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High relative humidity pre-harvest reduces post-harvest proliferation of Salmonella *in tomatoes*

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Abstract

Outbreaks of human illness caused by enteric pathogens such as *Salmonella* are increasingly linked to the consumption of fruits and vegetables. Knowledge on the factors affecting *Salmonella* proliferation on fresh produce therefore becomes increasingly important to safeguard public health. Previous experiments showed a limited impact of pre-harvest production practices on *Salmonella* proliferation on tomatoes, but suggested a significant effect of harvest time. We explored the data from two previously published and one unpublished experiment using regression trees, which allowed overcoming the interpretational difficulties of classical statistical models with higher order interactions. We assessed the effect of harvest time by explicitly modeling the climatic conditions at harvest time and by performing confirmatory laboratory experiments. Across all datasets, regression trees confirmed the dominant effect of harvest time on *Salmonella* proliferation, with humidity-related factors emerging as the most important underlying climatic factors. High relative humidity the week prior to harvest was consistently associated with lower *Salmonella* proliferation. A controlled lab experiment confirmed that tomatoes containing their native epimicrobiota supported significantly lower *Salmonella* proliferation when incubated at higher humidity prior to inoculation. The complex interactions between environmental conditions and the native microbiota of the tomato crop remain to be fully understood.

Keywords

Climate; Food safety; Human pathogens; Plant-pathogen interactions; Produce.

1 Introduction

Non-typhoidal *Salmonella enterica* (NTS) is one of the leading causes of foodborne disease burden worldwide (Havelaar et al. 2015). The World Health Organization estimated that in 2010, NTS caused over 150 million illnesses worldwide, resulting in nearly 120,000 deaths, mainly due to sepsis (Kirk et al. 2015). Recently, Scallan et al. (2015) confirmed that NTS was the dominant cause of foodborne disease burden in the United States, causing 1 million illnesses, 380 deaths, and 33,000 Disability-Adjusted Life Years (DALYs) per year. The majority of all NTS cases (94%) were assumed to be foodborne. While outbreaks of gastroenteritis linked to the consumption of well-known risky foods such as raw eggs and seafood have been declining, outbreaks associated with fruits and vegetables have increased (Gould et al., 2013; Kozak et al., 2013). Even though field surveys report that *Salmonella* and *Escherichia coli* are relatively uncommon in the pre-harvest crop production environment in the United States, fresh produce has been implicated in at least 130 outbreaks of gastroenteritis since 1996 (Centers for Disease Control and Prevention, 2013; Gould et al., 2013; Kozak et al., 2013; Mandrell, 2009). Raw tomatoes have been associated with at least 15 multi-state outbreaks of salmonellosis between 1990 and 2010, with traceback investigations suggesting that contamination occurred during production or processing (Bennett et al. 2015).

Even though plants have been suggested as alternate hosts for human enteric pathogens (Brandl et al., 2013), outbreaks of gastroenteritis linked to produce have been sporadic. This suggests that to lead to an outbreak, a number of factors must converge, resulting in a "perfect storm" scenario. Factors contributing to the perfect storm scenario include the presence of sources of pathogens and their vectors; genotype, maturity and physiological status of the crop and the pathogen; native plant microbiota capable of promoting or inhibiting human pathogens; the types and level of irrigation; and the use of soil amendments (Brandl, 2006, 2008; Brandl and Amundson, 2008; Franz and van Bruggen, 2008; Gu et al., 2013; Gutierrez-Rodriguez et al., 2012; Mandrell, 2009; Marvasi et al., 2015, 2014a, 2013; Moyne et al., 2011; Park et al., 2012; Poza-Carrion et al., 2013). How these factors interact and to what extent they contribute to the "perfect storm" is not clear. Knowledge on the factors affecting *Salmonella* proliferation on fresh produce therefore becomes increasingly important to safeguard public health. A better understanding of the role of the environmental conditions and production practices that affect susceptibility of fruits and vegetables to human pathogens

pre- and post-harvest may lead to the optimization of pre- and post-harvest operations to reduce the number and/or severity of the produce-associated outbreaks.

The impact of various farming practices on the microbiological quality of vegetables pre- and post-harvest has been evaluated. Different factors may contribute to *Salmonella* proliferation on fresh produce, including environmental conditions (such as regional differences, climate), pre- and post-harvest production factors, and genotype and physiological states of the crop and the pathogen (Marine et al., 2015; Marvasi et al., 2013; Pagadala et al., 2015). Because *Salmonella* and pathogenic *E. coli* are rare in the commercial fields in the United States, studies of the effects of crop production practices often rely on naturally-occurring indicators (such as generic *E. coli*). The presence of *E. coli* on tomatoes and leafy greens in the field correlated with the time of sampling, but not with regional differences or type of farming system (conventional versus organic) (Marine et al., 2015; Pagadala et al., 2015). However, Pagadala et al. (2015) reported that more *E. coli*-positive samples were detected in the conventional (rather than organic) tomato fields. Because contamination can occur at any point in the production cycle, it is also important to understand whether/how pre-harvest production practices can affect susceptibility of produce to human pathogens post-harvest. Recently, field experiments were carried out to determine the effects of the irrigation regime (Marvasi et al. 2013), nitrogen and potassium fertilization (Marvasi et al. 2014a), and iron and copper supplementation on the susceptibility of tomatoes to post-harvest proliferation of *Salmonella*. These studies confirmed the complex multifactorial nature of *Salmonella* proliferation, as evidenced by significant three-way interactions between production practices, time of harvest, crop genotype and maturity, and *Salmonella* strain. Furthermore, they suggested that time of harvest may have a dominant effect on *Salmonella* proliferation. The aim of this study was therefore to further explore these datasets using Classification and Regression Trees (CART), which allow overcoming the interpretational difficulties of classical statistical models when faced with higher order interactions. Furthermore, we aimed to explain the effect of harvest time by explicitly modeling the climatic conditions at the time of harvest and by performing additional confirmatory laboratory experiments.

2 Materials and Methods

2.1 Field production conditions

The set-up of the irrigation and nitrogen/potassium field studies are described in Marvasi et al. (2013) and Marvasi et al. (2014a). In brief, the irrigation field study imposed three different irrigation treatments two weeks prior to the onset of harvesting, with soil moisture targets for each treatment of 6, 10 and 12% volumetric water content. Additional experimental factors included tomato cultivar (three levels: Bonny Best, Florida-47, Solar Fire), tomato maturity at harvest (three levels: unripe, partially ripe, ripe), time of harvest (four levels: June 2011, June 2012, October 2012, October/November 2012), and inoculated *Salmonella* strain (two levels: type strain – *S. enterica* sv. Typhimurium 14028, or outbreak strains – an equal mix of *S. enterica* svs. Javiana, Montevideo, Newport and Braenderup which were associated with tomato outbreaks of salmonellosis). The nitrogen/potassium field study imposed three different nitrogen rates (168, 224, and 280 kg/ha N) and three different potassium rates (140, 210, and 280 kg/ha K) in 9 possible combinations. Additional experimental factors included tomato cultivar (two levels: Sebring, Solar Fire), tomato maturity at harvest (three levels: unripe, partially ripe, ripe), time of harvest (four levels: June 2011, June 2012, October 2012, October/November 2012), and inoculated *Salmonella* strain (two levels: type strain, outbreak strain). Irrigation and fertilization studies were carried out concurrently, in the same two locations (Citra in Central Florida and Live Oak, North Florida).

The iron/copper pesticides field study was set up in a similar way as the preceding ones. Seeds of tomatoes (cultivar Solar Fire) were purchased from Siegers Seed Co. (Holland, MI) and Harris Co. (Rochester, NY). Transplants were produced in an environmental chamber on the University of Florida campus, and then planted in the field. Experiments were conducted in the Spring production seasons June and July both 2014 and 2015 at the Plant Science Research and Education Unit IFAS, Citra (29°24'37.84"N; 82°10'12.14"W). The soil at the Citra site is Gainesville loamy sand (hyperthermic, coated typic quartzipsamments). Planting occurred in March 2013 and 2014. Plots consisted of a single row (7.6 m long) of 20 tomatoes. Generally recommended practices for Florida tomato production were used for this research, including polyethylene-mulched raised beds, soil fumigation with 50% methyl bromide: 50% chloropicrin, drip irrigation, pest control, and staking of plants (Olson et al., 2012). A cover crop (15 cm tall) of rye (*Secale cereale* L.) was rototilled in preparation for tomato production. The plots were fertilized with nitrogen, potassium and phosphate according to Freeman et al. (2012). The soil used for this experiment tested high in P so that no P fertilizer was used. The target total season amounts of N and K were 224 kg/ha each with 20% broadcast and incorporated in the bed prior to mulch application and 80% injected

through the drip irrigation system in 6 applications though the growing season. Irrigation was applied through drip-irrigation tubes, under the mulch to maintain volumetric soil water content (measured by time domain reflectometry) at 8-10% (Muñoz-Carpena, 2012). Early in the season, one irrigation event of 30 min per day was satisfactory to maintain optimal soil moisture but irrigation cycles were increased to three 30 min cycles starting 60 days after planting until the end of the season.

Iron/copper treatments were replicated three times in a randomized, complete-block design. Iron was applied as Fe-lignosulfonate (4% iron oxide, Interstate Products, Inc. Sarasota, FL, USA) and copper was applied as copper diamonia diacetate (8% metallic Cu, Southern Agricultural Products, Palmetto, FL, USA). According to the manufacturers' instructions, iron was applied at 0.17 kg Fe/ha per application and copper at 0.1 kg Cu/ha per application. Tomatoes were sprayed every two weeks, every six weeks or once 3 days prior to the harvest. The only Fe and Cu sprays received by the tomatoes were the specific treatments.

2.2 Tomato infections post-harvest

Harvested tomatoes were brought into the lab and inoculated with *Salmonella* through shallow wounds, typically within 2-24 h of the harvest, as previously described (Marvasi et al., 2015, 2014a). For the inocula, the type strain *S. enterica* Typhimurium ATCC14028 or a cocktail of strains (*S*. Javiana ATCC BAA-1593, *S*. Montevideo LJH519, *S*. Newport C6.3, *S*. Braenderup 04E01347, 04E00783, 04E01556) linked to the human outbreaks of salmonellosis were used as suggested by the Framework for Evaluation of Microbial Hazards (Harris et al., 2013, 2012). Strains were individually grown overnight at 37 °C in LB broth with shaking were washed three times in phosphate-buffered saline (PBS, pH 7.0), and the strains from the outbreaks were combined into a six-strain inoculum. These inocula were further diluted in PBS and 3 µl of the suspension (containing about 100 CFU) were spotted onto three shallow wounds (~ 1 mm) in the tomato epidermis. Infected tomatoes were incubated at 22 °C for a week. After incubation, tomatoes were blended in an equal volume of PBS using a stomacher (Sevard, West Sussex, UK) (200 rotations per minute for 1 min) and 50 µl of the suspensions were plated onto Xylose Lysine Deoxylate (XLD) agar (Beckton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 37 °C overnight. Proliferation was calculated by dividing the total CFU recovered from each tomato by the total CFU inoculated into each fruit. This allows accounting for differences in tomato sizes and for the fact that the

colonization of a tomato fruit by *Salmonella* is not even. The ratios were further subjected to the $\log_{10}$ transformation.

2.3 Effect of plate crowding on CFU recovery

We recognize that to obtain accurate counts, it is desirable to have 30-300 CFU/plate. However, when processing thousands of field samples, we invariably obtained plates with more than 300 CFU. Rather than discarding these data, we performed experiments to determine how to correct for the non-linearity of CFU counts on crowded XLD plates. Three tomatoes were inoculated with ~1,000 CFU of *Salmonella* Typhimurium 14028. Following incubation, tomatoes were stomached as above and each of the four ten-fold dilutions was plated onto XLD. CFUs were counted following overnight incubation at 37 ºC. The correction assumed that the observed count theoretically has to be proportional to the inoculum volume, which can be represented by a power curve: $y = \alpha x^{\beta}$. A power curve corresponds to a linear log-log curve, with the power curve coefficient β corresponding to the slope of the log-log curve: $\log y = \log \alpha + \beta \log x$. Without crowding, the slope should be equal to one. In presence of crowding, the slope will be lower than one, and the log(true count) can be obtained by dividing the log(observed count) by the slope. To obtain the slope factor β, we fitted a linear mixed effects model to the log(observed count) versus the log(dilution), with dilution series as random effect, using the lme4 package for R 3.3.0 (Bates et al., 2015; R Core Team, 2016).

2.4 Data analysis

We used regression trees to identify the experimental factors that were best able to explain the observed variation in *Salmonella* proliferation, defined as the $\log_{10}$-transformed ratio of *Salmonella* cells after and before inoculation. Models were fitted to the observed cell counts and to the overcrowding-corrected cell counts. The independent variables in the models were the experimental treatments (i.e., irrigation, fertilization, pesticide), tomato ripeness, tomato cultivar, *Salmonella* strain, and harvest time. To explore the effects of harvest time, we fitted additional regression tree models where harvest time was replaced by the underlying climatic variables. We obtained climate data up to one week prior to each harvest from the Florida Automated Weather Network (FAWN-IFAS, http://fawn.ifas.ufl.edu/). The data from 2011 were taken in Live Oak, while the data from 2012 and 2013 were taken in Citra. We selected climate variables with a biological implication and that can reliably be measured: temperature at 60 cm, solar radiation, total rainfall, relative humidity, and dew point. We calculated

average values for the preceding seven days and the preceding 24 h, which were explored in two separate regression tree models per dataset.

Regression trees are non-linear and non-parametric alternatives to classical statistical regression models that overcome problems of multicollinearity and higher order interactions (Speybroeck, 2012). Regression trees are part of the more general CART approach with classification trees allowing handling categorical outcomes and regression trees continuous variables. In this paper only regression trees were used as the outcome, $\log_{10}$ *Salmonella* proliferation, was a continuous variable. The construction of such trees begins with a parent node containing all observations. The regression tree algorithm then recursively iterates through all possible values of the experimental factors to find the best possible variable, as well as the best possible value of this variable, to split the parent node into two child nodes. In choosing the best splitter, the algorithm seeks to maximize the homogeneity (purity) within the two child nodes and thus the heterogeneity between both child nodes. The final result resembles an inverted tree and can be interpreted as a decision tree or classification system for the dependent variable. The tree visualizes discovered relationships and patterns in the data, but does not allow for interpretations in terms of statistical significance. However, overfitting is avoided by using a learning data set to prune the saturated tree and select the optimal tree with an appropriate fit to the learning data set.

Regression trees offer a way to deal with multicollinearity in an intuitively correct way. From two closely related variables, e.g., dew point and humidity, a regression tree will select only one variable as the most important (primary) splitter, but will also compute an importance measure reflecting a variable's ability to perform either as a primary splitter or as a so-called surrogate splitter. The values of all these improvements are summed over each node and totaled, and are then scaled relative to the best performing variable. Surrogate splitters closely mimic and predict the action of primary splitting variables. If one variable is not selected at several splits because it is the second most important variable each time it may not appear in the tree, but it will appear in the variable importance table, which ranks the variables based on their contribution in the construction of the tree (Liaw and Wiener 2002).

The regression trees and variable importance measures were generated using the rpart and randomForest packages for R 3.3.0 (Therneau et al. 2015; Liaw and Wiener 2002; R Core Team 2016).

2.5 Effect of tomato incubation at high relative humidity on subsequent proliferation of Salmonella

To determine what effects relative humidity pre-harvest plays in the ability of *Salmonella* to multiply in tomatoes post-harvest, we carried out confirmatory laboratory experiments. Tomatoes were incubated in a humidity chambers held at either 80-85% RH (high) or at 50-60% RH (ambient). Tomatoes were maintained at 22 ^{o}C. The humidity chambers were vented twice a day for 10 min to prevent accumulation of ethylene. Tomatoes were purchased from a local supplier, and were either greenhouse-grown (and sanitized post-harvest in chlorine-containing solution) or un-treated. The treatment (high or ambient humidity) was imposed for a week prior to the inoculation with *Salmonella*. Following the humidity treatment, tomatoes were inoculated with ~100 CFU of *S. enterica* sv Typhimurium 14028 that were spotted onto shallow (1 mm in diameter, 1-2 mm depth) wounds made in tomato epidermis. Post-inoculation with *Salmonella*, all tomatoes were incubated at ambient conditions (35-50% RH, 22 ^{o}C) for 7 days, after which they were stomached in PBS and processed as above for the enumeration of *Salmonella* CFU within fruit tissues. To remove native surface microbiota, tomatoes were treated as described by Marvasi et al. (2013).

3 Results

3.1 Experiments

The experiment to determine how to correct for CFUs on crowded plates resulted in a mean slope β of 0.833, significantly different from 1 ($P < 0.001$). The results presented here are based on this correction factor, while the results based on the original *Salmonella* counts are available in Appendix 1.

Fig. 1–3 show boxplots of the *Salmonella* proliferation observed in the three field studies. The average $\log_{10}$ *Salmonella* proliferation was 4.2 in the irrigation dataset (n=1,353), 4.8 in the nitrogen/potassium dataset (n=2,835), and 4.1 in the iron/copper dataset (n=2,406). Time series of temperature, solar radiation, total rainfall, relative humidity, and dew point prior to each harvest event are given in Appendix 2. Across experiments, temperatures at which tomatoes were harvested ranged from 4 °C to 38 °C, with the iron/copper experiment experiencing cold shocks (i.e., sudden drops in temperature) prior to harvest.

3.2 Regression trees

Regression trees confirmed time of harvest was the most important factor for explaining the observed variability in *Salmonella* proliferation, followed by tomato ripeness (with a relative importance of 30–40% of that of harvest time), while none of the experimentally imposed variables had a visible effect (Appendix 3). Climate variables were included in the model to explain the apparent associations between time of harvest and *Salmonella* proliferation. Humidity-related factors emerged as the most important factors (Fig. 4–6). In all three experiments, high relative humidity the week prior to harvest was consistently associated with less *Salmonella* proliferation with the breakpoint at 77-80% RH and explaining 1.3-1.9 $\log_{10}$ units of proliferation differential. Other factors related to humidity were also found influential, but their effects were less equivocal. Rainfall was of importance in the irrigation experiment, whereas a dew point ≥ 15 °C was associated with less proliferation in the nitrogen/potassium experiment, and a dew point < 23 °C was associated with less proliferation in the iron/copper experiment. Air temperature was of importance in two out of three experiments. The results of evaluating the impact of climatic factors one day before harvest were less consistent, although humidity related variables (relative humidity, dew point) were also important in this analysis. The most important non-climatic factor was tomato ripeness, with less *Salmonella* proliferation observed in unripe and partially ripe tomatoes. The effect of *Salmonella* strain was only evident in the iron/copper dataset, with the outbreak cocktail being associated with less *Salmonella* proliferation. The least important factors in explaining *Salmonella* proliferation were tomato cultivar and the experimental treatments.

3.3 Effects of humidity under laboratory conditions

As shown in Fig. 7A, no significant effect of humidity was observed when the native surface microbial communities were removed by a post-harvest sanitation treatment. In the follow-up experiments, untreated tomatoes, containing their native epimicrobiota were incubated under the same conditions. As shown in Fig. 7B, tomatoes that were incubated at higher humidity prior to the inoculation with *Salmonella* supported significantly lower proliferation of the pathogen than the tomatoes that were incubated at lower relative humidity.

4 Discussion

NTS is one of the major foodborne pathogens worldwide and in the United States. *Salmonella*, as well as other human pathogens, are rarely but routinely isolated from crop

production environments and field produce (Bell et al., 2015; Marine et al., 2015). Nevertheless, a significant number of the outbreaks of human salmonellosis linked to the consumption of fresh produce have been linked to farms and other production facilities (Bennett et al., 2015). As fresh produce is increasingly identified as a source of outbreaks, a better understanding of the role of crop production practices that affect susceptibility of crops to human pathogens pre- and post-harvest could eventually result in a significant reduction of the number and/or severity of the produce-associated outbreaks.

The impact of crop production conditions on microbiological safety of produce has been evaluated using three different approaches: 1) pathogens or avirulent surrogates were inoculated onto crops to determine whether production practices can distribute the pathogens throughout the field and how pathogens persist in the field under these conditions (Islam et al., 2004a, 2004b; Moyne et al., 2011; Williams et al., 2013); 2) naturally occurring indicator organisms were tracked under various cropping systems in order to extrapolate how human pathogens might behave under these conditions (Bell et al., 2015; Marine et al., 2015); and 3) fruits were inoculated post-harvest to determine whether different production conditions impact properties of produce making it more or less conducive to proliferation of the pathogen post-harvest (Marvasi et al., 2015, 2014a, 2013). The latter type of studies was the subject of this manuscript.

Our study confirms the complex interactions of factors affecting the proliferation of *Salmonella* on tomatoes post-harvest. We confirmed previous reports (Marvasi et al., 2014b, 2013) that tomatoes that are harvested mature green or as breakers are significantly less conducive to *Salmonella* proliferation. Even though consumers are thought to prefer vine-ripe tomatoes, microbiological consequences of allowing tomatoes to fully mature under the field condition must be carefully weighed. Furthermore, we confirmed that, by themselves, neither nitrogen or potassium fertilization, nor irrigation levels nor foliar sprays with Cu- or Fe-containing solutions had a major impact on how conducive tomatoes would be to proliferation of *Salmonella* if a contamination even occurred post-harvest. This has important consequences for both risk assessment and risk management. For risk assessment, our results imply that predicting consequences of field production practices on proliferation of *Salmonella* in the event of a post-harvest contamination of a particular crop is very difficult. Fig. 1 and 2 show that across experiments, *Salmonella* proliferation varies between 0.6 and 9.6 $\log_{10}$ units. A range of roughly 3.5–6.0 $\log_{10}$ units could be explained by the variables included in the regression trees but their effects were not consistent across experiments.

Under the field conditions, we did not observe an effect of the tomato cultivar on post-harvest susceptibility to *Salmonella*. However, it should be noted that unlike other studies in which dozens of tomato genotypes were compared (Han and Micallef, 2014; Marvasi et al., 2014b), only three tomato cultivars were compared in our study. Only relative humidity had a consistent effect, explaining a proliferation differential of 1.3–1.9 $\log_{10}$ units. Hence, a major part of the variability remains unexplained. Furthermore, the most important variables identified by our analysis are not readily available from routine observations made while growing or harvesting tomatoes. For risk managers, specifically tomato growers, our results imply that harvesting after a period of high humidity will decrease the potential *Salmonella* proliferation. It is unlikely that the physical and/or chemical changes associated with humidity itself had a major impact on the properties of the fruit: imposing high or low irrigation treatment did not predispose tomatoes to *Salmonella*. Only severe water congestion (which is unlikely to occur at the relative humidity that tomatoes experiences in these studies) increased *Salmonella* proliferation in tomato pericarps (Marvasi et al., 2013). The impact of high humidity pre-harvest on the subsequent proliferation of *Salmonella* in tomato fruit appears to be related to the presence of the native microbiota. Indeed, our follow-up laboratory experiments demonstrated that tomatoes that were surface disinfected prior to the humidity treatment supported the same levels of *Salmonella* proliferation.

The role of phytobacteria in both promoting and restricting proliferation of *Salmonella* and *E. coli* in and on plants has been well-documented (Brandl et al., 2013; Teplitski et al., 2011). Janisiewicz et al. (1999) provided the first evidence that a strain of *Pseudomonas syringae* (with previously characterized fungicidal properties) reduces proliferation of *E. coli* O157:H7 on wounded apples by 10-1,000 fold. Subsequent studies identified a number of native bacteria capable of reducing proliferation of *Salmonella* and pathogenic *E. coli* on produce (Allard et al. 2014; Cooley et al., 2006, 2003; Fett, 2006). Under the field conditions, treatment of tomatoes with systemic and foliar Cu-containing pesticides reduced abundance of γ-proteobacteria, including one of its antagonists (*Paenibacillus*) under the field conditions, thus impacting niche dynamics (Ottesen et al., 2015). While we did not assess changes in the tomato epimicrobiota following foliar treatments with copper and iron in our study, we did not observe any impact of this treatment pre-harvest on the ability of tomatoes to support *Salmonella* proliferation in a post-harvest contamination model. Even though it is clear that a number of environmental conditions and even some production practices impact native

microbiota of the tomato crop, the complex multi-partite interactions of these factors are still far from being fully understood.

5 Conclusion

To overcome the limitations of classical regression models, we used regression trees to explore the factors that affect *Salmonella* proliferation in three distinct experimental datasets. In line with previous studies, we confirmed the effect of tomato ripeness and the limited impact of production practices (such as varying levels of N, P fertilization, irrigation levels and overhead Cu- and Fe-containing sprays). By including information on climatic conditions prior to harvest, we identified the importance of humidity prior to harvest that was associated with decreased *Salmonella* proliferation, and thus showed a protective effect. The independent action of relative humidity was confirmed in a controlled laboratory experiment.

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Figure captions

Fig. 1. Post-harvest proliferation of *Salmonella* in tomatoes (cultivars Bonny Best [BB], Florida-47 [FL], and Solar Fire [SF]) grown under different irrigation treatments, i.e., D ("dry") = 6%, M ("medium") = 10% (recommended for tomato production), W ("wet") = 12% volumetric soil moisture contents imposed within two weeks of the first harvest. Four independent samplings were conducted, i.e., June 2011 (A), June 2012 (B), October 2012 (C) and October/November 2012 (D). Tomatoes were classified at harvest as unripe, partially ripe or ripe. At each sampling, tomatoes were infected with *Salmonella* Typhimurium ATTC 14028 or a cocktail of six outbreak-related *Salmonella enterica* strains. Upon completion of a 1-week incubation, *Salmonella* cells were recovered and proliferation was calculated as the ratio of counts after and before inoculation. The boxplots combine data for infections with both types of inocula.

Fig. 2. Post-harvest proliferation of *Salmonella* in tomatoes (cultivars Sebring [SE] and Solar Fire [SF]) grown under different fertilization treatments: N1 ¼ 168, N2 ¼ 224 (recommended), N3 ¼ 280 kg/ha; K1 ¼ 168, K2 ¼ 252 (recommended), K3 ¼ 336 kg/ha. Four independent samplings were conducted, i.e., June 2011 (A), June 2012 (B), October 2012 (C) and October/November 2012 (D). Tomatoes were classified at harvest as unripe, partially ripe or ripe. At each sampling, tomatoes were infected with *Salmonella* Typhimurium 14028 or a cocktail of six outbreak-related *Salmonella enterica* strains. Upon completion of a 1-week incubation, *Salmonella* cells were recovered and *Salmonella* proliferation was calculated as the ratio of *Salmonella* cells after and before inoculation. The boxplots combine data for infections with both types of inocula.

Fig. 3. Post-harvest proliferation of *Salmonella* in tomatoes (cultivar Solar Fire) grown under different pesticide treatments, i.e., 0.17 kg/ha Fe, 0.1 kg/ha Cu, equal combination of Fe and Cu and water (control), applied once prior the harvest, every 2 or every 6 weeks. Four independent samplings were conducted, i.e., July 1 2013 (A), July 8 2013 (B), June 26 2014 (C) and July 4 2014 (D). Tomatoes were classified at harvest as unripe, partially ripe or ripe. At each sampling, tomatoes were infected with *Salmonella* Typhimurium 14028 or a cocktail of six outbreak-related *Salmonella enterica* strains. Upon completion of a 1-week incubation, *Salmonella* cells were recovered and *Salmonella* proliferation was

calculated as the ratio of *Salmonella* cells after and before inoculation. The boxplots combine data for infections with both types of inocula.

Fig. 4. Regression tree (left) and relative variable importance (right) for $\log_{10}$ *Salmonella* proliferation in the irrigation dataset. Climatic variables are averages for the week prior to harvest (panel A) or the day before harvest (panel B). Abbreviations: cultivar = tomato cultivar; dewpoint = average dew point at 2 m (°C); irrigation = irrigation treatment {dry [D], medium [M], wet [W]}; rain = total rainfall at 2 m (cm); relhum = average relative humidity at 2 m (%); ripeness = tomato ripeness at harvest {unripe [unr], partially ripe [prt], ripe [rip]}; solar = average solar radiation at 2 m (W/m²); strain = *Salmonella* strain; t60 = average temperature at 60 cm (°C).

Fig. 5. Regression tree (left) and relative variable importance (right) for $\log_{10}$ *Salmonella* proliferation in the nitrogen/potassium dataset. Climatic variables are averages for the week prior to harvest (panel A) or the day before harvest (panel B). Abbreviations: cultivar = tomato cultivar; dewpoint = average dew point at 2 m (°C); nitrogen = nitrogen treatment; potassium = potassium treatment; rain = total rainfall at 2 m (cm); relhum = average relative humidity at 2 m (%); ripeness = tomato ripeness at harvest {unripe [unr], partially ripe [prt], ripe [rip]}; solar = average solar radiation at 2 m (W/m²); strain = *Salmonella* strain; t60 = average temperature at 60 cm (°C).

Fig. 6. Regression tree (left) and relative variable importance (right) for $\log_{10}$ *Salmonella* proliferation in the iron/copper dataset. Climatic variables are averages for the week prior to harvest (panel A) or the day before harvest (panel B). Abbreviations: c = copper treatment; dewpoint = average dew point at 2 m (°C); f = iron treatment; freq = iron/copper treatment frequency; rain = total rainfall at 2 m (cm); relhum = average relative humidity at 2 m (%); ripeness = tomato ripeness at harvest {unripe [unr], partially ripe [prt], ripe [rip]}; solar = average solar radiation at 2 m (W/m²); strain = *Salmonella* strain {type strain [T], outbreak cocktail [O]}; t60 = average temperature at 60 cm (°C).

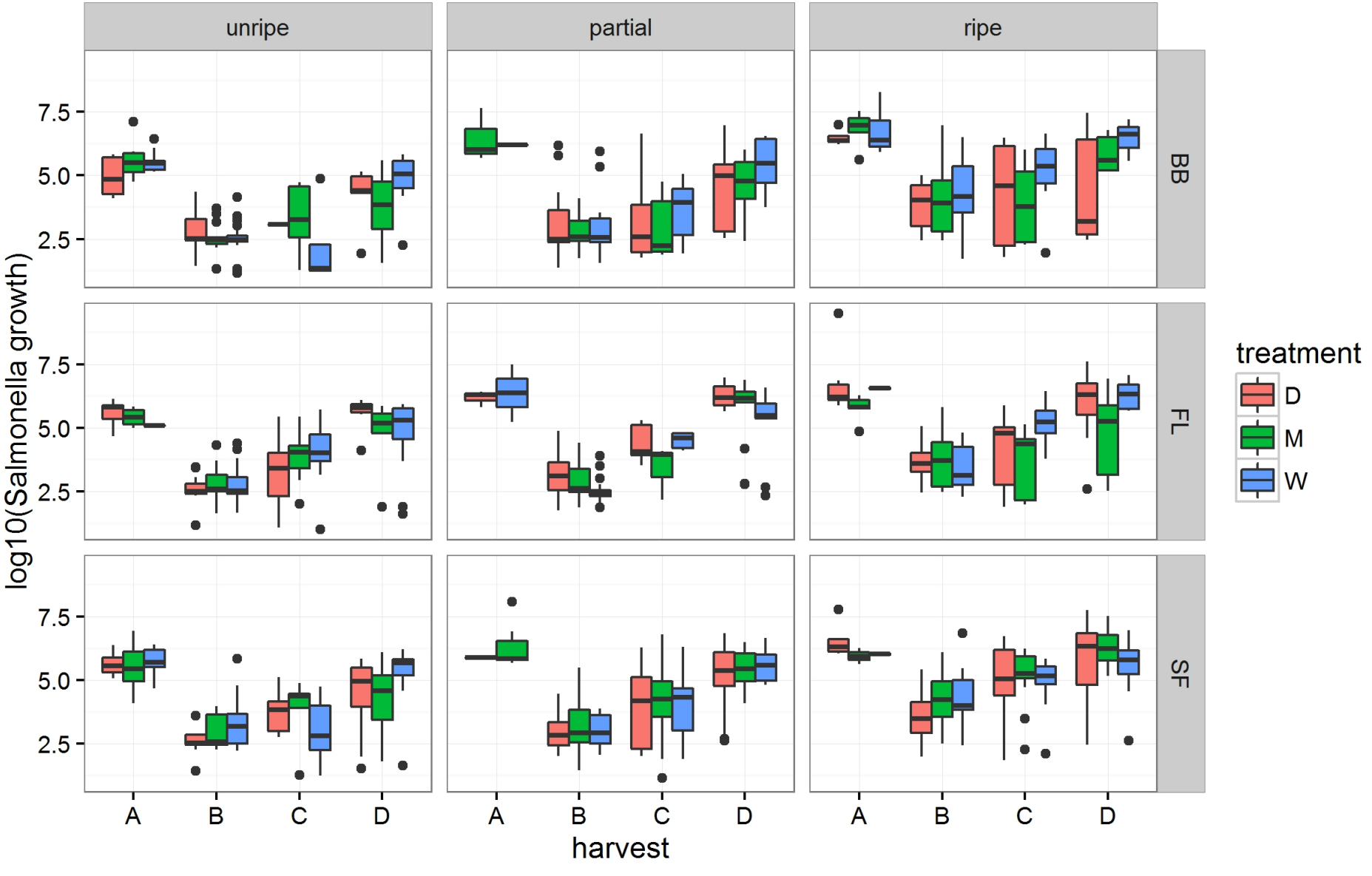
Fig. 7. Proliferation of *Salmonella enterica* sv Typhimurium 14028 in tomatoes. Tomatoes were incubated for a week at either 35-50% RH or 80% RH in a humidity chamber at 22°C, were then inoculated with *Salmonella* Typhimurium and incubated at 35-50% RH at 22°C. Tomatoes were either stripped of the native microbiota (panel A), or had native microbial communities (panel B).

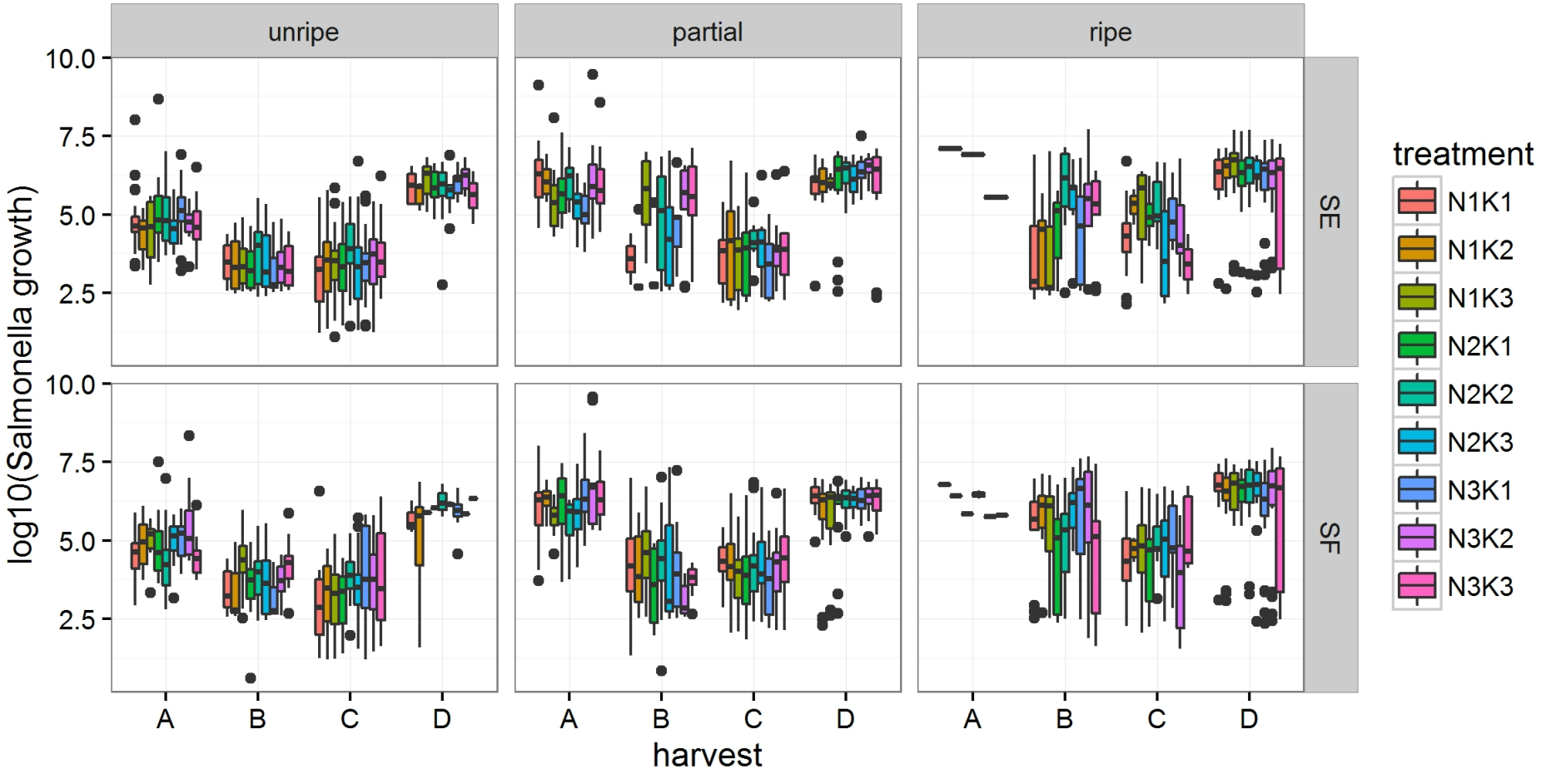
Supplementary data

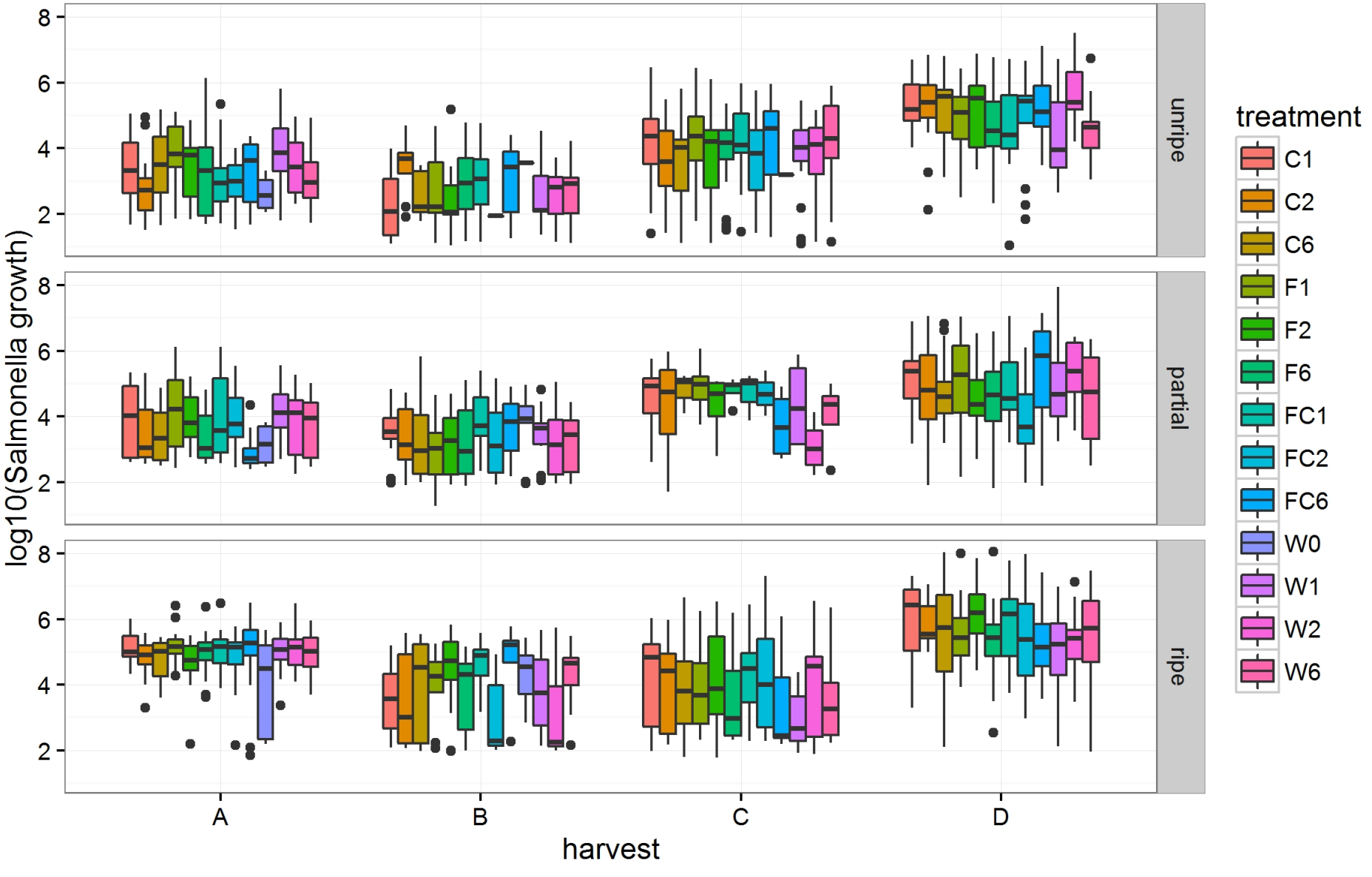
Appendix 1. Results based on the original *Salmonella* counts.

Appendix 2. Time series of temperature, dew point, relative humidity, total rainfall, and solar radiation prior to each harvest.

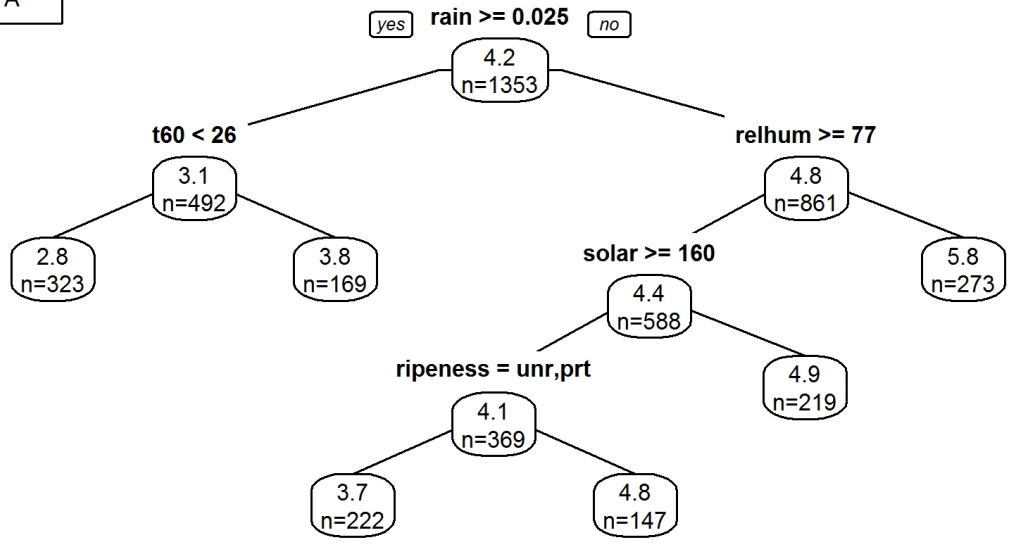
Appendix 3. Regression trees based on experimentally imposed variables and time of harvest.

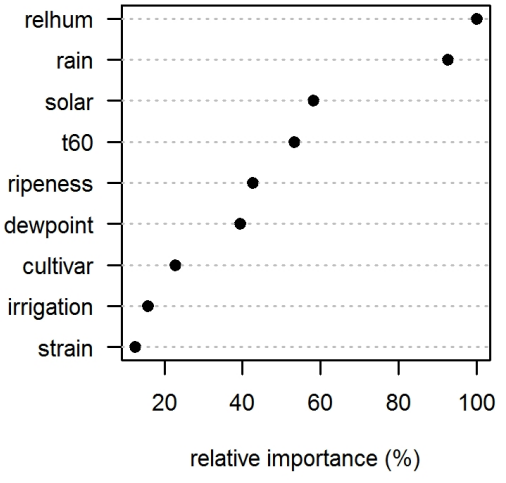

unripe
partial
ripe
BB
FL
SF
log10(Salmonella growth)
7.5
5.0
2.5
A
B
C
D
harvest
treatment
D
M
W


unripe
partial
ripe
SE
SF
log10(Salmonella growth)
10.0
7.5
5.0
2.5
A
B
C
D
harvest
treatment
N1K1
N1K2
N1K3
N2K1
N2K2
N2K3
N3K1
N3K2
N3K3

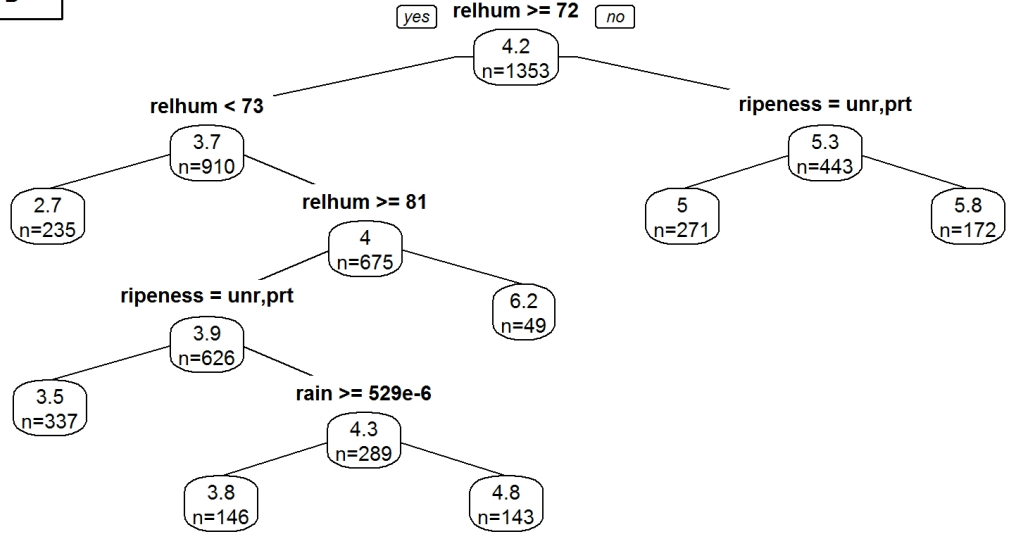

log10(Salmonella growth)
8
6
4
2
unripe
partial
ripe
A
B
C
D
harvest
treatment
C1
C2
C6
F1
F2
F6
FC1
FC2
FC6
W0
W1
W2
W6

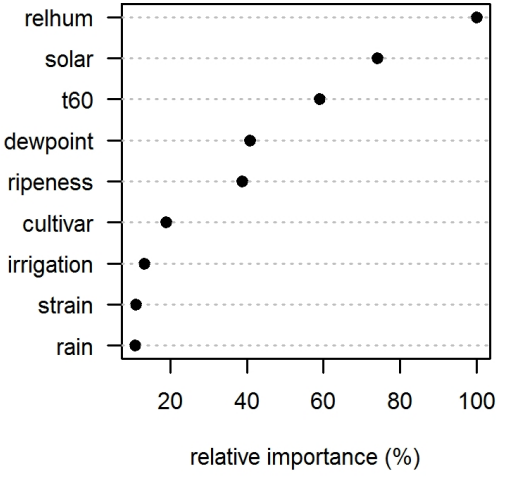
A


yes
rain >= 0.025
no
4.2
n=1353
t60 < 26
relhum >= 77
3.1
n=492
4.8
n=861
2.8
n=323
3.8
n=169
solar >= 160
5.8
n=273
4.4
n=588
ripeness = unr,prt
4.9
n=219
4.1
n=369
3.7
n=222
4.8
n=147

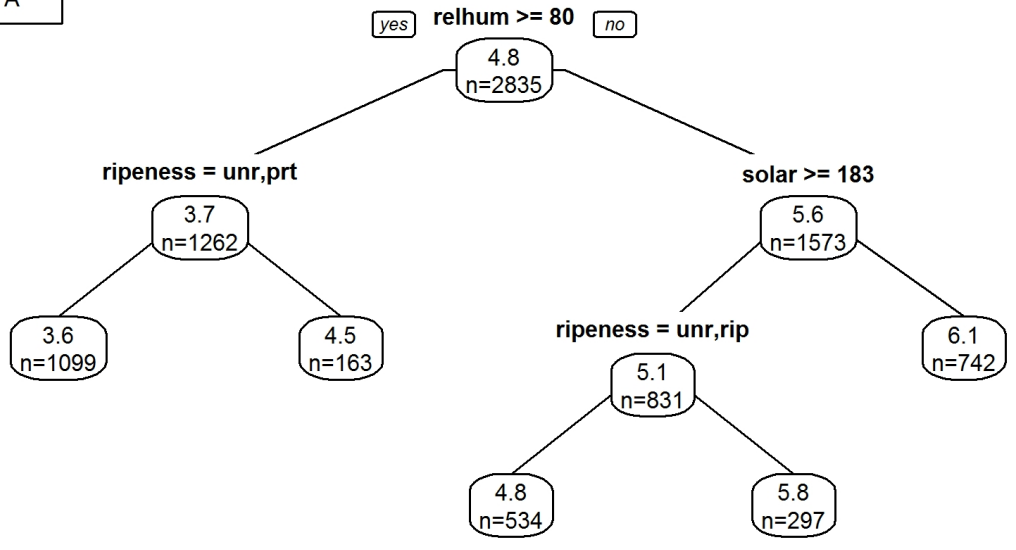

relhum
rain
solar
t60
ripeness
dewpoint
cultivar
irrigation
strain
20
40
60
80
100
relative importance (%)

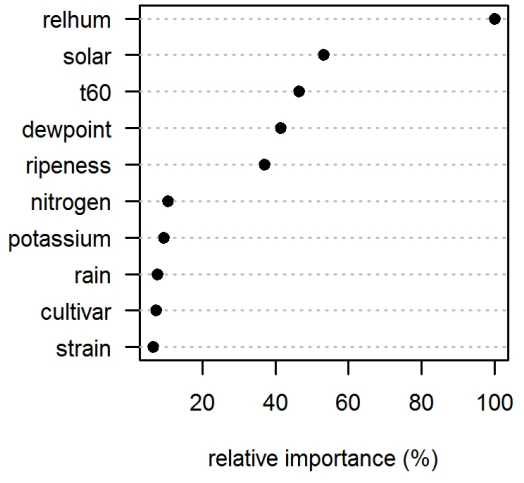
B


yes
relhum >= 72
no
4.2
n=1353
relhum < 73
ripeness = unr,prt
3.7
n=910
5.3
n=443
2.7
n=235
relhum >= 81
5
n=271
5.8
n=172
4
n=675
ripeness = unr,prt
6.2
n=49
3.9
n=626
3.5
n=337
rain >= 529e-6
4.3
n=289
3.8
n=146
4.8
n=143

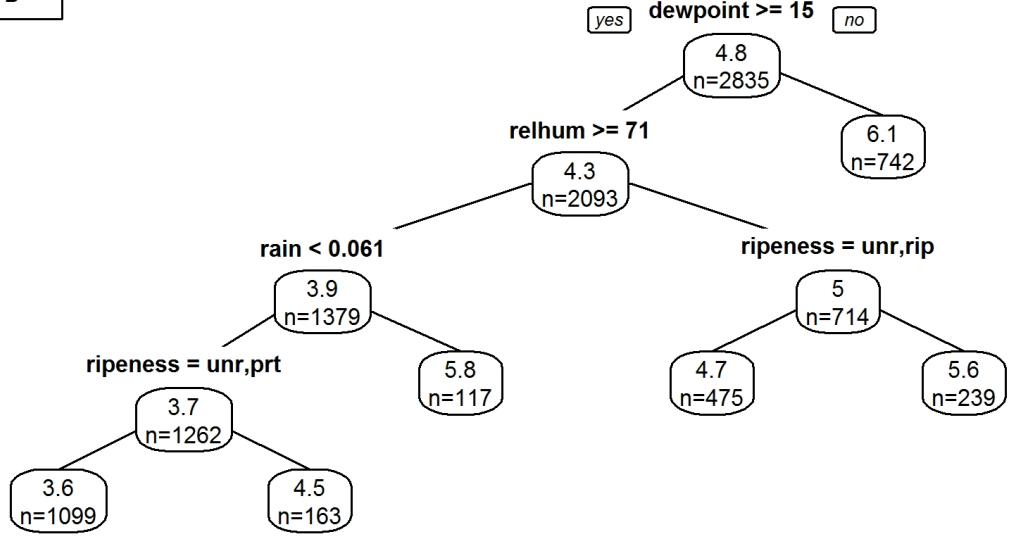

relhum
solar
t60
dewpoint
ripeness
cultivar
irrigation
strain
rain
20
40
60
80
100
relative importance (%)

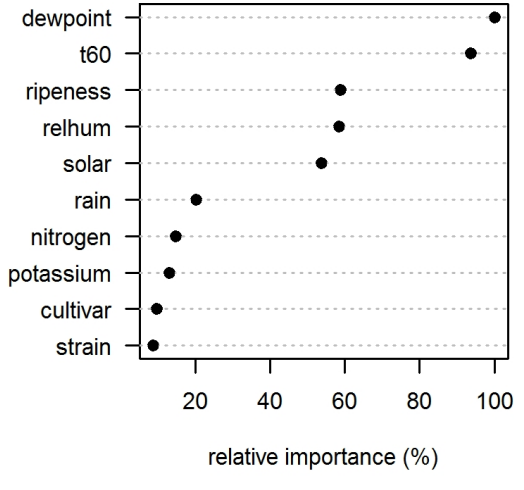
A


yes
relhum >= 80
no
4.8
n=2835
ripeness = unr,prt
3.7
n=1262
solar >= 183
5.6
n=1573
3.6
n=1099
4.5
n=163
ripeness = unr,rip
6.1
n=742
5.1
n=831
4.8
n=534
5.8
n=297

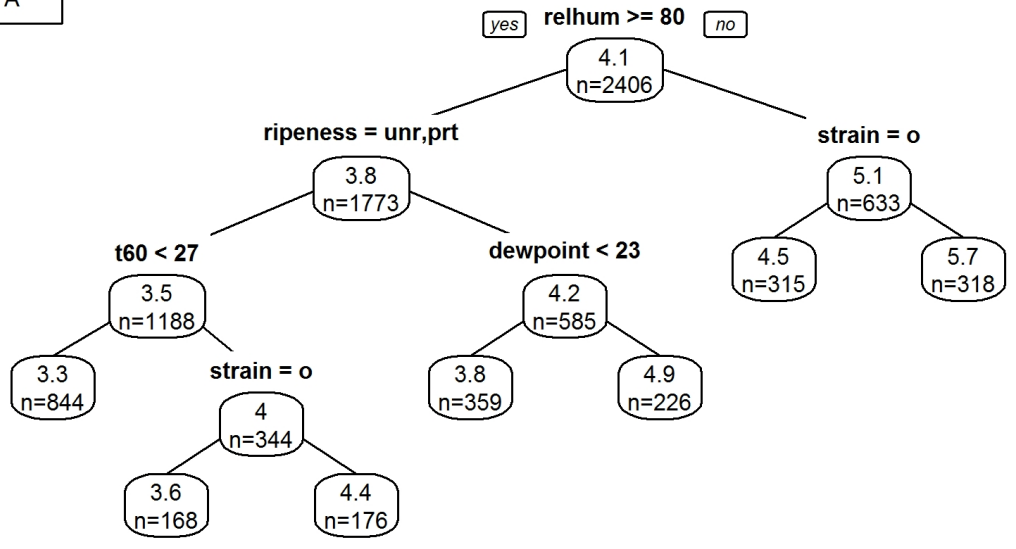

relhum
solar
t60
dewpoint
ripeness
nitrogen
potassium
rain
cultivar
strain
20
40
60
80
100
relative importance (%)

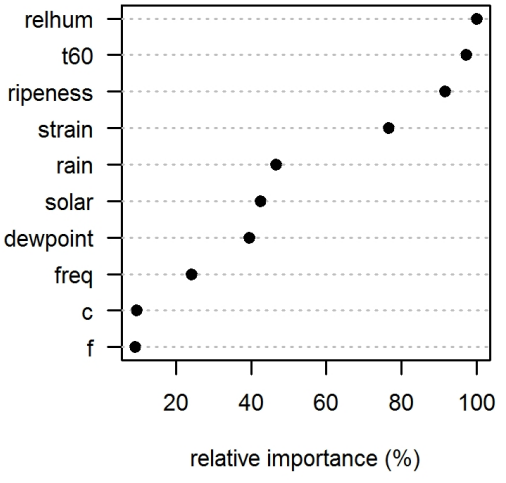
B


yes
dewpoint >= 15
no
4.8
n=2835
relhum >= 71
6.1
n=742
4.3
n=2093
rain < 0.061
ripeness = unr,rip
3.9
n=1379
5
n=714
ripeness = unr,prt
5.8
n=117
4.7
n=475
5.6
n=239
3.7
n=1262
3.6
n=1099
4.5
n=163

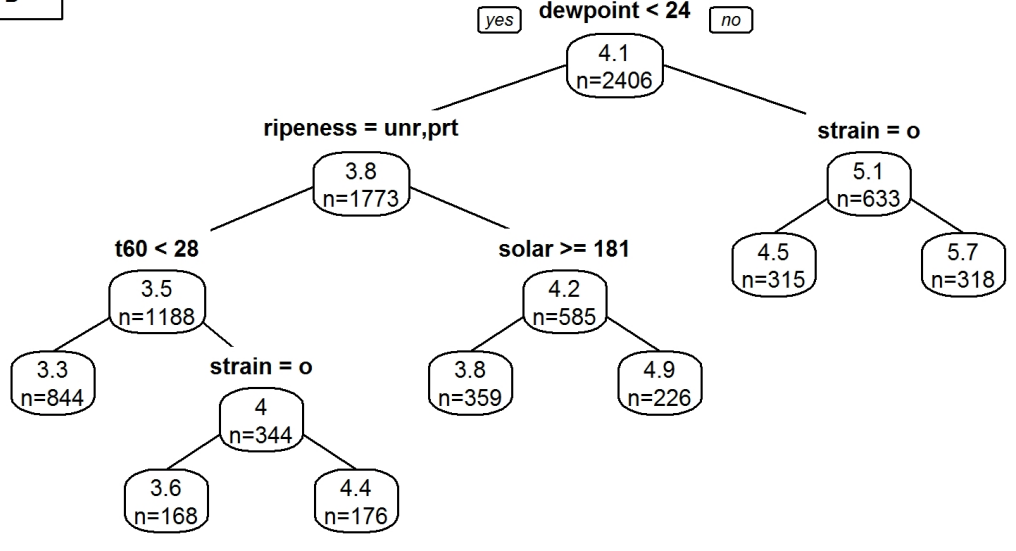

dewpoint
t60
ripeness
relhum
solar
rain
nitrogen
potassium
cultivar
strain
20
40
60
80
100
relative importance (%)

A


yes
relhum >= 80
no
4.1
n=2406
ripeness = unr,prt
strain = o
3.8
n=1773
5.1
n=633
t60 < 27
dewpoint < 23
4.5
n=315
5.7
n=318
3.5
n=1188
4.2
n=585
3.3
n=844
strain = o
3.8
n=359
4.9
n=226
4
n=344
3.6
n=168
4.4
n=176


relhum
t60
ripeness
strain
rain
solar
dewpoint
freq
c
f
20
40
60
80
100
relative importance (%)

B


yes
dewpoint < 24
no
4.1
n=2406
ripeness = unr,prt
strain = o
3.8
n=1773
5.1
n=633
t60 < 28
solar >= 181
4.5
n=315
5.7
n=318
3.5
n=1188
4.2
n=585
3.3
n=844
strain = o
3.8
n=359
4.9
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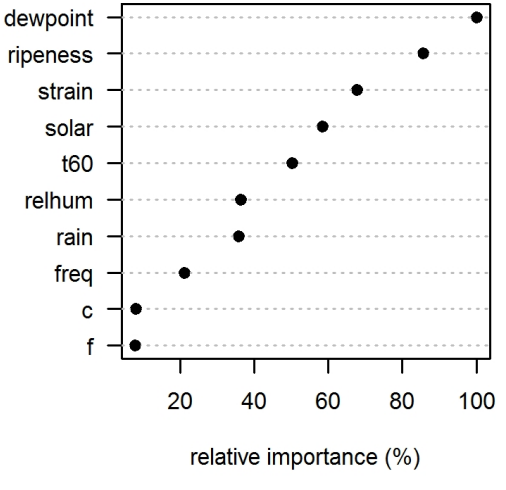
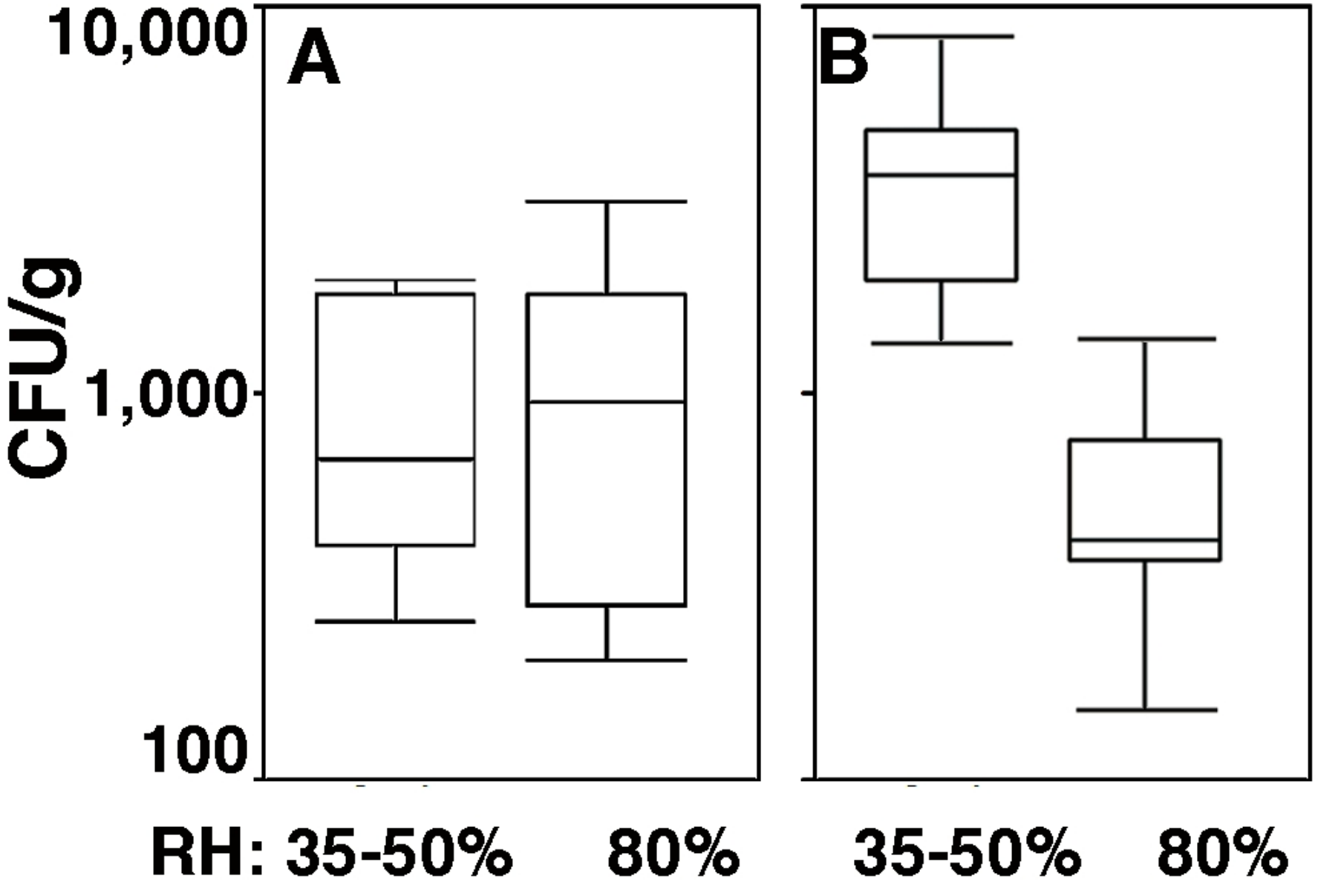
4
n=344
3.6
n=168
4.4
n=176


dewpoint
ripeness
strain
solar
t60
relhum
rain
freq
c
f
20
40
60
80
100
relative importance (%)

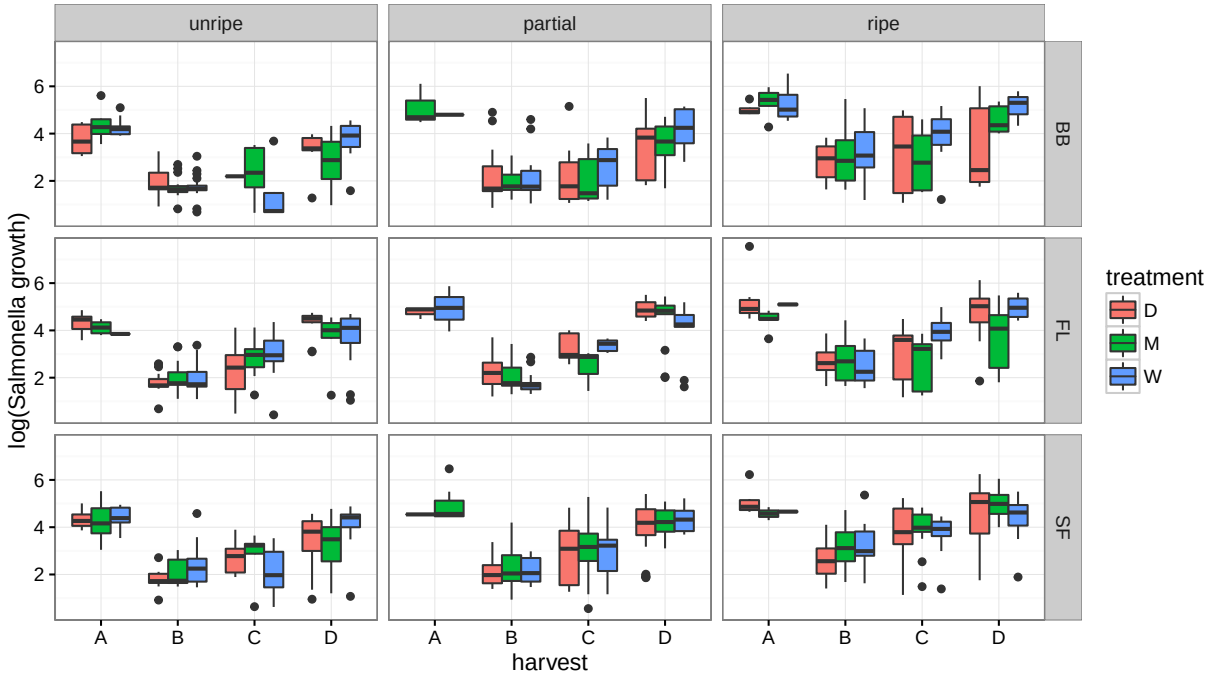

10,000
1,000
100
CFU/g
A
B
RH: 35-50%
80%
35-50%
80%

High relative humidity pre-harvest reduces post-harvest proliferation of *Salmonella* in tomatoes

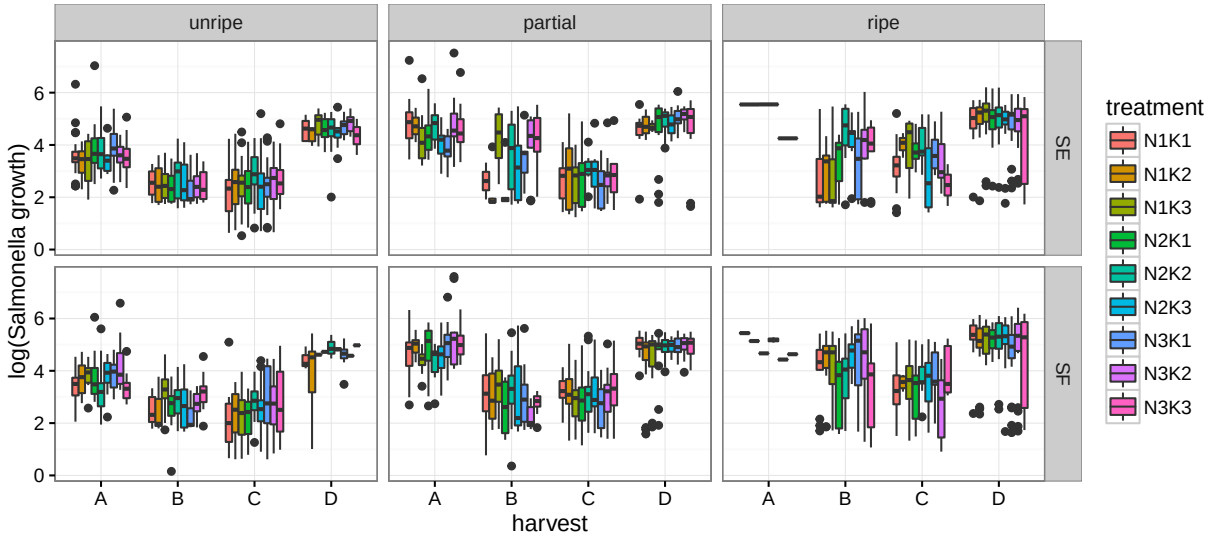
Appendix 1: Results based on the original *Salmonella* counts

Boxplots

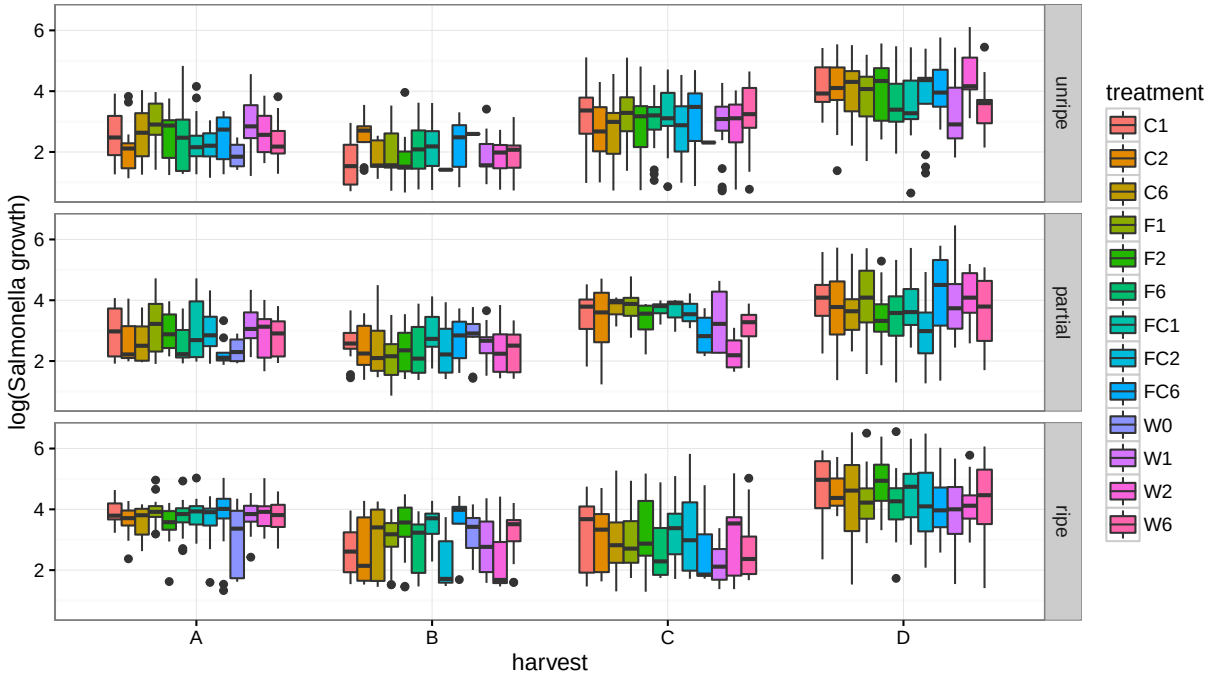
Irrigation dataset



Nitrogen/potassium dataset

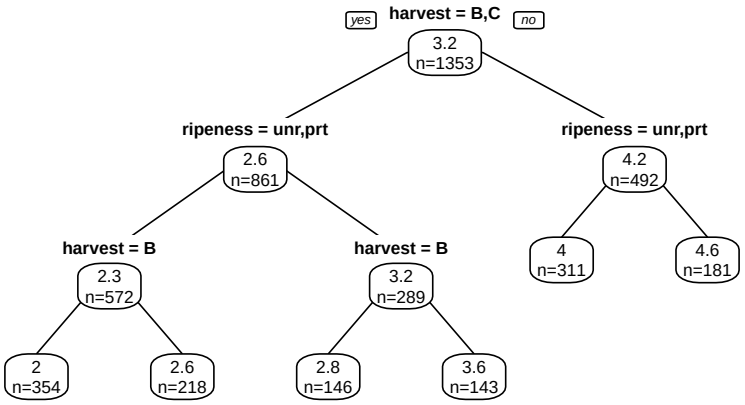


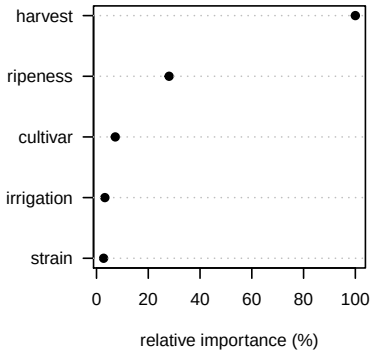
Iron/copper dataset



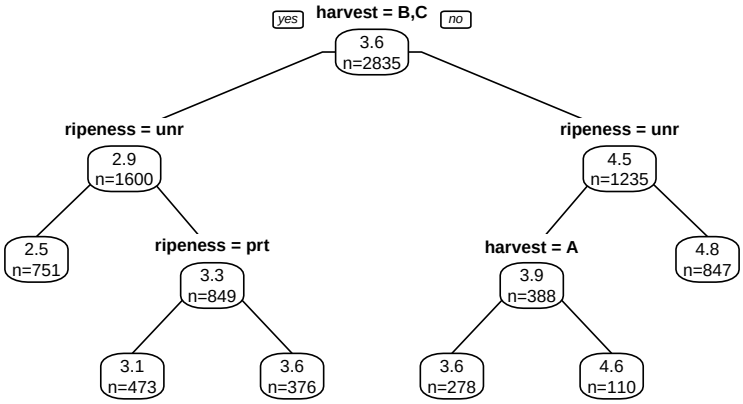
Regression trees – harvest time

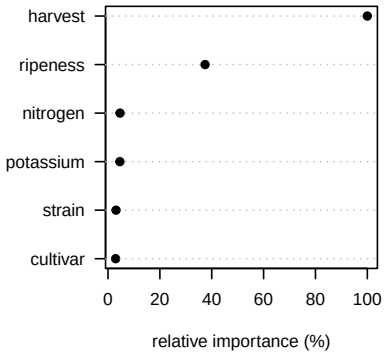
Irrigation dataset



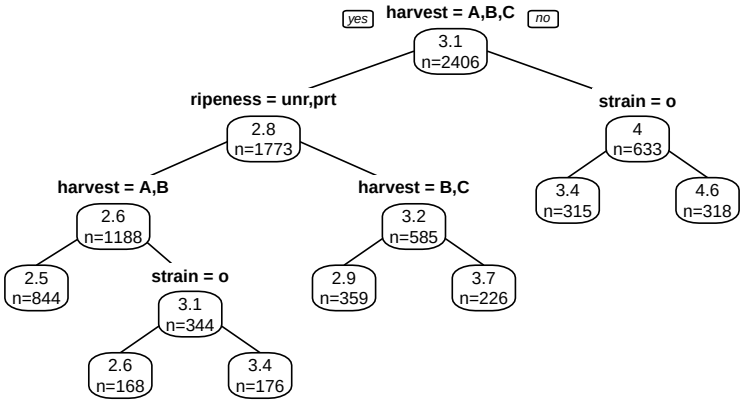


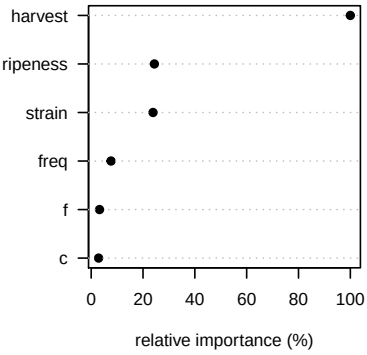
Nitrogen/potassium dataset





Iron/copper dataset

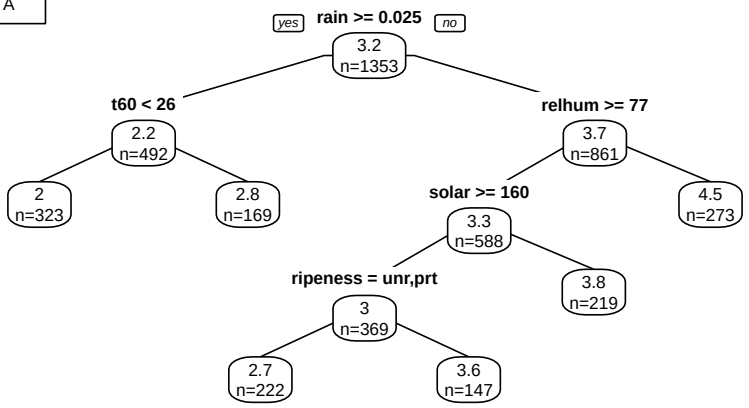


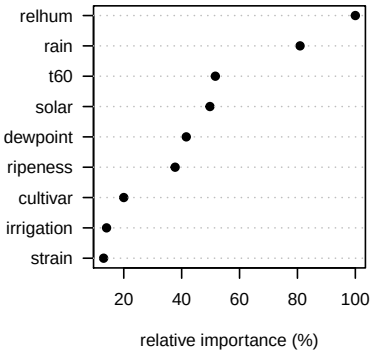


Regression trees – climatic data

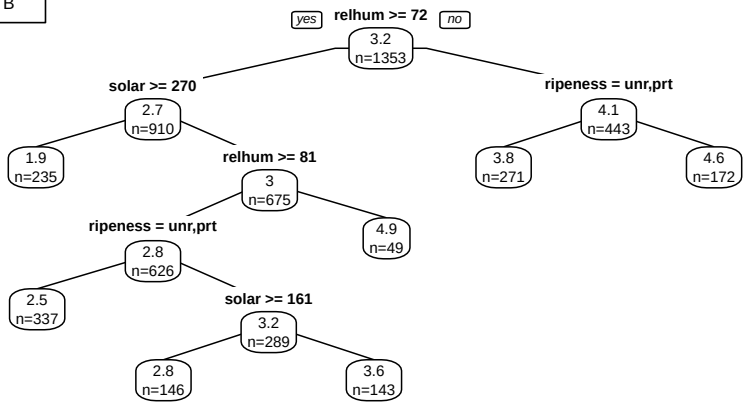
Irrigation dataset

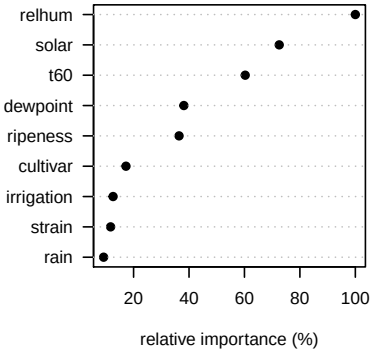
A





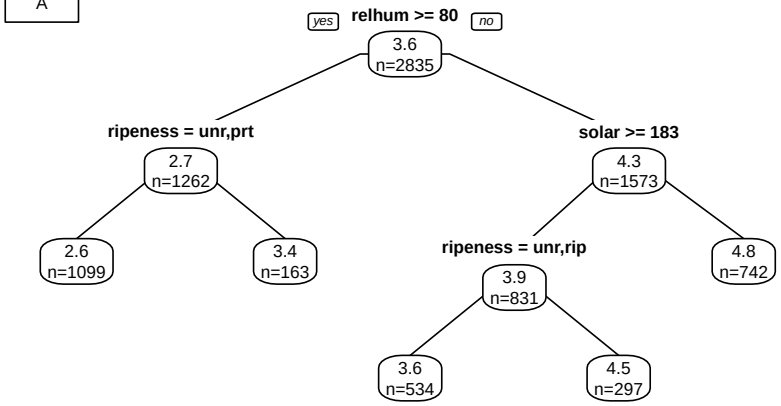
B

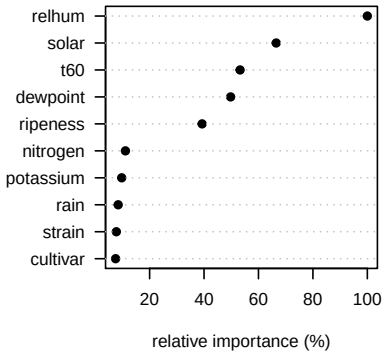




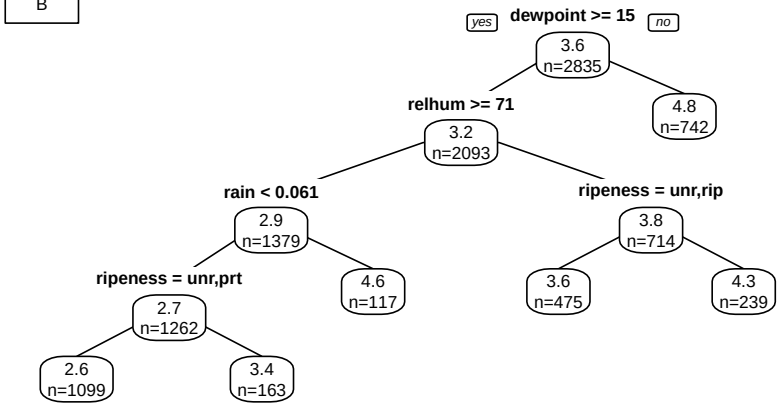
Nitrogen/potassium dataset

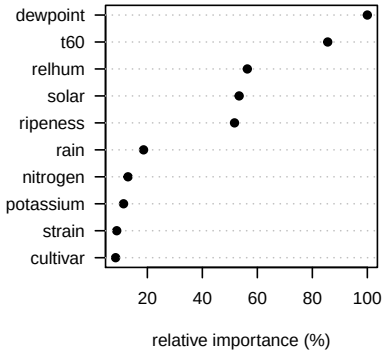
A





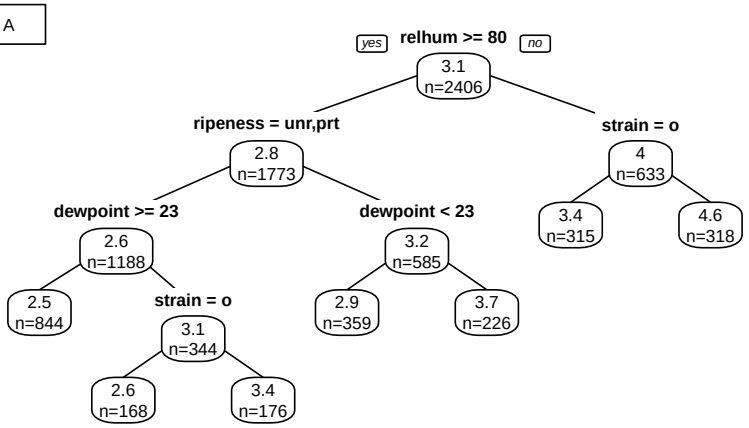
B

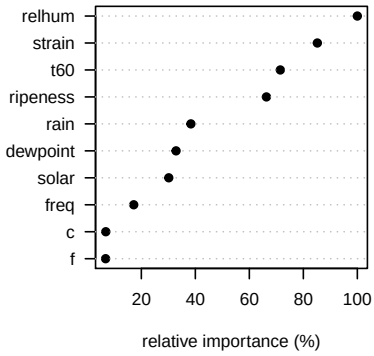




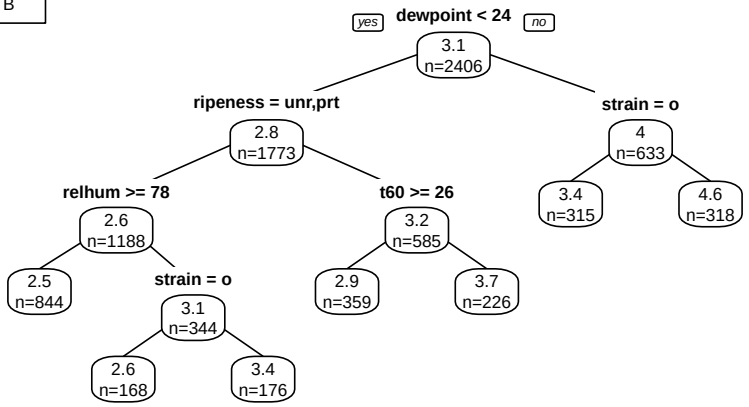
Iron/copper dataset

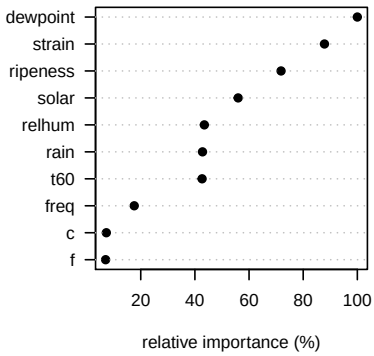
A





B





R session info

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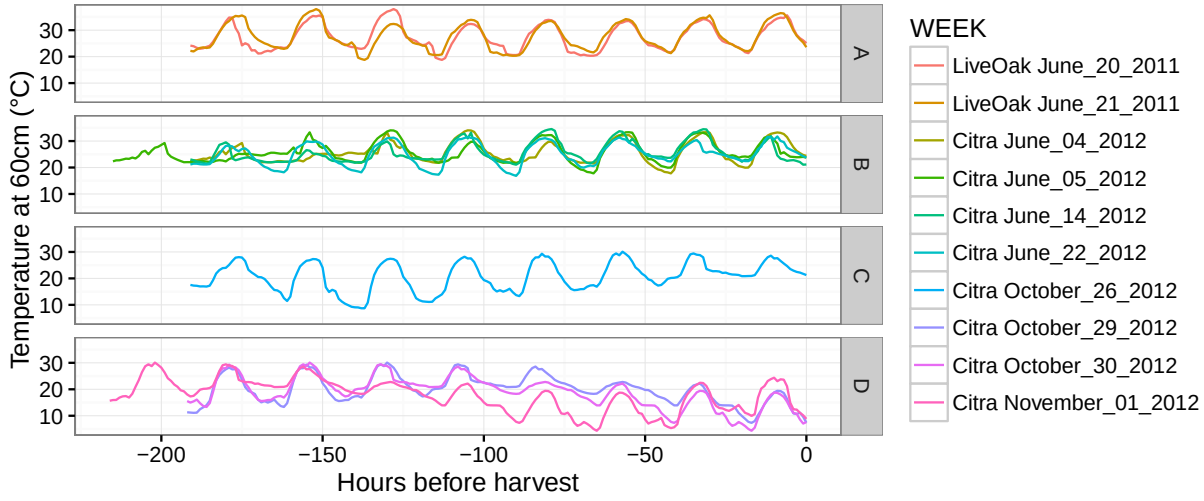
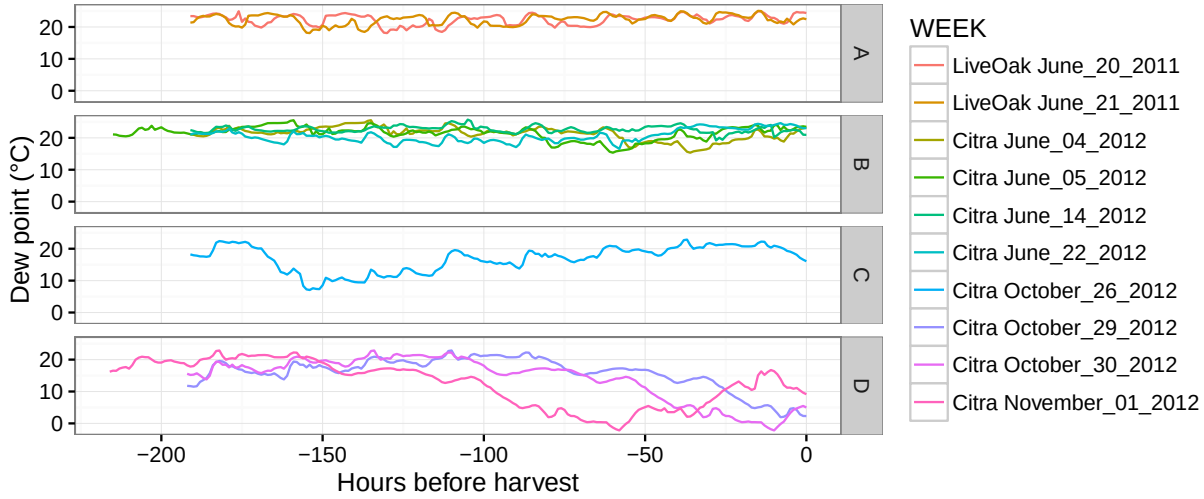
## R version 3.3.0 (2016-05-03)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 7 x64 (build 7601) Service Pack 1
##
## locale:
## [1] LC_COLLATE=Dutch_Belgium.1252  LC_CTYPE=Dutch_Belgium.1252
## [3] LC_MONETARY=Dutch_Belgium.1252 LC_NUMERIC=C
## [5] LC_TIME=Dutch_Belgium.1252
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] XLConnect_0.2-12     XLConnectJars_0.2-12 randomForest_4.6-12
## [4] rpart.plot_1.5.3     rpart_4.1-10         ggplot2_2.1.0
## [7] bd_0.0.11
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.6      knitr_1.14       magrittr_1.5     munsell_0.4.3
##  [5] colorspace_1.2-6 stringr_1.0.0    plyr_1.8.4       tools_3.3.0
##  [9] grid_3.3.0       gtable_0.2.0     htmltools_0.3.5  yaml_2.1.13
## [13] digest_0.6.9     rJava_0.9-8      reshape2_1.4.1   formatR_1.4
## [17] evaluate_0.9     rmarkdown_1.0    labeling_0.3     stringi_1.1.1
## [21] scales_0.4.0     foreign_0.8-66
```

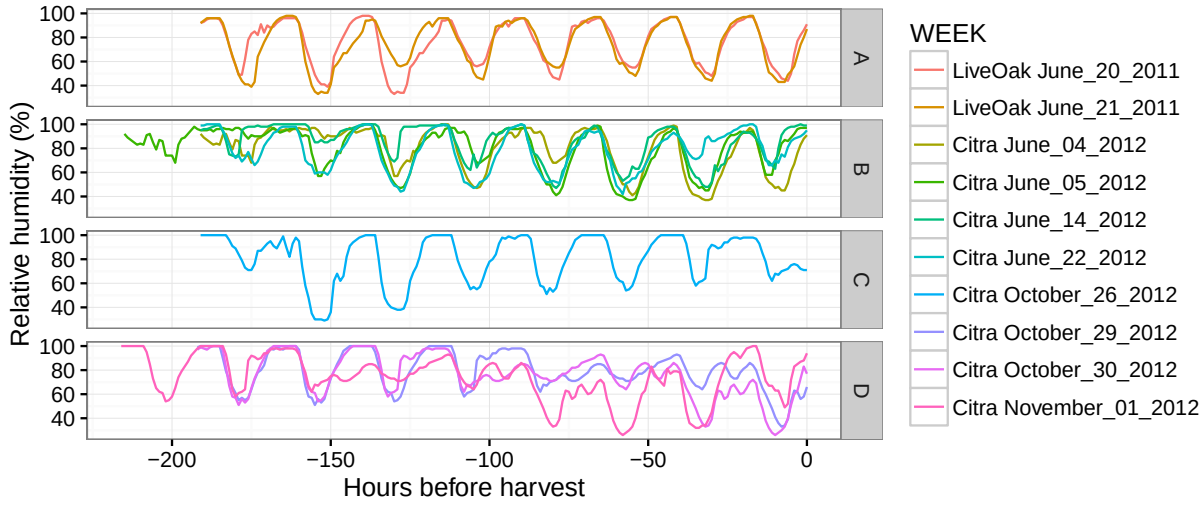
High relative humidity pre-harvest reduces post-harvest proliferation of *Salmonella* in tomatoes

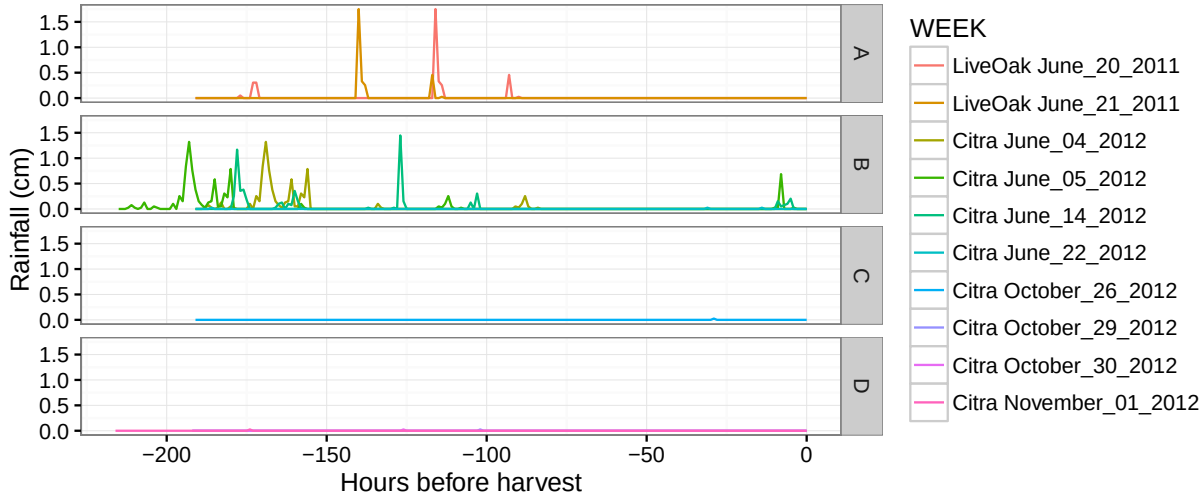
Appendix 2: Time series of temperature, dew point, relative humidity, total rainfall, and solar radiation prior to each harvest

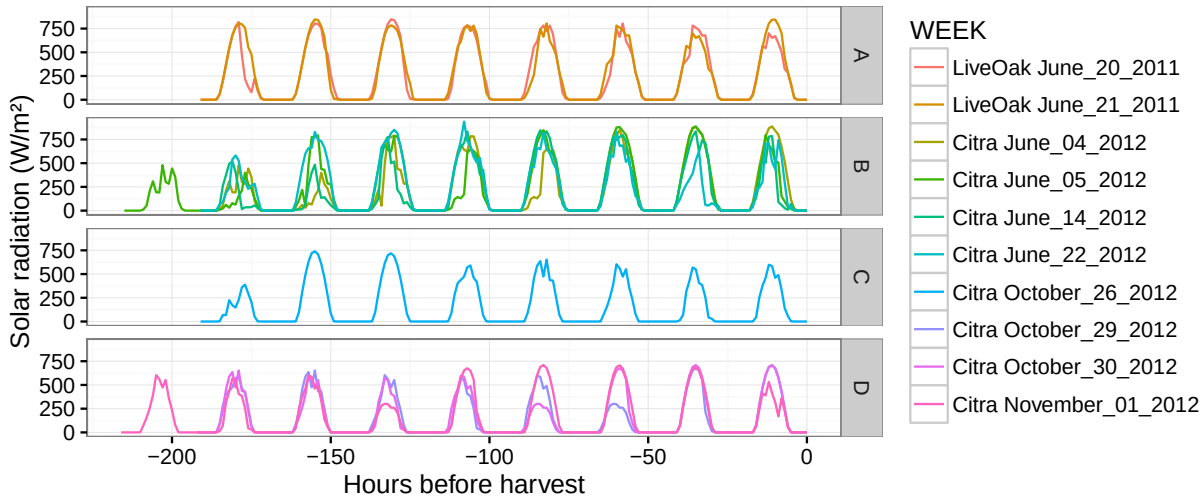
Irrigation dataset



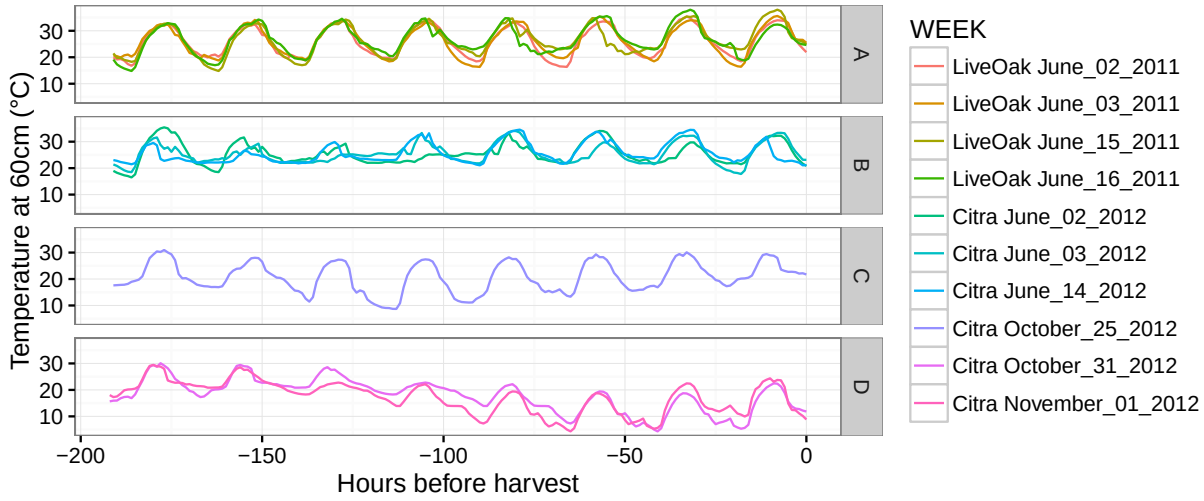


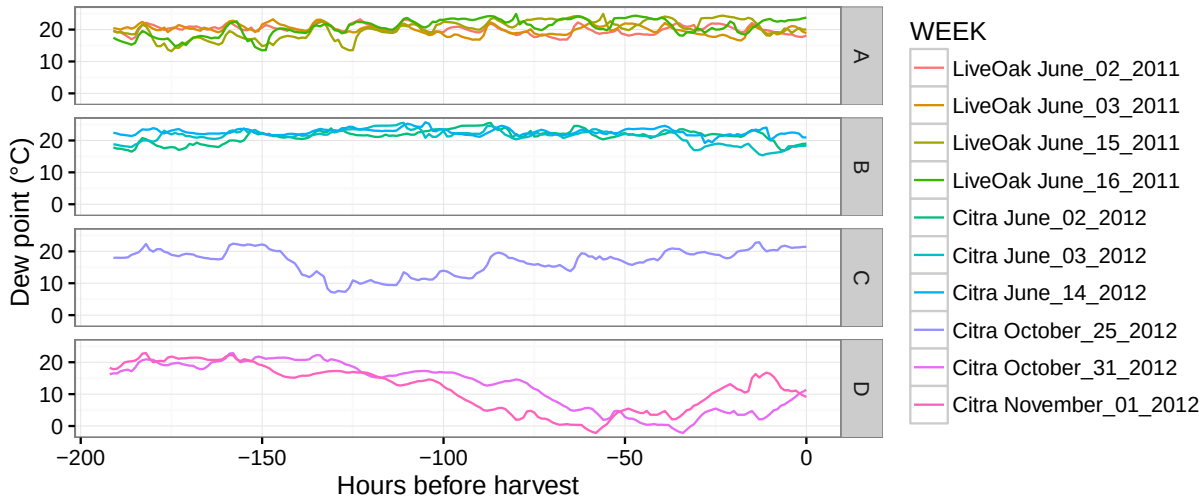

Relative humidity (%)
100
80
60
40
A
B
C
D
−200
−150
−100
−50
0
Hours before harvest
WEEK
LiveOak June_20_2011
LiveOak June_21_2011
Citra June_04_2012
Citra June_05_2012
Citra June_14_2012
Citra June_22_2012
Citra October_26_2012
Citra October_29_2012
Citra October_30_2012
Citra November_01_2012

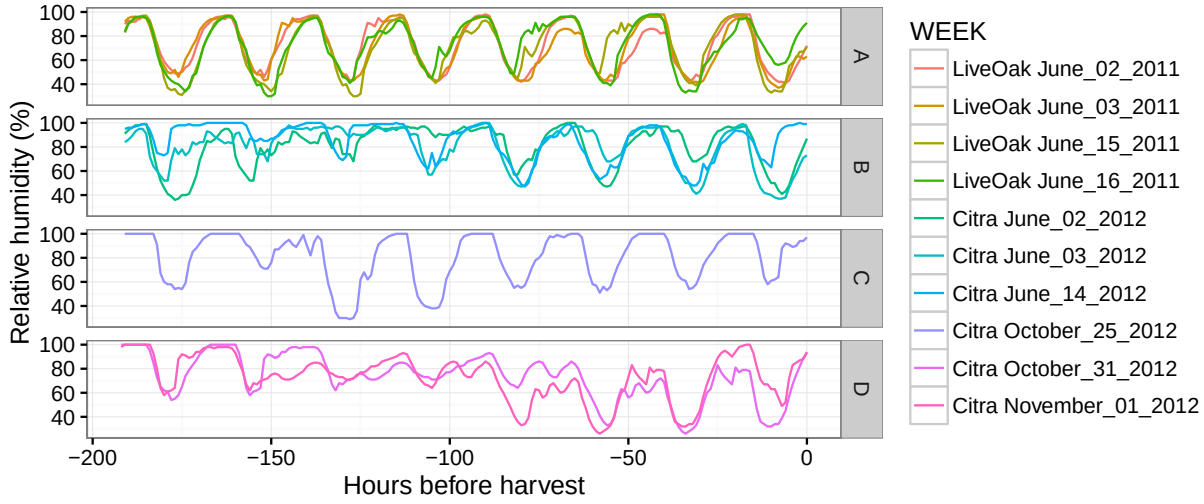

Rainfall (cm)
1.5
1.0
0.5
0.0
A
B
C
D
−200
−150
−100
−50
0
Hours before harvest
WEEK
LiveOak June_20_2011
LiveOak June_21_2011
Citra June_04_2012
Citra June_05_2012
Citra June_14_2012
Citra June_22_2012
Citra October_26_2012
Citra October_29_2012
Citra October_30_2012
Citra November_01_2012

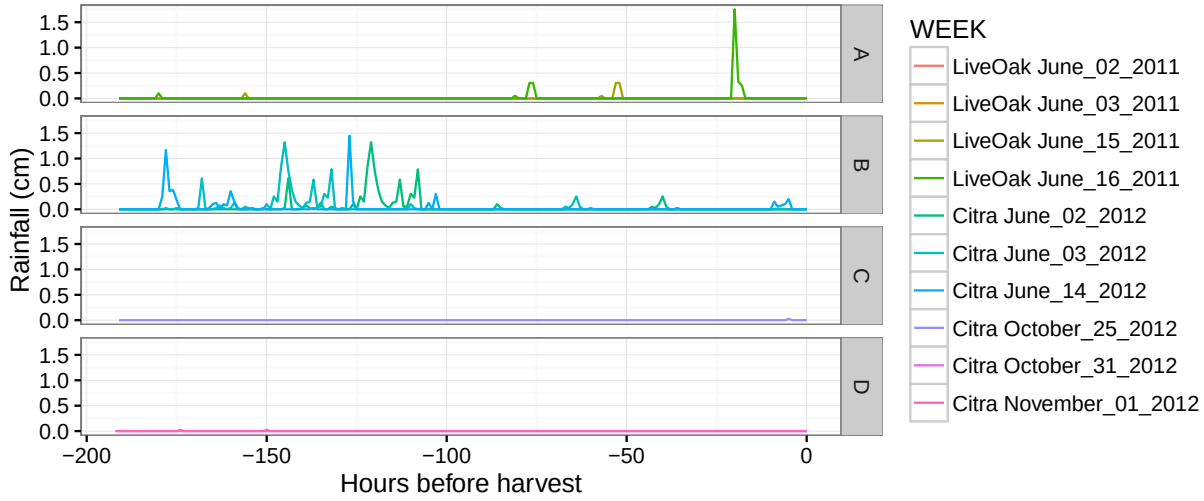

Solar radiation (W/m²)
750
500
250
0
A
B
C
D
−200
−150
−100
−50
0
Hours before harvest
WEEK
LiveOak June_20_2011
LiveOak June_21_2011
Citra June_04_2012
Citra June_05_2012
Citra June_14_2012
Citra June_22_2012
Citra October_26_2012
Citra October_29_2012
Citra October_30_2012
Citra November_01_2012

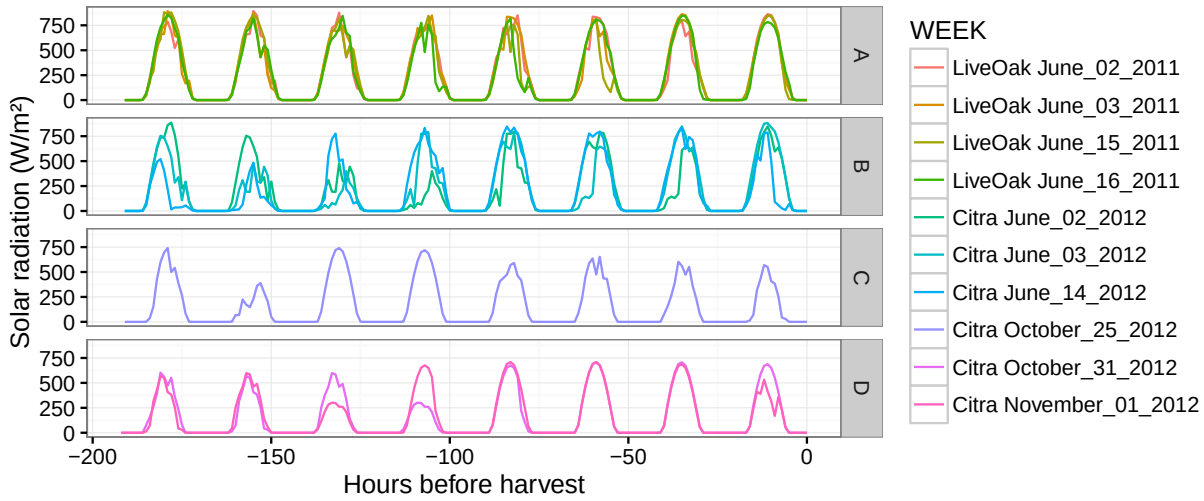
Nitrogen/potassium dataset



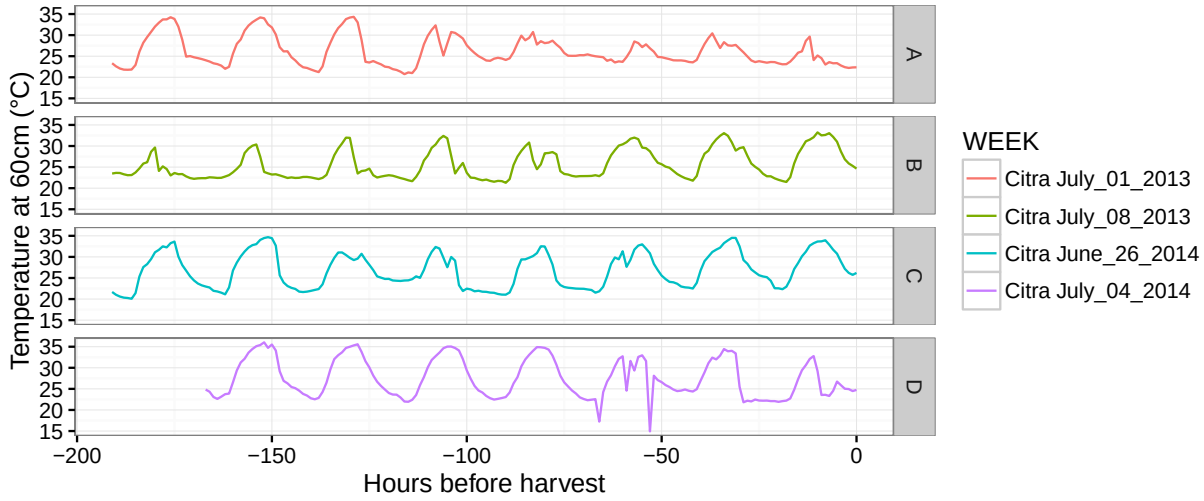


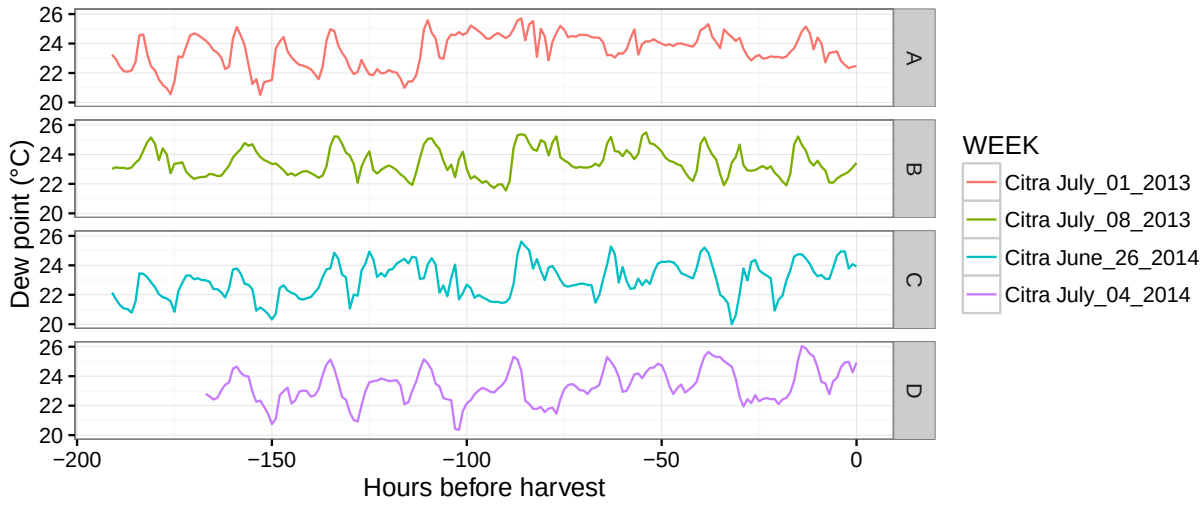


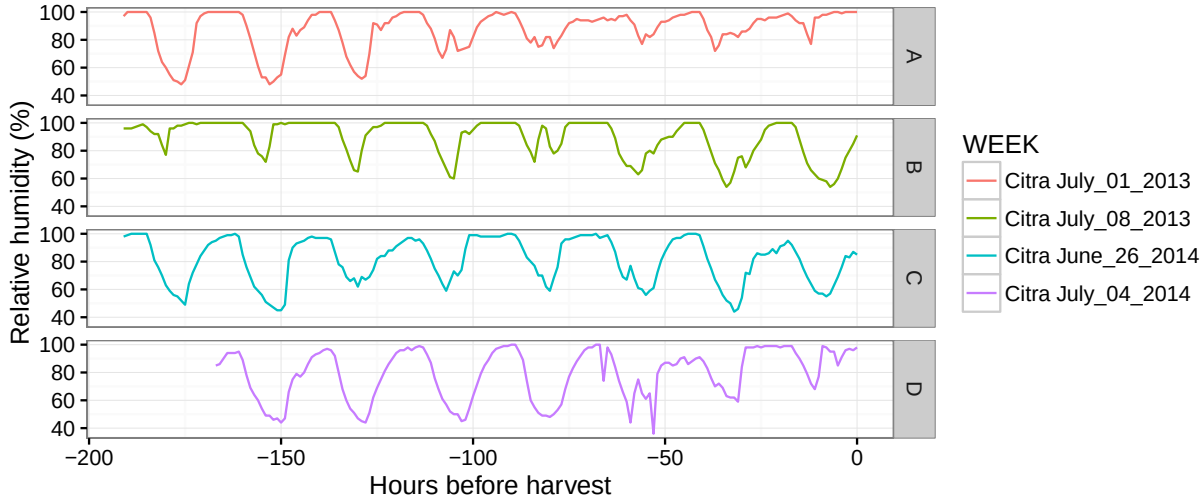

Rainfall (cm)
1.5
1.0
0.5
0.0
A
B
C
D
−200
−150
−100
−50
0
Hours before harvest
WEEK
LiveOak June_02_2011
LiveOak June_03_2011
LiveOak June_15_2011
LiveOak June_16_2011
Citra June_02_2012
Citra June_03_2012
Citra June_14_2012
Citra October_25_2012
Citra October_31_2012
Citra November_01_2012

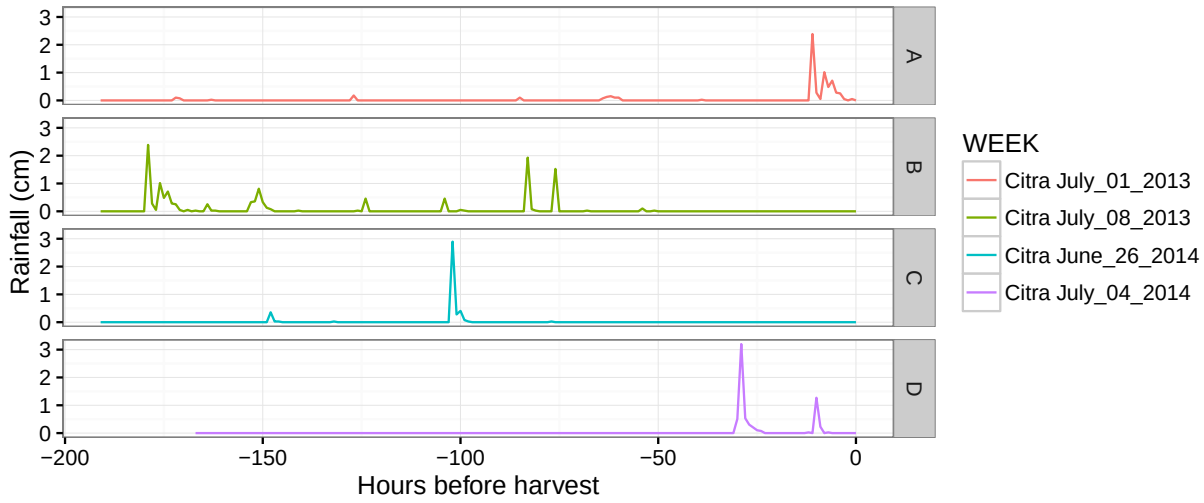

Solar radiation (W/m²)
750
500
250
0
A
B
C
D
−200
−150
−100
−50
0
Hours before harvest
WEEK
LiveOak June_02_2011
LiveOak June_03_2011
LiveOak June_15_2011
LiveOak June_16_2011
Citra June_02_2012
Citra June_03_2012
Citra June_14_2012
Citra October_25_2012
Citra October_31_2012
Citra November_01_2012

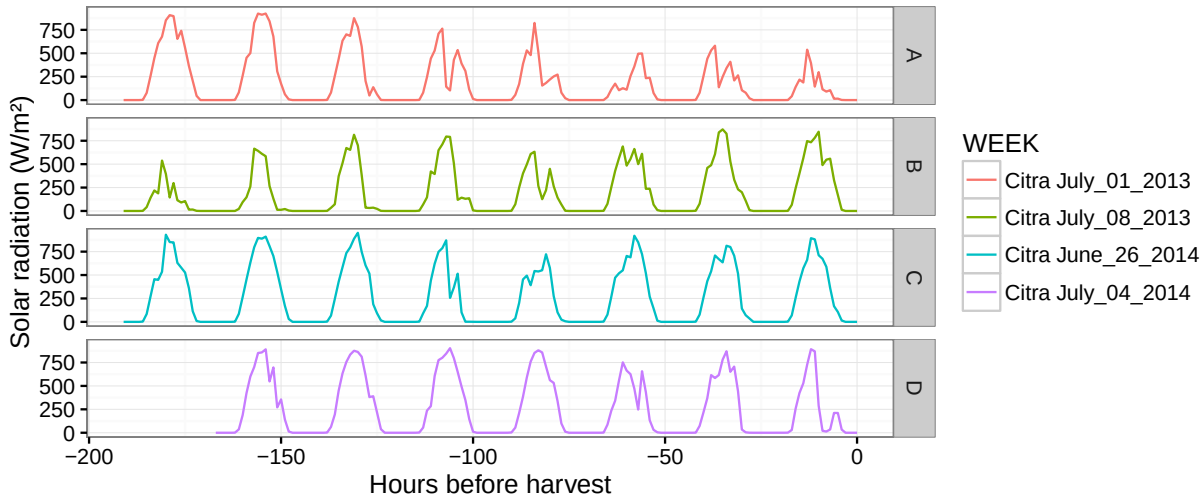
Iron/copper dataset








Rainfall (cm)
3
2
1
0
A
B
C
D
−200
−150
−100
−50
0
Hours before harvest
WEEK
Citra July_01_2013
Citra July_08_2013
Citra June_26_2014
Citra July_04_2014


Solar radiation (W/m²)
750
500
250
0
A
B
C
D
−200
−150
−100
−50
0
Hours before harvest
WEEK
Citra July_01_2013
Citra July_08_2013
Citra June_26_2014
Citra July_04_2014

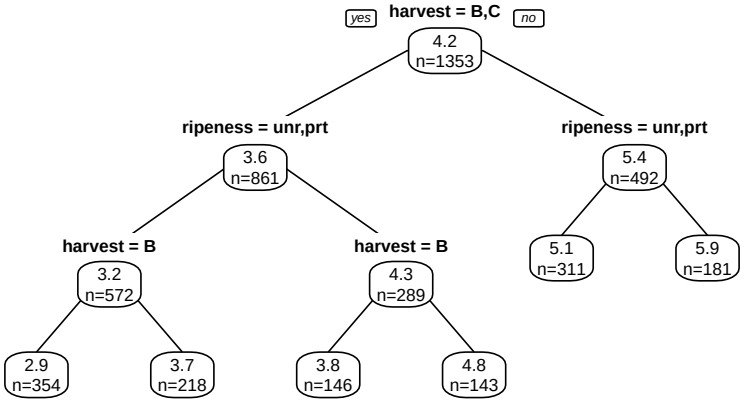
R session info

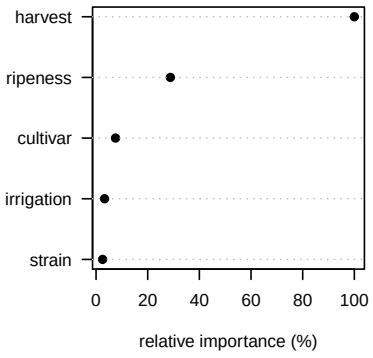
```
## R version 3.3.0 (2016-05-03)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 7 x64 (build 7601) Service Pack 1
##
## locale:
## [1] LC_COLLATE=Dutch_Belgium.1252      LC_CTYPE=Dutch_Belgium.1252
## [3] LC_MONETARY=Dutch_Belgium.1252     LC_NUMERIC=C
## [5] LC_TIME=English_United States.1252
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] ggplot2_2.1.0 bd_0.0.11
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.6      digest_0.6.9     plyr_1.8.4       grid_3.3.0
##  [5] gtable_0.2.0     formatR_1.4      magrittr_1.5     evaluate_0.9
##  [9] scales_0.4.0     stringi_1.1.1    reshape2_1.4.1   rmarkdown_1.0
## [13] labeling_0.3     tools_3.3.0      stringr_1.0.0    foreign_0.8-66
## [17] munsell_0.4.3    yaml_2.1.13      colorspace_1.2-6 htmltools_0.3.5
## [21] knitr_1.14
```

High relative humidity pre-harvest reduces post-harvest proliferation of *Salmonella* in tomatoes

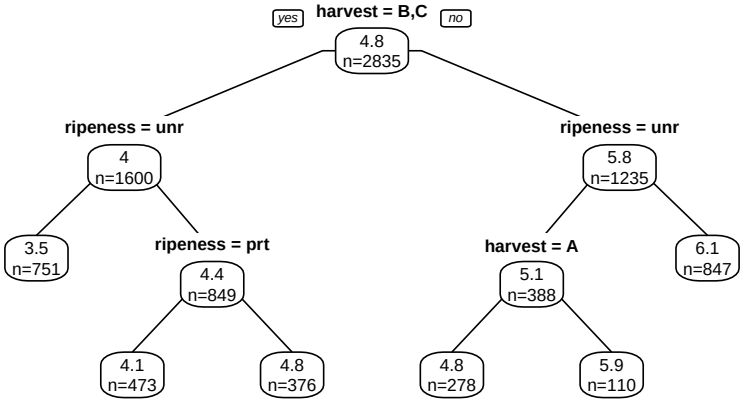
Appendix 3: Regression trees based on experimentally imposed variables and time of harvest

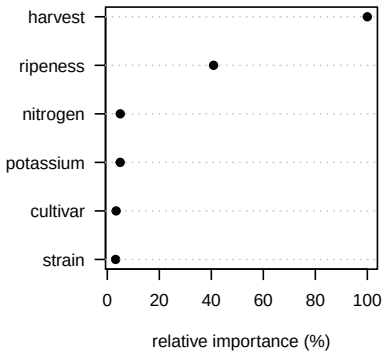
Irrigation dataset



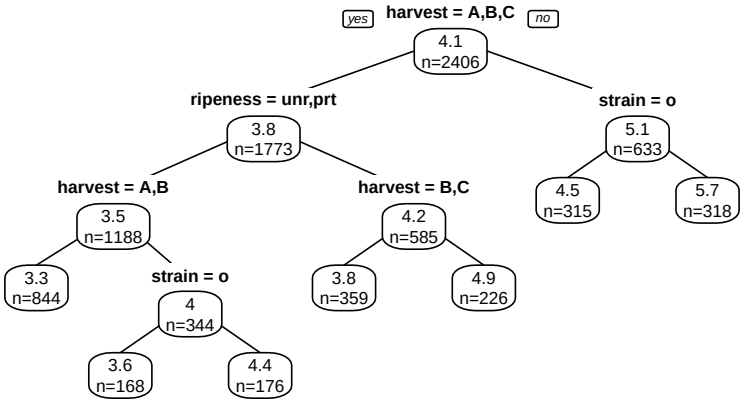


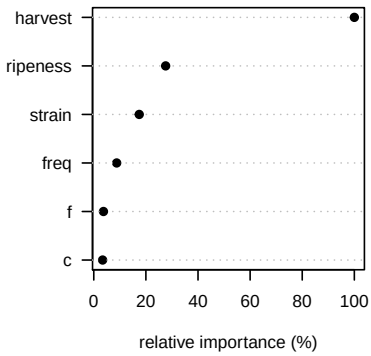
Nitrogen/potassium dataset





Iron/copper dataset





R session info

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## R version 3.3.0 (2016-05-03)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 7 x64 (build 7601) Service Pack 1
##
## locale:
## [1] LC_COLLATE=Dutch_Belgium.1252  LC_CTYPE=Dutch_Belgium.1252
## [3] LC_MONETARY=Dutch_Belgium.1252 LC_NUMERIC=C
## [5] LC_TIME=Dutch_Belgium.1252
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] ggplot2_2.1.0        XLConnect_0.2-12     XLConnectJars_0.2-12
## [4] randomForest_4.6-12  rpart.plot_1.5.3     rpart_4.1-10
## [7] bd_0.0.11
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.6      digest_0.6.9     plyr_1.8.4       grid_3.3.0
##  [5] gtable_0.2.0     formatR_1.4      magrittr_1.5     scales_0.4.0
##  [9] evaluate_0.9     stringi_1.1.1    rmarkdown_1.0    tools_3.3.0
## [13] stringr_1.0.0    foreign_0.8-66   munsell_0.4.3    yaml_2.1.13
## [17] colorspace_1.2-6 rJava_0.9-8      htmltools_0.3.5  knitr_1.14
```

Highlights

- *Salmonella* proliferation on tomatoes post-harvest is influenced by harvest time
- Humidity prior to harvest is associated with decreased *Salmonella* proliferation
- The independent action of humidity was confirmed in a controlled lab experiment
- The impact of humidity appears to be related to the presence of native microbiota