

Genetic variation in bitter pit and fruit calcium concentrations within a diverse apple germplasm collection

Richard K. Volz^{1,*}, Peter A. Alspach², David J. Fletcher³ & Ian B. Ferguson⁴

¹The Horticulture and Food Research Institute of New Zealand, Ltd., Hawkes Bay Research Centre, Private Bag 1401, Havelock North, New Zealand; ²The Horticulture and Food Research Institute of New Zealand, Ltd., Nelson Research Centre, PO Box 220, Motueka, New Zealand; ³University of Otago, Department of Mathematics and Statistics, PO Box 56, Dunedin, New Zealand; ⁴The Horticulture and Food Research Institute of New Zealand, Ltd., Mt Albert Research Centre, PO Box 190991, Auckland, New Zealand
(*author for correspondence: e-mail: rvolz@hortresearch.co.nz)

Received 18 November 2004; accepted 17 August 2005

Key words: cropping, disorder, fruit weight, mineral, over-dispersed data

Summary

Genetic variability in the apple [*Malus*] fruit disorder bitter pit and fruit calcium concentrations ([Ca]) was determined in 25 seedling families at each of two sites from 1999 to 2000 and again for one site, in 2001. Most trees were free of pit or had low pit incidence, which could be approximated by an over-dispersed binomial distribution. A genetic component to external and internal pit incidence was found across both sites and all years. This effect was irrespective of the use of several cropping factors, average fruit weight or fruit mineral concentrations as covariates in the analyses. Year and/or site always had substantial effects on pit incidence. Interactions of genetic effects with site or year were also detected with large differences in mean pit incidence between sites or years for some families but not others. No relationship was found between mean family pit incidence and mean family [Ca] or mean family harvest date. However fruit [Ca] and harvest date were usually important predictors of within-family variation in pit incidence. For any one seedling, family had the largest effect on bitter pit incidence followed by site and [Ca] and the smallest effect was that of harvest date. [Ca] showed a strong genetic component and estimated family means of [Ca] were consistent at different sites and years. These results suggest that susceptibility of apple genotypes to bitter pit in breeding programmes may be best assessed through screening across multiple sites and seasons. Fruit [Ca] might be useful as an indirect selection index for bitter pit within, but not among families.

Introduction

Bitter pit is a serious physiological disorder of apple fruit and is recognised as a major commercial problem for apple producers in many countries (Ferguson & Watkins, 1989). Its primary symptom is discrete pitting of the cortical flesh of the fruit, the pits being brown and desiccated. The pitting may occur on the skin surface and/or be located entirely beneath the skin, invisible from the outside. While bitter pit is usually observed in fruit after storage, its development is essentially pre-determined by factors influencing the growth of fruit on the tree, the major one being mineral (particularly

calcium) accumulation by the fruit; bitter pit incidence is associated with low calcium concentrations in the fruit flesh (Ferguson & Watkins, 1989; Ferguson et al., 1999).

Not surprisingly, there is a strong environmental influence on bitter pit development and its occurrence is often sporadic, varying between seasons, growing regions and amongst orchard lines (Ferguson & Watkins, 1989). Past research which sought to understand this environmental variation concentrated on the preharvest basis of fruit susceptibility, as well as on postharvest treatments, to predict and alleviate expression (Faust & Shear, 1968; Ferguson & Watkins, 1989; Ferguson

et al., 1999). Fruit mineral (especially calcium) accumulation during apple fruit development and bitter pit have been shown to be affected by such tree factors as crop load per tree (Ferguson & Watkins, 1992), fruit size (Perring & Jackson, 1975), the type of wood on which the fruit is borne (Volz et al., 1994), fruit number (Volz & Ferguson, 1999) and leaf area per spur (Jones & Samuelson, 1983) and water stress (Irving & Drost, 1987).

Breeding bitter pit resistant cultivars offers a better more permanent solution to the problem. We suspect that there is a genetic component to susceptibility based on comparisons of commercial cultivars (Ferguson & Watkins, 1989; Drazeta et al., 2001). Further, Korban and Swiader (1984) analysed the segregation ratios for bitter pit from three apple populations and concluded that two major dominant genes conferred bitter pit resistance and that such resistance was linked with high fruit [Ca]. However there has been little systematic work carried out to quantify if significant genetic relative to environmental variation exists in bitter pit susceptibility or fruit [Ca]. Such work would allow suitable breeding strategies to be devised that more effectively provide cultivars resistant to the disorder. In the following study, we sought to determine the genetic and environmental variations in bitter pit susceptibility and fruit [Ca] across a wide range of apple germplasm and the cropping and mineral (for bitter pit) factors that might explain this variation. Preliminary analyses of the first year's results have been presented elsewhere (Volz et al., 2001a,b). Here we present results from all three years of the study.

Materials and methods

Plant material

Part of a large apple germplasm collection assembled and established throughout New Zealand (Noiton et al., 1999) was used for the study. Seedling trees were derived from open pollinated seed that had been collected from several apple cultivars of *Malus × sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf. and one of *M. prunifolia* (Mill.) from eight countries. They were planted in 1995 at a spacing of 3 × 0.75 m as single-tree plots in randomized incomplete blocks of 20 trees, allocated using the 'Designer' software (K. Russell, University of Wollongong, NSW, Australia). Trees were evaluated at Havelock North (lat. 39°40'S, long. 176°53'E) in 1999, 2000, 2001, and at Riwaka

(lat. 41°04'S, long. 173°00'E) in 1999 and 2000. They received no calcium sprays or fertilizers, and were left unmanaged apart from regular applications of herbicide and pesticide sprays. Thirty of the 61 open-pollinated families that had at least three trees at both sites bearing more than ten fruit per tree were selected for use in 1999. Where possible, five trees per site were randomly selected from each of these families in 2000, and five families were added. Similar criteria were used for the 2001 harvest, but restricted to Havelock North where the target number of trees per family was raised to nine. The number of trees per family ranged from 2–6 (median 4) in 1999, 1–6 (median 4) in 2000 and 2–11 (median 8) in 2001.

Harvest and fruit evaluation

All fruit were harvested from each seedling when mature, assessed as the time when background color changed from green to yellow. Within 36 h of harvest, fruit were placed in cool-storage (air at 0.5 °C) at Havelock North. After eight weeks, fruit were transferred to 20 °C for one week, after which average fruit weight, bitter pit incidence and mineral assessments were carried out.

A maximum of ten fruit per seedling were randomly taken from each sample and weighed, and average weight per fruit calculated. For mineral assessments carried out in 1999 and 2001, a transverse equatorial tissue slice was cut across each of the ten fruit and two cortical plug samples taken from opposite side of each slice, immediately below the skin. These plugs were bulked together for each sample (Turner et al., 1977). Calcium, magnesium and potassium were analysed by digestion in nitric and perchloric acids and atomic absorption spectrophotometry. Data are expressed as mineral concentrations on a fresh weight basis.

For each seedling, all fruit were counted and sliced transversely three times (stem and calyx ends and middle). The number of fruit that had been affected by external (visible only from the outside) and internal (not visible from the outside) pit was recorded, so that the proportion (incidence) of fruit with pit could be calculated. Additional tree and cropping variables, which might influence bitter pit occurrence or mineral concentrations, were recorded immediately for each seedling before the first harvest in 2000 and 2001: trunk circumference from which trunk cross-sectional area (TCA) and total fruit number·TCA⁻¹ were calculated; the number of fruiting clusters with 1–2, 3–4 or 5–6

fruit-cluster⁻¹; the number of fruit on spurs, terminals or axillaries; and the number of fruit in each of three canopy locations (top, lower outer, and lower inner) were counted. The proportion of total fruit on the tree within each fruit number, wood type or canopy position category was then calculated.

Statistical analyses

The primary focus of the analysis was to investigate whether pit incidence varied among families, and if so, to what extent this could be explained by differences in covariates such as [Ca]. Our approach of using the maternal parent only to define family and therefore to estimate genetic variation, despite not knowing the number and source of pollen parents for each family, follows those of previous similar studies on *Pinus radiata* (Burdon et al., 1992) and apple (Alspach & Oraguzie, 2002; Bus et al., 2002; Luby et al., 2002).

Incidence can often be adequately modeled by transforming data to achieve approximate normality or using the binomial distribution as the probability model. However the high incidence of zeros (approximately two-thirds of the trees were free of disorder) in our data meant that some method of incorporating zero-inflation was desirable (Vieira et al., 2000). We therefore tested four underlying probability models: ordinary binomial, over-dispersed binomial (Williams, 1982), zero-inflated binomial (ZIB) (Hall, 2000; Vieira et al., 2000) and zero-inflated over-dispersed binomial (ZIBOver) (Vieira et al., 2000). In the ZIB and ZIBOver models, zero-inflation was modeled by using a mixture of Bernoulli and binomial or Bernoulli and over-dispersed binomial. A scale parameter (ϕ) was incorporated into over-dispersed models (Vieira et al., 2000) such that:

$$\text{Var}(Y_i) = n_i \pi_i (1 - \pi_i) [1 + \phi(n_i - 1)]$$

where Y_i is disorder incidence for the i th tree; π_i is the probability that a fruit of the i th tree exhibits the disorder, and n_i is the number of fruit examined on the i th tree.

For each probability model a maximal model was fitted, which involved all main effects and some first order year or site interactions. However, the precise model depended upon the subset of the data that was under consideration (e.g. mineral analyses were not available for the 2000 data and thus could not be included). This initial model was then reduced, one parameter at a

time, until the likelihood ratio (Burnham & Anderson, 2002) indicated a significantly poorer model than the previous one. For over-dispersed models, the scale parameter estimated from the initial model was used in all subsequent nested models (Collett, 1991; Vieira et al., 2000). The underlying distributional assumptions of the final model were checked using half-normal plots of the Pearson residuals with reference envelopes as suggested by Vieira et al. (2000). The over-dispersed binomial model was finally chosen for subsequent data analyses, as, in contrast to the ordinary binomial and ZIB models, its underlying distributional assumptions were valid and it was less complex than the ZIBOver model.

Initial generalised additive modelling (Venables & Ripley, 1994) indicated significant non-linearity using the raw [Ca] but not its inverse and so the latter was used in subsequent analyses. The average number of fruit per cluster was approximated from the proportions given by taking a weighted average of the mid-value for each category (e.g. 1.5 was used for the 1–2 fruit per cluster category). Since the three shoot proportions were not independent (they sum to 1), two independent and orthogonal shoot contrasts were chosen (Cornell, 1981) (similarly for fruit location).

Not all of the families were represented in each of the five site \times year combinations, and in some cases where they were, replication was only one or two trees. Furthermore, mineral analyses were not available in 2000, and the additional tree and cropping factors were not recorded in 1999. We therefore restricted the data to the 25 families common to all site.year (=five site \times year levels) combinations with five or more trees per family across any two combinations. Analysis of the pit data was undertaken on various subsets of the data from these 25 families:

1. 1999 and 2001: 342 genotypes. Giving the three-year.site combinations (Havelock, North 1999, Riwaka, 1999 and Havelock, North 2001) for which mineral analyses were available. Hence the initial model included family, year.site combination, inverse [Ca], harvest date, mean fruit weight [Mg], [K] and the year.site \times inverse [Ca] interaction;
2. 1999 and 2001a: 342 genotypes. Same as 1 but including only trees which did not have duplicate measures across the years, since trees at the different sites were of necessity different and many of the trees at the same site were different in the different years;

3. 2001 Havelock North: 165 genotypes. The only year and site for which mineral and cropping variates were available. The initial model included the main effects of 1 (excluding year.site) plus cropping variates: average number of fruit·cluster⁻¹, the two orthogonal shoot contrasts, the two orthogonal location contrasts and number of fruit·TCA⁻¹;
4. 2000 and 2001 Havelock North: 206 genotypes. This combination gave more data for examination of the cropping variates but could not include the minerals. The initial model included the main effects as in 3 (except for the minerals) with the addition of year;
5. All years: 408 genotypes. The initial model of necessity excluded minerals and cropping variates, and included only family, year.site, harvest date and mean fruit weight;
6. 1999 and 2000: 303 genotypes. Data for these two years were available from both sites that allowed for the year and site effect to be separated. Hence the initial model included family, year, site, harvest date, mean fruit weight, and family × site and year × site interactions.

Family effects on pit incidence were estimated from the appropriate model with other variates, when they occurred, set to their overall median value.

For data subsets 1, 2 and 3, inverse [Ca] was also modelled against the same potential candidates as pit incidence (except itself and the other minerals) along with the family × year.site interaction (data subsets 1 and 2). As with the pit data, stepwise model selection was performed using the likelihood ratio test. The underlying probability model was assumed to be normal and the adequacy of this assumption was checked by inspection of the residuals. No obvious problems were indicated.

All analyses were undertaken in R 1.8.1 (R Development Core Team, 2003).

Results

Bitter pit

Between 29 and 51% of apple seedlings had some symptoms of internal or external bitter pit in any one site.year combination (Table 1). While maximum incidence of pit for any one affected tree at any site ranged from 43–100%, in most cases affected trees had just a few fruit with pit. Mean incidence was less than 10%

Table 1. Basic statistics of bitter pit incidence, fruit weight and number per tree and fruit calcium concentrations at each site × year combination for 25 families common in all combinations

Variable	Site				
	Havelock North			Riwaka	
	1999	2000	2001	1999	2000
External pit					
Trees with pit (%)	29	36	36	34	36
Mean incidence (%)	3.4	4.2	2.2	7.8	4.1
Max. incidence (%)	72	50	43	100	44
Internal pit					
Trees with pit (%)	30	47	42	40	51
Mean incidence (%)	4.1	5.7	2.9	8.7	4.2
Max. incidence (%)	71	55	56	86	47
Average fruit weight (g)	103	97	94	76	78
Average fruit no/tree	40	76	95	27	77
[Ca] (mg 100 gFW⁻¹)					
Mean	2.3	–	2.6	3.2	–
Maximum	5.8	–	7.1	10.2	–
Minimum	1.0	–	1.1	1.1	–

and median incidence was always zero for both sites in all years.

Family and year.site were both important determinants of external and internal pit incidence across most data sets (Table 2; Figure 1). Only for Havelock North in 2001 was family not found to explain variation in external pit. Inverse [Ca] was an important predictor of pit in all data subsets where it could be included, with pit incidence declining with reducing inverse [Ca] (i.e. increasing [Ca]). External pit incidence also declined with earlier harvest dates in most data subsets, as it did with lower average fruit weight and increasing fruit number per TCA in some of these data subsets where mineral concentrations were not able to be included in the models (i.e. data subsets 4,5,6). Correlation coefficients between estimated family means for pit incidence and those for inverse [Ca], harvest date, fruit number·TCA⁻¹ or fruit weight did not differ significantly from zero ($P > 0.1$) for any of the year.site combinations for any of the relevant data subsets.

In order to gain an impression of the relative magnitude of the effects of the predictors, the over-dispersed model was used to give estimates of external pit incidence in 1999 at Riwaka and 2001 at Havelock North (the extremes of the year.site variates) for a range of families (low incidence ‘Paidés Ziemas Abols’, median incidence ‘Mleevskaya Krasavitsa’ and high incidence

Table 2. Variates retained (✓) in the final model for each data subset for both external and internal pit using the over-dispersed binomial

Data subset	Site and year	Pit type	Family	Year site ^a	Inverse [Ca]	Other main effects	Interactions
1 and 2	HN and R 1999, HN 2001	External	✓	✓	✓	harvest date [K]	
		Internal	✓	✓	✓		
3	HN 2001	External		–	✓		–
		Internal	✓	–	✓	fruit·TCA ^{-1b}	–
4	HN 2000 and 2001	External	✓	✓ ^c	–	harvest date	–
		Internal	✓		–	fruit·TCA ⁻¹	–
5	HN and R 1999, HN and R 2000, HN 2001	External	✓	✓	–	harvest date	–
		Internal	✓	✓	–	fruit weight	–
6	HN and R 1999, HN and R 2000	External	✓	✓ ^d	–	fruit weight	family × site
		Internal	✓	✓ ^e	–		family × site year × site

Note. A dash (–) indicates that the variate was not present in the initial model. HN = Havelock North, R = Riwaka.

^aYear × site combination.

^bTrunk cross-sectional area.

^cYear only (no site component in initial model).

^dSite only.

^eBoth year and site.

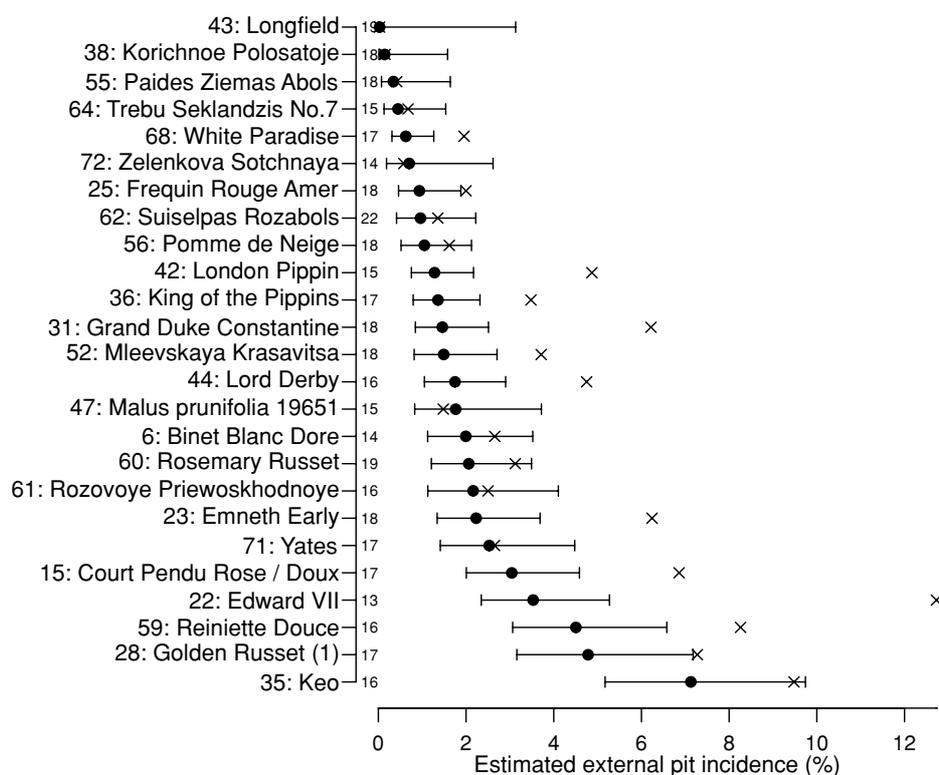


Figure 1. Family estimates (\pm one SE) of external pit using the final model from over-dispersed binomial on data subset 1. Inverse [Ca] and harvest date were set to their overall medians ($0.42 \times 100 \text{ gFW} \cdot \text{mg}^{-1}$ and 26 Feb., respectively) and year.site was 1999 at Havelock North. Crosses show arithmetic family means (across all year/sites). The number of trees per family is given to the right of the vertical.

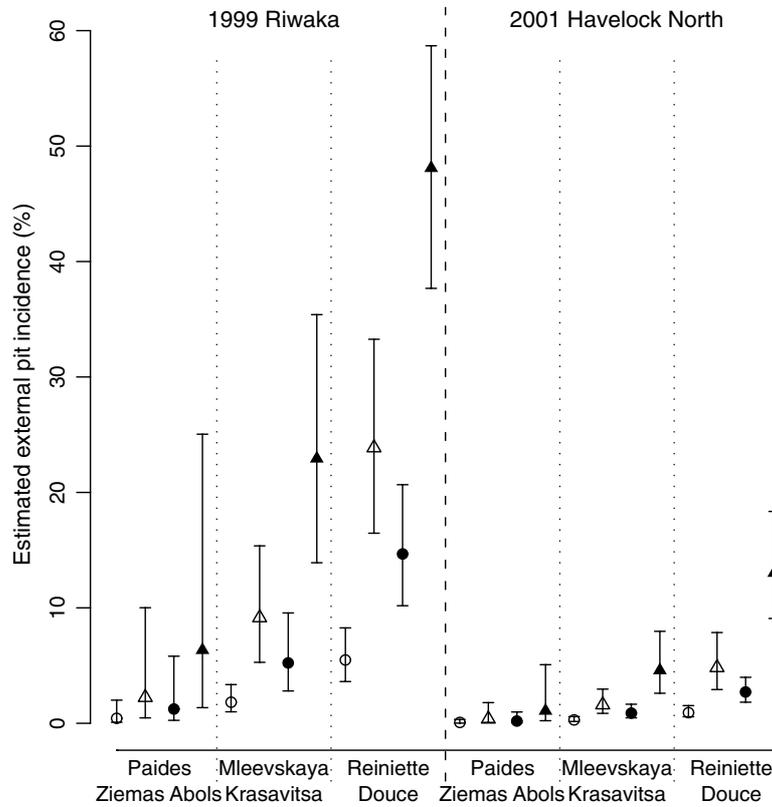


Figure 2. Estimates (\pm one SE) of incidence of external pit using the final model from over-dispersed binomial on data subset 1 for selected combinations: the extreme year, sites (1999 Riwaka and 2001 Havelock North), moderately extreme and median families ('Paides Ziemas Abols', 'Reiniette Douce' and 'Mleevskaya Krasavitsa' respectively) and the 10 and 90 percentiles for harvest date (3 Feb. (open symbols) and 26 Mar. (closed symbols), respectively) and inverse [Ca] (0.24 (circles) and 0.64 (triangles) $100 \text{ gFW} \cdot \text{mg}^{-1}$, respectively).

'Reiniette Douce') and the 10 and 90 percentiles for harvest date (3 Feb. and 26 Mar.) and inverse [Ca] (0.24 and $0.64 \times 100 \text{ gFW} \cdot \text{mg}^{-1}$) (Figure 2). For each estimate the non-specified variates were held at their median values (i.e. family 'Mleevskaya Krasavitsa', harvest date 26 Feb. and inverse [Ca] $0.42 \times 100 \text{ gFW} \cdot \text{mg}^{-1}$). There was over a ten-fold difference in estimated external pit incidence between the low incidence and high incidence families. Extremes of [Ca] or sites resulted in about a five-fold difference in external pit incidence, and the difference in incidence between early and late harvest dates was about three-fold.

Inclusion of the interaction of family with year.site (data subsets 1, 2 and 5), or both year and site together (data subset 6) in the initial model resulted in final models that violated the underlying distributional assumptions. However the family interaction with site was able to be included in the initial model in data subset 6 and it was retained in the final model (Table 2), where its effects were substantial ($P < 0.001$) (Fig-

ure 3). This was particularly so for families 15, 28 and 68 ('Court Pendu Rose/Doux', 'Golden Russet (1)' and 'White Paradise', respectively) and 35 and 36 ('Keo' and 'King of the Pippins', respectively). In contrast other families showed much greater consistency across the two sites (e.g. families 59 and 61 'Reiniette Douce' and 'Rozivoye Priewoskhodnoye'). The family \times year interaction was also significant ($P < 0.001$), when used in place of the family \times site interaction, but resulted in a final model with larger deviance. The average differences in incidence for the same trees assessed in different years were greater for those families also showing substantial differences between sites compared with other families (mean difference for families 15, 28, 68, 35 and 36 = 7.7 (26 trees) cf. 5.8 (101 trees) for the other families).

On an individual assessment basis, the relationship between external and internal pit incidence was positive ($r = 0.69$, $n = 641$), with only 16 (i.e. 2.5%) of the assessments differing by more than 20% incidence and

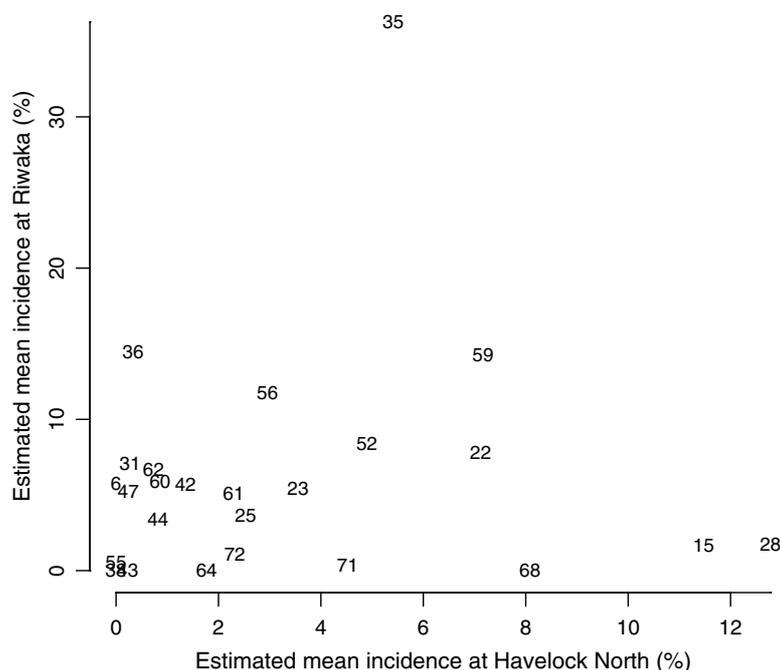


Figure 3. Mean incidence of external pit for each family at Havelock North and Riwaka estimated separately using the final over-dispersed binomial model of data subset 6. Families are identified by their numeric codes, which are shown in Figure 1.

with only family 55 ('Paides Ziemas Abols') exhibiting a marked difference between internal and external pit incidence (data not shown).

Calcium concentration

A wide range of fruit [Ca] was found across the seedlings in 1999 and 2001, from 1.0–10.2 mg·100 gFW⁻¹ (Table 1). Both family ($P < 0.001$) and fruit weight ($P < 0.07$) were retained in the inverse [Ca] models for all three data subsets 1, 2 and 3, and year.site was retained for data subsets 1 and 2 ($P < 0.001$). None of the other variates or their interactions contributed significantly to the fits, which indicates that the relative differences in inverse [Ca] among families were consistent across sites and years. A positive correlation was found between family means of inverse [Ca] and fruit weight averaged across both sites and years from data subset 1 ($r = 0.68$, $P < 0.001$). The arithmetic means of inverse [Ca] generally matched the estimates from the model, which were computed with fruit weight set to its median value (85.4 g), with differences in inverse [Ca] among families larger than those attributable to year site (Figure 4).

Discussion

Genetic effects on bitter pit

That bitter pit in apple is under some degree of genetic control (Smock & Neubert, 1950; Ferguson & Watkins, 1989; Drazeta et al., 2001) is confirmed in this study. Across years and sites, the maternal parent from which a seedling tree was derived partially determined whether fruit from the tree developed pit and the level of pit expression in that fruit. Further, relative to the other factors measured in this study, the genetic effect was the most substantial in affecting pit. Korban and Swiader (1984) also concluded that (external) pit assessed on fruit at harvest was a heritable trait after working on three scab resistant families of apple seedlings derived from controlled crosses, and further suggested that pit expression was controlled by two major genes.

Variable pit incidence within families was partially explained by fruit [Ca], confirming the important role of this mineral mediating environmental effects on pit. However little of the genetic differences in pit seems to be controlled by fruit [Ca]. Korban and Swiader (1984) also found higher fruit flesh [Ca] (and higher levels of

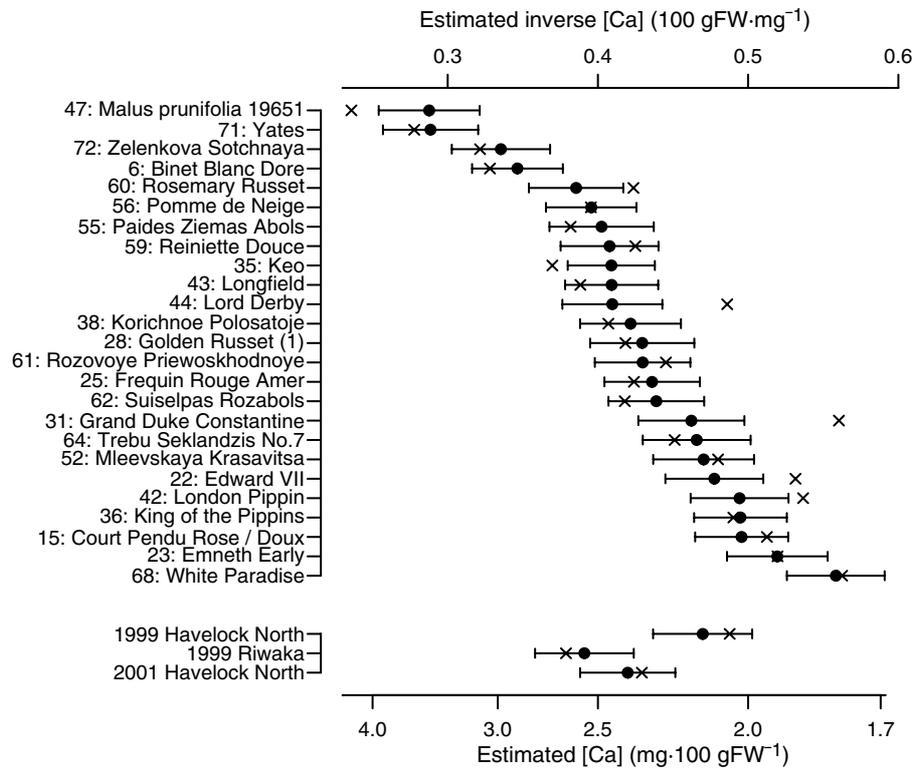


Figure 4. Estimates (\pm one SE) of inverse [Ca] from the linear model for data subset 1. Fruit weight was set to its overall median (85.4 g) and the family estimates were averaged over all year.site combinations. Similarly the year.site estimates were averaged over families. Crosses show arithmetic means.

boron with lower levels of magnesium and potassium) in pit resistant compared with pit susceptible seedlings within two seedling families, but relationships of fruit minerals with pit across families were not explored. Lewis (1980) could find no relationship between fruit mineral concentrations and pit susceptibility for nine apple cultivars. He suggested that only under conditions of low fruit calcium status was a genetic preponderance to pit manifested. However we were unable to investigate such interactions in our study.

As family effects on pit could never be replaced by fruit [Ca] (or any other mineral and cropping factor measured in this study), genetic effects on pit remain unexplained. Bitter pit susceptibility and its development are well known to be under the influence of many preharvest factors (Ferguson & Watkins, 1989; Saure, 1996; Ferguson et al., 1999). We were aware of the importance of these factors in possibly explaining genetic effects on pit. However our inability to do this demonstrates our still inadequate understanding of the complex nature of genetic effects on bitter pit.

This complexity is also demonstrated by the significant family \times site/year interaction for bitter pit expression. The estimated family mean of bitter pit for a given site was not necessarily a good indicator of the family mean at another site or in another year and suggests that genetic control of bitter pit is inconsistent across sites. Results of genetic studies on disorders such as bitter pit from a single site and/or year (Korban & Swiader, 1984; Volz et al., 2001a,b) must be treated with caution and, conversely, it is important to quantify genetic variation in disorder expression over several seasons and sites.

Genetic effects on fruit calcium concentrations

There were strong genetic effects on [Ca] that were consistent across the two years at Havelock North and across the two sites in 1999. Lewis (1980) also noted substantial variation in fruit [Ca] in nine commercial cultivars growing in the one environment, while studies on tomato (Li & Gabelman, 1990), snap

bean (Quintanca et al., 1996) and broccoli (Farnham et al., 2000) have all shown significant effects of genotype on Ca uptake by fruit, seed and flower head respectively.

The effect of family on fruit [Ca] was at least partially due to genetic differences in fruit size, where families with larger fruit accumulated relatively less Ca than smaller fruit. This “dilution” effect of increasing fruit size associated with lower [Ca] has been noted previously (Perring & Jackson, 1975), and is thought to reflect changing patterns of dry matter (phloem-borne) and Ca (xylem-borne) delivery to the fruit (Ferguson & Watkins, 1989; Lang, 1990).

Nevertheless, substantial genetic variation in [Ca] occurred that was also independent of fruit size. This variation could be due to genetic differences in the capacity to take up Ca from the soil and/or accumulate Ca in the fruit. Large tree-to-tree and within-tree variability in apple fruit [Ca] has often been observed (Ferguson & Triggs, 1990) and is associated with tree factors such as crop load, fruiting position, fruiting wood age or leaf/fruit ratios (Ferguson & Watkins, 1992; Volz et al., 1994, 1996; Volz & Ferguson, 1999). The environment in any one season heavily influences these physiological factors but we had suspected that these factors might also have contributed to the observed genetic differences in [Ca]. However such cropping factors failed to account for any family variation in [Ca]. Genetic variation in [Ca] may also be related to the vascular development of the fruit. Xylem functionality declines during the season (Lang, 1990; Drazeta et al., 2004) with greater reductions found in more pit-susceptible cultivars (Drazeta et al., 2001). However genetic effects on non-vascular modes of Ca transport cannot be discounted (Behling et al., 1982).

Conclusions

From a breeding perspective, the significant amount of genetic variation in pit expression found amongst families suggests that genetic improvement can be made in developing pit resistant apple cultivars. However selection for low bitter pit susceptibility needs to be carried out across several environments and over several years to confirm the initial phenotype (Fehr, 1987). The use of inverse [Ca] as an indirect selection measure for pit seems attractive given that it shows more consistent genetic variability compared with that found for pit. However our analysis suggests that the use of inverse [Ca] across families must be treated with caution in

that poor associations between mean family pit incidence and inverse [Ca] were found within sites and years. Nevertheless, given the strong relationship between [Ca] and pit within families it would still seem a useful variate to pursue, although possible linkages with small fruit size need further assessment.

Acknowledgments

We thank Murray Oliver for technical assistance, and Nihal DeSilva for helpful comments in the preparation of this manuscript.

References

- Alspach, P.A. & N.C. Oraguzie, 2002. Estimation of genetic parameters of apple (*Malus domestica*) fruit quality from open-pollinated families. *NZ J Crop Hort Sci* 30: 219–228.
- Behling, J.P., W.H. Gabelman & G.C. Gerloff, 1982. The distribution and utilization of calcium by two tomato (*Lycopersicon esculentum* Mill.) lines differing in calcium efficiency when grown under low-Ca stress. *Plant and Soil* 113: 189–196.
- Burdon, R.D., M.H. Bannister & C.B. Low, 1992. Genetic survey of *Pinus radiata*. 3: Variance structures and narrow-sense heritabilities for growth variables and morphological traits in seedlings. *NZ J For Sci* 22: 160–186.
- Burnham, K.R. & D.R. Anderson, 2002. Model selection and multi-model inference: A practical information-theoretic approach. 2nd ed. Springer, New York, USA.
- Bus, V.G.M., P.A. Alspach, M.E. Hofstee & L.R. Brewer, 2002. Genetic variability and preliminary heritability estimates of resistance to scab (*Venturia inaequalis*) in an apple genetics population. *NZ J Crop Hort Sci* 30: 83–92.
- Collett, D., 1991. Modeling Binary Data. Chapman and Hall, London, UK.
- Cornell, J.A., 1981. Experiments with Mixtures: Designs, Models, and the Analysis of Mixture Data. John Wiley and Sons, New York, USA.
- Drazeta, L., A. Lang, L. Morgan, R. Volz & P.E. Jameson, 2001. Bitter pit and vascular function in apples. *Acta Hort* 564: 387–392.
- Drazeta, L., A. Lang, A. Hall, R.K. Volz & P.E. Jameson, 2004. Causes and effects of changes in xylem functionality in apple fruit. *Ann Bot* 93: 275–282.
- Farnham, M.W., M.A. Grusak & M. Wang, 2000. Calcium and magnesium concentration of inbred and hybrid broccoli heads. *J Amer Soc Hort Sci* 125: 344–349.
- Faust, M. & C.B. Shear, 1968. Corking disorders of apples: A physiological and biochemical review. *Bot Rev* 34: 441–469.
- Fehr, W.R., 1987. Principles of cultivar improvement. Vol 1. Theory and technique. McGraw-Hill, Inc. Auckland, New Zealand.
- Ferguson, I.B. & C.M. Triggs, 1990. Sampling factors affecting the use of mineral analysis of apple fruit for the prediction of bitter pit. *NZ J Crop Hort Sci* 18: 147–152.

- Ferguson, I.B. & C.B. Watkins, 1989. Bitter pit in apple fruit. *Hort Rev* 11: 289–355.
- Ferguson, I.B. & C.B. Watkins, 1992. Crop load affects on mineral concentrations and incidence of bitter pit in 'Cox's Orange Pippin' apple. *J Amer Soc Hort Sci* 117: 373–376.
- Ferguson, I.B., R.K. Volz & A. Woolf, 1999. Preharvest factors affecting physiological disorders of fruit. *Postharvest Biol Tech* 15: 255–262.
- Hall, B.H., 2000. Zero-inflated Poisson and binomial regression with random effects: A case study. *Biometrics* 56: 1030–1039.
- Irving, D.E. & J.H. Drost, 1987. Effects of water deficit on vegetative growth, fruit growth and fruit quality in Cox's Orange Pippin apples. *J Hort Sci* 62: 427–432.
- Jones, H.G. & T.J. Samuelson, 1983. Calcium uptake by developing fruits. II The role of spur leaves. *J Hort Sci* 58: 173–182.
- Korban, S.S. & J.M. Swiader, 1984. Genetic and nutritional status in bitter pit-resistant and -susceptible apple seedlings. *J Amer Soc Hort Sci* 109: 428–432.
- Lang, A., 1990. Xylem, phloem and transpirational flows in developing apple fruits. *J Exp Bot* 41: 645–651.
- Lewis, T.L., 1980. The rate of uptake and longitudinal distribution of potassium, calcium and magnesium in the flesh of developing apple fruit of nine cultivars. *J Hort Sci* 55: 57–63.
- Li, Y. & W.H. Gabelman, 1990. Inheritance of calcium use efficiency in tomatoes grown under low-calcium stress. *J Amer Soc Hort Sci* 115: 835–838.
- Luby, J.J., P.A. Alspach, V.G.M. Bus & N.C. Oraguzie, 2002. Field resistance to fire blight in a diverse apple (*Malus* sp.) germplasm collection. *J Amer Soc Hort Sci* 127: 245–253.
- Noiton, D., M. Hofstee, P. Alspach, L. Brewer & C. Howard, 1999. Increasing genetic diversity for apple breeding: A preliminary report. *Acta Hort* 484: 105–107.
- Quintanca, J.M., H.C. Harrison, J. Nienhuis & J.P. Palta, 1996. Variation in calcium concentration among sixty S1 families and four cultivars of snap bean (*Phaseolus vulgaris* L.). *J Amer Soc Hort Sci* 212: 789–793.
- Perring, M.A. & C.H. Jackson, 1975. The mineral composition of apples. Ca concentration and bitter pit in relation to the mean mass per apple. *J Sci Food Agric* 28: 1493–1502.
- R Development Core Team, 2003. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Saure, M.C., 1996. Reassessment of the role of calcium in development of bitter pit in apple. *Aust J Plant Physiol* 23: 237–243.
- Smock, R.M. & A.M. Neubert, 1950. Apples and apple products. Interscience Pub., New York, USA.
- Turner, N.A., I.B. Ferguson & R.O. Sharples, 1977. Sampling and analysis for determining relationship of calcium concentration to bitter pit in apples. *NZ J Ag Res* 20: 525–532.
- Venables, W.N. & B.D. Ripley, 1994. Modern Applied Statistics with S-Plus. Springer-Verlag, New York, USA.
- Vieira, A.M.C., J.P. Hinde & C.G.B. Demétrio, 2000. Zero-inflated proportion data models applied to a biological control assay. *J Appl Stat* 27: 373–389.
- Volz, R.K., I.B. Ferguson, E.W. Hewett & D.J. Woolley, 1994. Wood age and leaf area influences fruit size and mineral composition of apple fruit. *J Hort Sci* 69: 385–395.
- Volz, R.K., D.S. Tustin & I.B. Ferguson, 1996. Mineral accumulation in apple fruit as affected by spur leaves. *Scientia Hort* 65: 151–161.
- Volz, R.K. & I.B. Ferguson, 1999. Flower thinning method affects mineral composition of 'Braeburn' and 'Fiesta' apple fruit. *J Hort Sci Bio* 74: 452–457.
- Volz, R.K., P.A. Alspach, A.G. White & I.B. Ferguson, 2001a. Genetic variability in apple fruit storage disorders. *Acta Hort* 553: 241–244.
- Volz, R.K., P.A. Alspach, A.G. White & I.B. Ferguson, 2001b. Genetic variability in mineral accumulation of apple fruit, pp. 92–93. In: W.J. Horst et al. (eds.). *Plant nutrition – food security and sustainability of agro-systems*. Kluwer Academic Publishers, The Netherlands.
- Williams, D.A., 1982. Extra-binomial variation in logistic linear models. *Appl Statist* 31: 144–148.