FOOD & BIOBASED RESEARCH
WAGENINGEN UR

GreenCHAINge Fruit & Vegetables

Work package 1: Formation of chocolate berries in white table grapes

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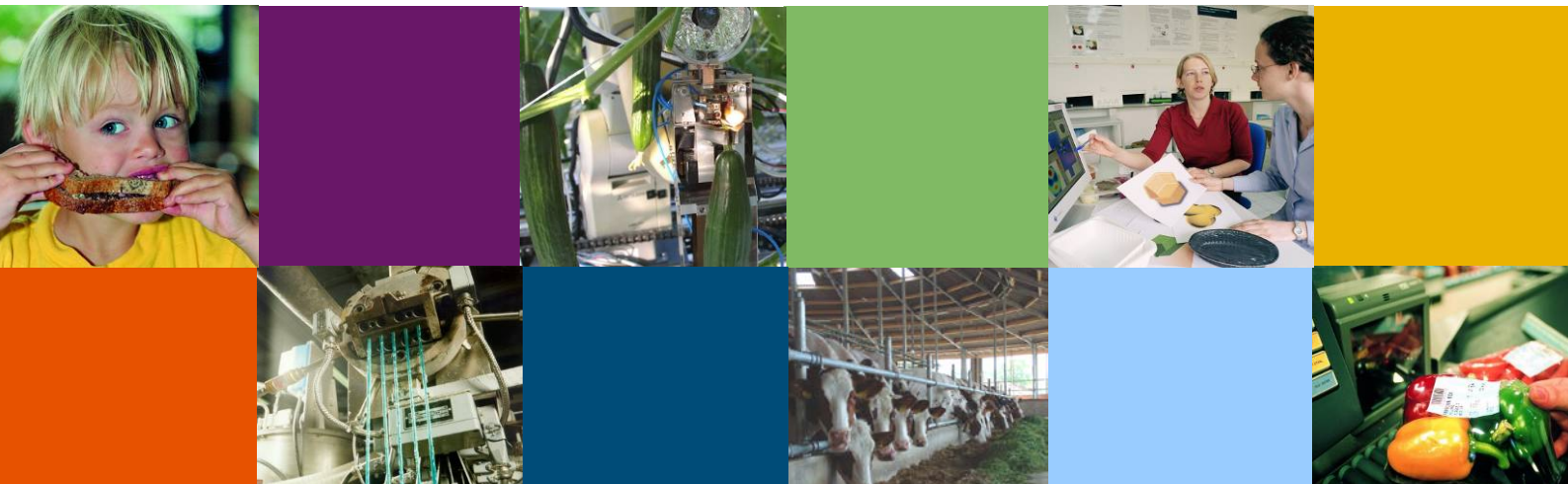
Rapport 1671



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Abstract

In the GreenCHAINge project Wageningen UR Food & Biobased Research (FBR) cooperates with the industry in order to get a better performance in quality for fresh fruits and vegetables. For table-grapes, one of the problems limiting shelf life is the formation of brown grapes, so-called 'chocolate berries', in a punnet. The objective is to understand what is causing the formation of this browning.

With this in mind, various classes of browning were identified and compared to the chocolate berry phenotype. Based on these findings, the hypothesis that mechanical damage causes the chocolate berry phenotype was tested using two types of mechanical stress: constant pressure and impact stress. While constant pressure showed no consequent increase in browning, the increased browning after impact stress indicates that impact stress may contribute to chocolate berry formation.

Content

1 Introduction

In the GreenCHAINge project Wageningen UR Food & Biobased Research coöperates with the industry to get a better performance in quality. The focus in work package 1 is on three important crops: green bean, table grape and mango. The main research questions are:

I. How can initial quality, shelf life and quality be measured and predicted?
II. What is the influence of environmental conditions (e.g. temperature and relative humidity) and handling on the quality and shelf life?
III. How can, by understanding specific product related problems, quality and shelf life be improved?

Regarding table grape, one of the main problems is brown discoloration of one, or a few berries per punnet, a.k.a. chocolate berries. Chocolate berries occur most often towards the end of the season and can be found in both ship- and airfreight from most of the sourcing countries, including South Africa, Greece and Chile.

Since the phenomena is not well understood, we first started a preliminary study to specify the problem and narrow down the possible approaches (chapter 2). Following this study, we tested the hypothesis that chocolate berries occur after mechanical stress (chapter 3).

2 Chocolate berry phenotype

2.1 Introduction

In order to drive some hypotheses for the cause of chocolate berry occurrence, we studied the chocolate berry phenotype.

2.2 Material and Methods

White table grapes cv. Thompson seedless arrived at FBR from Bakker Barendrecht (Ridderkerk, the Netherlands). The table grapes were packed in 500 gram punnets, which were numbered, photographed and observations were noted regarding the location and initial look of the brown grapes, as well as damage on both punnets and box. Brown berries were then removed from the punnets, placed in numbered dishes and observed more closely for visual damage on the skin, firmness of the brown spots and microbial presence. From a selected number of berries, pictures were taken using a microscope.

2.3 Results & Discussion

Based on our observations, we could divide the brown grapes in 6 different classes:

1. Dark brown, intact skin and dehydrated (figure 1a)
2. Light brown, soft and dent-like spots, called pitting (figure 1b)
3. Dented brown spots (figure 1c)
4. Sharply defined brown spots facing the punnet (figure 1d)
5. Brown spots around broken skin (often with microbial growth; figure 1e)
6. Reddish-brown, but firm, spots (figure 1f)

Many of the brown grapes and spots were located towards the punnet walls, particularly those belonging to types 4 and 5. Many of these grapes also showed broken skin (class 5), often with microbial growth (fungus or milky juice). Grapes with microbial growth were often light-brown, with darker brown around the infection site. Due to the location of these grapes and spots, it seems that they suffered from impact damage, most likely during handling and transport.

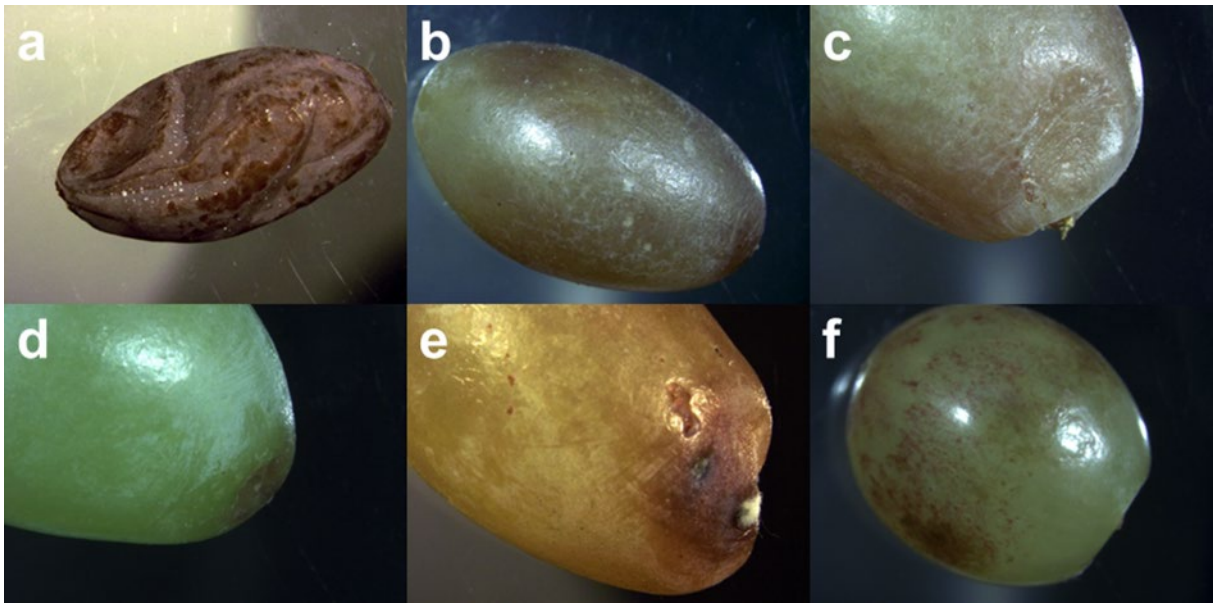


Figure 1: Different types of brown grapes: intact, dehydrated and dark brown (a), light brown, soft and dent-like spots (b), dented, light brown spots (c), sharply defined brown spot with collapsed tissue (d), brown spots around broken skin +/- microbial growth (e) and reddish-brown, but firm, spots (f).

Furthermore, it was suggested that an insect bite might be causing the phenotype. Within the batch of grapes, we found only two grapes showing a hole in their skin that may have been caused by an insect-bite (figure 2a & b). No insects or larvae were detected within the tissue beyond the hole. As such, while insect bites may cause browning, they do not seem to be causing the chocolate berry phenotype.

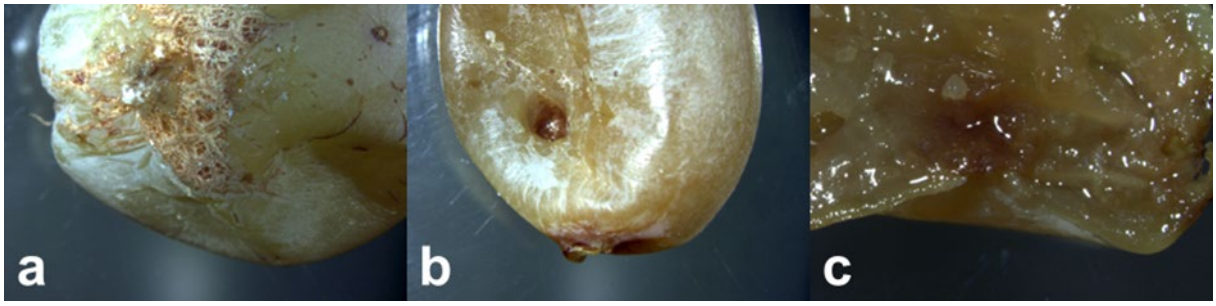


Figure 2: Light brown berries showing hole (a & b) and dissection of grape in figure b, showing brown tissue (c).

The typical phenotype that can be imagined from the name chocolate berry (type 1) showed no skin-damage and was dark brown and dehydrated (figure 1a). These berries were almost always located in between a tight bunch of grapes (figure 3d). Two of the other types of brown grapes (types 2 and 3) showed similar characteristics, but less intense. While type 2 berries were lighter brown and had grape-shaped soft spots under the skin (figure 3a), type 3 berries were darker brown, less hydrated and showed pitting (figure 3b). Finally, the most brown berries were dehydrated to the point that dents were obscured by wrinkles (figure 3c).

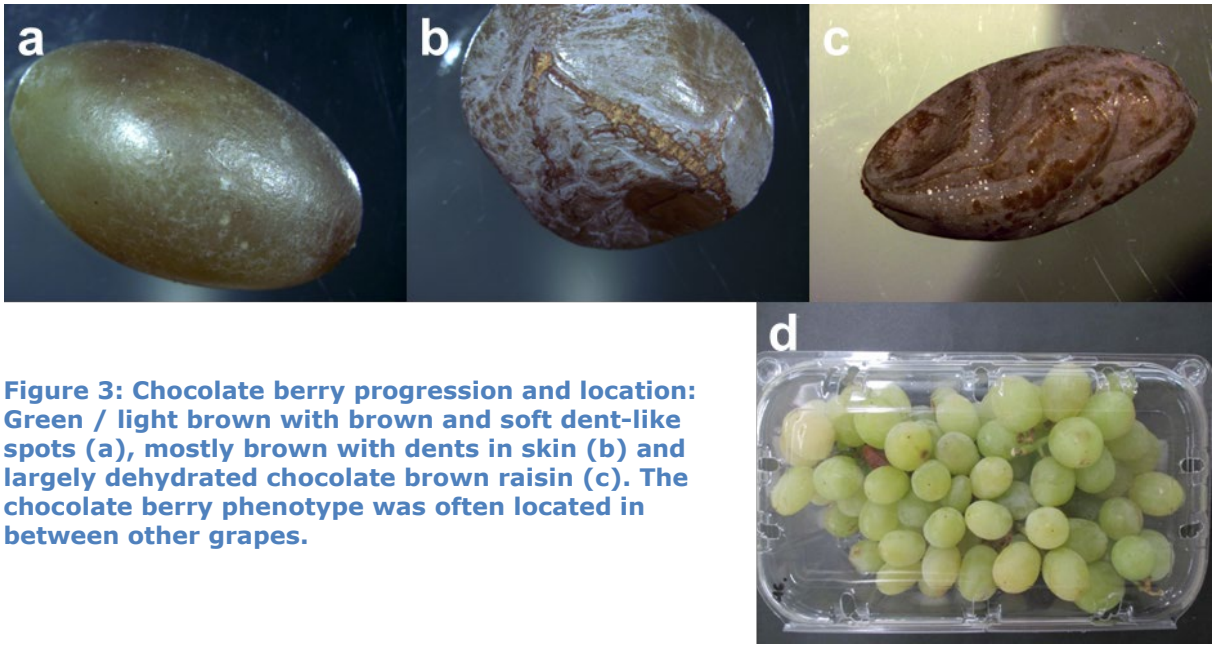


Figure 3: Chocolate berry progression and location: Green / light brown with brown and soft dent-like spots (a), mostly brown with dents in skin (b) and largely dehydrated chocolate brown raisin (c). The chocolate berry phenotype was often located in between other grapes.

2.4 Conclusion

Within a bunch of grapes, different types of brown berries can be observed. In most cases this seems due to mechanical damage and/or microbial attack. In a few cases, insect damage may be the basis of the deterioration. In some cases, a typical chocolate berry (brown, dehydrated berry) is observed in an otherwise intact bunch. At first glance, the chocolate berry phenotype seems to originate from an internal degradation process that leads to browning and finally desiccation.

2.5 Hypotheses

1. Most 'brown berry' symptoms are due to mechanical impact damage and/or microbial attack during harvest and distribution and can be avoided by proper handling.
2. Typical chocolate berries are formed during cultivation and should have been discarded upon packing the bunches as they may already be visible at harvest.
3. Typical chocolate berries are formed when grapes are packed too tight in the punnet. Pressure from the surrounding grapes causes internal stress, resulting in one of the grapes undergoing a controlled tissue degradation, starting from the spots where the pressure is exerted.

3 Table grape browning under mechanical stress

3.1 Introduction

To test the hypotheses that 'brown berry' symptoms are formed in response to mechanical impact damage or from being packed too tight in their respective packaging, we subjected grapes from lots containing chocolate berries to the two types of mechanical pressure stress: continuous and impact-based.

3.2 Methods

3.2.1 Product

Table grapes (Thompson seedless) arrived at FBR through Bakker Barendrecht (Ridderkerk, the Netherlands) and originated from different orchards in either Chili (Gesex and Subsole) or India (Freshtrop). From Gesex, the table grapes had been pre-packed in punnets (Black box) or been placed unpacked in a box (Green box). Subsole had packed their grapes into large bags, while Freshtrop had packed their grapes in punnets.

3.2.2 Treatments

Table grapes were subjected to either constant or impact pressure. Constant pressure was applied by placing bunches of grapes, still in the packaging with the lid removed, between Perspex plates with 1 kg of weight on the top plate (figure 1). The first lot of grapes under constant pressure was placed for 7 days at 4 °C and 82% RH, a second, smaller lot was placed for 7.5 days at -0.5 °C and 82% RH, followed by 3 days at 20 °C and 60% RH.

Impact pressure was applied on small branches, cut from the main bunch, by denting 4-10 individual grapes several times with a round metal hammer. Another branch, parallel on the bunch to the dented branch, was left undented as a reference. Pictures were taken after the grapes had been placed for 7.5 days at either -0.5 and 4 °C and afterwards placed for 3 days at 20 °C and 60% RH.

3.2.3 Assessments

Brown discoloration was assessed as either present or absent, excluding browning that showed both breached skin and microbial growth.

Brix from bundles of grapes was determined as the mean from 3 grapes from different parts of the bunch using a Refractometer (GMK-701R, Nie-Co-Products, Aalsmeer, the Netherlands).

3.3 Results & Discussion

Since the chocolate berry phenotype occurs in both ship- and airfreighted grapes, storage duration is unlikely to be a determinant cause in chocolate berry formation. Since air-freight typically does not last longer than one week, we deemed 7 days to be sufficient time to either subject the grapes to mechanical pressure stress or to let them recuperate after mechanical impact stress.

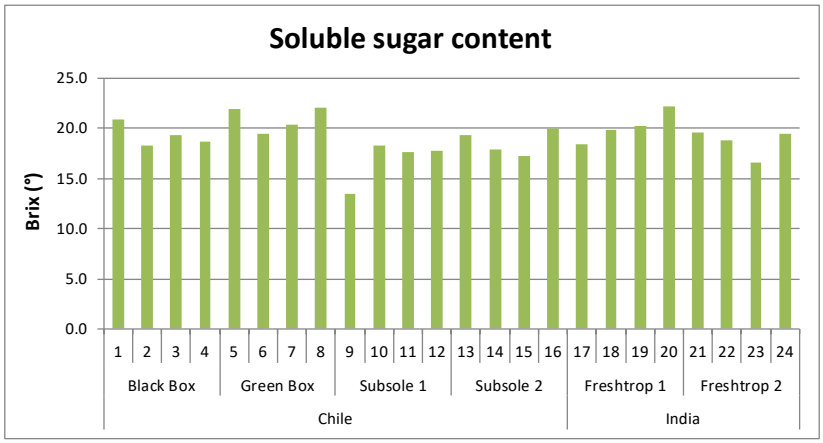
3.3.1 Mechanical pressure stress

To start the experiment, we placed bunches of grapes under 1kg of pressure using boxes that were weighted with water (figure 4). To prevent the boxes from sliding off the surface, we designed holders that consisted of two Perspex plates of which the top plate could freely move downwards, but was otherwise restricted. The bunches of grapes were placed between the two Perspex plates and the weighted boxes were placed on the top plate.

Figure 4: Application of constant pressure on bunches of grapes.

After 7 days at 4°C, the grapes were scored for browning and pictures were taken using a digital camera. After this, three grapes were taken from various locations within each bunch and used to measure the soluble sugar content (° Brix; figure 5).

In general, no clear chocolate berries were observed, apart from some browning due to fungal infection after skin- or stem-breach and some seemingly impact damage-related browning in punnets 20-24. The observed browning in these punnets did not coincide with differences in the soluble sugar content, as these punnets showed the same variation as the others. From punnets 21-24, the browned grapes were cut off the bundles and placed in an open box at 4°C to see whether the browning would spread through the grapes over time. After 11 days, only in a few cases these grapes showed a minor increase in the size of the brown spot (figure 6).



Grower		Punnet	Browning
Chile	Black Box	1	no
		2	no
		3	no
		4	no
	Green Box	5	no
		6	no
		7	no
		8	no
	Subsole 1	9	no
		10	no
		11	no
		12	no
	Subsole 2	13	no
		14	no
		15	no
		16	no
India	Freshtrop 1	17	no
		18	no
		19	no
		20	yes
	Freshtrop 2	23	yes
		22	yes
		23	yes
		24	yes

Figure 5: Soluble sugar content (° Brix) and minor browning in bundles of table grapes from different orchards and packaging types after having been 7 days under constant pressure at 4 °C. Data represent means of 3 grapes. Minor browning (table) of even a few table grapes was scored as either present (yes; red) or absent (no, white).

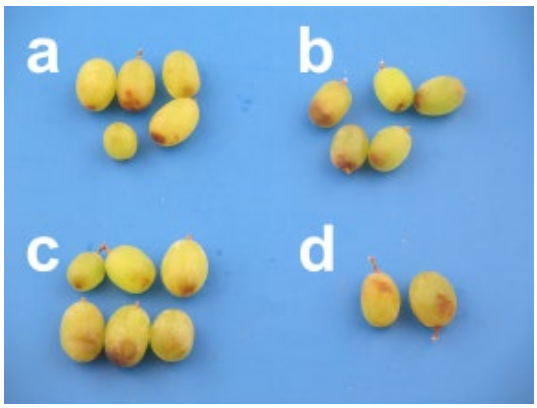


Figure 6: Browning in grapes from punnets 21 (a) - 24 (d) after 11 days at 4°C and 82% RH.

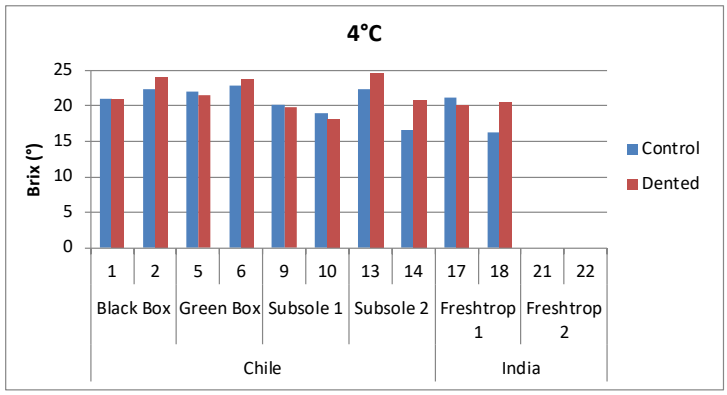
Since freezing temperatures could be theorized to affect the sensitivity of the grapes, we repeated this experiment at -0.5 °C, this time using only 2 replicates per origin. After 7.5 days, these bunches showed no clear formation of chocolate berries, but only some browning due to ripeness and fungal infections.

3.3.2 Mechanical impact stress

In his thesis, Kamfer (2014) reviews that disruption of cellular membranes may lead to internal browning, which links to our earlier observations of chocolate berries displaying grape-like dents (chapter 2). To test our hypothesis that chocolate berries are formed in response to mechanical impact damage, we took small branches of grapes from the bunches that had been placed at 4°C under pressure and subjected half of them to mechanical impact stress and placed them into open boxes at either -0.5 or 4°C. After 7.5 days, the branches were photographed (appendix A), scored for browning and used to determine the soluble sugar content (figure 7).

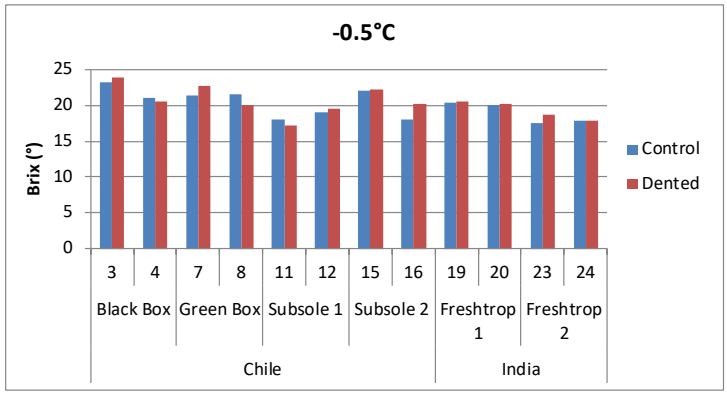
Figure 7 shows that impact pressure in most cases coincided with browning. This browning was unrelated to either storage temperature or soluble sugar content, appeared to occur mainly at the internal damage site (the dent) and did not always spread throughout the grapes. Interestingly, some of the dented branches did not show browning, despite being thoroughly dented. The soluble sugars of the grapes from punnets 21 and 22 at 4°C were not determined, although these grapes did show browning (appendix A).

a



Grower		Punnet	Browning Control	Browning Dented
Chile	Black Box	1	no	yes
		2	no	yes
	Green Box	5	no	no
		6	no	yes
	Subsole 1	9	no	no
		10	no	yes
	Subsole 2	13	no	yes
		14	no	yes
India	Freshtrop 1	17	no	yes
		18	no	yes
	Freshtrop 2	21	no	yes
		22	no	yes

b



Grower		Punnet	Browning Control	Browning Dented
Chile	Black Box	3	no	yes
		4	no	yes
	Green Box	7	no	yes
		8	no	yes
	Subsole 1	11	no	no
		12	no	no
	Subsole 2	15	no	yes
		16	no	yes
India	Freshtrop 1	19	no	no
		20	no	yes
	Freshtrop 2	23	no	yes
		24	no	yes

Figure 7: Soluble sugar content and minor browning in table grapes from different orchards and packaging types after being subject to impact pressure (control or dented) and having been stored at either 4 (a) or -0.5°C (b) for 7.5 days. Soluble sugar content data (° Brix) represent means of 2-3 grapes. Minor browning (table) was scored as either present (yes; red) or absent (no, white).

Since the grapes originated from lots in which chocolate berries had been found, factors such as overall growth conditions and microbial pressure both inside the grapes and on the grape surface are in theory unlikely to have influenced the browning. However, the tests were done on grapes that had already undergone and passed a period of transport. As such, the tested grapes may not have been as susceptible to become chocolate berries, especially when the time-period after picking and/or the handling during harvest and transport are involved in chocolate berry formation.

3.3.3 Temperature, packaging

Temperatures of -0.5°C and 4°C showed no clear differences in the observed browning. Similarly, no obvious packaging effects were observed with regard to the formation and spread of browning.

3.4 Conclusion

In this report, we aimed to understand the cause of chocolate berry occurrence. After an initial study to assess the chocolate berry phenotype; browning without skin breach or visible microbial influence; we made the hypotheses that 'brown berry' symptoms are formed in response to mechanical stress; specifically constant pressure or impact stress.
Constant pressure, such as may occur during 'too tight' packaging, did not show consequent browning of berries and therefore is unlikely to stimulate chocolate berry formation. Impact pressure on the other hand, such as may occur due to rough handling or transport, did show consequent berry browning without skin breach and microbial influence and therefore might stimulate the formation of chocolate berries. However, whether impact damage actually causes chocolate berry formation cannot be concluded from the current results.

3.5 Recommendations for further research

To assess whether impact damage is the underlying cause of chocolate berry formation, we recommend to involve the growers. Related to mechanical stress, we can ask growers to review their handling and transport processes and pinpoint the points where mechanical impact stress may occur. Following this, at these points in the chain, a number of boxes could then be handled "on the rough side" to see whether these influence chocolate berry formation.
To assess the occurrence of chocolate berry formation in relation to growth conditions, we could ask the growers to fill in a prepared questionnaire regarding growth conditions during the production year, their location and the transport to the harbour/airport. Such data could then be correlated to the occurrence of chocolate berries as visualized at Bakker Barendrecht.

3.6 References

Kamfer W.D. 2014. The effect of maturity and crop load on the browning and concentration of phenolic compounds of Thompson Seedless and Regal Seedless. Thesis published by Stellenbosch University, South Africa.

Acknowledgements

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Appendices

Appendix A:

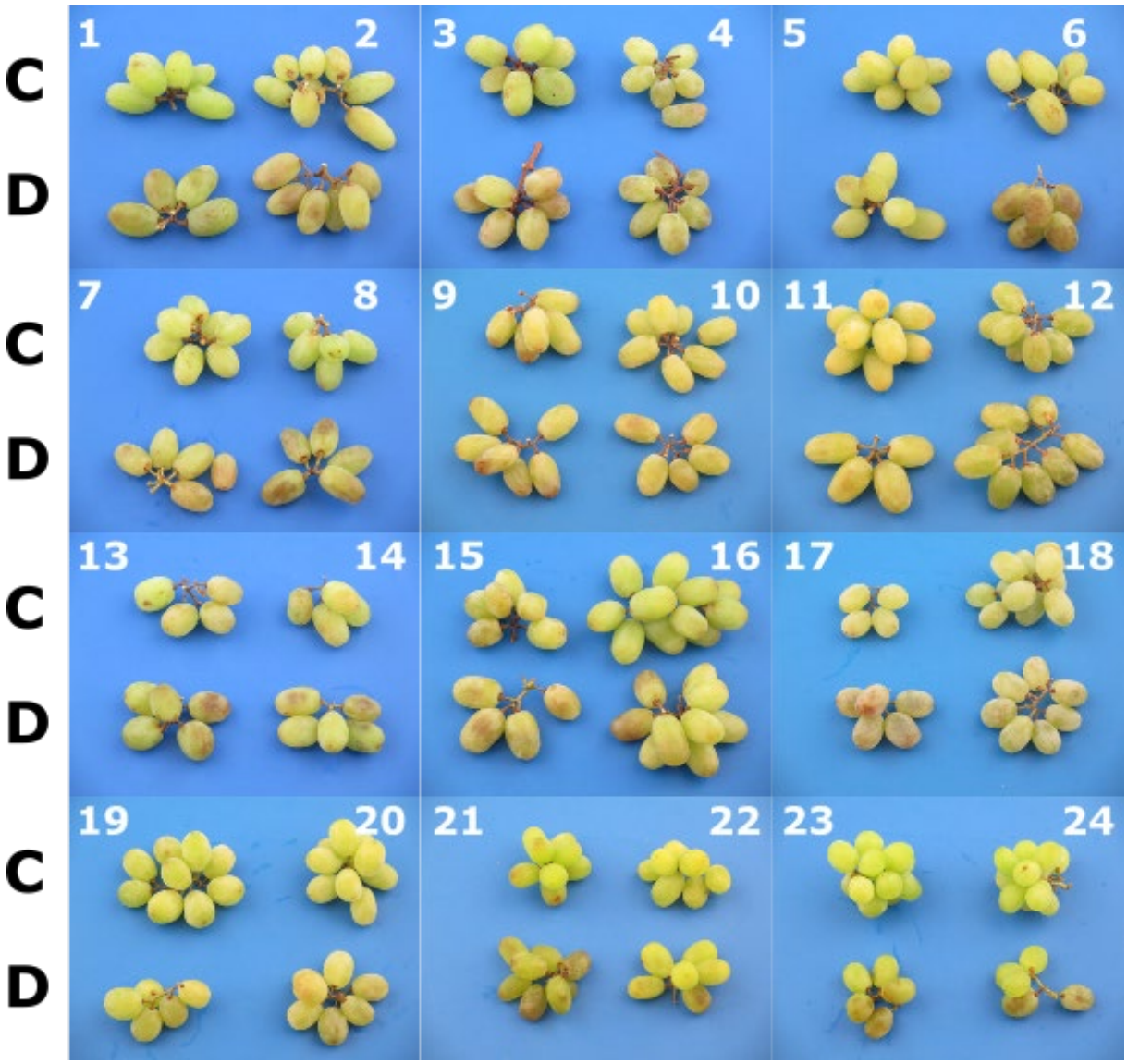

Figure 1: Browning caused by impact pressure in control (C) and dented (D) grapes. Numbers refer to the punnets the grapes originated from (see figure 1).

