Research Article

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Preharvest temperature affects chilling injury in dessert bananas during storage

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Abstract

BACKGROUND: The effect of temperature on chilling injury during fruit growth was studied in a new banana hybrid CIRAD925 in which seasonal variability in chilling susceptibility was observed when fruits were stored at 13 °C.

RESULTS: The relationship between the response to chilling (presence/absence) and the temperature during banana fruit growth was examined with a logistic regression model. An explanatory variable $X_{N,P}$ was defined as the mean temperature during a period, expressed in weeks, which began *N* week(s) after flowering and lasted *P* week(s). The model was calibrated with 143 bunches with a green life of 30 ± 5 days and validated with 156 bunches grown in six plots under different growing conditions. Chilling injury was best predicted by the mean temperature during the period beginning 1 week after flowering and lasting 5 weeks ($X_{1,5}$). Above a mean temperature of 24.1 °C in the period concerned, banana fruits had a 95% probability of chilling injury at 13 °C. Below a temperature of 23.4 °C, banana fruits only had a 5% probability of chilling injury.

CONCLUSION: The results provide a tool to predict chilling susceptibility in banana fruit whatever the thermal conditions in tropical regions.

Keywords: *Musa*; chilling injury; temperature; logistic regression; predictive model

INTRODUCTION

Chilling injury is the term used to describe physiological damage to fruit tissues resulting from the exposure of chilling-sensitive fruits to temperatures below a critical threshold.[1] In banana (*Musa* spp.), chilling injury can occur in unripe or ripe fruit and may reduce fruit quality and market value. Symptoms of chilling injury become apparent after a few days of storage at the injurious chilling temperature or after transfer of fruits to non-chilling temperatures. When chilling is not severe, which is true in the majority of cases in the conditions used by the banana export industry, the green fruit develops surface lesions such as pitting in the first layer of the green epicarp and the color of the peel becomes dull yellow to grayish-yellow or gray during ripening.[2] In ripe fruits, the characteristic banana flavor may fail to develop or an off-flavor may develop instead.[2] In their review, Aghdam *et al.*[3] summarized the biological processes caused by chilling injury as follows: low ratio of unsaturated fatty acids to saturated fatty acids, low levels of cell metabolic energy, the increased activity of the enzymes responsible for the degradation of unsaturated fatty acids increases damage to the cell membrane because of higher membrane lipid peroxidation and ultimately reactivates accumulation of oxygen species, all of which has an effect on susceptibility to chilling injury.

In the Cavendish and Gros Michel groups, chilling injury symptoms develop at temperatures below 12.5 °C.[4] To avoid this problem, bananas are shipped at a controlled temperature of 13–14 °C. The CIRAD925 (C925) hybrid was recently created in the framework of the banana breeding program by CIRAD, a French research center working with developing countries to tackle international agricultural and development issues. The hybrid chilled during shipping from the French West Indies to Rungis market, Paris, France in reefers regulated at 13 °C. Even more surprising, chilling susceptibility varied over the course of the year and the temperature during fruit growth appeared to be involved. In Guadeloupe, chilling tolerance was observed during the cool season (January–April) and in highland plots, whereas chilling injury occurred during the hot season and in lowland plots. To our knowledge, only Mattei[2] and Dadzie and Orchard[5] mentioned a link between chilling susceptibility and origin/growing conditions, but without directly establishing a link with temperature.

The objective of the present study was thus to investigate the effect of the preharvest temperature on the chilling susceptibility

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of banana fruit stored at 13 °C, and in particular to identify the fruit growth stage that is the most impacted. The relationship between preharvest temperature and response to chilling injury (presence/absence) was calibrated using a data set obtained from one plot and validated with a data set obtained from six other plots under different growing conditions. Our results provide a tool to predict chilling susceptibility based on the thermal conditions found at all production sites in the Caribbean.

MATERIAL AND METHODS

Experimental conditions

The cultivar used for this study was CIRAD925 (*Musa acuminata*, AAA group) (hereinafter C925). This cultivar was produced using conventional breeding techniques,[6] including diploid genitors previously doubled with colchicine.[7] It was selected particularly for its resistance to the leaf spot diseases *Mycosphaerella fijiensis* and *Mycosphaerella musicola*.[8] To calibrate the model, trials were carried in a CIRAD plot at Capesterre-Belle-Eau in Guadeloupe from December 2010 to August 2012. The weekly temperature in the field ranged from 22.3 to 26.1 °C. To validate the model, banana trials were conducted from November 2011 to January 2013 in six plots belonging to private farms in Martinique and Guadeloupe with quite different soil and climate conditions (Table 1). In these trials, the weekly temperature varied more (from 21.2 to 28.7 °C) than in the calibration trials. The cultivation practices (suckering, bunch trimming) were identical throughout the study period. All bunches were packed in polyethylene bags as soon as all female hands were identified (about 15 days after flowering). Depending on the amount of rainfall, 4–5 mm of water per day was supplied by drip irrigation. Mean daily temperatures were recorded with a temperature sensor (Tynitag, Gemini Data Loggers Ltd, Chichester, UK) placed inside a cylindrical PVC tube (internal diameter 8 cm) fixed 1.2 m above the ground in each plot. For each harvested bunch, the flowering date was recorded at bud burst, that is, when the inflorescence is pendulous and the bracts have not yet opened, along with the harvest date. Since no reference values were available on the optimal harvesting stage of C925, harvesting was carried out over a range of flowering-to-harvest periods lasting from 9 to 14 weeks. Only bunches with a green life of 30 ± 5 days were used for this study. The fruits were considered to have the same physiological age irrespective of the climatic conditions affecting the banana plants.[9] The number of harvested bunches and the flowering-to-harvest time are listed in Table 1 for each plot.

Measurement of chilling injury

The fourth proximal hand of each bunch was rinsed and immersed in fungicide (thiabendazole, 500 mg L^{-1}) for 1 min. Two median fruits were used for green life measured at 20 °C[10] and two other median fruits were used to measure chilling injury. The fruits were covered with a plastic bag with 20 mm respiration holes to prevent drying and placed on racks. The racks were stored in a climatic chamber at 13 ± 0.5 °C for 10 days to simulate transport conditions. At the end of this period, the fruits were removed and immediately examined. Visual symptoms of chilling injury were recorded after peeling the first layer of epicarp of the green fruit. Visual symptoms were characterized by cylindrical or lenticular brown or black pits (<1 mm in length). Fruits were identified as chilled when the density of pitting was >1 pit cm^{-2} and as not chilled when the density was <1 pit cm^{-2}. In 96% of the fruits with symptoms of chilling injury, the density was >50 pits cm^{-2}, indicating severe chilling injury.

Table 1. Geographical characteristics of plots

Plot	Location	Altitude (m a.s.l.)	Type of soil	Sampling period	Minimum weekly temperature (°C)	Maximum weekly temperature (°C)	Number of samples	Flowering-to -harvest time (weeks)	Used for
CIRAD	16° 04′ N 61° 36′ W	250	Gibbsite andosol	Dec 2010 – Aug 2012	22.3	26.1	143	9–14	Calibration
DUHAUMONT	14° 49′ N 61° 02′ W	20	Brown rust to halloysite	Nov 2011 – Oct 2012	24.6	28.2	32	9–14	Validation
EYMA	14° 50′ N 61° 07′ W	210	Recent andosol	Dec 2011 – Feb 2013	22.8	26.6	58	9–13	Validation
CHANGY	16° 04′ N 61° 34′ W	60	Halloysite to allophanic andosol	Jan 2012 – Oct 2012	24.6	28.7	18	9–12	Validation
SARDE	16° 05′ N 61° 34′ W	60	Ferrallitic	Mar 2012 – Nov 2012	24.7	28.6	28	10–13	Validation
FEFE	16° 03′ N 61° 36′ W	350	Allophanic andosol	Apr 2012 – Feb 2013	21.2	25.3	11	11–14	Validation
GRAND MARIGOT	16° 02′ N 61° 42′ W	500	Andosol	Apr 2012 – Feb 2013	21.7	25.3	9	9–12	Validation

Table 2. Flowering-to-harvest time (FTH) distribution

FTH (weeks)	Number of bunches	Number of bunches with an FTH of at least n[a] weeks
9	6	143
10	30	137
11	25	107
12	52	82
13	18	30
14	12	12
Total	143	156

[a] Ranged from 9 to 14.

Statistical analyses

Calibration model

The relationship between the binary response variable (1, chilling injury; 0, no chilling injury) and the temperature during banana fruit growth was examined with a logistic regression model. An explanatory variable $X_{N,P}$ was defined as the mean temperature during a period, expressed in weeks, which began N week(s) after flowering and lasted P week(s). To give an example, $X_{3,5}$ is the mean temperature calculated for each bunch from the 3rd week after flowering ($N=3$) to the 8th week after flowering ($8=3+5$, with $P=5$); $X_{0,9}$ is the mean temperature from flowering ($N=0$) to the 9th week after flowering ($9=0+9$). For each explanatory variable, a logistic regression model was built using the logit binomial function in the Rcmdr library in the statistical program R 2.15.1. The relative goodness of fit of the statistical model was measured with the Akaike information criterion (AIC). The best model was selected from the lowest AIC. To compare AICs, the degrees of freedom must be the same between models, i.e. the number of individuals (bunches) for each explanatory variable has to be the same. As flowering-to-harvest time ranged from 9 to 14 weeks, the number of individuals per explanatory variable decreased from 143 to 12 (Table 2). AICs were also calculated only for explanatory variables with the following conditions: an N parameter ranging from 0 to 8 and a P parameter ranging from 1 to 9, with $N+P\leq 9$ as the condition for P. The AICs were thus calculated and compared for 45 of the 105 possible explanatory variables.

The cut-off point was set at 0.5. When the probability of chilling injury was above 0.5, fruits were considered to be chilled; when the probability was below 0.5, fruits were considered to be not chilled. The performance of the calibration model was based on sensitivity, specificity and Youden's index. Sensitivity (SE) is the proportion of chilled fruits correctly predicted: SE = CCP/(CCP + CIP), where CCP is the number of chilled fruits correctly predicted and CIP is the number of chilled fruits incorrectly predicted. Specificity (SP) is the proportion of not chilled fruits correctly predicted: SP = NCCP/(NCCP + NCIP), where NCCP is the number of not chilled fruits correctly predicted and NCIP is the number of not chilled fruits incorrectly predicted. The efficacy of the model was tested with Youden's index (YI): YI = SE + SP − 1.[11]

Validation model

The logit function of the best model was validated using the 156 bunches harvested from six plots. The cut-off point was set at 0.5. When the probability of chilling injury was >0.5, fruits were considered to be chilled; when the probability was <0.5, fruits were considered to be not chilled. The robustness of the validation model is given by sensitivity, specificity and Youden's index.

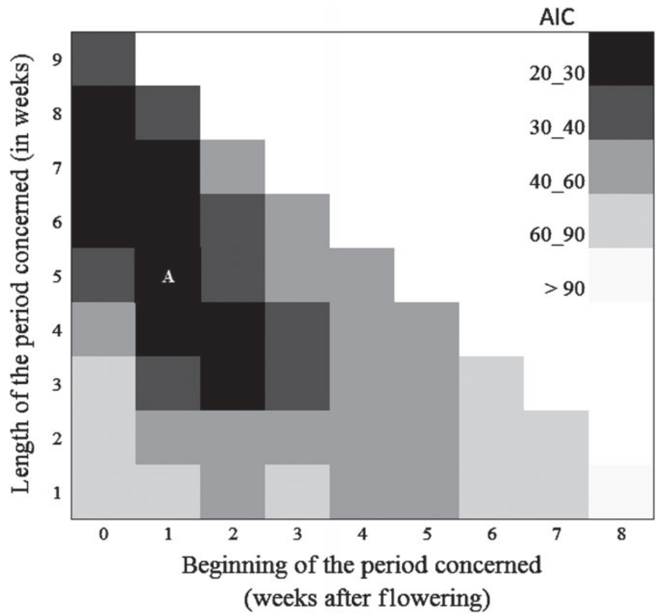

Figure 1. Distribution of Akaike information criterion (AIC) value for 45 explanatory variables. 'A' denotes the explanatory variable with the lowest AIC value (20.7).

Validation in real export conditions

Pallets with 10–30 boxes containing 17 kg per box of C925 bananas were regularly shipped from two plots (EYMA, DUHAUMONT) to FRUIDOR ripener at Rungis market (Paris, France). From November 2012 to May 2014, 16 pallets (11 from EYMA plot and five from DUHAUMONT plot) were shipped in reefers regulated at 13 °C. Upon arrival at the FRUIDOR ripener, two fruits were randomly selected from one of three boxes for measurement of chilling injury.

RESULTS

Best model to predict chilling injury

The AICs obtained from the 45 models ranged from 20.7 to 109.3 (Fig. 1). The lowest AIC (20.7) was obtained for the explanatory variable $X_{1,5}$. The best model to predict the chilling injury used the mean temperature during the period beginning 1 week after flowering and lasting 5 weeks (i.e. up to the 6th week after flowering).

The logistic regression equation of the model was

$$Y = 1/\left[1 + \exp\left(201.6 - 8.5X_{1,5}\right)\right] \quad (1)$$

Observed and predicted data are shown in Fig. 2. A mean temperature of 23.74 °C was found at the cut-off point equal to 0.5. Above a mean temperature of 24.1 °C during the period concerned, banana fruits had 95% probability of being chilled. Below a temperature of 23.4 °C, banana fruits only had a 5% probability of being chilled. Sensitivity was 0.958 (69/72) and specificity was 0.972 (69/71). Youden's index was 0.93. Youden's index was calculated for some relevant explanatory variables (Table 3). For calibration models with an AIC below 30 (using the explanatory variables $X_{0,6}$, $X_{1,4}$, $X_{0,7}$, $X_{1,6}$, $X_{2,4}$, $X_{0,8}$, $X_{1,7}$ and $X_{2,3}$),

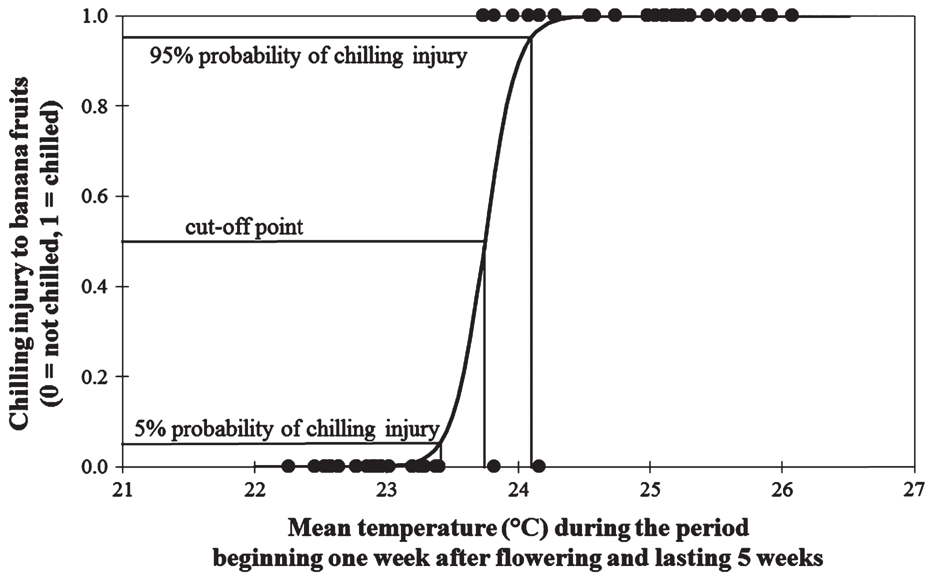

Figure 2. Calibration of relationship between chilling injury and mean temperature in plot during period beginning 1 week after flowering and lasting 5 weeks. Full circles represent all measurements in one plot (CIRAD). The curve represents the predicted logistic regression.

Table 3. Youden's index (YI) calculated for some relevant explanatory variables

Explanatory variable	AIC[a]	Calibration models		Validation models	
		NI[b]	YI	NI[b]	YI
$X_{1,5}$	20.7	143	0.93	156	1
$X_{0,6}$	22.9	143	0.92	156	0.98
$X_{1,4}$	25.6	143	0.93	156	0.87
$X_{0,7}$	25.8	143	0.93	156	1
$X_{1,6}$	26.0	143	0.93	156	1
$X_{2,4}$	26.7	143	0.93	156	0.98
$X_{0,8}$	27.4	143	0.93	156	1
$X_{1,7}$	29.6	143	0.93	156	0.96
$X_{2,3}$	29.7	143	0.93	156	0.98
$X_{0,9}$	30.6	143	0.93	156	1
$X_{0,10}$		137	0.93	138	0.97
$X_{0,11}$		107	0.86	97	0.92
$X_{0,12}$		82	0.77	58	0.68
$X_{0,5}$	39.6	143	0.92	156	0.98
$X_{2,5}$	32.1	143	0.92	156	1
$X_{3,5}$	43.2	143	0.92	156	0.92
$X_{4,5}$	51.2	143	0.92	156	0.92
$X_{5,5}$		137	0.88	138	0.76
$X_{6,5}$		107	0.86	97	0.72
$X_{7,5}$		82	0.37	58	0.62

[a] Akaike information criterion calculated only for models built with all individuals (143).
[b] Number of individuals used to build models.

Youden's indices were equal to those of the best model ($X_{1,5}$), i.e. 0.93. Youden's index decreased with an increase in P ($X_{0,9}$, $X_{0,10}$, $X_{0,11}$, $X_{0,12}$), i.e. when the period during which the temperature was taken into account for building models was longer. For $P = 5$, Youden's index decreased with an increase in N ($X_{1,5}$, $X_{2,5}$, $X_{3,5}$, $X_{4,5}$, $X_{5,5}$, $X_{6,5}$, $X_{7,5}$), i.e. when the time during which the temperature was taken into account was a long time from the date of flowering.

Model validation

In the validation trials, the mean temperature during the period beginning 1 week after flowering and lasting 5 weeks ranged from 22.4 to 28.1 °C. The previously calculated logistic regression Eqn (1) was applied to 156 observations. With a cut-off of 0.5 (i.e. a temperature of 23.74 °C), the presence/absence of chilling injury was well predicted by the model (Fig. 3). The sensitivity and the specificity were equal to 1, because chilled and not chilled fruits were all correctly predicted. Youden's index was equal to 1, showing that the test was perfect. As in the calibration models, Youden's index decreased with an increase in P or in N (with $P = 5$) (Table 3).

Prediction of risk of chilling injury and validation in real export conditions

The mean weekly temperatures in both plots (EYMA and DUHAUMONT) between 2012 and 2014 are given in Fig. 4. By computing the mean temperature during five consecutive weeks when the temperature was below 23.4 °C, we predicted the most favorable flowering periods in both plots for fruits that would be shipped at 13 °C with a 95% chance of no chilling injury. In the DUHAUMONT plot, where the temperature was systematically above 24 °C, chilling injury was systematically observed on each of the pallets shipped at 13 °C (Fig. 4). In the EYMA plot, no chilling injury was observed in pallets shipped at 13 °C when the flowering date was during or close to the period predicted to be insensitive to chilling injury (January 2013, January 2014, February 2014, April 2014) (Fig. 4). Aside from these periods and except for one shipment in November 2013, chilling injury was observed in all pallets shipped at 13 °C.

DISCUSSION

This study showed that exposure of C925 fruit to a low preharvest temperature (below 24 °C) causes acclimation of fruit to chilling injury during storage at 13 °C. Some whole fruit and green plant tissues have already been shown to better withstand low postharvest temperatures when they are subject to preharvest

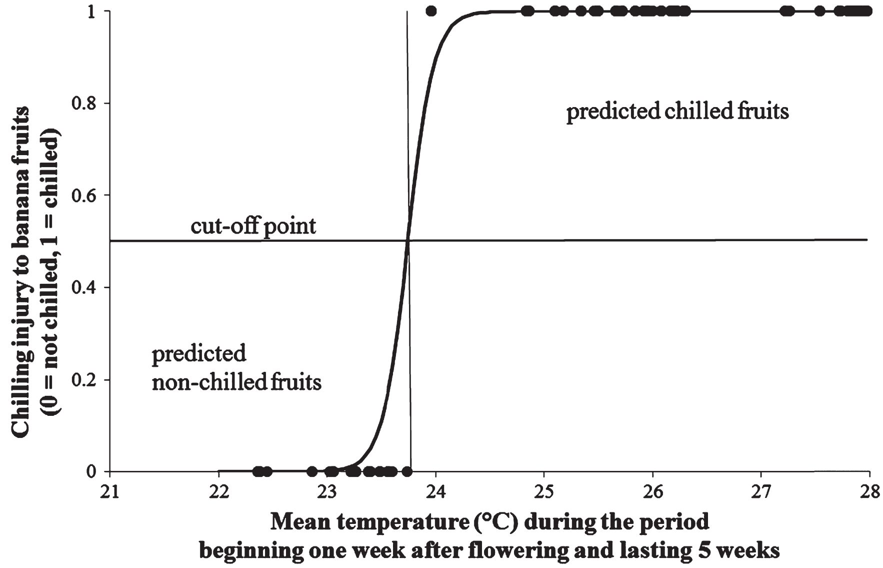

Figure 3. Validation of relationship between chilling injury and mean temperature in six plots during period beginning 1 week after flowering and lasting 5 weeks. The predicted logistic regression (curve) is given by Eqn (1). Full circles represent all measurements in six plots (CHANGY, DUHAUMONT, EYMA, FEFE, GRAND MARIGOT and SARDE)

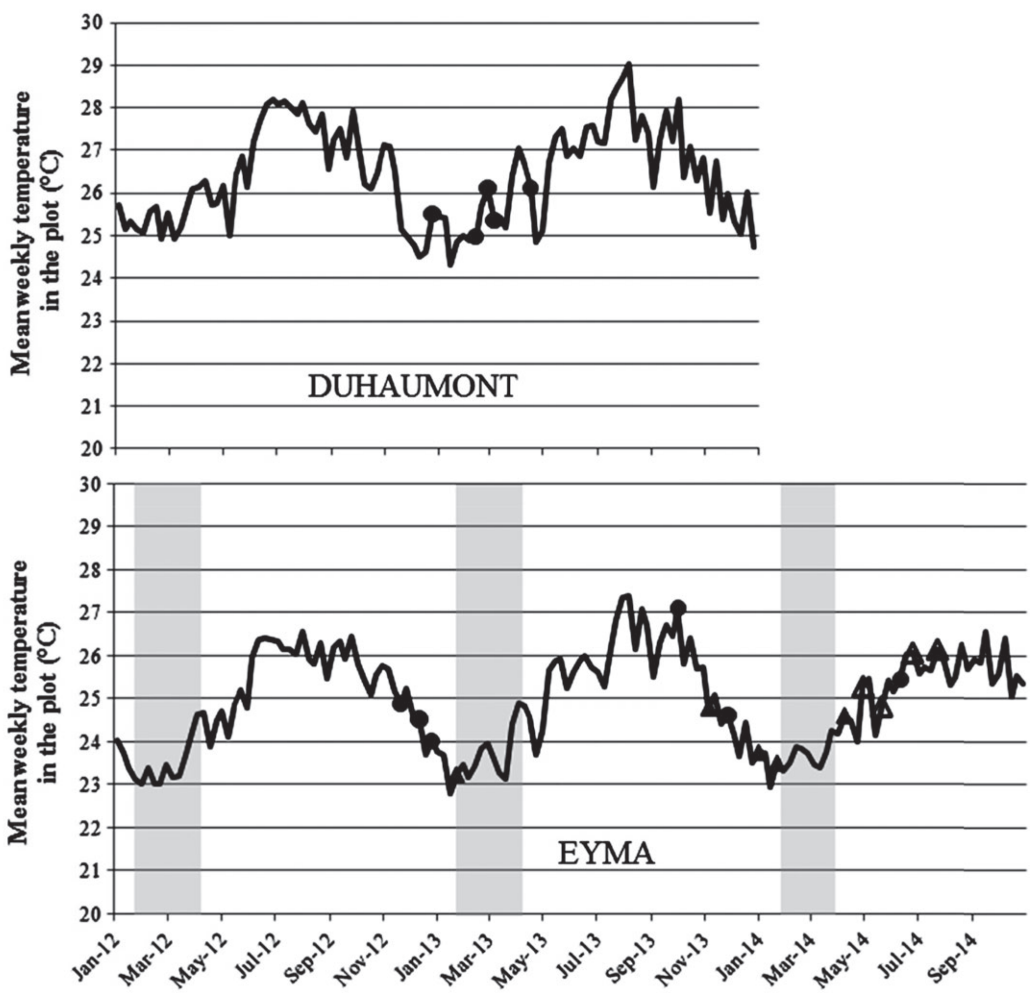

Figure 4. Prediction of risk of chilling injury (storage at 13 °C) as a function of mean weekly temperature in plot (°C) and flowering date. The gray areas represent the most favorable periods of flowering to ensure the shipping of fruits that will not be sensitive to chilling injury at 13 °C. C925 fruits were shipped in the real conditions in reefers regulated at 13 °C (full symbols) and at 15 °C (empty symbols). Circles show the occurrence of chilling injury and triangles the absence. The position of the symbol corresponds to the flowering date.

temperatures that are low but above those that induce injury. Examples include apple (*Malus domestica* L.), sweet basil (*Ocimum basilicum* L.) shoots, grapefruit (*Citrus paradisi* Macf.) and corn (*Zea mays* L.).[12–15] The chilling tolerance of fruits exposed to low preharvest temperatures may be linked to better cell membrane fluidity. Thomai *et al.*[13] found higher ratios of unsaturated fatty acids to saturated fatty acids and lower electrolyte leakage of apple peel tissue when fruits were exposed longer to a low temperature. Increases in linoleic acid in the flavedo of grapefruit were observed when the trees were subject to progressively cooler temperatures (from 25 to 5 °C) over a period of 11 weeks.[14] According to Aghdam *et al.*,[3] the increase in the degree of unsaturation of the membrane improves membrane fluidity. The increase in membrane fluidity reduces the tendency to switch from flexible liquid-crystalline to rigid solid-gel stages, resulting in improved resistance to chilling injury. More specifically, we observed that the period during which temperature most affected chilling susceptibility comprised the first weeks of fruit growth. Several elements suggest that this period has an impact on chilling injury. First, models built over the first 4 to 7 weeks near the beginning of flowering ($X_{1,5}$, $X_{0,6}$, $X_{1,4}$, $X_{0,7}$, $X_{1,6}$, $X_{2,4}$, $X_{2,5}$) better predicted the presence or absence of chilling injury than models based on the same interval but more toward the end of growth ($X_{4,5}$, $X_{5,5}$, $X_{6,5}$, $X_{7,5}$). Second, lengthening the period during which the temperature is taken into account, in particular beyond 9 weeks, reduces the accuracy of model prediction. The first weeks of fruit growth correspond to the cell division phase in banana.[16] During the cell division stage, the ratio of structural to dry matter started high but then progressively decreased during the cell filling stage.[17,18] This suggests that the cell membrane and cell wall are constructed primarily during cell division, which would explain why the thermal conditions during this period of fruit growth have the most impact on chilling susceptibility. Further studies on changes in lipid composition and fluidity of the cell membrane during fruit growth subjected to different thermal conditions are required to confirm this hypothesis.

The model predicted a low risk of chilling injury (<5%) when the mean temperature during the first weeks (1–5) of fruit growth was below 23.4 °C. Above 24.1 °C, the risk of chilling injury was extremely high (>95%). Based on the temperatures recorded in the field, it is therefore possible to predict before harvest whether or not fruits will be sensitive to chilling injury at 13 °C. In lowland farms (like DUHAUMONT) where the weekly temperature was above 24 °C, shipments of C925 in real export conditions during the coldest season confirmed the high risk of chilling throughout the year. In highland farms (like EYMA), we identified periods of flowering of fruits that can be shipped at 13 °C without risk of chilling injury, corresponding to the cold season (January–April in the Caribbean). In some cases, chilling injury was not observed, probably because the C925 boxes were located in a warmer area in the reefer.

The most obvious, easiest and cheapest way to avoid this physiological disorder in this hybrid is to increase the storage temperature during transport to a value that ensures no chilling injury, whatever the preceding thermal conditions in the field. Recent shipments of C925 fruits from the EYMA plot in the hot season showed that a temperature of 15 °C in the reefer completely eliminated the risk of chilling injury (Fig. 4). As C925 fruits are harvested at a growth stage that allows a green life of 30 ± 5 days, the increase in the temperature of the reefer was not prejudicial. Not putting polyethylene bags on bunches at flowering could also reduce chilling injury. According to Jullien *et al.*,[19] bagging bunches increases the temperature of the upper hands by about 2 °C. This could lengthen the period when banana fruits are not sensitive to chilling injury at 13 °C (Fig. 4), but it would increase the possibility of damage caused by insects.

CONCLUSIONS

The results of this study showed that the preharvest temperature during the first weeks of fruit growth affected the chilling susceptibility of banana peel during postharvest storage at 13 °C. We focused on the physiological mechanisms involved by analyzing changes in the lipid composition of membranes during fruit growth and impact during postharvest storage. Work is now under way to check the putative positive correlation between the preharvest temperature and the critical temperature below which chilling injury will occur during storage. More generally, we believe that what we observed in the C925 hybrid is likely true for all other cultivars, although at different temperature thresholds. Climate warming could thus require changing the postharvest temperature to be able to store banana fruits without the risk of chilling injury.

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