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Pre- and Postharvest Factors Control the Disease Incidence of Superficial Scald in the New Fire Blight Tolerant Apple Variety “Ladina”

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Abstract: Superficial scald is a physiological disorder that develops during cold storage affecting apples and causes substantial market losses. *Malus × domestica* cv. Ladina, a new scab resistant and fire blight tolerant variety, commercialized in 2012, shows a physiological disorder similar to superficial scald after storage. Here, we used different pre- and postharvest approaches to characterize the occurrence of these superficial scald symptoms in *Malus × domestica* cv. Ladina. Over a period of seven years, fruits from multiple orchards were stored for five to seven months and the occurrence of superficial scald was assessed in fruits after cold storage and controlled atmosphere (CA) storage. Apples picked at different stages of ripeness within the same year differed in superficial scald development. Additionally, superficial scald differed significantly between years and locations, strongly suggesting that maturity at harvest, weather during the growing season, and orchard management play important roles in scald occurrence. Treatment with 1-methylcyclopropene (1-MCP) after harvest, and storage in a dynamically controlled atmosphere (DCA) significantly reduced the occurrence of superficial scald, whereas storage under ultralow oxygen concentrations (ULO) showed mild but not significant effects. Low calcium concentrations in the fruit flesh and peel were associated with stronger superficial scald occurrence.

Keywords: apple variety; storage; physiological disorder; dynamic controlled atmosphere storage; 1-methylcyclopropene treatment; ultralow oxygen storage; X-ray fluorescence

1. Introduction

Malus × domestica cv. Ladina, a new variety, originated from a cross between *Malus × domestica* cv. Topaz and *Malus × domestica* cv. Fuji performed in 1999 at Agroscope in Wädenswil, Switzerland, and was first commercialized in 2012 [1]. Ladina has been selected as cultivar because of its genetic resistance against apple scab (*Venturia inaequalis*, Rvi6 resistance) and low susceptibility towards powdery mildew. Meanwhile, the variety *Malus × domestica* cv. Ladina has been successfully screened for its low susceptibility against fire blight (*Erwinia amylovora*) in shoot and flower inoculation tests in the glasshouse and orchards, respectively [2], increasing its potential for use in organic apple production systems. However, concern has arisen as *Malus × domestica* cv. Ladina has shown a considerable amount of superficial scald after a few months of storage (Figure 1).



Figure 1. Superficial scald on *Malus × domestica* cv. Ladina. Examples of superficial scald in apple variety *Malus × domestica* cv. Ladina in increasing degrees from left to right occurring after a storage period of more than three months and especially during the shelf life at 20 °C.

Other physiological disorders such as core flush and soft scald have rarely been observed and the variety is robust against fungal storage diseases such as grey mold (*Botrytis cinerea*), lenticel rots (*Neofabraea* spp.), and storage scab (*Venturia inaequalis*) (data not shown). Therefore, the amount of superficial scald is the only significant postharvest disorder, making it an important problem to manage under commercial production and marketing settings.

Superficial scald is a serious physiological disorder affecting apples and pears. This postharvest disorder develops after several months of cold storage and increases after removal from storage, causing a substantial loss of market value [3,4]. Symptoms appear as darkened areas on the peel and primarily affect the peel forming epidermis and hypodermis, with parenchymal layers rarely affected [5]. Susceptibility to superficial scald varies greatly by cultivar, climate, ripening stage, cultural practices, and postharvest conditions [6,7]. Despite intensive investigation, the biochemical mechanisms underlying superficial scald development remain largely unknown [3,8]. Superficial scald has been described as a special case of chilling injury induced by oxidative stress on secondary metabolites [9]. The oxidation products of α -farnesene include conjugated trienes [6,9] and 6-methyl-5-hepten-2-one [10], which seem to be more directly associated with superficial scald development than α -farnesene itself [11]. Furthermore, the synthesis of α -farnesene has been shown to be positively influenced by the ripening hormone ethylene, and hence ripening facilitates superficial scald development [12–14].

Several hypotheses on how to control the development of superficial scald exist. The lower abundance of ethylene under controlled atmosphere (CA) storage conditions delays the process of physiological disorders linked to ethylene production and ripening such as superficial scald [4,15]. Superficial scald occurrence has been further reduced by storage in a dynamic controlled atmosphere (DCA), [16]. Moreover, application of the competitive ethylene antagonist, 1-methylcyclopropene (1-MCP), blocks the response of plants to ethylene at very low concentrations after short-term exposure [4,11,17,18]. However, 1-MCP is not a naturally occurring chemical, and therefore does not meet the requirements for organic production systems. In contrast, DCA storage is a chemical-free technology that can be used in organic production [19,20]. Preharvest factors such as fruit maturity at harvest and the frequency of low temperature exposure over the ripening season have been shown to be important factors involved in the occurrence of superficial scald [21]. In addition, immature apples have been shown to develop more superficial scald, possibly due to a less efficient antioxidant system resulting in an inability to prevent the oxidation secondary metabolites [12]. Another indication of the extent of superficial scald development is the mineral supply of fruits. Although no underlying physiological mechanism has been established so far, low calcium and high potassium contents of apples have been associated with increased scald occurrence [21]. The apple peel has been shown to exhibit a high concentration of calcium relative to the flesh [22], and most of the cellular calcium was concentrated in the cell wall [23]. Low concentrations of calcium can cause cell membrane leakage and severe damage to the cell [24,25]. Calcium deficiency-related physiological disorders are thought to be predicted by the overall calcium concentration and its ratio to other elements, such as potassium, in

the skin and the fruit flesh [26]. Additionally, potassium has been shown to antagonize calcium uptake into the leaves, thus, lowering total calcium concentrations which indicates that there are possible interactions between these two minerals [27].

The objective of the present study was to characterize the influence of various pre- and postharvest factors on the incidence of superficial scald development on *Malus × domestica* cv. Ladina. These factors included (i) the duration of the storage period; (ii) preharvest factors such as varying growing seasons, orchard locations, and harvest dates; (iii) storage under ultralow oxygen conditions (ULO), DCA storage, and 1-MCP treatment; and (iv) potassium and calcium concentrations and their ratios in the apple fruits and skin.

2. Materials and Methods

Storage experiments of *Malus × domestica* cv. Ladina were conducted at Agroscope Wädenswil, Switzerland in research sized storage facilities with fruits from six consecutive growing seasons from 2013 to 2018. Fruits used for the trials originated from various locations and growers, and different orchards within locations. GPS data for the orchards are as follows: Wädenswil, WA (plots Wa64 and Wa66 47°13'15" N, 8°40'01" E, Wa39 47°13'27" N, 8°40'37" E), Güttingen, GU (Gu53 47°36'07" N, 9°16'37" E), Wülflingen, WU (47°30'50" N, 8°42'00" E), Bliedegg-Bischofszell, BI (47°30'09" N, 9°14'18" E), and Morges-Marcelin, MA (46°31'08" N, 6°29'02" E). Fruits from WA, GU, WU, and MA were treated with a standardized plant protection strategy representative for Swiss Integrated Pest Management while fruits from BI were treated under a Swiss organic management system. Fruits were stored in a cold store at 1 °C until further storage under the appropriate storage conditions. On average, fruits were stored in cold storage for 9 days before treatments started, however, never more than 23 days. To control for ripeness, basic apple quality parameters (i.e., firmness (kg/cm²) using a penetrometer equipped with a 11 mm punch and sugar content (° brix) using a refractometer per fruit plus acidity (titration) as a mean of 10 fruits) were assessed on the "Pimprenelle" machine (Setop Giraud Technology, Cavaillon, France) and the starch index was assessed visually prior to storage (data not shown). Afterwards, fruits were stored for 5 to 7 months before removal from storage. Basic apple quality parameters (firmness, total soluble solids (TSS), acidity) were measured immediately after removal from storage and again after 7 days of "shelf life" at 20 °C. Storage disorders and diseases of 20 fruits were visually assessed immediately after removal from cold store and after 7 days of "shelf life" at 20 °C. Exact harvest dates and dates of storage removal are specified (Table 1).

Standard controlled atmosphere (CA) conditions were set to 1 °C, 1% CO₂, 1% O₂, and 92% relative humidity. Additionally, variations to these conditions were added with the application of 1-MCP (Agrofresh, Philadelphia, PA, USA) in 2013 and 2014 (only to fruits from Wülflingen), the application of ultralow oxygen storage at 0.5% in 2014, and storage under dynamic controlled atmosphere (DCA) by measuring chlorophyll-a fluorescence using Harvest Watch sensors (DeLong et al., 2004) in 2017. Additionally, fruits were harvested at two different ripeness degrees in 2014 and at three different ripeness degrees in 2015 (Table 1).

Potassium and calcium concentrations and their ratio K/Ca were measured as suggested by the manufacturer of the atomic absorption spectrometer. Briefly, ten apples per sample were each divided into ten pieces. Four pieces of each apple were weighed, dried at 70 °C for 2 days, and ground. Then, 250 mg of fruit powder was dissolved in 4 mL nitric acid (69%). After 10 min, 3.5 mL hydrogen peroxide (30%) was added. After another 15 min, samples were digested in a microwave oven MLS-EM2, (Milestone, Sorisole, Italy). The K/Ca content of each sample was determined using an atomic absorption spectrometer (AAS) iCE3000 (Thermo Fisher, Waltham, MA, USA). Results were converted into mg/100 g fresh fruit.

For the fruits of the 2018 growing season, the incidence of superficial scald was quantified using an automatic iQS quality sorting system mounted on a small industrial scale fruit-sorting machine. Additionally, the disease incidence was scored visually on a scale from 1 to 5 and by calculating the percentage of fruits with disease per plot/orchard. The K/Ca content measured using X-ray fluorescence

analysis was performed on a CTX CounterTop XRF (Bruker, Berlin, DE) according to the instructions of the manufacturer. Fruits were placed on the measuring platform and mineral contents were measured using the fruit quality calibration provided by the manufacturer. Four points along the equator of the fruits were measured per fruit.

Table 1. Harvest dates and dates of storage removal of *Malus × domestica* cv. Ladina apples from different locations, years, and treatments, including estimated ripeness expressed as Streif index. (Streif index = firmness (kg/cm²) / (starch degradation (1–10) * total soluble solids (° brix)). Where not indicated otherwise, fruits were stored under controlled atmosphere (CA) conditions at 1 °C, 1% CO₂, 1% O₂, and 92% relative humidity (control treatment).

Plot	Harvest Date	Early Removal	Late Removal	Control	Treatment	Ripeness Index
Wa64	29.09.2013	03.02.2014	29.04.2014	CA	1-MCP	0.117
Wa66	01.10.2013	03.02.2014	29.04.2014	CA	1-MCP	0.086
Wa64	10.09.2014	18.02.2015	11.05.2015	CA	0.5% O ₂	0.116
Wa64	22.09.2014	18.02.2015	11.05.2015	CA	0.5% O ₂	0.08
Wa66	10.09.2014	18.02.2015	11.05.2015	CA	0.5% O ₂	0.111
Wa66	22.09.2014	18.02.2015	11.05.2015	CA	0.5% O ₂	0.079
Wu	05.09.2014	18.02.2015	11.05.2015	CA	1-MCP	0.097
Wu	13.09.2014	18.02.2015	11.05.2015	CA	1-MCP	0.082
Wa64	10.09.2015	01.02.2016	26.04.2016	CA	CA	0.19
Wa64	15.09.2015	01.02.2016	26.04.2016	CA	NA	0.15
Wa64	06.10.2015	01.02.2016	26.04.2016	CA	NA	0.1
Wa66	10.09.2015	01.02.2016	26.04.2016	CA	NA	0.182
Wa66	15.09.2015	01.02.2016	26.04.2016	CA	NA	0.15
Wa66	06.10.2015	01.02.2016	26.04.2016	CA	NA	0.1
BI	26.09.2016	21.02.2017	12.05.2017	CA	NA	0.145
Gu53	26.09.2016	21.02.2017	12.05.2017	CA	NA	0.105
Ma	22.09.2016	21.02.2017	12.05.2017	CA	NA	0.113
Wu	26.09.2016	21.02.2017	12.05.2017	CA	NA	0.116
BI	12.09.2017	19.02.2018	17.04.2018	CA	DCA	0.1
Gu53	20.09.2017	19.02.2018	17.04.2018	CA	DCA	0.1
Wa39	20.09.2017	19.02.2018	17.04.2018	CA	DCA	0.099
Gu53	14.09.2018	26.02.2019	09.04.2019	DCA	DCA + 1-MCP	0.109
Wa39	14.09.2018	26.02.2019	09.04.2019	DCA	DCA + 1-MCP	0.12

All statistical analyses were conducted using R version 3.5.1 (R Core Team 2018). Analysis of different harvest dates was performed using generalized linear models with a binomial error distribution. For the analysis of the influence of the growing season on scald incidence, only control treatments were chosen and analyzed in a generalized linear mixed-effects model (family = binomial) using “lme4” [28] with the occurrence of superficial scald as response variable, growing season as fixed factor, and plot as random factor. The incidence of postharvest treatment protocols such as ultralow oxygen storage, DCA, 1-MCP, and combined DCA + 1-MCP on superficial scald was compared using a generalized linear mixed-effects model with a binomial distribution. Treatment, time of removal from storage, and harvest date were incorporated as fixed factors, whereas plot was incorporated as a random factor. The significance of the fixed factors in all models was analyzed using likelihood ratio tests (LRT) (Chi-squared). A correlation test was performed on the K/Ca ratio at harvest and the incidence of superficial scald at early and late removal from storage. Furthermore, the K/Ca ratio was compared between damaged and undamaged fruits using an unpaired t-test. Measurements from different plots, years, and times of removal from storage were treated as replicates. Significance of differences in calcium and potassium concentrations in the skin and their ratios were tested using Kruskal–Wallis and Dunn post-hoc analyses.

3. Results

Immediately after removal from storage, no fruits showed signs of superficial scald. Superficial scald developed only after the fruits were exposed to a shelf life period of seven days at 20 °C. In total,

overall years and treatments, 17.7% of fruits removed from storage early (February) and 35.5% of fruits removed late (April) developed symptoms of superficial scald (Figure 1).

In all trials, occurrence of superficial scald was significantly greater, the longer fruits had been stored. The origin of the fruits showed a significant effect on the incidence of superficial scald (Figure 2a). In 2015, when fruits were harvested at three different ripeness degrees, the harvest date was crucial for the occurrence of superficial scald, occurring least in fruits that were harvested at the intermediate date (Figure 2b). Occurrence of superficial scald was significantly different between years. Fluctuations were considerable, with 2014 and 2015 showing low superficial scald incidence, whereas 2013, 2016, and 2017 showing increased occurrence of superficial scald (Figure 2c).

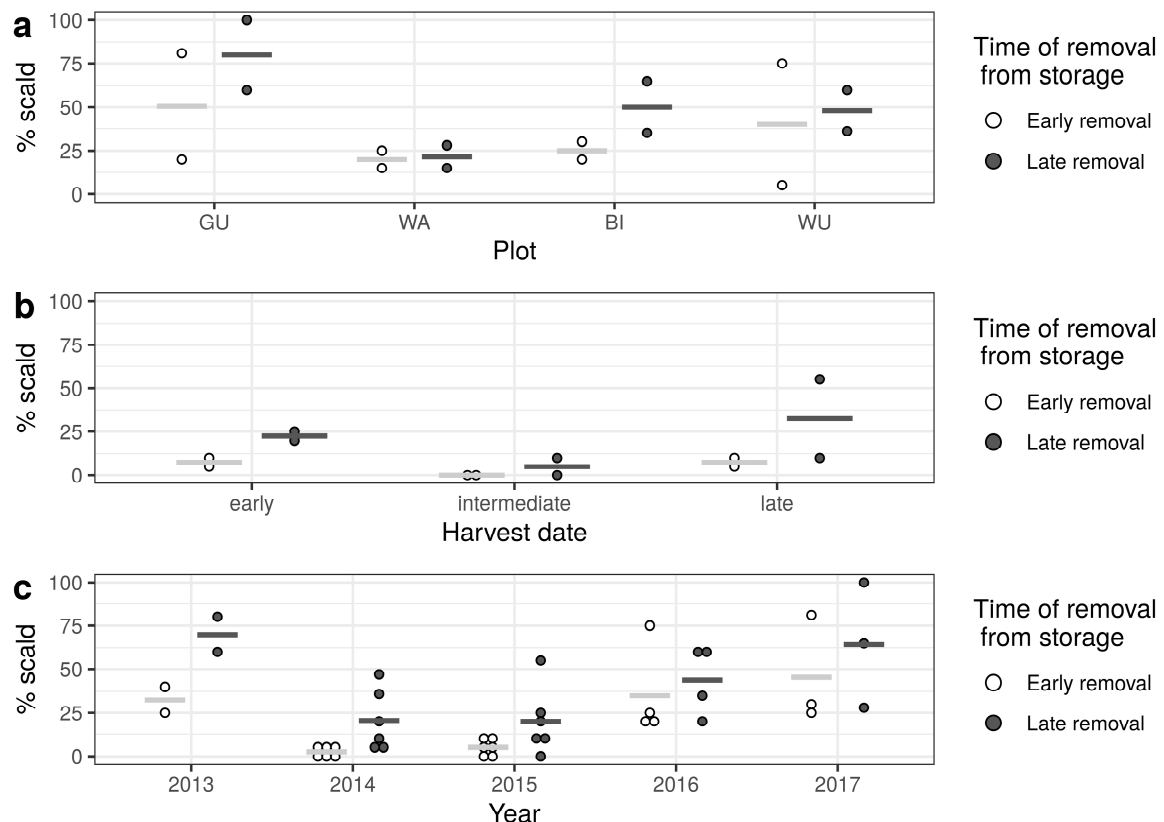


Figure 2. Preharvest factors influencing superficial scald development on *Malus × domestica* cv. Ladina. Percentage of superficial scald occurring in “Ladina” after 7 days “shelf life” at 20 °C when removed from storage after 5 months (early removal) or after 7 months (late removal). (a) Percentage of symptomatic fruits in four locations and two growing seasons (2016 and 2017); (b) Percentage of symptomatic fruits harvested at three different ripening degrees from two orchards in one location and one growing season (2015); (c) Percentage of symptomatic fruits from different growing seasons with fruits from all locations (2013 to 2018). All fruits displayed were stored under control conditions. The results of the statistical evaluations are displayed in Table 2. Dots represent measured data points and horizontal lines denoting the means.

An ultralow oxygen level storage (0.5% O₂) slightly but not significantly reduced the occurrence of superficial scald (Figure 3a). The storage under dynamic controlled atmosphere (DCA) significantly reduced superficial scald as compared with normal CA storage (Figure 3b), and there was a significant reduction of superficial scald incidence with the application of 1-methylcyclopropene (1-MCP) (Figure 3c). A combined application of 1-MCP and storage in DCA further reduced the occurrence of superficial scald incidence after storage (Figure 3d). All statistical results are in Table 2.

Table 2. Results of generalized linear models (family = binomial) comparing the occurrence of superficial scald between various treatments (1-MCP treatment, ultralow oxygen treatment, and DCA treatment) and the respective control, as well as the comparison between different harvest times, years, and locations within the control treatments, df = degree of freedom. Significance values ($p < 0.05$) for fixed effects were determined using LRT (Chi-squared) and are listed below in bold.

Treatment	Factor	df	LRT (χ^2)	p-value
Plot	Treatment	3	30	<0.001
	Time of removal	1	6.6	0.01
Harvest Date	Treatment	2	14.8	<0.001
	Time of removal	1	12	<0.001
Year	Year	4	27.3	<0.001
	Time of removal	1	18.6	<0.001
ULO	Treatment	1	1.7	0.202
	Time of removal	1	9.6	0.002
DCA	Treatment	1	6.6	0.007
	Time of removal	1	7.2	0.01
1-MCP	Treatment	1	5.8	0.016
	Time of removal	1	28.4	<0.001
DCA + 1-MCP	Treatment	1	14.1	<0.001
	Time of removal	1	3.4	0.07

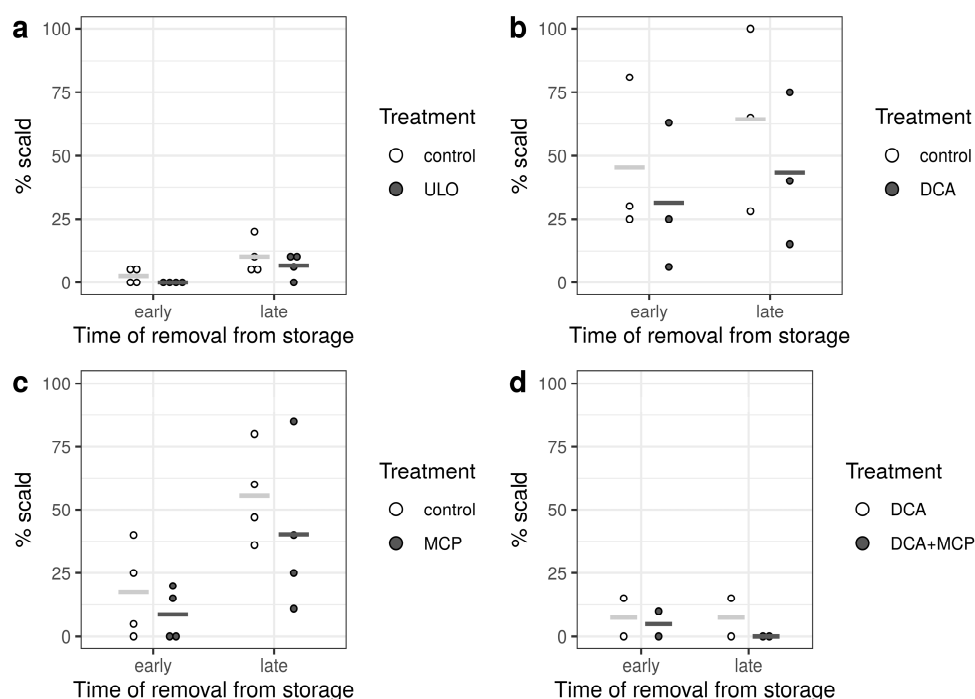


Figure 3. Postharvest factors influencing superficial scald. Percentage of fruits with superficial scald on *Malus x domestica* cv. Ladina stored under different storage conditions after 7 days “shelf life” at 20 °C when removed from storage after 5 months (early removal) or after 7 months (late removal). (a) Ultralow oxygen treatment (2014, two plots, two harvest dates each); (b) Dynamic controlled atmosphere storage (2017, three plots); (c) 1-MCP treatment (2013, two plots, two harvest dates each); and (d) combined DCA and 1-MCP treatment (2018, two plots). Dots represent measured data points and horizontal lines denoting the means.

The measured potassium/calcium (K/Ca) ratio at harvest showed a low correlation with percent of superficial scald at early or late removal from storage (Figure 4a, $r^2 = 0.0819$). However, the K/Ca ratios of damaged and undamaged fruits were significantly different from each other (Figure 4b, $p = 0.004$,

$t = 3.176$, $df = 21.43$). Symptomatic fruits showed a significantly higher K/Ca ratio in all plots except in plot BI where the mean K/Ca ratio was higher but not significant.

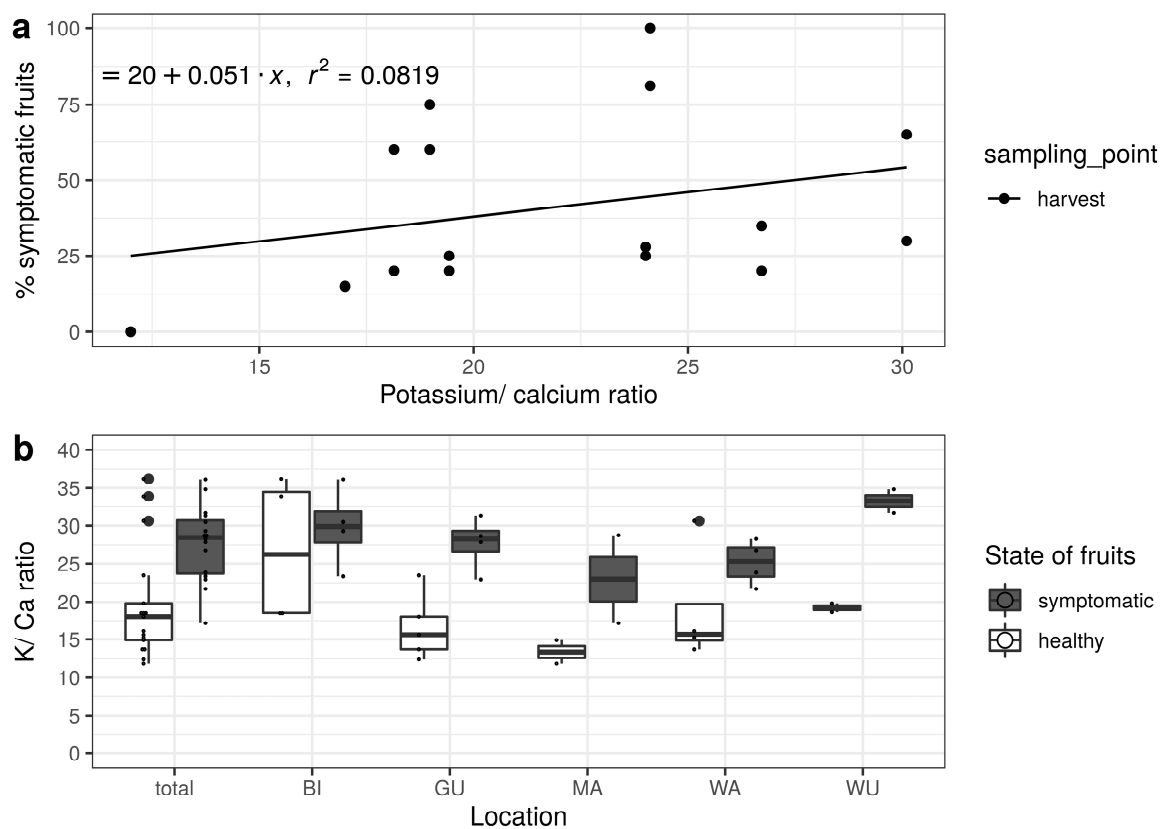


Figure 4. Potassium and calcium (K/Ca) ratios in fruits measured using AAS (atomic absorption spectroscopy) and correlations to superficial scald development. (a) Mean K/Ca ratio measured by AAS at harvest and percent of fruits with superficial scald at removal from storage showed a very poor correlation with $r^2 = 0.0819$; (b) K/Ca ratio in damaged and undamaged fruits after storage split by location, measured by AAS. Blidegg-Bischofszell (BI), Morges-Marcelin (MA), and Wülflingen (WU) were measured in 2016; Wädenswil (WA) in 2017 and 2018; and Güttingen (GU) in 2016, 2017, and 2018. K/Ca ratio was measured twice per plot each year, at two different storage removal dates.

Because the measurement of K/Ca concentrations in apples using AAS is labor intensive, the K/Ca concentrations on apples from five different plots were measured using X-ray fluorescence enabling a larger sampling size. The K/Ca ratios differed significantly between plots (Figure 5a). Potassium levels differed only slightly across plots (Figure 5b) but calcium levels showed significant differences (Figure 5c) affecting the K/Ca levels. The K/Ca ratios measured using XRF showed much lower values as compared with the AAS method, indicating a higher calcium and a lower potassium concentration in the skin as compared with the apple cortex tissue.

Previously, it was shown that the XRF technology has sufficient accuracy and resolution to measure the distribution of potassium and calcium concentrations throughout single fruits and due to its simple measurement principle allows for a much higher sample throughput as compared with AAS [28–30]. In order to test this claim and to measure K/Ca ratios more accurately, 20 to 30 fruits from five different orchards on four points along the equator of the fruits were measured. The red, sun-exposed side of the fruits showed a significantly lower K/Ca ratio and higher calcium concentration as compared with the green, shade-exposed side of the fruit. The other two sides in between the two extremes showed similar intermediate values (Figure 6).

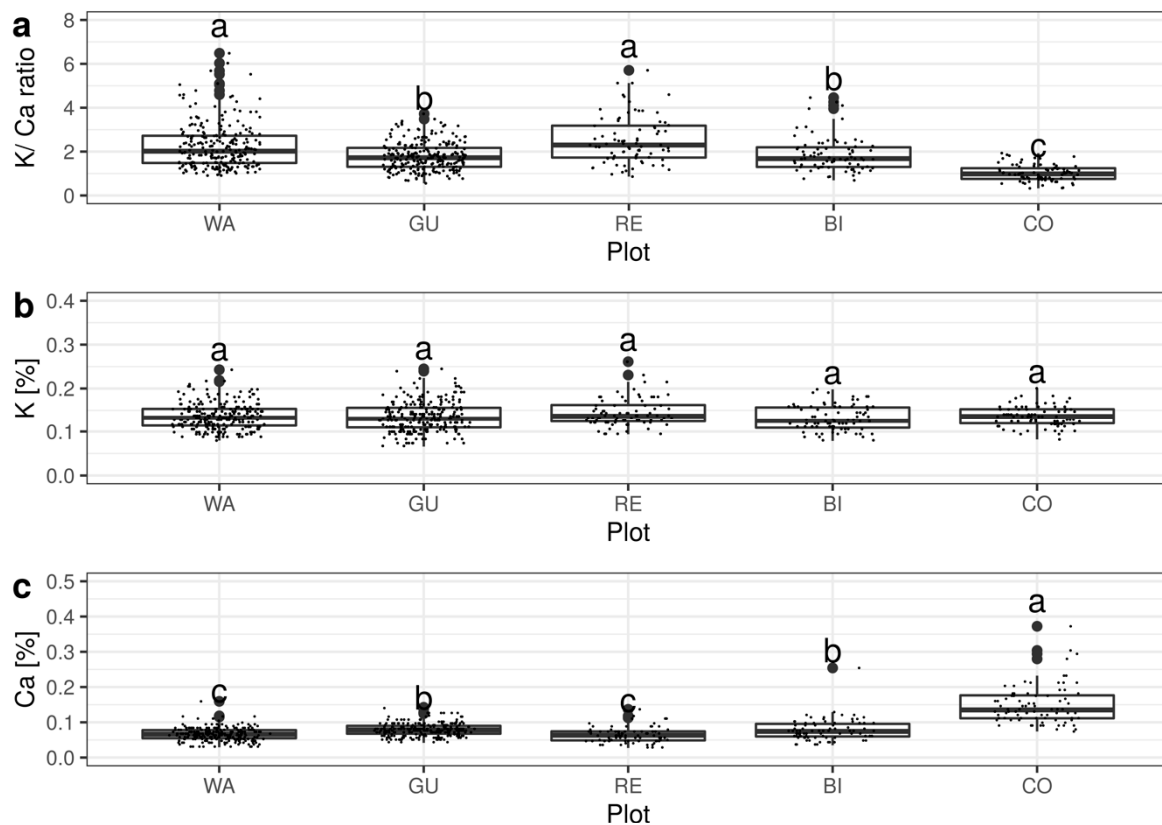


Figure 5. Potassium and calcium ratios in fruits measured using X-ray fluorescence (XRF). Mean K/Ca ratio (a), potassium (b), and calcium (c) concentrations measured using XRF for the trial 2018 to 2019. Letters denote significant differences after Kruskal–Wallis rank sum test and Dunn post-hoc analysis showing significant differences in calcium concentrations, and thus K/Ca ratios by orchard, while potassium values do not differ significantly.

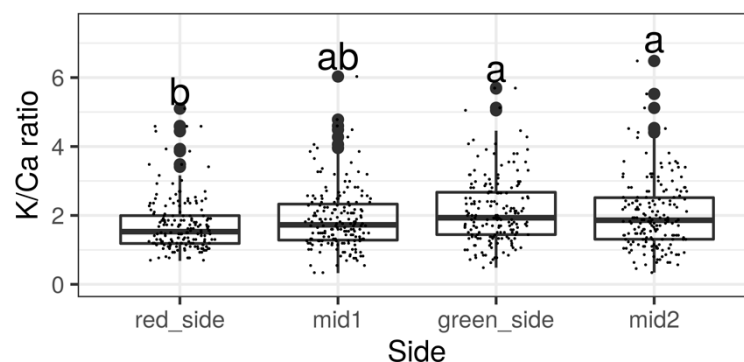


Figure 6. Spatial distribution of potassium and calcium ratios along the fruit equator. K/Ca ratio as measured on four sides per apple along the equator of the fruits using XRF for the trial 2018 to 2019. The red, sun-exposed side showed a significantly lower K/Ca ratio as compared with the green side facing the inside of the tree. The sections in between showed intermediate values. Significant differences in the K/Ca ratios were caused by significant differences in the calcium concentrations and constant levels of potassium levels, as already stated above with the different orchards. Letters denote significant differences after the Kruskal–Wallis rank sum test and Dunn post-hoc analysis.

The mean of superficial scald incidence correlated with the mean K/Ca ratio ($r^2 = 0.635$ to 0.741) depending on the type of disorder or disease quantification (Figure 7a–c). However, when plotted individually a small correlation coefficient $r^2 = 0.113$ was measured (Figure 7d).

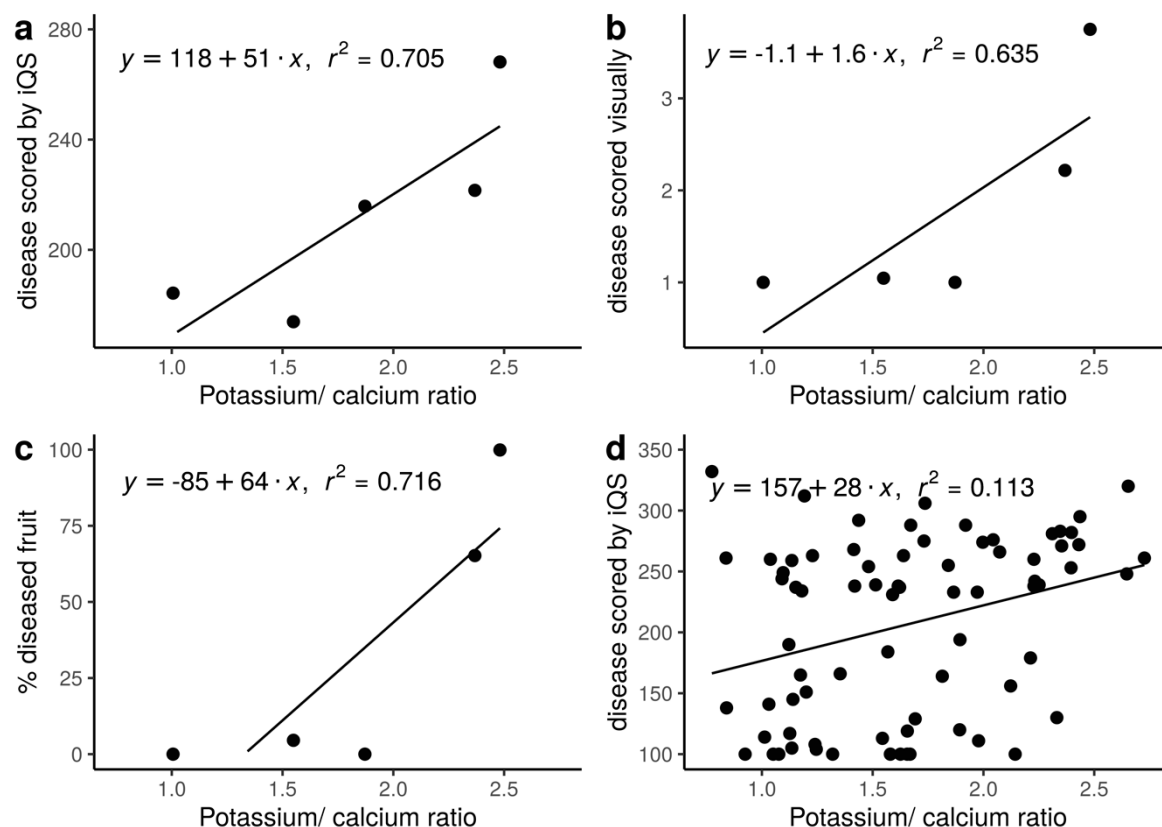


Figure 7. Correlations of K/Ca ratios and disease incidence on fruits. Mean K/Ca ratio as measured on four points across the equator for each fruit by XRF. The mean values per orchard were plotted against soft scald incidence measured using an automatic disorder and disease scoring on a commercial grading machine (a), plotted against superficial scald incidence scored visually by eye on a scale from 1 to 5 (b), plotted against percent of symptomatic fruits with superficial scald (c), and plotted for each single data point using the iQS quality values (d). When grouped by means, a high correlation coefficient (0.635 to 0.716) could be measured, whereas plotting all single data points showed a low correlation coefficient, indicating a high variability among the single fruits.

4. Discussion

In an attempt to characterize the incidence of superficial scald in *Malus × domestica* cv. Ladina to a deeper level, the disease incidence for fruits of different orchards, for fruits of different ripeness degrees harvested at varying harvest dates, and for different years has been monitored in this study. In 2014, early harvested fruits from two different orchards at the same location showed a trend towards developing superficial scald more frequently. Additionally, earlier harvested fruits from a different grower developed superficial scald significantly more often, confirming previous studies [7,31] that superficial scald is prone to early harvest dates. In 2015, apples from two different orchards were harvested at three different ripeness degrees. The different harvest dates significantly influenced the later occurrence of superficial scald, with the intermediate harvest date inducing the lowest incidence of superficial scald. Previously, it was speculated that immature apples could have a less efficient antioxidant system which makes them more susceptible towards superficial scald [31].

In a further analysis, superficial scald susceptibility of Ladina apples stored under the same storage conditions for multiple years (2013 to 2017) were compared. Susceptibility towards superficial scald was significantly greater the longer fruits were stored and differed considerably between years, confirming previous studies showing that the weather conditions during the growing season can greatly influence susceptibility towards physiological disorders such as superficial scald [32]. Compared to other years, fruits that were harvested in 2014 and 2015 showed a very low susceptibility towards

superficial scald, indicating that climatic differences between years could play an important role in the development of superficial scald. The weather conditions in Switzerland, in 2014 and 2015 in the last six weeks before harvest, showed a high number of hours below 10 °C as compared with the other years within this study. This corresponds with the study of [33], where it was shown that apples grown under warm and dry environmental conditions were generally more prone to superficial scald than apples that were grown in cooler climates. Previously, it was shown that susceptibility towards superficial scald can be predicted based on the frequency of low temperature exposure in autumn [12].

To decrease severity of superficial scald, different postharvest treatments and storage methods were tested over several years. Storing fruits under ultralow oxygen conditions showed a mild but not significant effect on the occurrence of superficial scald, contrary to previous studies [33]. The application of 1-MCP significantly reduced superficial scald on fruits (Figure 2a), supporting recent studies on other varieties [4,11,18], due to 1-MCP being an extremely effective competitive inhibitor of ethylene. Ethylene was shown to influence the content of α -farnesene in apple skin, and thus the development of superficial scald [18]. The reduction of superficial scald by 1-MCP was observed in fruits from all locations except from plot Wa64, where the overall occurrence of superficial scald of over 75% was high because fruits had been harvested very late that year (Table 1), and could have missed the appropriate ripeness degree for 1-MCP application, and thus influenced the results negatively. Dynamic controlled atmosphere storage showed similar results to the 1-MCP treatment (Figure 2b). Fruits from all orchards and growers showed a reduction of superficial scald when stored under DCA conditions as compared with regular CA conditions. These findings are consistent with studies by [34]. The reduction of superficial scald by application of 1-MCP or DCA storage suggests that the superficial scald in “Ladina” apples is an ageing problem, previously described as senescent scald [35].

No preharvest condition or postharvest method restrained superficial scald below a limit of detection after seven months of storage. *Malus × domestica* cv. Ladina seems to be a variety particularly susceptible to superficial scald, as was shown before for *Malus × domestica* cvs. Granny Smith and Red Delicious. Other cultivars, such as “Golden Delicious” and “Empire” seem to possess a natural robustness, suggesting a genetic basis for this particular physiological disorder [33]. However, the genetic reasons for these differences remain to be elucidated.

Mineral supply of fruits such as potassium and calcium can be an important precondition and even a main factor driving the superficial scald development. In this study, mean potassium/calcium (K/Ca) ratio at harvest was not predictive for superficial scald development. In the growing season 2017, plots Gu53 and Wa39 showed very similar K/Ca ratios; however, the respective fruits differed extremely in their amount of superficial scald after seven months of storage. Contrarily, the K/Ca ratio measurements after removal from storage, on damaged and undamaged fruits, showed clear differences for all orchards studied. While undamaged fruits had a rather low ratio, damaged fruits showed a significantly higher K/Ca ratio. This is in agreement with previous studies, where low calcium and high potassium levels were found to facilitate superficial scald occurrence [21]. This leads to the assumption, that the K/Ca ratio in fruits greatly varies within orchards and possibly even within single trees. In order to increase sample size, an experiment was performed measuring K/Ca concentrations using XRF. This technology allows for a much higher throughput but measures only the top 0.1 mm of the fruit skin (personal communication Bruker). Thus, the K/Ca ratios changed significantly from values of 10 to 40 measured using AAS on ground tissue, to values from 0.5 to 5. The potassium levels remained relatively constant with a 1.5 to 2 times increase in the skin as compared with the cortex. Calcium concentrations, however, increased by a factor of 10 to 15 indicating that in the majority of the fruits, calcium is abundant in much higher concentrations in the epidermis or hypodermis as compared with the cortex. The mean K/Ca values of the different plots correlated well with the amount of scald development but values from single apples showed a poor correlation. This could reflect the high variability of the calcium values within different apples and within trees and tree-to-tree variability, and thus would indicate a need for a better understanding of how the calcium distributes within the apple skin. Additionally, the results showed that the estimation of superficial scald could be

performed equally well by state-of-the-art grading machines and by visually categorizing fruits. This is an interesting perspective, especially for experiments with higher throughput, allowing to screen for very high numbers of fruits. In order to completely suppress superficial scald development for susceptible cultivars, further studies are necessary to establish more knowledge about the distribution of calcium within orchards, single trees, and even single fruits. Additionally, in depth analyses of biophysical processes, as done using X-ray computer tomography or magnetic resistance imaging on “Braeburn browning” disorder [36,37], biochemical processes using proteomics [38,39] and their genetic regulation using QTL mapping [40] or transcriptomic and metabolomic approaches [41], would facilitate a thorough understanding of this disorder in the near future allowing breeders to select for novel cultivars avoiding this disorder completely.

5. Conclusions

The novel scab resistant and fire blight tolerant apple variety *Malus × domestica* cv. Ladina can produce a form of superficial scald, which was shown to vary in extent depending on the growing season and ripeness degree at harvest. Storage under DCA, 1-MCP, and a combination thereof showed a promising reduction in the development of superficial scald. The distribution of potassium, calcium, and its ratio within the skin, but not in dried apple slices showed an effect on the development of senescent scald with low calcium values promoting the extent of the disorder.

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