Report

Research project on the impact of phytoplasma strains on the phenotypical expression of poinsettia varieties

Lars Henrik Jacobsen

Danish Institute of Agricultural Sciences
Department of Horticulture
Kirstinebjergvej 10
DK-5792 Årslev

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Introduction

The poinsettia, Euphorbia pulcherrima, is a well known plant that has already been cultivated for many years. The name poinsettia comes from a well known American botanist, Joel Robert Poinsett who introduced the plant in the USA in 1825. The introduced plant was different from the modern varieties; it grew into straight and tall shrubs. The first varieties were introduced in the 1920's. In 1967 the first free branching variety was introduced as 'Annette Hegg'. However, it took until the 1990's to prove that the free branching was caused by phytoplasma. Phytoplasma can be introduced in the poinsettia by so-called leaf hopper insects or by grafting on an infected plant.

The phytoplasmas are very small prokaryotes, which are related to bacteria, but in contrast to bacteria they do not have a cell wall. The phytoplasmas are found in the phloem cells of host plants. The phytoplasmas cannot be cultured on artificial media, and can only be maintained in their host plants. The fact that phytoplasmas cannot be cultured on artificial media has made the study of phytoplasmas very laborious and difficult.

Discussions concerning phytoplasma in PVR circles started in 1997 when applications for Euphorbia fulgens were filed, and where the applicant in the technical questionnaire had indicated that a 'vector' was introduced. Later it appeared that this 'vector' was phytoplasma, similar to the situation in poinsettia.

The problem with phytoplasma in relation to Plant Breeders' Rights is related to the definition of a plant variety in the UPOV convention (1) and the CPVO Basic regulation:

'A variety is a plant grouping within a single botanical taxon of the lowest rank…..'

Since the expression of the genotype is highly influenced by the phytoplasma that is not a part of the genotype of the plant there is a conflict in relation to phytoplasma infected varieties with the definition of a variety.

It is known that most, if not all, varieties of poinsettia on the market are infected on purpose with phytoplasma by the breeder.

In 2001 the CPVO decided to finance a first project in relation to phytoplasma. Mogens Nicolaisen from the Danish Institute of Agricultural Sciences, Flakkebjerg, performed a literature study, also including interviews with some breeders and scientists working in the field of phytoplasma.

One of the main conclusions of the literature study is that several strains of phytoplasma have been detected in poinsettia (2). These occur together in individual plants and probably to some extent in different combinations from variety to variety.

It has been speculated whether different populations of phytoplasmas from different poinsettia varieties could induce different phenotypes in the same poinsettia genotype. That is, can phytoplasma populations be used as a 'treatment' on the same poinsettia variety to obtain several varieties?

The literature study (2) was not able to answer the question. No scientific literature exists on the effect of different strains of phytoplasma on the phenotype of poinsettia.

The main subject for this study is: Will different populations of phytoplasma be able to induce different phenotypes of the same poinsettia genotype?

The phytoplasma experiment:

To investigate the effect of different phytoplasma populations on poinsettia varieties several sorts of plant materials are needed:

a) Rootstocks with different populations of phytoplasma.

b) Phytoplasma-free varieties

Phytoplasma-free plants will serve as recipients of phytoplasma and these plants will be evaluated for their phenotypes.

1. Selection of poinsettia rootstocks for the trial

First step in the experiment was to obtain different populations of phytoplasma.
25 varieties were selected for a pre-screening of poinsettia varieties for presence and variation in phytoplasma populations. The varieties were selected so that they represent the broadest variation in phytoplasma populations. So the criteria for the selection of plants for pre-screening were:

a) Origin: varieties from different breeders

b) Varieties originating from different countries

c) Both old and new varieties

As a result of the prescreening the following varieties were selected as rootstocks in 2003:

Annemie (75**)**: variety from Ecke
Maren (250): variety from Beckmann
Duecap (272): variety from Dümmen
Fiscor (313): variety from Flora-Nova
Lazzpopapaa (684): variety from Lazzeri

The above varieties were selected to cover the highest level of phytoplasma variation and cover as many breeders as possible. For details concerning the test for phytoplasma, see Nicolaisen, 2004 (3).

Since it was wanted that all main breeders should be present in the trial the following variety from Klemm was included in the trial as rootstock:

Klew01071 (763)

The varieties 'Red Fox j57' (741) and 'Red Fox 5115' (766) from Dümmen were also included in the trial. 'Red Fox j57' (741) had particular characteristics (deformed bracts and leaves) that were never observed in other varieties. 'Red Fox 5115' (766) was different from 'normal' poinsettias. Bracts and leaves were reflexed, bracts had a strong rugosity and petioles were very short and thick.

Since Klew01071 (763) and 'Red Fox j57' (741) did not give a sufficient phytoplasma signal in the pre-screening and we had added the variety Red Fox 5115 (766) that was not included in the pre-screening, the three varieties were resampled and retested by Research Center Flakkebjerg in December 2003. The result confirmed that phytoplasma was present in all 3 varieties. The phytoplasma content of the three varieties was very similar, only Klew01071 (763) showed a slightly different content.

Conclusion of the pre-screening: 8 rootstocks varieties were selected for the final trial:

75: Annemie
250: Maren
272: Duecap
313:Fiscor
684: Lazzpopapaa
763: Klew01071
741: Red Fox j57
766: Red Fox 5115
Phytoplasma was detected in all 8 rootstocks.

The number for each rootstock will be used in the following part of the report and it refers to each of the above-mentioned rootstocks.

2. Phytoplasma-free varieties

In September 2003 the following phytoplasma free plant materials were received from different breeding companies:

Christmas Cookie (KLEW01071)
Christmas Dream (KLEW01073)
Cortez (Fiscor)
Christine Zieger
Freedom
V-119
C-27 (Celebrate)
C-17

Three varieties were selected:

Christmas Cookie
Cortez
C-27

The varieties were tested for presence of phytoplasma in week 7, 2004 and no phytoplasma was detected (3). The selected phytoplasma-free varieties were used as plant material for grafting on the 8 selected rootstocks.

3. Grafting

The grafting was made in week 7 2004 after having received confirmation that the varieties were phytoplasma-free.

It was necessary to regraft some of the combinations, in week 10, 12 and 14 because not all combinations succeeded.

Samples from the grafted plants were taken several times during the spring/summer of 2004 and analysed for transmission of phytoplasma, see (3).

After the last test for phytoplasma transmission in August 2004, the final result of phytoplasma transfer showed:

a: 21 combinations were tested clearly positive and the mother plants showed branching.

b: 3 combinations cannot be used in the final experiment: 1 combination failed to graft, 1 combination had no phytoplasma transmission and 1 combination had a very low level of phytoplasma.

4. Final experiment

4.1 Set-up of trial

Combinations of rootstocks/varieties used in the final experiment are shown in Table 1.

Table 1: Combinations of rootstocks/varieties used in the experiment

Rootstock	C-27	Cortez	Christmas Cookie
272	X	X	
75	X	X	x
250	X	X	x
684	X	X	x
741	X	X	x
763	X	X	x
766	X	X	x
313			x

Each variety was placed on one bench in a greenhouse at Department of Horticulture, Aarslev, Denmark (Table 2). Each bench (Benches 1, 2 and 3) was divided into 12 blocks and each rootstock was randomized and was present at least one time in each block. Only plants in bench 1, 2 and 3 were used for recordings. The codings are shown in Table 3.

Table 2: Benches in greenhouse 5.4. Each number on benches 1-3 represent one plant

	bench 1	bench 2	bench 3	bench 4
Block	**C-27**	**Cortez**	**Christmas Cookie**	**C-27**
1	30 5 16 20 10 2 9 9	7 3 22 15 23 7 21 12	26 24 11 29 18 8 18 14	272
2	9 30 5 10 16 2 20 16	12 21 23 22 15 21 3 7	29 14 26 8 24 26 18 11	75
3	5 16 2 5 20 9 10 30	23 22 23 21 3 12 15 7	26 11 29 18 14 14 8 24	250
4	5 10 20 9 30 16 9 2	21 15 12 3 22 21 7 23	11 29 14 24 18 24 8 26	684
5	10 30 10 5 2 20 9 16	7 3 21 23 12 23 22 15	18 26 24 14 8 29 29 11	741
6	9 9 10 16 30 5 2 20	21 22 22 12 3 23 7 15	18 11 26 24 29 11 14 8	763
7	10 2 9 20 5 10 30 16	7 12 21 23 22 23 15 3	14 18 26 24 18 8 11 29	766
8	16 20 5 10 2 20 30 9	22 7 3 12 23 15 12 21	11 29 14 18 24 8 26 26	No phytoplasma
9	20 9 10 5 16 2 30 2	7 21 3 22 12 15 23 12	29 14 24 18 26 11 8 24	
10	10 5 16 30 20 9 2 16	15 21 7 22 3 12 12 23	24 18 24 8 14 26 11 29	
11	5 2 10 10 9 20 16 30	12 3 12 21 15 22 23 7	24 18 29 14 11 14 26 8	
12	10 30 5 9 5 16 20 2	12 7 15 23 21 7 22 3	8 14 29 26 8 24 18 11	

Table 3: Combinations of rootstocks/varieties used in the final experiment:

C-27 (Celebrate)	**G**	
Number	Rootstock	Name
2	272	Duecap
5	75	Annemie
9	250	Maren
10	684	Lazzpoapaa
16	741	red fox j 57
20	763	klew01071
30	766	red fox 5115
Cortez	**C**	
3	272	Duecap
7	75	Annemie
12	684	Lazzpoapaa
15	741	red fox j 57
21	763	klew01071
22	250	Maren
23	766	red fox 5115
Christmas Cookie	**A**	
8	75	Annemie
11	684	Lazzpoapaa
14	741	red fox j 57
18	763	klew01071
24	250	Maren
26	313	Fiscor
29	766	red fox 5115

For observation of relevant characteristics the experiment also included 3 benches where the rootstocks for each variety were placed in groups (8-10 plants per rootstock) for observations. See Table 2, bench 4 and Table 4. The purpose of the plants was not recording, only observation. Plants without phytoplasma were also present on those benches.

Table 4: Benches in greenhouse 5.3, Christmas Cookie and Cortez.

5.3

	Christmas Cookie	Cortez
No plants	75	272
	250	75
	684	250
	741	684
	763	741
	766	763
	313	766
	No Phytoplasma	No phytoplasma

The plants for the experiment were propagated in week 1, 2005. Short day treatment (14 hours darkness) started in week 5, 2005. Recordings were made in April 2005.

The plants in the experiment were not pinched.

Temperature set-point during propagation was 21ºC with ventilation at 24 ºC.

Temterature set-points during the trial: 21 ºC with ventilation at 24 ºC from week 5 to week 9 2005
20 ºC with ventilation at 23 ºC from week 9 to week 13 2005
18 ºC with ventilation at 21 ºC from week 14 to week 15 2005
17 ºC with ventilation at 20 ºC from week 16 2005

4.2 Recordings

To answer the question: Will the different populations be able to induce different phenotypes of the same genotype? – we decided to use characteristics that are known to be affected by phytoplasma infection, see Nicolaisen (2003). Characteristic 13.'Cyme width' was added after the expert meeting see page 10.

The following recordings were made:

1. Plant: branching	absent/present
2. Plant: number of branches	number (>1 cm)
3. Plant: height	cm, from pot rim to top of cyathia.
4. Stem: internode length	mm, average of 5 internodes.
5. Stem: diameter	mm, measured 3-4 cm above pot rim.

6. Leaf blade: intensity of colour of upper side: assessed by notes (1-9) very weak to very strong

7. Leaf blade: intensity of colour of lower side: assessed by notes (1-9) very weak to very strong

8. Leaf blade: development of lobes: assessed by notes (1-9) very weak to very strong

9. Bract: colour of upper side	RHS-colour chart
10.Bract: colour of lower side	RHS-colour chart
11.Time of opening of first cyathium	days from start of short day
12. Cyathium: persistence	days from start of short day
13. Cyme: width	mm

All recordings, (except cyathium persistence and time of opening of first cyathium) were made when the plants had approx. 3 open cyathia. Bract colour of upper and lower side was also recorded a second time approx. 4 weeks after the first observation of the colour.

Statistical analyses were made for the following Characteristics: Plant: number of branches, Plant: height, Stem: internode length, Stem: diameter, Time of opening of first cyathium, Cyathium: persistence and Cyme: width. A computer program from SAS Institute Inc. (4) was used for data processing.

Some of the characteristics are assessed with different states ('notes'). This assessment is described in 'Guidelines for the conduct of tests for distinctness, uniformity and Stability – Poinsettia' (5).

4.3 Expert meeting in Aarslev on 14 April 2005

The following experts participated in the meeting: Mrs Andrea Menne, Germany, Mrs Maria Zaleska and Mrs Malgorzata Pakulska, Poland, Mr Ton Kwakkenbos,CPVO, Mrs Lillie Andersen and Mr Lars Jacobsen, Denmark.

The experts visited the greenhouse trial and observed the plots of 8 plants of the different combinations. The weather conditions for observing the trial were excellent, there was no direct sunlight, so it was possible to observe the flower colour very well. The trial was well performed and the plants were in optimal condition for observation.

As first impression it was obvious that the different combinations within one variety grafted were very equal. No colour differences could be observed.

The experts were satisfied with the quality of the test in the greenhouse.

All experts agreed that the first measurements and visual observations made in the greenhouse did not show differences in the different graftings of 1 variety. The 'uniformity' was amazing. Clear differences were observed between grafted plants and the same variety of phytoplasma-free.

Only one exception was observed in one combination, C-27 grafted upon 684. There seems to be a difference in the diameters of the cymes/numbers of cyathia. The experts recommended that the measurement of the diameters of the cymes were added to the observations.

4.4 Results

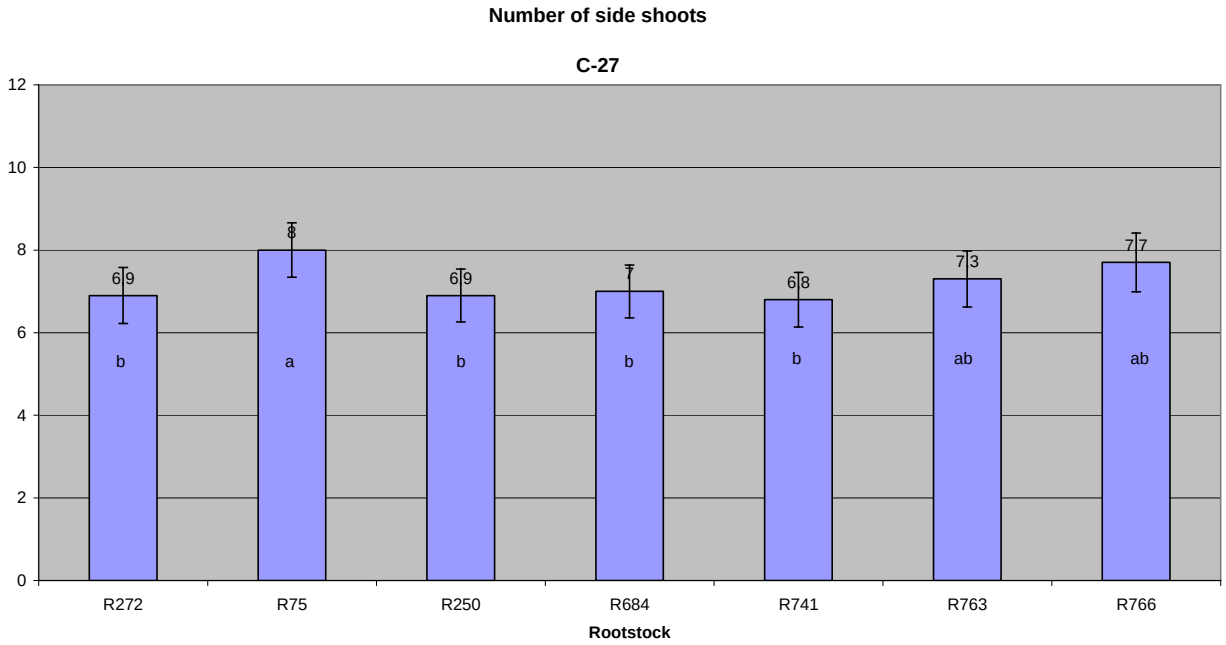
1. Plant: branching

There were no differences in the characteristic 'branching (absent/present)' between rootstocks for all three varieties. Branching was present in all plants in the experiment. Plants without phytoplasma had no branching at all for all three varieties.

All plants in the trial were not pinched.

2. Plant: number of branches

C-27
Numbers of branches on the variety 'C-27' are shown in figure 1.



Figur 1: Number of branches for the variety ‘C-27’ grafted on different rootstocks. Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

Rootstocks 272, 250, 684, 741, 741, 763 and 766 were not significantly different in the number of side shoots. Rootstock 75 had the highest number of side shoots (8,0) and was significantly different from rootstocks 272, 250, 684 and 741. However, the differences were rather small (approx. 1 side shoot on the average). The differences in number of side shoots correspond to less than 1 note. Notes are used for assessment in DUS-test of Poinsettia, See Upov Guideline TG/24/5 (5).

Cortez

Numbers of branches on the variety ‘Cortez’ are shown in figure 2.

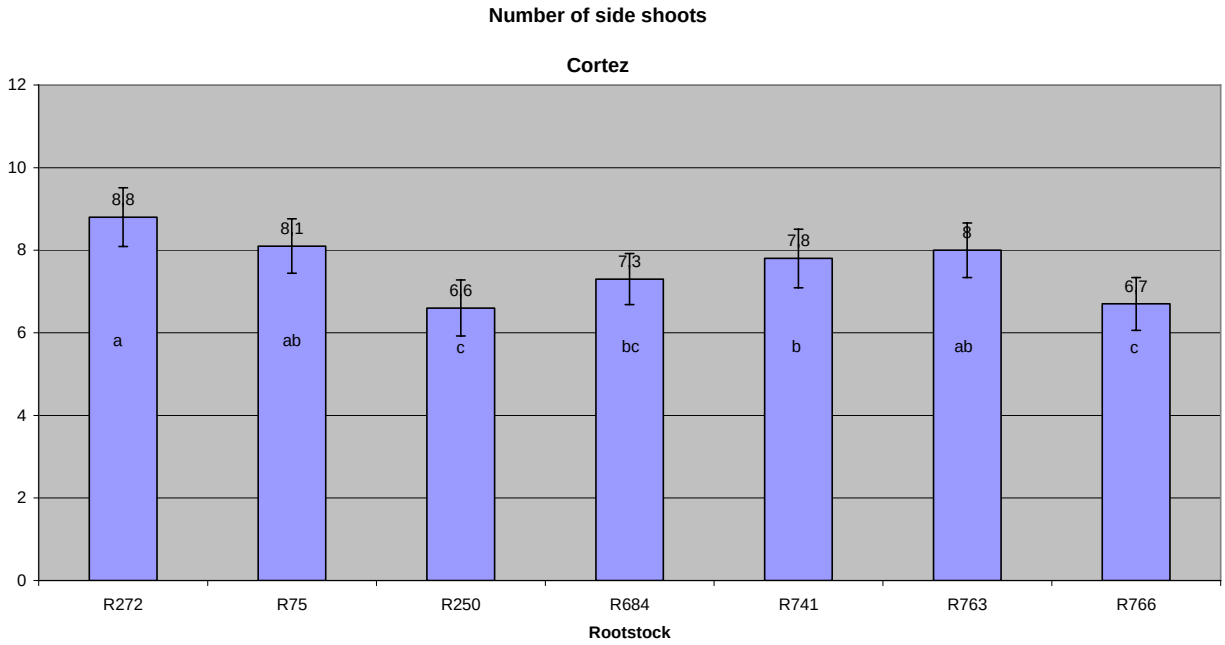


Figure 2: Number of branches for the variety ‘Cortez’ grafted on different rootstocks. Means with ±1,96 x standard error are indicated and means with significant differences are indicated with different letter

Rootstock 272 had the highest number of side shoots. However, rootstocks 272, 75 and 763 were not significantly different in the number of side shoots. 250 and 766 had the lowest number of side shoots, significantly lower than the other varieties except for rootstock 684.

However, the differences were small (maximum 2 side shoots). The maximum difference in number of side shoots correspond to 1 note.

Christmas Cookie

Numbers of branches on the variety ‘Christmas Cookie’ are shown in figure 3.

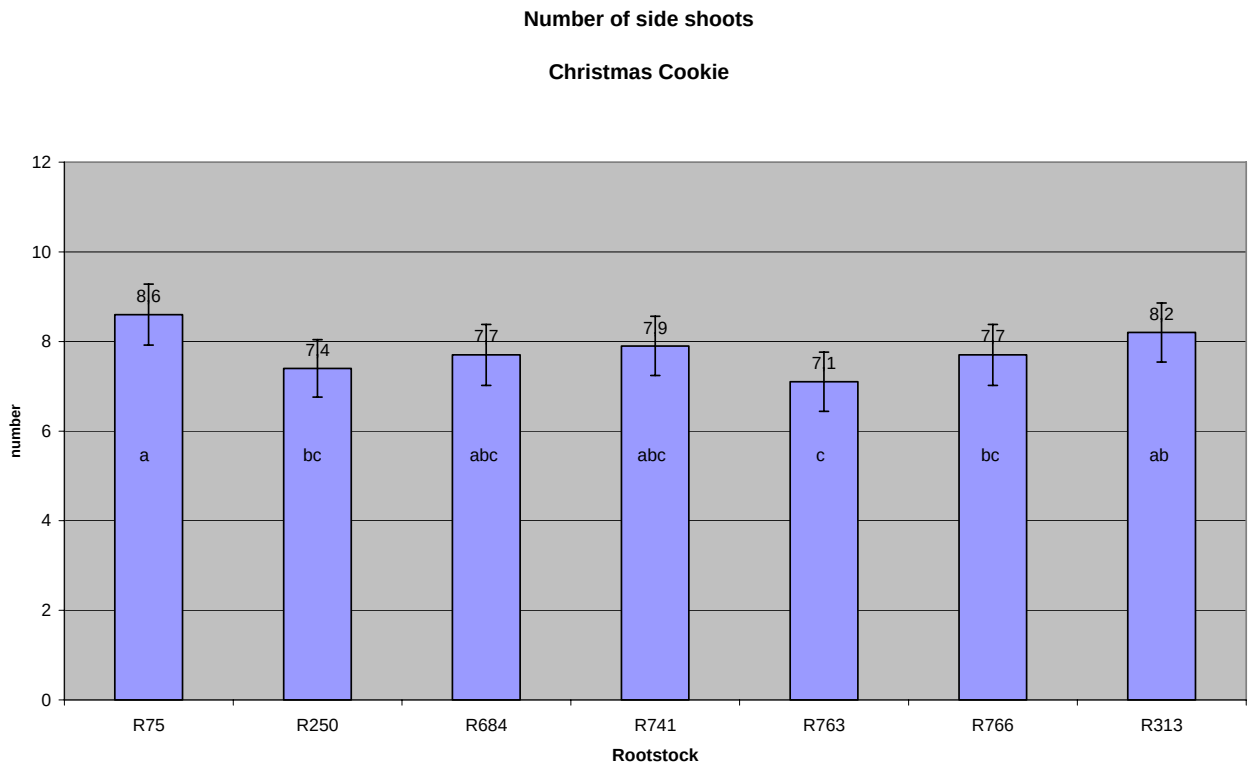


Figure 3: Number of branches for the variety ‘Christmas Cookie’ grafted on different rootstocks. Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

Rootstock 75 had the highest number of side shoots. However, rootstock 75, 250, 766 and 313 were not significantly different in the number of side shoots. 763 had the lowest number of side shoots, but it was not significantly different from 250, 284, 741 and 766.

However, the differences were small (maximum 1,5 side shoots). The differences in number of side shoots correspond to maximum 1 note.

3. Plant: height

C-27

There were no significant differences in plant height between rootstocks for the variety C-27 (figure 4).

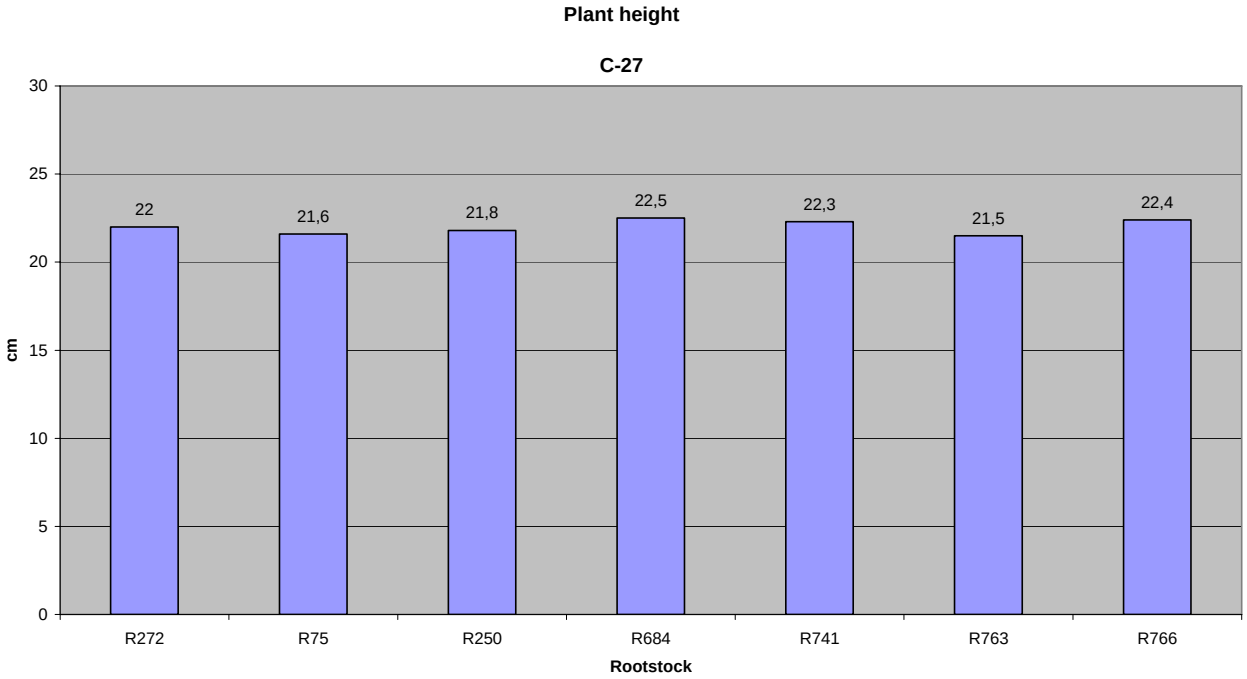
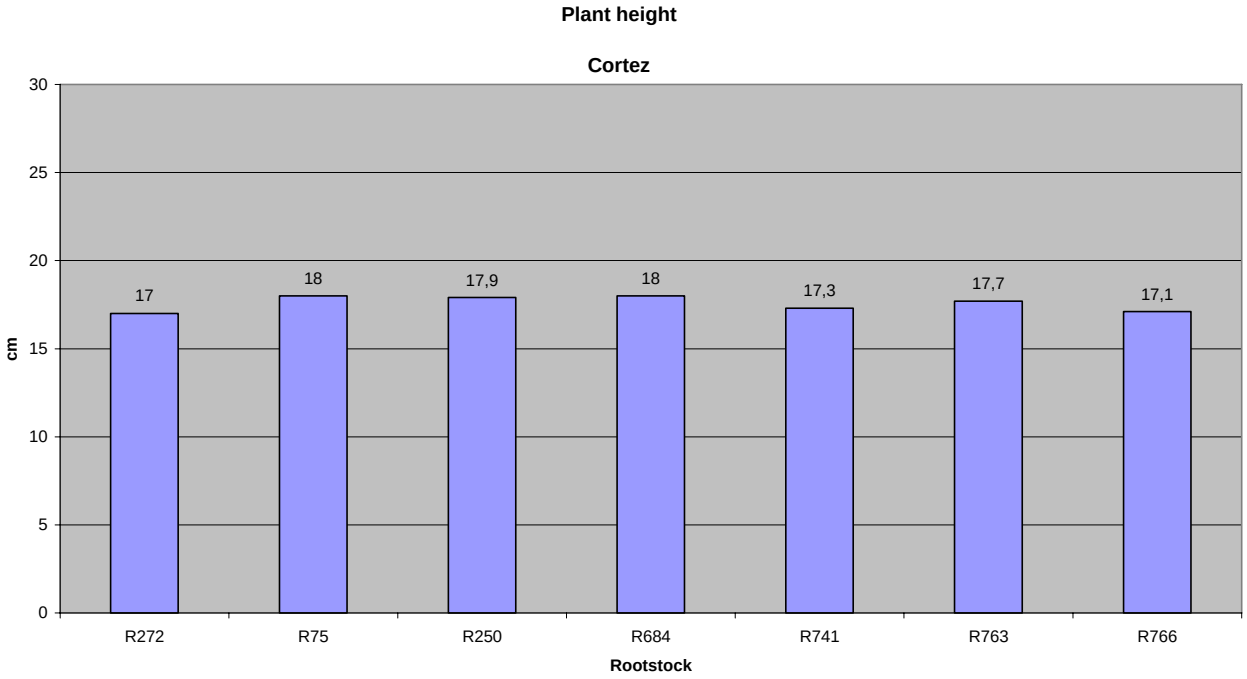


Figure 4: Plant height in cm for the variety ‘C-27’ grafted on different rootstocks

Cortez

There were no significant differences in plant height between rootstocks for the variety Cortez (figure 5).



Figur 5: Plant height in cm for the variety ‘Cortez’ grafted on different rootstocks

Christmas Cookie
There were no significant differences in plant height between rootstocks for the variety Christmas cookie (figure 6).

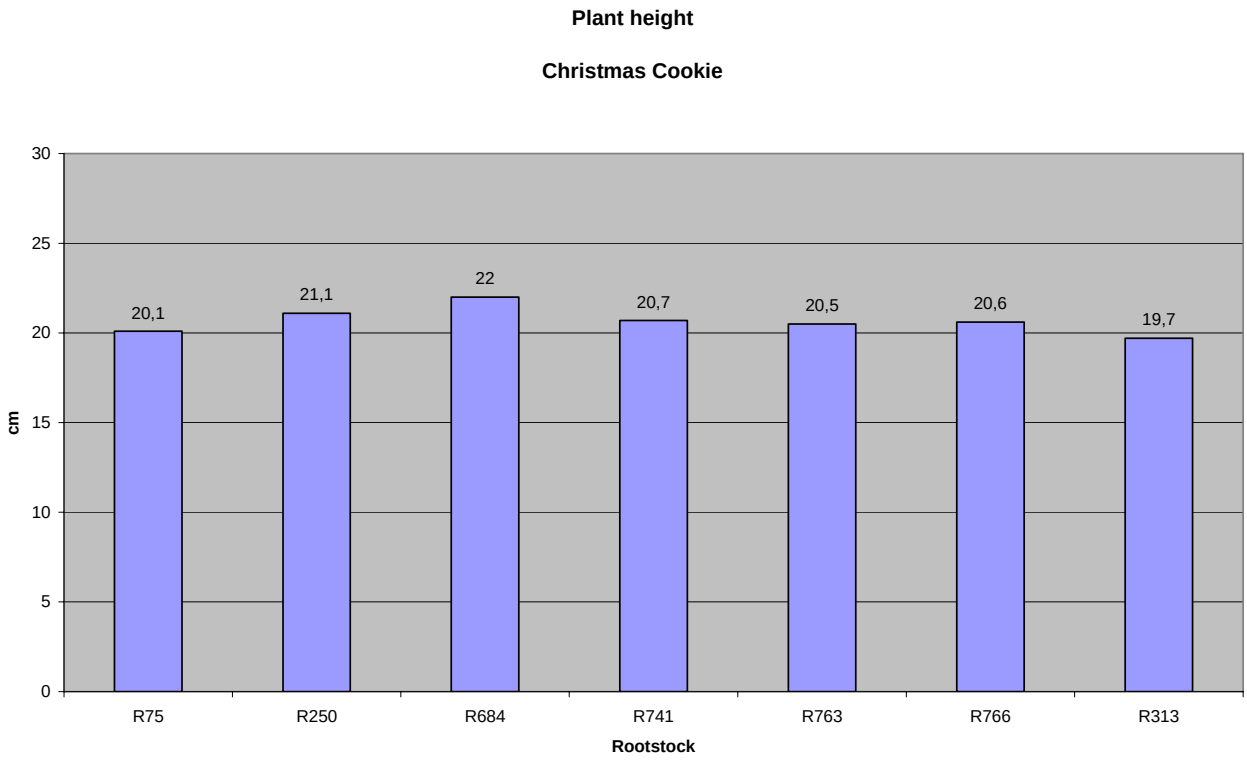


Figure 6: Plant height in cm for the variety 'Christmas Cookie' grafted on different rootstocks

4. Stem: internode length

C-27
There were no significant differences in internode length between rootstocks for the variety C-27 (figure 7).

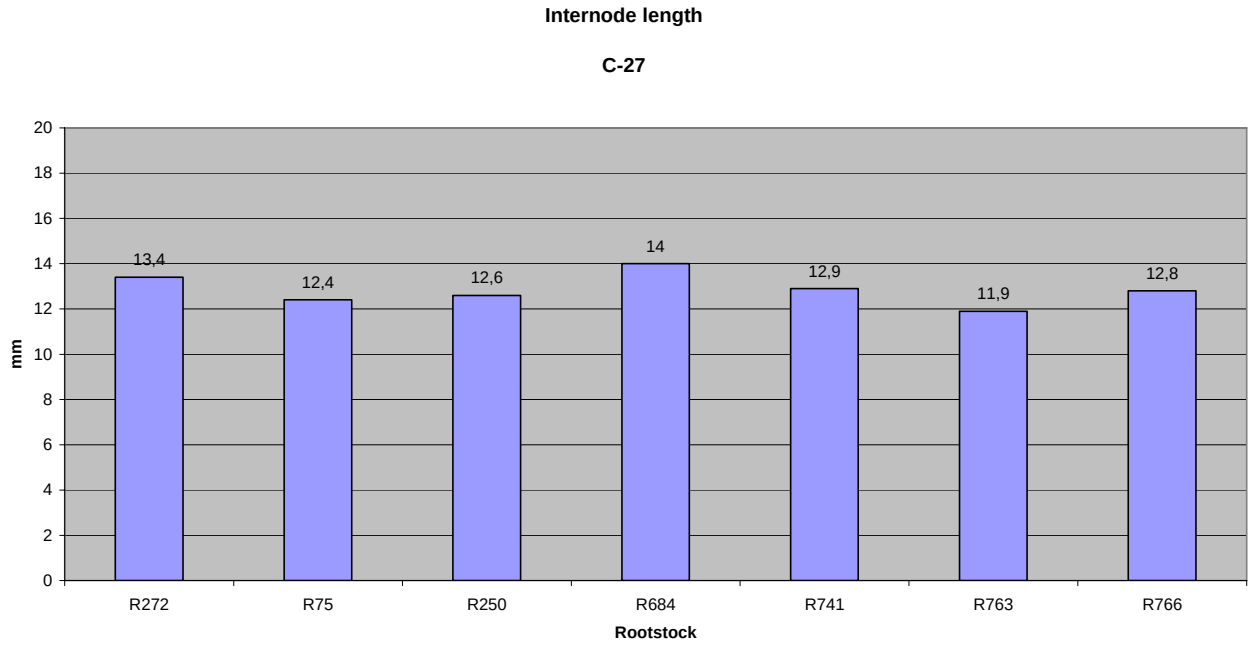


Figure 7: Internode length in mm for the variety ‘C-27’ grafted on different rootstocks.

Cortez

There were no significant differences in internode length between rootstocks for the variety Cortez (figure 8).

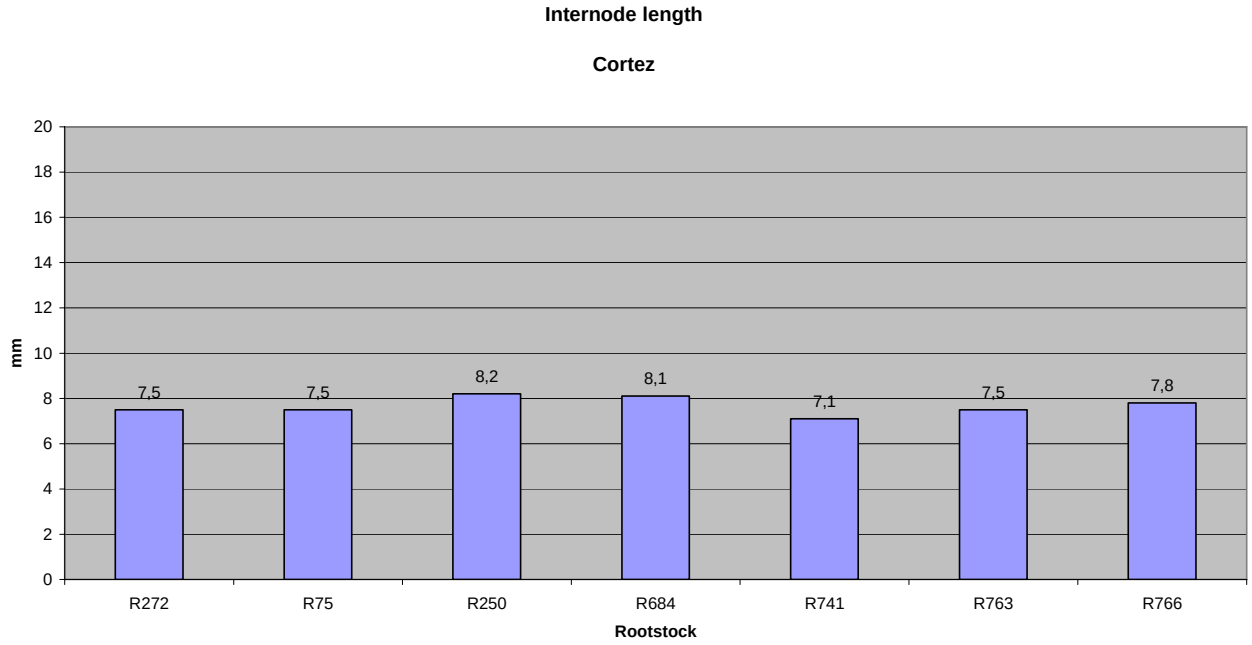


Figure 8: Internode length in mm for the variety ‘Cortez’ grafted on different rootstocks

Christmas Cookie

There were no significant differences in internode length between rootstocks for the variety Christmas Cookie (figure 9).

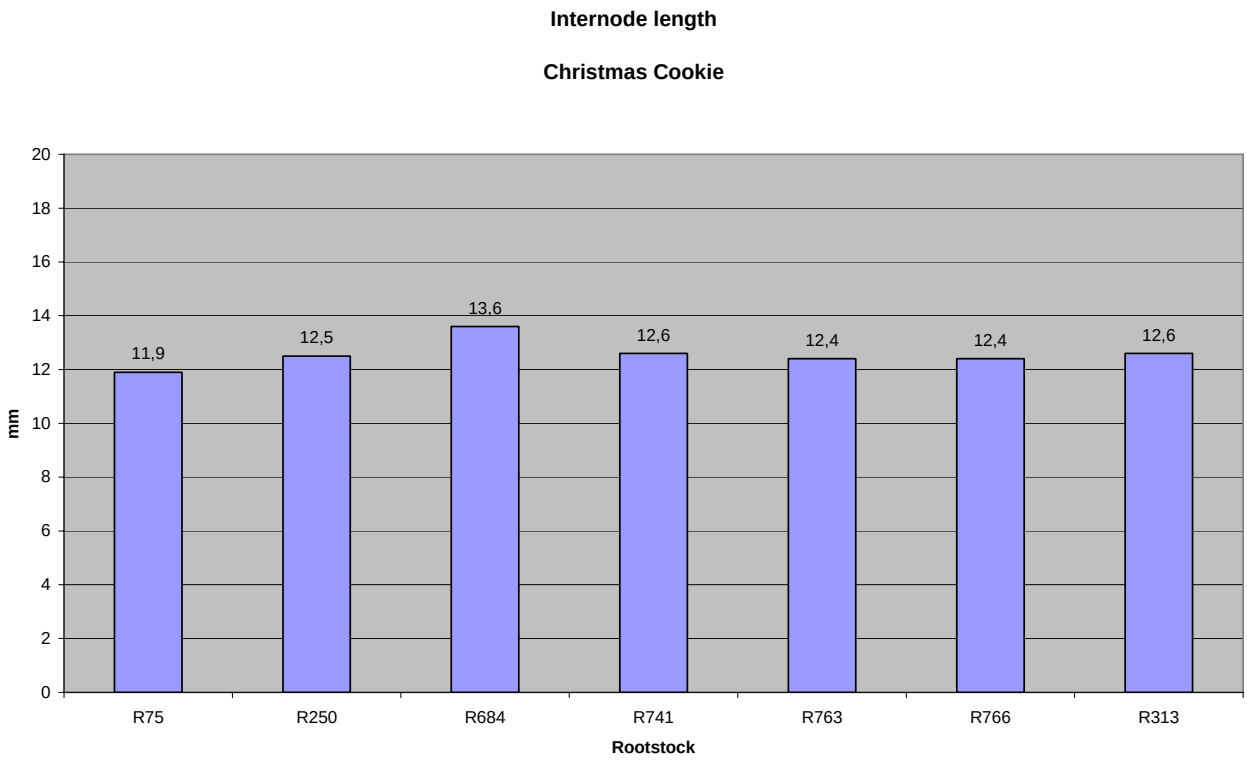


Figure 9: Internode length in mm for the variety ‘Christmas Cookie’ grafted on different rootstocks

5. Stem: diameter

C-27

The stem diameters of the variety C-27 are shown in figure 10.

Rootstock 250 had the largest stem diameter. However, rootstocks 250, 763 and 766 were not significantly different in the stem diameter. Rootstock 250 had a significantly larger stem diameter than rootstocks 272, 75, 684 and 741. However, the differences were small ranging from 0,4 mm to 0,7 mm.
Rootstock 272 had the smallest stem diameter, but it is not significantly different from rootstock 75 and 684.
Rootstocks 75, 684, 741, 763 and 766 were not significant different in stem diameter.

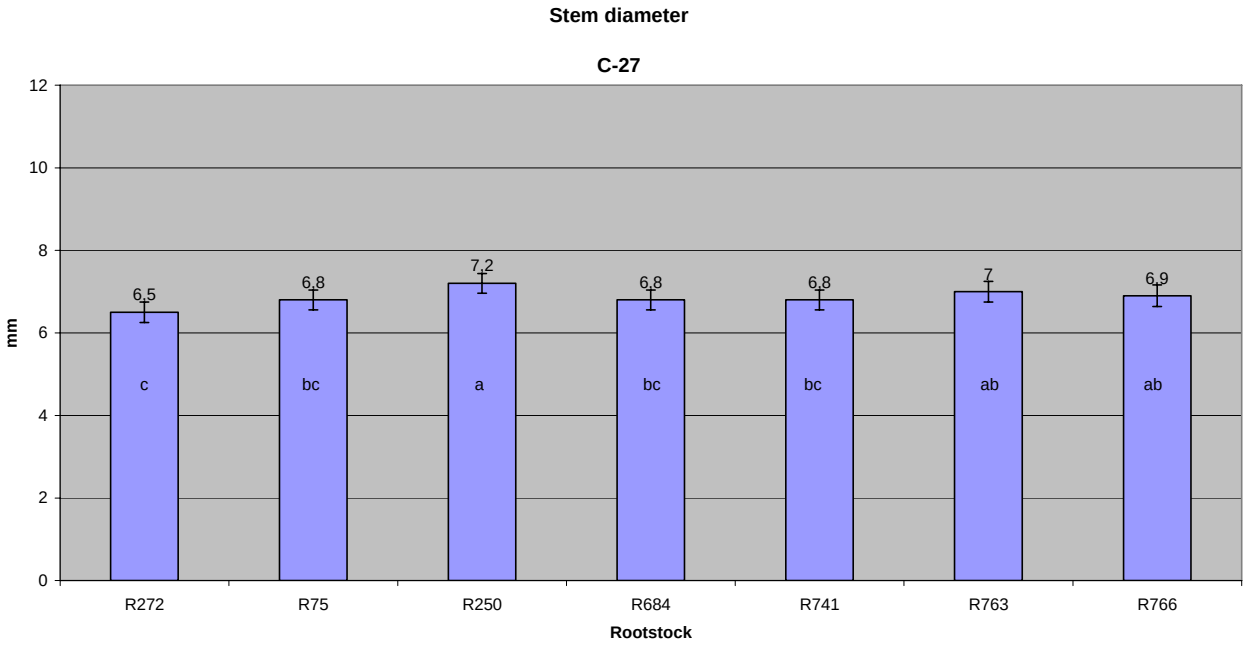


Figure 10: Stem diameter in mm for the variety C-27' grafted on different rootstocks. Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

Cortez

There were no significant differences in stem diameter between rootstocks for the variety Cortez (figure 11).

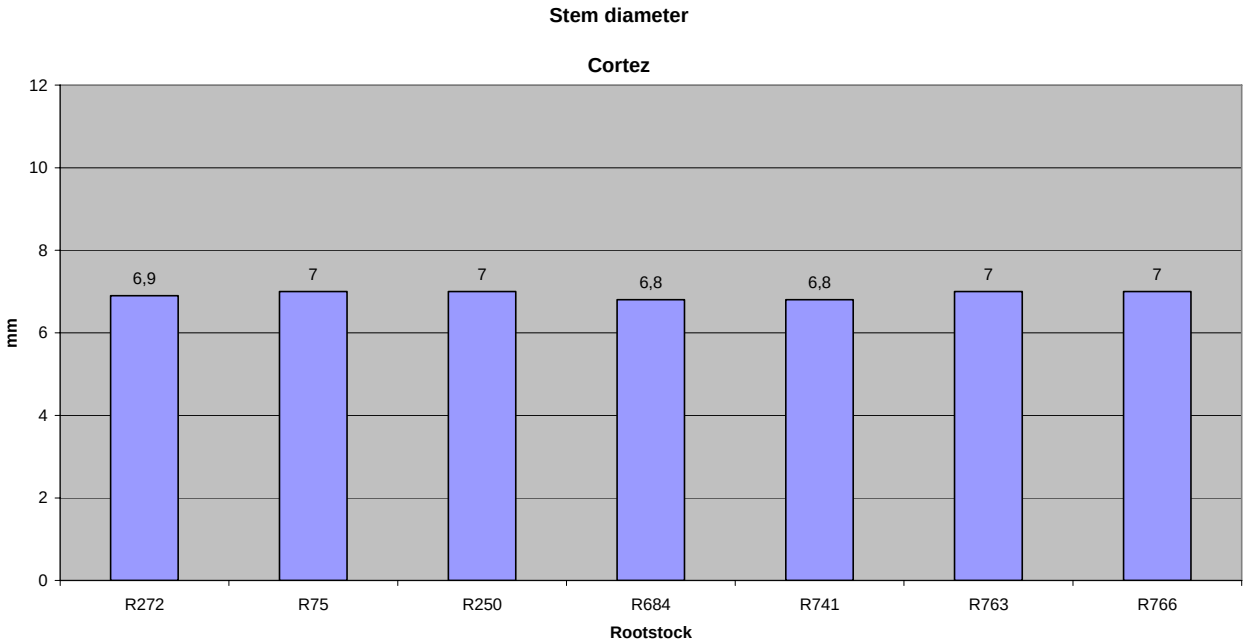


Figure 11: Stem diameter in mm for the variety 'Cortez' grafted on different rootstocks

Christmas Cookie

There were no significant differences in stem diameter between rootstocks for the variety Christmas cookie (figure 12).

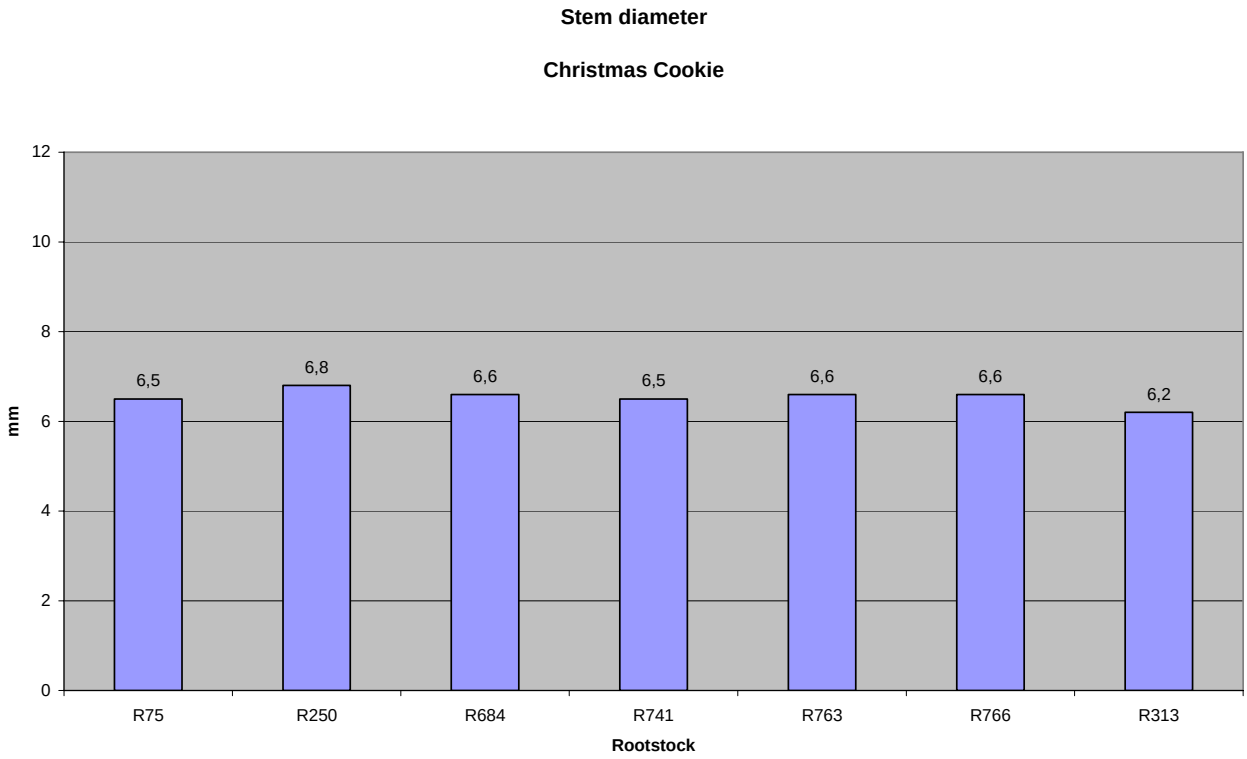


Figure 12: Stem diameter in mm for the variety 'Christmas Cookie' grafted on different rootstocks

6. Leaf blade: intensity of colour of <u>upper</u> side

C-27

No differences in the intensity of colour of upper side between rootstocks could be observed by visual side-by-side observations. The intensity of colour of upper side was medium (note 5)

Cortez

No differences in the intensity of colour of upper side between rootstocks could be observed by visual side-by-side observations. The intensity of colour of upper side was strong to very strong (note 8).

Christmas Cookie

No differences in the intensity of colour of upper side between rootstocks could be observed by visual side-by-side observations. The intensity of colour of upper side was medium to strong (note 6).

7. Leaf blade: intensity of colour of lower side

C-27
No differences in the intensity of colour of lower side between rootstocks could be observed by visual side-by-side observations. The intensity of colour of lower side was weak (note 3)

Cortez
No differences in the intensity of colour of lower side between rootstocks could be observed by visual side-by-side observations. The intensity of colour of lower side was medium to strong (note 6).

Christmas Cookie
No differences in the intensity of colour of lower side between rootstocks could be observed by visual side-by-side observations. The intensity of colour of lower side was weak to medium (note 4).

8. Leaf blade: development of lobes

C-27
No differences in the development of lobes between rootstocks could be observed by visual side-by - side observations. Each plant had leaves with lobes and leaves without lobes. The degree of developed lobes was assessed to be: weak (note 3).

Cortez
No differences in the development of lobes between rootstocks could be observed by visual side-by-side observations. All leaves with the same degree of developed lobes. The degree developed lobes was assessed to be: weak (note 3).

Christmas Cookie
No differences in the development of lobes between rootstocks could be observed by visual side-by-side observations. Each plant had leaves with lobes and leaves without lobes. The degree of developed lobes was assessed to be: weak to medium (note 4).

9. Bract colour of upper side.

The bract colour of upper side was observed two times:

a) first time when the plants had approx. 3 open cyathia

b) second time: 4 weeks after the first observation of the colour.

The colour was determined by RHS colour according to R.H.S. Colour Chart, 2001 published

by the Flower Council of Holland, Leiden. R.H.S.

C-27

No colour differences between rootstocks could be observed by visual side-by-side observations.

The RHS colour was closest to RHS colour 45A (Red) at the first observation and RHS 45B (Red) at the second observation.

Cortez

No colour differences between rootstocks could be observed by visual side-by-side observations.

The RHS colour of true bracts was closest to RHS colour 53B (dark Purple-Red) both at the first and the second observation. The RHS colour of the coloured leaf blades was closest to RHS colour 45A/53B (Red to dark Purple-Red) both at the first and the second observation.

Christmas Cookie

No colour differences between rootstocks could be observed by visual side-by-side observations.
The RHS colour of true bracts was closest to RHS colour 45B (Red) both at the first and the second observation. The RHS colour of the coloured leaf blades was closest to RHS colour 45B (Red) at the first observation and RHS colour 45A/B at the second observation.

10. Bract colour of <u>lower</u> side.

The bract colour of lower side was observed two times:

a) first time when the plants had approx. 3 open cyathia

b) second time: 4 weeks after the first observation of the colour.

The colour was determined by RHS colour according to R.H.S. Colour Chart, 2001 published by the Flower Council of Holland, Leiden. R.H.S.

C-27

No colour differences between rootstocks could be observed by visual side-by-side observations.

The RHS colour of the bracts was closest to RHS colour 47A/B (Red) at the first observation and RHS 47B (Red) at the second observation.

Cortez

No colour differences between rootstocks could be observed by visual side-by-side observations.

The RHS colour of true bracts was closest to RHS colour 53B (dark Purple-Red) at the first observation and RHS colour 53B/C (dark Purple-Red to dark Pink-Red) at the second observation. The RHS colour of the coloured leaf blades was closest to RHS colour 53B/C (dark Purple-Red to dark Pink-Red) both at the first and the second observation.

Christmas Cookie

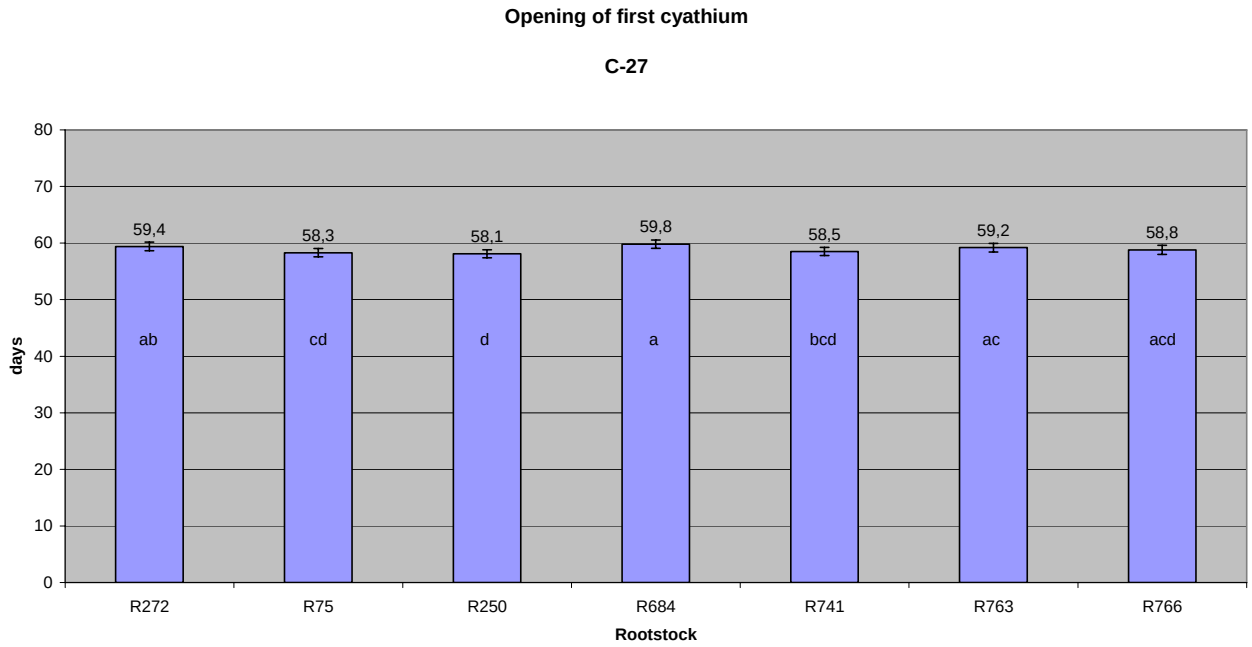
No colour differences between rootstocks could be observed by visual side-by-side observations. The RHS colour of true bracts was closest to RHS colour 47B (Red) at the first and the second observation. The RHS colour of the coloured leaf blades was closest to RHS colour 47B (Red) at the first observation and RHS colour 47A/B (Red) at the second observation.

11. Time of opening of first cyathium

C-27

Day from start of short day until opening of first cyathium for C-27 are shown in figure 13. Only small differences were observed – maximum difference in flowering time was less than 2 days between the rootstocks.

The experimental set up has obviously been very suitable for the detection of differences in flowering time. But statistical significance is not the same as significance in praxis. The maximum difference in flowering time corresponds to less than 1 note.



Figur 13: Days from start of short day until opening of first cyathium for the variety 'C-27' grafted on different rootstocks. . Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

Cortez

Days from start of short day until opening of first cyathium for Cortez are shown in figure 14. Only small differences were observed – maximum difference in flowering time between the rootstocks time was approx. 2 days between the rootstocks.

The experimental set-up has obviously been very suitable for the detection of differences in flowering time. But statistical significance is not the same as significance in praxis. The maximum difference in flowering time corresponds to less than 1 note.

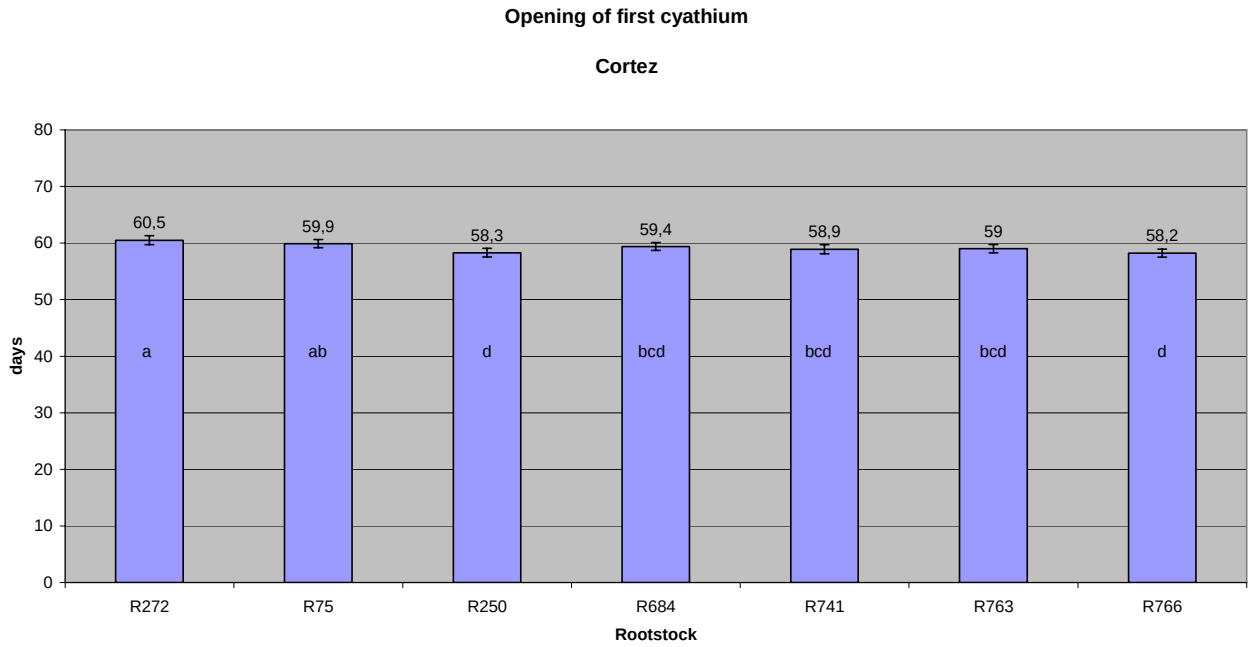
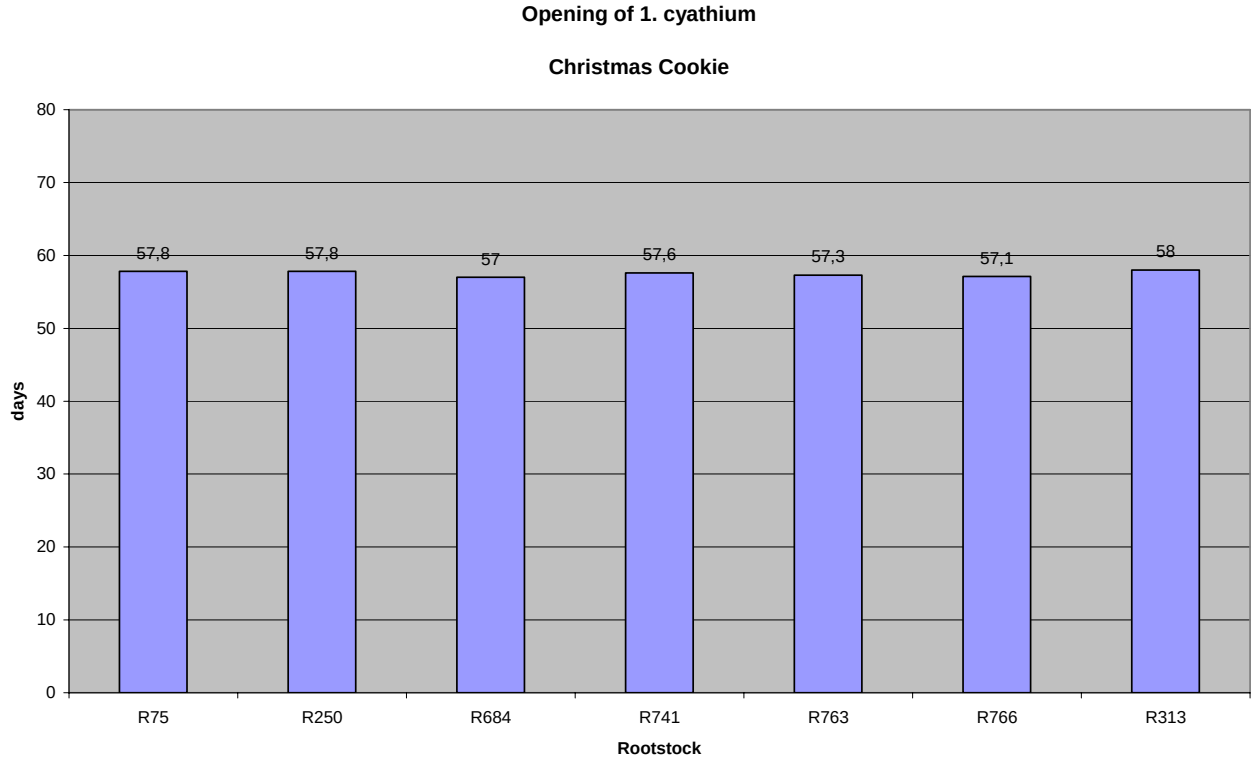


Figure 14: Days from start of short day until opening of first cyathium of ‘Cortez’ grafted on different rootstocks

Christmas Cookie

There were no significant differences in time of opening of first cyathium between rootstocks for the variety Christmas cookie (figure 15).

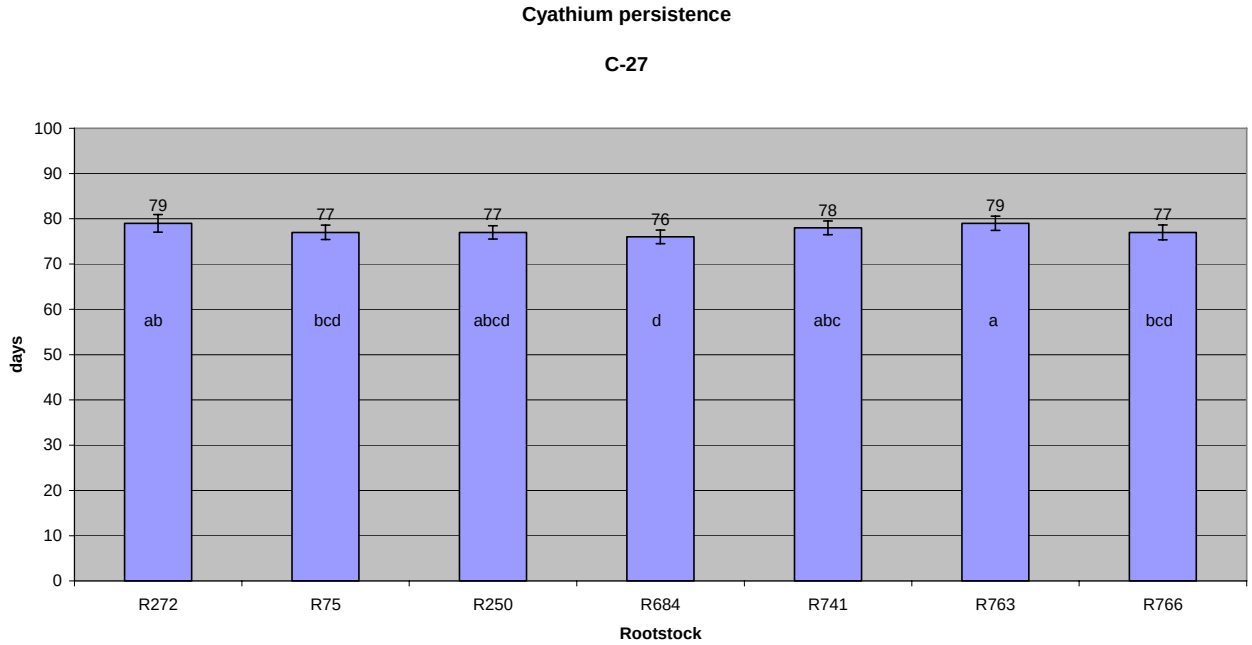


Figur 15: Days from start of short day until opening of first cyathium of ‘Christmas Cookie’ grafted on different rootstocks

12. Cyathium: persistence

C-27

Day from start of short day until drop of first cyathium for C-27 are shown in figure 16. Only small differences were observed – maximum difference were 3 days between the rootstocks.
The experimental set-up has obviously been very suitable for the detection of differences in differences in cyathium persistence. But statistical significance is not the same as significance in praxis. The maximum difference in days correspond to 1 note.
Rootstock 763 had the longest cyathium persistence and it was significant differently from rootstocks 75, 684 and 766. However, rootstock 763 was not significant different from 272 and 250.
Rootstocks 272, 75, 250,741 and 766 were not significantly different.



Figur 16: Days from start of short day until drop of first cyathium for the variety 'C-27' grafted on different rootstocks. Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

Cortez

Days from start of short day until drop of first cyathium for Cortez are shown in figure 17. Only small differences were observed – maximum difference between the rootstocks was 2 days.
The experimental set up has obviously been very suitable for the detection of differences in cyathium persistence. But statistical significance is not the same as significance in praxis. The maximum difference in days corresponds to less than 1 note.

Rootstocks 763 and 272 had the longest cyathium persistence and they are significant different from rootstocks 684 and 766. However, rootstocks 763 and 272 were not significantly different from75 and 741.

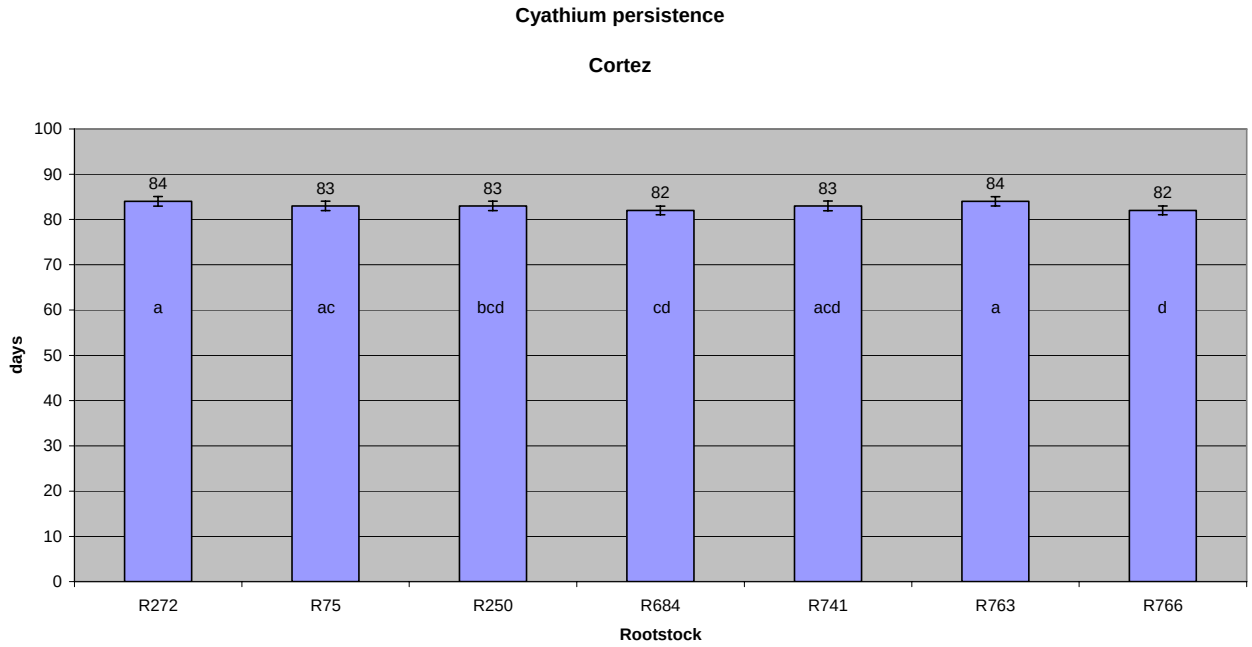
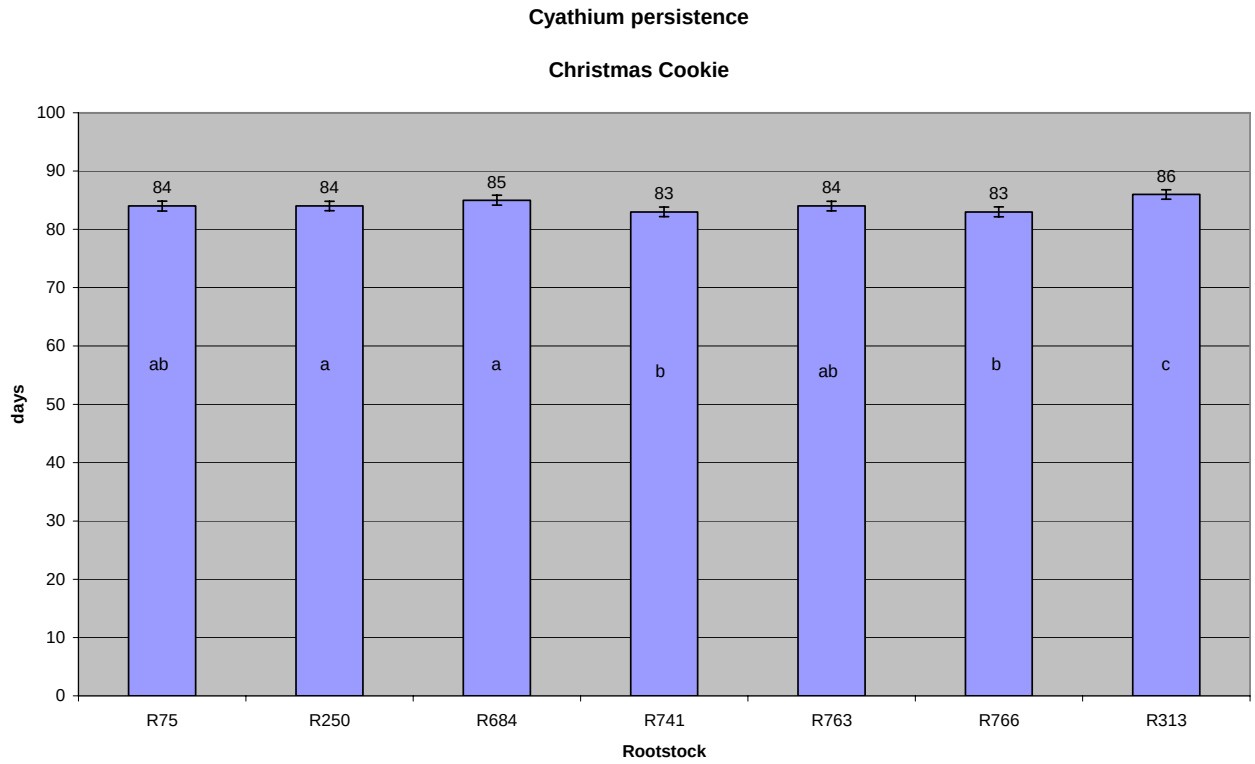


Figure 17: Days from start of short day until drop of first cyathium for the variety 'Cortez' grafted on different rootstocks. Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

Christmas Cookie

Days from start of short day until drop of first cyathium for Christmas Cookie are shown in figure 18. Only small differences were observed – maximum difference between the rootstocks was 3 days.The experimental set up has obviously been very suitable for the detection of differences in cyathium persistence. But statistical significance is not the same as significance in praxis. The maximum difference in days corresponds to 1 note
Rootstock 313 had the longest cyathium persistence and it was significantly different from all other varieties.
Rootstocks 75, 250, 684 and 763 were not significantly different.
Rootstocks 75, 741, 763 and 766 were not significantly different.



Figur 18: Days from start of short day until drop of first cyathium for the variety ‘Christmas Cookie’ grafted on different rootstocks. Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

13. Cyme: width

C-27

The diameters of Cyme for C-27 are shown in figure 19.
Rootstock 684 had the smallest cyme diameter and it was significantly different from all other varieties. The maximum difference in cyme width correspond to 2 note (differences between rootstock 684 and 250/741).
Only small differences were observed between the rest of the varieties. The differences in cyme width correspond to less than 1 note.

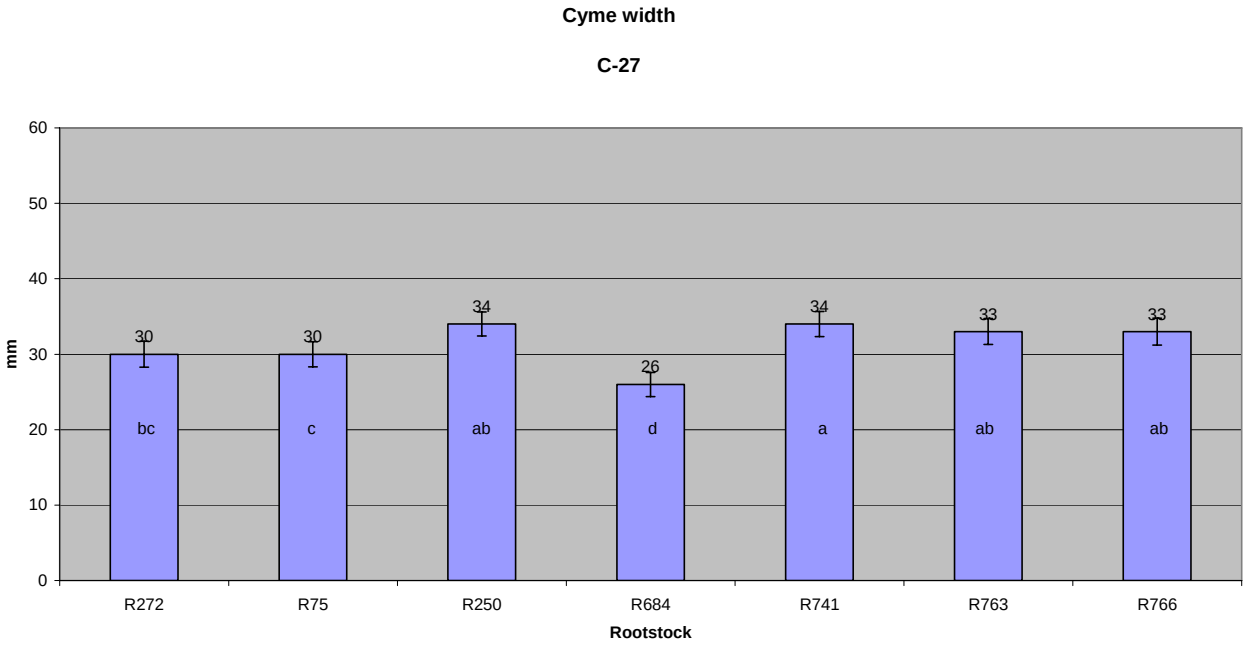


Figure 19: Cyme width in mm for the variety ‘C-27’ grafted on different rootstocks. Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

Cortez

The diameters of Cyme for Cortez are shown in figure 20.

Rootstock 766 had the largest cyme diameter and it was significantly different from all other varieties except rootstocks 763 and 250. The maximum difference in cyme width correspond to 1 note.

However, the differences are in general rather small even though significant differences are obtained in some combinations. The differences in cyme width correspond to less than 1 note.

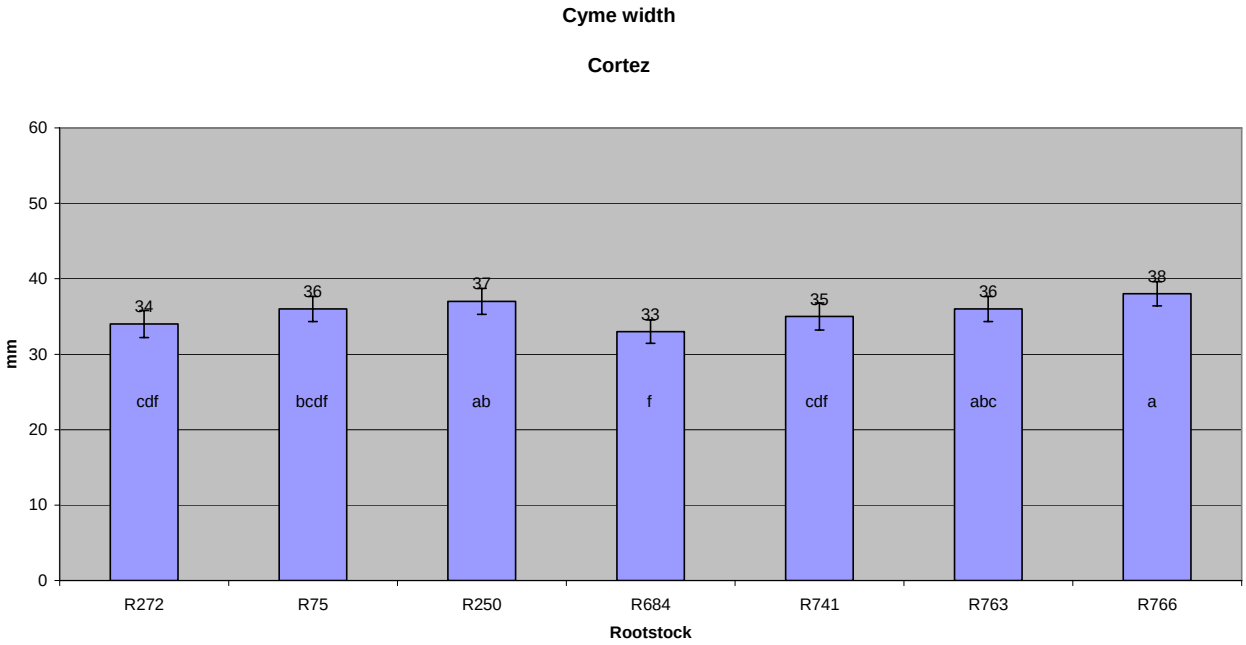


Figure 20: Cyme width in mm for the variety 'Cortez' grafted on different rootstocks. Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

Christmas Cookie

The diameters of Cyme for Christmas Cookie are shown in figure 21.
Rootstock 741 had the largest cyme diameter and it was significantly different from all other varieties except rootstock 766. The maximum differences in cyme width correspond to 1 note.

However, in general the differences were rather small even though significant differences are obtained in some combinations. The differences in cyme width correspond to less than 1 note.

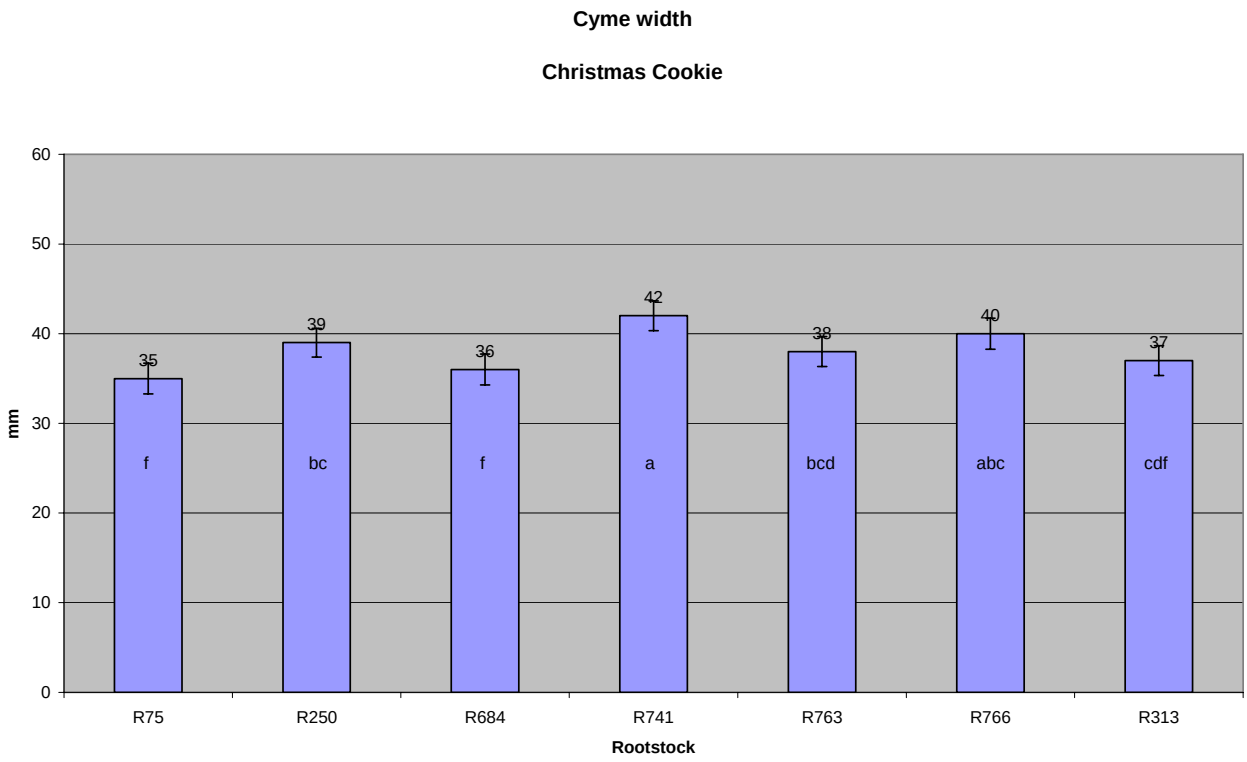


Figure 21: Cyme width in mm for the variety ‘Christmas Cookie’ grafted on different rootstocks. Means with ± 1,96 x standard error are indicated and means with significant differences are indicated with different letter

5. Conclusion

The differences in phenotypes between the different rootstocks (= different populations of phytoplasma) were small for all three varieties (C-27, Cortez and Christmas Cookie).

To separate two varieties in a DUS-test of ornamentals, differences in qualitative characteristics are essential. In this study, no differences in the following qualitative characteristics were observed: Bract colour of upper side, bract colour of lower side, leaf colour of upper side, leaf colour of lower side and development of lobes on leaves.

There were significant differences between rootstocks in the following quantitative characteristics: ‘Plant: number of branches’, stem: diameter of C-27, time of opening of first cyathium for the varieties C-27 and Cortez, ‘Cyathium: persistence’ and ‘cyme width’. However, even though the differences were significant, the differences in ‘number of branches, time of opening of first cyathium and ‘Cyathium: persistence’ were small. Calculated into ‘notes’ the differences were around 1 note or less.

Cyme width of rootstock 684 was significantly smaller than all the other rootstocks in variety ‘C-27’. The cyme width of 684 was also in the lower end for the varieties ‘Cortez’ and ‘Christmas Cookie’ but the differences were smaller.

There were no significant differences in ‘plant height’ and ‘internode length’ between the different rootstocks for any of the varieties.
There were no significant differences in ‘Stem: diameter’ for the varieties Cortez and Christmas Cookie and there were no significant differences in ‘time of opening of first cyathium’ for the variety Christmas Cookie.

6. Litterature

1: International convention for the proection of new plant varieties of plants of December 2, 1961, as Revised at Geneva on November 10, 1972, on October 23, 1978, and March 19, 1991,

2: Nicolaisen,M. (2003). Status quo of the knowledge on phytoplasma with the focus on Euphorbia pulcherrima and other ornamental plants. CPVO report.

3: Nicolaisen,M. (2004). Laboratory test during greenhouse trial. CPVO report.

4. SAS InstituteInc (1989), SAS/STAT User’s guide, Version 6, Fourth Edition, Volume 1, Cary, NC: SAS Institute Inc.;

5:‘Guidelines for the conduct of tests for distinctness, uniformity and Stability – Poinsettia’ (1981), UPOV document TG/24/5.