EVALUATION OF OPTIMUM DETECTION TIMES FOR AVOCADO SUNBLOTCH VIROID (ASBVd) DURING THE PHENOLOGICAL GROWTH STAGES OF AVOCADO

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ABSTRACT
Avocado sunblotch disease (ASBD) is an economically important disease of avocado, caused by the avocado sunblotch viroid (ASBVd). The disease can cause large yield losses, especially in asymptomatic trees. Currently, the most widespread infections are detected in asymptomatic trees in South African avocado orchards. Limited information is available on the effect of the time of sampling and tissue type (i.e. hardened-off leaves, new leaf flush, and flower buds) on ASBVd titer in field trees. These factors are important for reliable testing and could potentially increase the sensitivity of tests when pooled tree samples are tested.

INTRODUCTION

Considering the scarcity of information on the effect of time of sampling and tissue type (hardened-off leaves, young leaf flush, and flower buds) on ASBVd titer, the study was initiated to assist with optimal detection for ASBVd in field trees. No published literature is available where RT-qPCR has been utilised to investigate the effect of time of sampling and tissue type on ASBVd titer. A few studies, all using older detection techniques, reported that some tissue types can differ in ASBVd titer, whereas others found no differences. The aim of this study was to evaluate the effect of sampling time and tissue type during various phenological growth stages of avocado on ASBVd titer in mature avocado trees. Determination of the titer of ASBVd in the different phenological growth stages of avocado (Annexure A) will add value to the outcomes from diagnostic laboratories and determine optimum detection times throughout the year.

MATERIALS AND METHODS

Selection of field trees

Surveys to select ASBVd-infected trees were conducted in avocado blocks located at a commercial farm in Mbombela, Mpumalanga (Long: 30.928410887745482; Lat: -25.4292923547822). Four cultivars were surveyed to select plants for the study, including 'Fuerte', 'Edranol', 'Maluma Hass', and 'Hass'. A summary of the history of the selected cultivars is found in Table 1. The 'Fuerte' trees were planted in 1992, 'Edranol' trees in 1982, 'Maluma Hass' from 2016 to 2020 (the trees are different ages) and the 'Hass' trees in 2016/17. Therefore, old and young orchards were selected for the study. To identify the trees for this study, leaves from three trees in an orchard were pooled in the initial screening, followed by dsRNA extraction and RTqPCR (Zwane *et al*., 2023). Individual positive trees were identified with re-testing of positive pooled samples.

Sampling from different tissue types

Leaf and flower samples were collected from 'Fuerte', 'Edranol', 'Hass', and 'Maluma Hass' trees to determine detection in different tissue types. Collection of flower material was done towards the end of the flowering season and most of the flowers were already in the ovary growth stage. Photographic evidence to depict the stage of development was taken at the time of sampling.

Table 1: Planting history of the four selected cultivars in the study

	'Fuerte'	'Edranol'	'Maluma Hass'	'Hass'
Planting date	1992	1982	2016-2020	2016/2017
Rootstock	Duke 7	Unknown	Seedling	Duke 7
Spacing	6 x 7.5	6 x 7.8	8 x 3	8 x 4

Interpretation of results

The average Ct values of selected trees were used to determine the titer of ASBVd infection. Sampling times and results were plotted on the phenological growth stage chart (Fig. 4). Statistical analysis of the data will be done on results from the completed first seasonal cycle.

RESULTS AND DISCUSSION

Selection of field trees

In total, 213 'Fuerte' trees, 246 'Edranol' trees, 276 'Maluma Hass' trees, and 243 'Hass' trees were screened for the presence of ASBVd (results not shown but available on request). Of these, a total of 17 'Fuerte' trees (Table 1), 21 'Edranol' trees, 16 'Hass', and 20 'Maluma Hass' trees tested positive and were selected for further study. Statistically, a minimum of 16 trees are required per cultivar for the comparison. Analysis of variance will be done according to the experimental design and Fisher's protected Least Significant Differences will be used to separate means of significant source effects.

ASBVd detection in 'Fuerte' trees

Comparative results between different plant parts and phenological growth stages for selected 'Fuerte' trees are shown in Table 1, Annexure B. The average Ct values of tissue types collected at different stages show consistent detection of ASBVd throughout the phenological growth stages of the avocado trees (Fig. 1).

Flowers were collected at the end of the flowering season in October 2023 (Fig. 3A and B) and, in some cases, higher viroid titer, indicated by lower Ct values (lower than a Ct value of 14), were detected in the flowers of most trees (Table 1, Annexure B). The leaf material showed consistent levels of detection and the lower ASBVd concentration recorded in Samples 7, 8, and 17 was verified by detection in the flowers in October 2023.

Sample 17 had a low titer in the initial screen, a Ct value of 27.12, and the sample tested negative for ASBVd from December 2023 to March 2024 (Fig. 2). ASBVd was detected in Sample 17 in May, June and August 2024, and from October 2024 to January 2025 only detected in the old leaf samples. During July 2024 and September 2024, ASBVd was not detected in Sample 17. Samples 7 and 8 tested negative in the January and March 2024 samplings, but were positive again in May to June 2024 in the new leaf flush. They tested negative in July and September 2024, the same as for Sample 17. Samples 7 and 8 tested positive from October to December 2024 with higher ASBVd presence detected in January 2025 in Sample 7. However, no ASBVd was detected in Sample 8 during that period. Samples 4 and 5 had higher Ct values (27.02 and 26.55, respectively) during the fruit development stage in the January 2024 sampling. A year later, in January 2025,

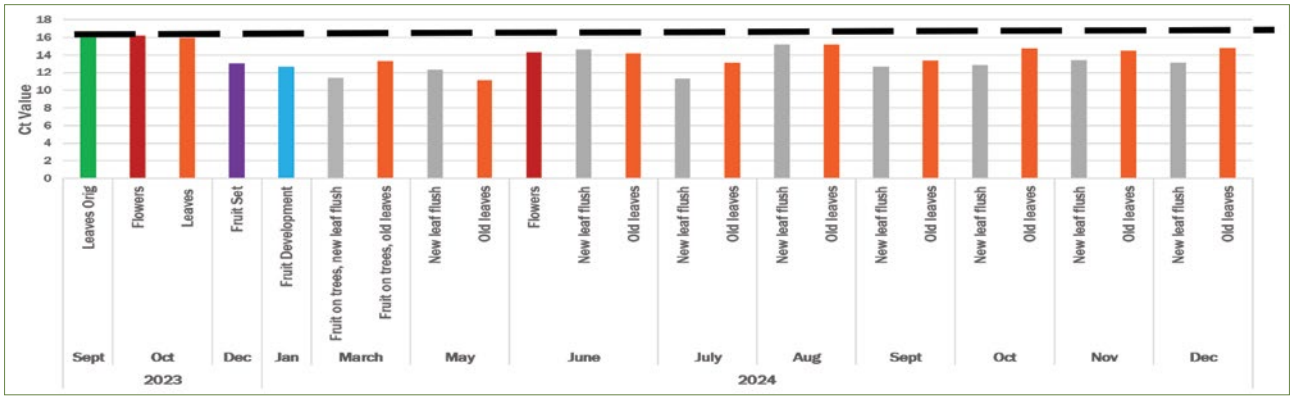


Figure 1: Average Ct values of 'Fuerte' at different stages/times of the year.

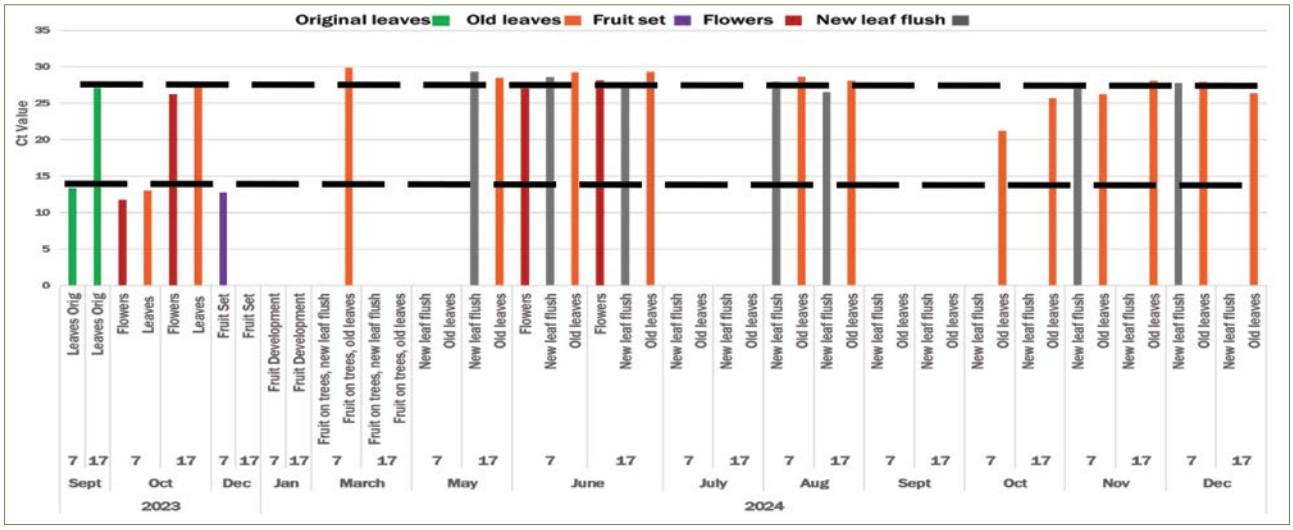


Figure 2: Average Ct values from two trees with an initial high and low ASBVd titer.

good detection was observed. The average Ct values indicated detection of the viroid throughout the September 2023 to January 2025 period, except for the samples discussed above (Fig. 1). Symptoms were seen on fruit (Fig. 3C) and individual monitoring of trees with fruit will be compared to the results. It is possible that Samples 7, 8, and 17 are symptomatic trees where ASBVd has an uneven distribution in the tree and therefore show uneven ASBVd detection.

ASBVd detection in 'Edranol' trees

Initially, twelve 'Edranol' samples were selected for monitoring, followed by the selection of nine more trees for the purpose of collecting a statistically sound dataset. 'Edranol' trees were tested at the end of the flowering stage and the initial ovary growth stage, indicating the beginning of fruit development (Fig. 4). There was good correlation between the Ct values detected in 'Edranol' trees in the leaf and flower samples of October 2023 (Table 4, Annexure C). The average Ct values of tissue types collected at different stages show consistent detection of ASBVd throughout the phenological growth stages of the avocado trees (Fig. 5).

Sample 15 had a higher Ct value of 25.57 during the fruit development stage in January 2024. ASBVd was not detected in Sample 15 in March 2024 from both old and young leaves when there were fruit on the trees, and no ASBVd symptoms were observed on the fruit in March 2024 (Fig. 4C). ASBVd was not detected in the new leaf flush in July, October, November, and December 2024 and the older leaf flush was a more reliable source for detection (Fig. 6).

ASBVd detection in 'Hass' trees

'Hass' trees were sampled at the beginning of the fruit set stage (Fig. 7) and detailed results are summarised in Annexure D. The average Ct value of 'Hass' trees showed detection throughout the year (Fig. 8) except for five trees with a low initial ASBVd titer (Samples 4, 5, 8, 11, and 13) (Fig. 9). There could be a seasonal effect in ASBVd detection in 'Hass' trees.

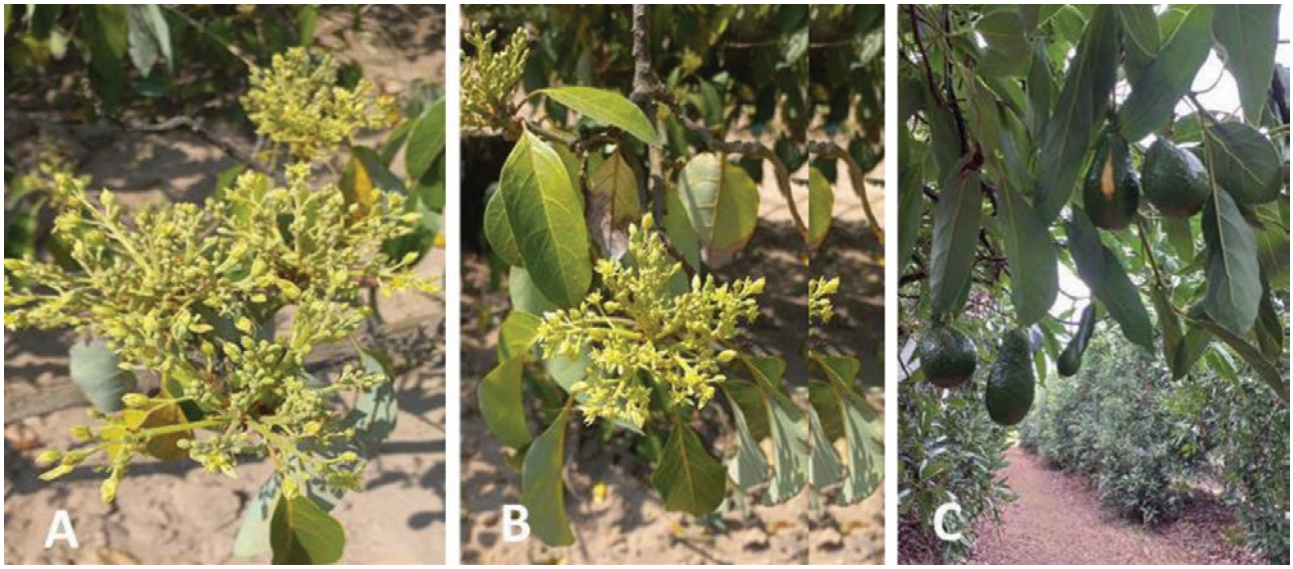


Figure 3: Photographic evidence of growth stage of 'Fuerte' trees at the time of sampling in October 2023 (A and B) and in March 2024 (C) when fruit with symptoms were seen.

Figure 4: Photographic evidence of growth stage of 'Edranol' trees at the time of sampling in October 2023 (A) and in March 2024 (C).

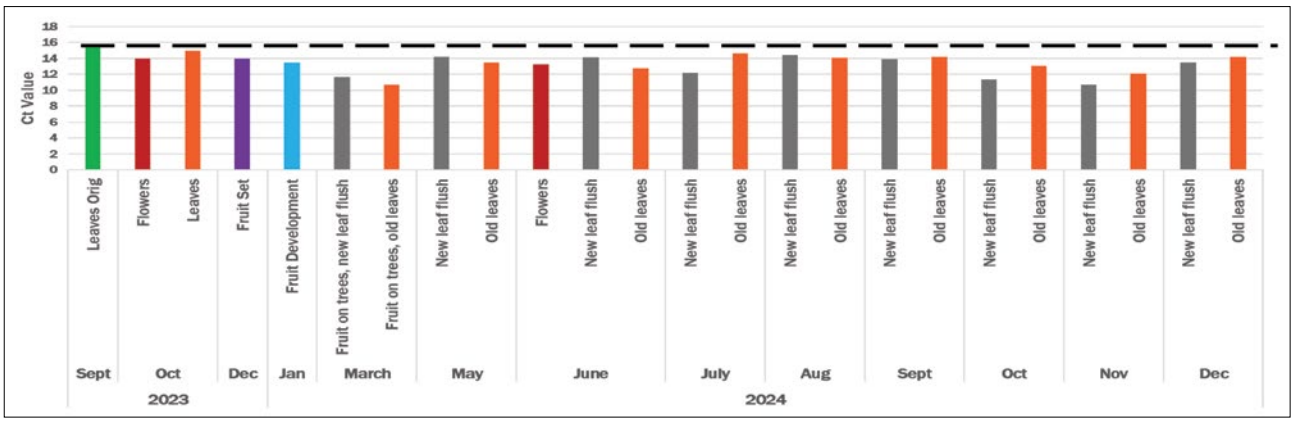


Figure 5: Average Ct values of 'Edranol' at different stages/times of the year.

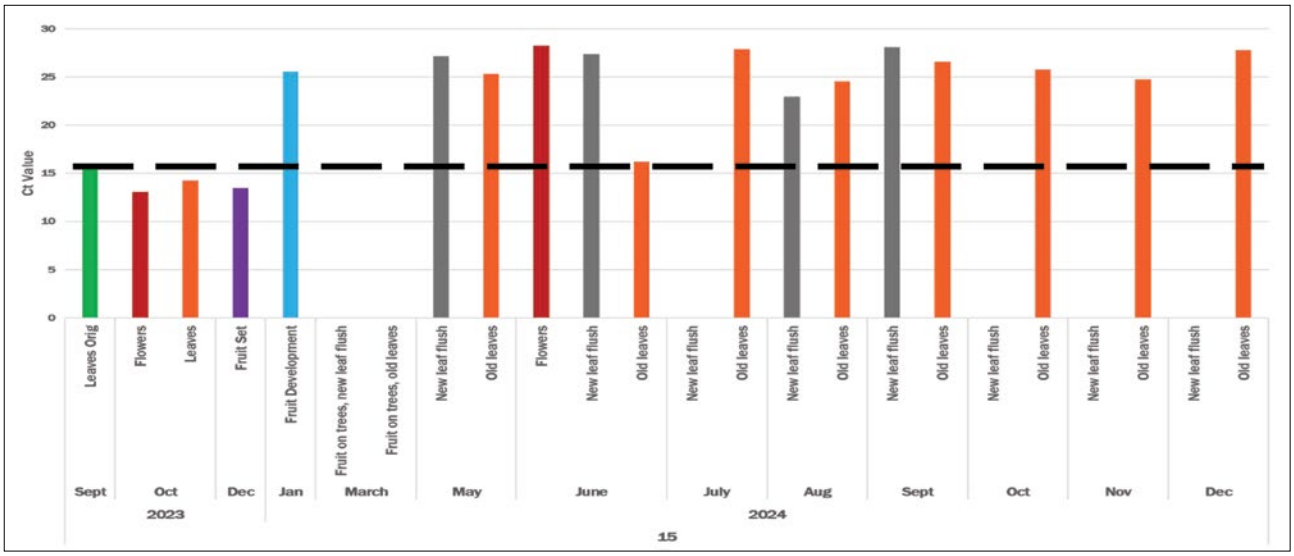


Figure 6: Average Ct values of 'Edranol', Sample 15 indicating times for detection.

Figure 7: Photographic evidence of growth stage of 'Hass' trees at the time of sampling.

It is noteworthy that the above-mentioned samples had a low titer with the initial screening results and ASBVd was detected in Samples 5, 8, 11, and 13 during the fruit development stage in January 2024. It is likely that these trees are symptomatic trees with an uneven distribution of ASBVd within the tree. Confirmation of symptomatic fruit on these trees will be done when trees bear fruit. No detection on leaves was possible after fruit development (Fig. 9).

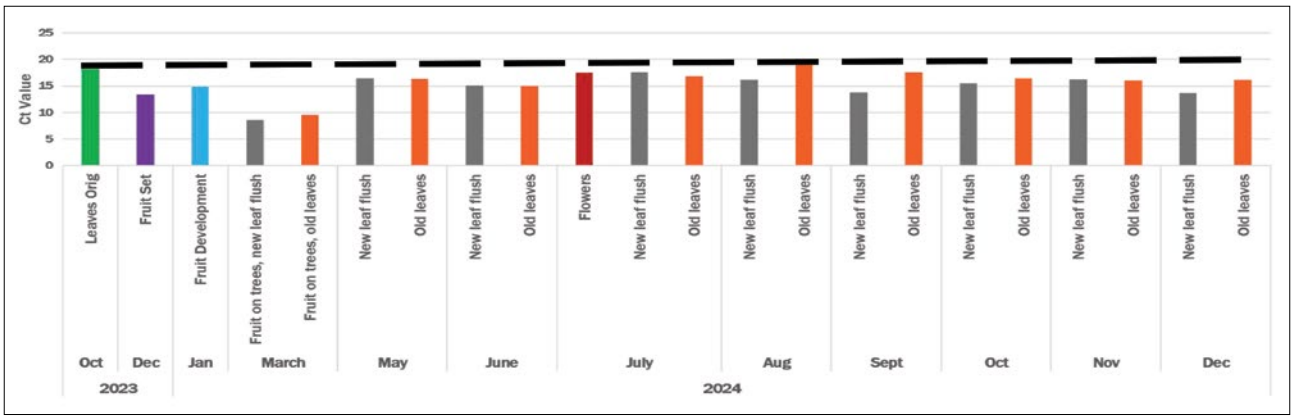


Figure 8: Average Ct values of 'Hass' at different stages/times of the year.

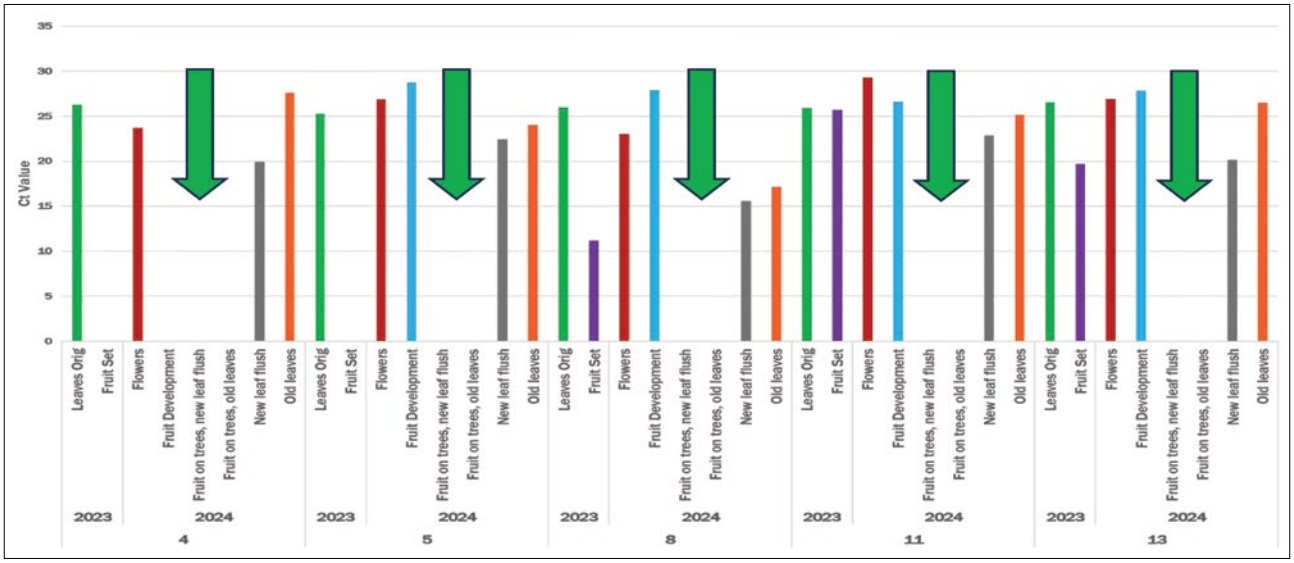


Figure 9: Average Ct values of 'Hass', Samples 4, 5, 8, 11, and 13 indicating times for detection. Green arrows indicate times when ASBVd was not detected.

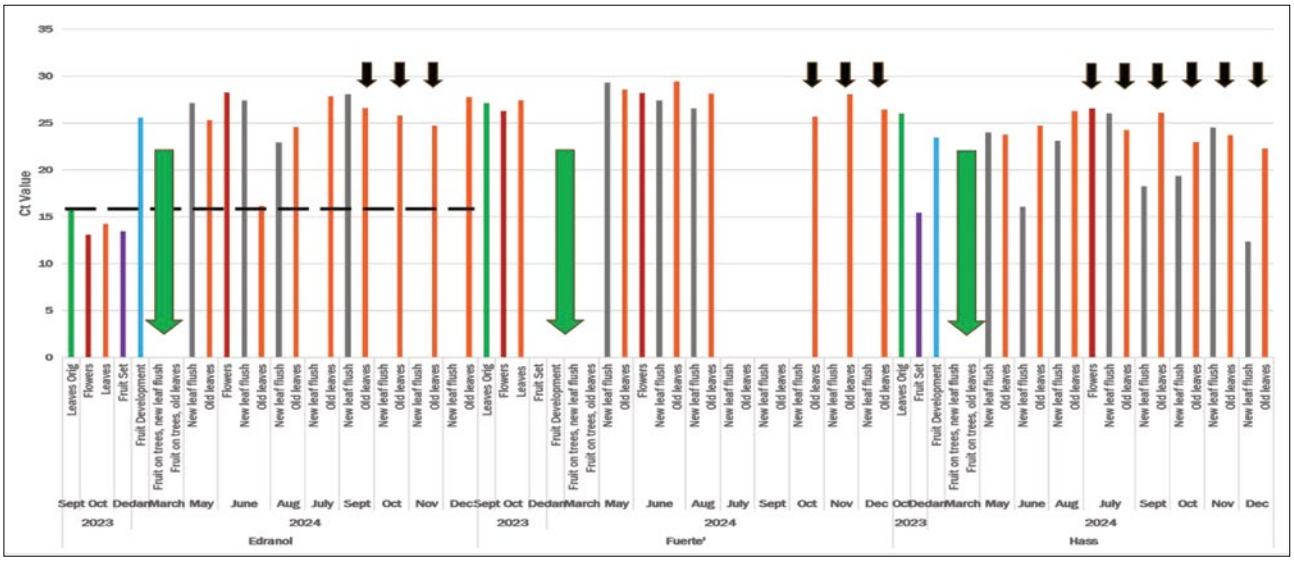


Figure 10: Average Ct values of seven low titer trees indicating the period for no detection with the green arrow and detection in older and younger leaves indicated by black arrows.

Comparison of ASBVd detection from seven low titer trees from different cultivars

When detections in seven trees with an initial low titer were compared to each other, similar times were identified when ASBVd cannot be detected (Fig. 10). This period where there was no detection was consistent across all seven trees, indicating that during the period of fruit development and fruit set no detection is possible in the leaves of these trees. This is indicated by the green arrows in Figure 10. A difference between detection in young and old leaves was also seen. In 'Edranol' and 'Fuerte' trees, the older leaves had reliable ASBVd detection, but in 'Hass' trees, the younger leaves had a slightly higher titer for ASBVd detection, as indicated by the black arrows in Figure 10.

ASBVd detection in 'Maluma Hass' trees

During the initial screening of 'Maluma Hass' trees, low ASBVd titers were detected in leaf samples and these trees were included in the study to monitor the detection behaviour of trees infected with low levels of ASBVd. However, when leaves were tested in October 2023, detection could only be verified in material from the last stages of flowering and fruit set. Very low viroid titers were detected in most trees, and only in flowers. Detection in the leaves will be verified in upcoming tests to investigate possible seasonal variation of viroid titer in 'Maluma Hass'. It will be interesting to monitor if an increase in viroid titer can be detected over time in the selected 'Maluma Hass' trees. In March 2024, all trees tested negative when younger and older leaves were sampled. The lack of detection can be an indication of a seasonal effect or a very low ASBVd titer in these trees. Monitoring in 'Maluma Hass' trees is ongoing.

CONCLUSION AND DISCUSSION

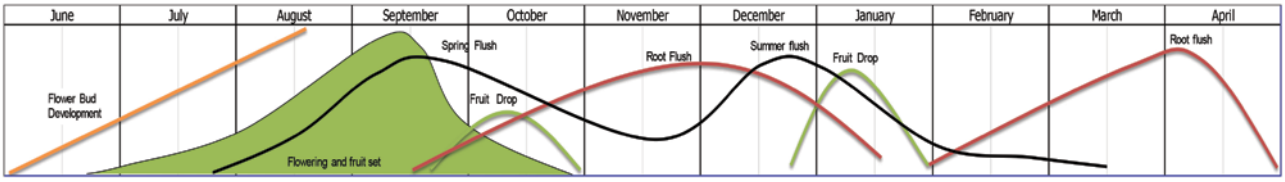
'Fuerte', 'Edranol', 'Maluma Hass', and 'Hass' trees were selected for this study. The ASBVd results from September 2023 to January 2025 are included per cultivar in this report. The average Ct values in 'Fuerte', 'Edranol', and 'Hass' trees showed detection throughout the phenological growth stages of the selected trees. However, in trees with an initial low ASBVd titer, ASBVd is not detectable at certain stages, especially during the period when fruit is on the tree. In these low titer trees, the older leaves were a more reliable source for detection in 'Fuerte' and 'Edranol' trees and in 'Hass', both young and older leaves gave reliable detection. These trees are probably related to the symptomatic trees detected in avocado orchards.

The way forward for the project includes the screening of the trees in the current data set for a second season and identifying younger orchards to repeat and compare the ASBVd detection trends. At the end, the method to plot the data on the phenological growth stages chart will be done (Annexure A). When other cultivars are included in the study, a conclusion can be made if detection charts per cultivar are necessary.

REFERENCES

ZWANE, Z.R., GUBBA, A. & JOOSTE, A.E.C. 2023. The effect of avocado sunblotch disease (ASBD) on tree morphology, fruit maturity, yield and quality of 'Hass' avocado in South Africa. 2023. *European Journal of Plant Pathology*, 167: 287-299 (2023). https://doi.org/10.1007/s10658-023-02705-z

Annexure A: The phenological growth stages of avocado



Annexure B: 'FUERTE' results

Table 1: Ct values of leaves and flowers collected from 'Fuerte' trees from September 2023 to March 2024

CODE		SEPT 2023	OCT 2023		DEC 2023	JAN 2024	MARCH 2024	
		FIRST SCREEN	LEAVES	FLOWERS	FRUITSET	FRUIT DEVELOPMENT	FRUIT ON TREES NEW LEAVES	FRUIT ON TREES OLD LEAVES
Sample no	Tree no	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value
1a)	R1T1	13.53	13.68	11.27	13.59	12.68	14.43	11.93
2 (5a)	R1T14	14.80	12.36	12.86	12.68	11.88	14.04	11.36
3 (5b)	R1T15	13.32	12.34	13.57	10.53	11.70	12.60	11.81
4 (20a)	R2T1	13.67	13.74	12.87	10.22	27.02	13.95	13.72
5 (20b)	R2T2	21.99	22.05	25.49	15.46	26.55	12.78	13.85
6 (31a)	R2T44	14.82	13.11	11.45	10.48	13.52	15.14	14.17
7 (34b)	R2T54	13.39	13.04	11.70	12.74	-	-	29.90
8 (35c)	R2T58	14.61	26.94	25.95	25.09	-	-	-
9 (36a)	R2T59	13.28	13.08	11.98	15.05	14.68	13.72	11.52
10 (48b)	R3T38	13.74	13.74	12.83	12.10	14.51	12.44	13.22
11 (50a)	R3T44	14.04	14.02	12.54	14.94	15.44	14.13	13.22
12 (63b)	R4T33	13.36	14.21	12.88	12.85	15.45	14.28	13.38
13 (64c)	R4T37	20.38	15.84	27.09	16.42	14.24	16.56	13.58
14 (68a)	R4T47	14.30	13.60	11.27	12.32	13.04	13.56	13.96
15 (68b)	T4T48	14.26	14.16	12.10	12.63	11.76	13.83	13.25
16 (1b)	R1T2	27.21	17.93	23.61	14.93	13.42	12.83	27.71
17 (63c)	R4T34	27.12	27.43	26.28	-	-	-	-
Average Ct values		16.35	15.95	16.22	13.87	15.42	13.8	14.0

Table 2: Ct values of leaves and flowers collected from 'Fuerte' trees from May 2024 to August 2024

SEPT 2023	MAY 2024		JUNE 2024			JULY 2024		AUGUST 2024	
Selected trees	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	FLOWERS	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH
Sample no	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value
1 (1a)	12.19	12.99	12.81	12.81	9.26	11.30	11.11	12.62	11.52
2 (5a)	13.44	13.73	11.45	11.81	10.16	11.81	11.26	11.89	12.58
3 (5b)	11.75	10.32	10.75	11.00	10.60	11.80	13.43	12.11	13.14
4 (20a)	11.78	11.77	11.83	13.62	11.07	12.24	12.10	12.52	12.79
5 (20b)	12.29	14.03	13.04	14.22	18.03	12.68	13.13	14.29	11.83
6 (31a)	12.11	12.61	11.88	13.45	10.32	13.06	12.49	12.81	13.10
7 (34b)	-	-	29.28	28.58	27.08	-	-	28.64	28.00
8 (35c)	-	16.67	-	11.94	10.86	28.71	-	27.40	28.44
9 (36a)	20.85	11.19	11.33	11.75	11.03	12.55	12.61	11.49	12.45
10 (48b)	-	-	11.30	11.58	12.78	12.10	13.15	10.50	10.77
11 (50a)	13.85	12.69	13.84	12.33	10.49	16.13	13.41	11.60	12.44
12 (63b)	13.25	12.98	13.43	12.20	12.82	12.50	12.33	13.08	10.23
13 (64c)	13.78	12.45	11.31	12.48	12.17	13.71	14.51	12.52	14.93
14 (68a)	13.13	12.09	11.68	10.96	10.67	14.14	13.38	11.28	12.30
15 (68b)	12.30	11.41	11.25	11.54	10.58	13.48	14.48	13.29	12.98
16 (1b)	-	16.15	26.95	21.36	26.61	27.12	25.92	14.47	14.54
17 (63c)	28.54	29.32	29.38	27.37	28.17	-	-	28.11	26.53
Av Ct Value	14.5	14	15	14.6	14.3	14.9	13.8	15.2	15.2

Table 3: Ct values of leaves and flowers collected from 'Fuerte' trees from September 2024 to January 2025

	SEPTEMBER 2024		OCTOBER 2024		NOVEMBER 2024		DECEMBER 2024		JANUARY 2025	
Selected trees	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH
Sample no	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value
1 (1a)	14.09	14.59	11.39	15.89	11.08	12.07	12.20	10.88	10.25	11.63
2 (5a)	13.11	13.55	12.96	13.39	12.65	11.52	11.96	13.30	12.79	12.30
3 (5b)	13.90	14.45	13.50	12.45	11.75	12.24	11.05	11.18	11.48	12.51
4 (20a)	13.79	13.34	12.75	13.19	11.29	12.80	12.12	11.82	13.09	11.78
5 (20b)	16.09	15.08	13.06	14.18	15.24	13.82	15.31	12.39	9.67	11.15
6 (31a)	13.78	15.92	13.04	14.08	12.24	11.94	12.63	12.48	13.66	13.67
7 (34b)	-	-	21.29	-	26.24	27.33	27.95	27.75	25.69	25.82
8 (35c)	28.27	17.63	28.06	29.02	29.10	27.27	29.19	24.86	-	-
9 (36a)	13.42	13.56	10.81	13.13	10.33	9.66	10.23	10.84	11.60	11.61
10 (48b)	14.27	13.72	12.00	13.81	11.20	13.06	11.97	13.68	11.38	13.01
11 (50a)	15.45	15.43	12.50	12.39	10.77	12.45	11.62	12.75	11.62	10.11
12 (63b)	12.75	13.80	12.62	13.59	9.94	11.66	12.47	11.90	12.47	11.83
13 (64c)	14.94	12.84	12.41	15.50	13.26	12.19	12.13	12.59	11.82	16.38
14 (68a)	12.41	12.30	12.91	12.07	10.57	15.22	11.61	12.42	13.26	11.98
15 (68b)	12.32	14.99	12.55	12.41	10.26	11.15	11.77	12.35	11.47	12.36
16 (1b)	18.26	14.30	13.33	13.36	12.56	13.96	11.38	11.84	10.92	10.98
17 (63c)	-	-	25.70	-	28.09	-	26.42	-	27.63	28.73
Av Ct Value	15.1	14.4	14.7	14.6	14.5	14.3	14.8	13.9	13.7	14.1

Annexure C: 'EDRANOL' Results

Table 4: Ct values of leaves and flowers collected from 'Edranol' trees from September 2023 to March 2024

CODE		SEPT/ OCT 2023	OCT 2023		DEC 2023	JAN 2024	MARCH 2024	
		FIRST SCREEN	LEAVES	FLOWERS	FRUITSET	FRUIT DEVELOPMENT	FRUIT ON TREES NEW LEAVES	FRUIT ON TREES OLD LEAVES
Sample no	Tree no	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value
1 (7c)	R1T27	17.99	13.26	10.77	11.27	11.51	13.45	13.05
2 (9a)	R2T38	14.13	N/A	N/A	12.14	13.18	12.54	12.43
3 (13c)	R3T33	14.11	N/A	N/A	13.06	13.28	12.90	12.52
4 (30c)	R4T12	16.97	14.89	13.22	14.42	12.42	11.47	11.95
5 (20a)	R4T35	14.34	N/A	N/A	15.04	13.68	11.25	10.89
6 (20b)	R4T36	14.64	N/A	N/A	13.36	14.05	11.56	11.52
7 (20c)	R4T37	13.08	N/A	N/A	13.06	12.81	12.19	10.65
8 (21a)	R4T38	12.73	N/A	N/A	14.45	12.71	10.89	10.76
9 (39a)	R5T11	17.33	14.31	14.01	11.62	12.65	11.52	11.29
10 (44b)	R5T28	15.11	15.48	15.22	11.94	12.55	10.68	10.69
11 (26b)	R5T36	14.93	N/A	N/A	15.29	13.55	12.60	10.71
12 (26c)	R5T37	12.62	N/A	N/A	13.30	12.22	11.91	11.99
13 (52a)	R6T25	15.04	14.50	13.13	19.19	11.14	11.53	10.88
14 (54a)	R7T6	15.97	14.90	13.68	11.89	13.33	12.16	12.30
15 (54b)	R7T8	15.73	14.24	13.07	13.48	25.57	-	-
16 (58b)	R7T27	15.33	13.87	13.68	12.91	11.70	11.28	11.50
17 (40c)	R7T43	14.23	N/A	N/A	14.73	12.98	12.37	11.84
18 (64c)	R8T24	13.92	13.41	13.73	13.48	11.65	11.01	11.37
19 (65a)	R8T25	14.46	13.37	13.49	12.26	12.20	12.62	11.00
20 (74b)	R9T25	15.70	13.12	12.38	12.56	12.23	11.49	10.93
21 (75a)	R9T26	16.31	23.49	21.61	22.99	14.60	22.64	13.33
Average Ct values		14.98	14.9	13.99	13.93	13.33	12.40	11.58

Table 5: Ct values of leaves and flowers collected from 'Edranol' trees from May 2024 to August 2024

SEPT 2023	MAY 2024		JUNE 2024			JULY 2024		AUGUST 2024	
Selected trees	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	FLOWERS	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH
Sample no	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value
1 (7c)	11.63	12.33	12.33	12.17	10.95	14.15	12.55	12.56	13.78
2 (9a)	11.69	12.88	12.34	13.06	11.22	13.11	14.48	11.46	12.87
3 (13c)	12.22	12.67	12.71	13.37	10.75	12.24	12.85	11.08	12.39
4 (30c)	11.33	12.99	11.63	12.25	11.46	13.48	13.57	24.57	27.01
5 (20a)	11.98	11.34	12.54	-	11.98	12.61	12.97	27.90	25.54
6 (20b)	13.16	13.48	13.12	14.27	10.25	13.62	15.75	11.98	11.31
7 (20c)	13.12	13.15	11.29	11.69	13.39	14.11	14.90	11.47	10.35
8 (21a)	11.80	12.66	13.40	12.18	11.22	13.61	15.31	13.10	11.37
9 (39a)	11.15	11.67	13.87	14.05	14.12	13.63	14.52	13.37	10.49
10 (44b)	11.33	12.35	13.18	13.34	12.03	12.27	14.36	12.24	13.07
11 (26b)	13.99	13.22	12.28	13.11	10.96	13.65	13.34	12.06	12.06
12 (26c)	11.67	15.99	13.04	12.80	11.94	13.11	12.15	12.23	11.50
13 (52a)	14.16	11.69	12.44	13.15	10.74	13.27	12.35	10.30	13.19
14 (54a)	14.40	15.16	12.59	14.15	11.01	13.48	13.02	12.90	11.25
15 (54b)	25.33	27.14	16.19	27.38	28.25	27.87	-	24.55	22.92
16 (58b)	12.38	11.17	11.88	12.21	10.52	11.42	12.03	11.47	11.57
17 (40c)	13.86	11.79	12.29	13.48	-	13.84	13.53	11.38	10.59
18 (64c)	11.81	11.38	10.77	12.19	12.85	13.29	13.57	12.22	12.06
19 (65a)	11.74	12.43	11.58	12.12	11.92	13.60	15.28	11.45	13.41
20 (74b)	12.41	12.71	11.92	11.42	11.63	13.36	11.64	11.40	11.14
21 (75a)	14.12	19.87	14.35	15.42	13.33	15.79	13.10	12.08	14.85
Av Ct value	13.1	13.7	12.6	13.6	12.5	14.07	12.9	13.9	13.9

Table 6: Ct values of leaves and flowers collected from 'Edranol' trees from September 2024 to January 2025

	SEPTEMBER 2024		OCTOBER 2024		NOVEMBER 2024		DECEMBER 2024		JANUARY 2025	
Selected trees	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH
Sample no	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value
1 (7c)	12.74	12.96	11.62	12.25	9.90	10.63	12.95	15.80	13.06	13.48
2 (9a)	10.77	12.19	11.71	11.46	10.02	11.41	15.39	15.62	13.45	15.71
3 (13c)	12.08	13.36	12.53	13.40	10.02	12.08	13.28	14.07	12.56	12.94
4 (30c)	14.10	14.41	10.66	12.18	10.82	13.57	12.50	13.29	12.92	13.38
5 (20a)	12.95	12.06	12.32	10.75	9.74	12.47	11.77	15.33	14.12	12.98
6 (20b)	12.65	12.64	12.38	13.31	10.79	11.02	15.68	13.91	14.22	13.41
7 (20c)	13.35	13.59	11.54	14.25	11.47	14.94	12.57	15.17	14.19	13.96
8 (21a)	12.14	12.15	12.72	14.29	10.67	11.30	14.56	14.18	13.48	13.24
9 (39a)	12.13	12.87	11.73	13.11	10.87	13.38	12.92	14.26	-	-
10 (44b)	12.47	11.39	13.54	12.40	10.83	10.72	14.29	15.43	14.04	15.67
11 (26b)	14.48	12.38	11.26	12.60	10.66	11.51	15.43	16.23	15.2	14.09
12 (26c)	12.34	13.97	12.27	11.85	11.37	13.25	14.01	15.21	11.26	13.45
13 (52a)	12.51	12.71	9.86	10.81	12.64	12.20	11.85	12.45	15.59	12.1
14 (54a)	14.06	13.27	11.23	11.90	10.75	10.98	12.25	15.99	12.64	13.48
15 (54b)	26.58	28.06	25.79	-	24.75	-	27.77	-	15.54	27.06
16 (58b)	10.06	10.47	11.94	11.57	10.30	13.13	12.34	16.30	13.23	13.93
17 (40c)	10.57	11.60	11.58	12.38	11.77	12.48	13.40	15.52	12.29	13.33
18 (64c)	11.89	11.93	11.17	12.11	10.88	10.63	14.40	13.24	13.35	15.72
19 (65a)	12.08	12.11	11.31	12.95	12.45	11.31	11.71	14.41	11.77	13.03
20 (74b)	10.38	12.05	11.39	11.11	11.33	11.81	11.81	13.66	13.83	13.42
21 (75a)	21.38	14.43	16.15	16.37	9.42	10.45	16.14	16.71	12.16	13.5
Av Ct value	13.4	13.4	12.6	12.5	11.5	12.0	14.1	14.8	13.4	14.4

Annexure D: 'HASS' Results

Table 7: Ct values of leaves and flowers collected from 'Hass' trees from September 2023 to March 2024

CODE		OCT 2023	DEC 2023	JAN 2024	MARCH 2024	
		FIRST SCREEN	FRUITSET	FRUIT DEVELOPMENT	FRUIT ON TREES NEW LEAVES	FRUIT ON TREES OLD LEAVES
Sample no	Tree no	Ct value	Ct value	Ct value	Ct value	Ct value
1 (6b)	R3T2	12.78	8.66	10.01	11.55	10.41
2 (6c)	R3T3	26.52	23.33	10.96	-	26.71
3 (8a)	R3T7	10.53	13.27	-	11.06	11.87
4 (11a)	R4T1	26.29	-	-	-	-
5 (11b)	R4T2	25.29	-	28.78	-	-
6 (11c)	R4T3	10.94	11.72	10.54	11.98	12.26
7 (14b)	R4T11	12.62	10.18	10.76	12.96	11.07
8 (14c)	R4T12	25.96	11.19	27.89	-	-
9 (30a)	R6T19	13.79	11.99	14.37	13.07	12.93
10 (34a)	R7T4	12.59	10.38	12.04	12.09	12.48
11 (34b)	R7T5	25.93	25.70	26.61	-	-
12 (40a)	R7T22	17.81	Very positive	10.93	14.06	13.25
13 (57a)	T9T10	26.55	19.71	27.81	-	-
14 (57b)	R9T11	14.13	21.79	14.82	13.66	14.65
15 (60c)	R9T21	18.56	24.97	22.09	22.70	15.02
16 (78c)	R10T33	12.18	7.91	10.75	13.68	11.83
Average Ct values		18.27	15.44	17.02	13.68	13.95

Table 8: Ct values of leaves and flowers collected from 'Hass' trees from May 2024 to August 2024

SEPT 2023	MAY 2024		JUNE 2024		JULY 2024			AUGUST 2024	
Selected trees	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	FLOWERS	OLD LEAVES	NEW LEAF FLUSH
Sample no	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value
1 (6b)	9.92	9.85	9.60	10.88	14.01	10.41	8.35	12.60	11.31
2 (6c)	27.02	25.52	-	29.98	28.59	27.95	29.18	25.47	28.38
3 (8a)	9.83	10.48	10.78	11.04	11.32	9.91	12.29	12.86	12.29
4 (11a)	26.11	25.61	27.04	27.44	27.91	24.95	23.72	27.81	26.05
5 (11b)	27.16	27.69	25.12	29.38	24.92	27.25	26.87	25.25	26.12
6 (11c)	8.89	9.56	11.78	10.69	10.25	8.86	10.36	15.16	13.17
7 (14b)	9.85	10.87	11.56	11.83	9.40	12.38	11.24	15.02	13.24
8 (14c)	13.87	10.81	13.31	28.13	11.54	28.52	23.04	26.63	28.05
9 (30a)	10.15	10.60	11.56	11.34	11.71	11.42	10.64	12.06	13.44
10 (34a)	9.44	10.15	11.33	11.21	10.74	11.44	9.23	12.13	12.28
11 (34b)	24.85	26.42	25.98	-	26.84	24.57	29.26	24.16	29.05
12 (40a)	8.98	9.89	11.65	11.06	11.42	9.95	11.16	11.24	11.61
13 (57a)	25.92	27.02	28.51	-	23.91	26.06	26.91	29.49	-
14 (57b)	20.01	11.99	14.71	21.18	11.61	12.41	12.86	25.81	20.35
15 (60c)	19.38	25.83	15.16	14.62	24.49	24.89	22.71	27.93	-
16 (78c)	9.18	10.54	11.11	11.74	11.38	9.88	11.33	13.26	12.34
Av Ct value	16.2	16.4	15.94	17.18	16.9	17.6	17.4	19.8	18.4

Table 9: Ct values of leaves and flowers collected from 'Hass' trees from September 2024 to January 2025

	SEPTEMBER 2024		OCTOBER 2024		NOVEMBER 2024		DECEMBER 2024		JANUARY 2025	
Selected trees	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH	OLD LEAVES	NEW LEAF FLUSH
Sample no	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value	Ct value
1 (6b)	10.38	11.28	10.90	13.50	13.23	15.14	10.85	11.25	10.42	11.7
2 (6c)	24.07	13.08	29.39	26.61	24.18	25.75	25.75	26.82	29.14	-
3 (8a)	9.16	10.29	10.81	11.74	10.00	12.13	10.87	11.37	12.41	12.74
4 (11a)	28.09	28.47	28.36	-	25.55	26.77	29.89	-	26.05	25.02
5 (11b)	25.98	-	26.64	27.04	25.38	28.89	11.75	12.86	27.51	-
6 (11c)	9.04	9.12	11.09	11.68	9.26	10.12	25.60	26.16	11.03	11.5
7 (14b)	8.86	10.60	10.21	10.50	10.07	9.51	10.17	12.34	10.8	11.65
8 (14c)	27.02	-	12.08	15.37	15.51	13.55	16.94	-	27.54	29.26
9 (30a)	10.91	12.06	10.20	11.34	9.27	10.19	10.10	11.46	12.5	13.32
10 (34a)	9.68	10.46	9.66	11.63	9.38	9.68	9.63	11.02	10.75	10.64
11 (34b)	24.39	26.50	21.73	26.00	26.66	26.36	26.67	24.16	26.51	27.14
12 (40a)	9.92	10.56	9.88	10.93	10.66	11.10	9.87	10.74	10.42	11.84
13 (57a)	26.62	27.98	25.97	28.48	25.43	26.89	26.03	24.64	27.66	14.43
14 (57b)	22.19	13.24	13.13	11.20	9.91	10.73	11.42	12.27	10.64	14.23
15 (60c)	23.20	27.42	23.11	21.38	21.20	11.50	12.13	13.60	21.64	11.49
16 (78c)	11.33	10.20	9.79	10.97	9.88	11.32	10.50	10.71	11.61	11.29
Av Ct Value	17.5	15.80	16.4	16.5	16.0	16.2	16.1	15.7	17.9	15.4