Vitis **27**, 133—141 (1988)

Viticultural and Oenological Research Institute, Stellenbosch, Republic of South Africa

The ontogeny and development of *Vitis vinifera* L. cv. Chenin blanc inflorescence in relation to phenological stages[1])

by

J. J. SWANEPOEL and E. ARCHER

Die Ontogenie und Entwicklung der Infloreszenzen bei der Rebsorte Chenin blanc (*Vitis vinifera* L.) in Beziehung zu phänologischen Stadien

Zusammenfassung: Die Ontogenie und Entwicklung der Infloreszenzprimordien und die Blütenbildung wurden mit Hilfe der Rasterelektronenmiskroskopie untersucht. Anlage und Differenzierung der ersten und zweiten Primordien in den Knospen der basalen Nodien erfolgten von Mitte Oktober (12—15 d vor der Blüte) bis Ende November (40 d nach der Blüte). Zwischen der Anlage der ersten und zweiten Infloreszenz innerhalb einer Knospe lag eine Zeitspanne von 3 Wochen. Die zweiten Primordien wurden nach der Ausdifferenzierung der ersten Primordien angelegt.

Die einzelnen Blütenteile werden in der Reihenfolge Kelch, Krone, Staubblätter, Stempel angelegt; sie waren innerhalb von 20 d nach Sichtbarwerden der Infloreszenz vollständig entwikkelt.

Key words: inflorescence, flower, differentiation, growth, morphology, phenology.

Introduction

The fertility of grapevine buds is an important factor in determining size, quality and profitability of crops. The development of inflorescence primordia and factors affecting it has been the subject of worldwide study for more than a century (SRINIVASAN and MULLINS, 1981). The ontogeny and development of inflorescences and flowers in various grapevine cultivars comprise three well defined phases, viz. phase 1 : the initiation of anlagen, phase 2 : the formation of inflorescence primordia (differentiation) and phase 3 : the development of flowers (PEROLD 1927; WINKLER and SHEMSETTIN 1937; MAY 1964; CAROLUS 1971; SRINIVASAN and MULLINS 1976, 1981).

Various methods have been employed for determining the time of occurence of these phases. With the aid of optical microscopy, MAY (1964) linked the time of initiation to a phenological stage and reported that the forming of inflorescences in buds in the middle part of the shoot terminates about 3 weeks after flowering. According to RAO and MUKHERJEE (1970), inflorescence formation takes place 66—74 d after pruning in Pusa Seedless.

In a field study, LAVEE *et al.* (1967) found a correlation between the number of leaves and the time of induction of inflorescence. 18—21 leaves, acropetal to the buds examined, were necessary in both Alphonse Lavallée and Sultanina to complete induction. BOCHINOVA-BONEVA (1975) reported the commencement of bud development when 5—7 nodes were present on the shoot, while leaf and internode primordia appeared after the 7th—9th nodes were formed. The critical period for the formation of inflorescence primordia occured with the development of the 14th—18th internodes. According

[1]) Part of Ph.D. thesis to be presented by the senior author to the University of Stellenbosch. Promotor: Prof. P. G. GOUSSARD.

to PRATT (1979), however, the first recognizable inflorescence primordium appeared when the shoot had 13 expanded leaves while 6—7 nodes were present in the primary buds.

The formation of the different floral parts (phase 3) occurs almost simultaneously (BARNARD and THOMAS 1933; WINKLER and SHEMSETTIN 1937; SCHOLEFIELD and WARD 1975; SRINIVASAN and MULLINS 1981) while, according to ALLEWELDT and BALKEMA (1965), ALLEWELDT and ILTER (1969) and AĞAOĞLU (1971), the first calyx primordium appeared in the cluster primordia at the end of summer. Although the formation of flower primordia is retarded in winter, further development thereof was reported by AĞAOĞLU (1971). The differentiation of inflorescence is then completed after budburst in the following spring.

To quantify the effect of different environmental factors on fertility and to optimize the influence of these factors, it is important to know the developmental pathway and time of occurence of different stages of inflorescence formation. The objective of this study was therefore to link the developmental pathway of inflorescences to the phenological stages of the grapevine.

Materials and methods

One shoot from each of five *Vitis vinifera* L. cv. Chenin blanc vines was collected weekly from the beginning of budburst (September) to veraison (January) for two consecutive years. The vines were grafted onto 99 Richter, grown on the Nietvoorbij Experimental Farm, Stellenbosch (33° 54' S; 18° 52' E and 146 m above sea level) and trellised on a three-strand Perold system as described by ZEEMAN (1981). Bud scales, hair and leaf primordia of all the buds on a shoot were removed under a stereomicroscope. The apex and adjacent primordia were excised, fixed in a 6 % glutaraldehyde in 0.1 M sodium cacodylate buffer solution (pH 6.8) and stored in this solution at 3 °C. Flower primordia were sampled in the vineyard and were fixed in the same solution. The specimens were dehydrated in a graded acetone series and critically point dried under a CO_2 atmosphere at 35 °C and a pressure of 8 560 kPa. They were then placed on aluminium stubs, sputter-coated with gold and studied with a scanning electron microscope.

Results and discussion

The ontogeny and development of the reproductive organs were clearly perceptible in the different phases as described by SRINIVASAN and MULLINS (1981).

Abb. 1: Bildung der zugespitzten Blattanlage (LP) mit Blattschuppen (SC) an beiden Seiten.

Abb. 2: Sonderung der Infloreszenzanlage (AL) Mitte Oktober. Man beachte ihre stumpfe Beschaffenheit.

Abb. 3: Beginnende Differenzierung der Infloreszenanlage (AL) 14 d nach ihrem ersten Auftreten.

Abb. 4: Abgeschlossene Differenzierung der ersten Infloreszenz (I) in der basalen Knospe und Bildung einer zweiten Anlage (11. November).

Abb. 5: Erstes Auftreten der zweiten Infloreszenzanlage (AL) in Knospe 8 am selben Tag wie in Abb. 4.

Abb. 6: Zwei ausdifferenzierte Infloreszenzanlagen (I) am 50. d nach ihrem ersten Auftreten (7. Dezember).

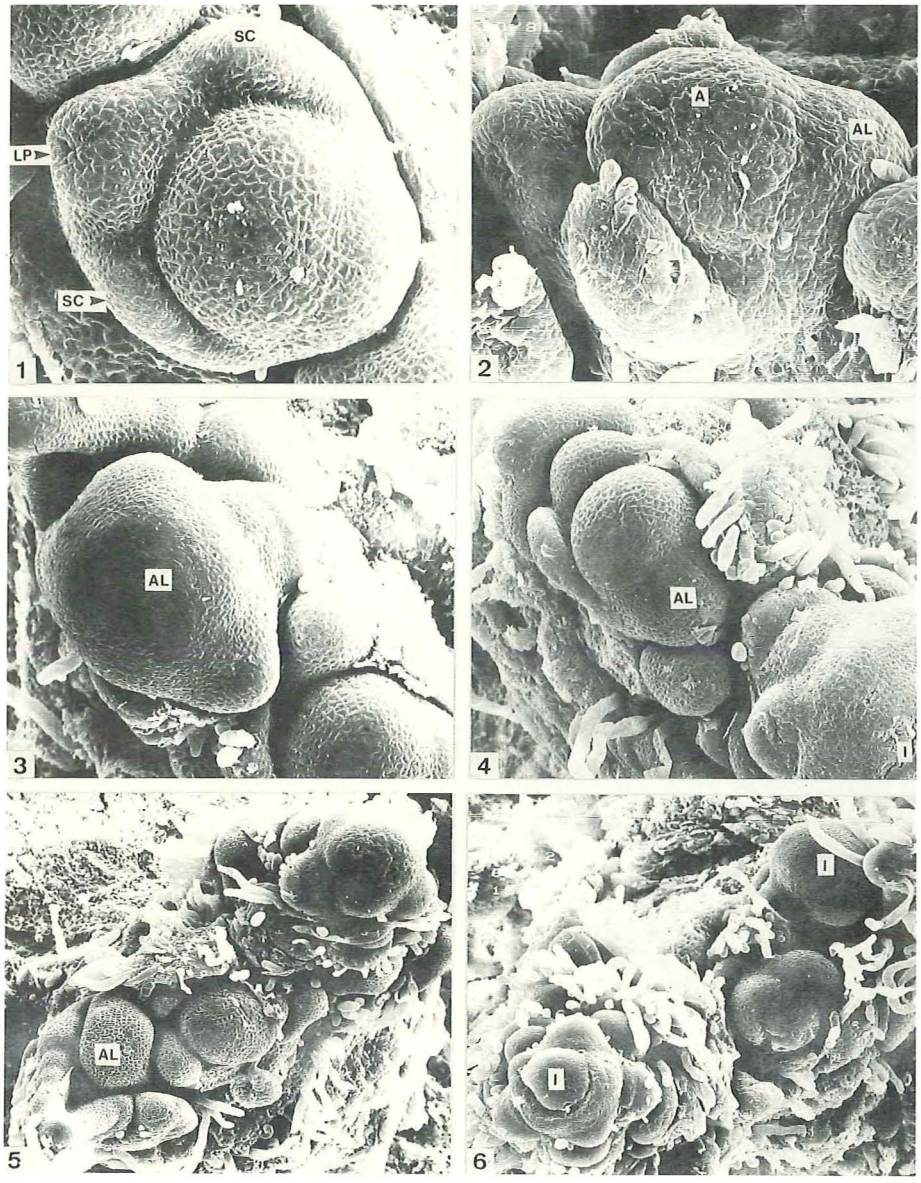

Fig. 1: Formation of leaf primordium (LP) which is sharp-pointed with stipular-scales (SC) on both sides.

Fig. 2: Separation of anlage (AL) from the apex (A) in mid October. Note the blunt appearance of the anlage.

Fig. 3: Commencement of anlage (AL) differentiation 14 d after the initiation.

Fig. 4: Differentiation of the first inflorescence (I) in the basal bud completed while a second anlage (AL) is being formed (11 November).

Fig. 5: Initiation of the second anlage (AL) in bud position 8 on the same date as in Fig. 4.

Fig. 6: Two differentiated inflorescence primordia (I) on the 50th d after initiation (7 December).

Time of initiation and differentiation

Leaf primordia were distinguishable as sharp-pointed structures with stipular scales (Fig. 1). Anlagen, however, were observed as blunt, broad, obovate structures lacking stipular scales (Fig. 2). The separation of anlagen from the apex, which is the earliest indication of the commencement of reproductive growth in the grapevine, was clearly perceptible.

Commencement of anlagen initiation in basal buds (Fig. 2) was observed 12—15 d before anthesis (start of bloom, mid October). After 7 d initiation was completed in all the buds investigated at nodes 1 and 2, while it was taking place at nodes 4 and 5. At this stage, 12 expanded leaves were present on the shoot while the vines were starting to bloom.

Anlagen which separate from the apex are, however, not necessarily potential inflorescences. Depending on certain conditions, they may become inflorescence or tendril primordia. According to SRINIVASAN and MULLINS (1976), the anlage forms a bract primordium and inner and outer arms. If conditions are not inducive to inflorescence formation these two arms cease to divide and usually give rise to tendrils after budburst in the following season. Divisions of the inner and outer arms of the anlagen are thus indicative of the differentiation of inflorescence. Multiple divisions were observed for the first time in some buds on the 4 basal nodes 14 d after the initiation of the anlagen (full bloom, beginning November) (Fig. 3). The appearance of this fully developed inflorescence primordium after a further 7 d is rather like a bunch of grapes in which each berry-like branch primordium is a protuberance of undifferentiated meristematic tissue. From this meristematic tissue the flowers will be differentiated in the following season. At this stage, anlagen formation in buds 7 and 8 was taking place.

25 d from the onset of initiation (end of bloom), the differentiation of anlagen in the 4 basal buds was completed while a second anlage was being initiated (Fig. 4). Thus, differentiation of the first anlagen occurs a few days before initiation of the second anlagen. This initiation was observed in more than one bud position simultaneously (Fig. 5). During this period, initiation of the first anlagen was observed at node 14. At this stage, 16 expanded leaves were present on the shoot.

The differentiation of the second anlagen commenced approximately 21 d after the first anlagen had started differentiating. If conditions were inducive for the differentiation of the second anlagen, both inflorescence primordia (Fig. 6) were perceptible on the 15th d after initiation of the first anlagen in the basal buds.

The above mentioned evidence points to an initiation and differentiation of the first anlagen in an acropetal succession rather than the simultaneous initiation in a number of buds. The time period of approximately 14 d for the initiation of the first anlagen in buds 7 and 8 and 25 d for bud 14 points to a time lapse of approximately 2 d between initiation of anlagen in consecutive buds. The initiation of the second anlagen in more than one bud position could explain the findings of SKIPINA (1976) who reported the starting of bud initiation on the middle part of the shoot expanding firstly in a proximal and then in a distal direction.

A similar initiation pattern emerged in the following year, the only exception being the onset of initiation about a week earlier due to an earlier season. The time between different developmental stages, however, coincided with that found in the previous year.

Formation of flowers

The development of phase 3 occurs within 10—15 d of appearance of the inflorescence. On each branch primordium 5—6 flower primordia are formed, which corre-

sponds with the finding of PRATT (1971) who described the bunch as a trichasium. Contrary to results reported by AĞAOĞLU (1971), no calyx formation was found in the inflorescence before the bud entered winter dormancy (Fig. 7). The bracts subtending the flower primordium (Fig. 8) could have led to this conclusion in light microscope studies. Flower primordia were, however, in different stages of development on the

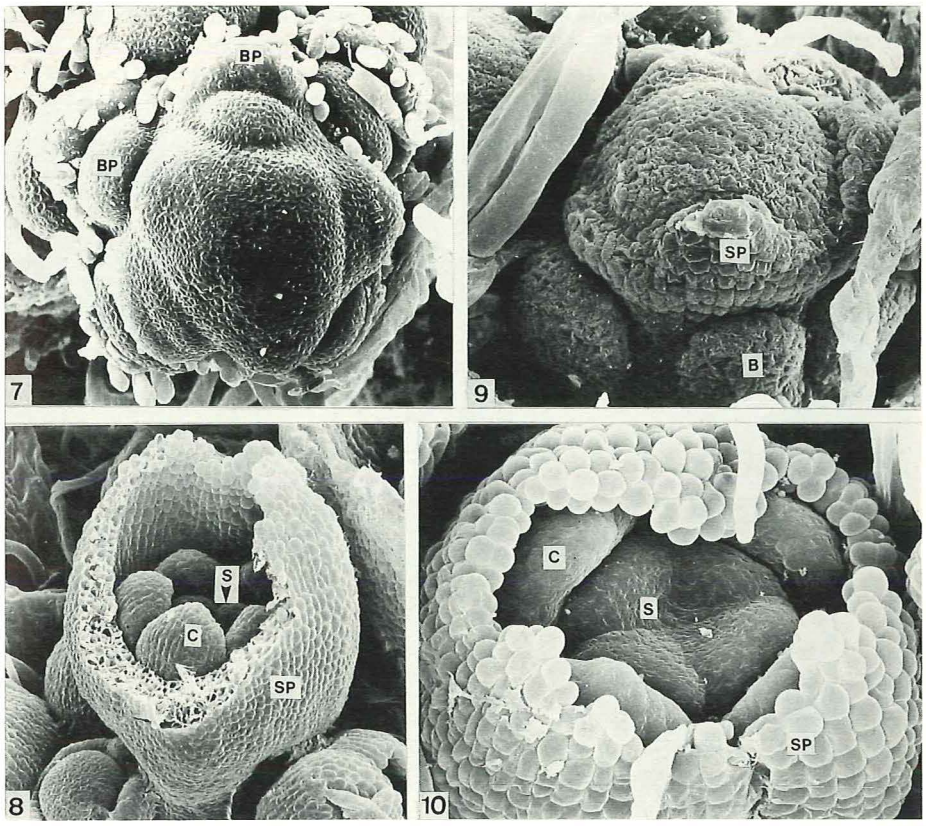

Fig. 7: Inflorescence primordium before the bud enters winter dormancy. No calyx primordium is observed on the branch primordium (BP).

Fig. 8: Different stages of flower primordia development on the same branch primordium. C = Corolla, S = stamens, SP = sepals.

Fig. 9: Continuous ring of sepals (SP) formed before appearance of inflorescence on the shoot. B = Bract.

Fig. 10: Incomplete cap of calyx (SP) formed over the initiating corolla (C) and stamens (S).

Abb. 7: Infloreszenzprimordien vor Eintritt der Knospe in die Winterruhe. An der Anlage des Infloreszenzastes (BP) ist keine Anlage des Kelches festzustellen.

Abb. 8: Verschiedene Entwicklungsstadien der Blüte an derselben Astanlage. C = Blütenkrone, S = Staubblätter, SP = Kelchblätter.

Abb. 9: Geschlossener Ring der Kelchblätter (SP) vor dem Erscheinen des Blütenstandes am Trieb. B = Deckblatt.

Abb. 10: Unvollständige Kappe der Kelchblätter (SP) über der sich entwickelnden Blütenkrone (C) und den Staubblättern (S).

same branch primordium, e.g. the corolla (petals) had already been formed and the stamens initiated while in the adjacent flower primordium no sign of the corolla primordium was present (Fig. 8). It is thus evident that the flowers on the inflorescence do not reach the same stage of development simultaneously.

Sepal (calyx) initiation commenced prior to the appearance of the inflorescence primordium on the shoot after budburst. A continuous ring of tissue (Fig. 9) was formed which covered the whole flower primordium. Consequently it formed an incomplete cap over the initiating corolla and stamens (Fig. 10).

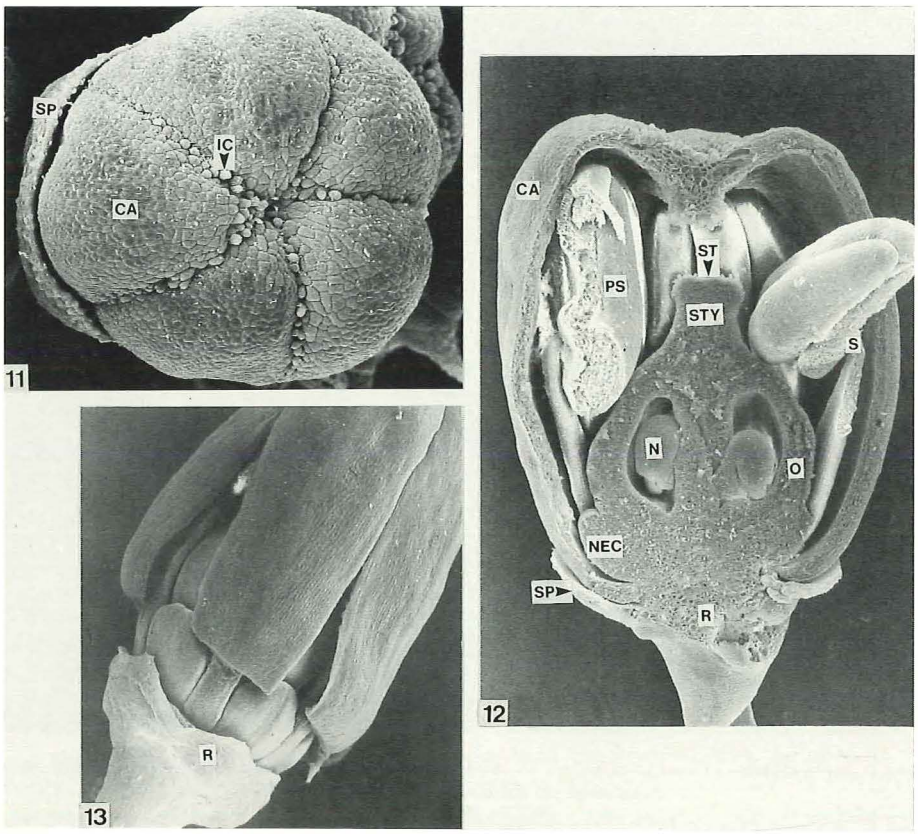

Fig. 11: Calyptra (Ca) pushing through the sepals (SP). IC = Interlocking cells.

Fig. 12: Fully developed flower prior to cap fall. — CA = Calyptra, N = nucellus, NEC = nectary, O = ovary, PS = pollen sack, R = receptacle, S = stamen, SP = calyx, ST = stigma, STY = style.

Fig. 13: The loosening of the calyptra from the receptacle (R) marks the onset of flowering.

Abb. 11: Die Calyptra (Ca) drängt sich zwischen den Kelchblättern (SP) hindurch. IC = Verbindungszellen.

Abb. 12: Voll entwickelte Blüte vor dem Abwerfen des Käppchens. — CA = Calyptra, N = Nucellus, NEC = Nectarium, O = Fruchtknoten, PS = Pollensack, R = Blütenboden, S = Staubblatt, SP = Kelch, ST = Narbe, STY = Griffel.

Abb. 13: Die Ablösung der Calyptra vom Blütenboden (R) kennzeichnet den Blühbeginn.

The petals (corolla) which developed just after the calyx, became lobed and started growing together. Almost simultaneously the stamens were initiated and started to elongate. As the five petals elongated, cells were formed at the margins which interlocked with similar cells on the margins of adjacent petals. This coalescence of the petals caused the formation of a calyptra which elongated and pushed through the sepals (Fig. 11). The calyptra served as protecting tissue for the developing stamens and pistil. Approximately 20 d after the appearance of the inflorescences the floral parts, as described by amongst others PEROLD (1927), AĞAOĞLU (1971) and WINKLER *et al.* (1974), were fully developed and clearly perceptible (Fig. 12).

During cap fall, the calyptra loosened from the base, the petals curled upwards (Fig. 13) and when the calyptra fell off the stamens were exposed. According to PRATT (1971), cap fall can be ascribed to temperature-induced changes in the turgor of the interlocking marginal cells.

Conclusions

The ontogeny and developmental stages of inflorescence primordia were easily detected with the aid of scanning electron microscopy. It was therefore possible to link the morphological stages of inflorescence formation with certain phenological stages of the vine (Fig. 14).

Depending on the season, initiation of the first anlagen at the basal bud occured from 15 d before bloom (mid to end of October) while 12 expanded leaves were present on the shoot. During full bloom (14—21 d after initiation), differentiation of these anlagen could be observed, while the second anlagen, providing favourable conditions exist, were initiated just after this differentiation (21—25 d from initiation of the first anlagen). These anlagen differentiate 25 d after the first.

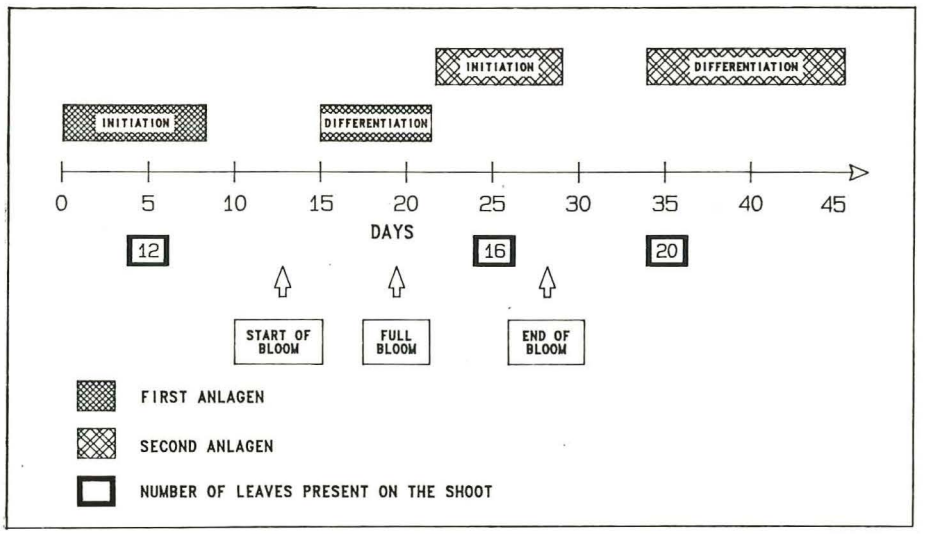

Fig. 14: A diagrammatic representation of inflorescence formation in *Vitis vinifera* L. cv. Chenin blanc.

Diagramm der Infloreszenzentwicklung von *Vitis vinifera* L. cv. Chenin blanc.

When the bud entered winter dormancy no flower primordia were evident. The formation of floral parts occured almost simultaneously and was in the order calyx, corolla, stamens and pistil. The differentiation of these parts was completed within 10—15 d after the appearance of the first inflorescence on the shoot, while after 20 d they were fully developed.

From this study it was possible to observe when and how inflorescence and floral development in the grapevine took place. However, it was not possible to identify the exact time of induction. This must be verified in future studies by examining how and when different factors exert their influence on fertility.

Summary

The ontogeny and development of inflorescence primordia and flower formation were studied with the aid of scanning electron microscopy. Initiation and differentiation of the first and second inflorescence primordia in each bud at the two basal nodes occured in the period middle October (12—15 d before bloom) to end November (25 d after bloom). A time lapse of 3 weeks between initiation of the first and second anlagen in a bud was observed. The second anlagen were initiated just after the differentiation of the first.

Floral parts were formed in the order calyx, corolla, stamens and pistil and within 20 d after appearance of the inflorescence these parts were fully developed.

Acknowledgements

The assistance of the personnel of the SEM unit, FFTRI, Stellenbosch, is gratefully acknowledged.

Literature cited

AĞAOĞLU, Y.A.; 1971: A study on the differentiation and the development of floral parts in grapes (*Vitis vinifera* L.). Vitis **10**, 20—26.

ALLEWELDT, G.; BALKEMA, G. H.; 1965: Über die Anlage von Infloreszenz- und Blütenprimordien in den Winterknospen der Rebe. Z. Acker- Pflanzenbau **123**, 59—74.

— — ; ILTER, E.; 1969: Untersuchungen über die Beziehungen zwischen Blütenbildung und Triebwachstum bei Reben. Vitis **8**, 286—313.

BARNARD, C.; THOMAS, J. E.; 1933: Fruit bud studies. II. The Sultana: Differentiation and development of the fruit buds. J. Counc. Sci. Ind. Res. Austral. **6**, 285—294.

BOCHINOVA-BONEVA, I.; 1975: Studies on the morphophysiology of the grapevine. II. Morphogenesis of the winter buds along the main shoot [Bulg.]. Gradinar. Lozar. Nauka **12**, 110—118. [Abstr.: Hort. Abstr. **47**, 1338.]

CAROLUS, M.; 1971: Description des stades du développement des primordia inflorescentiels durant l'organogenèse des bourgeons latents de la vigne (*Vitis vinifera* L. var. Merlot). Connaiss. Vigne Vin **5**, 163—173.

LAVEE, S.; REGEV, U.; SAMISH, R. M.; 1967: The determination of induction and differentiation in grape vines. Vitis **6**, 1—13.

MAY, P.; 1964: Über die Knospen- und Infloreszenzentwicklung der Rebe. Wein-Wiss. **19**, 457—485.

PEROLD, A. I.; 1927: A Treatise on Viticulture. MacMillan, New York.

PRATT, CHARLOTTE; 1971: Reproductive anatomy of cultivated grapes — a review. Amer. J. Enol. Viticult. **22**, 92—109.

— — ; 1979: Shoot and bud development during the prebloom period of *Vitis*. Vitis **18**, 1—5.

RAO, V. N. M.; MUKHERJEE, S. K.; 1970: Studies on pruning of grape. III. Fruit bud formation in Pusa Seedless grapes (*Vitis vinifera* L.) under Delhi conditions. Vitis **9**, 52—59.

SCHOLEFIELD, P. B.; WARD, R. C.; 1975: Scanning electron microscopy of the developmental stages of the Sultana inflorescence. Vitis **14**, 14—19.

SKIPINA, K. P.; 1976: Characteristics of bud morphogenesis in the grapevine cultivar Karaburnu in the sub-tropical zone [Russ.]. Nauch. Trudy NII Gorn. Sadovod. Tsvetovod. **23**, 89—93. [Abstr.: Hort. Abstr. **48**, 3327.]

SRINIVASAN, C.; MULLINS, M. G.; 1976: Reproductive anatomy of the grapevine (*Vitis vinifera* L.) : Origin and development of the anlagen and its derivatives. Ann. Bot. (London) **38**, 1079—1084.

— — ; — —; 1981: Physiology of flowering in the grapevine — a review. Amer. J. Enol. Viticult. **32**, 47—63.

WINKLER, A. J.; COOK, J. A.; KLIEWER, W. M.; LIDER, L. A.; 1974: General Viticulture. University of California Press, Berkeley.

— — ; SHEMSETTIN, E. M.; 1937: Fruit bud and flower formation in the Sultanina grape. Hilgardia **10**, 589—600.

ZEEMAN, A.; 1981: Oplei. In: BURGER, J. D.; DEIST, J. (Eds.): Wingerdbou in Suid-Afrika, 185—201. VORI, Stellenbosch.

Eingegangen am 3. 12. 1987

J. J. SWANEPOEL
V.O.R.I.
Private Bag X5026
Stellenbosch
7600
Republic of South Africa