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Use of a growth regulator (Prohexadione-Ca) and summer pruning as post symptom rescue treatments following a fire blight infection during bloom.

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Full Title:	Use of a growth regulator (Prohexadione-Ca) and summer pruning as post symptom rescue treatments following a fire blight infection during bloom.
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Abstract:	Fire blight of apples (*Erwinia amylovora*) can be a serious disease depending on production area and year. Bloom is the most frequent starting point for infection and the primary focus for sprays which can prevent infection, or of growth regulators such as prohexadione-Ca (ProCa) to limit spread. Because of the sporadic cases, many growers opt to forego bloom sprays when disease pressure is low. When symptoms do appear, quickly pruning out affected branches is currently the most effective control measure. Our goal was to determine if ProCa applied when symptoms appear instead of during bloom when infection risks are predicted could be used to limit disease spread, and if this would interact with summer symptom removal. The study was carried out in 2018 and 2019 at the IRDA orchard in Saint-Bruno-de-Montarville, Québec. Plots inoculated during bloom were allocated to treatments in which diseased branches were either pruned during summer or only in winter, and trees sprayed with ProCa starting at bloom, when symptoms appeared, or not. Potential for disease progression was assessed by inoculating shoots and rating them for disease severity and ooze production. Significantly less wood was removed from trees pruned during the summer as opposed to winter, but total yield was reduced. ProCa had no effect on the amount of dead wood pruned, but still reduced disease severity. We found ProCa reduced ooze on inoculated shoots and that post symptom timing was as effective as the bloom spray. Delaying sprays until symptoms appear could become an alternative strategy to reduce summer spread of fire blight.
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Response to Reviewers:	Dear Editor, I first want to express my deepest gratitude for the extensive review work. The comments and suggestions of both reviewers greatly improved the manuscript. Most the comments probably don't require a formal answer and I simply included them in the revised version. I must admit that I could not understand some markings of editing on the PDF and I tried to improve the text wherever these occurred. Reviewer #1 also included comments in the PDF which expressed opinions I don't completely share. I added nuance to the text to bridge the gap. In these comments, Reviewer #1 asked that we explain why we used a mix of strains for inoculation. I'm not convinced this is relevant to the scope of this paper or that is matters in any way. We could omit

	this detail, but this is what we do as a routine practice. Some comment boxes appeared empty and I don't understand what they were meant for. There were also some disagreements between reviewers. Reviewer #1 generally crossed out subtitles in the Results section, whereas Reviewer #2 asked to make these more descriptive. I opted for the later. A bit more problematic is that reviewer #1 was very critical of plots and statistics whereas reviewer #2 only had positive comments about these aspects of the manuscript. I must express some disagreement with reviewer #1, but it's possible I simply did not understand what was meant. In general, I am very open to modifying the Figures, but frankly don't agree that box plots would be adequate. For each treatment combination we observed 4 trees, so we have 4 values. In this case, I believe individual values should be presented, otherwise the reader might be left with the impression we display more values than we have. The topic is nicely discussed here: https://statisticsbyjim.com/basics/graph-groups-boxplots-individual-values/ Similarly, lines are commonly used to help visualize interactions, even across categorical predictors. Interactions are not obvious otherwise. We chose the most basic and popular format. For instance, this tutorial (look at figure 7.3) https://bookdown.org/max/FES/detecting-interaction-effects.html I also respectfully disagree that the data analysis is an 'unneeded surplus'. Considering this paper dealt with repeated measures and count data some consideration of statistical models was unavoidable. I understand that this makes the paper longer and more difficult to read and I did invest time to make it more straightforward and shorter. If you feel I should make more changes, I could do this promptly. Again, thank you for this opportunity. Best regards, Vincent Philion

Use of a growth regulator (prohexadione-Ca) and summer pruning as post symptom rescue treatments following a fire blight infection during bloom.

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Abstract

Fire blight of apples (*Erwinia amylovora*) can be a serious disease depending on production area and year. Bloom is the most frequent starting point for infection and the primary focus for sprays which can prevent infection, or of growth regulators such as prohexadione-Ca (ProCa) to limit spread. Because of the sporadic cases, many growers opt to forego bloom sprays when disease pressure is low. When symptoms do appear, quickly pruning out affected branches is currently the most effective control measure. Our goal was to determine if ProCa applied when symptoms appear instead of during bloom when infection risks are predicted could be used to limit disease spread, and if this would interact with summer symptom removal. The study was carried out in 2018 and 2019 at the IRDA orchard in Saint-Bruno-de-Montarville, Québec. Plots inoculated during bloom were allocated to treatments in which diseased branches were either pruned during summer or only in winter, and trees sprayed with ProCa starting at bloom, when symptoms appeared, or not. Potential for disease progression was assessed by inoculating shoots and rating them for disease severity and ooze production. Significantly less wood was removed from trees pruned during the summer as opposed to winter, but total yield was reduced. ProCa had no effect on the amount of dead wood pruned, but still reduced disease severity. We found ProCa reduced ooze on inoculated shoots and that post symptom timing was as effective as the bloom spray. Delaying sprays until symptoms appear could become an alternative strategy to reduce summer spread of fire blight.

Keywords: Erwinia amylovora, Malus x domestica, Apogee, prohexadione-Ca, plant growth regulator

Introduction

In many areas where apples are grown, fire blight caused by the bacterium *Erwinia amylovora*, is a sporadic but potentially devastating disease. Severe outbreaks can kill entire orchards in one season (Vanneste 2000). Under Québec conditions, infections occur primarily during bloom when warm weather conducive to bacteria multiplication on the stigma of contaminated flowers is followed by wetting which facilitates transfer of bacteria to the hypanthium, where entry occurs. In absence of resistance, timely sprays during bloom of streptomycin can very effectively prevent infection. However, market concerns over the use of antibiotics, their ban in organic production (Johnson and Temple 2013), and resistance in some areas have prompted the need for alternative approaches. Provided good timing, one effective possibility is to spray Blossom Protect (Kunz and Donat 2013), a formulation of antagonist strains of *Aureobasidium pullulans* with an acidic buffer. In effect, most spray recommendations against this disease are timed during bloom ahead of symptoms. In some areas, software such as Maryblyt (Turechek and Biggs 2015), Cougarblight (Smith and Pusey 2011), and RIMpro (Philion and Trapman 2011) are used to help time these sprays, reducing the spray inputs compared to systematic sprays. Since fire blight inoculum is infrequent in any given orchard, predictive models tend to err with false positive prognosis and result in unnecessary sprays. The sporadic nature of this disease causes many growers to question preventative sprays, especially in orchards with no history of the disease (Norelli et al. 2003). Many growers opt not to spray during bloom, especially in old mature blocks or less susceptible cultivars which can withstand some disease pressure. Unfortunately, new inoculum sources can appear, for instance following changes in local landscape, and can trigger sudden outbreaks. Neglecting bloom sprays can thus periodically result in widespread disease. Bacterial ooze from cankers or produced on the new disease foci can be spread by insects, wind, and rain to new shoots that are then infected through wounds formed especially during storms, most notably following hail. Disease spread to younger blocks of high economical value is most problematic as they can be killed by the disease within weeks. Risks subside when shoot growth ends (Smith 2013), but the time that terminal buds form is influenced by a number of factors including tree vigour (Forshey and Elfving 1989). Infections can spread for several weeks after blossom symptoms appear and before growth ends. The growth regulator prohexadione-Ca (ProCa) applied starting at bloom or as soon as pink stage (Wallis and Cox 2019) is effective at reducing disease spread on shoots in the summer (Yoder et al. 1999; Cox, et al. 2019), and has been advised for this use to growers for close to two decades (Turecheck et al. 2001; Norelli et al. 2003; Charest and Philion 2006). Unfortunately, cost and side effects of ProCa such as growth stunting and thinning inhibition (Greene 2008), have limited its adoption. Moreover, the pre bloom or bloom sprays occur before infection is confirmed and precludes the possibility of initiating sprays only when needed. Past studies have concluded ProCa isn't useful when treatments are initiated post-symptomatically, about a month after bloom infection (Schupp et al. 2002). In contrast, treatments initiated immediately after infection but before symptoms become apparent can be valuable (Aćimović et al. 2019), but still suffers from the limitation that sprays are applied before disease is observed. Even though reacting to a fire blight outbreak instead of preventing it is risky, growers can likely benefit from knowing what options are available when symptoms appear.

Our interest lies in the short time window of about 10 days between the first signs of the disease (ooze production), early symptoms (wilting), until necrosis becomes prevalent and the treatments are no longer considered cost effective. Rescue treatments during this period of early symptoms are not typical but were recently recommended (Cox et al. 2019) based on observations by growers, advisors, and early results from our research (Cox, pers. comm.). Although late ProCa applications may not be sufficient as the sole strategy against fire blight, we hypothesized that the cell thickening effect of ProCa which prevents infection (McGrath et al. 2009) could also reduce ooze exudation of infected shoots, thus providing some benefit by limiting inoculum spread. Since forecast models can be used to help pinpoint when symptoms appear, we also aimed at initiating sprays upon early signs of oozing or shoot wilting (2-3 weeks after infection) as opposed to spray on well-established necrotic lesions. To our knowledge, these aspects were not considered in past studies. Since quick summer pruning aimed at symptom removal is a common and effective recommendation for limiting disease spread (Toussaint and Philion 2008), we decided to test the separate and combined effects of ProCa and pruning. Our hypothesis was that rescue ProCa and pruning would have a synergistic effect to slow disease progress and reduce the amount of pruning required following an outbreak. Our goal was to evaluate the benefits of both late ProCa treatments and summer pruning for fire blight management in mature trees.

Materials and methods

Experimental setup

The study was carried out in 2018 and 2019 at the IRDA research orchard in the Mont-Saint-Bruno National Park in Québec (45°32’36’’N, 73°20’33’’W, 160 m). Plots of 2 consecutive mature trees (2006 ‘Royal Cort’ on M106 rootstock) of 2.7 m canopy height (total tree = 3.2 m) with a spacing of 3.65 m x 3.65 m, were randomly allocated to 6 different treatments and replicated in 4 blocks (CRBD). Blocking was based on the natural sloping of the orchard and proximity of the forest which slightly impacted speed of bloom. For two years, the same plots were either pruned (A, C, E) or unpruned (B, D, F) in the summer, and either sprayed with ProCa starting at bloom (C, D), upon early bloom symptom detection (E, F), or left unsprayed (A, B). Symptom removal in the unpruned plots was done during the winter. The trial also included a grower standard without ProCa but sprayed during bloom with streptomycin and pruned in the summer.

ProCa treatments

ProCa sprayed trees received either three applications of Apogee (BASF, 27.5% ProCa) starting at 100% bloom or two applications with a 1.5x dose starting two days after blossom symptoms started to appear in the control, about two weeks after bloom. The last spray was applied two weeks later (Table 1). Thus, the same total quantity of ProCa was applied to trees of the different sprayed treatments. The higher dose was derived from the registered dilute rate per application (max 45 g/ 100L) (Apogee® Plant growth regulator registration 28042, 2015), adjusted for tree size (approx. 60% of the maximum (3000 L/ha)), and applied in a low water volume spray. All treatment applications were made with a custom airblast tunnel sprayer equipped with Weber (Bodman, Germany) tangential fans with air speed adjusted to canopy (Triloff et al. 2013). Vertical booms with 10 Albuz ATR 80 hollow cone “yellow” nozzles, each delivering 0.823 L/min, sprayed the trees from both sides. The sprayer was operated at 5.92 km/h and delivered approximately 460 L/ha. Treated buffer trees along the row prevented drift to test trees, whereas drift to adjacent rows was prevented with a plastic shield on each side of the sprayer.

Bloom inoculation

In both years, flower clusters from all trees were counted at pink stage, and whole trees were inoculated during bloom by misting a suspension of the pathogen using the same sprayer used for treatments. In 2018, inoculation was done once at 100% bloom, two hours after the bloom ProCa spray. The sprayer was operated at 2.9 km/h delivering 920

L/ha of a suspension of 1E+6 CFU/ml. In 2019, trees were inoculated approximately at 100% king bloom (May 26th), 50% of full bloom (May 28th), and 90% bloom (May 30th), with 450 L/ha of a suspension adjusted to 3E+5 CFU/ml. Inoculum was obtained by resuspending in potassium phosphate buffer (pH = 6.5) fresh cultures on KingB agar of a 1:1 mix of local strains of *E. amylovora*.

Data collection

Pruning, harvest, growth: Between June 6 and July 27 in 2018, and June 12 and July 10 in 2019, infected clusters were enumerated, and diseased tissue was removed from the pruned treatments on 4 occasions in both years until disease progress subsided as annual growth ceased. Symptoms from the three unpruned treatments were cut out during the following winter (2018), or after harvest (2019). Summer sanitation cuts of the pruned treatments were made ahead of visible symptoms, cutting back to older wood rather than a set distance, according to recommendations made to growers (Philion 2015). Pruning shears were not sterilized but were carefully handled not to come into contact with symptoms (Toussaint and Philion 2008). Winter cuts were aimed at clearing trees from dead wood, and canker edges were scrapped down to the wood xylem instead of removing the branch whenever possible. For all treatments, individual tree cuttings were air dried and weighed. Because pruning of the streptomycin grower standard was not the focus and was minimal in 2018, cut branches from this treatment were only weighed in 2019 and not included in the main analysis. Similarly, since growth reduction was not the aim of the project and not expected for late ProCa sprays, it was not formally estimated in 2018. In 2019, the length of the annual growth of 20 shoots haphazardly selected in unsprayed control plots was measured at the time the late ProCa treatment was applied, and again after harvest in all plots. This was used to estimate the growth reduction observed in ProCa treated plots. At harvest for both years, the total number of fruits and overall yield was recorded per tree and visually rated for defects such as russeting.

Summer inoculation: In both years, healthy actively growing shoots (18-25 per tree) in each plot of the 6 main treatments were purposely inoculated about two weeks after blossom symptom appeared (June 15th 2018 and June 26th 2019) by transversely bisecting the two youngest leaves with a pair of scissors dipped in a suspension of 1E+9 CFU/ml of the pathogen prepared as above. About a week later, on June 22th 2018 and July 5th 2019, each of these shoots was rated for disease severity observed as a combined leaf and shoot necrosis score (0 = absence, 1 = limited to central vein of inoculated leaves, 2 = extending to petiole, 3 = reaching shoot, 4 = reaching other leaves and so the shoot was apparently dead) and the length of the necrotic area was recorded. Shoots were also assessed for presence or absence of bacterial exudate, and if alive: still actively growing, or not.

McIntosh inoculation: In a separate trial in 2019, the same summer inoculation was applied to adjacent mature McIntosh trees. In this experiment, trees were not inoculated at bloom but were sprayed with ProCa on the same dates and at the same rate as the main experiment and using the same experimental design. Shoot bisecting inoculations were done on June 26th, July 12th, and August 21st. Inoculated shoots were observed once per inoculation on July 5th, 22nd, and August 30th, 2019 respectively. However, shoots remaining from the August inoculation were again observed immediately after bloom of the following year (May 29th, 2020).

Data analysis The R language (R Core Team 2019) was used to handle data using the “tidyverse” packages (Wickham et al. 2019) and to fit all models. Infected and total cluster counts and the number of fruits per tree were analysed as Poisson count models. Overdispersion was handled when required using observation level random effect (Harrison et al. 2018) (GLMER) or GLMER.NB (negative binomial error distribution) (Venables and Ripley 2002; Bates et al. 2015). Clusters per tree was used as an offset for infected cluster models. The disease severity score of inoculated shoots was analyzed per year with a cumulative link model (CLMM) using the “ordinal” package (Christensen 2019). Shoot growth status and exudate presence were analyzed using logistic regression (GLMER)(Bates et al. 2015). For these models, odds ratio (OR) is presented. Inoculated shoot necrosis length, quantity of diseased wood pruned out, fruit yield, and shoot growth were analysed as continuous variables. In all models, the effect of pruning, ProCa timing, and their interactions were treated as fixed effects. Sampled trees (plots) and blocks were used as random effects in mixed models. However, in the absence of block effect the analysis was done as a completely randomized design (CRD). Subsamples were averaged per tree before plot level analysis. Since repeated measurements were taken in the same plots for two years, correlation was addressed using the GLS function (Pinheiro et al. 2019) and different structures including treating time as a split plot were compared using Akaike information criterion (AIC). Models with AIC differences less than 2 were assumed to have a similar fit. For all models, fits using maximum likelihood (ML) were used to compare model fixed effects. Test of effects were done with likelihood ratios (LR), for which the chi-square statistics ($\chi2$), difference in the number of parameters for the two models (degrees of freedom) and associated P-values were reported. Wald confidence intervals were used for individual levels.

Results

Blossom inoculation: Disease pressure following inoculation was very heavy in both years. In 2018, ooze first became visible on blossoms on June 4th. In 2019, ooze first appeared on flower clusters on June 10th, whereas obvious wilting symptoms became visible on June 12th. In both years blossom blight oozing and wilting was visible about 2 weeks after infection, whereas typical necrosis symptoms appeared several days after the first ProCa spray was in place. Symptoms were noticed 2-3 days later than forecasted by the RIMpro software in both years. Final flower cluster fire blight incidence per plot in pruned plots was between 76% and 89% irrespective of year (GLMER.NB: $\chi2 = 0.47$(1 df), $P = 0.49$) or use of ProCa (GLMER.NB: $\chi2 = 0.17$(1 df), $P = 0.68$). More exudate was seen on clusters in 2019 than 2018 (GLMER: OR =2, $\chi2 = 21.6$(1 df), $P < 0.001$), but independent of ProCa (GLMER: $\chi2 = 0.4$ (1 df), $P = 0.53$). Although infection was successful both years, the number of flower clusters available for infection in 2019 was much reduced as a result of the 2018 infection damage and we explored the possibility that summer pruning and ProCa sprays interacted in the process. The effect was slightly worse for trees pruned during the season (GLMER : OR=1.9, $\chi2 = 4.1$ (1 df), $P = 0.043$), whereas ProCa treated trees in 2018 had a little more clusters in 2019 than those left unsprayed (GLMER : OR= 1.8, $\chi2$ = 3.8 (1 df), P = 0.051). No interaction (pruning * ProCa) was observed ($\chi2$ =0.1) and ProCa timing had no effect ($\chi2$ =0.4).

Pruning wood removal: The effect of ProCa, summer pruning, and year on the amount of diseased wood removed are shown in Fig. 1. Among different models, the compound symmetry correlation structure (using GLS) and the equivalent LME model using individual plots as random effect gave the lowest AIC models and was used to address the yearly repeated measures in the same plot. Starting with the null model, and sequentially adding terms, we observed that pruning in summer (GLS: $\chi2$ = 5.2 (1 df), P = 0.022), year (GLS: $\chi2$ = 13.6 (1 df), P < 0.001), the year * pruning interaction (GLS: $\chi2$ = 6.8 (1 df), P = 0.009) best described the amount of wood removed. ProCa sprays taken globally, or at different timings, or with interactions with year or pruning treatments, did not contribute to the models (P >0.2 in all cases); implying ProCa did not impact dry weight of pruned off fire blight brush.

The parcimonious model parameters and estimates are presented in Table 2. Although the effect was slightly different between the two years, we observed overall after 2 years that less wood was removed from trees pruned during the season (3.5 kg/tree) than from trees cleaned of symptoms only after harvest (5.9 kg/tree). It’s likely that the reduced amount of diseased wood removed in 2019 resulted from the fewer number of flower clusters available for infection in the second year. Although we did not conduct a detailed economic analysis, we estimated that pruning required approximately 1 hr per tree in winter, whereas cleaning in season cost 3-4 hours per tree because it required multiple passages. Although streptomycin sprays reduced cluster incidence in the grower standard (not shown), sprays were insufficient in 2019 and did not have a big impact on the amount of diseased wood pruned (about 1.3 kg/tree in 2019).

Harvest: Fruit yield (Fig. 2) was also affected by the treatments and yearly correlation best addressed with compound symmetry using GLS. Since yield indirectly reflects a count (number of apples), residuals showed heteroscedasticity which was modeled with a variance function (varExp). Starting with the null model, trees left unpruned in summer (GLS: $\chi2$ = 24.6 (1 df), P < 0.001) and pooled treatments sprayed with ProCa (GLS: $\chi2$ = 6.3 (1 df), P = 0.01) had a higher yield. ProCa timing (GLS: $\chi2$ = 12.8 (1 df), P < 0.001) was significant, with bloom sprays resulting in a higher yield than rescue post symptom sprays. The year effect taken alone did not contribute to the model (GLS: $\chi2$ = 1.4 (1 df), P = 0.24), but was included because the effect of ProCa sprays varied with year (GLS: $\chi2$ = 6.7 (2 df), P = 0.035). Other interactions did not improve the model. The yield model and parameter estimates are presented in Table 3. In summary, after 2 years the cumulative yield per tree varied from about 5 kg/tree for trees pruned during the season and either not sprayed or sprayed late with ProCa, to close to 9 kg/tree when ProCa was sprayed at bloom. For trees pruned only after harvest, total average yield varied from 11 kg/tree for unsprayed trees to about 19 kg/tree when sprayed at bloom, with late sprays intermediate at 14 kg/tree. Considering this orchard should produce about 25 kg/tree per year, losses to fire blight varied from 60 to 90%. A low overall proportion of fruits harvested had russeting at various levels (1.4%), and ProCa treatments may have contributed (GLM: OR= 2.1, $\chi2$ = 2.2(1 df), P = 0.14) (not illustrated). ProCa application, spray timing, pruning, year, and their interactions had no observable effect on average fruit size per plot (GLM: $\chi2 < 1$ (1 df), $P > 0.6$ in each case) (not illustrated).

Shoot growth inhibition: When the post symptom ProCa treatments were initiated in 2019, shoots in the control already averaged 21 cm. At the end of September, control shoots measured 38 cm, whereas ProCa treated shoots were 9 cm shorter (GLM: $\chi2 = 418$(1 df), $P < 0.001$) measuring on average between 28 and 30 cm depending on treatment (not illustrated). ProCa timing, pruning or their interaction did not affect shoot length (GLM: $\chi2 < 2$ (1 df), $P > 0.6$ in each case).

Summer inoculation: The effect of ProCa and pruning treatments on the severity score of inoculated shoots is presented in Fig. 3. The effect on exudate production (Fig. 4), necrotic length, and growth status (Fig. 5) were considered separately. Although CLMM and GLMER models cannot account for repeated measures, we applied the correlation structure observed for all the other observations. Thus, the severity scores, exudate production and growth status were analyzed as mixed effect models with time as split plot. The overall disease score was higher in 2019 than 2018 (CLMM: LR = 191 (1 df); $P < 0.001$), presumably in part because disease had more time to progress in shoots between inoculation and observation (7 days in 2018 and 9 days in 2019). The disease score of shoots from plots sprayed with ProCa was lower (CLMM: LR = 19.5 (1 df); $P < 0.001$) than for the unsprayed, and the ProCa effect was greater in 2019 (interaction) (CLMM: LR = 6.3 (1 df); $P = 0.011$). The pruning effect was different between years (interaction) (CLMM: LR = 21.9 (2 df); $P <0.001$). Summer pruning reduced the disease score in 2018 but had no effect in 2019. An observation made 5 days later on the 2018 shoots (not shown) presented the same effect of ProCa, but no pruning effect. There was no interaction between ProCa sprays and pruning (CLMM: LR = 7.6 (1 df); $P= 0.27$) or higher order interaction. The effect of ProCa (odds ratio (OR) approx. 4x in 2018 and 8x in 2019) was the same for both spray initiation timings (CLMM: LR = 1.8 (2 df); $P= 0.41$). Model summary is presented in table 4.

Exudate (ooze) was more prevalent in 2019 than 2018 (GLMER: $\chi2 = 34.7$(1 df), $P < 0.001$) and ProCa sprays reduced the probability of observing exudate on the inoculated shoots (GLMER: OR=7.4, $\chi2 = 30.8$(1 df), $P < 0.001$), with a stronger effect in 2019 (GLMER: OR=1.8, $\chi2 = 5$(1 df), $P = 0.025$) (Table 5). ProCa spray timing (GLMER: $\chi2 = 0.3$(2 df), $P = 0.84$) had no effect. Pruning and other interactions also did not affect exudate presence (GLMER: $\chi2 < 0.5$ (1 df), $P > 0.5$ in all cases). Treatment effects on inoculated shoot necrotic length paralleled their effect on exudate presence (not illustrated). Necrotic length in the control was longer in 2019 (GLS: $\chi2 = 20$ (1 df), $P < 0.001$) than 2018 and was reduced with ProCa sprays (GLS: $\chi2 = 28.5$ (1 df), $P < 0.001$), with a stronger effect in 2019 (GLS: $\chi2 = 19$ (1 df), $P < 0.001$). As for exudate, there was no effect of pruning, ProCa timing, or their interactions on necrotic length. Again, comparison between GLS models revealed that yearly correlation was addressed with compound symmetry.

Shoots inoculated in 2018 that were alive at the time of rating were more likely to be still growing on pruned trees (Fig. 5), whereas growth had ceased on most shoots at the time of rating in 2019. After adjusting for the year effect (GLMER: $\chi2 = 200(1\ df)$, $P < 0.001$), pruning (GLMER: $\chi2 = 4(1\ df)$, $P = 0.04$) and their interaction (GLMER: $\chi2 = 8.5(1\ df)$, $P = 0.004$), we observed that ProCa also contributed to the growth status model of the shoots, but only in 2019 for unpruned shoots (Year * Pruned * ProCa interaction GLMER: OR = 11, $\chi2 = 9.2(4\ df)$, $P = 0.057$) where it caused growth to stop more than in the untreated (Table 6). However, ProCa timing did not improve the model (GLMER: $\chi2 = 5.6(4\ df)$, $P = 0.23$).

McIntosh shoot inoculation: Terminal shoot growth of the separate summer inoculation trial averaged 19 cm at the time ProCa applications were initiated in the post-symptom strategy. On June 26th, all shoots were still growing at the time of the first inoculation. Disease incidence on July 5th in the control shoots was 97% and 49% of shoots were already dead. The effect of ProCa on the disease score was evident (CLMM: $\chi2 = 8.7(1\ df)$, $P = 0.003$), however the effect of spray timing was not (CLMM: $\chi2 = 1.3(1\ df)$, $P = 0.25$). Shoots from trees treated with ProCa were more likely (OR= 7.2) to have a disease score lower than shoots from untreated trees (not illustrated). Presence of exudate on the inoculated shoots paralleled the disease score observations. Control trees of the first inoculation had 64% of inoculated shoots displaying exudate, in contrast to 17% of shoots from trees sprayed with ProCa (GLMER: OR=8.8, $\chi2 = 19\ (1\ df)$, $P < 0.001$). Again, spray timing did not affect exudate (GLMER: $\chi2 = 0.45\ (1\ df)$, $P = 0.5$). Necrosis length on shoots with disease score >2 followed the same pattern: ProCa treated shoot necrosis (2.6 cm) was shorter (LMER: $\chi2 = 6.5(1\ df)$, $P = 0.01$) than in the control (5.9 cm), and no timing effect was observed (LMER: $\chi2 = 0$). This translated in a lower shoot mortality for ProCa treated trees (11%) (GLMER: OR= 9.7, $\chi2 = 10.7(1\ df)$, $P = 0.001$), again not related to spray timing (GLMER: $\chi2 = 0.9\ (1\ df)$, $P = 0.35$).

On July 12th, we observed that 92 to 99% of McIntosh shoots had already stopped growing, notwithstanding the ProCa treatments. In consequence, we approximately reflected the growth status during the 2nd inoculation and insured that 95% of the inoculated shoots had stopped growing. As a result, very few symptoms appeared (5% incidence in the control) and ProCa had no effect on the disease score (CLMM: $\chi2 = 0.1\ (1\ df)$, $P = 0.75$). For the third and last inoculation in late August, all shoots had ceased growth and only one shoot out of the 240 inoculated (3 treatments * 4 trees * 20 shoots/tree) showed limited necrosis on one inoculated leaf. Overall incidence fell to 0.4% of shoot infection. None of the shoots of the second and third inoculations displayed exudate. Shoots from the 1st and 2nd inoculations of 2019 were not further observed. However, shoots from the 3rd inoculation showed some necrosis in the Spring of 2020. Once rated for disease score, it appeared that ProCa treated shoots (19 % incidence) were less affected (CLMM: OR = 3, $\chi2 = 4.7\ (1\ df)$, $P = 0.03$) than the control (42% incidence), notwithstanding timing (CLMM: $\chi2 = 0.05\ (1\ df)$, $P = 0.83$). This was apparent despite regular winter maintenance which removed about half the shoots in the ProCa trees and 70% in the control, presumably because they appeared diseased.

Discussion

Our results showed that initiating ProCa sprays immediately when fire blight becomes visible is almost as effective as bloom sprays to reduce shoot susceptibility. Results also showed that ProCa reduces ooze production on infected shoots. These findings may help fill a gap in fire blight control strategies. Since forgoing or badly timing bloom sprays and resistance can lead to fire blight outbreaks (Schupp et al. 2002; Norelli et al. 2003), there's a need for post-symptom fire blight management options. Our own streptomycin grower standard which resulted in numerous strikes in 2019 exemplifies the issue. Thus, the potential severity of damage to trees that follows outbreaks, and risk of spread to neighbouring orchards remain a concern. Since Québec growers are legally responsible for damage to neighboring orchards (Loi sur la protection sanitaire des cultures 2008), the need for strategies which prevent disease spread extends beyond the consequences for the individual grower.

Unfortunately, post-symptom control options are limited, and sprays are typically dismissed because of lack of efficacy (Norelli et al. 2003). The idea of using ProCa post-symptomatically is not new (Schupp et al. 2002). Rosenberger who coauthored that paper suggested it may still hold promise; which prompted the current study. The possibility of spraying only when needed (after symptoms are confirmed) while avoiding the negative impact of early ProCa sprays on thinning can be useful to growers. Although our data could not distinguish bloom and post symptom spray efficacy, it's more than likely that timing played a crucial role in our results and that any delay in spraying would translate in the reduction of efficacy observed by our predecessors (Schupp et al. 2002). The difference of about two weeks between ooze apparition or early wilting (that can go unnoticed), and the typical necrotic symptoms which appear a month after infection is both key and a limit to the applicability of our results; the strategy of post symptom sprays relies on early scouting so that sprays can be applied quickly. Although the scouting efforts could be minimized based on a symptom forecast date, this may not be feasible on large farms.

Another limit is that ProCa requires time before it becomes effective. Similarly to Yoder (1999), we observed ProCa reduced disease on shoots infected less than 2 weeks after treatment whereas Fernando and Jones (1998) reported some efficacy after one week. This still leaves a window during which secondary spread can occur but considering the sprays may be useful until about mid-July, post-symptom ProCa could shorten the overall period of susceptibility by half.

Since one of the goals of fire blight control in summer is preventing spread to neighbour orchards, the main benefit of ProCa sprays may be related to its effect on ooze production. For both years and two cultivars our results suggest ProCa strongly inhibits ooze production. Although we haven't seen ooze reduction mentioned in previous papers, Fernando and Jones (1998) reported reduced epiphytic populations of *E. amylovora* on ProCa treated shoots and less spread to uninoculated shoots, two parameters that were not evaluated in our study. Exudate reduction is likely a side effect of the toughening of shoots previously reported (McGrath et al. 2009).
Growth reduction in relation to ProCa sprays is usually the most striking effect observed in trials. On mature cropping trees such as those used in our trial this effect is positive,

but undesirable on young trees until they fill their space. Since growth inhibition and fire blight control are linked for sprays applied at bloom or later, post symptom ProCa sprays may not be the preferred fire blight management strategy for young trees, although fire blight damage including potential tree death may outweigh the impact of temporary growth stunting. Surprisingly, our late ProCa treatment resulted in the same growth inhibition as the standard bloom timing, despite the fact shoots were already half of their final size at the time of spraying. This goes against current guidelines that suggest early sprays are needed to obtain the full benefit of growth inhibition (Greene 2008).

The McIntosh inoculation trial illustrated that fire blight risk drops when shoots stop growing. Unfortunately, we did not track terminal bud formation on uninoculated shoots in our main trial, and only observed a limited effect of ProCa on terminal bud set in unpruned plots in 2019. It's difficult to establish if the reported effect (Bubán et al. 2004) was masked because of inoculation. Similarly, although we did observe an effect of ProCa on return bloom (Costa et al. 2004), the effect was possibly masked by the extent of fire blight in our plots. Although this wasn't evaluated, it's likely the effect of ProCa on shoot symptoms observed the next spring may be an indication that wood invasion was reduced and that less active cankers were formed. Since the late season cankers are more prone to have indeterminate margins and be more active the following year (Beer 1979), post symptom rescue ProCa treatments may also have an impact on the spread of disease the following year (Aćimović et al. 2019). However, this carryover effect on shoots had no effect on blossom blight severity or exudate production on flowers the year following the treatments. The dose used in our experiment (819 g/ha $\simeq$ 12 oz/A) is lower than even the lowest dose (6 oz /100 gal or 18 oz/A) used post infection in other studies (ex: Aćimović et al. 2019). It's possible a higher dose could result in better efficacy, but we chose the dose in accordance to the label restrictions and adjusted for tree size. Since the cost of ProCa treatments is a limiting factor for adoption, spraying only when symptoms appear and at the lowest effective rate is likely optimal.

Pruning out symptoms is currently the de facto standard for fire blight control in summer; but pruning can be impractical, cost prohibitive, and even detrimental when strikes are too numerous (Zwet and Keil 1979; Toussaint and Philion 2008). Our results reflect this contradiction and shed little new insight on this conundrum. Although pruning out symptoms in early summer caused less damage to trees, early removal of diseased wood resulted in a lower yield and partially inhibited fruit bud initiation as seen by the reduced number of fruit clusters. Pruning manuals warn against early summer pruning because of this effect (Forshey et al. 1992). The immediate yield impact and higher pruning costs even favored not pruning trees during the season. However, the experiment didn't last long enough to show the effect of pruning on the long-term yield of trees. Presumably, the more wood removed on the trees pruned after the season in 2018 and 2019 would have an impact on the yield in 2020 and beyond, as fruit buds are mostly on older wood.

Extensive pruning in 2018 following the initial outbreak most likely slowed the formation of the terminal bud in the summer pruned plots as seen by the growth status of shoots, whereas the lower summer eradication pruning in 2019 probably didn't provoke a delay in growth termination. Tree pruning did temporarily reduce shoot susceptibility in 2018, but since this effect was not observed in 2019 it is possible this direct effect is masked as disease progresses. It could be simply a side effect of temporary reduced evapotranspiration on the heavily pruned trees. In summary, our observations favor quick summer pruning interventions only when the amount of wood involved is small compared to tree size and unlikely to offset tree balance or substantially affect yield. Summer pruning performed to limit disease spread by eliminating the ooze inoculum should probably be replaced by ProCa sprays, at least in heavily diseased orchards. Since our experiment was done on relatively mature trees, tree death because of fire blight was unlikely. On younger trees, summer rescue pruning only has a marginal effect on the probability of tree mortality (Toussaint and Philion 2008).

Interestingly, our results showed that pruning and ProCa sprays had complementary effects. ProCa and pruning had independent, sometimes additive effects (ex: yield, disease score) but contrary to our hypothesis, were mostly not synergistic. During the course of this project, only one ProCa x Pruning interaction was observed (growth status) and the effect was minor. Our results confirm observations by Schupp et. al. (2002) that post symptom ProCa sprays have virtually no impact on the amount of wood pruned from trees. This may be explained by the absence of weather events conducive for natural secondary spread in our trial. Heavy storms or hail trauma events would presumably have a similar effect to shoot inoculation and result in reduced damage where ProCa is applied.

Our trial wasn't meant to compare the efficacy of bloom sprays and post-infection interventions. When conditions are conducive for disease, effectively preventing infection at bloom is better than limiting damage post infection. However, if the trees are mature and are unlikely to be killed by the disease and if the probability of bloom infection is low, it may be more cost effective not to spray at bloom and manage the summer blight risk accordingly. A thorough risk assessment is beyond the scope of this paper as includes many aspects (McRoberts et al. 2011) but may be a topic of interest.

Future trials on fire blight control measures may also consider the use of the disease score rating, which helped quantify pruning effects that were not detected through other observations, and was quicker than measuring necrotic length. Similarly, directly rating presence/absence for exudate and shoot growth status were also quick and could help assess the potential for inoculum spread and give insight on the infection risk. There remains many aspects of early post-symptomatic sprays of ProCa which require further study. For instance, we did not evaluate the effect of ProCa on rootstock infection or tree survival (Schupp et al. 2002), as our focus was not on young trees. It's also likely that growing conditions in Québec may explain in part why ProCa post bloom was effective in our study and not in the southern part of New York (Schupp et al. 2002), and more work would be needed to circumscribe the climate and growing conditions that are needed for post-bloom ProCa efficacy. Nonetheless, ProCa soon immediately after blossom blight symptoms appears could become a useful tool to reduce summer spread of fire blight in Québec.

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Compliance with Ethical Standards:

Vincent Philion and Valentin Joubert declare they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

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Figure legends

Fig. 1. Yearly wood removal following fire blight infection in relation to eradication pruning strategy and prohexadione-Ca (ProCa) spray timing. Treatment means per pruning

strategy were linked across ProCa spray strategies to help visualize interaction between effects.

Fig. 2. Yearly apple harvest following fire blight infection in relation to eradication pruning strategy and prohexadione-Ca (ProCa) spray timing. Treatment means per pruning strategy were linked across ProCa spray strategies to help visualize interaction between effects.

Fig. 3. Disease severity score distribution of inoculated shoots from plots either not pruned (Control) or pruned in summer (Pruned) and either unsprayed, sprayed with prohexadione-Ca (ProCa) starting at bloom, or when blossom symptoms first appeared. Apparently healthy shoots of all plots were inoculated 9 days (2018) and 14 days (2019) following the first ProCa treatment timing and observed 7 days (2018) or 9 days (2019) later. Disease score was based on necrosis extent (0 = absence, 1 = limited to central vein of inoculated leaves, 2 = extending to petiole, 3 = reaching shoot, 4 = reaching other leaves)

Fig. 4. Fraction of inoculated shoots presenting ooze in relation to eradication pruning strategy and prohexadione-Ca (ProCa) spray timing. Treatment means per pruning strategy were linked across ProCa spray strategies to help visualize interaction between effects.

Fig. 5. Fraction of live inoculated shoots still actively growing when rated for disease severity. Treatment means per pruning strategy were linked across ProCa spray strategies to help visualize interaction between effects.

Table 1. ProCa application rate and timing

Spray treatment	Application rate (g/ha)	Spray timing		
		Bloom	Symptom occurence[b]	Last spray[c]
A,B				
C,D	546	x	x	x
E,F	819		x	x

[a] 100% bloom (May 23rd 2018, and May 29th 2019)

[b] Ooze and or wilting present (June 6th 2018, and June 12th 2019)

[c] June 21st 2018, and June 26th 2019.

Table 2. Summary of the GLS model describing dry weight of diseased wood removal following ProCa applications and summer pruning interventions.

Model terms[a]	Estimate[b]	S.E.[c]	T[d]	P-value
(Intercept)	2.0	0.4		
Unpruned	1.9	0.6	3.3	0.002
Year 2019	-0.5	0.4	1.4	0.155
Unpruned: Year 2019	-1.4	0.5	2.7	0.010

[a]Significant main effects and interactions. Rho value for correlation between repeated measures calculated by the gls model was 0.604.

[b] Coefficients of the fixed-effect parameters (kg/tree).

[c] S.E. = standard error

[d] t-value (parameter estimate/standard error) and associated probability.

Table 3. Summary of the GLS model for yield following rescue ProCa applications and summer pruning interventions.

Model terms[a]	Estimate[b]	S.E.[c]	T[d]	P-value
(Intercept)	3.2	0.8		
Unpruned	3.5	0.8	4.5	<0.001
Year 2019	-1.5	1.1	1.4	0.16
ProCa Bloom: Year 2018	1.0	1.3	0.8	0.44
ProCa Symptom: Year 2018	-0.4	1.0	0.4	0.71
ProCa Bloom: Year 2019	3.0	1.3	2.2	0.03
ProCa Symptom: Year 2019	0.9	0.8	1.1	0.29

[a]Significant main effects and interactions. Rho value for correlation between repeated measures calculated by the gls model was -0.356.

[b] Coefficients of the fixed-effect parameters (kg/tree).

[c] S.E. = standard error

[d] t-value (parameter estimate/standard error) and associated probability.

Table 4. Summary of the CLMM model describing the disease severity score of shoots inoculated in summer following ProCa applications and summer pruning interventions.

Model terms[a]	Estimate[b]	S.E.[c]	z[d]	P-value
Year 2019	2.6	0.2	10.7	<0.001
Year 2018 : ProCa	-1.5	0.3	4.3	<0.001
Year 2019 : ProCa	-2.1	0.3	6.0	<0.001
Year 2018 : Unpruned	0.8	0.3	2.5	0.014
Year 2019 : Unpruned	-0.3	0.3	0.9	0.38

[a]Significant main effects and interactions.

[b] Log of the odds ratio

[c] S.E. = standard error

[d] z-value (parameter estimate/standard error) and associated probability.

Table 5. Summary of the GLMER binomial model describing presence of exudate on shoots inoculated in summer following ProCa applications and summer pruning interventions.

Model terms[a]	Estimate[b]	S.E.[c]	z[d]	P-value
(Intercept)	0.8	0.2	3.3	0.001
Year 2019	1.1	0.2	4.8	<0.001
ProCa	-2.0	0.3	6.9	<0.001
ProCa : Year 2019	-0.6	0.3	2.2	0.027

[a]Significant main effects and interactions.

[b] Log of the odds ratio

[c] S.E. = standard error

[d] z-value (parameter estimate/standard error) and associated probability.

Table 6. Summary of the GLMER binomial model describing shoot growth status of inoculated shoots following ProCa applications and summer pruning interventions.

Model terms[a]	Estimate[b]	S.E.[c]	z[d]	P-value
(Intercept)	0.3	0.5	0.5	0.61
Year 2019	-3.6	0.9	4.1	<0.001
UnPruned	-1.0	0.7	1.4	0.17
ProCa	0.3	0.6	0.5	0.60
Unpruned:Year 2019	3.1	1.0	3.1	0.002
ProCa : Year 2019	0.4	0.9	0.4	0.68
Unpruned:ProCa	-0.2	0.9	0.2	0.85
Unpruned:ProCa: Year 2019	-2.4	1.2	2.1	0.04

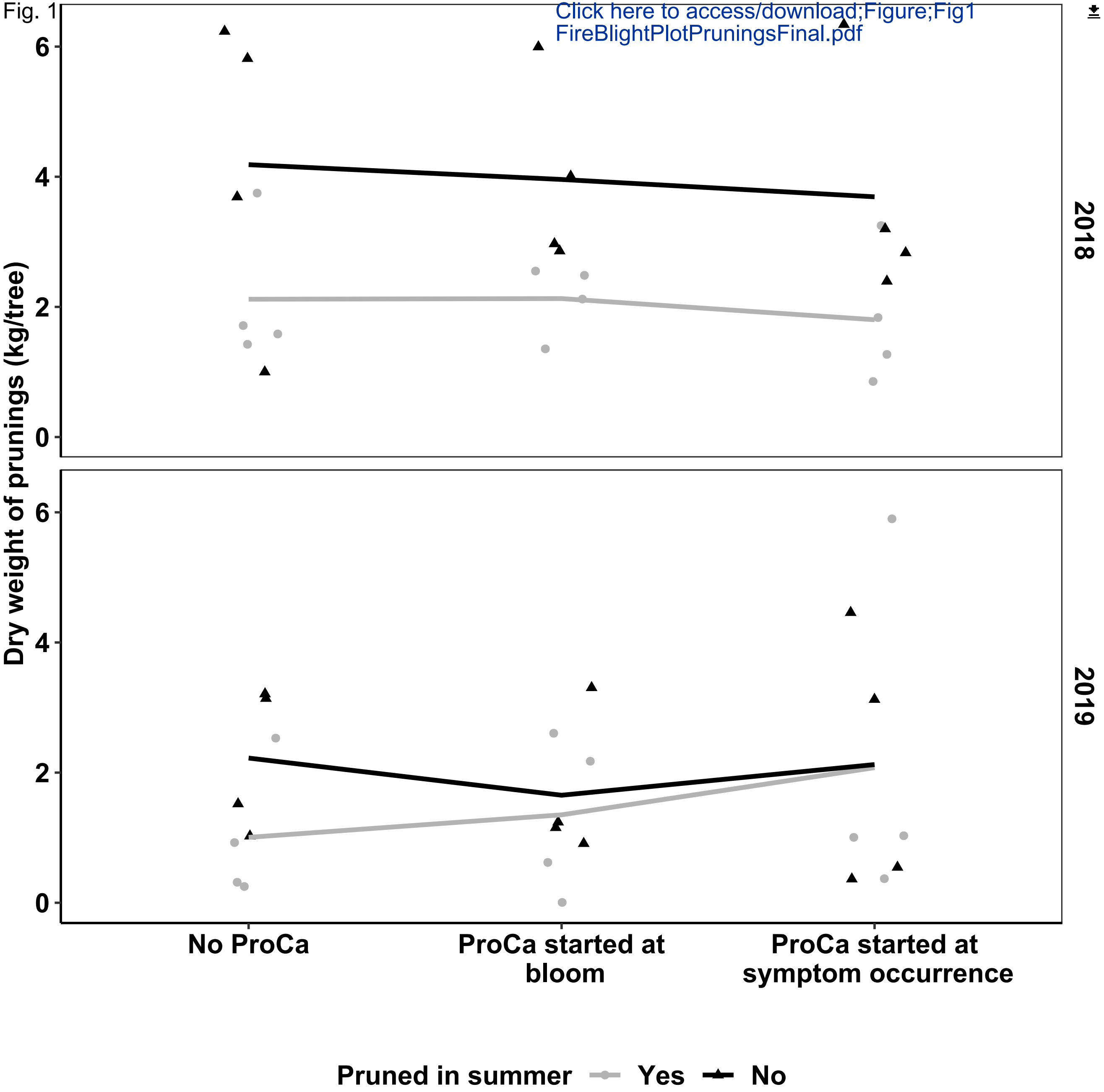
[a]Significant main effects and interactions.

[b] Log of the odds ratio

[c] S.E. = standard error

[d] z-value (parameter estimate/standard error) and associated probability.

Fig. 1



Dry weight of prunings (kg/tree)
0
2
4
6
2018
2019
No ProCa
ProCa started at bloom
ProCa started at symptom occurrence
Pruned in summer
Yes
No

Fig. 2

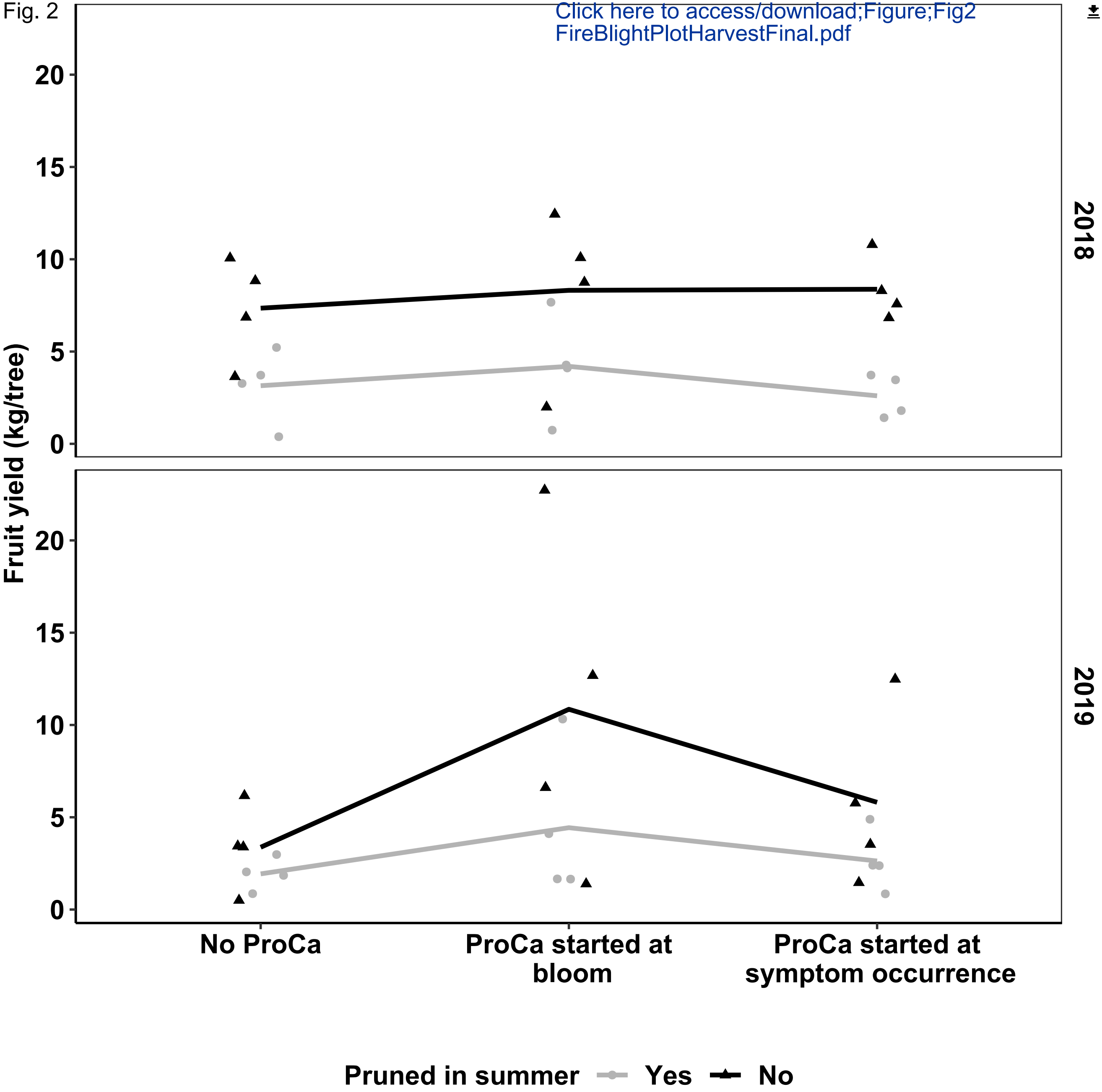

Fruit yield (kg/tree)
20
15
10
5
0
2018
2019
No ProCa
ProCa started at bloom
ProCa started at symptom occurrence
Pruned in summer
Yes
No

Fig. 3

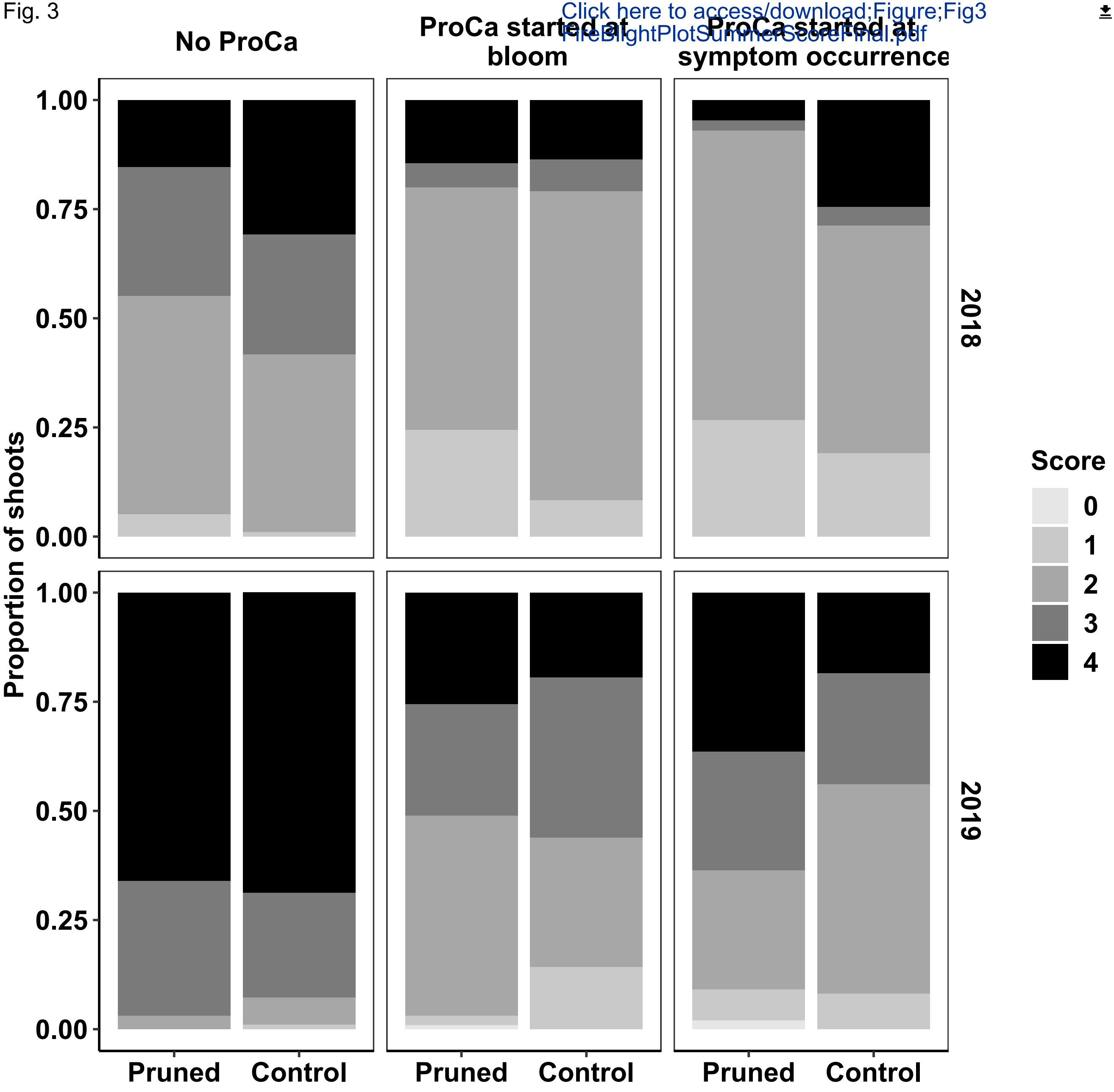

No ProCa
ProCa started at bloom
ProCa started at symptom occurrence
Proportion of shoots
1.00
0.75
0.50
0.25
0.00
2018
2019
Pruned
Control
Score
0
1
2
3
4

Fig. 4

Click here to access/download;Figure;Fig4 FireBlightPlotSummerOoze.pdf

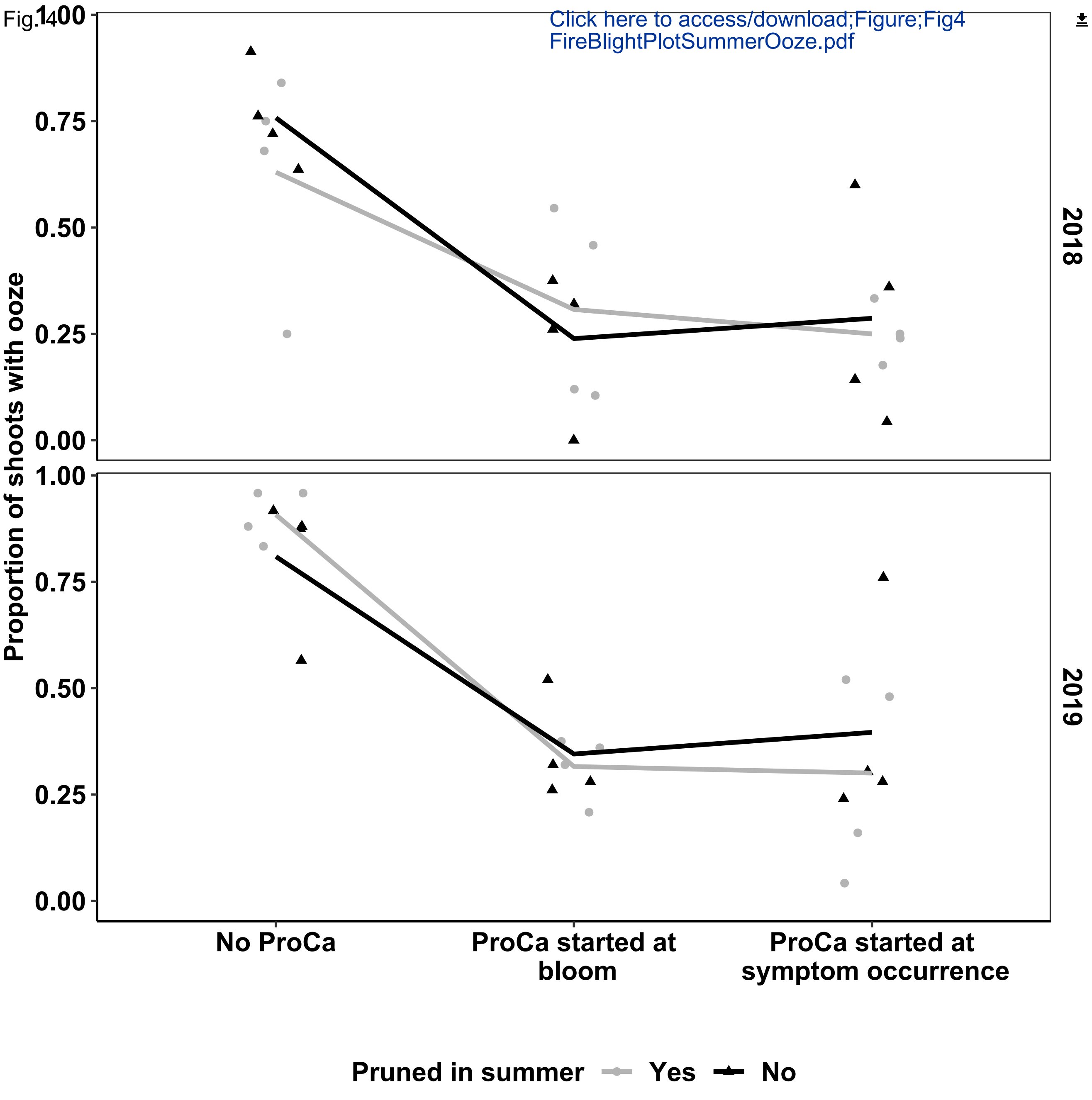
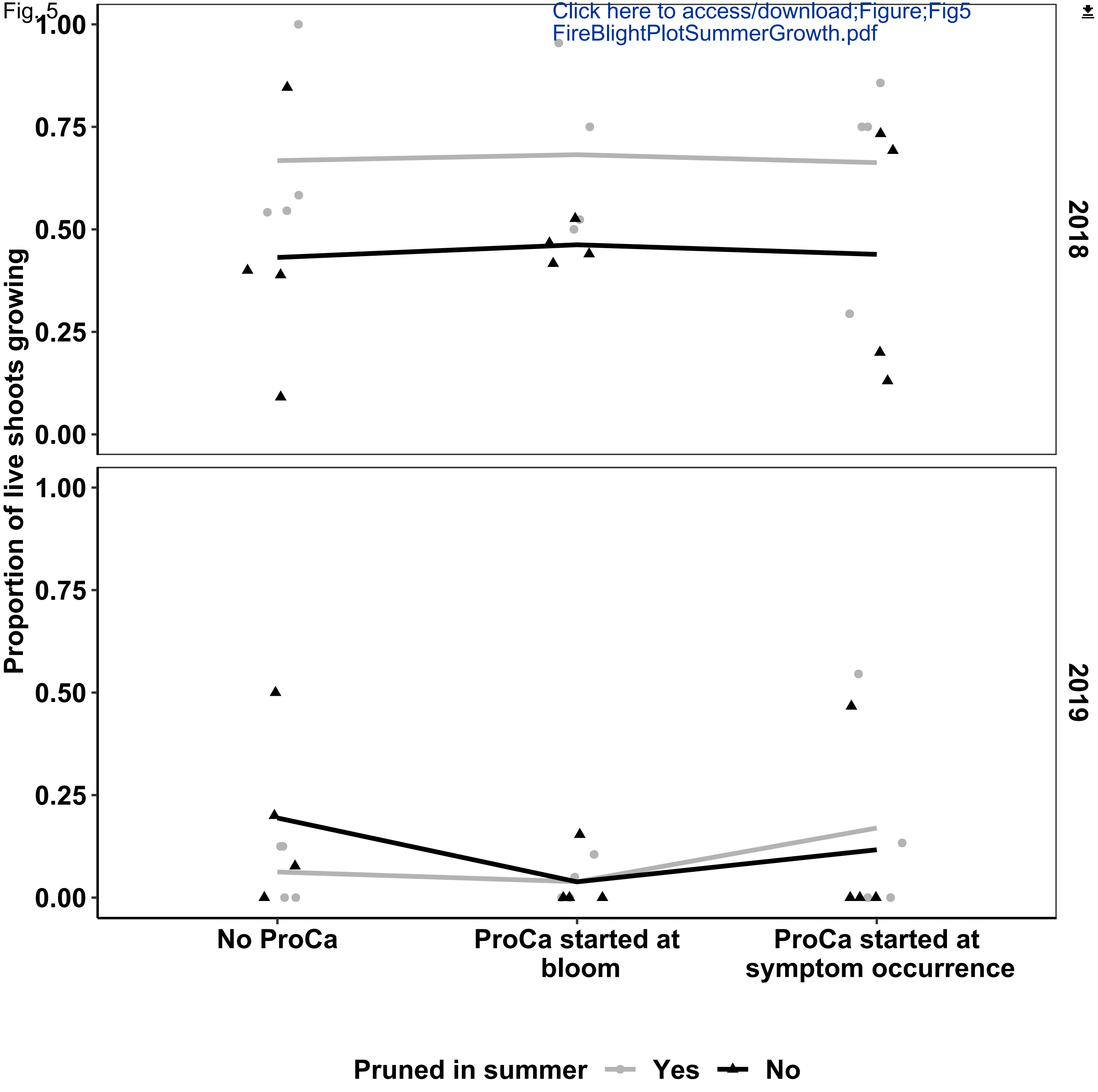


Fig. 5


Proportion of live shoots growing
1.00
0.75
0.50
0.25
0.00
2018
2019
No ProCa
ProCa started at bloom
ProCa started at symptom occurrence
Pruned in summer
Yes
No