Integral procedure to predict bitter pit in ‘Golden Smoothee’ apples based on calcium content and symptom induction.

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Abstract

Bitter pit has been described as one of the most important physiological disorders of apple fruit whose symptoms appear late in the season and during storage and which can cause high economic losses. The negative relationship between bitter pit and mineral contents in fruit led to develop prediction models. However, these models are based on mineral content close to harvest and can only provide valuable information at harvest. Aim of this study was to assess for three years the accuracy of different bitter pit prediction methods along fruit development, based on either Ca content in fruit (at 90 days before harvest and at harvest) or induction of symptoms (Mg infiltration, ethephon dip, passive method) at three levels: the overall accuracy, the accuracy of bitter pit incidence prediction (true positive rate), and the accuracy of non-bitter pit prediction (true negative rate). These three accuracies were considered together to know the real prediction risk of each method. From the results obtained, we proposed and validated, for other two years more, a detailed protocol to predict bitter pit which incorporated fruitlet analysis at 90 DBH, in order to gain prediction time, and the passive method at 40 and 20 DBH, in order to reduce the proportion of negative samples that were incorrectly classified.

Keywords: bitter pit, *Malus* × *domestica*, calcium disorders, prediction accuracy, false positives, false negatives.

1. Introduction

Bitter pit has been described as one of the most important physiological disorders in different apple cultivars and growing regions (Al Shoffe et al., 2019). The symptoms consist of circular sunken lesions on fruit of 1 to 4 mm and bitter in taste. They are, generally, in the calyx end in flesh and peel, but often found scattered in the flesh of the fruit (Jemrić et al., 2016). The symptoms can appear in the orchard late in the season and/or during storage, which causes high economic losses.

Bitter pit has been associated with nutrient imbalance during apple fruit development, especially calcium (Ca) deficiency. Mineral imbalance might produce a decrement in Ca levels and/or increment of potassium (K), magnesium (Mg) and nitrogen (N) concentrations, thus affecting cell membrane permeability and progressive cell death. Certain climatic and cultural conditions can contribute to increase bitter pit risk. Highlighted factors related with its increase are early harvest (Al Shoffe, 2020), poorly drained fine-textured soils (Sió et al., 2018), light cropping (Van Der Boon, 1980), excessive tree vigor (Baugher et al., 2017; Terblanche et al., 1979), excessive nitrogen nutrition (Fallahi et al., 1997; Kim and Ko, 2004), and moisture stress (Goode and Ingram, 1971).

Applications of Ca on fruit can reduce bitter pit development; nevertheless, the results are highly variable and not always complete (Torres et al., 2017b). Because of the absence of an effective control method for bitter pit, some studies have focused on its prediction before harvest. A reliable method of prediction at an early stage would allow implementing corrective actions in the field in order to reduce bitter pit incidence, such as to increase Ca sprays or decreasing N and/or K fertilization. But even if the prediction were only possible close to harvest time, it would have a great potential to manage the fruit storage and reduce economic losses.

The relationship between mineral nutrition and bitter pit incidence has led to develop prediction models (Ferguson and Triggs, 1990). These models are based on Ca alone close to harvest (Ferguson et al., 1979; Perting and Sharples, 1975) or its relation with other minerals such as Mg (do Amarante et al., 2018; do Amarante et al., 2013), K (do Amarante et al., 2018) or N (Baugher et al., 2017). But these models can only provide valuable information just before harvest. In this regard, early season fruitlet analysis has been pointed out by some authors in order to gain time (Brooks, 2000; Drahorad and Aichner, 2000; Torres et al., 2017a). In a previous study, we suggested that when Ca content at 60 days after full bloom (about 90 days before harvest) is above a threshold (11 mg Ca 100 g^{-1} fresh weight), the bitter pit incidence is unlike to develop (Torres et al., 2017a). However, mineral content is not the only criterion for the occurrence of bitter pit, and not always explains the incidence or absence of symptoms in a particular orchard (do Amarante et al., 2020).

As an alternative to mineral based methods to predict bitter pit, some authors have suggested approaches for forcing symptoms before they naturally appear (Torres and Alegre, 2010; Torres et al., 2015). Vacuum infiltration with 0.05–0.1 M $MgCl_2$ for 2 minutes has been used by the Chilean industry to trigger symptoms and predict bitter pit incidence (Burmeister and Dilley, 1994; Retamales et al., 2000; Retamales and Valdes, 2001). Dips of ethephon at 2000 mg L^{-1} have been also proposed to advance the onset of symptoms and predict bitter pit (Eksteen et al., 1977; Lötze et al., 2010; Lötze and Theron, 2006). But these methods are difficult to implement by growers or packing houses and, currently, they are conducted only by specialized laboratories. In a previous paper, we presented a simple method named passive method to force the appearance of bitter pit before harvest in ‘Golden Smoothee’ apples (Torres et al., 2015). Recently, Al Shoffe et al. (2019) reported the success of the passive method when used in ‘Honeycrisp’ apples.

Mostly, when working in bitter pit prediction, only the overall accuracy is dealt, obviating the error analysis, which lead to bias results. Aim of this study was to assess the accuracy of different bitter pit prediction methods, based on either Ca content in fruit or induction of symptoms, at three levels: the overall accuracy, the accuracy of bitter pit incidence prediction (true positive rate), and the accuracy of non-bitter pit prediction (true negative rate). These three accuracies should be considered together to know the real prediction risk of each method. This analytical structure allowed to study bitter pit prediction in a wider way, and to increase the objectification of accuracy of analysed methods. Finally, we propose a new integral procedure where mineral and induction methods to predict bitter pit are combined to help growers and advisors to make decisions within distinct orchards.

2. Materials and methods

The study had two parts. In the first part (from year 1 to 3) we assessed and compared the accuracy rates of different bitter pit prediction methods based on Ca content and induction of symptoms in fruit. In the second part of the study (year 4 and 5), we validated an integral protocol proposed from the results obtained in the first part.

2.1. Part 1: assessment of individual prediction methods

2.1.1. Orchards and management

The first part of study was carried out during three seasons in ten commercial orchards of ‘Golden Smoothee’ grafted on M.9 rootstock, with different bitter pit susceptibility antecedents. All the orchards were mature, located in the Lleida area (NE Spain), and tree spacing was approximately 4 m × 1.2–1.4 m. Orchards were managed under standard cultural practices of pruning, fertilization, irrigation and crop management. No calcium applications were carried out during the years of study.

2.1.2. Analysis of Ca content in fruit and bitter pit incidence

To analyse the Ca concentration in fruit, a 40-fruit sample per orchard was collected at 90 days before harvest (DBH) and at harvest. Apples were taken from 40 selected trees (20 fruit per side) with standard crop loads and vigor. One average-sized, undamaged apple was taken from each tree at a height of 130–170 cm above the ground. Two longitudinal opposed slices per fruit were analysed, excluding the core and seeds. Each sample was weighed, dried, and then re-weighed to know the percentage of dry mass. This dried tissue was then ground, and a sub-sample was wet-digested in a microwave oven (Millestone MCR) with concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). The Ca concentration was determined using inductively coupled Plasma-optical emission spectroscopy (ICP-OES). Ca concentration in flesh weight (FW) was calculated from the percentage of dry mass of the initial sample and expressed as mg of Ca per 100 g FW. A high risk of bitter pit (positive classification) was considered when the Ca concentration at 90 DBH was < 11 mg 100 g^{-1} FW or < 6 mg 100 g^{-1} FW at harvest; on the contrary, a low risk of bitter pit (negative classification) was considered (Torres et al., 2017a; Terblanche et al., 1980).

2.1.3. Induction of symptoms (ethephon dips, Mg infiltration, and passive method) and storage treatment

A 40-fruit sample per orchard were collected at 40 and 20 DBH to test induction of bitter pit symptoms by ethephon dips, Mg infiltration, and the passive method. We used the same sampling method as the one used in the Ca content analysis. Samples for ethephon dips were dipped in an ethephon solution (2000 ppm) for five minutes; subsequently, fruit were put in plastic trays and left at room temperature (approximately 20–25 °C and 40–45% relative humidity) to develop bitter pit-like symptoms. Mg infiltration samples were treated under vacuum (250 mm Hg) for two minutes with a solution that contained 0.10 M $MgCl_2$, 0.4 M sorbitol as osmotic, and 0.01% Tween-20 as surfactant in accordance with the recommendations of Chilean apple exporters (Retamales and Valdes, 1996), and afterwards fruit were left at room temperature. Passive method samples were left untreated at room temperature, under the same conditions as the ethephon dips and Mg infiltration samples (Torres et al., 2015).

Seven to ten days after, fruit were individually inspected for external signs of superficial bitter pit-like symptoms. The percentage of fruit with bitter pit-like symptoms was calculated for each treatment and orchard. The classification was carried out according to the method used by Torres et al., (2015), where samples with incidences $\geq$ 10% were considered of high bitter pit risk (i.e. positive classification). Samples with incidences $<$ 10% were treated as negative classification.

2.1.4. Assessment of bitter pit at postharvest and actual orchard classification

To evaluate the incidence of actual bitter pit at postharvest, a 100-fruit sample from each orchard was collected at commercial harvest when the starch index was between 7 and 8 (starch chart EC-Eurofru). The percentage of bitter pit associated with each sample was recorded after four months of storage at 0 °C and at 80% relative humidity, plus further seven days period at room temperature (approximately 20–25 °C and 40–45% relative humidity). The orchards were classified as positive (high level of bitter pit) when bitter pit incidence was $\geq$ 10%, and as negative (low level of bitter pit) when bitter pit incidence at post-harvest was < 10% (Torres at al., 2015; Torres et al., 2017b). The obtained values were then classified according to Table 1.

2.4. Part 2: validation of the integral method to predict bitter pit

From the obtained results above, we developed an integral procedure where the mineral-method based on Ca content at 90 DBH and the induction method based on the passive method at 40 and 20 DBH were combined to predict bitter pit. This procedure was validated in two different seasons (seasons 4 and 5) on twenty-two different 'Golden Smoothee' commercial orchards (12 orchards in the season 4 and 10 orchards in the season 5) in the Lleida area (NE Spain). Sampling and assessments were carried out as described above.

2.5. Data Analysis

Overall accuracy (ACC) and error rate (ERR) were calculated for each predictive method. ACC was calculated as the ratio between the correctly classified samples to the total number of samples. ERR represents the number of misclassified samples from both positive and negative classes. They were calculated as follows:

$$ACC = \frac{TP + TN}{TP + TN + FP + FN}$$

$$ERR = 1 - ACC$$

True positive rate (TPR) represents the proportion of the positive samples that were correctly classified, and it was estimated according to the following formula:

$$TPR = \frac{TP}{TP + FN}$$

True negative rate (TNR) represents the proportion of the negative samples that were correctly classified, and it was estimated according to the following formula:

$$TNR = \frac{TN}{TN + FP}$$

TPR and TNR are considered as two kinds of accuracy, the first for actual positive samples and the second for actual negative samples. The false positive rate (FPR) and false negative rate (FNR) were also calculated for each prediction method. FPR represents the proportion of the negative samples that were incorrectly classified. The FNR is the proportion of positive samples that were incorrectly classified. Both FPR and FNR were calculated according to the following formulas:

$FPR = 1 - TNR$

$FNR = 1 - TPR$

ACC depends on the TPR and TNR as well as the fraction of observations in each category, and thus can be a misleading indicator of method success from dataset with different number of positive and negative examples, as occurred in the validations. In this case, a better overall measure of accuracy is given by Youden’s index (YI) (Madden, 2006). The formula of YI combines the TPR and TNR into one measure which summarizes the performance of the test. The YI metric is ranged from 0 when the test is poor to 1 for a perfect predictor. YI was calculated as:

$YI = TPR + TNR - 1$

ACC and classification rates (TPR, TNR, FPR, FNR) of each prediction method assessed for the first part of the study were modelled using linear mixed effect models to determine whether the methods generated different accuracies of classification and to determine if accuracies were different for negative, positive and overall classifications. When the main effect (predictive method) was significant, Tukey's HSD test at P values ≤ 0.05 was applied simultaneously to the set of all pairwise comparisons. Single degree of freedom and polynomial contrast were also performed to compare specifics groups among the tested methods for bitter pit prediction. Residual analysis (normal distribution of residuals) was performed to ensure that model assumptions were met. Data were analysed using the JMP statistical software package (Version 12; SAS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Bitter pit incidence range

Throughout the first period of the study there was a wide range of bitter pit incidence across orchards, with about half of the orchards with high incidence and the other half with low incidence (Figure 1). There were 31% of the orchards with less than 5% of bitter pit, 19% of the orchards with 5–10 % bitter pit incidence, 19% of the orchards with 10–15% incidence, 3% of the orchards with 15–20% of bitter pit, and then 28% of the orchards with more than 25% of bitter pit incidence. Finally, 50% of the observations were classified as negative ones (low incidence of bitter pit) and the other 50% as positive ones (high incidence of bitter pit). This wide range of bitter pit incidence conferred validity and robustness to the study, by using a balanced data set (50/50 distribution).

3.2. Overall, positive and negative accuracies

There were no significant differences between prediction methods when comparing the ACC, with 67% on average (Figure 2). However, significant differences between methods were observed when comparing the accuracies for each classification rate (TNR, FPR, TPR and FNR).

The Ca content at harvest was the prediction method with the highest FPR (81% of negative samples incorrectly classified) and the lowest TNR (19% of negative samples correctly classified), with significant differences when comparing it to the induction methods (Figure 2). The FPR from Ca content analysis at 90 DBH tended to be lower (and TNR higher) than Ca content at harvest, but without significant differences. Conversely, the passive method at both 20 and 40 DBH, the Mg infiltration at 40 DBH, and the ethephon dips at 20 DBH, had the lowest FPR (8–16%), and the highest TNR (84–92%), without significant differences among them, and with significant differences when compared to the Ca content at harvest.

The Mg infiltration and passive methods, both at 40 DBH, had the highest FNR (62–65%) and the lowest TPR (35–38%), followed by the ethephon dips (50%) at 40 and 20 DBH, Mg infiltration at 20 DBH (39% and 61%), passive method at 20 DBH (33% and 67%), the Ca content at 90 DBH (14% and 86%), and the Ca content at harvest (0% and 100%) (Figure 2).

The results of the different contrasts performed to compare specific groups of methods are discussed below.

3.2.1. Mineral vs induction methods

There were no significant differences in terms of ACC between mineral and induction prediction methods (Table 2). Induction methods such as the passive, Mg infiltration, and ethephon dips were also reported by Al Shoffe et al. (2019) to have as good or even higher accuracies than mineral analysis. When comparing within negative samples, the induction methods had higher TNR than the mineral methods (84% *vs* 33%). On the other hand, the mineral methods obtained higher TPR than induction methods (93% *vs* 51%). Consequently, the risk of false-positive results was higher using mineral methods than induction methods, whereas the risk of false-negative results was higher when using induction methods. Baugher et al. (2017) observed that their proposed two-variable regression model to predict bitter pit throughout shoot length and N/Ca rate in peel, predicted quite well the percentage of fruit developing bitter pit on trees with less than 50% bitter pit, but the model underpredicts bitter pit for trees with higher levels of observed bitter pit.

3.2.2. Mineral methods: 90 DBH vs harvest

There were no significant differences between the prediction accuracies at 90 DBH or at harvest when using the mineral method to predict bitter pit (Table 2). However, both timings produced higher FPR than FNR (53–81% *vs* 0–14%). Therefore, when Ca content was above the reference threshold suggested low bitter pit incidence, but when Ca content was below the threshold did not imply high incidence of bitter pit. Other mineral models to predict bitter pit potential have been suggested, either involving Ca content in fruit as one single variable model (Ferguson et al., 1979; Lanauskas and Kvikliene, 2006) or more complex models using the ratios of fruit Mg and/or K and/or N to Ca (Baugher et al., 2017; Dris et al., 1998), but all to be used at advanced fruit stages .

Our results showed that early in the season, the threshold value at 90 DBH was an indicator of bitter pit risk as good as the threshold value at harvest. The use of fruitlet analysis early in the season at 90 DBH could allow to gain time and then implement corrective actions if needed. However, it is important to know the type of error that this method entails.

3.2.4. Induction methods: passive method, ethephon dips and Mg infiltration

No significant differences were observed for the ACC, TNR and TPR among the induction methods. Bitter pit-like symptoms were visible within 5−7 days of the initial application in all methods (Figure 3). There was no increase in either size or incidence of bitter pit symptoms beyond 10 days after treatment. Fruits infiltrated with Mg showed more pronounced symptoms than when the other methods (ethephon or passive) were used (Figure 3). Mg infiltration method could produce Mg-induced pits as a result from breakdown caused by Mg toxicity (Burmeister and Dilley, 1991). Al Shoffe et al. (2019) also reported difficulty to distinguish bitter pit from toxicity symptoms on the skin when using Mg infiltrations in 'Honeycrisp' apples.

Among the induction methods, passive method offered the best alternative for bitter pit prediction since it was easy to implement and offered a low-cost solution. Unlike the other alternatives, this method did not require the use of either reactive products or specialized equipment. The passive method has also been reported to show consistent results in 'Honeycrisp' apples (Al Shoffe et al., 2019). However, we observed a high FNR for the passive method, as well as for the other induction methods (47–51%) (Table 2). This implies a high proportion of positive orchards at postvarvest that were incorrectly classified as negative at preharvest. In previous studies, we already reported bitter pit under-estimation when using the passive method (Torres et al., 2015). On the other hand, the passive method had the lowest rate of misdetection of negative orchards (FPR = 8%). Thanks to its easiness and robustness, passive method can also be helpful for research and research-related activities about bitter pit development and its control in 'Golden' apples.

3.2.3. Induction methods: 40 vs 20 DBH

All inductionmethods predicted bitter pit risk with high accuracies already at 40 DBH (Table 2). However, the ACC and the TPR were higher when the methods were used at 20 DBH rather than at 40 DBH (ACC: 72% *vs* 64%; TPR: 60% *vs* 42%). This suggests that prediction improves when using induction methods closer to harvest by decreasing the cases of false negatives. In a previous study where these methods were tested, we also observed higher bitter pit-like symptoms, and better linear correlations between bitter pit at postharvest and at 20 DBH rather than at 40 DBH (Torres et al., 2015). Other studies also reported good relationship between bitter pit at postharvest and bitter pit induced up to 20 DBH (Eksteen et al., 1977; Lötze et al., 2010). However, we considered that sampling at 40 DBH can be very useful in cases where corrective measures need to be used. No significant differences were observed for the TNR between timing when the induction methods were used, and high levels of bitter pit-like symptoms at preharvest were usually associated with high levels of bitter pit at postharvest in any sampling time or season. Early sampling at 40 DBH would allow growers to know the potential risk of bitter pit at least one month before harvest. Therefore, if a high risk of bitter pit is detected at that moment, there would be enough time to adopt measures for bitter pit control.

3.3. Integral prediction program

Based in the results of the first part of the study, we proposed a bitter pit prediction protocol which encompassed fruitlet analysis at 90 DBH (i.e. about 60 days after full bloom), in order to gain prediction time, and then the passive method at 40 and 20 DBH, in order to reduce the proportion of negative samples that were incorrectly classified (Figure 4). This is very useful in terms of avoiding unnecessary corrective actions to control bitter pit and would be valued as a method that might improve orchard sustainability.

As mentioned above, the use of fruitlet analysis at 90 DBH would be a better suitable option to gain time and then implement corrective actions to reduce bitter pit incidence if needed. If Ca content in fruit at 90 DBH is $\geq$ 11 mg 100 g^{-1} FW, we can confirm that there will be low risk of bitter pit incidence. On the other hand, if Ca content is < 11 mg 100 g^{-1} FW we recommend adopting corrective actions such as a $CaCl_2$ spray program applied every 10–14 days (Torres et al., 2017b). However, since mineral analysis had high FPR associated with it, the use of an additional method with low FPR would be advised. In this regard, the induction methods provided lower FPR than the mineral fruitlet analysis at 90 DBH and, among the induction methods, the passive method would be the most suitable option that can be easily used by growers. Therefore, we used the passive method from 40 DBH to confirm the risk of bitter pit in the orchards that were already classified as high-risk by the mineral analysis at 90 DBH. Then, if the orchard was classified again as high bitter pit risk, the corrective actions should be intensified (e.g. increasing the number of $CaCl_2$ sprays and carrying a $CaCl_2$ dip treatment after harvest). On the other hand, if the orchard was classified as low bitter pit risk at 40 DBH, the corrective actions could be reduced (e.g. reducing the number of $CaCl_2$ sprays). At 20 DBH we used again the passive method for the samples that were classified as negatives at 40 DBH. In this case, if the test provided again a low risk of bitter pit, no more corrective actions would be recommended. On the contrary, they should be intensified (increasing the number of $CaCl_2$ sprays and a $CaCl_2$ dip treatment after harvest would be suggested).

We validated this protocol for other two years (years 4 and 5) in 12 orchards at the year 4, and 10 different orchards at the year 5 (Table 3). When both years were analysed together, there was an ACC of 86% with a FPR and FNR of 13% and 17%, respectively (i.e. a TPR of 83 % and a TNR of 88%). But in this case, the fraction of negative orchards was twice as the portion of positives. Therefore, a better overall measure of the accuracy was given by YI which was of 0.71 for the two years. In general, the YIs of the integral program were improved in comparison to the YIs of each individual method (from 0.41–0.71 to 0.63–0.88). We must point out that the accuracy of the methods and this protocol might vary between cultivars, regions and/or different crop and storage technology. However, this protocol could be the first step to take when adopting prediction methods and bitter pit management in other situations. By using this protocol, we optimize the corrective actions to reduce bitter pit and, consequently, we will be becoming more sustainable. The idea of being more sustainable in agrarian activity is grown in the last years to improve the ecological impact of agriculture.

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References

Al Shoffe, Y., J.F. Nock, Baugher, T.A., Marini, J.R., C.B. Watkins. 2020). Bitter pit and soft scald development during storage of unconditioned and conditioned 'Honeycrisp' apples in relation to mineral contents and harvest indices. Postharvest Biol. Technol. 160, 111044.

Al Shoffe, Y., J.F. Nock, Y. Zhang, L.-w. Zhu, and C.B. Watkins. 2019. Comparisons of mineral and non-mineral prediction methods for bitter pit in 'Honeycrisp' apples. Sci. Hortic. 254, 116-123.

Baugher, T.A., R. Marini, J.R. Schupp, and C.B. Watkins. 2017. Prediction of bitter pit in 'Honeycrisp'apples and best management implications. HortScience 52(10), 1368-1374.

Brooks, J. 2000. Early season fruitlet analysis as a predictive tool. Acta Hortic. 564, 225-228.

Burmeister, D.M. and D.R. Dilley. 1991. Induction of bitter pit-like symptoms on apples by infiltration with Mg+ 2 is attenuated by Ca+ 2. Postharvest Biol. Technol. 1(1), 11-17.

Burmeister, D.M. and D.R. Dilley. 1994. Correlation of bitter pit on northern soy apples with bitter pit-like symptoms induced by Mg2+ salt infiltration. Postharvest Biol. Technol. 4(4), 301-308.

do Amarante, C., A. Miqueloto, C. Steffens, T. Maciel, V. Denardi, L. Argenta, and S. de Freitas. 2018. Optimization of fruit tissue sampling method to quantify calcium, magnesium and potassium contents to predict bitter pit in apples. Acta Hortic. 1194, 487-492.

do Amarante, C.V., J.P.G. Silveira, C.A. Steffens, S.T. de Freitas, E.J. Mitcham, and A. Miqueloto. 2020. Post-bloom and preharvest treatment of 'Braeburn'apple trees with prohexadione-calcium and GA4+ 7 affects vegetative growth and postharvest incidence of calcium-related physiological disorders and decay in the fruit. Sci. Hortic. 261, 108919.

do Amarante, C.V.T., A. Miqueloto, S.T. de Freitas, C.A. Steffens, J.P.G. Silveira, and T.R. Corrêa. 2013. Fruit sampling methods to quantify calcium and magnesium contents to predict bitter pit development in 'Fuji' apple: A multivariate approach. Sci. Hortic. 157, 19-23.

Drahorad, W. and M. Aichner. 2000. Development and evaluation of a bitter pit prognosis model in apple orchards in the south Tyrol (northern Italy). Acta Hortic. 564, 91-96.

Dris, R., R. Niskanen, and E. Fallahi. 1998. Nitrogen and calcium nutrition and fruit quality of commercial apple cultivars grown in Finland. J. Plant Nutr. 21(11), 2389-2402.

Eksteen, G., L. Ginsburg, and T. Visagie. 1977. Post-harvest prediction of bitter pit. Deciduous Fruit Grower 27(1), 16-20.

Fallahi, E., W.S. Conway, K. Hickey, and C.E. Sams. 1997. The role of calcium and nitrogen in postharvest quality and disease resistance of apples. HortScience 32(5), 831-835.

Ferguson, I., M. Reid, and M. Prasad. 1979. Calcium analysis and the prediction of bitter pit in apple fruit. N. Z. J. Agric. Res. 22(3), 485-490.

Ferguson, I, Triggs, C.M. 1990. Sampling factors affecting the use of mineral analysis of apple fruit for the prediction of bitter pit. N. Z. J. Crop Hortic. Sci., 18 (1990), 147-152

Ferguson, I., R. Volz, and A. Woolf. 1999. Preharvest factors affecting physiological disorders of fruit. Postharvest Biol. Technol. 15(3), 255-262.

Goode, J. and J. Ingram. 1971. The effect of irrigation on the growth, cropping and nutrition of Cox’s Orange Pippin apple trees. J. Hortic. Sci. 46(2), 195-208.

Jemrić, T., I. Fruk, M. Fruk, S. Radman, L. Sinkovič, and G. Fruk. 2016. Bitter pit in apples: pre-and postharvest factors: a review. Span. J. Agric. Res. 14(4), 15.

Kim, M.-S. and K.-C. Ko. 2004. Effects of forms and levels of nitrogen and levels of calcium on bitter pit incidence in 'Fuji' apples (Malus domestica Borkh.). Hortic. Sci. Technol. 22(2), 200-205.

Lanauskas, J. and N. Kvikliene. 2006. Effect of calcium foliar application on some fruit quality characteristics of 'Sinap Orlovskij' apple. Agron. Res. 4(1), 31-36.

Lötze, E., K. Theron, and J. Joubert. 2010. Assessment of pre-harvest physiological infiltration methods for predicting commercial bitter pit in 'Braeburn' and 'Golden Delicious'. Acta Hortic. 868, 347-352.

Lötze, E. and K.I. Theron. 2006. Existing pre-harvest predictions and models for bitter pit incidence. SA Fruit J. 5, 20-25.

Madden, L.V. 2006. Botanical epidemiology: some key advances and its continuing role in disease management. Eur. J. Plant Pathol. 115(1), 3-23.

Perring, M.A. and Sharples, R.O. (1975), The mineral composition of apples. Composition in relation to disorders of fruit imported from the southern hemisphere. J. Sci. Food Agric., 26: 681-689.

Retamales, J. and C. Valdes. 2001. Chileans find better way to predict bitter pit. Good Fruit Grower 52(2), 35-38.

Retamales, J.B., L. León, and K. Tomala. 2000. Methodological factors affecting the prediction of bitter pit through fruit infiltration with magnesium salts in the apple cv. 'Braeburn'. Acta Hortic. 564, 97-104.

Retamales, J.B. and C. Valdes. 1996. Avances en la prediction de bitter pit en manzanas. Rev. Fruticola 17(3), 93-97.

Sió Torres, J., À.D. Bosch Serra, J. Rosera, J. Chiné, and J. Boixadera Llobet. 2018. Características de los suelos y su fertilidad en relación con la incidencia de bitter pit en plantaciones de manzanas Golden. ITEA-Información Técnica Ecónomica Agraria 114(3), 206-222.

Terblanche, J., K. Gürgen, and I. Hesebeck. 1979. An integrated approach to orchard nutrition and bitter pit control. Acta Hortic. 92, 71-82.

Terblanche, J.H., K.H. Gürgen, and I. Hesebeck. 1980. An integrated approach to orchard nutrition and bitter pit control. Acta Hortic. 92, 71-82.

Torres, E. and S. Alegre. 2010. Predicting bitter pit in'Golden Smoothee'apples. Acta Hortic. 934, 861-864.

Torres, E., I. Recasens, G. Àvila, J. Lordan, and S. Alegre. 2017a. Early stage fruit analysis to detect a high risk of bitter pit in ‘Golden Smoothee’. Sci. Hortic. 219, 98-106.

Torres, E., I. Recasens, J. Lordan, and S. Alegre. 2017b. Combination of strategies to supply calcium and reduce bitter pit in ‘Golden Delicious’ apples. Sci. Hortic. 217, 179-188.

Torres, E., I. Recasens, J.M. Peris, and S. Alegre. 2015. Induction of symptoms pre-harvest using the ‘passive method’: An easy way to predict bitter pit. Postharvest Biol. Technol. 101, 66-72.

Van der Boon, J. 1980. Prediction and control of bitter pit in apples. I. Prediction based on mineral leaf composition, cropping levels and summer temperatures. J. Hortic. Sci. 55(3), 307-312.

TABLES

Table 1. Classification criteria for bitter pit. Positive value (+) was given when bitter pit incidence was ≥10%, and negative value (-), when bitter pit incidence <10%.

Pre-harvest	Post-harvest	Classification
+	+	True positive (TP)
–	+	False negative (FN)
–	–	True negative (TN)
+	–	False positive (FP)

Table 2. Polynomial contrast to compare specifics groups of methods for bitter pit prediction: overall accuracy (ACC), true negative rate (TNR), true positive rate (TPR), false positive rate (FPR), and false negative rate (FNR) for specifics groups of methods to predict bitter pit. Mineral methods include analysis of Ca content in fruit at harvest and 60 days after full bloom (DAFB). Induction methods include ethephon dips, Mg infiltration, and the passive method at 20 and 40 days before harvest (DBH). Data values represent the mean average of three years. ^{ns} Nonsignificant (*P* > 0.05).

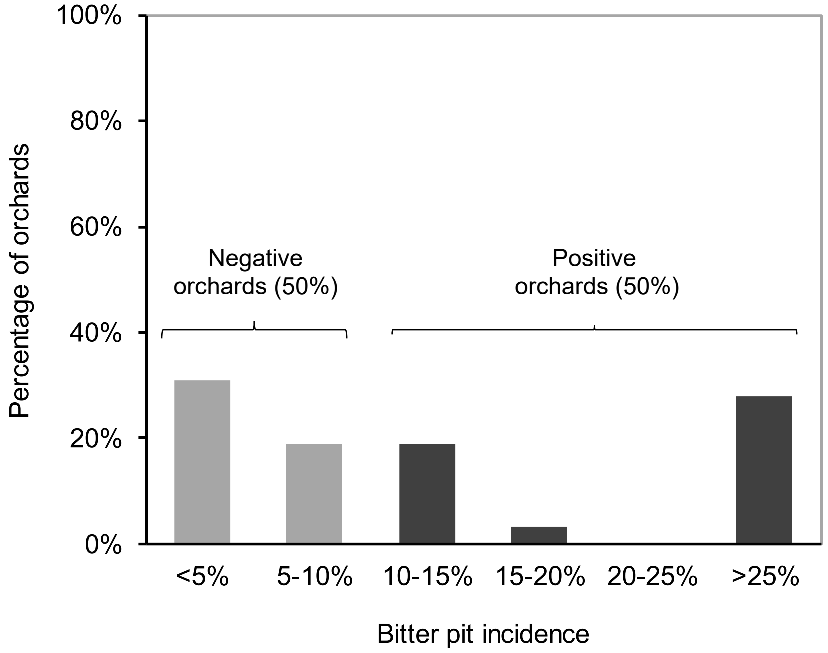
Prediction type	Method	Timing	ACC	TNR	TPR	FPR	FNR
Mineral			0.65	0.33	0.93	0.67	0.07
Induction			0.68	0.84	0.51	0.16	0.49
P			*ns*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Mineral	Ca	90 DBH	0.67	0.47	0.86	0.53	0.14
		Harvest	0.62	0.19	1.00	0.81	0.00
		P	*ns*	*ns*	*ns*	*ns*	*ns*
Induction	Ethephon		0.66	0.78	0.49	0.22	0.51
	Mg		0.67	0.82	0.50	0.18	0.50
	Passive		0.72	0.92	0.53	0.08	0.47
	P		*ns*	*ns*	*ns*	*ns*	*ns*
		40 DBH	0.64	0.85	0.42	0.15	0.58
		20 DBH	0.72	0.83	0.60	0.17	0.40
		P	*0.018*	*ns*	*0.048*	*ns*	*0.048*
Method × timing	*P*		*ns*	*ns*	*ns*	*ns*	*ns*

Table 3. Accuracy rates of the integral protocol to predict bitter pit: overall accuracy (ACC), true negative rate (TNR), true positive rate (TPR), false positive rate (FPR), false negative rate (FNR), and Youden's index (YI) for an integral procedure (IP) to predict bitter pit (Fig. 4) using the analysis of Ca content in fruit at 60 days after full bloom (Ca 90 DBH) and the passive method (PS) at 20 and 40 days before harvest (DBH), as well as the number of orchards classified as positives and negatives in each step and the actual occurrence of bitter pit (AO)

Year	Method	Total orchards	Possitive orchards	Negative orchards	ACC	TNR	TPR	FPR	FNR	YI
4	Ca 90 DBH	12	10	2	0.50	1.00	0.40	0.00	0.60	0.40
	PS 40 DBH	10	2	8	0.80	0.75	1.00	0.25	0.00	0.75
	PS 20 DBH	8	2	6	0.75	0.83	0.50	0.17	0.50	0.33
	IP	12	4	8	0.83	0.88	0.75	0.13	0.25	0.63
	AO[1]		4 (3)	8 (7)						
5	Ca 90 DBH	10	7	3	0.60	1.00	0.43	0.00	0.57	0.43
	PS 40 DBH	7	1	6	0.71	0.67	1.00	0.33	0.00	0.67
	PS 20 DBH	6	1	5	0.83	0.80	1.00	0.20	0.00	0.80
	IP	10	2	8	0.90	0.88	1.00	0.13	0.00	0.88
	AO[1]		3 (2)	7 (7)						
4 and 5	Ca 90 DBH	22	17	5	0.55	1.00	0.41	0.00	0.59	0.41
	PS 40 DBH	17	3	14	0.76	0.71	1.00	0.29	0.00	0.71
	PS 20 DBH	14	3	11	0.79	0.82	0.67	0.18	0.33	0.49
	IP	22	6	16	0.86	0.88	0.83	0.13	0.17	0.71
	AO[1]		7 (5)	15 (14)						

[1] Actual number of orchards (hits)

FIGURES



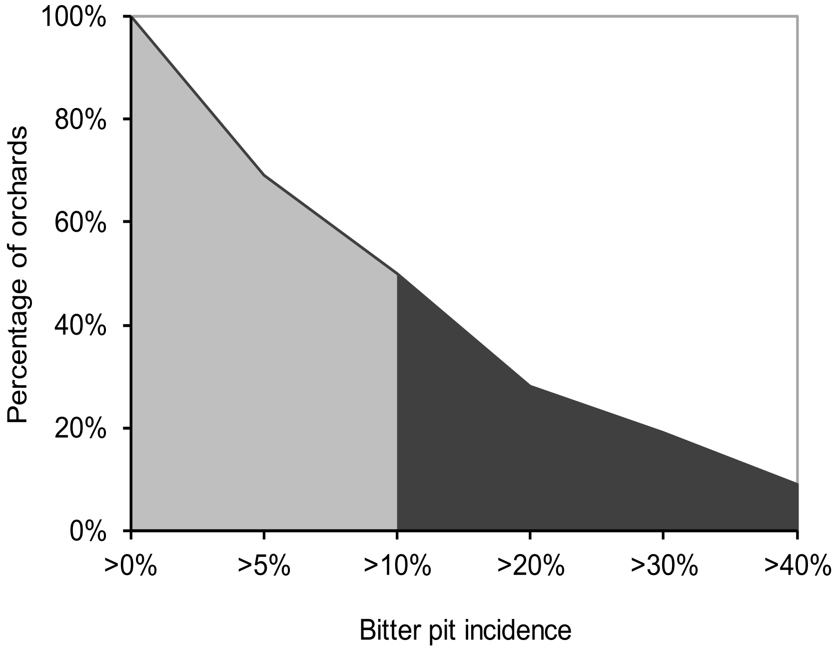


Figure 1. Bitter pit incidence distribution: percentage of orchards with different bitter pit incidence ranges over the three years of the study. Positive value was considered when bitter pit incidence was ≥10%, and negative, when bitter pit incidence <10%. Data bars represent the mean average of three years.

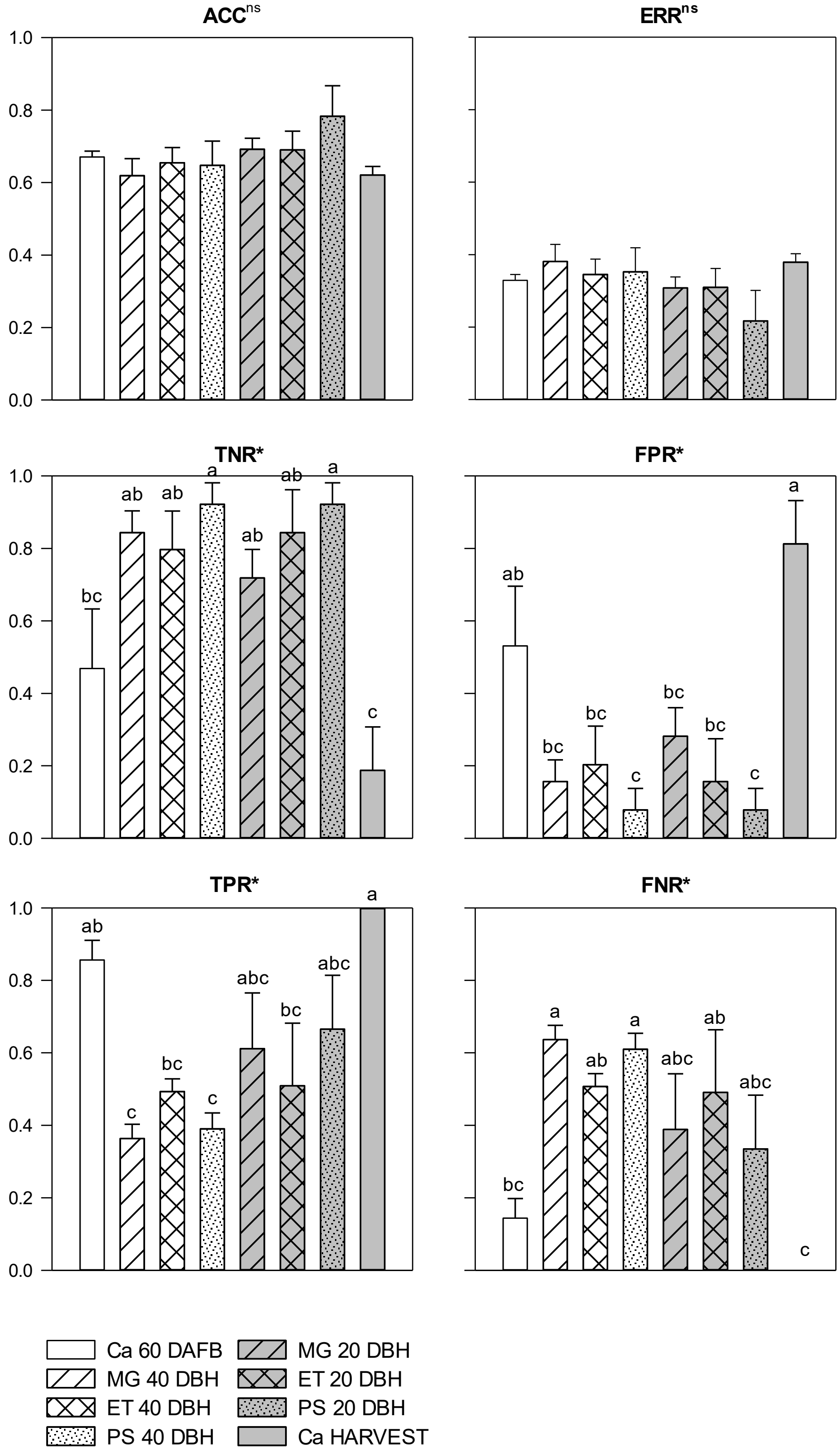


Figure 2. Accuracy rates of individual methods to predict bitter pit: overall accuracy (ACC), error rate (ERR), true negative rate (TNR), false negative rate (FNR) true positive rate (TPR) and false positive rate (FPR), for each prediction method to predict bitter pit. Methods include analysis of Ca content in fruit 60 days after full bloom (DAFB) and at harvest, ethephon dips (ET), Mg infiltration (MG), and the passive method (PM) at 20 and 40 days before harvest (DBH). Data bars represent the mean average of three years. Error bars indicate standard error. * Different letters denote significant differences between treatments (Tukey's honestly significant difference, $P \leq 0.05$). ^{ns} Nonsignificant at $P \leq 0.05$.

Figure 3. Induction of symptoms: view of bitter pit-like symptoms of the passive method 5 (left) and 10 (right) days after sampling (above). View of bitter pit-like symptoms from samples of the same orchard treated with the different induction methods tested to predict bitter pit 15 days after sampling: passive method (PS), ethephon dip (ET) and Mg infiltration (MG) (below).

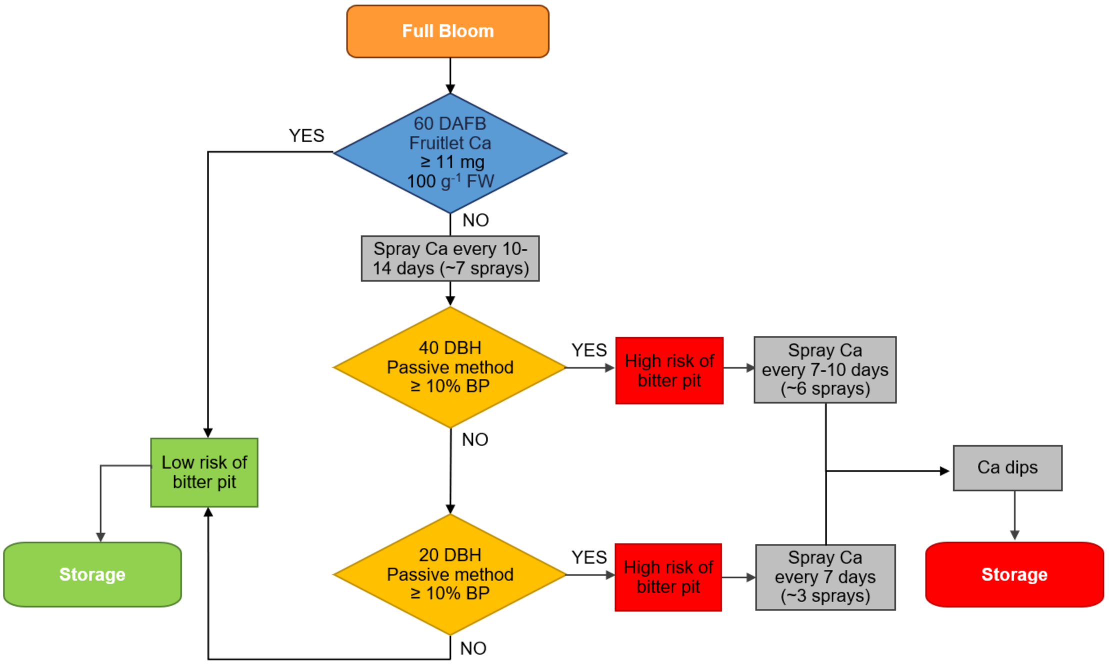


Figure 4. Flow diagram for bitter pit (BP) prediction on 'Golden' apple orchards. FW ≡ Fresh weight. DAFB ≡ Days after full bloom. DBH ≡ days before harvest. The suggested corrective actions ($CaCl_2$ sprays and Ca dips) are recommended according to the study of Torres et al. (2017b).