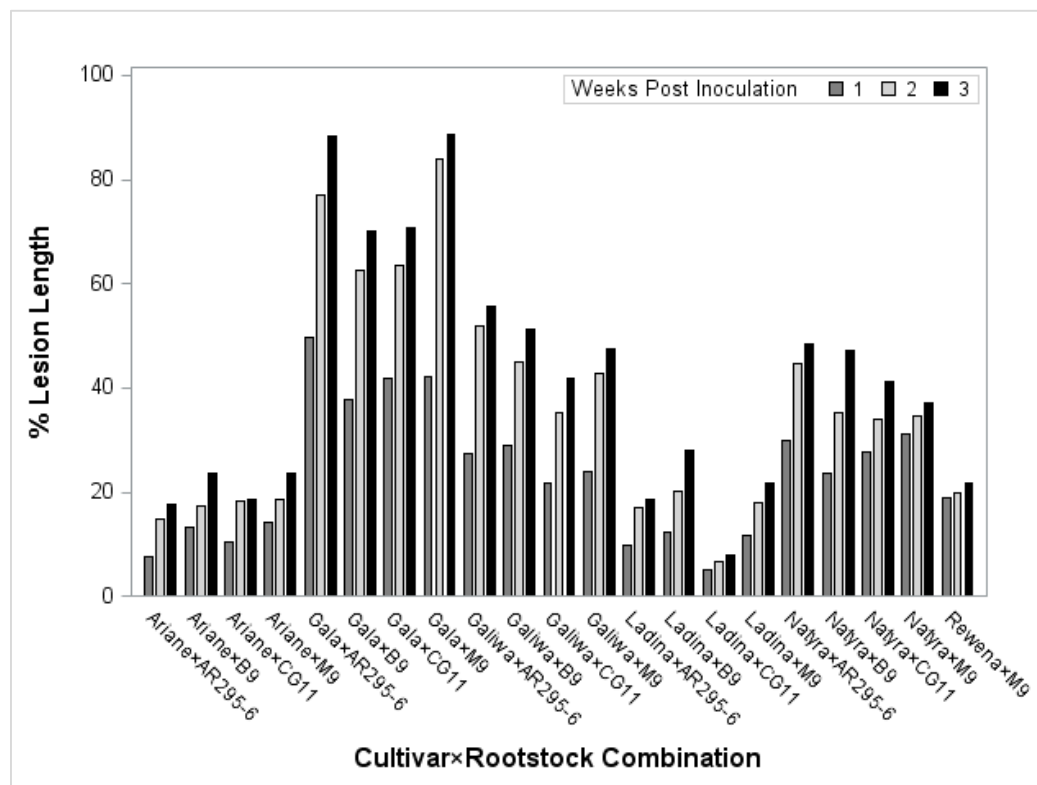


Figure 7: Dynamics of lesion length development of different apple cultivar \times rootstock combinations. LS mean values of relative lesion length as assessed for combinations in the period 2012–2013. Model: cultivar, rootstock, week, cultivar \times rootstock, cultivar \times week, rootstock \times week, cultivar \times rootstock \times week.

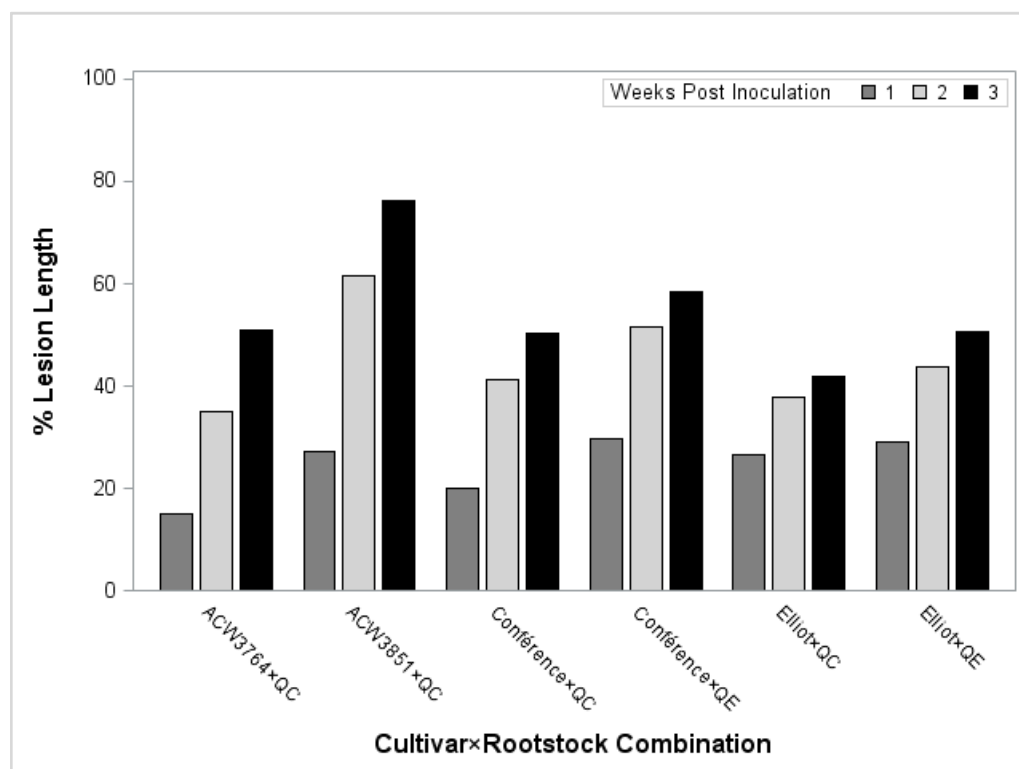


Figure 8: Dynamics of lesion length development of different pear cultivar \times rootstock combinations. LS mean values of relative lesion length as assessed for combinations in the period 2012–2013. Model: cultivar, rootstock, week, cultivar \times rootstock, cultivar \times week, rootstock \times week, cultivar \times rootstock \times week.

4.2. Apple cultivar × rootstock combinations tested in single years

4.2.1. 2010

In the first year of the experiment, 66 out of 155 plants (43%) have been excluded from statistical analysis. Thereof two crcs entirely: Gala × B.9 and Galiwa × G.11, as they both suffered from insufficient shoot growth (17 plants were < 10 cm), complete rootstock deficit, and losses related to bench grafting. The analyzed 11 combinations (89 individual plants) revealed a significant cri effect ($P = 0.0002$). This indicated that both single factors affect each other so that the pattern of the fire blight tolerance of cultivars and rootstocks changes depending on the particular combination. Additionally, both single factors were significant cultivar ($P < 0.0001$) and rootstock ($P < 0.0001$). The statistical significance of the single factors cultivar and rootstock indicated significant differences among the analyzed cultivars and rootstocks.

With a lesion length of 8.1% and 10.9%, the 2 most tolerant rated combinations were Rewena × G.41 and Rewena × G.11 (Figure 9 and annex Table 27). Furthermore, on each rootstock separately, all crcs with Rewena showed significantly lower necrotized shoot lengths compared to the other cultivars. Galiwa × G.41 (33.7%) revealed a more than 58 percent points lower lesion length compared to all other crcs with Galiwa and Gala that range from 88.0 to 98.6%. However, it should be mentioned that only 4 Galiwa × G.41 plants could be used for the statistical analysis.

The rootstock sensitivity was assessed by applying a Dunnett's test for each cultivar separately. Thereby, all crcs of the respective cultivar were pairwise compared to a susceptible reference: the respective cultivar grafted onto M.9. A significant rootstock sensitivity was found for Galiwa ($P < 0.0001$) and Rewena ($P = 0.010$). Both cultivars revealed a higher tolerance against *E. amylovora* bacteria when grafted to G.41 as compared to M.9. In contrast to that, no significant rootstock effects could be found for crcs with Gala ($P = 0.330$).

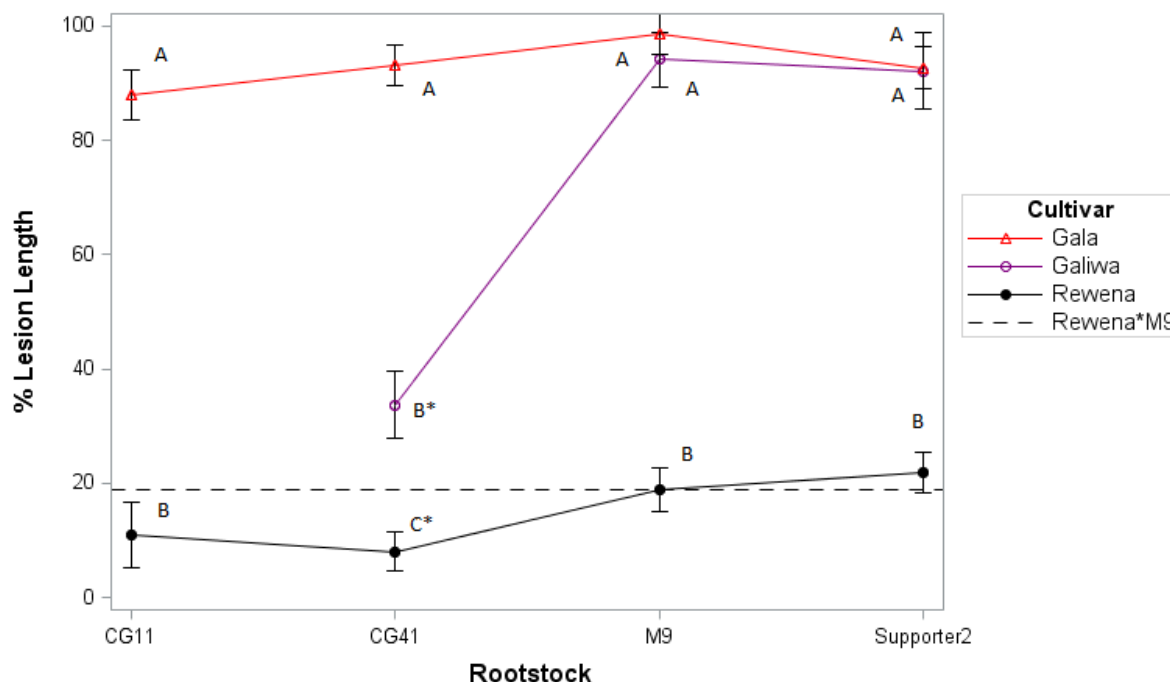


Figure 9: Apple cultivar \times rootstock combination analysis (2010): Interaction curves displaying LS-means values of relative lesion length 3 wpi; $\alpha = 0.05$. Bars of the data are representing the standard error. For calculations data transformed by square root. Letter display following a post-ANOVA Tukey test. Differing capital letters indicate significant differences between cultivars on the respective rootstock (vertical difference). Asterisk marks follow a post-ANOVA Dunnett test. A letter marked with an asterisk indicates a significant difference to the respectively same cultivar grafted on M.9 (horizontal significance). Model: cultivar, rootstock, cultivar \times rootstock.

Rootstock shoot inoculation of rootstocks grafted onto M.9, revealed significant differences ($P < 0.0001$). Both Geneva rootstocks (G.41 and G.11) showed the significantly lowest relative shoot necrosis with 1.0 and 3.4% necrotized shoot length, followed by B.9 (17.4%) which was significantly different to the more than threefold higher data of M.9 (61.4%) and Supporter 2 (65.0%) (Table 10).

Table 10: Apple analysis of rootstock shoot inoculation (2010) of rootstocks grafted onto M.9. Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: rootstock (on top). For calculations data transformed by third root. Means with the same letter are not significantly different.

Rootstock	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
Supporter	12	A	65.0 (\pm 6.0)
M.9	10	A	61.4 (\pm 6.5)
B.9	12	B	17.4 (\pm 6.0)
G.11	9	C	3.4 (\pm 6.9)
G.41	10	C	1.0 (\pm 6.9)

4.2.2. 2011

Compared to 2010, the plant material tested in 2011 was diluted by the cultivar Rustica and the rootstocks B.9 and AR 295-6. In return, the rootstock G.41 was not anymore included in the experiment. 91 out of 240 plants (38%) were excluded from the statistical analysis. As less than

3 plants of Gala \times AR 295-6 and Galiwa \times AR 295-6 had provided usable data, these combinations were not considered for statistical analysis. The high exclusion rate is attributed to lacking rootstock material of AR 295-6 and insufficient shoot growth of its combinations. The remaining 18 combinations (149 plants (62%)) revealed a significant cri ($P = 0.005$). In contrast to the significant factor cultivar ($P < 0.0001$), the factor rootstock was not significant ($P = 0.153$). The most fire blight tolerant combinations were Rewena \times B.9 with 19.7% and Rewena \times G.11 with 24.6% lesion length (Figure 10 and annex Table 28). As in 2010, all crcs with Rewena obtained significantly lower values compared to the other cultivars on each rootstock separately. A major group consisting of all other tested combinations had the highest shoot necrosis.

Assessing the rootstock influence for each cultivar separately, Gala revealed a significant rootstock sensitivity ($P = 0.042$) with a higher relative lesion length grafted onto B.9 than compared to M.9. This year, and in particular with Rewena, the cri was close to a significant level ($P = 0.054$): on B.9 with 19.7% and G.11 with 24.6% lesion length Rewena was less fire blight susceptible than on M9 with 51.0% and Supporter with 56.5% infected wood length. Although the other tested cultivars revealed no significant rootstock sensitivity (Galiwa $P = 0.247$ and Rustica $P = 0.792$), Galiwa showed a similar pattern as in 2010 with a lower susceptibility of 20 percentage points grafted onto G.11 compared to M.9.

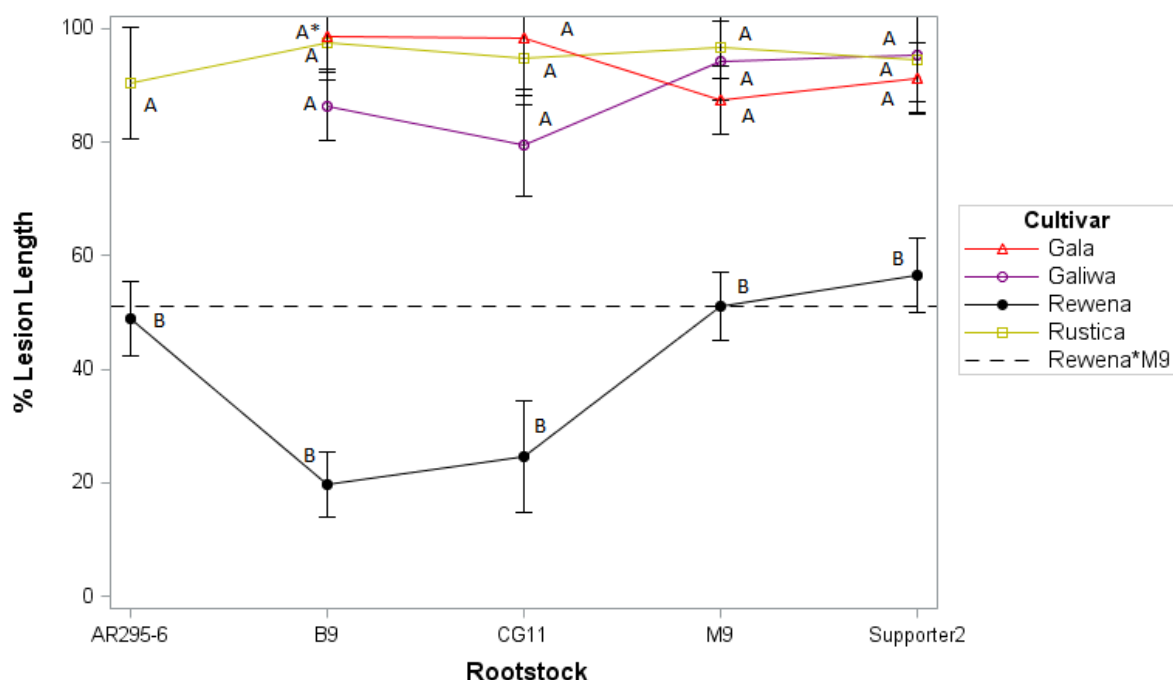


Figure 10: Apple cultivar \times rootstock combination analysis (2011): Interaction curves displaying LS-means values of relative lesion length 3 wpi; $\alpha = 0.05$. Bars of the data are representing the standard error. For calculations data transformed by square root. Letter display following a post-ANOVA Tukey test. Differing capital letters indicate significant differences between cultivars on the respective rootstock (vertical difference). Asterisk marks follow a post-ANOVA Dunnett test. A letter marked with an asterisk indicates a significant difference to the respectively same cultivar grafted on M.9 (horizontal significance). Model: cultivar, rootstock, cultivar \times rootstock.

Following shoot inoculation of rootstocks grafted on M.9, the factor rootstock was significant ($P < 0.0001$). Ranging from 8.2% to 25.2% the rootstocks G.11, B.9, and AR 295-6 were significantly more tolerant to aninfection with *E. amylovora* bacteria than M.9 (62.6%) and Supporter 2 (73.0%) (Table 11).

Table 11: Apple analysis of rootstock shoot inoculation (2011) of rootstocks grafted onto M.9. Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: rootstock (on top). For calculations data was not transformed. Means with the same letter are not significantly different.

Rootstock	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
Supporter 2	12	A	73.0 (\pm 6.1)
M.9	12	A	62.6 (\pm 6.1)
AR 295-6	12	B	25.2 (\pm 6.1)
B.9	12	B	21.1 (\pm 6.1)
G.11	12	B	8.2 (\pm 6.1)

4.2.3. 2012

Since the groups of crcs tested in 2012 and 2013 were identical, a thorough analysis of the combined dataset is conducted in the following chapter for the whole period.

With Ariane, Ladina and Natyra, three new cultivars were introduced to the experiment in 2012, where only 16% (29 of 210) of plants needed to be eliminated from the statistical analysis. Thus, all groups of crc could be statistically assessed. As in the previous year, the cri ($P = 0.003$) and the factor cultivar ($P < 0.0001$) were significant. However, the factor rootstock was not significant ($P = 0.142$). With a value of 13.4% the most tolerant crc was Ladina \times G.11, which showed a sharp decline compared to the other crcs with Ladina (Figure 11 and annex Table 29). Furthermore, crcs with Ariane displayed low necrotized shoot lengths irrespective of the rootstock that ranged from 21.1% to 32.8%. Extending from 51.2% to 87.1% a major group of 14 combinations that comprises all crcs with Gala, Natyra, and Galiwa as well as Ladina \times B.9 and Ladina \times M.9, revealed the highest susceptibility to an *E. amylovora* infection.

The cultivar specific assessment of rootstock sensitivity highlights the above mentioned. With a result of 13.4%, Ladina \times G.11 obtained a 37.8 percentage points lower relative lesion length than the susceptible reference Ladina \times M.9 (51.2%), that was significantly lower ($P < 0.0001$). The other cultivars revealed no significant rootstock sensitivity Ariane ($P = 0.100$), Gala ($P = 0.508$), Galiwa ($P = 0.725$), Natyra ($P = 0.706$).

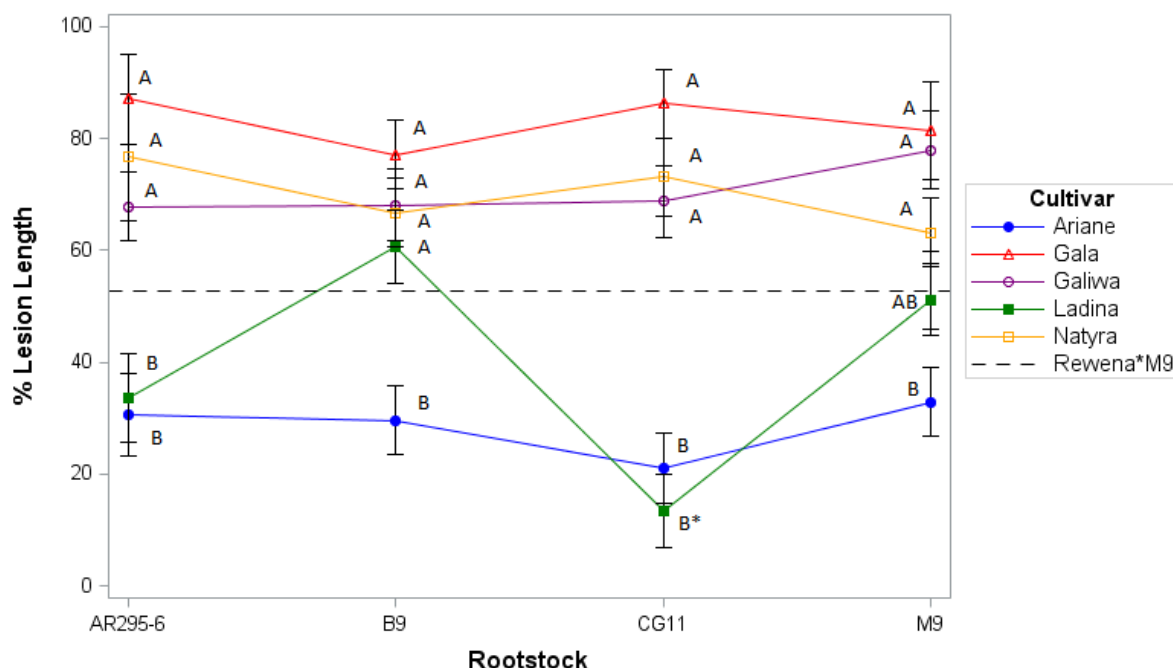


Figure 11: Apple cultivar \times rootstock combination analysis (2012): Interaction curves displaying LS-means values of relative lesion length 3 wpi; $\alpha = 0.05$. Bars of the data are representing the standard error. For calculations data not transformed. Letter display following a post-ANOVA Tukey test. Differing capital letters indicate significant differences between cultivars on the respective rootstock (vertical difference). Asterisk marks follow a post-ANOVA Dunnett test. A letter marked with an asterisk indicates a significant difference to the respectively same cultivar grafted on M.9 (horizontal significance). Model: cultivar, rootstock, cultivar \times rootstock.

Equally to the previous years, the statistical analysis of direct shoot inoculation of rootstocks grafted onto M.9 revealed significant differences ($P < 0.0001$). G.11 had the lowest relative shoot necrosis with 7.8%, significantly different to intermediate results of AR 295-6 (25.5%) and B.9 (26.6%), which were still 45 percentage points below the significantly highest value revealed by M.9 (71.6%) (Table 12).

Table 12: Apple analysis of rootstock shoot inoculation (2012) of rootstocks grafted onto M.9. Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: rootstock (on top). For calculations data was transformed by third root. Means with the same letter are not significantly different.

Rootstock	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
M.9	10	A	71.6 (\pm 4.1)
B.9	10	B	26.6 (\pm 4.1)
AR 295-6	10	B	25.5 (\pm 4.1)
G.11	10	C	7.8 (\pm 4.1)

4.2.4. 2013

With the same crcs tested as in 2012 and an exclusion rate of 17% (36 of 215 plants) the experimental starting position in 2013 was similar to the previous year. Accordingly, all combinations could be statistically considered. All tested factors, the cri ($P = 0.018$) as well as the single factors cultivar ($P < 0.0001$) and rootstock ($P = 0.005$), were statistically significant. As in 2012, Ladina \times G.11 revealed the lowest relative shoot necrosis with 3.2%. Furthermore,

it obtained the significantly lowest results on G.11 compared to all other tested cultivars. Moreover, all other crcs with Ladina showed low values in the range of Rewena × M.9 (9.7%). Intermediate lesion lengths were recorded for all crcs with Galiwa, Natyra and Ariane, whereas highest levels of shoot necrosis were found for crcs with Gala (60.6% to 94.3%).

In addition to the above mentioned, Gala revealed a significant rootstock effect ($P = 0.004$). With necrotized shoot lengths of 60.6% and 68.4%, both Gala × G.11 and Gala × B.9 were significantly more fire blight tolerant than Gala × M.9 (94.3%). Ladina was almost significantly rootstock sensitive and confirmed the trend of the previous year ($P = 0.068$). Although the recorded shoot necrosis of Ladina × M.9 with 6.9% was remarkably low in 2013, it was still 3.7 percentage points more susceptible than Ladina × G.11 (3.2%). The cultivars Galiwa ($P = 0.076$) and Natyra ($P = 0.065$) expressed marginal significant rootstock effects. However, just as in 2010 and 2011, Galiwa was more tolerant grafted onto a Geneva rootstock (Galiwa × G.11 (28.1%)) as compared to M.9 (Galiwa × M.9 (33.5%)). In contrast to that, Ariane was not sensitive to rootstock influences ($P = 0.475$).

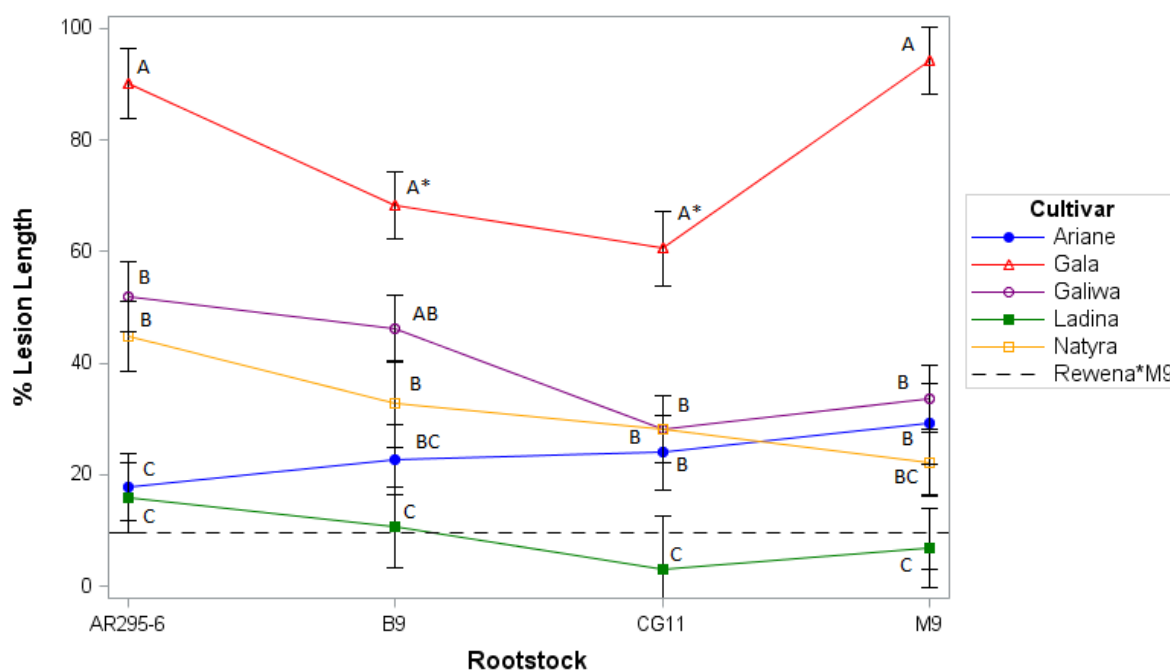


Figure 12: Apple cultivar × rootstock combination analysis (2013): Interaction curves displaying LS-means values of relative lesion length 3 wpi; $\alpha = 0.05$. Bars of the data are representing the standard error. For calculations data transformed by 4th root. Letter display following a post-hoc Tukey test. Differing capital letters indicate significant differences between cultivars on the respective rootstock (vertical difference). Model: cultivar, rootstock, cultivar × rootstock.

The F-test indicated significant differences ($P < 0.0001$) among the rootstocks grafted to M.9 that have been directly shoot inoculated. With a necrotized shoot length of 5.5%, G.11 had significantly lower values than AR 295-6 with 17.0% and M.9 with 33.0% (Table 13). Additionally B.9 (9.3%) and AR 295-6 were significantly different to M.9.

Table 13: Apple analysis of rootstock shoot inoculation (2013) of rootstocks grafted onto M.9. Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: rootstock (on top). For calculations data was transformed by third root. Means with the same letter are not significantly different.

Rootstock	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
M.9	10	A	33.0 (\pm 2.6)
AR 295-6	8	B	17.0 (\pm 2.9)
B.9	9	B C	9.3 (\pm 2.7)
G.11	10	C	5.5 (\pm 2.6)

4.3. Apple cultivar \times rootstock combinations analyzed in two-year periods

Due to the annually fluctuating constitution of tested combinations, a full factorial analysis of relative shoot length over the whole experimental period was not feasible. Hence, the data was arranged in three experimental periods (2010–2011, 2011–2012, and 2012–2013) to approach an analysis that includes an annual effect. This approach provides 2 advantages as compared to single year analysis: First of all, it allows to assess whether the annual results are temporally stable, and secondly it increases the statistical power by analyzing a higher number of observations. A full factorial F-test led to heterogeneous results for the three periods (Table 14). In contrast to the investigated periods 2010–2011 and 2011–2012, the interaction of cultivar \times rootstock was only significant in the period 2012–2013. However, in 2010–2011 and 2011–2012 solely 8, respectively 7, combinations have been investigated. This allows only a narrow scope for possible cri effects. In contrast to that, 21 combinations could be statistically assessed for the period 2012–2013. The reason for this is that the exact same group of combinations has been tested in 2012 and 2013. Accordingly, also the threefold interaction of cultivar \times rootstock \times year was only significant in the last period. A significant threefold interaction indicates a reciprocal effect of all 3 factors. Rootstock as single factor was only significant in 2012–2013. The single factors cultivar and year were significant in all 3 periods. Year as significant factor reveals that the mean values of relative necrosis differ significantly between the investigated years. However, the factor year alone provides no information if the cultivar or rootstock pattern differs between the years. The question of a changing pattern is covered by the twofold interactions of each factor with year. The interaction of cultivar \times year was significant for the periods 2010–2011 and 2012–2013, but the rootstock \times year interaction was not significant for any of the three periods. This reveals rather temporal fluctuations in the fire blight tolerance pattern of cultivars, but a temporally stable pattern of fire blight tolerance of the tested rootstocks.

Table 14: Apple cultivar × rootstock combination analysis of two-year periods: P-values following F-tests for lesion length mean comparison of combinations that have been tested in respective periods (3 wpi; $\alpha = 0.05$).

Model: cultivar, rootstock, year, cultivar*rootstock, cultivar*year, rootstock*year, cultivar*rootstock*year.

Period	N	Comb.	Cult.	Rootst.	Year	Cult.× Rootst.	Cult.× Year	Rootst.× Year	Cult.×Rootst.× Year
2010– 2011	124	8	< .0001	0.1208	0.0144	0.1529	0.0008	0.7910	0.2310
2011– 2012	122	7	0.0058	0.7526	< .0001	0.0920	0.7509	0.4035	0.6304
2012– 2013	356	21	< .0001	< .0001	< .0001	0.0011	< .0001	0.1804	0.0066

4.3.1. 2010–2011

The post-ANOVA Tukey test of the significant factor cultivar indicated a difference between Rewena with a mean relative lesion length of 32.3% and the 2 foremost cultivars Gala and Galiwa with 91.8% and 91.0% lesion length (Table 15). Concerning the significant factor year, the mean value integrating all crcs in 2010 (66.4%) was significantly lower than in 2011 (77.0%).

Table 15: Apple analysis of cultivars (2010–2011): Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock, year. For calculations data was not transformed. Means with the same letter are not significantly different.

Cultivar	N	Letter Display	LS Means Lesion Length (± Standard Error) [%]
Gala	54	A	91.8 (± 2.7)
Galiwa	21	A	91.0 (± 4.6)
Rewena	49	B	32.3 (± 2.9)

4.3.2. 2011–2012

As in 2010–2011, the cultivar Rewena (49.8%) had a significantly lower shoot necrosis than Gala (88.0%) and Galiwa (79.4%) (Table 16). Summarizing all crcs, the combined mean value in 2011 (77.0%) was significantly higher than in 2012 (66.6%).

Table 16: Apple analysis of cultivars (2011–2012): Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock, year. For calculations data was not transformed. Means with the same letter are not significantly different.

Cultivar	N	Letter Display	LS Means Lesion Length (± Standard Error) [%]
Gala	53	A	88.0 (± 2.6)
Galiwa	50	A	79.4 (± 2.7)
Rewena	19	B	49.8 (± 5.2)

4.3.3. 2012–2013

In 2012–2013, 356 plants divided in 21 crcs have been statistically analyzed. The two most tolerantly assessed cultivars were Ariane and Ladina. Ranging from 22.4% to 27.0%, Ariane revealed a stable low-level shoot necrosis which – irrespective of the rootstock – remained

below the tolerant reference Rewena \times M.9 (31.3%). In contrast to the homogeneous results of crcs with Ariane, Ladina had an accentuated low necrotized shoot length of 10.3% grafted onto G.11. Intermediate lesion lengths were found for crcs with Galiwa (47.3% to 60.3%) and Natyra (40.6% to 54.0%), although Natyra \times M.9 with a lesion length of 40.6% was not significantly different to the most tolerant crcs with M.9 (Ariane \times M.9 (27.0%) and Ladina \times M.9 (31.8%)). Crcs with Gala revealed the highest susceptibility with necrotized shoot lengths ranging from Gala \times B.9 with 72.8% to Gala \times M.9 with 90.0%.

In addition to the cultivar Ladina ($P = 0.016$), Gala indicated a significant rootstock effect ($P = 0.020$). With a lesion length of 10.3%, Ladina \times G.11 was significantly different to Ladina \times M.9 (31.8%), and Gala \times B.9 (72.8%) had a lower shoot necrosis compared to the susceptible reference Gala \times M.9 with 90.0%. This is in line with the single year assessment of 2013. Apart from Gala and Ladina, the remaining 3 cultivars (Ariane ($P = 0.630$), Galiwa ($P = 0.529$), and Natyra ($P = 0.622$)) revealed no significant rootstock sensitivity.

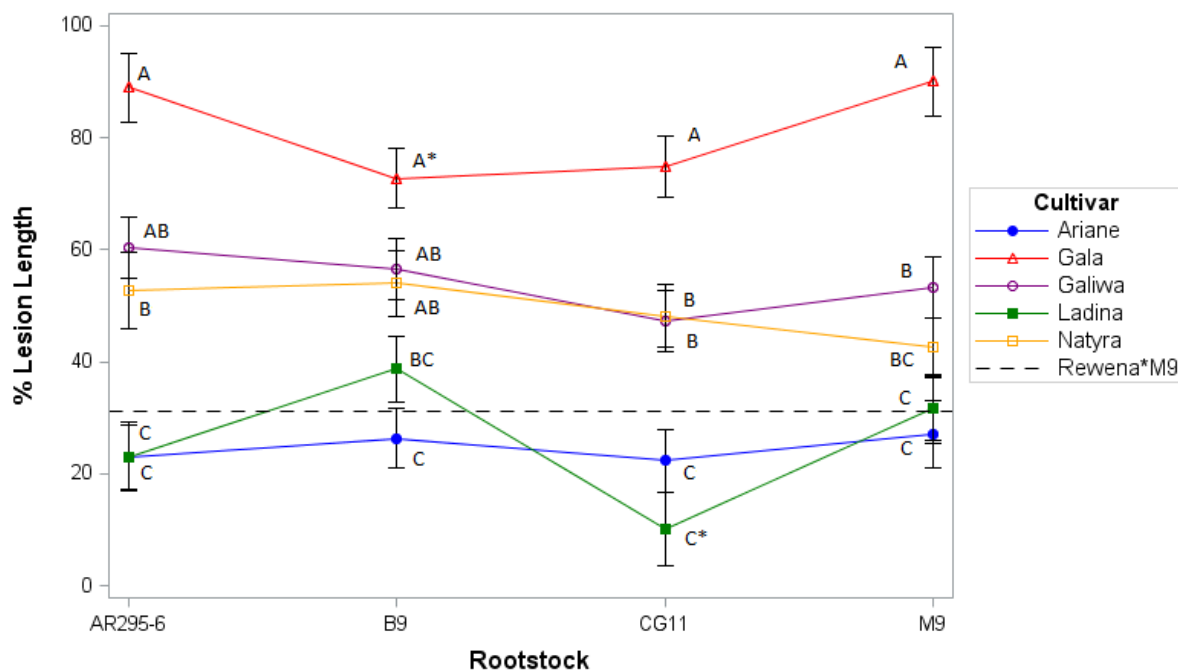


Figure 13: Apple cultivar \times rootstock combination analysis (2012–2013): Interaction curves displaying LS-means values of % lesion length 3 wpi; $\alpha = 0.05$. Bars of the data are representing the standard error. For calculations data transformed by third root. Letter display following a post-ANOVA Tukey test. Differing capital letters indicate significant differences between cultivars on the respective rootstock (vertical significance). Asterisk marks follow a post-ANOVA Dunnett test. A letter marked with an asterisk indicates a significant difference to the respectively same cultivar grafted on M.9 (horizontal significance). Model: cultivar, rootstock, year, cultivar \times year, rootstock \times year, cultivar \times rootstock.

The boxplots displayed in Figure 14 allow a closer look to the effect of the interaction among the 4 rootstocks tested in 2012–2013 and the cultivar Ladina. Bearing a standard deviation of 31% and a result range of 98%, the results of Ladina \times B.9 are exceptionally heterogeneous. In contrast to that, particularly the combination Ladina \times G.11, obtained relatively homogeneous results (standard deviation 8%, result range 23%). Moreover, it revealed the lowest value of necrotized shoot length of all tested crcs in 2012–2013.

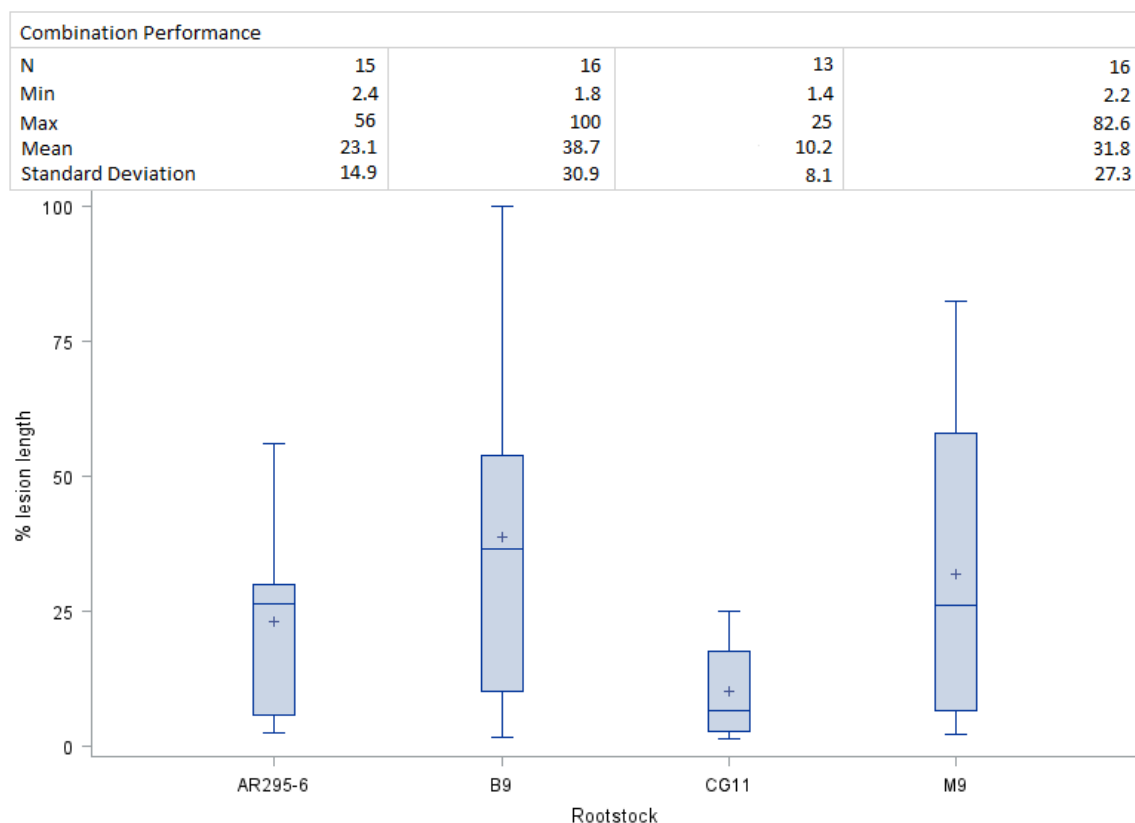


Figure 14: Apple cultivar \times rootstock combination analysis (2012–2013): Box plots of the cultivar *Ladina*, tested on different rootstocks.

4.4. Pear cultivar \times rootstock combinations tested in single years

4.4.1. 2010

The first year of pear testing was characterized by a high exclusion rate from the statistical analysis: 61 of 96 plants (64%). The main reasons for plant exclusion were affinity problems between cultivars and rootstocks. Due to a residual number of less than 3 plants after plant exclusion, the combinations *Conférence* \times OHF 87 and *Elliot* \times QC were rejected for the analysis. The remaining 6 combinations (*Conférence* \times QC, *Elliot* \times OHF 87, *Hortensia* \times QC, *Roksolana* \times OHF 87, *Roksolana* \times QC, *Uta* \times QBA 29) did not allow a statistical analysis for the cri. However, single factor testing of differences among cultivars ($P = 0.094$) and rootstocks ($P = 0.824$) were not significant.

In line with the factor rootstock when grafted with a cultivar, also direct shoot inoculation of rootstocks grafted on QC (OHF 87, QC) indicated no significant differences ($P = 0.301$).

4.4.2. 2011

In contrast to 2010, only a small number of 3 plants from 58 plants (5%) were eliminated for statistical purposes. Thus, all tested combinations could be assessed. With the cri ($P < 0.0001$) and the single factors cultivar ($P = 0.0001$) and rootstock ($P = 0.023$), all tested parameters were significant. The lowest lesion lengths were found for both crcs with Elliot (Elliot \times OHF 87 (24.7%), Elliot \times QC (33.2%)) as well as ACW 3851 \times QC with a shoot necrosis of 32.1%. With 58.3%, Conférence \times QC had a lesion length twice as high as the above mentioned while ACW 3851 \times OHF 87 (93.4%) even revealed a three times higher shoot necrosis than the group with the lowest values (Table 17).

A significant rootstock sensitivity ($P = 0.0001$) was recorded for the cultivar ACW 3851. It showed a higher tolerance against *E. amylovora* bacteria grafted onto QC (32.1%) than grafted onto OHF 87 (93.4%) (- 61.3% shoot necrosis). In contrast to that, Conférence ($P = 0.456$) and Elliot ($P = 0.258$) were not affected by significant rootstock influences.

Table 17: Pear cultivar \times rootstock combination analysis (2011): Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock, cultivar \times rootstock. For calculations data transformed by 4th root. Means with the same letter are not significantly different.

Combination	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
ACW 3851 \times OHF 87	8	A	93.4 (\pm 6.8)
Conférence \times QC	12	A	58.3 (\pm 5.6)
Conférence \times OHF 87	7	A B	51.7 (\pm 7.3)
Elliot \times QC	11	B	33.2 (\pm 5.8)
ACW 3851 \times QC	12	B	32.1 (\pm 5.6)
Elliot \times OHF 87	7	B	24.7 (\pm 7.3)

The shoot inoculation of rootstocks grafted onto QC indicated a significantly lower infected shoot length for OHF 87 with 17.7% compared to QC with 46.0% ($P < 0.0001$, Table 18).

Table 18: Pear analysis of rootstock shoot inoculation (2011) of rootstocks grafted onto QC. Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: rootstock (on top). For calculations data square root transformed. Means with the same letter are not significantly different.

Rootstock	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
QC	12	A	46.0 (\pm 3.6)
OHF 87	11	B	17.7 (\pm 3.7)

4.4.3. 2012

In 2012, 4 cultivars (ACW 3764, ACW 3851, Conférence, and Elliot) in combination with 3 rootstocks (OHF 87, QC, and QE) amounted to 12 investigated crcs. With a total of 35 out of 120 plants, 29% of plants were rejected for statistical analysis. The main reason for exclusion was insufficient shoot growth (19 plants), followed by plants accidentally omitted during

inoculation (8 plants) and not engrafted plants (8 plants). Thereby, Elliot \times QE with 7 plants and Conférence \times OHF 87 with 5 plants, suffered most plant losses.

In contrast to the significant factor rootstock ($P = 0.0003$), the interaction of cultivar \times rootstock ($P = 0.056$) and the factor cultivar ($P = 0.254$) were not significant. With a shoot necrosis of 60.7%, cultivars grafted onto OHF 87 revealed a one-third lower lesion length than cultivars grafted onto QE (89.5%), while cultivars on QC showed an intermediate susceptibility against *E. amylovora* bacteria (74.8%) (Table 19).

Table 19: Pear rootstock analysis (2012): Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock. For calculations data not transformed. Means with the same letter are not significantly different.

Rootstock	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
QE	25	A	89.5 (\pm 4.8)
QC	36	A B	74.8 (\pm 3.9)
OHF 87	24	B	60.7 (\pm 4.9)

With ACW 3764 ($P = 0.015$), ACW 3851 ($P = 0.036$) and Elliot ($P = 0.042$), all cultivars except Conférence ($P = 0.222$) revealed significantly different results depending on the rootstock they were grafted onto. With lesion lengths of 53.8% and 55.6%, the cultivars ACW 3764 and ACW 3851 showed a significantly lower relative necrosis grafted onto OHF 87 as compared to QC (ACW 3764 \times QC (81.6%), ACW 3851 \times QC (93.4%)). Elliot \times QE was completely devastated by *E. amylovora* bacteria (100% necrotized shoot length), while Elliot \times QC was significantly more tolerant (51.9%).

Shoot inoculation of rootstocks grafted on QC led to significant differences among the tested rootstocks. With an infected shoot length of 34.2%, a significantly lower shoot necrosis was assessed for OHF 87 compared with QC (58.0%) (Table 20).

Table 20: Pear analysis of rootstock shoot inoculation (2012) of rootstocks grafted onto QC. Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: rootstock (on top). For calculations data not transformed. Means with the same letter are not significantly different.

Rootstock	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
QC	10	A	58.0 (\pm 4.2)
QE	10	A B	47.8 (\pm 4.2)
OHF 87	10	B	34.2 (\pm 4.2)

4.4.4. 2013

Likewise to 2012, the 4 cultivars ACW 3764, ACW 3851, Conférence, and Elliot combined with the rootstocks QC and QE, were intended for the pear screening in 2013. However, unlike to the previous year, OHF 87 was substituted with OHF 11. Among the eliminated 34 of 120 plants (28%), the 2 combinations ACW 3764 \times QE and ACW 3851 \times QE were excluded from the analysis. This was mainly due to missing plant material (21 plants), followed by insufficient

shoot growth (nine plants), accidentally omitted inoculation (2 plants), 1 broken shoot tip, and 1 dead plant. With 16 of 20 plants missing at the beginning of the experiment, especially the two excluded crcs suffered from lacking plant material.

The cri ($P = 0.032$) and the factor cultivar ($P = 0.0002$) were significant. Ranging from 23.2% to 28.6% lesion length, a group of five crcs revealed the highest fire blight tolerance: ACW 3764 \times QC, Elliot \times OHF 11, Elliot \times QC, Conférence \times OHF 11, and Conférence \times QC (Figure 15 and annex Table 32). This group was significantly different to the most susceptible crc, ACW 3851 \times QC with 60.9% necrotized shoot length. Intermediate values were found for Conférence \times QE (33.1%), Elliot \times QE (34.2%), ACW 3851 \times OHF 11 (46.4%), and ACW 3764 \times OHF 11 (51.5%).

A significant rootstock effect was only indicated for the cultivar ACW 3764 ($P = 0.022$). With an infected shoot length of 23.2%, the most tolerantly rated crc, ACW 3764 \times QC, revealed a lower shoot necrosis than ACW 3764 \times OHF 11 with 51.5%.

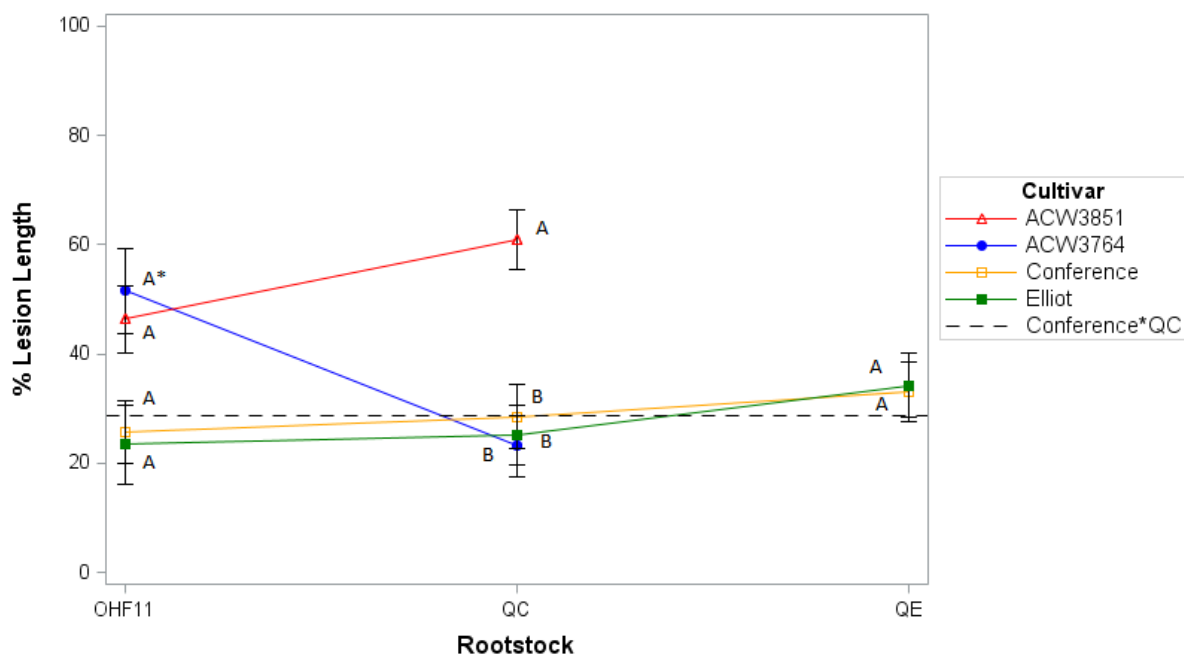


Figure 15: Pear cultivar \times rootstock combination analysis (2013): Interaction curves displaying LS-means values of relative lesion length 3 wpi; $\alpha = 0.05$. Bars of the data are representing the standard error. For calculations data transformed by square root. Letter display following a post-ANOVA Tukey test. Differing capital letters indicate significant differences between cultivars on the respective rootstock (vertical difference). Asterisk marks follow a post-ANOVA Dunnett test. A letter marked with an asterisk indicates a significant difference to the respectively same cultivar grafted on M.9 (horizontal significance). Model: cultivar, rootstock, cultivar \times rootstock.

With a lesion length of 7.2%, the rootstock OHF 11 showed a significantly lower shoot necrosis than QC (21.5%) and QE (27.6%) following shoot inoculation of rootstocks grafted on QC ($P < 0.0001$, Table 21).

Table 21: Pear analysis of rootstock shoot inoculation (2013) of rootstocks grafted onto QC. Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: rootstock (on top). For calculations data transformed by 4th root. Means with the same letter are not significantly different.

Rootstock on QC	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
QE	10	A	27.6 (\pm 2.7)
QC	10	A	21.5 (\pm 2.7)
OHF 11	9	B	7.2 (\pm 2.8)

4.5. Pear cultivar \times rootstock combinations analyzed in two-year periods

In the period 2010–2011, only 2 combinations (Conférence \times QC and Elliot \times OHF 87) were tested. Hence, a factorial analysis for this period was not feasible. In the two testable periods (2011–2012 and 2012–2013), the factors cultivar, year, and rootstock \times year were significant (Table 22). The cri and the factor rootstock were only significant in 2012–2013. Furthermore, the threefold interaction of cultivar \times rootstock \times year was significant in 2011–2012.

Table 22: Pear cultivar \times rootstock combination analysis of two-year periods: P-values following F-tests for lesion length mean comparison of combinations that have been tested in respective periods (3 wpi; $\alpha = 0.05$).

Model: cultivar, rootstock, year, cultivar \times rootstock, cultivar \times year, rootstock \times year, cultivar \times rootstock \times year.

Period	N	Comb.	Cult.	Rootst.	Year	Cult. \times Rootst	Cult. \times Year	Rootst. \times Year	Cult. \times Rootst. \times Year
2010–2011	25	2	--	--	--	--	--	--	--
2011–2012	102	6	< .0001	0.8160	0.0004	0.4184	0.3781	0.0060	< .0001
2012–2013	101	6	< .0001	< .0001	< .0001	0.0428	0.0270	0.0014	0.0937

4.5.1. 2011–2012

Significant differences of the threefold interaction cultivar \times rootstock \times year for 2011–2012 are displayed in Table 23. The data emphasizes the heterogeneity of the results in both years. In contrast to mean values in 2012 which covered a range of 41.5%, results in 2012 were more extreme – particularly in the lower data field – and covered a range of 68.7% (+ 27.2%). Notably striking is the relative necrosis increase of ACW 3851 \times QC in 2012 compared to 2011 (+ 61.3%). It is the only combination that is significantly different within the 2 years, although also the mean values of other combinations considerably oscillated. Furthermore, the significant factor year indicates the annual heterogeneity of mean values. The combined mean values of all combinations separated in the two years differ by 19.1% (2011: 48.9%, 2012: 68.0%).

Table 23: Pear cultivar \times rootstock combination analysis (2011–2012): Mean comparison of LS-mean values of relative lesion length 3 wpi Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock, year, cultivar \times rootstock, cultivar \times year, rootstock \times year, cultivar \times rootstock \times year. For calculations data transformed by square root. Means with the same letter are not significantly different.

Combination	Year	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
ACW 3851 \times OHF 87	2011	8	A	93.4 (\pm 8.2)
ACW 3851 \times QC	2012	9	A	93.4 (\pm 7.7)
Conférence \times OHF 87	2012	5	A B	77.7 (\pm 10.3)
Conférence \times QC	2012	9	A B	72.4 (\pm 7.7)
Conférence \times QC	2011	12	A B C	58.1 (\pm 6.7)
Elliot \times OHF 87	2012	7	A B C	57.0 (\pm 8.7)
ACW 3851 \times OHF 87	2012	6	A B C	55.6 (\pm 9.4)
Elliot \times QC	2012	9	A B C	51.9 (\pm 7.7)
Conférence \times OHF 87	2011	7	A B C	51.7 (\pm 8.7)
Elliot \times QC	2011	11	B C	33.2 (\pm 7.0)
ACW 3851 \times QC	2011	12	C	32.1 (\pm 6.7)
Elliot \times OHF 87	2011	7	C	24.7 (\pm 8.7)

4.5.2. 2012–2013

The combinations of 4 cultivars (ACW 3764, ACW 3851, Conférence, and Elliot) and 2 rootstocks (QC and QE) have been tested in 2012 and 2013. However, due to the exclusion of ACW 3764 \times QE and ACW 3851 \times QE in 2013, these combinations could not be considered for the analysis of this period. Assessing the remaining 6 crcs, with 38.7% Elliot \times QC revealed the highest *E. amylovora* tolerance – significantly different to the most susceptible crc: ACW 3851 \times QC with 77.2% lesion length and Elliot (64.5%) along with Conférence (61.9%), both grafted to QE (Table 24). Intermediate lesion lengths were found for Conférence \times QE (61.9%), ACW 3764 \times QC (52.4%), and Conférence \times QC (50.5%), which were all significantly different to the most susceptible crc ACW 3851 \times QC.

Table 24: Pear cultivar \times rootstock combination analysis (2012–2013): Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock, cultivar \times rootstock. For calculations data was not transformed. Means with the same letter are not significantly different.

Combination	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
ACW 3851 \times QC	19	A	77.2 (\pm 3.5)
Elliot \times QE	12	A B	64.5 (\pm 4.8)
Conférence \times QE	18	B	61.9 (\pm 3.6)
ACW 3764 \times QC	19	B C	52.4 (\pm 3.5)
Conférence \times QC	18	B C	50.5 (\pm 3.6)
Elliot \times QC	15	C	38.7 (\pm 4.0)

5. Discussion

Due to the distinct practice-orientation of this study, the discussion is divided in two sections: a methodic-scientific and a practice-related discussion. Thus, both target groups can be addressed specifically and in detail.

5.1. Practical Discussion

5.1.1. Cultivar × rootstock interaction

In this experiment, the fire blight tolerance of crcs was assessed. In 4 single year assessments, the cri was significant in all years for apples and in 2 years for pears. This indicates that testing crcs is essential to rate the fire blight tolerance of grafted apple and pear trees. Fruit tree growers should be advised to select a tolerant combination. In order to preselect promising plant material for testing combinations, separate fire blight assessments of cultivars (e.g. Korba et al. 2008, Nybom et al. 2012, Perren et al. 2013, Sobiczewski et al. 2014) and rootstocks (e.g. Norelli et al. 2003a, Russo et al. 2008, Kockerols et al. 2009, Aichholz 2012) are crucial.

5.1.2. Apple

Particularly striking is the low shoot necrosis development by Ladina × G.11 in 2012 and 2013. Moreover, the cultivars Galiwa and Rewena (2010) developed relatively low shoot necrosis grafted on the tested Geneva rootstocks (G.11 and G.41). In line with the observed cri effects in this study, Jensen et al. (2012) observed significant differences between 3 year old Gala trees grafted on 7 different rootstocks, 15 days after leave inoculation. Trees grafted on a Geneva rootstock (CG 30) revealed the lowest shoot necrosis, significantly different to B.9 (post-ANOVA lsd test). Furthermore, results obtained by Kellerhals et al. (2014b) and Schöneberg et al. (2015) underline the outcome for Ladina of this study. Kellerhals et al. (2014b) compared the fire blight tolerance of different cultivars grafted on M.9 after shoot inoculation in the greenhouse and after artificial flower inoculation in an open air protected orchard. In both tests, the cultivar Ladina was assessed as fire blight robust. In the greenhouse experiment, Ladina achieved a shoot necrosis of <25% compared to Gala, and in the orchard test only 5% of the inoculated flowers resulted in a shoot necrosis 28 days after inoculation (susceptible reference: Gala 65%). Furthermore, Leumann et al. (2013) examined a natural occurring fire blight incidence in an organic orchard trial in Richterswil, Switzerland. They observed that Ladina trees became infected but could be rehabilitated by removing the infected parts. In contrast, infected Gala trees in the same orchard had to be cleared completely.

In addition to Ladina × G.11, the cultivar Ariane showed a high fire blight tolerance irrespective of the rootstock combined with. This corresponds to results found in literature regarding greenhouse tests and field observations (Brown and Maloney 2008; Laurens et al. 2005; Weibel

and Häseli 2015). Schöneberg et al. (2015) likewise classified Ariane as very tolerant after artificial shoot inoculation under greenhouse conditions. However, following blossom inoculation in an open air orchard, Ariane was classified as susceptible, similar to the susceptible reference cultivar Gala (62% of infected flower clusters – relative to Gala) (Schöneberg et al. 2015). Hence, Ariane is an exemplary cultivar for antithetic fire blight screening results, depending on the way of inoculation. To receive a reliable statement about the fire blight tolerance of specific plant material, both shoot inoculation and flower inoculation are necessary. Ariane is available to fruit growers as a club variety (Weibel and Häseli 2015).

Gala was verified as highly fire blight susceptible in every year, the rootstock influence proving negligible. This confirms results of other studies referring to shoot and flower inoculation, which make use of Gala solely as a fire blight susceptible reference cultivar (Kellerhals et al. 2014a; Leumann et al. 2013; Perren et al. 2013; Schöneberg et al. 2015; Silvestri and Egger 2011). No significant rootstock influence was assessed in 2010 and 2012, and although Gala was rated significantly more tolerant grafted on G.11 and B.9 compared to M.9 in 2013, no significant differences for G.11 were detectable in any other year of the experiment. Furthermore, in 2011 Gala grafted on B.9 was – contradictory to results gained in 2013 – more susceptible than grafted on M.9. Therefore, no explicit recommendation for the selection of a specific rootstock can be made for Gala.

With stable, low level results, Rewena confirmed its status as a fire blight tolerant cultivar. However, a significantly more tolerant result was obtained when it was grafted on G.41, compared with M.9 in 2010. When Rewena is considered in experiments, it is commonly used as tolerant reference cultivar (Silvestri and Egger 2011, Egger et al. 2013, Perren et al. 2013, Schöneberg et al. 2015).

Rustica was only tested in 2011, but it revealed a remarkably high fire blight susceptibility, namely in the same range of Gala, which is concordant to literature findings (Weibel and Häseli 2015). Natyra was slightly more tolerant than Galiwa across all rootstocks. Both obtained intermediate necrosis values between Gala on the upper end and Ladina, Rewena and Ariane at the lower end. This is in compliance with results obtained by Franck and Kellerhals (2010), and Egger et al. (2013). Regarding Natyra and Galiwa, no rootstock specific tolerance was reported. However, Egger et al. (2013) recommended to graft Natyra onto a more vigorous rootstock than M.9. Both Geneva (11 and 41) rootstocks tested proved appropriate. An additional positive effect of Geneva rootstocks in contrast to M.9 is the lower risk of rootstock blight (Robinson et al. 2004).

Contrary to the results of this study, Russo et al. (2007) observed no significant cri for the development of rootstock blight after artificial inoculation of the scion cultivar. Development of rootstock blight is especially critical in young trees since bacteria are able to move rapidly from infected scion wood into the rootstock, where rootstock infections lead to complete tree loss (Robinson et al. 2004). However, the crcs were significantly affected by the rootstock

employed regarding their rootstock blight susceptibility. All tested cultivars (Gala, Honeycrisp, and Golden Delicious) grafted on M.9 revealed an elevated probability to develop rootstock blight compared to a major group of tested rootstocks, including B.9, G.11, and G.41 (Russo et al. 2007). These findings confirm to results of this study, in particular Galiwa and Rewena grafted on G.41 (2010), Ladina grafted onto G.11 (2012), and Gala \times B.9 as well as Gala \times G.11, which were all significantly different from the respective cultivar \times M.9. Furthermore, this is in line with results for direct rootstock inoculation, which showed M.9 to be highly susceptible in all years.

Constant results can be reported for direct rootstock shoot inoculation of rootstocks grafted on M.9. The highest tolerance could be assigned to Geneva rootstocks (G.11 and G.41), followed by intermediate tolerance of AR 295-6 as well as B.9, and the least tolerant rootstocks Supporter 2 and M.9. These results can be validated by means of several literature findings. Following non-grafted rootstock leaf inoculation, a similar pattern of fire blight susceptibility (M.9 > B.9 > CG 16) was observed by Russo et al. (2008). Based on the same methodic approach, Norelli et al. (2003a) obtained results in line with this study (B.9, M.9 > G.11, G.41). The only exception was B.9, which was slightly more susceptible and not significantly different from M.9. Furthermore, Kockerols et al. (2009) reported similar rootstock results. The shoot necrosis of non-grafted M.9 rootstocks that they examined amounted to 65% compared to 5-10% for Geneva rootstocks (G.11, G.41, G.16). However, these results are in contrast to findings of Aichholz (2012) which indicate B.9 as more susceptible compared to M.9, following direct inoculation.

5.1.3. Pear

Compared with the amount of apple cultivar and rootstock fire blight screenings, there are only a few published studies regarding pears. In a greenhouse experiment with artificial shoot inoculation, Perren et al. (2013) tested pear cultivars grafted on QBA 29 on their fire blight tolerance. As a susceptible reference cultivar, Conférence (~ 45% lesion length) was assessed as intermediately tolerant and Elliot (~ 38% lesion length) as tolerant based on lesion length values 3 wpi. The cultivar Conférence is generally rated as fire blight susceptible (Jackson 2003; Weber and Fischer 2005; Weibel and Häseli 2015).

Direct rootstock inoculation revealed a higher tolerance of the tested OHF rootstocks compared with Quince (C and Eline) rootstocks. This is in line with literature findings. Originating from fire blight resistant parentage (Old Home and Farmingdale), OHF rootstocks are generally assessed as moderately blight tolerant (Jackson 2003; Stebbins 1995; Zimmer 2003).

5.1.4. Results of two-year periods

Besides the introduction of the temporal factor year, the perennial data analysis offers an increased number of observations and therefore a higher statistical power.

Based on the results of the single year assessments, it is striking that only the period 2012–2013 of the assessed apple and pear periods revealed a significant cri. This is due to two reasons of which the first is most important: The composition of crcs that have been tested in single years did not allow perennial comparisons of all tested combinations since some crcs were only included in the experiment for one year. This reduces the number of tested groups and thus the likelihood to observe interaction effects. The apple rootstock G.41, for example, was only tested in 2010 while the pear rootstock OHF 11 was solely introduced in 2013. The period 2012–2013 was the only time span of the apple experiment with the same composition of combinations. Thus, all 21 combinations tested were considered, in contrast to eight combinations in 2010–2011 and 7 combinations in 2011–2012. In addition, the second reason for less cri in perennial periods is that values are more balanced as the number of observations per combination increases.

The results of perennial testing indicate an annual variability. The factor year was significant in all tested periods of apple and pear. Regarding the apple results, an annual variability is indicated for the cultivars whereas rootstock results were stable. In contrast, pear results indicate a stable cultivar performance and fluctuating rootstock patterns. Furthermore, the annual effect is highlighted by two significant threefold interactions of cultivar \times rootstock \times year for apple (2012–2013) and pear (2011–2012). Temporal variability of fire blight tolerance was also observed in other studies. Joos et al. (2012) partly reported annually conflicting results in a two-year test of old apple cultivars under greenhouse conditions. Sobiczewski et al. (2014) recorded both, annually conflicting and consistent results in testing different apple cultivars grafted on M.9 under greenhouse conditions for a three-year period. Annually fluctuating results of fire blight tolerance experiments of apple and pear trees might be associated with differences in shoot vigor, environmental conditions occurring in the greenhouse (e.g. aphid infection), and diversity in the genetic tolerance of tested individual plants (Sobiczewski et al. 2014).

A review of the methodical procedure and the obtained results reveals that a stable composition of crcs over perennial periods in connection with a sufficient amount of plants per combination enhances the statistical power of the fire blight tolerance assessment. Testing the same combinations in subsequent experiments, leads to a more balanced and significant observation of perennial cri effects.

5.1.5. Overall assessment

Designing an orchard system is very complex. Accordingly, the selection of an apple or pear cultivar for cultivation is a most vital decision (Hester and Cacho 2003; Rozman et al. 2015). Since the decision for a rootstock or cultivar already involves several criteria and is very complex, the selection of a most adequate cultivar \times rootstock combination in a specific environment is even more complex.

There are several interdependent factors that influence an orchardist's decision for a specific crc. A crucial aspect is the plant materials' adaptation to local abiotic site conditions, such as soil type, temperature, radiation, precipitation regime, frost occurrence or periods of drought. Furthermore, the crc should suit the intended orchard management (e.g. orchard system, tree density, irrigation system, fruit thinning). Therefore, horticultural aspects such as tree vigorousness (height, width, and canopy volume), development of root suckers, yield (cumulative yield, cumulative yield efficiency), fruit size, fruit quality, and shelf life need to be considered.

The marketability is a key factor for the selection of a cultivar. In this regard, fruit appearance (size, body color), inner quality (taste, physical properties), recognition factor, and “the certain something” are important aspects (Rozman et al. 2015; Rühmer 2014a). There are different approaches to introduce new cultivars to the grower-retailer-consumer chain. As in the case of Ariane, cultivars can be marketed as a club variety under strict stipulations regarding the whole food-chain and additionally be accompanied by a sophisticated marketing strategy. Ariane, a selection of the INRA program in Angers, France is marketed by the company S.A.S. POMALIA and distributed as part of a series under the generic trademark “Les Naturianes” (Laurens et al. 2005). To attain access to the Swiss market for new cultivars with desirable features (e.g. scab resistance, fire blight tolerance), FiBL developed two distinct tools in collaboration with actors along the food chain (organic apple growers and retailers), which are already at hand. The first one, called Flavor Group Concept, provides buyers with information on the taste of the unknown apple in the supermarket. 3 flavors are distinguished: mild to sweet, spicy-tart and predominantly tart. The second tool is called variety team: representatives of retailers, growers and scientists form a consortium that mutually chooses promising cultivars, which are then experimentally produced on 1 ha for a period of 4 years. The fruits of the third and fourth year are sold in a test-selling at the involved retailer. The consortium then meets again and decides whether the cultivar should be produced further or not. The financial risk of this approach is shared by all members of the consortium (Weibel and Leder 2007).

Pest and disease features of the tree likewise determine the fruit grower's selection of a crc. Besides fire blight, prevalent diseases that need to be considered in economic apple and pear production are two fungal diseases: scab (Apple: *Venturia inaequalis* COOKE, Pear: *Venturia pirina* ADERHOLD) and powdery mildew (*Podosphaera leucotricha* E.S. SALMON) (Jackson 2003). Major insect pests are codling moth (*Cydia pomonella* L.) in apple production and woolly aphid (*Eriosoma lanigerum* HAUSMANN) in apple and pear production (Jackson 2003). Particularly in organic production, where the application of synthetic products is prohibited, the cultivar × rootstock tolerance or resistance to diseases plays an important role (IFOAM 2014). Besides fire blight tolerant cultivars, scab resistant cultivars are especially recommended for cultivation in organic systems (Weibel and Häseli 2002; Weibel et al. 2005). Additionally, rootstock tolerance against pests, diseases and weed competition is of emphasized interest in

organic systems (Weibel and Häseli 2002, Weibel et al. 2005, Weibel et al. 2008). Furthermore, the selection of an adequate pear cultivar is impaired by a low number of potentially marketable cultivars, and special attention needs to be paid to affinity problems with quince rootstocks and pear cultivars (Weber and Fischer 2005, Weibel et al. 2005).

Fire blight tolerance is a major piece of the puzzle when it comes to the recommendation of apple and pear crcs. However, other important characteristics need to be taken into account as well in order to gain a holistic assessment of the utilized plant materials. Other important factors of cultivars and rootstocks considered in the experiment are summarized in Table 2, 3, (apple), and 6, 7 (pear).

5.2. Scientific Discussion

5.2.1. Cultivar × rootstock interaction

In the pathosystem of *E. amylovora* and Rosaceous plants, no avirulence gene and equivalent plant resistance gene have been detected (Vrancken et al. 2013). Thus, no gene for gene resistance has yet been reported. In contrast, several plant defense reactions have been identified: A rapid increase of reactive oxygen species (ROS), a change in levels of secondary metabolites derived from the phenylpropanoid-flavonoid pathway, a change in levels of plant hormones, the production of pathogenesis-related proteins, and the abundance of phytoalexins was recorded in infected apple and pear trees (Vrancken et al. 2013).

Just as the plant defense mechanisms, the rootstock induced fire blight tolerance is ambiguous. However, microbiological methods, such as the microarray analysis, have recently improved analytical opportunities. By microarray analysis, Jensen et al. (2012) identified possible compounds, such as *sdh* and the protein calnexin, that evoke a rootstock induced fire blight tolerance. Identifying compounds that indicate fire blight tolerance would allow for a preselection of promising plant material before inoculation assessments. This would accelerate the identification of tolerant combinations and save costs.

5.2.2. Experimental Design

As previously described, the experiment took place under controlled environmental conditions inside the greenhouse. The plants were distributed into two types of randomized racks, each single rack containing 20 plants. Each rack type was represented 10 times – amounting to an overall number of 400 single plants. However, since the racks were randomized two times and each rack type was duplicated 10 times, all tested trees were placed in the same position in the racks. This may lead to neighboring and bordering effects between the crcs (Laso Bayas (01.07.2015), Personal Communication). For instance, a possible bordering effect could be enhanced lighting conditions of plants stored at the edge of the racks, resulting in an increased rate of photosynthesis and thus more vigorous plants. However, vigorously growing shoots of

apple and pear trees allow a more rapid progression of *E. amylovora* infection than slowly growing shoots (Hepaksoy et al. 1999; Ozrenk et al. 2012; Sobiczewski et al. 2014). In this case, the bordering effect may lead to comparatively increased necrosis values and an overestimation of fire blight susceptibility of the crc. On the other hand, conceivable neighbouring effects inside the racks could be different lighting conditions of combinations, e.g. adjacent to invigorously growing combinations. To eliminate any possible spatial trend, a full randomization of agricultural experiments is highly recommended (Piepho et al. 2013).

5.2.3. *E. amylovora* strain

Among other influencing factors, the bacterial strain used is affecting the degree of disease symptoms. Although *E. amylovora* bacteria depict a very homogeneous species of plant pathogenic bacteria regarding its biochemical and genetic properties, strains vary in their virulence to the same host genotype, their serology, their susceptibility to bacteriophages and their level of sensitivity to streptomycin (Momol and Aldwinckle 2000; Puławska and Sobiczewski 2012; Sobiczewski et al. 2008). Due to its high virulence against apple and pear trees and its natural abundance in Switzerland, the bacterial strain *Erwinia amylovora* ACW 610 was used for inoculation in this experiment. Moreover, this strain was widely applied in previous experiments regarding the tolerance of plant material against *E. amylovora* (Egger et al. 2013; Khan et al. 2006; Perren et al. 2013; Rezzonico and Duffy 2007; Schöneberg et al. 2015; Silvestri and Egger 2011). Sobiczewski et al. (2008) reported significantly different results of the same host genotype inoculated with different single *E. amylovora* strains. However, inoculating a mixture of all strains utilized did not lead to different results compared to results obtained with each single strain (Sobiczewski et al. 2008). Furthermore, Jensen et al. (2012) observed a strain dependent fire blight susceptibility of Gala × M.9 combinations after scion inoculation. The inoculation of strain mixtures can therefore increase the reliability of the assessed combinations' fire blight tolerance. Therefore, to verify the results obtained in this study, promising plant material needs to be counter-checked with other regional *E. amylovora* strains.

5.2.4. Inoculation methods

Legal security demands, that are meant to prevent the discharge of *E. amylovora* to the open environment, are very high. Particularly experiments including artificial blossom inoculation outside the greenhouse are difficult to realize. Thus, due to the status of *E. amylovora* as quarantine organism in Switzerland, an open air protected orchard allowing blossom inoculation was missing, but is now in use since 2013 (Schöneberg et al. 2015). Therefore, just as conducted in this study, the main mode of assessing plant materials' tolerance to fire blight is artificial shoot inoculation inside the greenhouse (Horner et al. 2014). Although Steiner (2000) states that shoot infection poses the main risk in tree nurseries, under practical orchard

conditions, bacteria are mainly entering the plants through natural openings in the flowers, such as nectarhodes (van der Zwet et al. 2012, Kellerhals et al. 2014a). Comparing the results obtained of testing apple cultivars for their fire blight tolerance with either shoot inoculation or blossom inoculation, Schöneberg et al. (2015) reported a weakly positive correlation of both testing methods. Schöneberg et al. (2015) described it as a conforming trend, although some cultivars were more tolerant when they were assessed after shoot inoculation rather than after blossom inoculation. Horner et al. (2014) observed no significant correlation between results obtained with inoculation of shoots or blossoms. They reported alternating results of the progeny of Royal Gala \times Malus robusta, i.e. either results obtained from shoot or blossom inoculation seemed to verify a higher tolerance. It might be an explanation that quantitative trait loci involved in shoot tolerance differ from those associated with blossom tolerance (Horner et al. 2014). In line with this, only a weak correlation between the tolerance of blossoms and shoots of Gala trees was reported by Thibault and Le Lezec (1990). Testing the fire blight tolerance of pear cultivars, Honty (2010) also reported conflicting results obtained from shoot and blossom inoculation. Peil et al. (2014) generally concluded that the fire blight tolerance is overestimated when natural infection occurs in an orchard while the susceptibility of plants is overestimated when shoots are artificially inoculated in the greenhouse. In order to provide a substantive statement of a genotype's fire blight tolerance, both inoculation methods should be employed (Horner et al. 2014, Schöneberg et al. 2015).

5.2.5. Latent infestation

Although fire blight tolerant assessed crcs do not express any disease symptoms within the time span of the experiment, there is scientific evidence that *E. amylovora* bacteria can be abundant in significant amounts in their asymptomatic tissue (Baumgartner et al. 2012; Joos et al. 2012). LoGiudice et al. (2006) did indeed isolate bacteria from grafted B.9 rootstocks out of asymptomatic tissue.

When these bacteria multiply, the bacterial build-up of the latent infestation might serve as the inoculum for secondary infection in orchards or fruit-growing regions which host different crcs. Thus within these areas, particularly susceptible tree combinations might be endangered. Since latent infestations cannot be visually detected, they might pose an even greater risk for tree growers who cultivate tolerant and susceptible trees in close vicinity.

5.2.6. Climate Change

Adaptation to climate change depicts an additional challenge for breeders as well as apple and pear growers. Higher temperatures in combination with a more variable precipitation regime and a higher frequency of extreme weather conditions are forecasted for regions in temperate Europe (Hirschi et al. 2012). Following a climate change scenario of Hirschi et al. (2012), no significant increase in fire blight infection days in northern Switzerland was projected.

However, climate change affects apple and pear production systems holistically. In a 48-year observation, apple spring phases annually occurred between 0.1 and 0.3 days earlier across Europe (Ahas et al. 2002). Chmielewski et al. (2004) observed an advanced flowering period of apple trees of 2.2 days per decade. A preponed flowering period may increase the risk of late spring frosts damaging apple blossoms (Chmielewski et al. 2004). Thus, cultivars that exhibit a rather late blooming stage may become increasingly interesting. Extreme weather conditions – drought and flooding – along with storms and thunderstorms are going to occur more frequently. Therefore, more distinct rooting systems as good anchorage, resulting in an avoidance of uprooting and increased economic viability under non-irrigated conditions, are becoming increasingly important. In the course of ongoing climate change, Sugiura et al. (2013) even detected long-term changes in fruit quality parameters such as a higher sugar content, and a decrease in acid concentration and fruit firmness. To assess prospective promising crcs, aspects related to climate change need to be necessarily considered.

6. Conclusions

Due to the distinct practice-orientation of this study, the conclusions are divided into two sections: methodic-scientific conclusions and practical conclusions. Thus, the intended target groups can be addressed directly and the knowledge transfer from science to practice can be improved. Furthermore, the hypothesis that were compiled in chapter 2.5 are examined. To get an overview they are recapitulated below:

- (1) Apple and pear rootstocks can have a significant effect on the *E. amylovora* tolerance of the scion (cultivar).
- (2) With highly *E. amylovora* susceptible cultivars, rootstocks cannot prevent severe fire blight damage.
- (3) The results are consistent for several years.

6.1. Scientific conclusions

6.1.1. Cultivar × rootstock interaction

The cri effects were significant in every year of the experiment for apples and in two of three years in the pear screening. Thus, hypothesis (1) fully conforms to the apple results. Moreover, with an exception in 2012, when it was almost significant ($P=0.06$), it also accorded with the pear results. Accordingly, the fire blight tolerance screening of crcs is necessary. However, to select probable fire blight tolerant crcs, the separate screening of cultivars and rootstocks is of major importance.

Although the critical tolerance reaction in crcs is yet insufficiently investigated, microbiological methods, such as the microarray analysis, offer entirely new analytical opportunities. As an output of this, Jensen et al. (2012) identified candidate compounds for a rootstock induced fire blight tolerance. As an integrated measure, tracing these compounds in prospective cultivar × rootstock tolerance screenings may help to determine a compound that could serve as an indicator for fire blight tolerance in the future. In this regard, sorbitol dehydrogenase enzymes and the protein calnexin are of particular interest (Jensen et al. 2012).

6.1.2. Perennial composition of combinations

With the exception of 2012 and 2013, the composition of crcs displayed variation from year to year. Due to this, a collective perennial statistical analysis of all combinations that took place in the experiment was not feasible. However, the conducted analysis of two-year periods revealed that the year itself and interactions of cultivar and rootstock with year were significant in both, apple and pear experiments. This indicates an erratic annual arrangement of the tested combinations. Therefore, hypothesis (3) must be rejected. Thus, a reliable assessment of a combination that is only tested for one year is not viable. In upcoming fire blight tolerance

screening experiments the group of tested combinations needs to be constant for a minimum of 3 years to take into account the observed temporal variability.

6.1.3. Method of inoculation

Comparing the results obtained by way of shoot inoculation in this study with documented results of blossom inoculation, there are partly conflicting assessments of a cultivar's fire blight tolerance (Schöneberg et al. 2015). In this context, Horner et al. (2014) hypothesized a different genetic background involved in apple trees for the expression of fire blight tolerance to shoot and flower infection. Under natural conditions, the main mode of *E. amylovora* infection occurs via openings in the flowers, such as nectarhodes (van der Zwet et al. 2012). Thus, testing both infection paths in an integrated approach is the method of choice (Schöneberg et al. 2015). A preselection of fire blight tolerant trees assessed by time-saving shoot inoculation, associated with a subsequent floral tolerance screening of most promising crcs, offers most reliable conclusions.

6.1.4. Bacterial strain

In this study the single Swiss *E. amylovora* strain ACW 610 was used in a concentration of 10^9 cfu ml⁻¹. Different *E. amylovora* strains vary in their virulence to the same host genotype and may generate different results in the fire blight tolerance assessment of host plants (Sobiczewski et al. 2008). In contrast, the inoculation of a strain mixture did not lead to different results compared to each single strain (Sobiczewski et al. 2008). The inoculation of strain mixtures increases the reliability of the fire blight tolerance assessment of crcs. Thus, to verify the results obtained in this study, promising plant material needs to be counter-checked with other regional *E. amylovora* strains, such as both the strains Ea 797 and Ea 815 isolated on *Malus* in Germany (Bantleon 2012; Sobiczewski et al. 2014).

6.1.5. Full Randomization

The trees were kept in 2 types of randomized racks. However, each rack type was subsequently reproduced ten times. To avoid any spatial trend, all racks should be fully randomized in upcoming experiments (Piepho et al. 2013).

6.2. Practical conclusions

6.2.1. Recommended apple and pear combinations

The significant cri of apple and pear underlines the importance for fruit growers to thoroughly consider the crc regarding its fire blight tolerance rather than solely the cultivar's or rootstock's fire blight attributes. In general, the impact of the cultivar is more pronounced than the significance of the rootstock. However, a significant rootstock effect to the cultivar was

observed for some cultivars, but not for all of them. Thereby, with the exception of Galiwa in 2010, the fire blight tolerance of a susceptible cultivar could generally not be improved by a graftage with a tolerant rootstock. This corresponds to hypothesis (2).

This implies that on the one hand, a robust rootstock can contribute to a tolerant combination, together with a tolerant cultivar, but on the other hand, a robust rootstock cannot add up to a tolerant combination grafted to a susceptible cultivar. Nevertheless, a robust rootstock grafted to a susceptible cultivar can improve the fire blight tolerance of the entire tree, and – avoiding rootstock blight – it definitely increases the chance of tree survival after fire blight infected parts of the cultivar are pruned away (Robinson et al. 2004). However, in return the results indicated that a robust rootstock may (e.g. Ladina), or may not (e.g. Ariane), increase the fire blight tolerance of an already tolerant cultivar.

Ladina \times G.11 was the most promising apple combination assessed in this study. It achieved the lowest rate of shoot necrosis in both of the single years it was tested in. It revealed a decline compared to the other Ladina combinations that was significantly different from Ladina \times M.9 in 2012 and marginally significant in 2013. Even in the summarized analysis of the data 2012–2013, where – due to a higher sample size – results are more balanced, Ladina \times G.11 was significantly different from Ladina \times M.9. Furthermore, the cultivar Ladina itself has evinced good fire blight tolerance after both flower and shoot inoculation (Schöneberg et al. 2015). This is particularly important since it is hypothesized that there might be different quantitative trait loci responsible for the tolerance against shoot or floral infection (Horner et al. 2014), which means that plants possibly can express a high tolerance against one of the pathways while they might be susceptible to the other. Moreover, Ladina achieved good results regarding its yield characteristics, especially the share of class I apples and it does not tend to biennial bearing (Leumann et al. 2013). It is scab resistant (Vf), marginally susceptible to powdery mildew and available from an organic nursery in Switzerland (Leumann et al. 2013; Rühmer 2014a; Weibel and Häseli 2015). Furthermore, Ladina was preferred over Gala regarding its taste features and its overall performance (Leumann et al. 2013). First promising practical experience with the cultivar Ladina has been gained since 2007, when Ladina trees were planted in 6 orchards in Switzerland (4 managed according to guidelines of integrated production and 2 managed organically) (Leumann et al. 2013; Weibel and Häseli 2015). Moreover, Ladina showed good growth characteristics in a tree nursery, similar to those of Topaz (Nursery Scherrer, Egnach (19.08.2015), Personal Communication). However, reported storage difficulties from January onwards constitute a problem that calls for further examination (Weibel and Häseli 2015). G.11 showed fire blight tolerance to both direct inoculation and inoculation of the scion cultivar and was assessed tolerant against phytophthora and the replant disease (Fazio et al. 2014; Kockerols et al. 2009; Russo et al. 2007). In addition to that, it proofed a higher yield efficiency than M.9, is comparably vigorous and has the potential to increase the monetary benefit compared to M.9

(Fazio et al. 2014; Pfeiffer 2014). On a small scale, G.11 is commercially available (e.g. Jansen, NL).

Currently Ladina \times G.11 is only available to fruit growers in an outmost limited extent, whereas Ladina \times M.9 is temporarily most widely available on the market for fruit growers (Nursery Brugger, Bozen (20.08.2015) and nursery Scherrer, Egnach (19.08.2015), Personal Communication). Furthermore, despite a high loss rate in 2015, Ladina \times G.41 is currently propagated, and – given that the supply of G.11 is more stable – Ladina \times G.11 is intended for prospective propagation. If the demand for Ladina \times G.11 will increase, nurseries will be able to serve the market (Nursery Brugger, Bozen (20.08.2015), Personal Communication)

Summing up, Ladina \times G.11 is a very promising combination regarding horticultural aspects, its disease, and pest features and its marketability. It can therefore be recommended for experimental cultivation. Based on results obtained with G.41 in 2010, Ladina \times G.41 should be included in upcoming screenings. Moreover, Ladina \times G.41 is already included in experimental propagation in a Swiss organic nursery (Nursery Scherrer, Egnach (19.08.2015), Personal Communication).

Another combination that revealed promising results was Galiwa \times G.41 in 2010. Its shoot necrosis was significantly lower than Galiwa \times M.9 (-60.5 percentage points). To the detriment of the study, only 3 plants of this combination could be considered in the analysis, which is limiting the statistical validity of the results for this combination. Due to this, Galiwa \times G.41 should be considered in forthcoming scientific fire blight tolerance screenings.

In contrast to Ladina, the as tolerantly assessed cultivar Ariane did not show any rootstock affinity regarding its fire blight tolerance. However, Schöneberg et al. (2015) found that Ariane was highly susceptible to blossom infection with *E. amylovora*. Although Ariane has promising horticultural features and exhibited very low shoot necrosis in this study, it cannot be recommended for its fire blight tolerance in the same way as Ladina \times G.11 since the bacterial pathway through flowers is the most common one under natural conditions.

In contrast to the apples, no specific pear crc constantly showed a low relative shoot necrosis. Nevertheless, as assumed, the lowest lesion lengths in 2011 and 2013 were found for the tolerant reference cultivar Elliot grafted on OHF (87 and 11).

This study substantially revealed the effect of cultivar \times rootstock interaction regarding the fire blight tolerance of grafted apple and pear trees. It has highlighted Ladina \times G.11 as a fire blight tolerant apple combination that can be recommended for experimental cultivation.

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Annex

I. Tables

Table 25: Origin of the apple rootstocks utilized in the experiment

Rootstock	Year	Origin
AR 295-6	2011–2012	Ducarros nursery, Villers-Cotterets, France
B.9	2010–2013	Agroscope, Wädenswil, Switzerland
G.11	2010–2012	Dali tree, Angers, France
G.11	2013	Gebr. Jansen nurseries, the Netherlands
G.41	2010	Dali tree, Angers, France
M.9	2010–2013	Agroscope, Wädenswil, Switzerland
Supporter 2	2010–2011	F. Weibel

Table 26: Origin of the pear rootstocks utilized in the experiment

Rootstock	Year	Origin
OHF 11	2013	Brigitte Astier, Mondragon, France
OHF 87	2010–2012	Brigitte Astier, Mondragon, France
QBA 29	2010	F. Weibel
QC	2010–2013	Agroscope, Wädenswil, Switzerland
QE	2012–2013	F. Weibel

Table 27: Apple cultivar \times rootstock combination analysis (2010): Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock, cultivar \times rootstock. For calculations data transformed by square root. Means with the same letter are not significantly different.

Combination	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
Gala \times M.9	11	A	98.6 (\pm 3.5)
Galiwa \times M.9	6	A	94.2 (\pm 4.8)
Gala \times G.41	11	A	93.2 (\pm 3.5)
Gala \times Supporter 2	10	A	92.7 (\pm 3.7)
Galiwa \times Supporter 2	3	A	92.2 (\pm 6.8)
Gala \times G.11	7	A	88.0 (\pm 4.4)
Galiwa \times G.41	4	B	33.7 (\pm 5.8)
Rewena \times Supporter 2	11	B C	21.8 (\pm 3.5)
Rewena \times M.9	10	B C	18.9 (\pm 3.7)
Rewena \times G.11	4	C D	10.9 (\pm 5.8)
Rewena \times G.41	12	D	8.1 (\pm 3.4)

Table 28: Apple cultivar \times rootstock combination analysis (2011): Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha=0.05$). Model: cultivar, rootstock, cultivar \times rootstock. For calculations data transformed by square root. Means with the same letter are not significantly different.

Combination	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
Gala \times B.9	12	A	98.5 (\pm 5.7)
Gala \times G.11	5	A B	98.3 (\pm 8.8)
Rustica \times B.9	9	A	97.6 (\pm 6.6)
Rustica \times M.9	12	A	96.8 (\pm 5.7)
Galiwa \times Supporter 2	4	A B	95.2 (\pm 9.9)
Rustica \times G.11	6	A B	94.7 (\pm 8.1)
Rustica \times Supporter 2	7	A B	94.6 (\pm 7.5)
Galiwa \times M.9	8	A B	94.4 (\pm 7.0)
Gala \times Supporter 2	10	A B	91.2 (\pm 6.3)
Rustica \times AR 295-6	4	A B C	90.5 (\pm 9.9)
Gala \times M.9	11	A B	87.4 (\pm 6.0)
Galiwa \times B.9	11	A B	86.4 (\pm 6.0)
Galiwa \times G.11	5	A B C	79.4 (\pm 8.8)
Rewena \times Supporter 2	9	B D	56.5 (\pm 6.6)
Rewena \times M.9	11	C D	51.0 (\pm 6.0)
Rewena \times AR 295-6	9	C D	48.9 (\pm 6.6)
Rewena \times G.11	4	D E	24.6 (\pm 9.9)
Rewena \times B.9	12	E	19.7 (\pm 5.7)

Table 29: Apple cultivar \times rootstock combination analysis (2012): Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha=0.05$). Model: cultivar, rootstock, cultivar \times rootstock. For calculations data not transformed. Means with the same letter are not significantly different.

Combination	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
Gala \times AR 295-6	6	A B	87.1 (\pm 8.0)
Gala \times G.11	10	B	86.3 (\pm 6.2)
Gala \times M.9	5	A B	81.5 (\pm 8.7)
Galiwa \times M.9	8	A B	77.9 (\pm 6.9)
Gala \times B.9	10	A B	77.1 (\pm 6.2)
Natyra \times AR 295-6	3	B C D	76.7 (\pm 11.3)
Natyra \times G.11	8	A B	73.2 (\pm 6.9)
Galiwa \times G.11	9	B C	68.7 (\pm 6.5)
Galiwa \times B.9	9	B C	68.2 (\pm 6.5)
Galiwa \times AR 295-6	10	B C	67.9 (\pm 6.2)
Natyra \times B.9	10	B C	66.8 (\pm 6.2)
Natyra \times M.9	10	B C D	63.2 (\pm 6.2)
Ladina \times B.9	9	B F	60.6 (\pm 6.5)
Rewena \times M.9	8	B E F	52.8 (\pm 6.9)
Ladina \times M.9	9	A C E F	51.2 (\pm 6.5)
Ladina \times AR 295-6	6	C F G	33.7 (\pm 8.0)
Ariane \times M.9	10	D F G	32.8 (\pm 6.2)
Ariane \times AR 295-6	7	D F G	30.7 (\pm 7.4)
Ariane \times B.9	10	F G	29.6 (\pm 6.2)
Ariane \times G.11	10	E G	21.1 (\pm 6.2)
Ladina \times G.11	9	G	13.4 (\pm 6.5)

Table 30: Apple cultivar \times rootstock combination analysis (2013): Mean comparison of LS-mean values of relative lesion length 3 wpi. Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock, cultivar \times rootstock. For calculations data transformed by 4th root. Means with the same letter are not significantly different.

Combination	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
Gala \times M.9	10	A	94.3 (\pm 5.7)
Gala \times AR 295-6	9	A B	90.2 (\pm 6.0)
Gala \times B.9	10	A B C	68.4 (\pm 5.7)
Gala \times G.11	8	A D	60.6 (\pm 6.4)
Galiwa \times AR 295-6	9	A D E	51.9 (\pm 6.0)
Galiwa \times B.9	10	B D F	46.1 (\pm 5.7)
Natyra \times AR 295-6	9	B D F	44.7 (\pm 6.0)
Galiwa \times M.9	10	C D F I	33.5 (\pm 5.7)
Natyra \times B.9	6	C D G H	32.7 (\pm 7.3)
Natyra \times G.11	10	D H	28.2 (\pm 5.7)
Galiwa \times G.11	10	D H	28.1 (\pm 5.7)
Ariane \times G.11	8	D G H J	24.0 (\pm 6.4)
Ariane \times B.9	9	E F G H J	22.8 (\pm 6.0)
Natyra \times M.9	10	E F G H J	22.1 (\pm 5.7)
Ariane \times AR 295-6	6	F H K	17.8 (\pm 5.7)
Ariane \times M.9	10	H I K	17.4 (\pm 7.3)
Ladina \times AR 295-6	9	H I K	16.0 (\pm 6.0)
Ladina \times B.9	7	H K	10.6 (\pm 6.8)
Rewena \times M.9	8	G K	9.7 (\pm 6.4)
Ladina \times M.9	7	J K	6.9 (\pm 6.8)
Ladina \times G.11	4	K	3.2 (\pm 9.0)

Table 31: Apple cultivar \times rootstock combination analysis (2012-2013): Mean comparison of LS-mean values of relative lesion length 3 wpi Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock, year, cultivar \times rootstock, cultivar \times year, rootstock \times year, cultivar \times rootstock \times year. For calculations data transformed by third root. Means with the same letter are not significantly different.

Combination	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
Gala \times M.9	15	A	90.0 (\pm 6.1)
Gala \times AR 295-6	15	A	89.0 (\pm 6.1)
Gala \times G.11	18	A B	74.9 (\pm 5.6)
Gala \times B.9	20	A B	72.8 (\pm 5.3)
Galiwa \times AR 295-6	19	A B C	60.3 (\pm 5.2)
Galiwa \times B.9	19	A B C D	56.6 (\pm 5.4)
Natyra \times B.9	16	A B C D E	54.0 (\pm 5.9)
Galiwa \times M.9	18	A B C D E	53.3 (\pm 5.6)
Natyra \times AR 295-6	13	A B C D E	52.7 (\pm 6.8)
Natyra \times G.11	18	B C D E F	48.2 (\pm 5.6)
Galiwa \times G.11	19	B C D E F	47.3 (\pm 5.4)
Natyra \times M.9	20	C D E F	40.6 (\pm 5.4)
Ladina \times B.9	16	C D E F	38.7 (\pm 5.9)
Ladina \times M.9	16	E F G	31.8 (\pm 5.9)
Rewena \times M.9	16	E F G	31.3 (\pm 5.9)
Ariane \times M.9	16	D E F G	27.0 (\pm 5.9)
Ariane \times B.9	19	E F G	26.4 (\pm 5.4)
Ariane \times AR 295-6	17	F G	23.1 (\pm 5.7)
Ladina \times AR 295-6	15	E F G	23.1 (\pm 6.1)
Ariane \times G.11	18	F G	22.4 (\pm 5.6)
Ladina \times G.11	13	G	10.3 (\pm 6.5)

Table 32: Pear cultivar \times rootstock combination analysis (2013): Mean comparison of LS-mean values of relative lesion length 3 wpi Post-ANOVA Tukey test ($\alpha = 0.05$). Model: cultivar, rootstock, cultivar \times rootstock. For calculations data transformed by square root. Means with the same letter are not significantly different.

Combination	N	Letter Display	LS Means Lesion Length (\pm Standard Error) [%]
ACW 3851 \times QC	10	A	60.9 (\pm 5.5)
ACW 3764 \times OHF 11	8	A B	51.5 (\pm 7.8)
ACW 3851 \times OHF 11	5	A B	46.4 (\pm 6.2)
Elliot \times QE	9	A B	34.2 (\pm 5.8)
Conférence \times QE	10	A B	33.1 (\pm 5.5)
Conférence \times QC	9	B	28.6 (\pm 5.8)
Conférence \times OHF 11	9	B	25.7 (\pm 5.8)
Elliot \times QC	10	B	25.2 (\pm 5.5)
Elliot \times OHF 11	6	B	23.4 (\pm 7.1)
ACW 3764 \times QC	10	B	23.2 (\pm 5.5)

II. Declaration on oath

I, Matthias Schluchter, born on August 18th, 1985, matriculation number 549024,

hereby declare on my honor that the attached declaration,

Master Thesis,

has been independently prepared, solely with the support of the listed literature references, and that no information has been presented that has not been officially acknowledged.

Supervisors: Prof. Dr. Ralf Voegelé, Dr. Franco Weibel

Thesis topic: Four year assessment of the cultivar \times rootstock interaction on fire blight tolerance

Semester: 7

I declare, here within, that I have transferred the final digital text document (in the format doc, docx, odt, pdf, or rtf) to my mentoring supervisor and that the content and wording is entirely my own work. I am aware that the digital version of my document can and/or will be checked for plagiarism with the help of an analyses software program.

Matthias Schluchter

Stuttgart-Hohenheim, November 2015