Review of banana green life throughout the food chain: from auto-catalytic induction to the optimisation of shipping and storage conditions

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ABSTRACT

Banana green life (GL) is the time between harvesting and the start of natural ripening. GL could be considered as a major quality criterion, as it defines whether or not a fruit is suitable for export and marketing. The ending of GL, when climacteric crisis occurs, is characterized by autocatalytic ethylene production. Ethylene synthesis and regulation are described. The main methods for determining Gl are based on detecting the CO_2 peak or a decrease in green pigments, using a spectrometer (NDVI) for the latter. Temperatures during fruit growth and Black Sigatoka disease are the main pre-harvest factors affecting GL. The former can be managed by applying the thermal sums concept and the latter by adequate field practices. The effects of the main exogenous storage parameters (storage temperature, relative humidity, concentration of ethylene and O_2/CO_2 ratio in the atmosphere) could be modelled in some cases. The most effective solutions for extending GL rely on either developing coatings using new preservative compounds, or designing packaging capable of controlling the temperature, CO_2/O_2 ratio and ethylene concentrations in the environment close to the fruits. It was demonstrated that 1-MCP is not relevant for increasing GL. A global and integrated approach involving the overall optimization of pre- and post-harvest factors needs to be applied to maintain green fruit until voluntary/artificial ripening is induced.

Keywords: banana; green life; ethylene; shipping; storage

1. Introduction

Banana is the fourth most consumed product in the world and approximately 50 billion tonnes of Cavendish bananas are produced globally every year (FAO, 2018). Bananas are not grown in Europe and thus need to be imported from the production sites, which are mainly located in Central and Latin America, and in West and Central Africa. Bananas are harvested green and ripened in market areas. Fruits that are allowed to ripen on the tree often split, and tend to be mealy. The pre-climacteric period after harvesting is vitally important for importers and ripeners, because bananas are transported before they are ripened.

During this period, mature green fruit have a low basal respiration rate and ethylene production is almost undetectable. This period is also called the “green life” (GL) and the longest practical “pre-climacteric period” is sought (Hailu et al., 2013b). The length of this period is influenced by several factors, such as temperature, relative humidity (RH) or the surrounding ethylene concentration. At the onset of fruit ripening in climacteric fruits, such as banana, a rapid rise in respiration and autocatalytic ethylene production is observed. Indeed, at this key point, a cascade of enzymes implicated in the ethylene synthesis pathway occurs, initiating the onset of fruit ripening, hence the end of the GL period. The first section of this review is therefore devoted to the fruit itself, with special focus on ethylene synthesis auto-induction mechanisms, the physiology of the fruit, and the correlative prediction of banana GL.

GL prediction is of great scientific, but also economic interest, so a dedicated section will focus on the different approaches and ways of evaluating this quality factor.

Field conditions largely affect ripening onset and numerous publications have studied the influence of agronomic conditions (temperature, fungal pressure, etc.). Indeed, fruit growing conditions, or disease control on plants in the orchard, will lessen or increase the GL period and therefore need to be closely considered.

Numerous post-harvest treatments and technologies, such as controlled or modified atmospheres, have been developed to maintain GL duration as much as possible, and thereby limit potential losses during the fruit shipping and delivery stages. A section will be dedicated to this and a special section will be developed on management at reefer level.

Lastly, post-harvest technology trends for the future will be proposed in order to raise stakeholder awareness of the need to develop novel and safe ways of optimizing GL in the coming decades.

2. Green life at fruit level

2.1. Green life ending and occurrence of the auto-catalytic ethylene process

As clearly depicted by Dominguez and Vendrell (1993), the ripening of climacteric fruits such as banana shows a rapid rise in respiration and autocatalytic ethylene production. In these fruits, ethylene is considered the trigger because exogenous ethylene induces ripening and inhibition of its synthesis prevents it. They established that the regulation of ethylene biosynthesis seems to depend on ACC (1-aminocyclopropane-1-carboxylic acid) availability, but also on the capacity of the tissue to convert ACC to ethylene.

The pathway for ethylene biosynthesis was clearly depicted in apple, and other fruits such as avocado, banana, and tomato by Kende (1993). At the onset of fruit ripening, the expression of multiple ACC synthase genes is activated, resulting in increased production of ACC, which is then oxidized to ethylene by ACC oxidase. In most cases, it is the ACC synthase activity that

88 determines the rate of ethylene biosynthesis. In banana fruit, ethylene biogenesis in the naturally
89 ripening fruit is initiated by some factors involved in the regulation of the ageing process. To
90 establish the succession of events occurring from the green to the ripening stage, and therefore
91 to gain a better understanding of the banana GL ending process, the study by Areas *et al.* (1988)
92 is worth looking at in detail. Ethylene synthesis was enhanced by using protein synthesis
93 inhibitors meaning that these inhibitors affected the equilibrium mechanisms of ripening at
94 tissue level. In fact, the capacity for ethylene production, and therefore the climacteric peak, is
95 often associated with the integrity of the cell membrane integrity. Maintenance of the ethylene
96 concentration in the fruit tissues under its threshold level during fruit GL might ultimately be a
97 result of an intensive repair process that is highly dependent on protein synthesis (Areas et al.,
98 1988). The biosynthesis of ethylene from methionine involved two key steps: *i*) the ATP
99 (Adénosine-TriphosPhate) break, which affects the conversion of methionine into S-
100 adenosylmethionine (SAM) and *ii*) ACC oxidase activation, which affects the conversion of
101 ACC into ethylene. After harvesting of the fruit, ACC and ethylene production are low in
102 banana fruit during the pre-climacteric phase. About 0.20 $nl.g^{-1}.h^{-1}$ of ethylene and 0.25 $nmol.g^{-1}$
103 ACC were detected in immature banana during the pre-climacteric period (Li and Huang,
104 1988) and their levels remained nearly constant up to the onset of ripening. At the onset of
105 ripening, meaning the end of GL, ethylene production and ACC levels increase markedly, with
106 ethylene production reaching a peak before the respiratory climacteric (Li and Huang, 1988).
107 Trace amounts of endogenous ethylene may be responsible for the gradual elevation of ACO
108 (ACC-oxidase) activity during the pre-climacteric stage (Gao *et al.*, 1990).

109

110 *2.2. Changes in biochemical compounds throughout fruit GL*

111

There are only a few papers dealing with changes in fruit composition throughout the GL period. There are 7 stages in the banana fruit ripening process, with GL covering the first two stages with, for the Cavendish *cv.*, a sugar content close to 0%, a constantly high starch content (23 to 25%) and CO_2 respiration ~ 4 mg $CO_2.kg^{-1}.h^{-1}$ (Mees, 2017). The only visible parameter is chlorophyll declining gradually during the green life period and making the peel turn a deep green to green with a faint hint of yellow.

There is no starch hydrolysis during GL and thus we could not relate the changes in fruit GL to histological changes such as starch hydrolysis (Jullien *et al.*, 2001). The water-soluble tannin content of banana pulp decreased and then increased during ripening (Chang and Hwang, 1990), while that of banana peel decreased continuously during storage, especially during ripening. A possible relationship between critically low levels of free indole-3-acetic acid (IAA), the onset of starch degradation and therefore banana GL, was suggested by Purgatto *et al.* (2002). In relation to starch degradation during banana ripening, IAA levels can be considered as one of the regulatory factors, or at least a compound that can efficiently delay the onset of the ripening process.

2.3. Impact of exogenous factors on the auto-induction of ethylene synthesis

2.3.1. Relative humidity storage effect.

With storage at 20 °C, the impact of water loss on GL (d) could be estimated by linearization, the resulting equation being GL (d) = 22.15 – [5.3 x % Weight Loss per day] ($p < .001$) (Littmann, 1972). As a result, for a shipping time of ~10 d and a total fruit weight loss of 10% after shipping, GL was reduced from 22 to 16 d. Finger *et al.* (1995) also confirmed the

effects of water loss on banana fruit GL (*Musa accuminata* Colla), a water loss of 5% leading to a shortening of GL in pre-climacteric fruits. In fact, ethylene production in pre-climacteric fruits was stimulated by water stress, causing an increase in respiratory metabolism, such as observed in vegetative tissues. It is likely that the ethylene induced by water stress triggered autocatalytic ethylene production by the fruit and the climacteric rise of respiration. Indeed, it is commonly accepted that maintaining fruits at a low relative humidity (RH) accelerates water loss and also causes earlier changes in respiration and ethylene production (Burdon *et al.*, 1994). A gradual increase in the ACO activity of pulp and peel stored at a high RH was also observed.

2.3.2. Storage temperature effect.

At room temperature, fruit respiration is maintained at the normal level, but if the fruit is stored at a lower temperature, fruit respiration will be reduced, resulting in a decreased of ATP, hence reduced ethylene biosynthesis. The purpose of storage temperature control is therefore to limit fruit respiration by suspending ethylene biosynthesis, thereby extending fruit post-harvest life, including green life. Fruit GL bears an inverse linear relationship to the temperature at which fruit are kept. An average regression equation established on the average of 4 experiments was determined (Wills *et al.*, 2001) as GL (day) = [- 0.97 x θ] + 93 (θ storage temperature in °F). Thus, at 55 °F (12.8 °C) and 59 °F (15 °C), the expected GL was estimated at 40 and 36 days respectively, meaning that a 1 °F increases during storage reduces potential GL by one day. Blake and Peacock (1971) extended these former findings to higher temperatures (60 °F (15.5 °C) to 96 °F (35.5 °C)) and obtained the following regression equation GL (d) = $10^{[(-0.02529 \times T\,°F) + 2.898)]}$ meaning that at 15.5 °C (60 °F) the estimated GL of the fruit was 24 days. A 12 days difference in GL was therefore obtained applying the two

158 regression equations mentioned above, highlighting the precaution that should be taken if using
159 it.

160 The high sensitivity of banana fruit to the duration and intensity of low temperatures that
161 could provoke chilling injury (Jackman *et al.*, 1988) should be kept in mind at all times to
162 effectively match the constraints in the banana supply chain. Indeed, considering the shipping
163 duration (usually 10 to 20 days), hence the long cold-stress induced, the air-shipping
164 temperature of the reefer at 12.5-13 °C should really be considered as a critical value, hence a
165 limit for possibly extending GL. With a view to curtailing post-harvest losses and extending
166 fruit availability, Gonge *et al.* (2013) studied the combined effect of harvest maturity stages and
167 storage temperatures throughout the shipping of bananas (cv. Grande Naine). The greater the
168 physiological age is at harvest the lower is the GL (Table 1). Indeed, harvesting bunches at 90%
169 of the total maturity allows the GL to increase to 2 or 3 days, this behaviour being confirmed
170 whatever the storage temperature (14 or 16°C). Even if potentially interesting in terms of GL
171 extension, 12 °C implies chilling injury and is therefore not recommended. Joas (1987)
172 estimated the potential GL reduction at 7-8% of GL for a 1 °C higher storage temperature,
173 whatever the maturity at harvest, and therefore partially confirmed the data previously
174 presented (~ 4 days lost from 12 to 14 °C storage T °C). As a conclusion at this point, the faster
175 the fruit can be cooled after harvesting, then the longer would be the expected GL, the fruit
176 being less sensitive to any ethylene present in the post-harvest chain (Turner and Fortescue,
177 2012).

178 Table 2 presents an overview of the regression equations established to estimate the
179 potential GL of banana depending on the limiting temperature and CO_2 conditions. Averaging
180 the equations resulting from different studies, at 13°C the estimated GL is ~35 days and is
181 reduced by 3 days for a 1 °C increase in storage, which tallies well with the 7-8% GL time
182 reduction for 1 °C higher proposed by Joas (1987).

183

184 2.3.3. Light effect.

185 Although not specifically demonstrated for banana, it has been reported for many years
186 that light decreases the amount of ethylene produced (Sisler and Wood, 1988). The level of
187 ethylene production could be stimulated above the dark level when both CO_2 and light were
188 supplied. While maintaining the fruit at an ethylene concentration below 0.005 ppm, banana
189 GL was reduced by ~50% when the fruit was exposed to light. The response did not seem to be
190 dependent on the physiological age at harvest and was greatest when exposure started
191 immediately after harvest (Peacock, 1972). Although this aspect is not relevant for bananas as
192 they are of course shipped in boxes in the reefer, therefore in the dark, this tendency could help
193 in understanding fruit green life behaviour in relation to mechanisms induced by ethylene.

194

195 2.3.4. Effect of O_2 and CO_2 concentration during storage.

196 The submitted hypothesis in view of the apparent role of CO_2 in facilitating the
197 conversion of ACC into ethylene was that illuminated thylakoid membranes also convert ACC
198 into ethylene, and that this conversion can be stimulated by added bicarbonate. However, since
199 this reaction only occurs in the light, it is presumably free radical-mediated and non-enzymatic.
200 The review by Sisler and Wood (1988) tried to clear up the controversial effect of CO_2, finding
201 that low levels of carbon dioxide regulate ethylene synthesis and higher levels inhibit ethylene
202 action. It seems, in fact, that CO_2 counteracts the effects of ethylene in many cases but it can
203 also stimulate ethylene production by increasing the amount of ACC compared to ethylene, and
204 by increasing the activity of that enzyme (Bufler, 1986).

205 The importance of oxygen concentration in the storage atmosphere for controlling banana
206 fruit ripening is very important. It is indeed possible to prevent any synthesis of ethylene, above
207 the minute amounts found emanating from unripe fruit, by storing the fruit in oxygen
208 atmospheres containing not more than 7.5% oxygen at the normal ripening temperature (18 °C).
209 Under such conditions, the oxygen tension is sufficient to prevent either injury and will not
210 impart taste or flavour to the fruit.

211 Combined storage in reduced oxygen and increased carbon dioxide reduced respiratory
212 activity during storage and thereby boosted banana GL. The lowest weight loss was established
213 at 2% O_2 and 4 to 8% CO_2, with the weight loss for 14 storage days being reduced by more than
214 37% using this controlled atmosphere (Hailu *et al.*, 2013). We can confirm that this specific gas
215 composition helps to optimize banana GL during storage. Indeed, Jedermann *et al.* (2014b)
216 confirmed that elevated CO_2 (5%) and low O_2 (2%) concentration in storage vessels led to an
217 increase in average green life of 4 d at 15 °C and 11 d at 18 °C.

218 To obtain these required O_2 and CO_2 target values, use of the controlled or modified
219 atmosphere is the only way of maintaining this gas composition throughout the shipping phase.
220 Modified atmosphere (MA) packaging, created by the respiration of the fruit itself inside a bag
221 having specific O_2/CO_2 permeability, is recognized as an easy and inexpensive method of
222 extending banana GL compared to the controlled atmosphere. Chamara *et al.* (2000) used Low
223 Density Polyethylene bags (LDPE) as a packaging system to extend storage life, thus creating
224 a modified atmosphere able to extend the storage life of banana (cv. Kolikuttu). Stewart *et al.*
225 (2005) improved this system by using silicone membrane and diffusion channel systems to
226 provide suitable conditions for optimum storage of Giant Cavendish bananas. As a result, they
227 achieved levels of 3.5% CO_2 and 3% O_2 in about 10 d using this membrane, with these gaseous
228 conditions being rather close to optimum conditions for banana (2% O_2 and 4 % CO_2, Hailu et
229 al. (2013)).

230 Table 3, extracted from the study by Jedermann et al. (2014b), can be considered as a
231 good summing up of the relative humidity, temperature and modified atmosphere effect at
232 reefer level. Using extreme conditions, namely 18 °C in dried air as opposed to 15 °C in a wet
233 atmosphere at 5% CO_2, reduced fruit GL to around one third of its original potential.

234 Considering that the RH inside Low Density Polyethylene bags during the shipping of
235 fruits with a conventional perforation density of 0.01 $dm^2.m^{-2}$ (4 holes in a conventional
236 polybag), is higher than 99%, each additional degree during storage from 15 to 18 °C reduces
237 GL by 5 d or more. The detrimental effect of a low RH is indeed again highlighted, since at 15
238 °C the measured banana GL will drop from 48 to 42 d in normal air at a 50% RH. Lastly, the
239 positive effect of a high CO_2 concentration (5%) in terms of GL extension seems to be more
240 intense at a high storage temperature (18 °C).

241

242 2.3.5. Effect of ethylene concentration during storage.

243 Ethylene, which is auto-catalytically synthesized at levels as low as 0.01 $\mu l.L^{-1}$, triggers
244 the ripening process (Prasanna *et al.*, 2007). It should be noted that while this observation is
245 commonly accepted, the threshold of ethylene concentration likely to initiate the ripening
246 process is debatable. Indeed, the ethylene concentration needed to initiate banana fruit ripening
247 is highly dependent on the physiological age at harvest (sometimes called maturity at harvest)
248 and on the storage temperature. In fact, it is unambiguous that there is no threshold of ethylene
249 concentration for ripening, but instead that several factors such as ethylene contact time. The
250 physiology of the fruit at harvest or the temperature background are the key factors delimiting
251 fruit GL (Wills *et al.*, 1999). Indeed, for a long time, a lower ethylene limit that could initiate
252 fruit ripening was discussed and the limit of 0.1 ppm (Inaba and Nakamura, 1988) was
253 commonly accepted in the banana chain for many years. The study by Wills et al. (2001)

confirmed banana to be one of the most ethylene-sensitive climacteric fruits. By fixing two limits, a 10% loss in storage life that could be tolerable in most marketing situations, and a 30% loss that could limit marketing opportunities, the authors established a regression equation at 20°C for banana GL (d) = [- 9.24 x Surrounding Ethylene Concentration] + 3.3. It appears therefore that the marketing limit (30%) is reached with less than 0.03 ppm, while the tolerable marketing limit (10% loss) will be at less than 0.01 ppm. Note that this value is not so far from the limit of detection used in this paper, which was 0.005 ppm. Furthermore, the authors established that 0.1 ppm, formerly accepted as a limit ethylene value, provoked a ~50% reduction in GL under these conditions.

A reduction in surrounding ethylene concentration as a trigger to maintain or extend banana GL was extensively studied by Wills et al. They investigated the impact of a wide range of ethylene concentrations, ranging from rather high to ultra-low, during storage using different banana cultivars from Australia (Wills et al., 1999). It appeared that, whatever the cultivar, GL at 20 °C does not exceed 4 days at 1 ppm of ethylene, while rising to ~ 11 d at 0.1 ppm, ~24 d at 0.01 ppm and, lastly, more than 33 d at 0.001 ppm. However, these extremely low ethylene concentrations are not reachable in the food chain, even using specific ethylene absorbers in the cardboard box, or at reefer level. Wills and Golding (2015); Wills *et al.* (2014) quantified the relationship between ethylene concentration, temperature and GL of Australian-grown Cavendish bananas. Fruits were stored at 15, 20 or 25 °C in air containing 1.0, 0.1, 0.01 or 0.001 ppm ethylene, and green life was taken as the number of days to reach the respiratory climacteric. They found that banana green life decreased with increasing storage temperatures and ethylene concentrations according to the relationship: GL (d) = 5.74 - (21.81 x X) - (0.82 x X^2) - (0.097 x Z) + (0.55 x Z), where X = log of ethylene concentration (ppm) and Z = storage temperature (°C). These authors concluded that it would not seem unreasonable to expect that

keeping bananas at a low ethylene concentration would give a similar post-harvest life as at low temperatures in current commercial situations, where ethylene levels can readily accumulate.

3. GL determination and forecasting

If we consider that GL is the time interval between the harvest date and the initiation of the climacteric phase. This associated end-phase is therefore correlated both with an increase in respiratory intensity with CO_2 exhausted from 10-20 mg $CO_2.kg^{-1}.h^{-1}$ to 60-120 mg $CO_2.kg^{-1}.h^{-1}$, and with degreening of the peel associated with chlorophyll degradation. Any determination methods are based on these physiological changes. Chillet *et al.* (2008) detected the climacteric peak of a banana placed in a semi-permeable container, through an analysis of O_2 and CO_2 levels resulting from fruit respiration. This method allowed accurate measurement (± 1 d) of GL compared to methods based on fruit colour or decrease in texture. Anyway, the successive phases: ethylene auto-induction and respiration peak, and green colour and texture decreasing range no more than 2 d, this time day difference being far less the GL distribution among the fruits of a single bunch.

New spectrometric methods have been developed to limit the two main limitations of the methods described above. These spectral approaches are not destructive and can therefore be applied directly on the fruit still attached to the bunch in the orchard; they give an immediate response while the former method needs, by principle, to wait for the end of GL. Among criteria for determining the end of green life, the parameter NDVI (normalized difference vegetation index) was measured with a hand-held spectrometer (Pigment Analyser, CP Falkensee, Germany) (Praeger *et al.*, 2013). NDVI indicates chlorophyll content in plant material and is calculated from the reflected light intensity I in the following manner: $NDVI = (I_{780\,nm} - I_{660\,nm})$

/ ($I_{780\ nm}$ + $I_{660\ nm}$) (Richardson *et al.*, 2002; Zude *et al.*, 2009). The NDVI values for green bananas at ripening stage 1, just after harvest, vary from 0.3 to 0.2, while at the end of GL NDVI falls between +0.05 and -0.05. This pigment analyser, which gives the NDVI value, could therefore be considered as a potential tool for directly and instantly giving a good estimation of the potential GL of the fruit in the orchard. It should however be validated on large fruit samples including different physiological ages at harvest, disease severity or shipping conditions.

4. GL optimization in the orchard

In general, any action that involves physiological stress on a fruit causes a reduction in the pre-climacteric period, which can be associated with increased ethylene production resulting from the stress. Extremes of temperature, fungal infection, exposure to light, dehydration and mechanical injury are all common causes of stress during normal commercial operations that reduce the pre-climacteric period of banana.

4.1. Pre-harvest factors affecting GL in the field

4.1.1. Nutrients and agronomic conditions management.

Excess nitrogen produces crops with smaller fingers and bunches, and reduced their conformity. Bunches can be poorly filled and have a reduced GL if harvest occurs to late (Robinson, 1996). For instance, Srikul and Turner (1995) reported that fertilising with 450 kg $N.ha^{-1}.year^{-1}$ increased the relative fruit growth rate of cultivar "Williams" (Cavendish

subgroup) bananas by 7% ($P < 0.05$) and hasten the relative loss of GL by 46% ($P < 0.05$). From 0 to 300 kg $N.ha^{-1}.year^{-1}$, N had no significant effects on fruit growth or GL. Robinson (1996) also mentioned that excess of manganese in leaves leads to a reduction in green shelf-life, thus contributing to the disorder known as "ripe mixture". Lastly, calcium, known to increase the shelf-life of some fruits, such as apples does not seem to be efficient on bananas (Tixier *et al.*, 2010). Lastly, calcium is known to strengthen cell tissue and thus can increase the shelf-life of some fruits, such as plum fruits (Sinha *et al.*, 2019). However, it does not seem to be the case with bananas as Tixier et al. (2010) showed that applications of calcium in the field, by fertilization or spraying, had no influence on the GL of Cavendish and CIRAD 920 hybrid bananas.

As revealed by Mahmoud and Elkashif (2003), plant spacing has a significant impact on fruit GL. The wider spacing resulted in significantly longer green life than the closer spacing in both the plant and ratoon crops. This was because plants grown at a wider spacing produced large, well developed bunches with large fingers which were more likely to have a longer green life than those produced at a closer spacing.

4.1.2. Water stress.

Water management is one of the first factors to consider in expressing the potential of banana. Excess water weakens fruit and may result in a short GL (Lassoudière, 2007). Conversely, a water deficit imbalances the fruit growth/development ratio, thus fruits will be often harvested at a higher physiological stage which means a lower GL. Srikul and Turner (1995) reported that a soil water deficit can reduce GL by 48% ($P < 0.05$) in a semi-arid environment. Indeed, exposure of the roots to drying soil changes the chemistry of the fruit and

347 could eventually cause a small increase in respiration rate.

348

349 4.1.3. Management expertise.

350 Among pre-harvest factors that could reduce fruit GL, an important necrotic leaf area
351 and/or a low leaf/fruit ratio on the plant are the two main parameters. Indeed, when this ratio is
352 unfavourable, it will take longer to reach the minimum commercial fruit size, fruit harvesting
353 will be consequently delayed and finally GL reduce

354 In a study of the factors affecting the post-harvest life of plantains, Ferris *et al.* (1993)
355 found that out of the genotype, mechanical damages is the most significant factor that affect
356 GL at the pre-harvest stage. Indeed, increasing peel permeability to water vapour, this exposes
357 the fruit to increased water stress, which initiates early ripening.

358

359 *4.2. GL and harvest point optimization*

360 Physiological age could be a good indicator of the fruit GL at harvest (Jullien *et al.*, 2008).
361 For a long time, the harvest point was determined by the fruit grade measurement directly on
362 the bunch or by the fruit age in number of weeks after flowering (Marin *et al.*, 1996). For
363 instance, the minimum Cavendish fruit grade (diameter measured in the middle section
364 perpendicular to the plane) in the French West Indies is 32 mm but it can rise to 36-39 mm for
365 the *Musa Goldfinger* variety with an adequate GL (Thompson, 2011). These methods
366 unfortunately do not take into account the seasonal effect, temperature variation or altitude. An
367 approximate linear relation (R^2 =-0.87) between GL and the bunch development period of
368 different cultivars and clones was already established (Ganry and Meyer, 1975). The established
369 regression coefficient for the cv. Valery was meaning, for instance, that for harvesting 95 d

after flowering, the estimated fruit GL is 28 d at 13.5 °C and that a one-week delay in bunch harvesting would reduce GL by approximately 3 d.

On the basis of the harvest thermal-sum method (i.e. daily sum of average temperature at the orchard since inflorescence emergence with a zero basis at 14°C) (Umber *et al.*, 2011), these former limitations could be avoided. An harvest point really representative of the outer temperature and of the minimum Cavendish growth temperature could be provided. To ensure a GL of not less than 25 d at 98% RH, the recommended thermal sum for Cavendish is 900 ± 50 dd (degree.day) (Ganry and Chillet, 2008). Jullien et al. (2001) confirmed that fruit GL correlates well with bunch age expressed in dd accumulated since inflorescence emergence. In actual production conditions, the harvest determination method used in the FWI to ensure EU banana export potential is a compromise between the grade measurement and thermal sum methods. Indeed, to ensure an optimized yield, but avoiding any risk of ripening in the reefer during sea-shipping, the thermal sum is only used as a limit not to be exceeded, but bunches could be earlier harvested if the grade of the fruit reaches the required value.

4.3. GL optimization at bunch level

Banana bunch covering using thin plastic (low density polyethylene) at flowering is a physical protection method that improves the visual quality of fruit by promoting skin coloration and reducing blemishes. It can also change the micro-environment for fruit development, which can have several beneficial effects on internal fruit quality (Santosh *et al.*, 2017). Jullien et al. (2008) showed that a difference of 2 °C between the air temperature inside and outside the bag was measured. They also showed that plastic bags induce a difference in accumulated dd between older fruits at the top of a bunch and younger fruits at the bottom. The

394 earlier the bagging date, the larger is the difference in dd, and therefore in GL. Research reports
395 on fruit bagging have given contradictory information on the effect of bagging on both the
396 physical and compositional quality of fruits. Kimani *et al.* (2017) mentioned that bunch covers
397 did not significantly influence GL ($p>0.05$).

398 The solution of 'sealed' bunch cover should be emphasized since this hermetic bag adds
399 the effect of modified atmosphere (MA) (Johns and Scott, 1989a). Atmospheres of 1.5-3% O_2
400 and 5-10% CO_2 have been claimed to be optimum for modified atmosphere within the sealed
401 bag storage of pre-climacteric bananas at 20 °C. However, the MA requirements of growing
402 bunches could differ markedly from the MA storage requirements of harvested fruit (Johns and
403 Scott, 1989a). Unfortunately, leakage is almost unavoidable, even under shed storage
404 conditions and consequently, the selective permeability of the polyethylene will be offset by
405 the non-selective nature of the leaks (Wade and Graham, 1987). The bunch/bagging effect on
406 GL was improved by Johns and Scott (1989b), who established that the inclusion of KMnO4
407 in sealed covers, creating a modified atmosphere at bunch level, increased GL by an average of
408 8 d.

409

410 *4.4. Seasonal effect*

411 According to Marin et al. (1996) and despite of similar grade and age, GL was different
412 between fruit harvested at different times of the year. Fruit harvested at the hot and dry season
413 had a longer green life than those harvested during “rainy” winter in Costa-Rica. Seasonal
414 transition seems to have an impact on the reduction of the pre-climacteric period for fruit
415 developing rapidly initially and maturing slowly (summer/winter transition). The effect of
416 climatic factors was later studied by Bugaud *et al.* (2007), who did not find any correlation
417 between climatic conditions and the GL of bananas harvested at a constant physiological age.

These results well matched with those previously obtained by Bugaud *et al.* (2006); Chillet *et al.* (2006), who showed that climatic variations had no significant impact on the GL of bananas harvested at the same temperature sum. It seems, in fact, that the shortening of GL during the hot wet season was probably more due to the higher Sigatoka disease pressure during that season.

4.5. Black Sigatoka disease management

Sigatoka Disease (SD) and Black Leaf Streak Disease (BLSD), caused by the pathogenic fungi *Mycosphaerella musicola* and *Mycosphaerella fijiensis*, respectively, are the only biotic stress in the field known to have an impact on GL (Chillet et al., 2006). Both pathogens affect banana leaves, thus reducing the photosynthetic surface area, but also affect fruit physiology and reduce post-harvest GL, up to field-ripe fruit in case of higher infestations (Castelan *et al.*, 2012). A number of studies were conducted to understand this effect, but until recently most of them had been conducted on SD. As BLSD is more aggressive, and is becoming predominant in most tropical countries (De Lapeyre de Bellaire *et al.*, 2010), a few specific studies are available on its relation with GL.

4.5.1. A reduction related to disease damages.

A reduction in GL is often related to an indirect effect of SD, through the reduction of leaf area. The fewer leaves present on the plant during the flowering-harvest period, the lower the GL is. A minimum of 3 to 4 leaves at harvest for minimum quality fruit and to ensure fruit shipping potential is commonly recommended. Ramsey *et al.* (1990) and Daniells *et al.* (1994) even

441 stated that a minimum of 10 leaves at harvest was necessary to prevent premature ripening, with
442 all bunches having fewer than 4 viable leaves at harvest were field-ripened. This share
443 decreased logistically as the number of viable leaves at harvest increased up to 10 leaves. A
444 reduction in the number of leaves below 6 leaves after flowering through severe leaf pruning
445 was also linked to a reduction in GL by Robinson *et al.* (1992). As a consequence, the number
446 of leaves at the harvesting stage seemed a practical criterion that is easy to control in the field
447 for evaluating potential fruit GL, and for limiting the ripening risk during transit (Ramírez *et*
448 *al.*, 2008). Until now, exporters have often determined a number of leaves as a threshold
449 (usually 4 to 5 viable leaves at harvest) beyond which fruit should not be exported, or the harvest
450 grade must be reduced, to supposedly avoid fruit ripening during transit. However, this criterion
451 has proved to be highly empirical, and has often led to unsuitable decisions.

452 The qualitative descriptions of SD levels related to GL reduction was known for a long
453 time. Premature ripening occurred at very low spotting levels, at least eight leaves without
454 spotting should remain at harvest to ensure an optimized GL (Stover, 1974). Yet, this level is
455 seldom reached under production conditions, and only in regularly sprayed fields and/or under
456 dry climatic conditions. However, it should be noted that in most of these first studies, fruits
457 were harvested following a calliper grade threshold and/or the number of weeks between
458 flowering and harvest, without considering their physiological age. It is possible that, in some
459 cases, fruits from infested plants took more time to reach harvest grade, and thus were
460 considerably more mature than indicated by size and appearance at the time of harvest, with a
461 lower potential GL.

462 Taking into account the physiological age of the fruit at harvest by the thermal sum
463 method, Ganry and Chillet (2008) proved that quantitative evaluations of the disease level were
464 more correct in predicting GL than the qualitative or indirect effects of the disease. Chillet *et*
465 *al.* (2009) confirmed that SD had a direct effect on banana GL through the harvesting of fruits

466 at the same physiological age. Under these conditions, GL decreases as SD pressure increases,
467 even though the fruits have an identical diameter, proving that the reduction in GL is not caused
468 by a trophic effect, but rather by a direct effect of the disease. The best disease level indicators
469 for GL are quantitative: the disease severity indexes at flowering and at harvest. Even though
470 BLSD is more aggressive than SD, the same results were obtained with BLSD, with no greater
471 reduction in GL than for SD (Castelan et al., 2012). A linear negative relationship between
472 disease parameters and GL was further established for SD by Castelan *et al.* (2013): the SD
473 effect is gradual and continuous, with no threshold effect. Such work has not yet been conducted
474 for BLSD, but it can be assumed that the same observation would be made.

475 In addition to the severity of the disease, the duration of the disease is also critical for its
476 impact on GL. Under southern tropical conditions in Brazil, with a longer flowering-harvest
477 period (FHP), the reduction in GL was drastic in plots highly infested by both SD and BLSD,
478 with only field-ripened fruits at the time the bunches reached 960 dd (Castelan et al., 2012).
479 Thus, the longer the disease stays on the plant, the greater is the reduction in GL.

480

481 4.5.2. Potential of agronomic practices as a tool to limit this stress.

482 Some sanitary practices, such as deleafing (or leaf pruning), which consists in
483 systematically eliminating all necrotic parts of the leaves by cutting them off, proved to be
484 efficient in limiting the negative effect of SD and BLSD on GL. Necrotic leaf removal from
485 SD-infested plants led to a large reduction in SD effects on fruit quality, preventing premature
486 ripening (Chillet *et al.*, 2013). This technique allowed the harvesting of bunches with as few as
487 3 leaves at the time of harvest. The same observation was made by Guillermet *et al.* (2016) on
488 BLSD under both humid and dry tropical conditions. Under these conditions, strict elimination
489 of necrotic tissue, and adequate cultural practices including optimal plant growth and harvesting

490 time, allowed the exporting of bunches with as few as no leaves at harvest, without ripening in
491 transit.

492 These results show that systematic removal of necrotic parts of leaves can be an efficient
493 technique for limiting production losses. It furthermore reveals that the presence of necrosis
494 from SD or BLSD is responsible for GL reduction, whatever the number of leaves at harvest.
495 Thus, this criterion might not be the most appropriate for evaluating bunch suitability for export.
496 It also highlights the existence of a potential "stress signal" emitted by necrosis, and directed
497 to the bunch, responsible for the reduction in GL. The longer this signal is emitted, hence the
498 longer the necrosis stays on the plant, the greater is the reduction in GL (Castelan et al., 2012).
499 The timing of the signal also seems critical.

500

501 4.5.3. Markers of GL reduction and hypothesis on the nature of the stress.

502 For both SD and BLSD, GL reduction is associated with an altered maturation process in
503 the fruit. Although the initial rate of ethylene production is identical in fruits from infected or
504 uninfected plants, fruits from infested plots show higher emissions of CO_2 and C_2H_4 production
505 at the time of the climacteric peak (Castelan et al., 2012). This elevation is higher for BLSD
506 than for SD. BLSD is also involved in modifications to the starch metabolism during ripening,
507 and the enzymatic activities linked to its degradation (Saraiva *et al.*, 2013).

508 So far, only hypotheses can be put forward regarding the origin of this "stress signal".
509 The synthesis of metabolites by the fungus could have a direct impact on fruit physiology. Some
510 toxins, such as juglone, are secreted by *M. fijiensis* and involved in the BLSD infection process
511 (Chuc-Uc *et al.*, 2011; El Hadrami *et al.*, 2005). Ethylene is also produced by diseased tissues,
512 but is unlikely to be transported very far in the plant, and is probably quickly lost in the air.
513 Secondary plant metabolites involved in host-pathogen relationships could also impact fruit

514 ripening, as has been shown for methyl-jasmonate and salicylic acid on other pathosystems
515 (Fan *et al.*, 1998). Lastly, changes in hormone balances, such as indole-3-acetic acid and
516 gibberellic acid following pathogen infection, may also have an effect on fruit quality (Saraiva
517 et al., 2013). It is probable that these factors are not exclusive, but act in synergy on processes
518 leading to GL reduction.

519 To conclude, Sigatoka diseases are an essential factor to consider in most banana
520 producing countries in order to manage fruit post-harvest quality. A better understanding of
521 changes in the physiological metabolism induced by these diseases could enable adequate
522 practices, in order to avoid the stress or interrupt its transmission to the fruit. More generally,
523 additional work is needed on BLSD in order to quantify its impact on GL, and potentially adapt
524 management practices.

525

526

527 **5. Post-harvest fruit treatment to optimize GL potential**

528

529 *5.1.Fruit damages and post-harvest fungal diseases effect*

530

531 Peel abrasion was proved to be negatively correlated to fruit GL (Santana Llado and
532 Marrero Dominguez, 1997). These authors varied mechanical injury by peeling the fruit surface
533 at different degrees of severity (0, 1 and 4 cm^2) and observed GL shortening, which was also
534 reflected in the onset and peak production of ethylene. Again, the stress generated by the

mechanical injuries could impact the oxidative status of the fruit, enhance cell membrane degradation and, as a consequence, reduce the potential fruit GL.

Several fungi at the post-harvest stage also have the potential to produce ethylene and therefore have a negative impact on fruit GL. The *in vitro* ethylene production capacity of *Colletotrichum musae*, the main agent causing banana anthracnose (De Lapeyre de Bellaire and Mourichon, 1997), was confirmed and explained by Daundasekera *et al.* (2003); Fukuda *et al.* (1993). In fact, it seems that the fungus has a dual effect: producing ethylene itself, but also acting as a stress factor and indirectly inducing ethylene biosynthesis in the plant (Bleecker and Kende, 2000). Daundasekera et al. (2003) suggests the ability of *C. musae* to use host banana tissue to provide substrates for ethylene production but, at this point, the relative contribution of pathogen-produced ethylene versus host system I and system II (autocatalytic) ethylene production in this pathogen-host relationship is not known and remains to be studied. The fungal and fruit peeling injury effect is worth discussing with regard to oxidative protein degradation and to the subsequent auto-catalytic fruit ripening process. Indeed, it can be suggested that any external peel aggression could be translated as stress factors, thereby triggering auto-catalytic ethylene synthesis and consequently resulting in reduced fruit GL potential.

5.2. Hormonal compounds and gas treatment

Auxins, kinins and gibberellins have been found to regulate aging processes in many plants, including fruits. Particularly, dipping whole banana fruit in gibberellic acid (GA_3) solution was found to postpone the climacteric peak rise and therefore to extend GL (Archana and Sivachandiran, 2015; Osman and Abu-Goukh, 2008). A concentration of the dipping

559 solution at 1.10^{-4} M was proved to increase GL from 13 (without GA_3) to 18 d at 20 °C and
560 under saturating RH conditions. It should, however, be pointed out that the concentration of
561 GA_3 actually fixed in the tissue is not known, with peel and mainly cuticular wax acting as a
562 hydrophobic barrier. Huang and Jiang (2012), using another plant growth regulator,
563 forchlorfenuron (CPPU), increased by 2 d the GL of banana stored at 25 °C and at 75-95% RH.
564 The combined effects of CPPU (phenylurea type molecule) and GA_3 were also studied by
565 Huang *et al.* (2014). The most beneficial effect was obtained by dipping the fruits in a mixture
566 composed of 10 $mg.L^{-1}$ of CPPU and 50 $mg.L^{-1}$ of GA_3: The treatment delayed the occurrence
567 of the peak of respiration but had a steady ethylene production rate at the green stage. The
568 molecular mechanism was not known and is still to be proved.

569 Nitrous oxide (N_2O), although not tested on banana but on other climacteric fruits
570 (tomato, avocado), also blocks or delays ACC and ACO activity, and could be worth
571 considering (Gouble *et al.*, 1995). N_2O, which is an anaesthetic, is chemically neutral and non-
572 toxic and therefore clearly differs from the very toxic but efficient ethylene oxide in extending
573 GL (Williams *et al.*, 2003). As a consequence, autocatalytic ethylene evolution is inhibited and
574 GL therefore extended. Again, although this treatment is promising, it should be considered
575 with regard to food legislation, which does not accept this gas treatment for fruits and
576 vegetables.

577

578 *5.3. 1-Methylcyclopropene (1-MCP) treatment*

579

580 An exciting new strategy for controlling ethylene production, and thus fruit ripening and
581 senescence, especially of climacteric fruits, as well as the senescence of vegetative tissues, has
582 emerged with the discovery and commercialization of the inhibitor of ethylene perception, 1-

583 methylcyclopropene (1-MCP) (Jiang *et al.*, 2004; Watkins, 2006). 1-Methylcyclopropene is a
584 cyclopropene derivative initially used as a synthetic plant growth regulator. It is structurally
585 related to the natural plant hormone ethylene and is used commercially to slow down the
586 ripening of fruit and to help maintain the freshness of cut flowers. The use of cyclopropenes to
587 inhibit ethylene action was patented by Sisler and Blankenship (1996). Macnish *et al.* (2000)
588 established an extension of the GL of bananas stored at 20 °C when treated with 15 $\mu L.L^{-1}$ 1-
589 MCP and subsequently exposed to ethylene. Jiang *et al.* (1999b) showed that exposure for 24
590 h at 0.5 or 1 $\mu L.L^{-1}$ 1-MCP at 20 °C extended GL from 16 to 31 d compared to control fruits.
591 Jiang *et al.* (1999a) demonstrated that 1 $\mu L.L^{-1}$ 1-MCP for 1 h at 20 °C delayed the ripening
592 effects induced by 100 $\mu L.L^{-1}$ ethylene. Lastly, Golding *et al.* (1998) showed that the
593 effectiveness of 1-MCP on banana fruit was closely related to its colour stage. A commercial
594 breakthrough in 1-MCP application technology resulted from the formulation of 1-MCP as a
595 stable powder in which it is complexed with γ-cyclodextrin, so that 1-MCP is easily released as
596 a gas when the powder is dissolved in water (Edgington *et al.*, 2013; Zhang *et al.*, 2013).

597 Despite good results for 1-MCP delaying fruit softening (Bagnato *et al.*, 2003), soluble
598 solid accumulation and colour development, an uneven ripening of peel following treatment
599 with 1-MCP has been reported (De Martino *et al.*, 2007; Harris *et al.*, 2000). This uneven
600 ripening of the peel appears when 1-MCP is applied after ethylene treatment to bananas that
601 have not completely lost their green peel colour, and results in a dull-grey appearance.
602 Moreover, these symptoms are often combined with areas on the peel that remain green, similar
603 to blotchy ripening of tomatoes. It has been observed on banana fruit treated with ethylene at
604 ripening stage 2, before or after 1-MCP treatment, that respiration was completely inhibited by
605 1-MCP, whereas ethylene was slightly affected and this response affected the quality
606 characteristics of bananas on a different level (DeMartino *et al.*, 2004). In particular, the
607 occurrence of the yellow colour was completely delayed whenever the 1-MCP treatment was

608 carried out. Similar results, but with a lower response, were obtained when treating bananas
609 with 1-MCP at a more advanced ripening stage.

610 It therefore clearly appears that using 1-MCP in the banana food chain will present many
611 drawbacks in terms of logistical *vs.* quality improvement:

612 - Gassing fruit with 1-MCP at the green stage after harvesting at the production site
613 causes some fruits, depending on their maturity on harvesting, to display severe
614 visual defects (uneven green discoloration) when ripening is induced by ethylene
615 after shipping, whatever the ethylene concentration and contact time (Feygenberg *et*
616 *al.*, 2010).
617 - Gassing fruit with 1-MCP at colour stage 2 (light green) after ripening induction
618 with ethylene is effective in greatly slowing down and even blocking fruit ripening.
619 As the fruit cannot be consumed at this ripening stage, this option is not acceptable
620 (DeMartino et al., 2004).
621 - Gassing fruit with 1-MCP at colour stage 3-4 (green-yellow) after ripening
622 induction with ethylene is not effective in slowing down ripening (DeMartino et al.,
623 2004).

624 As a conclusion, since the effectiveness of 1-MCP varies with fruit maturity, and in any
625 commercial consignments there is a mixture of fruit maturity, 1-MCP has limited commercial
626 potential for the storage of unripe Cavendish bananas (Harris et al., 2000). The study by Golding
627 et al. (1998) confirmed that the application of 1-MCP significantly delayed and disrupted
628 degreening, even when propylene was applied continuously, but that incomplete and uneven
629 yellowing of the peel was a feature of fruit treated with 1-MCP fruit. Indeed, although yellowing
630 is initiated by ethylene, completion of the process involves enzymes whose biosynthesis may
631 be irreversibly disrupted by 1-MCP.

632

633 *5.4. Fruit coating and dipping*

634

635 Recently, increased efforts have been made to develop coatings using new preservative
636 compounds that could generate a modified atmosphere by creating a semipermeable barrier
637 against oxygen and carbon dioxide (Kudachikar *et al.*, 2011). Among them, Maqbool *et al.*
638 (2011b) applied an edible coating composed of 10% gum Arabic plus 1% chitosan. Coated
639 fruits had far fewer cracks, showed a smoother surface, and fruit GL was extended for up to 33
640 d. This aqueous dispersion of sucrose esters of fatty acids, and the sodium salt of
641 carboxymethylcellulose, indeed modifies the internal atmosphere of the fruit and delays the
642 subsequent climacteric phase. Lastly, coating with Arabic gum was improved using 0.4%
643 cinnamon oil (Maqbool *et al.*, 2011a). Indeed, by controlling *Colletotrichum Musae*, the main
644 fungus encountered after the harvest on banana, these authors indirectly preserved potential
645 banana GL (cf. § 5.1.). Basil oil was also shown to maintain banana GL by controlling fungal
646 attacks that could develop during shipping (Anthony *et al.*, 2003; Siriwardana *et al.*, 2017).

647 As salicylic acid has been shown to affect the biosynthesis and action of ethylene, the
648 effects of salicylic acid were explored (Srivastava and Dwivedi, 2000). By dipping the fruits in
649 500 µM of salicylic acid for 6 h, this compound acted as an ethylene antagonist and prolonged
650 fruit GL by up to 3 d at 25 °C.

651 Lastly, although heat treatments (thermotherapy) have been used for over a century to
652 free plant materials from pathogens, this post-harvest treatment of fruit could also be used to
653 modify fruit responses to other stresses and maintain fruit quality during storage (Paull and
654 Chen, 2000). The detrimental effect on final fruit quality was, however, not really highlighted

655 in these studies; heat treatment at more than 55 °C definitely *degrades* the subcellular cuticular
656 wax layer and therefore increases peel browning at the ripening stage.

657

658 **6. GL optimization throughout the banana cold chain**

659

660 *6.1. Reefer, pallet box and carton levels scale*

661

662 Although palletized bananas have mainly been transported on dedicated vessels in the
663 past, transportation is now shifting to containers on third-party vessels. While such
664 transportation enables a more flexible reaction to fluctuating market volumes, shipping by third-
665 party vessels in rented refrigerated containers (reefers) can cause more quality-related
666 problems, unless adequate care is taken to maintain optimum transport conditions (Jedermann
667 et al., 2014b).

668 Refrigerated containers, or "reefers", as they are regularly known, are of particular interest
669 to shipping companies and similar organizations, because they permit relatively high freight
670 rates and margins that are not possible with dry containers. The temperature settings of the
671 reefer are prescribed in a range of 13 to 18 °C. In the case of goods with a high water content
672 and 2nd order biotic activity (BA 2), such as fruit and vegetables in which respiration processes
673 predominate, ventilation has not only to ensure that temperature and humidity requirements are
674 met, but also gas exchanges. Ventilation ensures that the necessary oxygen is supplied and that
675 harmful gases (carbon dioxide, ethylene) are removed.

676 The supply chain of the banana trade is a typical cold chain. The banana farm is the
677 starting point of the banana cold chain, and the final customer is the end point (Figure 1 from
678 Chen and Notteboom (2012)).

679 As bananas are exported over long distances, requiring days of sea shipment to reach the
680 ripening location and thereafter the consumer location, fruit physiology and exogenous gas
681 sensitivity need to be addressed jointly with the industrial chain and logistical requirements.
682 Indeed, it takes between 5 and 35 d for bananas to reach the final customers, depending on the
683 area considered. If any bananas ripen during transportation, they not only cannot be sold, but
684 also endanger the whole container of bananas. Jedermann *et al.* (2013); Jedermann *et al.*
685 (2014a); Jedermann et al. (2014b); Praeger et al. (2013) evaluated some of the suggested
686 innovations integrating logistical food chain constraints. Ethylene management should be
687 particularly discussed regarding the structure of the cold chain and integration of the
688 "neighborhood" of each entity (cluster, carton, pallet box). Indeed, during shipping, each
689 banana cluster cannot be individually considered, as there are other clusters in the same polybag
690 that very probably come from another bunch and therefore have a different physiology and fruit
691 maturity at harvest. This consideration can be scaled up to the carton or pallet box levels: each
692 one impacting on and being impacted by its neighbors in terms of the temperature and gas
693 composition of the surrounding atmosphere. Inside the box, the bananas are usually protected
694 by a polyethylene bag to prevent dehydration. Indeed, post-harvest packaging of fruits in
695 cartons lined with polyethylene film resulted in a longer GL (Elkashif *et al.*, 2010). The bag
696 may either have some small holes to allow penetration of a controlled atmosphere (CA) into the
697 box, provided by the ship, or may be completely sealed to generate a higher CO_2 concentration
698 and lower O_2 concentration by respiration of the fruit.

699 Controlling the temperature, CO_2, O_2 and ethylene concentrations at reefer, pallet box and
700 carton levels is a real challenge and numerous studies and innovative technologies have been
701 developed to achieve the required parameters to optimize banana GL.

702

703 *6.2. Modified Atmosphere Packaging (MAP)*

704

705 MAP is one of the most accepted methods for extending the shelf life of perishable and
706 semi-perishable food products by altering the relative proportions of atmospheric gases
707 surrounding the produce. However, the high capital cost of gas packaging machinery to control
708 the atmosphere at the reefer scale has limited the use of this technology. This gives rise to the
709 concept of active packaging (Sen *et al.*, 2012) Besides being independent of package
710 permeability and gas packaging equipment, this packaging system is more rapid and accurate
711 than traditional MAP. The concept of active packaging is one of the emerging technologies in
712 food packaging. It has been defined as a system in which the product, the package and the
713 environment interact in a positive way to extend GL or to achieve some characteristics that
714 cannot be obtained otherwise (Miltz *et al.*, 1995). The active system can be an integral part of
715 the package or be a separate component placed inside the package and different substances
716 capable of either absorbing or releasing a specific gas control the atmosphere inside the
717 package. Active packaging components can work as either an absorbing or releasing system.

718

719 *6.3. GL optimization at carton level*

720

721 Polyethylene bags are extensively proposed as a trigger, not only to protect fruits from
722 external damage but also as a technology to delay ripening of bananas during storage and
723 thereby increase GL. Commonly, potassium permanganate was added to these polyethylene
724 bags as an ethylene absorbent to delay the potential GL even more. The most popular method
725 at carton level is the oxidation of ethylene by potassium permanganate ($KMnO_4$), adsorbed on
726 an inert carrier with a large surface area, such as silica gel, alumina, and activated carbon. The
727 stripping chemical mechanism is rather simple, as described here: $3\ C_2H_4 + 12\ KMnO_4 \rightarrow 12$
728 $MnO_2 + 12\ KOH + 6\ CO_2$. Considering the low basal level of ethylene *vs.* the CO_2 (~ 1 ppm of
729 ethylene / 4 % of CO_2) concentration, the release of 2 moles of CO_2 for 1 mole of ethylene does
730 not have a great impact on the controlled atmosphere composition (Mangaraj and Goswami,
731 2009). Chamara et al. (2000) extended the GL of bananas cv. Kolikuttu' from 4 to 20 d at 25 °C
732 and 85% RH by packing them in LDPE bags (75 µm) complemented with ethylene scavenger.
733 Thus, GL could be greatly boosted using a modified atmosphere naturally obtained through the
734 equilibrium between natural fruit respiration and PELD permeability and ethylene scavengers.
735 Consequently, packing bananas as individual hands in LDPE (0.075 mm) bags with a 1:1
736 surface to weight ratio ($cm^2.g^{-1}$) containing 50 mL of saturated potassium permanganate
737 absorbed onto suitable porous matrices could be recommended to increase GL.

738 Passive modified atmosphere (2% O_2 and 5% CO_2) packaging of bananas completed with
739 an ethylene scrubber was found to increase fruit GL five-fold compared to fruits stored without
740 wraps. Again, this gas composition is a result of the tryptic: fruit quantity/surface area of
741 available film ratio, CO_2 exhausted at a given temperature, and gas permeability of the PEHD
742 polybag. The combined effect of ethylene and CO_2 scrubber in the polyethylene bag during
743 fruit shipping at 13 °C was tested by Chauhan *et al.* (2006). The increase in GL was only 2 d
744 more using the two absorbers, while ethylene absorber alone led to an increase in GL from 8 to
745 13 days. It appears that the concentration of ethylene surrounding the fruit is really the more

746 strategic parameter to be controlled during shipping until the initiation of the ripening stage, to
747 ensure an optimized GL.

748 Another approach was using only material permeability to ensure the required atmosphere
749 during shipping to maintain the potential GL. These materials included Banavac®, also called
750 unvented polybag (Narayana *et al.*, 2002) which consists of 0.4 mm thick polyethylene bags in
751 which the carbon dioxide content is raised to 5%, the oxygen content is reduced to 2%
752 ("modified atmosphere"). The ethylene that arises is absorbed by adding potassium
753 permanganate. However, Banavac® bags must be torn open before the ripening can be initiated,
754 the high CO_2 concentration accumulating during ripening making this action necessary. The
755 need to rip open the bags before gassing results in added labor costs.

756 In recent years, laser micro-perforation of the polybags has been the most promising
757 technology to ensure a passive but steadily modified atmosphere. Indeed, these
758 micro-perforations allow for the transmission of oxygen, CO_2 and ethylene gases into and out
759 of the polybags (Varriano-Marston, 2008). Given the ethylene permeability of this wrap, this
760 technology provides a package that does not have to be ripped open to initiate the ripening
761 cycle. This approach was further optimized by Balasubramanian *et al.* (2013b), who provided
762 a polymeric film, which comprised one or more copolymers of ethylene with a polar monomer,
763 in which the oxygen transmission rate of the said wrap was 8,000 to 16,000 $cm^3.h^{-1}$. These
764 perforated plastic bags could display non-uniform perforation density with the density of the
765 lower zone being greater than that of the upper zone (Balasubramanian *et al.*, 2013a).

766

767 *6.4. Temperature and RH control at reefer level*

768

769 At the industrial level in the reefer, Jedermann et al. (2014b) found an exponential
770 function for the relationship between storage temperature T and the average GL for the
771 temperature range between T = 12 °C and T = 30 °C: GL (d) = 159.86 $e^{-0.124\,T}$ in normal air
772 within a polyethylene bag et 98% RH. At an optimum storage temperature of 13 °C, the green
773 life lasted about 30 d and for each 10 °C temperature increase, the green life was reduced by a
774 factor of Q_{10} = 3.46. Green life decreased to one week at a storage temperature of 25 °C. The
775 parameters of this equation should be modified depending on whether fruits are stored with or
776 without CO_2 control. Thus, it becomes GL (d) = 122.14 $e^{-0.09\,T}$ if the CO_2 level reaches 5% or
777 more, meaning the estimated fruit GL at 15 °C rises from 25 d to 32 d at 5% CO_2 (Jedermann
778 et al., 2013; Mees, 2017). Still at reefer level, Lang *et al.* (2011) proposed an interesting
779 synthesis of the temperature, RH and CO_2 effect on banana GL during sea exports from Latin
780 America to Europe (Table 3).

781 While the high sensitivity of banana fruits to ethylene was shown at laboratory level
782 (individual banana fingers or clusters), the management and optimization of GL at reefer level
783 needs to consider all the key parameters as a whole. Indeed, and as an example, one 18 kg box
784 of yellow bananas produces enough ethylene in 2.2 d to increase the concentration in the whole
785 container with a free air volume of 47.4 m^3 to a critical threshold of 0.1 ppm, if the air flaps are
786 closed, and no ethylene is released into the atmosphere (Jedermann et al., 2014b).

787 Controlling, optimizing and estimating the internal fruit temperature at reefer level is a
788 real challenge. The speed of the cooling process depends largely on the quality and age of the
789 cooling equipment, but surprisingly there is no preferred location for the hottest point inside the
790 container (Jedermann et al., 2014b). As an example, after 8 d of shipping with a set-point fixed
791 at 13.9 °C, the temperature difference could reach 18 °C in an old reefer while being only 14.5-
792 15 °C in a new one. Cooling efficiency is therefore a key parameter that needs to be clearly
793 defined. Furthermore, trucks or rail cars are frequently not refrigerated, so bananas only start

794 their cooling process once loaded into the vessel hatch. Cooling delays can range from 4 to 36
795 h. This cooling delay is frequently considered one of the causes of premature ripening in transit.

796 Delays in cooling can have a marked influence on banana ripening during subsequent
797 transit (Madrid, 1996). For fruit exposed to 12 h of cooling delay (30 °C instead of 14 °C, steady
798 RH at 95%), the GL duration dropped by 12%, while GL was reduced by ~20% for fruit exposed
799 to 24 h of cooling delay. Average shipment and delivery times from the tropics to the ripeners
800 are around 18 – 20 days, so that losses of 4 to 6 days of green life can have a significant impact
801 on the 'ripe and turning' incidence.

802 The Cargo Loss Prevention Information Handbook (Handbook, 2003) is very useful for
803 knowing the temperature rise during banana transport if the cooler system of the reefer is
804 damaged. As an example, 20 t of bananas that are temperature stabilized at 13 °C in a reefer
805 will have their temperature reach 16.5 °C after only 48h. Given the impact of temperature rises
806 on banana GL, this damage will undoubtedly provoke fruit ripening in the reefer, but also tend
807 to increase the average temperature of the ship. To avoid such uneven ripening initiation, Haass
808 *et al.* (2015) developed the so called "Intelligent Container". This Intelligent Container is able
809 to calculate the green life of its cargo and this leads to the feasibility of quality driven
810 distribution. Thus, banana losses should be decreased and both transport costs and carbon
811 emissions should be reduced even further. The use of chlorophyll fluorescence in fruit and
812 vegetable storage (HarvestWatch^{TM}) could be a good solution for optimizing the O_2
813 concentration in a dynamic controlled-atmosphere (DCA) (Prange *et al.*, 2012).

814

815 *6.5. GL optimization through innovative technology at reefer level*

816

817 Temperature-related problems are mainly caused by an imbalance between generated and
818 removed heat. Jedermann et al. (2013) identified the width of the gaps between pallets as the
819 major influence on the spatial temperature profile. The best cooling was achieved with a 4 cm
820 wide gap between pallets and a gap from the wall, therefore meaning that optimized stowage
821 of pallets is needed, but also that the reefer's wall should not be flat but present instead some
822 regular notches. Although developed on citrus fruit, the study by Defraeye *et al.* (2015a) is
823 worth discussing. The existence of gaps between the pallets led to significant airflow short-
824 circuiting, which reduced the cooling rate at all box heights on the pallet, but it improved
825 cooling uniformity between the boxes to some extent. Simulations showed that low airflow
826 rates typical for refrigerated containers do not only induce slower fruit cooling, compared to
827 Forced-Air Precooling (FAC) airflow rates, but also the cooling heterogeneity between
828 different layers of boxes (in height) and between individual fruits in a single box is greater. In
829 addition, the existence of gaps between pallets invokes airflow short-circuiting, leading to
830 greatly reduced fruit cooling rates.

831 Optimized airflow circulation to reduce ethylene concentration inside and outside the box
832 and thereby maintain banana GL is achieved with fan optimization at the polybag, box and
833 pallet levels. Indeed, *i*) polyethylene bags should not have fewer than 4 pinholes (0.01 $dm^2.m^{-2}$)
834 for 20 kg of banana fruit; *ii*) boxes are provided with perforations to ensure proper flow of
835 cooling air (perforation not less than 8% of the total area) and these boxes should be perfectly
836 aligned to ensure airflow passage; *iii*) pallet stowage should be optimized. To optimize
837 ventilation using carton perforations, Sanabria *et al.* (2003) improved the ventilation pattern by
838 developing a shade box with the usual plurality of ventilation apertures, but with those apertures
839 being in communication when cartons were regularly stowed, thereby advantageously
840 improving airflow through an entire palletized load of containers.

As maintaining market quality is of vital importance for the success of the banana industry, it is necessary not only to cool the product, but to cool it as quickly as possible after harvest. Since the rate of respiration is influenced by temperature, precooling to remove the field heat before storage will reduce the respiration rate, hence deterioration will decline accordingly (Brosnan and Sun, 2001). The process of precooling is the removal of field heat, which arrests the deterioration process, so as to maintain a high level of quality that ensures customer satisfaction (Brosnan and Sun, 2001). Forced air cooling was developed to accommodate products requiring relatively rapid removal of field heat immediately after harvest. Forced air or pressure cooling is a modification of room cooling and is accomplished by exposing packages of produce to a higher air pressure on one side than on the other. This technique involves particular stacking patterns and the baffling of stacks, so that the cooling air is forced through (rather than around) the individual containers. Consequently, a relatively small pressure difference between the two sides of the containers exists, resulting in good air movement and excellent heat transfer, hence faster cooling.

The economic cost of this high-speed temperature reduction should however be pointed out. If it is essential that the heat load be removed quickly, whatever the cooling technique used.

A poorly ventilated, or worse still, unventilated container of bananas with rapid respiration rates can quickly change the atmosphere, reversing the normal levels of oxygen and carbon dioxide in less than 24 hours. New reefer containers are bottom-air delivery units, which means that air is constantly supplied from the bottom of the container through the T-bar floor. Cool air circulates through and around the cargo as it absorbs cargo heat. In order to remove the gas generated by respiration, thereby reducing ethylene concentration surrounding the fruit and maintaining GL up to 28 days, it is recommended that fresh air be exchanged from the outside to the inside at a rate of 30 $m^3.h^{-1}$ for banana.

865 Although closely related to ventilation efficiency, dedicated reefers have been developed
866 to specifically reduce exogenous ethylene. The main requirement during storage identified by
867 the agrifood sector is 'online' monitoring of ethylene in the ppb range with low-cost devices in
868 the storage chambers (Janssen *et al.*, 2014). Indeed, if gas sensing systems for CO_2 fruit
869 monitoring in containers were already available, they would enable more reliable predictions
870 of GL by measuring the ethylene concentration inside a container. The "intelligent" container
871 developed by Lang et al. (2011) might be a solution to this challenge. The online control of
872 ethylene, hence GL, seems to be such a challenge that Herdeman (1997) proposed bypassing
873 this challenge by only reducing ethylene once the bananas were stabilized at the desired holding
874 temperature.

875 Different methods of ethylene removal (ventilation, catalytic oxidation, photochemistry
876 under UV−C light, and $KMnO_4$) were evaluated (Lawton, 1991). As ambient levels in the sea
877 transport environment rarely exceed 0.01 ppm, and as the best available method for the removal
878 of ethylene has so far been ventilation with air, the costs and benefits of ethylene removal
879 technologies are worth evaluating (Keller *et al.*, 2013). Compared with the other ethylene
880 oxidation methods, the photocatalytic oxidation process can be considered as a very promising
881 and reliable technology, combining the efficiency of catalytic oxidation with both low energy
882 consumption and low heat load resulting from UV irradiance. Using TiO_2, photocatalytic
883 processes chemically oxidize organic compounds into carbon dioxide and water at near-ambient
884 temperatures to complete mineralization. It has proven to be an efficient method for removing
885 ethylene from horticultural storage facilities in order to extend the postharvest storability of
886 produce, and can further be used in combination with other means, such as cold storage,
887 controlled atmospheres, or modified atmosphere packaging (Keller et al., 2013). Since
888 photocatalytic material is not consumed during the oxidation process, there is no need for the

889 replacement and disposal of depleted treatment units. It can be applied to both large and small-
890 scale storage units and used for low and high ethylene concentrations.

891

892 ## 7. Conclusion

893

894 This review definitely brought out the need to manage GL as a whole, meaning that from
895 the orchard to unripe banana delivery after shipping, many preventive actions should be taken
896 and optimized parameters observed. Indeed, the auto-induced climacteric peak could be
897 considered as an internal physiological factor mainly dependent on cultural practices when
898 external conditions modulate these ripening mechanisms.

899 Table 4 proposes several recommendations and correlated banana chain good practices to
900 ensure optimum GL in the orchard, packing-house and reefer. While not exhaustive, this table
901 tries to clearly depict the key parameters and how they should be managed. To be really
902 effective in optimising fruit GL, these "good practices" should all be adopted totally, in order
903 to achieve a synergetic effect up to ripening induction by exogenous ethylene in the ripening
904 room.

905 For these best practices to be applicable to all other regions, good control of both Black
906 Sigatoka and harvesting points is required in any event, whatever the country of origin and the
907 context. Post-harvest technologies should be considered as a trigger and not as a ready-to-apply
908 solution.

909 As an overall perspective for the future, it would seem that an integrated approach is
910 needed, considering GL as the challenge. Indeed, whatever the production area and the transit

911 time, a continuum between producers, post-harvest methods and shipping arrangements will be
912 necessary to counteract how Black Sigatoka affects GL.

913 As technologies evolve, stakeholders will be able to pilot the post-harvest life of the fruit
914 using intelligent containers that would adapt the composition of the atmosphere and the
915 temperature to the cargo. In any event, research is required to try and estimate fruit GL after
916 harvest as accurately as possible, using spectral and non-invasive technologies for instance.
917 This will considerably change all our approaches to GL optimization and will considerably
918 reduce fruit losses after shipping.

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TABLE 1. Effect of maturity and storage temperature on banana green life (d) (cv. Grande naine) (taken from Gonge et al. (2013)).

Level of maturity	**Storage T °C**			**Mean green life (d)**
	12 °C	***14 °C***	***16 °C***	
75%	35	28	21	28
90%	29	24	16	23
100%	26	21	14	20

1194

1195 **TABLE 2.** Regression equations of estimated green life (GL) duration (d) according to the range of temperatures and

1196 the CO_2 concentration.

Regression equations	**Reference**	**Estimated GL at 13 °C**	**Estimated GL at 15 °C**	**Limiting conditions**
GL (d) = [- 0.97 x T °F] + 93	Peacock and Blake (1970)	40	36	13 < T °C < 22 Normal air
GL (d) = $10^{[(-0.02529 \times T\ °F) + 2.898)]}$	Blake and Peacock (1971)	32	25	15.5 < T °C < 35.5 Normal air
GL (d) = $159.86\ e^{-0.124\ T\ °C}$	Jedermann et al. (2014b)	32	25	12 < T °C < 30 Normal air
GL (d) = $122.14\ e^{-0.09\ T\ °C}$	Mees (2017)	38	32	12 < T °C < 30 CO_2 > 5%

1197

TABLE 3. Green life[a] of Cavendish bananas harvested in spring 2012 and stored under different relative humidity (RH), T °C, and CO_2 concentration conditions (taken from Jedermann et al. (2014b)).

	Effect of RH			**Effect of elevated CO_2**	
RH	**50-60% normal air**	**80% normal air**	**98%, < 2% CO_2**	**98%, < 2% CO_2**	**98%, 5% CO_2**
T 15 °C	42 ± 4	37 ± 5	48 ± 2	57 ± 1	**61 ± 2**
T 18 °C	**23 ± 7**	28 ± 4	32 ± 2	30 ± 4	49 ± 17

[a]only hand-top position GL is presented.

1208

1209 **TABLE 4.** Summary of recommendations and correlated banana chain good practices to ensure optimum green life at the orchard, packing-house
1210 and reefer levels of intervention.

Levels of intervention	Recommendations(s)	Good practice(s) to set up	Key publication(s)
At orchard level	Limit stress induced by fertilization and drought (when harvest at a specific fruit size/grade)	Low soil N supply and no soil water deficiency	Srikul and Turner (1995)
	Control of Sigatoka diseases	Elimination of necrotic parts of the leaves	Chillet et al. (2013)
	Optimisation of the harvest point: compromise yield and GL	Harvest stage at 900 ± 50 dd	Ganry and Chillet (2008)
At packing house level	Avoid peel abrasion after harvest	Do not injure the fruits during de-handing and cluster fruit preparation steps	Santana Llado and Marrero Dominguez (1997)

	Control fungal attack	Use natural or chemical treatments that prevent or limit *Colletotrichum Musae* during shipping	Daundasekera et al. (2003)
	Limit ethylene exhausting as much as possible	If possible, pre-cool fruit immediately after harvest	Defraeye *et al.* (2015)
		Limit cooling delay (< 4 h)	Madrid (1996) Herdeman (1997)
	Reduce ethylene accumulation + Control the temperature at 13.5 ± 0.5 °C	Optimise ventilation at pallet and reefer level	Jedermann et al. (2014b) Jedermann *et al.* (2013)
		Shade storing of the reefers	Rodríguez-Bermejo *et al.* (2007)
	Limit water loss	Maintain RH > 95-98%	Jedermann et al. (2014b)
At ripening room level	Avoid heterogeneous ripening induced by "ethylene pollution"	Proceed to the ripening step without delay	Jedermann *et al.* (2014a)

1211

Itemized list of Figures:

FIGURE 1. Schematic representaion of the banana cold-chain (from Chen and Notteboom (2012)).

FIGURE 2. Theoretical pathway and controlling parameters of banana GL.

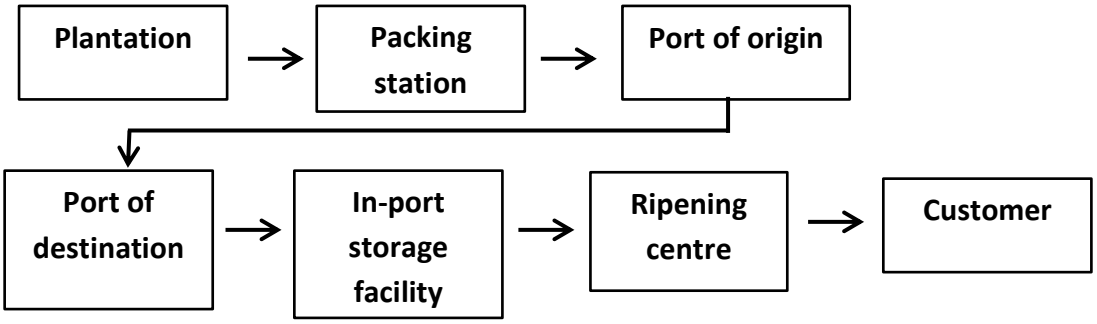
Plantation
Packing station
Port of origin
Port of destination
In-port storage facility
Ripening centre
Customer

FIGURE 1.

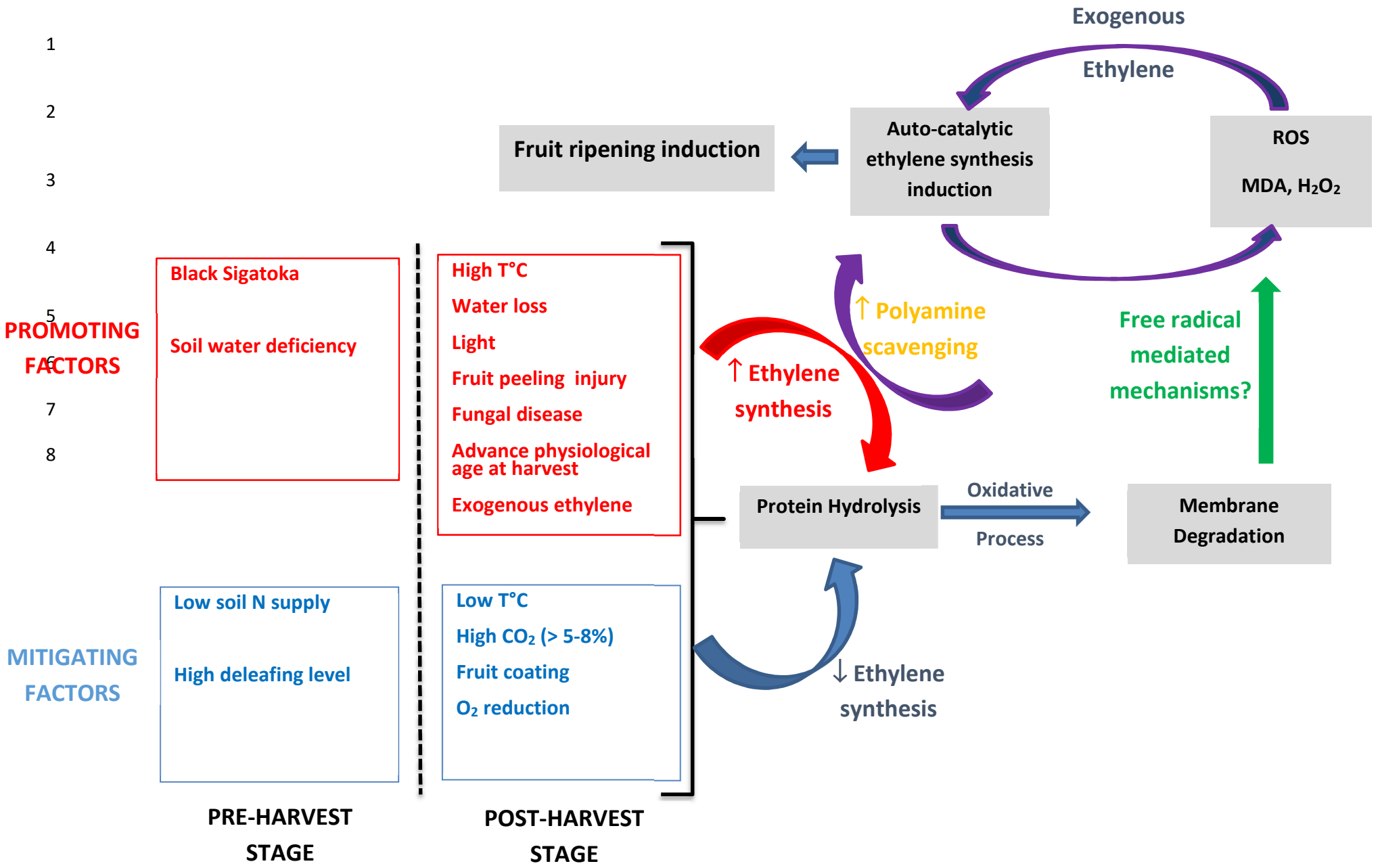
Exogenous
Ethylene
Fruit ripening induction
Auto-catalytic ethylene synthesis induction
ROS
MDA, H_2O_2
PROMOTING FACTORS
Black Sigatoka
Soil water deficiency
High T°C
Water loss
Light
Fruit peeling injury
Fungal disease
Advance physiological age at harvest
Exogenous ethylene
↑ Polyamine scavenging
↑ Ethylene synthesis
Free radical mediated mechanisms?
Protein Hydrolysis
Oxidative
Process
Membrane Degradation
MITIGATING FACTORS
Low soil N supply
High deleafing level
Low T°C
High CO_2 (> 5-8%)
Fruit coating
O_2 reduction
↓ Ethylene synthesis
PRE-HARVEST STAGE
POST-HARVEST STAGE

FIGURE 2