Could Ethylene Metabolism in Lemon Fruit Influence Peteca Incidence?

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Abstract

Peteca of lemon is a postharvest physiological disorder resulting in the collapse of the oil glands. Subsequently, the oil leaks into the adjacent tissue and causes a darkened depression. The occurrence can be severe, without any specific pre- or postharvest practises to employ to reduce the incidence. Over several seasons the incidence of peteca was investigated to identify factors that influence the incidence of the disorder. The first observation was the highly erratic incidence between seasons as well as within an orchard in a season, with the early fruits being highly susceptible. In experiments to identify postharvest factors which influence the peteca incidence, 3 ppm ethylene and 5% CO_2 were applied in a continuous flow-through system (20°C for 3 d). The CO_2 treatment resulted in significantly higher incidence compared to the rest. Following on from these results, postharvest Ethephon (2-Chloroethyl phosphonic acid) (200 and 400 mg/L) and AVG (aminoethoxy-vinylglycine) (400 and 800 mg/L) applications to fruit resulted in a significant reduction in peteca. In the subsequent season, the same treatments were applied in an orchard one week before harvest and a similar reduction in peteca was recorded. Ethylene production from these fruits after harvest was measured and showed a transient spike in fruits from the Ethephon treatment. The results collected could indicate a protective action of ethylene in reducing rind sensitivity to peteca. It is hypothesised that if the internal ethylene synthesis is increased prior to harvest in sensitive fruit, i.e. immature fruit, a reduction in peteca can occur.

Keywords: *Citrus limon*, physiological disorder, oil gland, ethylene

INTRODUCTION

Peteca of lemon fruit is a postharvest physiological disorder associated with the collapse of an oil gland, which generally occurs from harvest until cold storage. Peteca reduce the fruit quality due to the unsightly sunken light brown lesions that develop in the rind (Cronje, 2007). Incidence of peteca in lemon fruit has been recorded as early as 1924 in the USA in lemon fruits shipped from Italy, from which it appears the name was adopted from *petecchia* as it was known in Italy (Fawcett, 1936).

Economically significant incidences of peteca have been recorded in most of the lemon producing regions viz. Tucuman, Argentina (Torres Leal, 2004), Chile (Undurraga et al., 2009), Australia (Wild, 1991), Lebanon (Khalidy et al., 1969) and California in the USA (Fawcett, 1936). In South Africa peteca have a negative impact every year for lemon producers in the inland warm and humid summer rainfall areas (Limpopo, Kwazulu-Natal and Mpumalanga provinces), as well as in the cooler coastal areas with summer (Eastern Cape) or winter (Western Cape) rainfall (Cronje, 2005).

The first symptom of peteca can be seen as early as 3-5 days after harvest and develops before the fruit has been put onto the packline. It consists of darkening of an individual oil gland and if the flavedo is removed the brown oil gland is visible with discoloured albedo tissue directly below. After a few days the rind tissue surrounding the collapsed oil gland sunks and adjacent oil glands can also collapse. This collapse of the oil gland can occur in green and yellow fruit but normally the sunken lesion is clearly seen in fruit with a yellow rind (Cronje, 2007). The sunken lesion develops without any physical or mechanical damage to the epidermal rind tissue, as seen in oleocellosis, which results in the leaking of the oil gland onto the rind epidermis and degeneration of the cortical cell contents.

Fig. 1. Peteca of lemon fruit is associated with the collapse of the oil gland and result in phytotoxic damage to the rind.

The susceptibility of lemon fruit to this disorder has been associated with various pre and postharvest factors. In South Africa low rainfall during the three months prior to harvest as well as cold/wet conditions (dramatic changes in day night temperature) close to harvest, have been seen to result in higher incidence of peteca, and concurs with the marked increase of peteca incidence reported in Tucumán after an abrupt temperature fall in the week prior to harvest (Torres Leal, 2004). The same conditions (low temperature and high relative humidity) was mentioned by Wild (1991) and Artes et al. (1983) to be deleterious and Undurraga et al. (2006) identified rainfall on lemon during harvest in Chile to be problematic in the development of peteca. Undurraga et al. (2002) reported that rind colour, and therefore fruit rind maturity, play a role in fruit susceptibility and reported that yellow fruits have a greater incidence compared to fruits in the "silver" stage. The incidence of peteca in South Africa is generally associated with a higher occurrence during the initial weeks in the picking window, with a dramatic reduction in subsequent weeks (Fig. 2).

Peteca has been associated with calcium (Ca) imbalances in the rind (Khalidy et al., 1969), and the involvement of Ca-content in the rind as well as Ca-oxalate crystals has been investigated by Storey and Treeby (2002). They found in peteca affected areas of the rind 23% higher Ca an 30% lower Boron. However, they could not find any direct evidence of Ca-oxalate crystal growth being a causal factor of cell damage seen in peteca, a view supported by Undurraga et al. (2009).

Postharvest conditions can aggravate peteca in lemon fruit, and Wild (1991) found the incidence increased after citrus wax application (commercial polyethylene based citrus wax with 16% solids) as well as fruit brushing. In contrast, storage for 3-5 days after harvest (no handling) as well as degreening, decreased the incidence of peteca. Cold storage at 3°C of yellow coloured lemons resulted in higher peteca incidence compared to "silver" coloured fruit storage at 3 and 7°C (Undurraga et al., 2009). Undurraga et al. (2009) hypothesised that there is a relationship between peteca and some physiological stress indicators such as hydrogen peroxide and peroxidase activity.

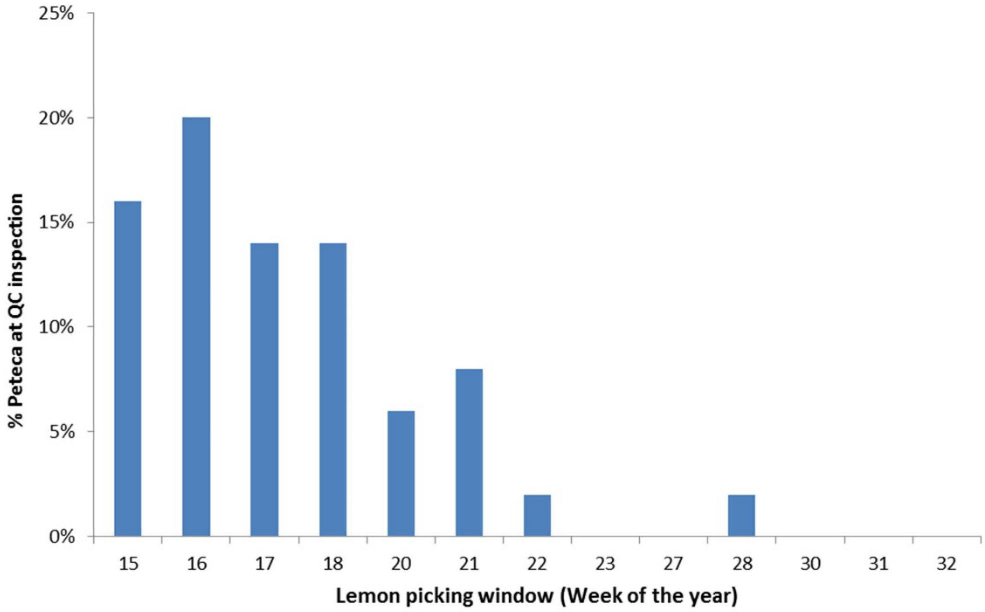

Fig. 2. Incidence of peteca as reported at a citrus packhouse in Groblersdal, Mpumalanga, South Africa during 2012 (source Steve Turner, Core fruit).

Strategies to control peteca incidence are an important research area for the South African lemons producers. However, the erratic incidence of this disorder with large variations between season, orchards and within orchards in one season, have made it difficult to fully elucidate the impact of pre- and postharvest factors on this physiological disorder. The influence of ethylene applied in degreening rooms are always suspected to affect citrus fruit quality negatively, however contradictory reports were received from the South African lemon packhouses on the impact of ethylene (degreening) on peteca incidence. Therefore, the aim of this study was firstly to determine the influence of postharvest ethylene application and secondly the efficacy of pre-harvest manipulation of fruit ethylene metabolism on peteca incidence.

MATERIALS AND METHODS

During four consecutive seasons various experiments were conducted to determine the influence of ethylene on the incidence of peteca of lemon fruit. In 2009 fruits were harvested from a commercial 10 year old 'Eureka' lemon (*Citrus limon* Burk.) orchard in Simondium, Western Cape. The fruits were of the same size and colour and were divided into 5 lots of 30 fruits. The 30 fruits were put in closed plastic containers connected to a continuous flow-through system, through which the gas treatments were applied i.e. normal air, 3 ppm ethylene, or 5% CO_2 at 20°C for 3 days. In addition 30 fruits were kept in a closed plastic container. After treatments fruits were evaluated for peteca incidence (% peteca per treatment). The experiment was repeated 3 weeks later from the same trees.

In the second experiment, pre-harvest manipulation of fruit ethylene production was done in the Eastern Cape (Addo) in 2010, 2011 and 2012 and in Mpumalanga (Groblersdal) in 2012 in commercial 'Eureka' lemon orchards, 8-10 years old. In each area the orchards used were within a 10 km radius. Foliar applications of Ethephon (2-Chloroethyl phosphonic acid) (low, 200 mg/L and high, 400 mg/L) and AVG (aminoethoxy-vinylglycine) (low, 400 mg/L and high, 800 mg/L) were done to single tree replicates (n=8) in a complete randomised block design, one week before the first planed commercial harvest date. Buffer trees were left open between treatments which were applied with a motorised knapsack sprayer at 10 L/min. After one week the fruit were harvested and 10 fruits per replicate stored in closed plastic bags to "force" peteca incidence. This technique of forcing fruit at room temperature stem from the result in 2009, where significantly higher peteca developed in closed plastic containers (Table 1). After storage for 14 days at ambient temperature (~20°C) in closed plastic bags, fruits were scored for peteca according to this index:

$$Peteca\ index\ (0-2) = \sum \frac{[Peteca\ (scale\ 0\text{–}2) \times number\ of\ fruit\ in\ each\ class]}{Total\ number\ of\ fruit\ in\ rep} \quad (1)$$

Table 1. The influence of continuous gas applied to lemon fruit from Simondium Western Cape in 2009, for 3 days at 20°C. Fruits were evaluated for peteca 1 day after treatments.

Treatment	% Peteca incidence	
	Sample 1	Sample 2 (3 weeks later)
Normal air (control)	18	0
5% CO_2	21	0
3 ppm ethylene	0	0
Closed container	41	0

The ethylene production was measured in 2012 at harvest time and 1 week later from one orchard in the Eastern Cape. For each of the 8 replicates, 5 fruits were placed in sealed bottles for 5 hours. Samples were taken from the closed bottles with air tight syringes and the ethylene concentration were measured with a flame ionization gas chromatograph (Varain, Model 3300, Varian instrument Group, Palo Alto, California, USA). The volume of the oranges was determined as well as the weight to determine the ethylene production expressed as µl $C_2H_4.kg^{-1}.h$.

The data were analysed with SAS (data were analysed using a one-way ANOVA (SAS v. 6.12, SAS Institute, Carry, NC,) to test the significance ($P \leq 0.05$).

RESULTS

The fruits that were closed in a plastic container had the highest incidence of peteca (41%) compared to the 21% observed in those treated with 5% CO_2 and the 18% in those treated with air. In contrast, the 3 ppm ethylene gas treatment resulted in no peteca developed (Table 1). Repeating the experiment, from the same trees three weeks later, with the same gaseous treatments resulted in no peteca developing in the fruit. This difference in incidence between sampling dates indicate a possible influence of fruit maturity on sensitivity.

In the second part of the project, the pre-harvest foliar applications of Ethephon and AVG from 2010 to 2012 reduced the incidence of peteca of lemons in four out of the seven orchards (Figs. 3A-C). However, variation in efficacy occurred between seasons, production areas and orchards. During 2012 in Groblersdal both of the orchards used showed no treatment effect (Fig. 2B). In addition, the difference in peteca incidence between the two orchards in this production area were pronounced i.e. 0.25-0.35 in orchard 1 and ±0.05 in orchard 2 (Fig. 3B). This variation in incidence is also seen in Figure 3C with peteca incidence in the controls varying from 0.2 to 0.7.

The ethylene measurement of fruit after harvest and 1 week later indicate that the Ethephon treatment resulted in a significant spike in ethylene production (Fig. 4). The AVG treatment, known to reduce ethylene synthesis, resulted in measurable levels of ethylene, although similar to those of the control treatment.

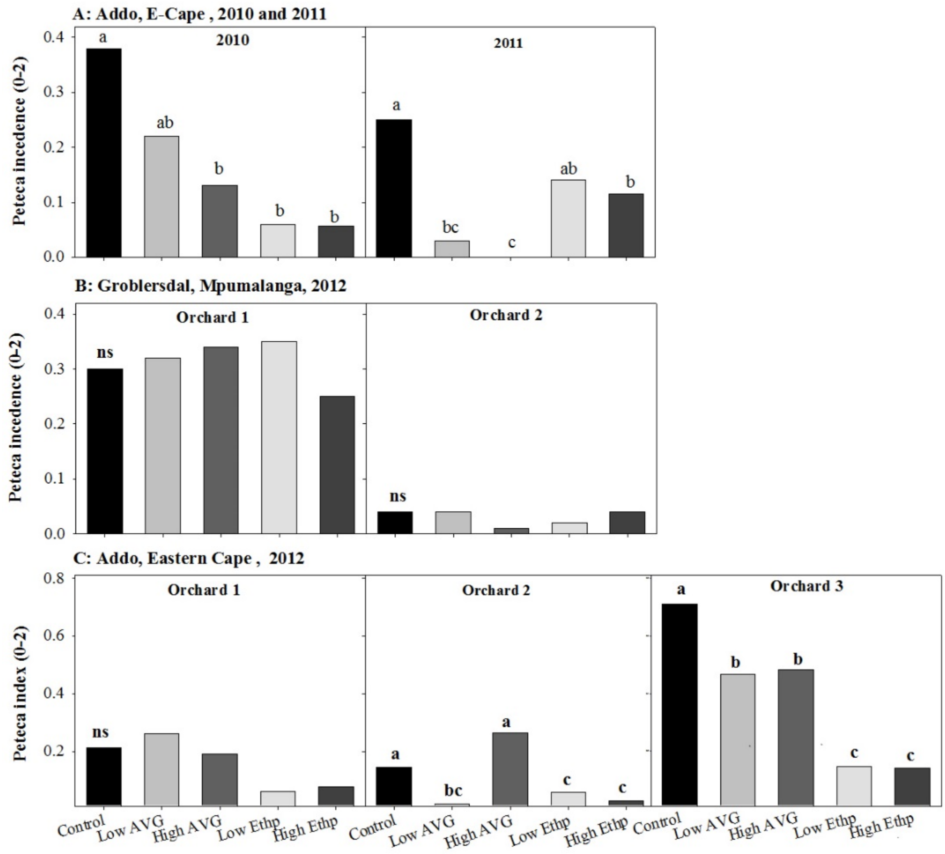

Fig. 3. Incidence of peteca in lemon fruits in A: Addo, Eastern Cape (2010 and 2011), B: Groblersdal Mpumalanga (2012) and C: Addo, Eastern Cape (2012), after pre-harvest foliar application of Ethephon (2-Chloroethyl phosphonic acid) (low, 200 mg/L and high, 400 mg/L) and AVG (aminoethoxy-vinylglycine) (low, 400 mg/L and high, 800 mg/L) 1 week before harvest (n=8). After harvest fruits were stored at 20°C for 2 week in closed plastic bags prior to scoring for peteca incidence ($P \geq 0.05$).

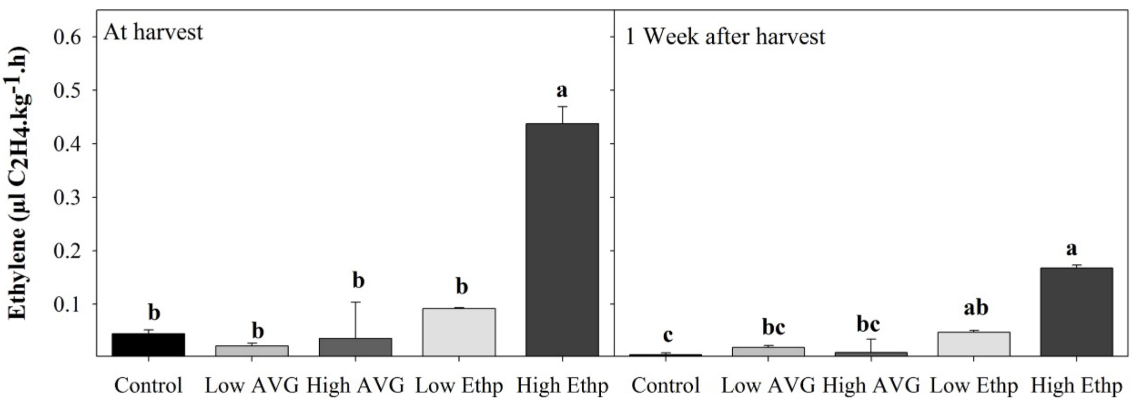

Fig. 4. Ethylene measured at harvest and 1 week after harvest of lemon fruits in Addo, Eastern Cape (2011), after pre-harvest foliar application of Ethephon (2-Chloroethyl phosphonic acid) (low, 200 mg/L and high, 400 mg/L) and AVG (aminoethoxy-vinylglycine) (low, 400 mg/L and high, 800 mg/L) 1 week before harvest (n=8) ($P \geq 0.05$).

DISCUSSION

The incidence of peteca of lemon fruit has previously not been associated with ethylene metabolism in the fruit, but rather with aspects such as Ca content in the rind (Khalidy et al., 1969) and postharvest treatments (Wild, 1991). However, the change of peteca incidence in an orchard from one week to the next in 2009, could indicate that Ca is not the main factor to determine peteca sensitivity, as the nutrient content in the rind do not change at that late maturity stage. In addition, the reduction in incidence of peteca after application of Ethephon as well as AVG, followed by a transient change in fruit ethylene synthesis do offer a new direction of research into elucidating the causal mechanism of this disorder.

The hormone ethylene is known to be involved in the senescence processes in fruit as well as the development of cellular degradation associated with stress conditions, albeit it can also protect fruit against stress conditions causing tissue damage (Yang and Hoffman, 1984; Lafuente and Sala, 2002). Cajuste and Lafuente (2007) suggested that this endogenous hormone could induce protective properties of the citrus fruit rind against physiological disorders. In addition, ethylene metabolism has also been associated with the accumulation of defence properties against *Penicillium digitatum* infection (Marcos et al., 2005). The mechanisms underlying the reduced sensitivity to some physiological rind disorders of citrus after exposure to ethylene has not been elucidated, but it is suspected that an increased protective action due to up-regulating of rind phenolic metabolism may be involved, although additional defence mechanisms could also be induced by ethylene pre-treatment (Cajuste and Lafuente, 2007).

The reduction in peteca incidence after the pre-harvest manipulation of the fruit ethylene metabolism, as well as the reduction after ethylene gas application could indicate the possible induction of protective processes in the flavedo due to the higher ethylene prediction. It should be noted that the high and low Ethephon treatments resulted in leave drop after application (±20 and ±10%, respectively), but did not negatively affect the tree condition the following season. Currently the impact of peteca is managed by RSA lemon producers and packhouses with a strategy that include the following three aspects. The fruits in the first part of the picking window are more susceptible compared to later in the season: therefore moving harvest back two weeks can reduce the incidence dramatically even in the same orchard. Secondly, after a cold front, no fruit will be harvested for at least 3-5 days to reduce fruit rind turgor. The third aspect that has successfully reduced the incidence is the storing of fruits for 4-7 days in a shaded well-ventilated area after being harvested, with minimum handling of the fruit. However, this strategy limits but do not prevent the incidence of peteca. Therefore reducing the sensitivity of fruit to peteca by preharvest treatments would be a valuable tool to reduce the negative impact of peteca on lemon.

In conclusion peteca of lemon is a physiological disorder associated with the collapse of the oil gland. The incidence of peteca varies significantly between seasons, orchards and whit in an orchard in one season. It is thought that immature fruit (first pick) as well as cold conditions prior to harvesting could negatively influence peteca incidence. The pre-harvest application of Ethephon and AVG, known to alter the rind ethylene metabolism, has resulted in significant reduction of peteca over three seasons. However, the causal mechanisms as well as factors influencing fruit susceptibility to peteca are far from being elucidated. In addition, further research as to concentration and timing of application of these chemical needs to be undertaken.

Literature Cited

Cajuste, J.F. and Lafuente, M.T. 2007. Ethylene-induced tolerance to non-chilling peel pitting as related to phenolic metabolism and lignin content in 'Navelate' fruit. Postharvest Biol. Technol. 45:193-203.

Cronje, P.J.R. 2005. Peteca spot of lemons. SA Fruit J. 3:26-28.

Cronje, P.J.R. 2007. Postharvest Rind Disorder of Citrus Fruits. Citrus Research International, Nelspruit, South Africa.

Fawcett, H.S. 1936. External stains, spots, pits and eruptions on citrus fruit. p.486-490. In: Citrus diseases and their control. Mcgraw-Hill book Co., New York.
Khalidy, R., Jamali, A. and Bolkan, H. 1969. Causes of the Peteca spot disease of lemons occurring in Lebanon. Proc. 1st Int. Citrus Symp. 2:1253-1261.
Lafuente, M.T. and Sala, J.M. 2002. Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rind staining of 'Navelina' orange (*Citrus sinensis* L. Osbeck) fruit. Postharvest Biol. Technol. 25:49-57.
Marcos, J.F., Gonzalez-Candelas, L. and Zacarìas, L. 2005. Involvement of ethylene biosynthesis and perception in the susceptibility of citrus fruits to *Penicillium digitatum* infection and the accumulation of defence-related mRNAs. J. Exp. Bot. 56:2128-2193.
Storey, R. and Treeby, M.T. 2002. Cryo-SEM study of early symptoms of peteca spot in 'Lisbon' lemon. J. Hort. Sci. Biotechnol. 77:551-556.
Torres Leal, G.J., Lamelas, C.M. and Forciniti, J. 2004. Meteorological conditions during preharvest and postharvest management affecting peteca incidence in lemon from Tucuman, Argentina. Proc. Int. Soc. Citricult. 3:1110-1111.
Undurraga, P., Olaeta, J.A., Luttges, C.G. and Suarez, R. 2002. Efecto del calibre y madurez de limones cv. Fino 49, sobre el desarrollo de peteca en almacenamiento refrigerado. Siminete 72:35-40.
Undurraga, P., Oleata, J.A., Retamales, J.B. and Brito, A. 2006. Efecto de inmersiones en calico sobre peteca en limones amarillos y plateados, cosechados despues de una lluvia y almacenados en refrigeración. Agric. Tecnica (Chile) 66:3012 (in Spanish with English abstract).
Undurraga, P., Oleata, J.A., Retamales, J.B., Escobar, J. and Toso, A.M. 2009. Effect of maturity and storage temperature on the development of peteca in lemons (*Citrus limon* (L.) Burm. F.) cv. Eureka. Scientia Hort. 122:56-61.
Wild, B.L. 1991. Postharvest factors governing the development of peteca spot on 'Meyer' lemons. HortScience 26:287-289.
Yang, S.F. and Hoffman, N.E. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. 35:155-189.