



A meta-analysis using a logit non-linear mixed effects model for 'Hass' avocado postharvest performance data

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ABSTRACT

'Hass' avocado fruit quality data collected from storage trials in New Zealand during the 2002, 2003 and 2004 seasons have been assimilated into a model to describe relationships between the postharvest environment (storage temperature and duration), fruit dry matter at harvest, and the incidence of pathological and physiological disorders in fruit after cold storage. Similar or related disorders were grouped into three categories: disorders in unripe fruit, pathological disorders of ripe fruit (rots) and physiological disorders of ripe fruit. A logistic non-linear mixed effects model and its simplified version with normal errors on the logit linear predictor gave a good general description of relationship between postharvest disorders and dry matter at harvest, storage temperature and storage duration, but not for predictions of disorder for individual orchards. The capacity of the model to predict disorder incidence was limited largely by the variability among orchards and seasons. It is concluded that while the mixed effects model does describe well the relationships of the disorder categories with storage temperature, storage duration and dry matter, any predictions of disorder at the orchard level are likely to have large prediction errors because of the high variability among orchards and seasons. The challenge in the future is to determine, and incorporate into the model, those factors that contribute to the large variation among orchards and seasons.

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1. Introduction

The postharvest quality of 'Hass' avocado (*Persea americana*) fruit may be reduced by pathological (rots) or physiological disorders that develop during storage and/or ripening. The degree to which rots or physiological disorders develop postharvest is affected by the storage conditions, including duration and temperature, and also the maturity of the fruit at harvest (Hofman et al., 2002; Yahia and Woolf, 2011). Storage duration may have either beneficial or detrimental effects on fruit quality. Early season fruit that would naturally take a long time to ripen benefit from a short period of storage after which the fruit ripen more rapidly. It has previously been shown that extended ripening periods are associated with higher incidences of rot (Hopkirk et al., 1993). In contrast, storing fruit for too long at any time of the season can result in an increased incidence of rots and physiological disorder. For New Zealand grown 'Hass' fruit it appears that quality deteriorates when fruit age (time after harvest) exceeds 32 days (Dixon et al., 2003a).

'Hass' avocado fruit are chilling sensitive with both the skin and the flesh showing symptoms of chilling injury (Hofman et al., 2002; Yahia and Woolf, 2011). Skin chilling injury occurs as discrete patches of discoloured skin whereas in the flesh the symptom is a diffuse discolouration, or greying, of the flesh termed diffuse flesh discoloration (DFD). The two symptoms are not linked; skin injury can occur well before DFD, with DFD being associated with the fruit starting to ripen at storage temperatures rather than a simple time by temperature relationship.

Fruit quality after storage is also dependent on fruit maturity at harvest (Dixon et al., 2003b). Avocado fruit maturity is often discussed in terms of dry matter (as a proxy for oil content) although dry matter is not a true reflection of the physiological changes associated with fruit maturation, such as the change in pre-climacteric period. The use of dry matter as a harvest index is based on a minimum eating quality, and has been found not to be useful in determining later stages of maturity (Hofman et al., 2000). However, as avocado fruit continue to accumulate oil whilst on the tree, the measurement of dry matter does give some measure of change in the fruit during the season. The use of fruit at different times during the harvest season is of particular significance for New Zealand grown fruit which may be harvested over a period of up to 6 months starting when the fruit reach a dry matter of 24%.

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In 2002, 2003 and 2004 the New Zealand Avocado Industry Council (AIC) undertook research into the occurrence of postharvest quality disorders in 'Hass' avocado fruit. Fruit were harvested at different times in the season and then stored at a range of temperatures for different durations. The incidence and severity of disorders were recorded in fruit both at the end of storage, whilst the fruit were still unripe, and when ripe after holding at 20 °C using the standard New Zealand industry criteria (Dixon, 2003). This research has been reported in part in the New Zealand Avocado Growers' Association Annual Research Report for 2004 (Dixon et al., 2004). In this paper, a single model is presented that describes the development of postharvest disorders on the basis of the postharvest environment, i.e. storage temperature and duration, across orchards and seasons.

2. Methods

2.1. Description of the data

A brief outline of the trials from 2002, 2003 and 2004 is given below. In all trials, sample size was 100 fruit per orchard per treatment.

2002: Two trials were undertaken investigating the effects of storage duration and storage temperature. For the storage duration trial, fruit were harvested from 3 orchards (designated O1, O2 and O4) on 3 occasions (2–5 September 2002, 29 October–1 November 2002 and 10–14 February 2003). Fruit were stored for 0, 1, 2, 3, 4, 5 and 6 weeks at 4 °C and ripened at 20 °C. For the storage temperature trial, fruit were harvested from 3 orchards (O1, O2 and O4) on 4 occasions (4 September 2002, 31 October 2002, 7 January 2003 and 7 March 2003). Fruit were stored for 0 and 4 weeks at 2, 3, 5 and 7 °C and ripened at 20 °C.

2003: One trial was undertaken in which fruit were harvested from 3 orchards (O2, O3 and O4) on 3 occasions (21–24 October 2003, 16–18 December 2003 and 9–11 February 2004). Fruit were stored for 0, 4 and 6 weeks at 2 and 5 °C and ripened at 20 °C.

2004: One trial was undertaken in which fruit were harvested from 3 orchards (O1, O2 and O4) on 3 occasions (18–22 October 2004, 8–12 December 2004 and 10–11 February 2005). Fruit were stored for 0, 4 and 6 weeks at 2 and 5 °C and ripened at 20 °C.

Fruit quality was assessed at the end of storage whilst the fruit were still unripe, and also when ripe after holding at 20 °C. Fruit were assessed for incidence and severity of disorders according to the scales described in the New Zealand Avocado Industry Council fruit assessment manual 2003 (Dixon, 2003). The most common disorders in unripe fruit at the end of storage were discrete patches (DP) and fuzzy patches (FP), and in ripe fruit, stem end rot (SER), body rot (BR), external rot (ER), vascular browning (VB) and diffuse flesh discoloration (DFD).

2.2. Interpretation of disorder categories

Similar or related disorders were grouped into three categories: disorders in unripe fruit, pathological disorders of ripe fruit (rots) and physiological disorders in ripe fruit. In unripe fruit there were DP or FP. DP are often described as physiological chilling damage whereas FP are considered to be the initial stages of fungal infection, but which may occur on chilling damaged tissue. Hence, both symptoms may arise from a common cause, and they have been combined as a single category of disorder in unripe fruit.

In ripe fruit there may be SER, BR and ER, which have been combined in creating a category of pathological disorders. Physiological disorders in ripe fruit tend to be either diffuse or discrete flesh discoloration or flesh adhesion to the seed. These disorders have been combined to create a physiological disorders category.

Fruit with disorder were included for modelling where the disorder was >5% severity. This filtering excluded a proportion of the data by not including the lowest severity of incidence.

Initially, data were examined by harvest date, however, given the large seasonal differences in dry matter accumulation in the fruit, it was decided that dry matter would be a more consistent basis (compared to date) on which to develop the model.

2.3. Model formulation and fitting

Exploratory plots indicated a dependence of disorders on storage temperature, storage duration as well as dry matter. The relationship with dry matter appeared to be approximated by a quadratic equation, hence the inclusion of dry matter as a covariate in the resulting model. The basic model was developed by sequentially fitting to each season's data, but the final general model allowed for variability among both orchards and seasons as a single random effect. While more precise models could be developed for some individual seasons, such models would have limited applicability to other seasons or orchards. The concept of 'Chill units' (CU) is introduced to derive an empirical composite variable that takes into account both storage temperature and duration, as explained below.

For a given Season \times Orchard \times Storage Temperature \times Storage Duration, let the random variable Y_{ijkl} denote the number of disordered fruit in a sample of size n_{ijkl} . It is assumed that $Y_{ijkl} \sim \text{Binomial}(\pi_{ijkl}, n_{ijkl})$, where π_{ijkl} is the probability of incidence for the given disorder. The probability π_{ijkl} is modelled by considering the logit of the probability to be a linear function as follows:

$$\log \left\{ \frac{\pi_{ijkl}}{1 - \pi_{ijkl}} \right\} = \eta_{ijkl} = \mu + L_{ij} + \beta_1 CU_{ijkl} + \beta_2 DM_{ij} + \beta_3 DM_{ij}^2$$

where μ = overall mean of the logit linear predictor, L_{ij} = effect of ij th fruit line (i.e. of j th grower in i th season), DM_{ij} = %Dry matter of ij th line, CU_{ijkl} = normalised 'chill units' accumulated when ij th line is stored in k th temperature for l number of weeks, and β_1, \dots, β_3 are the regression coefficients. The line effect is assumed to be random, but all other effects to be fixed, hence $L_{ij} \sim N(0, \sigma_L^2)$ i.e. L_{ij} is assumed to be normally distributed with mean = 0, and a variance of $\sigma^2 L$.

In the above model the concept of 'Chill units' is introduced to derive a composite variable that takes into account the storage temperature and the duration. If the chill units can be calculated from storage temperature and duration without having to estimate any parameters from the data, the above model is a generalised linear mixed model. The model formulated here, however, assumes a non-linear relationship between Chill units, and temperature and duration, hence the model now becomes a non-linear one. It is hypothesised that fruit accumulates 'Chill units' over time when stored at temperatures below a base temperature. The 'Chill units' accumulation over time is expressed as $CU = 1 - e^{-kt}$ where t = time (weeks), and k = a temperature dependent rate constant given by

$$k = 1 - \left(\frac{T - T_L}{T_U - T_L} \right)^c \quad \text{for } T_L \leq T \leq T_U.$$

where T_L and T_U are the lower and upper limits of temperature between which 'chill units' accumulate, and c = constant coefficient. Based on knowledge of similar biological systems T_L and T_U have been fixed at 2 °C and 14 °C, respectively. Note that the 'Chill units' are normalised to be between (0, 1).

The use of 'Chill units' is a common approach to dealing with exposure to temperatures, either for heat or cold exposure, and have been used traditionally to model biological responses including heat accumulation for insect development (e.g. Maiorano et al.,

2012) or cold exposure for plant development such as budbreak (e.g. Luedeling and Brown, 2011). Our approach uses a slight modification of this concept by constraining the 'Chill units' to an asymptotic maximum and normalising.

The logistic non-linear mixed effects model detailed above was arrived at after other possibilities for the link function had been examined, e.g. a complementary log-log model, but it gave very similar results and was not superior in any way. Both these appropriate link functions change the disorder proportion from a bounded value (0–1) to an unbounded one. On the logit scale values are negative for $P < 0.5$, positive for $P > 0.5$, and 0 at $P = 0.5$.

The model assumes dry matter, temperature, and storage time are fixed effects, whereas combinations of orchards and seasons constitute a random effect. Chill units have been used to describe the temperature the fruit have been exposed to in storage, with a larger value resulting from a longer exposure or lower temperatures within the temperature bounds specified. It was hypothesised that fruit accumulate 'Chill units' over time when stored at temperatures below a base temperature. Different constant base temperatures for computing the chill unit component were tried out in the non-linear part of the model, and some did not converge for all three disorder categories we wished to cover. Of those that did converge, the models gave very similar predictions to those using the temperature bounds specified earlier. The base temperature constants used in the model were selected to reflect the biological situation rather than being estimated from the data.

More parsimonious models with fewer parameters were fitted but there was no general form that was applicable to all seasons and temperatures. Hence the general form of the model described above was kept the same across all disorders to ascertain effects of storage temperature, storage duration and dry matter on disorders.

The model was initially fitted for one disorder category using the NLMIXED procedure in SAS Software (SAS Institute Inc, 2000–2004) assuming binomial errors. However, it was observed that using a logit transformation of the incidence data and assuming normal errors, a non-linear mixed effects model using the nlme package (Pinheiro et al., 2005) with the 'R' statistical software (R Development Core Team, 2005), gave almost identical results. Had the numbers of fruit used been much below the 100 that were used, the normality assumption could not have been justified. The model hereinafter is referred to as 'the logit non-linear mixed effects model'.

3. Results and discussion

3.1. Disorders in unripe fruit

The statistical significance of the parameter estimates used in the model to describe the incidence of unripe fruit defects (physiological and pathological) are given in Table 1. The μ parameter estimate (i.e. orchards and seasons) was not significant, indicating the model could be applicable to a wide range of growers and seasons. All other parameter estimates were significant, indicating significant impacts of dry matter, temperature and time of storage.

The shape of the relationship between unripe fruit disorders and dry matter was markedly U-shaped at 2 °C, with higher disorder incidence at low or high dry matters, but with a minimum at about 28% dry matter (Fig. 1). At temperatures above 2 °C, the incidence of disorders was lower, and only tended to increase in incidence at > 30% dry matter with no difference among low dry matters. The incidence of unripe fruit disorders was higher at lower temperatures, with a larger difference between fruit stored at 2 or 3 °C than between other temperatures. Increased storage duration increased the disorder incidence, although the increase in disorders was not uniform through the 6 weeks storage period (Fig. 2). Instead, there

Table 1

Significance of the parameter estimates from the logit mixed effects model in describing the effect of dry matter, storage temperature and duration on the incidence of disorders in unripe 'Hass' avocado fruit at the end of storage.

Parameter ^a	Estimate	Std error	DF ^b	t-Value	P-value
Fixed					
μ	9.7297	7.2307	188	1.34	0.180
β_1	5.5595	0.3156	188	17.61	0.000
β_2	-1.2130	0.4916	188	-2.46	0.015
β_3	0.0225	0.0083	188	2.71	0.007
c	0.0706	0.0102	188	6.93	0.000
Random					
σ^2_L	0.3566				

β_1 is the linear coefficient of regression for chill units.

β_2 is the linear coefficient for regression on dry matter.

β_3 is the quadratic coefficient for regression on dry matter.

c is a parameter that describes the relationship between the temperature rate constant with the actual temperature.

σ^2_L is the variance of the random effect of the combinations of orchards and seasons.

^a μ is the overall mean of the linear predictor on the logit scale.

^b Degrees of freedom.

was a steady increase in disorders up to 4 weeks of storage, with little further increase for the subsequent 2 weeks of storage.

The response of the unripe fruit disorders for fruit harvested at different dry matter contents to storage temperature and duration may be interpreted as 2 components of the disorder occurring at high or low dry matter. The disorder in low dry matter fruit is

Table 2

Significance of the parameter estimates from the logit mixed effects model in describing the effect of dry matter, storage temperature and duration on the incidence of pathological disorders in ripe 'Hass' avocado fruit.

Parameter ^a	Estimate	Std error	DF ^b	t-Value	P-value
Fixed					
μ	19.9948	7.9804	188	2.5	0.013
β_1	2.2009	0.3445	188	6.39	0.000
β_2	-1.6997	0.5418	188	-3.1	0.002
β_3	0.0307	0.0091	188	3.37	0.001
c	0.1202	0.0421	188	2.86	0.005
Random					
σ^2_L	0.7725				

β_1 is the linear coefficient of regression for chill units.

β_2 is the linear coefficient for regression on dry matter.

β_3 is the quadratic coefficient for regression on dry matter.

c is a parameter that describes the relationship between the temperature rate constant with the actual temperature.

σ^2_L is the variance of the random effect of the combinations of orchards and seasons.

^a μ is the overall mean of the linear predictor on the logit scale.

^b Degrees of freedom.

Table 3

Significance of the parameter estimates from the logit mixed effects model in describing the effect of dry matter, storage temperature and duration on the incidence of physiological disorders in ripe 'Hass' avocado fruit.

Parameter ^a	Estimate	Std error	DF ^b	t-Value	P-value
Fixed					
μ	-12.8617	8.8883	188	-1.45	0.150
β_1	2.6211	0.3939	188	6.65	0.000
β_2	0.2451	0.6050	188	0.41	0.686
β_3	-0.0010	0.0102	188	-0.100	0.922
c	0.3024	0.1253	188	2.41	0.017
Random					
σ^2_L	0.0001				

β_1 is the linear coefficient of regression for chill units.

β_2 is the linear coefficient for regression on dry matter.

β_3 is the quadratic coefficient for regression on dry matter.

c is a parameter that describes the relationship between the temperature rate constant with the actual temperature.

σ^2_L is the variance of the random effect of the combinations of orchards and seasons.

^a μ is the overall mean of the linear predictor on the logit scale.

^b Degrees of freedom.

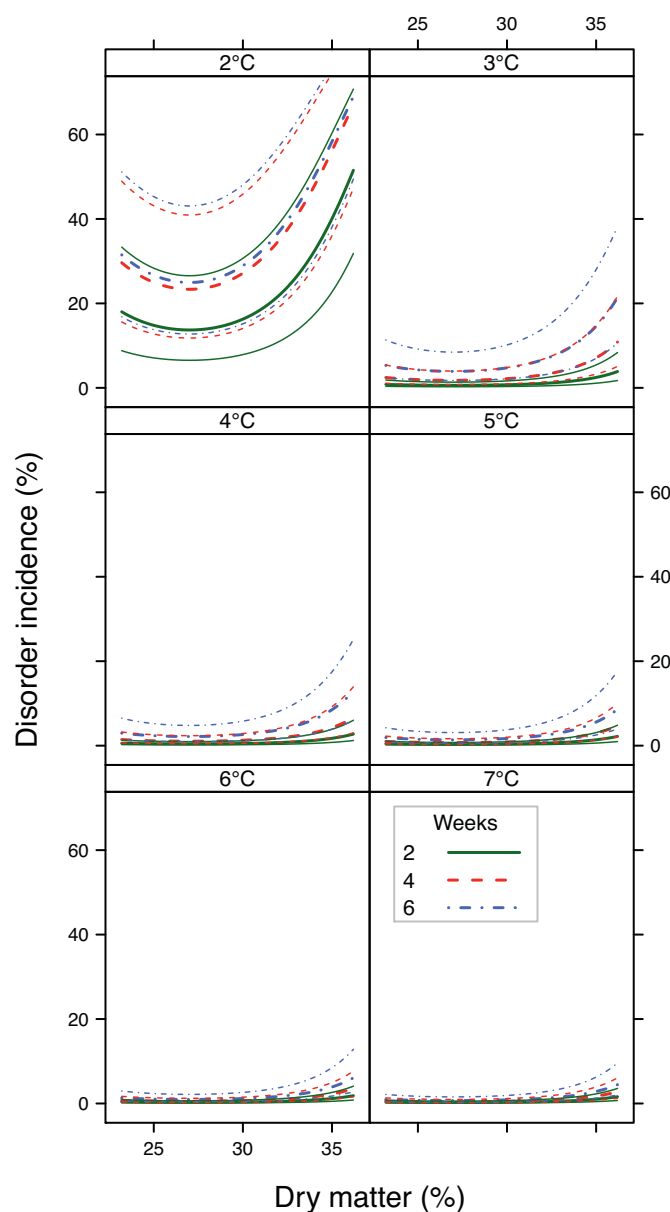


Fig. 1. Logit non-linear mixed effect model description of disorder incidence in unripe 'Hass' avocado fruit after storage at 2, 3, 4, 5, 6 or 7 °C for fruit harvested at different dry matter. Fitted lines are derived from the model combining orchards and seasons, with the effect of 2, 4 or 6 weeks storage represented within each storage temperature panel. At each temperature, the data represented are the fitted means (bold lines) and the 95% confidence interval of the random effects (fine lines). Details for the model are given in Table 1.

most temperature sensitive, being limited largely to fruit stored at 2 °C, whereas the disorder in high dry matter fruit was still present at temperatures above 2 °C, but in decreasing amounts. The disorder at 2 °C may be the result of temperature sensitivity, i.e. chilling injury, which fits with the general relationship found between maturity and chilling sensitivity, in which less mature fruit are more sensitive to chilling (Paul, 1990). This relationship is also seen in avocados (Hofman et al., 2002; Yahia and Woolf, 2011). The disorder incidence at high dry matters may be more associated with advanced or over-maturity.

3.2. Pathological disorders in ripe fruit

The statistical significance of the parameter estimates used in the model to describe the incidence of ripe fruit rots are given in

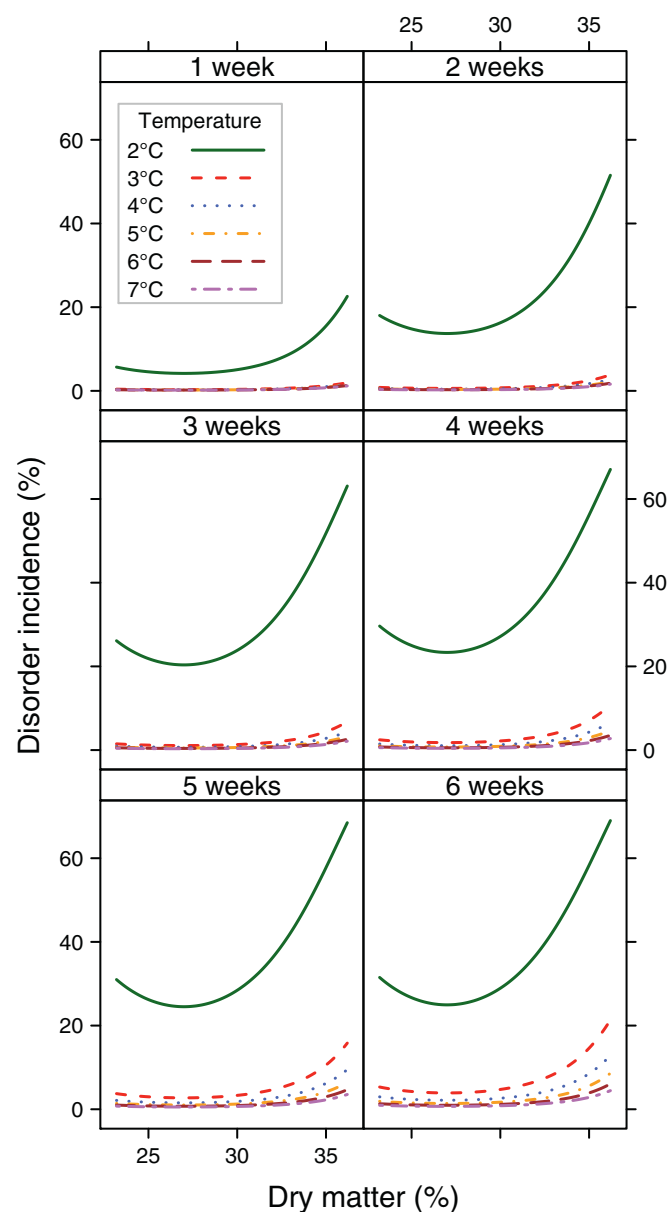


Fig. 2. Logit non-linear mixed effects model description of disorder incidence in unripe 'Hass' avocado fruit after storage for 1, 2, 3, 4, 5 or 6 weeks for fruit harvested at different dry matter. Fitted lines are derived from the model combining orchards and seasons, with the effect of storage temperature (2, 3, 4, 5, 6 or 7 °C) represented within each storage week panel. Details for the model are given in Table 1.

Table 2. The μ estimate was statistically significant indicating that fruit from different seasons and/or orchards have substantially different numbers of ripe fruit rots, possibly indicative of seasonal weather impacts or of grower orchard control practices. All other parameter estimates were also significant, indicating significant impacts of dry matter or temperature and time of storage.

The relationship between rot incidence and dry matter was U-shaped, and was most pronounced in fruit that had been stored at 2 °C (Fig. 3). The U-shaped response was not symmetrical, there being a greater increase in rot incidence at high dry matter than at low dry matter. The incidence of rots in ripe fruit was markedly higher in fruit that had been stored at 2 °C than at higher temperatures, with the incidence gradually decreasing as storage temperature increased. The incidence of rots increased with longer storage duration, there being a uniform increase with time for all temperatures except 2 °C (Fig. 4). At 2 °C, the rot incidence increased

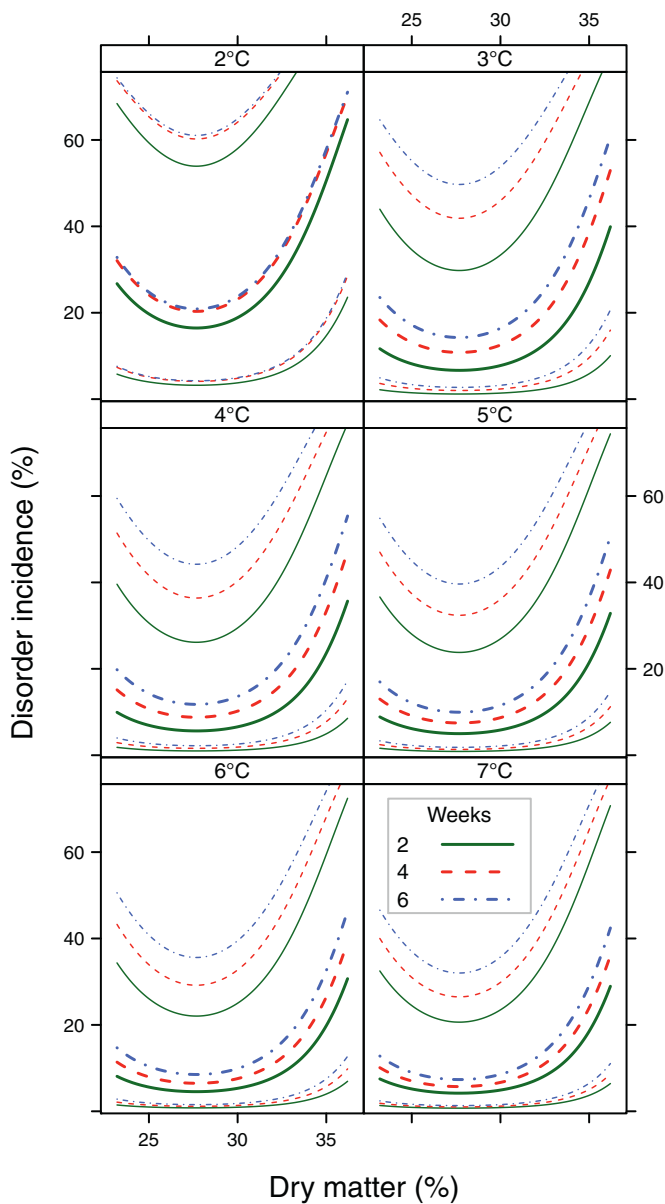


Fig. 3. Logit non-linear mixed effects model description of pathological disorder incidence in ripe 'Hass' avocado fruit after storage at 2, 3, 4, 5, 6 or 7 °C and ripening at 20 °C for fruit harvested at different dry matter. Fitted lines are derived from the model combining orchards and seasons, with the effect of 2, 4 or 6 weeks storage represented within each storage temperature panel. At each temperature, the data represented are the fitted means (bold lines) and the 95% confidence interval of the random effects (fine lines). Details for the model are given in Table 2.

between 2 and 4 weeks of storage, but increased little between 4 and 6 weeks of storage. As storage duration increased so did the degree of separation between the rot incidences at individual storage temperatures.

The higher incidence of rots in fruit stored at 2 °C compared with other temperatures may be interpreted as there being rot development on physiologically chill damaged fruit tissues, which would be greatest in the less mature (low dry matter) fruit. In the absence of disordered or damaged tissue, rot growth would be expected to be lower at lower temperatures. Alternatively, after storage at 2 °C the time taken to ripen at 20 °C is longer than for fruit stored at higher temperatures and longer ripening times have previously been associated with higher rot incidences. However, the higher rot incidences in the high dry matter fruit are more difficult to rationalise; possibly the result of more advanced fruit being more

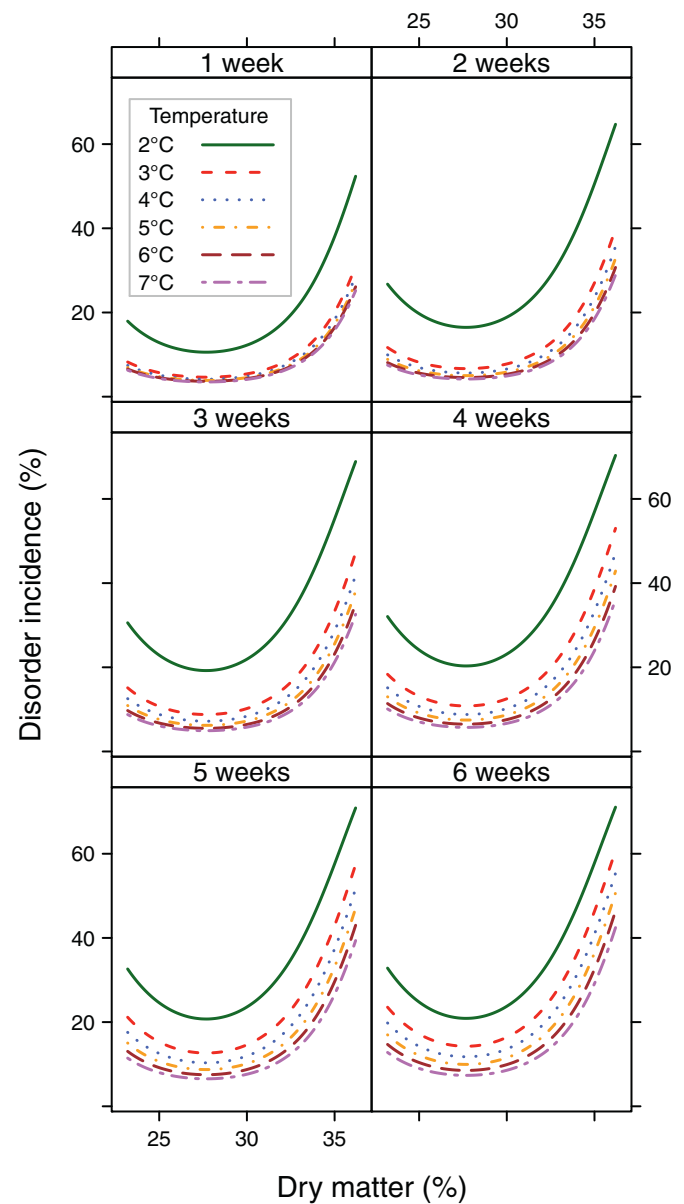


Fig. 4. Logit non-linear mixed effects model description of pathological disorder incidence in ripe 'Hass' avocado fruit after storage for 1, 2, 3, 4, 5 or 6 weeks and ripening at 20 °C for fruit harvested at different dry matter. Fitted lines are derived from the model combining orchards and seasons, with the effect of storage temperature (2, 3, 4, 5, 6 or 7 °C) represented within each storage week panel. Details for the model are given in Table 2.

susceptible to rot, or a result of increased inoculum load in the orchard, and being coincident with a high incidence of unripe fruit disorders.

3.3. Physiological disorder in ripe fruit

The statistical significance of the parameter estimates used in the model to describe the incidence of ripe fruit physiological disorders is given in Table 3. It is evident that the model does not describe physiological defects very successfully. The lack of statistical significance of the β_2 and β_3 parameter estimates indicate that maturity (as measured by dry matter) has little effect on the incidence of physiological defects and only the time and temperature of storage is having an effect. There is no significant difference among seasons or orchards (μ parameter). The ability of the model to describe pathological defects but not physiological defects is

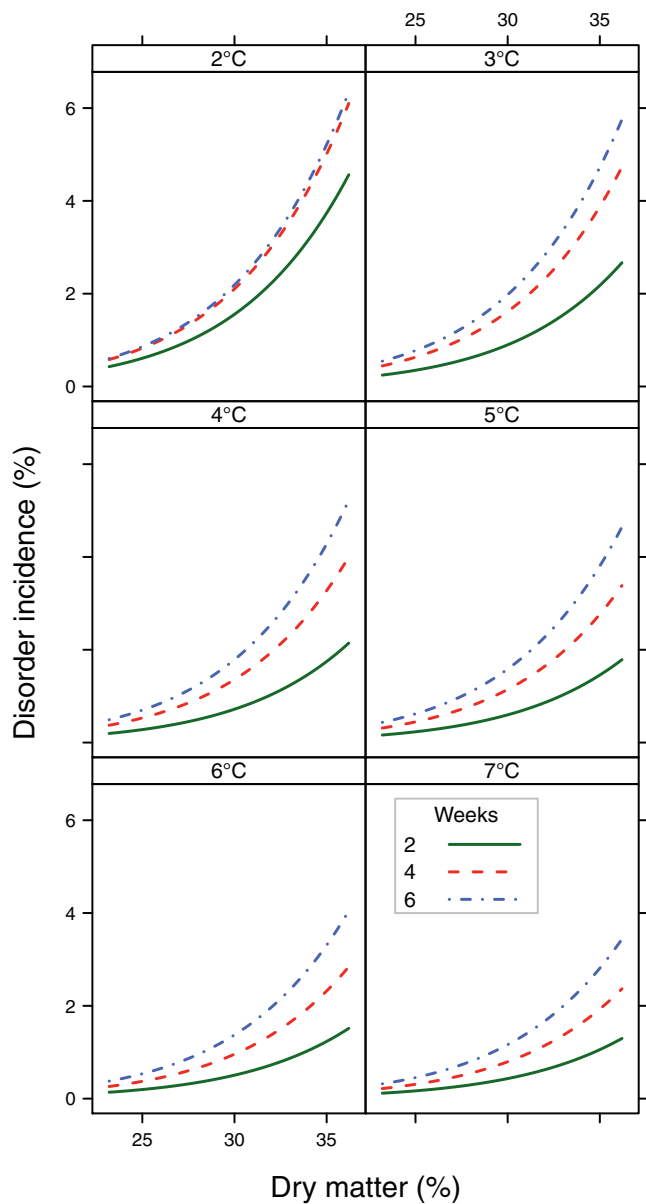


Fig. 5. Logit non-linear mixed effects model description of physiological disorder incidence in ripe 'Hass' avocado fruit after storage at 2, 3, 4, 5, 6 or 7 °C and ripening at 20 °C for fruit harvested at different dry matter. Fitted lines are derived from the model combining orchards and seasons, with the effect of 2, 4 or 6 weeks storage represented within each storage temperature panel. Details for the model are given in Table 3.

possibly indicative of the different underlying causes of the two defect categories.

It was considered that a more elaborate model, such as including a term for the inverse of dry matter, could make better predictions of physiological disorders, however, none of those that were tested were more successful than the existing logit model.

The incidence of physiological disorders was higher in fruit harvested with higher dry matter and higher in fruit that were stored at lower temperatures and for longer durations (Figs. 5 and 6). The trend for higher physiological disorders in higher dry matter fruit is not supported statistically from the significance of the coefficients in Table 3. The lack of statistical significance of the dry matter trend may be the result of low incidences of disorder and the variability among samples. There was little difference in physiological disorder incidence among storage temperatures in the range 3–6 °C after one or two weeks of storage, but separation among temperatures

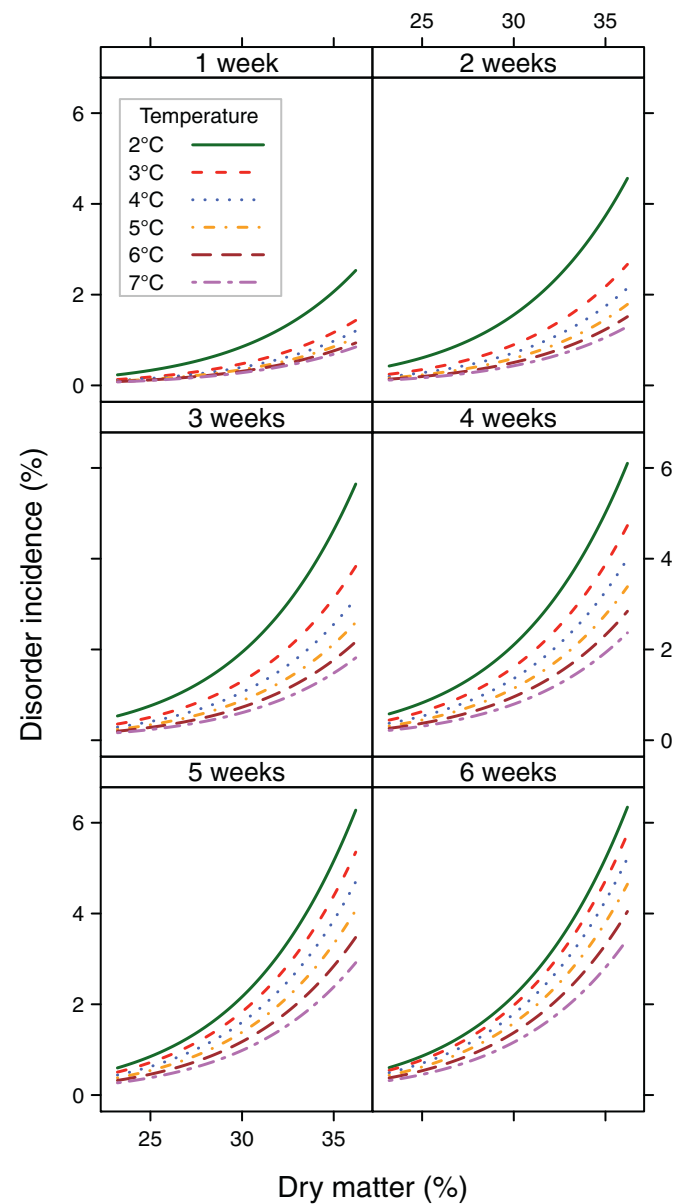


Fig. 6. Logit non-linear mixed effects model description of physiological disorder incidence in ripe 'Hass' avocado fruit after storage for 1, 2, 3, 4, 5 or 6 weeks and ripening at 20 °C for fruit harvested at different dry matter. Fitted lines are derived from the model combining orchards and seasons, with the effect of storage temperature (2, 3, 4, 5, 6 or 7 °C) represented within each storage week panel. Details for the model are given in Table 3.

increased with longer storage periods. Overall, disorder incidence increased with storage duration, although at 2 °C there was little further increase in the number of disorders after 4 weeks of storage.

3.4. Accuracy of models and statistical discrimination between samples

Models using the coefficients calculated as described were used to predict the defects for all samples and the results compared with the actual data. Unripe, pathological and physiological disorders were overestimated by a factor of 2 in 9%, 28%, and 4% respectively of the samples in this study and underestimated by a factor of 2 in about 20% of the samples for all three defect categories. The greater propensity to overestimate the pathological disorders could be due to the fact that the samples were more variable for this response and thus a model which tries to be general is less successful.

The degree of statistical discrimination detectable between the incidences of disorder in two populations as measured by samples of fruit is dependent on the sample size, the disorder incidences and the statistical significance level allowed. The sample size of 300–400 fruit per treatment provides for some statistical discrimination between samples when disorder incidences are below 10%. Small increases in sample size make little difference to the power of detection of differences between samples. The power is the statistical probability of detecting a difference by the experimental design, given that the difference exists at the specified significance level. A 0.8 power of detection is an 80% probability that the statistically significant difference will be detected. Doubling the sample size from 400 to 800 fruit would reduce the interval required for statistical separation at $P=0.05$ from 0.4–7.4% to 1.0–5.9%.

4. Conclusion

It is concluded that while the logit non-linear mixed effects model does describe the relationship of the disorder categories with storage temperature, storage duration and dry matter, the model may not be useful as a predictor of disorder at the orchard level because of the high variability among orchards and seasons. Hence the model is good as a general description of the relationship between postharvest disorders and dry matter at harvest, but not for specific orchard predictions of disorder. The challenge in the future is to determine, and incorporate into the model, those factors that contribute to the large variation among orchards and seasons.

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