Induction of symptoms at pre-harvest using the 'Passive Method': An easy way to predict bitter pit

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Running title: Bitter Pit Prediction Based on Symptom Induction Methods

ABSTRACT

An effective method for predicting bitter pit incidence implemented before harvest could be a useful instrument for both the fruit industry and growers. Between 2009 and 2011, various methods for the prediction of bitter pit were evaluated at 60, 40 and 20 days before harvest (dbh) and at a commercial harvest in 'Golden Smoothee' apples. Four methodologies, two new ones (the development of natural bitter pit before harvest through the 'passive method' and bagging detached fruit before commercial ripening), and two of known efficacy under other conditions (infiltration with magnesium salts and maturity enhancement with ethephon dips) were assessed. To estimate the predictive accuracy of each method, bitter pit-like symptoms were related to the presence of bitter pit at postharvest. The 'passive method' assesses the bitter pit-like symptoms that appear naturally in fruit once they are picked from the tree, and left at room temperature during its evaluation. The 'passive method', as well as the infiltration with magnesium salts and ethephon dips, recognized bitter pit-like symptoms approximately five to seven days after sampling (at 40 dbh) and showed significant correlation with the incidence of bitter pit after three months of cold storage. The 'passive' and 'ethephon' methods were also validated over two additional seasons in 30 and 16 different orchards, respectively. The results of these validations supported the efficacy of using the 'passive method' as the main method for predicting bitter pit, without having to use either reactive products or specialized equipment.

KEYWORDS: *Malus domestica*, Prediction of Disorders, Calcium Disorders, Passive Method, Ethephon Dips, Mg Infiltration, Bagging Fruit.

1. INTRODUCTION

Bitter pit is the main physiological disorder affecting apples (*Malus domestica* Borkh). Currently, the most effective way to reduce bitter pit is by spraying the fruit with calcium (Ca) during the growing season (Ferguson et al., 1999; Lotze and Theron, 2007; Peryea et al., 2007; Casero et al., 2010). Although this treatment can significantly reduce the incidence of bitter pit, it cannot eradicate the disorder. The responses of fruit crops to foliar Ca sprays may vary; therefore, fruit growers often obtain inconsistent results after applying treatments (Serrano et al., 2004; Manganaris et al., 2006; Val et al., 2008; Fernandez et al., 2009). The negative impact of bitter pit could be reduced if its incidence could be predicted or previously assessed. An effective prediction of bitter pit would allow for measures to be taken to reduce its incidence by either increasing the application of Ca sprays or by reducing the application of fertilization involving nitrogen and potassium (Retamales and Lepe, 2000). If predictions could be made close to harvest time, it would be helpful to packing houses that have to make management decisions: whether to use Ca salt dips, design appropriate storage strategies, etc. (Manganaris et al., 2005; Torres and Alegre, 2012).

The main predictive method that has been used for many years to assess the risk of bitter pit, is based on analysis of Ca content in fruit, and also on that of nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg) contents, and their relationship with Ca content in fruit (Autio et al., 1986; Wolk et al., 1998). However, the concentration of Ca is usually very low and variable, both within the same fruit and between fruit on the same tree; as a result, large sample sizes are necessary to assess the risk of bitter pit (Ferguson and Triggs, 1990). Even so, this does not always explain the incidence, or absence, of symptoms in a particular orchard. There are, however, other methods which are based on inducing symptoms before they naturally occur.

An inversely proportional relationship between Ca and Mg established the basis for developing a prediction method based on infiltrating fruit with $MgCl_2$ (Burmeister and Dilley, 1993; Retamales et al., 2000). Burmeister and Dilley (1993) speculated that extracellular Mg supplied by infiltration could disrupt cellular homeostasis via the key enzymes that regulate intracellular Ca. This would result in the appearance of visible spots reminiscent of bitter pit after 10-15 days (bitter pit-like symptoms). They suggested that the pitting symptoms induced by Mg infiltration are physiologically synonymous with the bitter pit disorder. Since 1997, this methodology has been applied commercially in some packing houses in Chile for the 'Gala', 'Braeburn', and 'Fuji' varieties (Retamales and Valdes, 2001). Nevertheless, very few studies have been conducted on 'Golden' apples, which is the most important cultivar in Europe (Casals and Iglesias, 2013). It is therefore possible that the effectiveness and/or accuracy of the methodology could differ between regions and/or cultivars (Retamales et al., 2000).

Another method for predicting bitter pit involves the acceleration of maturity through the application of ethylene. Ethylene plays an essential role in regulating the ripening process of climacteric fruit (Recasens et al., 2004) and is also known to enhance the expression of bitter pit-like symptoms in apples (Eksteen et al., 1977). Two ethylene-related methods were studied by Eksteen et al. in South Africa during the 1970s (Eksteen et al., 1977; Lotze and Theron, 2006; Lotze et al., 2010): the Ginsburg method, in which apples were treated with 1% of acetylene gas; and the Bangerth method, in which they were immersed in a water solution containing 0.2% ethephon. The results obtained showed that the Ginsburg method tended to be less accurate in the majority of cases. Lötze et al. (2010) compared the effectiveness and accuracy of the Mg infiltration and the Bangerth method to predict the incidence of bitter pit in 'Golden Delicious' and 'Braeburn' apples in South Africa, with the Bangerth method proving more effective.

Another possible method involves bagging fruit before the climacteric peak. It is possible to advance the climacteric onset (onset of ethylene production) by exposing pre-climacteric fruit to the ethylene that is self-produced when they are placed inside closed bags (Mattheis, 1996; Recasens et al., 2004). However, this effect has not yet been assessed. In contrast, reductions in bitter pit at post-harvest have been reported in association with modified atmospheres and low O_2 levels (Khan et al., 2006; Torres and Alegre, 2012). Pesis et al. (2010) and Val et al. (2009) showed that pre-treatments prior to cold storage involving low O_2 and short-term storage at 20 ºC were able to reduce the incidence of apple scald and bitter pit during cold storage.

Another method to predict bitter pit involves assessing bitter pit that appears naturally in fruit picked from the tree before harvest, and keeping the fruit at room temperature. Prior to the study, we observed that it was possible to observe symptoms of bitter pit in some apple orchards, in fruit that had dropped to the ground approximately three weeks before harvest. From this, we developed the hypothesis that apples picked from the trees before harvest could offer a good method for predicting bitter pit. We have called this way of predicting bitter pit the 'passive method' because there is no need to use any product or additional treatment to trigger the mechanism of bitter pit development. In the 1970s, England and Larsen (1973) conducted a study involving the varieties 'Goldspur' and 'Wellspur', in which the incidence of bitter pit was evaluated in apples removed from the tree before harvest, and before there were any visible symptoms on the apples on the tree. This study suggested that the incidence of storage bitter pit could be observed 1-3 weeks prior to harvest, but no analyses were conducted to assess the relationship between the incidence of pre- and post-harvest bitter pit. Despite being an easy and inexpensive way for predicting bitter pit, the method has not been tested in commercial orchards and, to the best of our knowledge, similar studies have not been conducted to date.

The aim of this study was to find a good methodology for predicting the incidence of bitter pit, after harvest and under the typical conditions found in Northeast Spain, choosing either one of the existing methodologies (infiltration with magnesium salts and maturity enhancement with ethephon dips) or a new one (development of natural bitter pit through the 'passive method' and the effect of bagging fruit picked before commercial ripening). This is the first time that the 'passive method' and the effect of bagging fruit are tested and that all four approaches are compared to each other.

2. MATERIAL AND METHODS

2.1. Plant material

The first experiment was conducted over three consecutive seasons (2009, 2010 and 2011) in 10 commercial orchards growing 'Golden Smoothee' apples (*Malus domestica* Borkh) in the Lleida area (NE Spain), with antecedents of different levels of susceptibility to bitter pit. All of the orchards studied were mature and their trees were normally spaced (approximately 4 × 1.2 m), grafted onto M9 rootstock and fully irrigated. They all received standard cultural practices in terms of pruning, fertilization, irrigation and crop management.

2.2. Fruit sampling

All of the orchards were sampled four times in two of the three seasons: 2009 and 2010, and three times in 2011. In the first two seasons (2009 and 2010), four 40-fruit samples were collected from each orchard at 60, 40, and 20 days before harvest (dbh) and at the commercial harvest. In the third season (2011), the samples were taken at 40 and 20 dbh and at the commercial harvest when the starch index was within 7-8 (starch chart EC-Eurofru). Each sample was composed of 40 apples without symptoms of bitter pit.

Apples were taken from 40 selected trees with standard crop loads and vigour. One average-sized, undamaged apple was taken from each tree at a height of 130-170 cm above the ground.

2.3. Sample preparation and treatments

Each 40-fruit sample taken from each orchard received a different treatment. These were performed as follows:

- Infiltration of apples with $MgCl_2$ (MG): the first 40-fruit sample was treated under vacuum conditions (250 mm Hg) during 2 min with a solution of 0.10 M $MgCl_2 \times 6\ H_2O$, 0.4 M sorbitol as osmoticum, and 0.01% Tween-20 as a surfactant, in accordance with the recommendations of Chilean apple exporters (Retamales and Valdes, 1996). The apples were subsequently placed on plastic fruit trays and left at room temperature (approximately 25 °C) to allow for the development of bitter pit-like symptoms. This method was evaluated for the three seasons (2009-11).
- Ethephon dips (ET): the second 40-fruit sample was dipped in an ethephon solution (2000 ppm) for 5 min. The apples were then left at room temperature as described above. This method was evaluated for the three seasons (2009-11).
- Passive method (PS): the third 40-fruit sample was left untreated at room temperature, under the same conditions as described above. This method was also evaluated for the three seasons (2009-11).
- Bagging of fruit (BG): the fourth 40-fruit sample was packaged on two plastic 20-fruit trays. Each tray was then left inside a transparent plastic bag without holes, which was subsequently sealed, and left at room temperature. To maintain the atmosphere inside the bag, the subsequent assessments were performed

visually without opening the bag. This method was evaluated in two of the seasons (2009 and 2010).

To detect the point at which the incidence or severity of bitter pit-like symptoms stopped increasing, all of the apples contained in each sample were individually examined at intervals of 5-7 days over a 40-day period for any external signs of superficial bitter pit-like symptoms. The incidence of bitter pit was calculated as the percentage of fruit with bitter pit-like symptoms. The severity of bitter pit was assessed by classifying each apple according to a grading of its bitter pit-like symptoms (Figure 1): level 0, healthy fruit; level 1, or slight, when the fruit had between 1-6 pits on its surface; level 2, or moderate, when less than one-third of the surface of the fruit was affected (approximately between 7-15 pits per fruit); and level 3, or severe, when more than one-third of the surface of the fruit was affected (> 15 pits per fruit).

2.4. Storage treatment

To evaluate the incidence of bitter pit at post-harvest, another 80-fruit sample was collected from each orchard at the maturity status recommended for long-term storage. The percentage of bitter pit associated with each sample was recorded after three months of storage in air at 0 ºC and at 80% relative humidity plus a further seven days at 20 ºC and 45% relative humidity.

2.5. Validation at the commercial scale

The PS and ET methods were validated in two seasons (2011 and 2012), and in relation to 30 and 16 commercial orchards, respectively, which were different from those used above. They also received standard cultural practices in terms of pruning, fertilization and irrigation. Two 40-fruit samples were collected from each orchard at 40 and 20 dbh in 2011, and at 40 dbh in 2012. One 40-fruit sample was treated according to the PS method, while the other was treated according to the ET method. Another 80-fruit sample was collected at harvest to evaluate the percentage of post-harvest bitter pit per orchard, with the same methodology as described above.

2.6. Data Analysis

Data were analysed by linear correlation using the percentages of bitter pit-like symptoms (BPLS) associated with each method (MG, ET, PS, BG) at pre-harvest, as the independent variable. The dependent variable was the percentage of bitter pit (BPAS) after four months of storage in air at 0 ºC. Correlations were calculated for each method-sampling time combination and reported for each year. We then calculated the coefficient of determination (R^2) and the level of significance of each linear regression model. The data were analysed using the JMP® 8.0.1 programme (SAS Institute Inc., 2009).

3. RESULTS AND DISCUSSION

3.1. Development of bitter pit-like symptoms

Bitter pit-like symptoms were visible on the apples treated by all methods within five days of the initial application of the method. Beyond 10 days after treatment, there was no increase in either the size or incidence of lesions in most of the methods. The only exception was the BG method; in this case, there was a tendency for symptoms to develop more slowly than in the other methods (Figure 2). Other researchers (Burmeister and Dilley, 1991; Lotze et al., 2010) had previously observed that beyond 10 days after Mg infiltration or ethylene treatment, bitter pit-like symptoms tended not to increase. Nevertheless, the recommendation made by packing houses in Chile involved performing evaluations 16 days after Mg infiltrations (Valeria Lepe and Jose AntonioYuri personal communication). Our results suggest the possibility of predicting bitter pit using either the MG, ET or PS methods within 7-10 days of treatment.

However, we observed some differences between methods regarding the severity and/or development of symptoms, which are discussed below.

Bagging apples at the pre-climacteric stage did not increase either the incidence or severity of bitter pit; in fact, it did quite the opposite. The BPLS associated with the BG method were less pronounced for all sample groups and seasons. Other methods consisting of modifying the atmosphere and achieving low O_2 levels to reduce post-harvest bitter pit have also been reported (Hewett, 1984; Hewett and Thompson, 1989; Khan et al., 2006; Val et al., 2009; Pesis et al., 2010). Pesis et al. (2010) and Val et al. (2009) reported that low O_2 and 20 ºC short-term pre-treatments prior to cold storage, could help to reduce bitter pit during cold storage. This bitter pit reduction could be due to a decrease of the respiratory rate. Recasens et al. (2004) demonstrated that respiratory rate and ethylene production correlate negatively with Ca content in apples, and Sharma et al. (2012) reported that rates of ethylene production and respiration were lower in disorder-free fruit than in disorder-affected fruit. On the other hand, Saure (2014) considered the susceptibility to stress, rather than Ca deficiency, as the main cause of the development of bitter pit. According Saure (2014), the influence of stress on the occurrence of 'Ca-related disorders', such as bitter pit, could be based on an increased production of reactive oxygen species (ROS) in the fruit. ROS are known to trigger cell death, which is characterized by a progressive loss of membrane integrity and release of cellular constituents (Van Breusegem and Dat, 2006; Saure, 2014). The bagging could reduce the oxidative stress into the fruit and, therefore, decrease the production of ROS and the incidence of BPLS.

Unlike the bagging, the BPLS associated with the MG method were more pronounced than with the other methods, especially closer to harvest. There were more pits per fruit; therefore, a greater fruit surface was affected. Researchers do not agree as to whether

Mg-induced pits are directly comparable with those associated with the natural bitter pit disorder (Burmeister and Dilley, 1991). It has been suggested that Mg-induced pits result from a breakdown caused by either Mg toxicity at the point of entry into the fruit (Ferguson and Watkins, 1989), or by polyphenol oxidase (PPO) activity (Hopfinger et al., 1984). The Mg could generate an increase of ROS as result of PPO activity, and cause cell death (Saure, 2014). Results from Burmeister and Dilley (1991), using 0.18 M $MgCl_2$ for infiltration, supported the hypothesis that Mg-induced pits are quite similar to those associated with the actual bitter pit disorder when the $MgCl_2$ concentration was 0.18 M. Our results at concentrations of 0.10 M indicate that Mg-induced pits are also quite similar to those associated with BPAS and that the major differences with the other methods are in the severity of the symptoms rather than in the incidence.

No relevant differences were observed between the other two methods, ET and PS, in terms of the development of symptoms. We should, however, underline that ethephon-dipped fruit, after 5-7 days of treatment, had a yellower background colour than other treatments. This helped us to observe bitter pit-like lesions more clearly, thanks to the contrast between the green colour of the pits and the yellow of the background. From our existing data, it is not possible to conclude whether applying ethephon dips stimulated the appearance of BPLS because we observed that BPLS were also enhanced if the apples were left at room temperature without any additional treatment (PS method).

According to our results, final bitter pit potential could be determined within 7-10 days of the application of the method, whether using the MG, ET or PS method. This implies that bitter pit could potentially be predicted between 6-9 days earlier than the Chilean method based on Mg infiltration, or 4-7 days earlier than the South African method based on ethephon dips. This would offer important benefits for both farmers and packing houses as they would have more time for decision making.

3.2. Accuracy of the predictions in relation to sampling time

There were no significant correlations between the prediction methods at 60 dbh and BPAS, either in 2009 or 2010 because of the lack of BPLS at this early sampling stage (Table 1). According to our observations, the lack of accuracy of the methods at 60 dbh could be because the sampled fruit were still at an unripe stage. As fruit maturity is an important factor in the subsequent development of bitter pit (Ferguson and Watkins, 1989; Prinja, 1990), this could have masked the full appearance of BPLS. At this sampling time, it was not possible to improve either the accuracy of prediction or the incidence of BPLS by accelerating fruit maturation with ethephon. It is evident that sampling at 60 dbh is not useful for predicting bitter pit, and for this reason we decided not to use it in the next season.

At 40 dbh, the MG, ET, and PS methods produced significant linear correlations with BPAS, with $R^2 \geq 0.4$ ($P < 0.05$) over the three seasons (Table 1). When all of the years were analysed together, the MG, ET and PS methods applied at 40 dbh also showed significant linear correlations with BPAS. However, the maximum visual expression of bitter pit-like symptoms and the best correlations did not occur until 20 dbh, ($R^2 = 0.7$, $P < 0.01$) (Table 1). Moreover, when we analysed the 2009 and 2010 seasons separately, the best correlation was also observed at 20 dbh. Other researchers have obtained good linear correlations between BPLS at pre-harvest up to 20 dbh and the BPAS (Eksteen et al., 1977; Lotze et al., 2010). Nevertheless, from our data, it can be observed that both the ET and PS methods at 20 dbh produced non-significant linear correlations with BPAS in one of the seasons (2011), whereas at 40 dbh, the linear correlations were always statistically significant in the three seasons and regardless of the method used.

At harvest, the linear correlations between the MG, ET, and PS methods with BPAS were significant for all three seasons ($P < 0.05$), but with a lower R^2 than at 20 dbh in 2009 and particularly in 2010 (Table 1). The apparent lack of accuracy for the 2010 harvest could be due to the decrease in the incidence of bitter pit. The mean percentage of BPAS in 2010 season was approximately 7%, whereas in 2009 and 2011 the corresponding values were 21% and 17%, respectively. Other trials with incidences of bitter pit below 10% also produced low correlation coefficients (Lotze et al., 2010). The lower accuracy at harvest could be improved by considering the apples with evident signs of bitter pit on the tree. Another cause of the apparent lack of accuracy at harvest could be the fruit maturity stage. Most reports have shown that, in general, bitter pit is more severe in early-picked than in later-picked apple fruit (Perring, 1986; Volz et al., 1993; Prange et al.) Therefore, BPLS could also be more severe in early-picked than in later-picked apple fruit.

From our data, we observed that the orchards in which BPLS appeared earliest were those with a high incidence of BPAS, regardless of the method used. Furthermore, there were no cases of high levels of BPLS before harvest associated with low levels of BPAS in any sampling time or season. Therefore, early sampling (and always after 60 dbh) could perhaps detect orchards with a high risk of bitter pit, at least qualitatively. Consequently, sampling at 40 dbh seems to be the best option both in terms of earliness and accuracy. Sampling at 40 dbh would allow growers to know the potential risk of bitter pit development approximately 30 days before the expected harvest date. If a high risk of bitter pit could be detected at that moment, there would be enough time to take measures to reduce the incidence of bitter pit (Casero et al., 2010).

3.3. Accuracy of predictions in relation to the method used.

There were no significant correlations between the BG method and BPAS in any season when these were separately analysed (2009 and 2010). When the two years were correlated together, the BG method showed significant linear correlations with BPAS ($P < 0.01$), but with a R^2 lower than 0.4, whereas the other methods reached $R^2 = 0.7$. The lack of accuracy in relation to the BG method was due to the lack of BPLS, as discussed in the preceding subsection (3.1). It is evident that the BG method is not useful for predicting bitter pit, and for this reason we decided not to use it in the next season.
In determining the most accurate of the remaining three methods (MG, ET and PS) analysed for predicting bitter pit, our results suggest that they would all be sufficiently accurate for commercial purposes. However, the variability between methods was high in terms of accuracy (Table 1). In the 2009 season, the ET method applied at 40 dbh showed the highest coefficient of determination with respect to BPAS ($R^2 = 0.60$, $P < 0.05$), whereas in the 2010 and 2011 seasons, this was associated with the PS method ($R^2 = 0.61$ and 0.62, respectively, $P < 0.01$). At 20 dbh, the highest correlations in the 2009 and 2010 seasons were obtained with the ET method ($R^2 = 0.89$ and 0.66, respectively, $P < 0.01$), and in 2011 with the MG method ($R^2 = 0.64$, $P < 0.01$). At harvest, the MG method produced the highest coefficient of determination for the 2009 season ($R^2 = 0.73$, $P < 0.01$) and the 2011 season ($R^2 = 0.73$, $P < 0.01$), and the PS method for the 2010 season ($R^2 = 0.44$, $P < 0.05$). Even so, our results with the MG and ET methods were quite similar to those obtained in Chile during the 1990s (Mg infiltration $R^2 = 0.67$ to 0.87; ethephon $R^2 = 0.60$ to 0.84) (Retamales and Valdes, 1996), and better than those reported by Lötze et al. (2010) for ethephon dips in the ‘Golden Delicious’ cultivar. Regarding the latter, the different sample size between both trials (40-fruit samples vs. 20-fruit samples) could have influenced the results.
Obtaining a wide range of incidence of bitter pit among the different samples might also

have had a positive influence on the accuracy of the models. The ranges that we obtained were quite wide: from 0% to 59% (2009), from 0% to 23% (2010), and from 0% to 38% (2011).

A prediction method would only be applied commercially by packing houses and/or growers if it is easy to implement and offers a low-cost solution. For these reasons, the PS method seems to offer a better alternative for bitter pit prediction. Unlike the other alternatives, this method does not require the use of either reactive products or specialized equipment. However, the accuracy of the method may vary from cultivar to cultivar, from region to region (Retamales et al., 2000; Lotze et al., 2010), and also between seasons, as we have seen in this study, but its simplicity suggests that it could be the first real commercial approach for predicting bitter pit before harvest. The method should therefore be evaluated in different regions and with different cultivars to determine its accuracy of prediction under varied conditions.

3.4. Validation of the Passive and Ethephon Methods in commercial orchards

The results of the validations of the PS and ET methods in different commercial orchards in the 2011 and 2012 seasons showed a clear and significant relationship with BPAS, regardless of the sample time (40 and 20 dbh) and season (2011 and 2012). In all of the cases studied, the PS and ET methods produced significant R^2 with BPAS ($R^2 > 0.6$, $P < 0.01$), without any important differences between the two methods (Figure 3). The accuracy of the validations was even better than in previous tests; this could be attributable to the use of more orchards and, as a consequence, a wider range of incidence of bitter pit (from 0% to 80%). As explained in the discussion in the preceding subsections, sampling at 40 dbh and using the PS method would therefore offer the best alternative. In terms of practical use and taking an incidence scale of three degree (<5% or low; 5%-10% or medium; >10% or high), the percentage of orchards well classified were 71% in 2011 and 62% in 2012. The rest of orchards were all under-estimated. The results indicate that we should assume the risk of having some orchards with a high incidence of BPAS if no BPLS are recorded by the PS method, but that the identification of significant BPLS levels (>5%) are a guarantee of high incidence of BPAS. From all of the above it can be stated that neither the PS method nor the other methods would permit an accurate quantitative prediction of bitter pit, but they would allow a qualitative prediction of BPAS.

4. CONCLUSION

We conclude that the ‘passive method’ (PS) offers a reliable and relatively inexpensive method for assessing the risk of bitter pit in ‘Golden Smoothee’ apples after storage between 10-30 days before harvest. Furthermore, unlike the other methods studied, the PS method requires neither reactive products nor specialized equipment. This makes it a more interesting option than the other methods for commercial and/or industrial purposes. Nevertheless, the method would need to be assessed for different regions and for different cultivars before it can be adopted on a wider scale.

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FIGURES AND TABLES

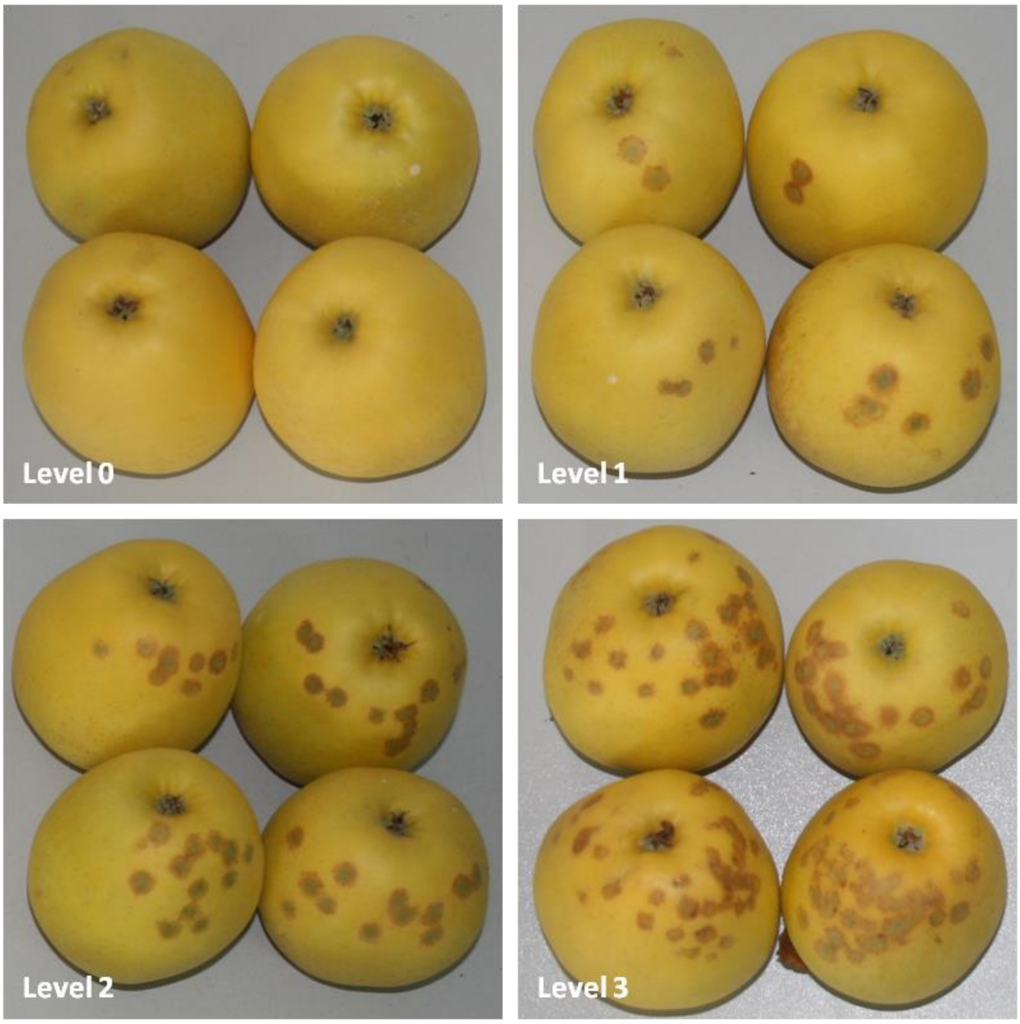

Figure 1. Scale to assess the severity of bitter pit symptoms. Level 0 (no-bitter pit): without symptoms; Level 1 (slight): one to six pits on surface; Level 2 (moderate): one-third of the surface of the fruit affected (approximately between 7-15 pits per fruit); Level 3 (severe) one-third of the surface of the fruit affected (> 15 pits per fruit).

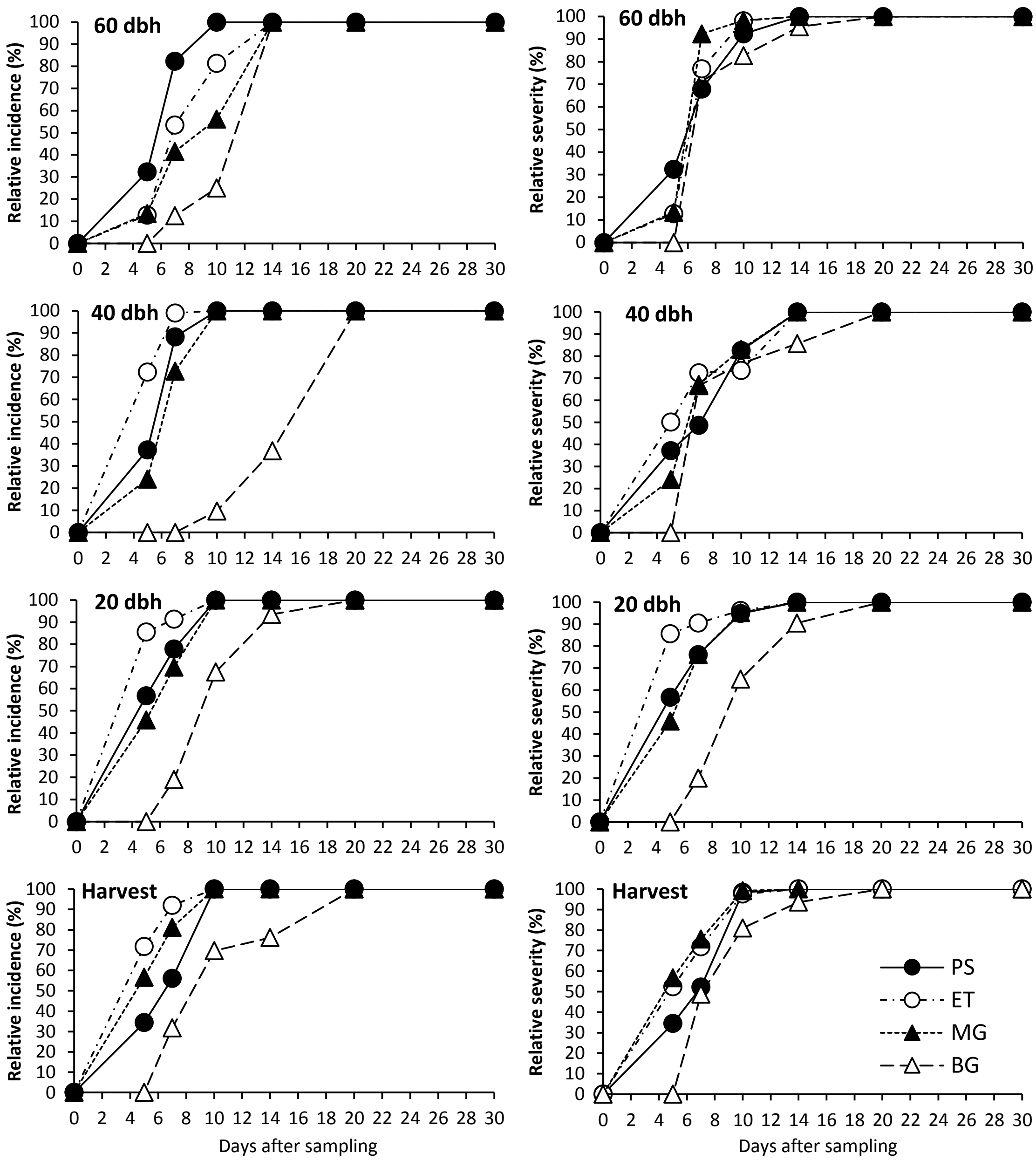

Figure 2. Progress curves of the relative incidence (relative to the incidence at 30 days after sampling; left) and relative severity (relative to the severity at 30 days after sampling; right) of bitter pit-like symptoms beyond the treatment to predict bitter pit: passive method (PS), ethephon dips (ET) infiltration with $MgCl_2$ (MG), and bagging of fruit (BG). A graph for each sample date: 60, 40 and 20 days before harvest (dbh) and at commercial harvest. Each data point represents the mean for all of the orchards over two seasons (2009 and 2010).

Table 1. Correlations between bitter pit after storage and bitter pit-like symptoms using the passive method (PS), ethephon dips (ET), infiltrations with $MgCl_2$ (MG) and bagging of fruits (BG) during three seasons (2009, 2010 and 2011), and sampling at 60 days before harvest (dbh), 40 dbh, 20 dbh and harvest time.

Method	2009		2010		2011		All seasons	
	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
60 dbh								
MG	0.023	n.s.	0.075	n.s.	-	-	0.069	n.s.
ET	0.061	n.s.	0.100	n.s.	-	-	0.000	n.s.
PS	0.062	n.s.	0.000	n.s.	-	-	0.004	n.s.
BG	0.004	n.s.	0.000	n.s.	-	-	0.048	n.s.
40 dbh								
MG	0.398	0.043	0.567	0.005	0.435	0.043	0.315	0.002
ET	0.599	0.024	0.542	0.010	0.417	0.044	0.297	0.002
PS	0.564	0.032	0.614	0.003	0.623	0.004	0.599	<.001
BG	0.064	n.s.	0.246	n.s.	-	-	0.017	n.s.
20 dbh								
MG	0.814	0.001	0.599	0.003	0.638	0.006	0.754	<.001
ET	0.887	<.001	0.656	0.001	0.312	n.s.	0.656	<.001
PS	0.792	0.001	0.601	0.003	0.375	n.s.	0.670	<.001
BG	0.303	n.s.	0.119	n.s.	-	-	0.395	0.002
Harvest								
MG	0.733	0.003	0.350	0.054	0.645	0.022	0.581	<.001
ET	0.465	0.043	0.320	0.054	0.424	0.041	0.376	<.001
PS	0.603	0.014	0.439	0.019	0.458	0.003	0.527	<.001
BG	0.164	n.s.	0.144	n.s.	-	-	0.296	0.013

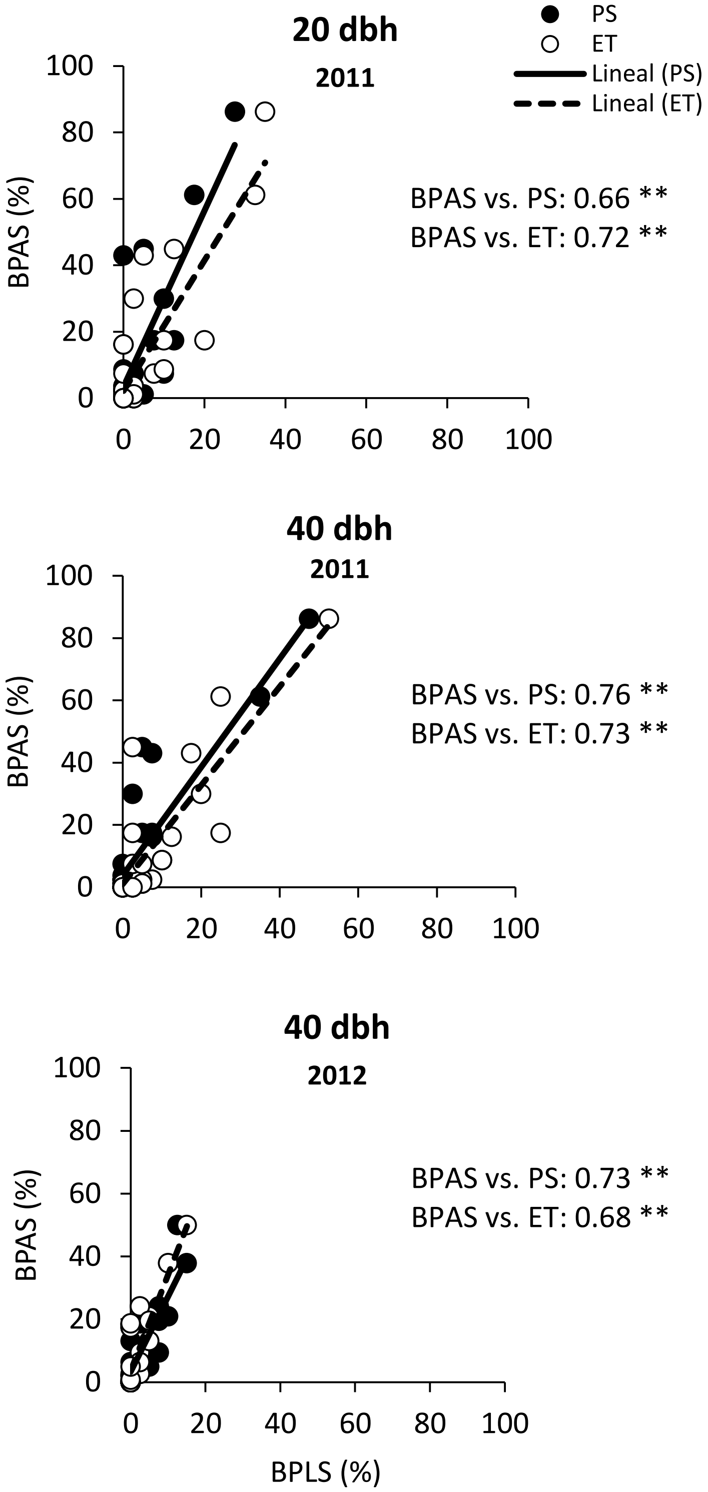

Figure 3. Lineal relationships and coefficients of determination (R^2) between the incidences of bitter pit after storage (BPAS) and of bitter pit-like symptoms (BPLS) using the passive (PS) and ethephon (ET) methods in two seasons (2011 and 2012). In the 2011 season, the pre-harvest samples were collected 40 and 20 days before harvest (dbh) and in the 2012 season only at 40 dbh.

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