

Regulation of Flowering in the Grapevine (*Vitis vinifera* L.)

M.G. MULLINS¹

Introduction

Detailed information on the control of flowering is available for numerous annual and biennial plants, including species of little or no economic importance, but such information is scarce for many of the world's major crop plants. Fruit trees, grapevines, and most other broad-leaved woody perennials are among the plants which have received relatively little attention in this respect.

There are several reasons for this paradox. First, trees and vines are inconvenient experimental material. They are large and difficult to manage in glasshouses or growth rooms. Seedlings of most woody perennials have a protracted juvenile or nonflowering phase and adult, sexually mature individuals produce flowers only once a year. With normal techniques the turnover of experiments is slow, and this has severely limited the acquisition of knowledge on the physiology of flowering in trees and vines.

Second, the control of flowering was first studied in plants which are highly responsive to changes in light or temperature. Subsequently, much research on the mechanisms by which apices are transformed from the vegetative to the reproductive mode of development has been with plants specially selected for sensitivity to photoperiod or to vernalization, such as *Xanthium*, *Pharbitis*, *Lolium*, *Hyoscyamus*, *Nicotiana*, *Kalanchoë*, and *Bryophyllum*. These are relatively small, fast-growing, and easily grown herbaceous plants.

Third, there is the tacit assumption that the information which emerges from research on "plants of convenience" has wide applicability. Textbooks in plant physiology, for example, seldom refer to the fact that flowering in many species, including economically important plants, is regulated by mechanisms which are either unknown or which seem to lack a close relationship with the familiar light- or temperature-controlled systems.

This paper describes the control of flowering in the grapevine, the most widely grown of fruit plants (10.3×10^6 ha in 1976). Special attention is given to effects of cytokinins, but the discussion will not be limited to a single class of growth substance because flowering in the grapevine is a sequence of events widely separated in time and now known to involve different growth substances.

Mention will be made of the structural peculiarities of grapevines and of the origin of inflorescences. It will be shown that whilst the route to flowering in *Vitis* is signifi-

¹ Department of Agronomy and Horticultural Science, University of Sydney, N.S.W. 2006, Australia

cantly different from that of the more commonly studied herbaceous species there are, nevertheless, some parallels in the roles of growth substances in the regulation of flowering.

Origin of Inflorescences

The complex morphology of the grapevine shoot system and the origin of inflorescences (2, 5, 6, 20, 21) have been clarified recently by scanning electron microscopy (28). In brief, flowering in the mature grapevine is normally a three-step process. The first step is the formation of *Anlagen* or "uncommitted primordia" by the apices of specialized lateral buds (latent buds) on shoots of the current season. Next, the *Anlagen* develop either as inflorescence primordia or as tendril primordia, and shortly thereafter the latent buds enter into dormancy. In some circumstances *Anlagen* may produce shoot primordia instead of inflorescences or tendrils. Finally, the formation of flowers from the inflorescence primordia occurs at the time of bud burst in the next season.

Formation of Anlagen and Tendrils

The apex of the latent bud produces up to five leaf primordia (Fig. 1a) and then divides into two almost equal parts (Fig. 1b). The part opposite the youngest leaf primordium is the *Anlage* (28). The first step in the differentiation of the *Anlage* is the formation of a bract (Fig. 2). Next, the apex subtended by the bract divides to form two branches or arms. This is a critical stage in the reproductive development of the



Fig. 1a. Structure of the latent bud before Anlagen formation. The apex (A) has produced four leaf primordia (LP 1-4). X 100

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grapevine because two-branched Anlagen have the potential to produce inflorescence primordia, tendril primordia, or shoot primordia (Fig. 2).

The control of shoot formation from Anlagen (28, 31) will not be considered further here. In the case of flowering, however, it is evident that control can be exercised at two levels. The first is a coarse control and involves the formation of Anlagen. The second is a finer level of control and concerns the switching of the two-branched Anlage into either the inflorescence or the tendril pathway.



Fig. 1b. Anlagen (AN) formation. X 200

Gibberellin is involved in both Anlagen formation and in the determination of Anlagen development (31). When container-grown vines are treated with gibberellic acid (GA_3 , 8–30 μM) there is premature sprouting and elongation of latent buds and precocious formation of Anlagen, i.e., the first Anlagen are formed at the 2nd or 3rd nodes from the base of the stem instead of at the 4th or 5th nodes as is normal. Anlagen formed in GA -treated plants grow only into tendrils and formation of inflorescence primordia is inhibited. Anlagen formation and tendril elongation are suppressed by chlormequat (2-chlorethyltrimethyl ammonium chloride), an inhibitor of GA synthesis (13).

Differentiation of Inflorescences

Effects of Exogenous Cytokinins

A characteristic of *Vitis* is that tendrils and inflorescences are homologous organs, both arising from the same Anlage. Tendrils are generally regarded as vegetative appendages which provide support for climbing plants, but grapevine tendrils can be regarded as potential reproductive organs.

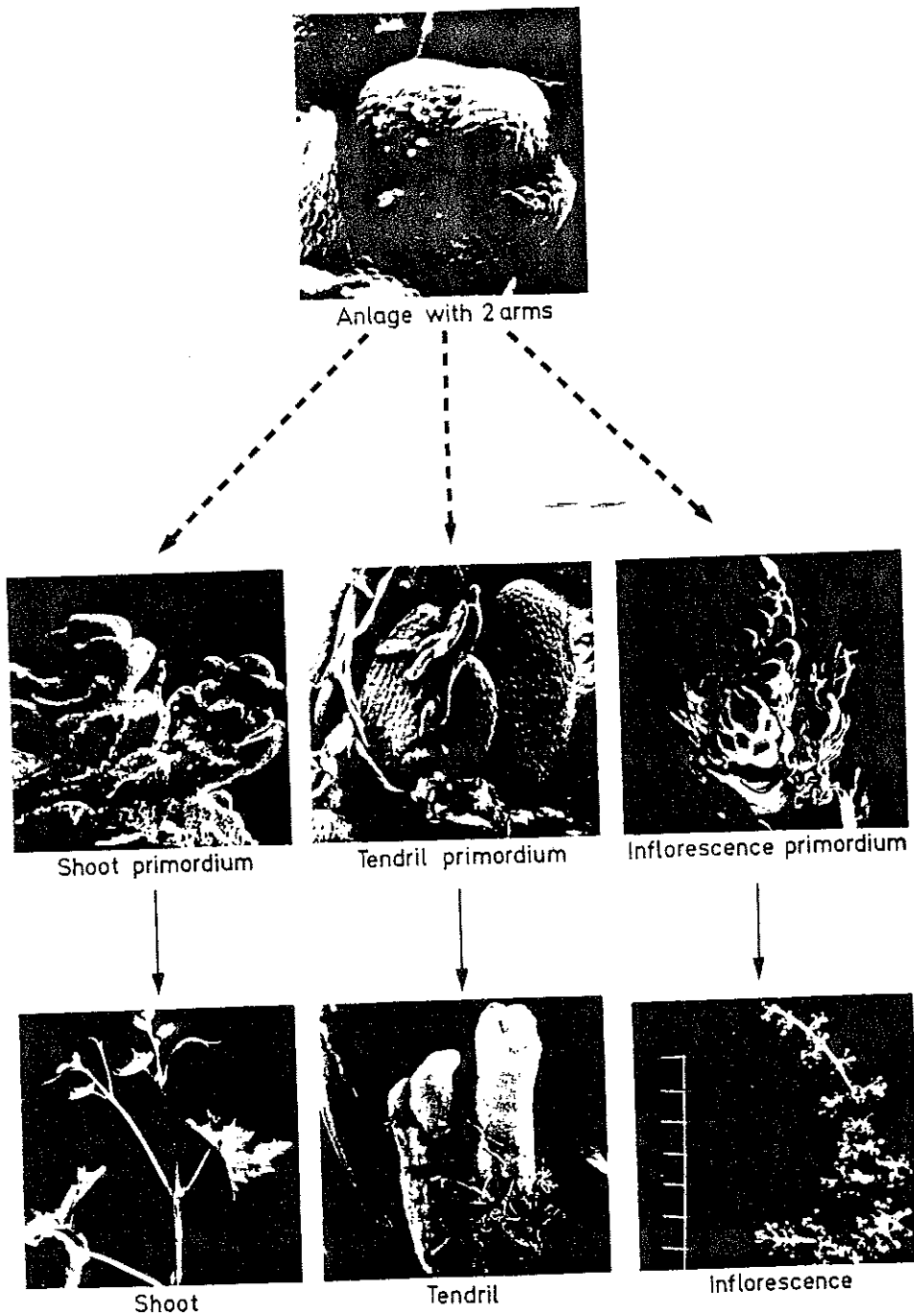


Fig. 2. Pathways of Anlagen development. Anlage with 2 arms, $\times 125$; shoot primordium, $\times 35$; tendril primordium, $\times 75$; inflorescence primordium, $\times 20$; shoot, $\times 0.15$; tendril, $\times 10$; inflorescence, $\times 0.25$

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Anlagen which undergo repeated branching give rise to inflorescence primordia (2) but those which produce only two or three branches give rise to tendrils (28). It follows that the control of inflorescence formation in grapes hinges upon the control of branching of Anlagen (or of tendrils). This process has been studied by growing isolated apices and tendrils in aseptic culture with various growth substances (29) and by application of growth substances to the apices and tendrils of container-grown plants (30, 31).

Isolated tendrils were induced to branch profusely, and they grew into inflorescences or inflorescence-like structures when cultured in vitro with the cytokinins benzyladenine (BA), 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H purine (PBA), or zeatin riboside. There was normal development in vitro of calyx, corolla (calyptra) stamens and pistils, but micro- and megasporogenesis were absent. With normally grown plants inflorescences and fruits with viable seeds were produced in place of tendrils when shoot apices of a *Vitis vinifera* cultivar (Muscat of Alexandria) and of a hybrid grape (*V. vinifera* × *V. rupestris*), were given repeated applications of PBA (50–200 μ M).

The ability of cytokinin to transform tendrils into inflorescences is not restricted to cultivars of the grapevine but is applicable also to grapevine seedlings. By repeated application of cytokinin (BA or PBA) to the first-formed tendrils of grape seedlings, flower initials have been induced to form within four weeks of germination. These results, which are still preliminary, raise the prospect of greatly reducing the generation time in grapevine breeding.

To summarize, inflorescence formation in the grapevine appears to involve a weakening of apical dominance in Anlagen or in young tendrils. The effects of cytokinins on branch formation which are observed in vitro and in vivo are similar in character to responses to cytokinins in other correlative phenomena (19).

Environmental Factors and Inflorescence Formation

The effects of environmental factors on inflorescence formation in the grapevine have been studied in the field and in plants grown in controlled conditions (4). There are several reports that the mode of development of Anlagen is regulated by temperature conditions during the differentiation of latent buds. In the cultivar Muscat of Alexandria (syn. Muscat Gordo Blanco), for example, high temperatures (30°C) favor the formation of inflorescence primordia by Anlagen but low temperatures favor the formation of tendril primordia (5). The mechanism by which temperature affects inflorescence formation in *Vitis* is unknown, but knowledge of the effects of exogenous growth substances indicates that temperature may exert its effect on flowering by modifying the levels of endogenous cytokinin(s). This view is supported by evidence of increasing cytokinin production with increasing temperature (1) and by qualitative differences in cytokinins in the xylem sap of Sultana vines which were grown at either 20°C or 30°C (25).

Differentiation of Flowers

When dormant latent buds are activated in spring the inflorescence primordia which were formed in the previous summer undergo a period of rapid development. Each branch of the inflorescence primordium divides many times to give groups of flower primordia. Depending on the cultivar the flower primordia are formed in groups of 3 or 5 (24, 28).

Flower formation from the inflorescence primordium is a cytokinin-controlled process. There is high cytokinin activity in the xylem (bleeding) sap of the grapevine during bud burst and flowering (18, 26) and there is evidence that the cytokinin produced by roots is involved in the regulation of flower development. Inflorescence primordia in hardwood cuttings of the grapevine atrophy if bud burst precedes the emergence of adventitious roots, but normal inflorescences are formed when cuttings are propagated by a technique which ensures root formation in advance of bud burst (14). Rootless cuttings require exogenous cytokinin (BA or PBA) for normal differentiation of inflorescences (15, 16).

Other effects of exogenous cytokinins in *Vitis* include the induction or promotion of pistil development in male vines (17) and the promotion of fruit set in grape cultivars (35). An effect of cytokinin and of roots in promoting femaleness in dioecious species has been reported recently by Chailakhyan and Khryanin [(10); see also the following paper in this volume].

The Control of Flowering

The concept of a single trigger for flowering is inappropriate in the grapevine because inflorescence formation is regulated at two levels — that of formation and that of differentiation of Anlagen. The theory that the floral stimulus involves two complementary stimuli, as suggested by Carr (7), Chailakhyan (8, 9), and Evans (11) is a more acceptable working hypothesis.

According to Thimann (32), flowering is simply a developmental process under the control of the interplay of hormones. Zeevaart (36) made a distinction between annual and perennial plants by proposing that the requirement for a specific balance of hormones for flower formation is readily applicable to woody perennials but less attractive as a hypothesis for control of flower formation in herbaceous plants. Evidence is accumulating that in the grapevine gibberellin and cytokinin are the principal regulators of flowering.

Chailakhyan (9) has suggested that gibberellins are involved in the formation and growth of floral stems or inflorescence axes. The responses of grapes to exogenous GA and chlormequat are consistent with this view. Gibberellin is necessary for the formation of inflorescence axes (initiation of Anlagen) and for the growth of inflorescence axes (two-branched stage of the Anlagen).

The observation that gibberellins are inhibitors of flowering in many fruit species (12) requires qualification in the case of *Vitis*. The role of GA in flowering in grapevines varies with the stage of development of the latent bud. At an early stage GA is a

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promoter of flowering because Anlagen formation is a GA-requiring process. Later, GA acts as an inhibitor of flowering because it directs the Anlagen to form tendrils.

Cytokinins are implicated in the control of many aspects of reproduction in the grapevine (15–17, 28–31), including the branching of Anlagen to form inflorescence primordia and the differentiation of flowers. The mechanism by which cytokinins exert these effects is unknown but may be associated with the partitioning of assimilates. It has been demonstrated in many plants, including *Vitis vinifera*, that cytokinins are strong mobilizers of assimilates to the site of application. In another perennial, *Bougainvillea*, Tse et al. (33) showed that PBA-induced accumulation of ^{14}C -assimilates was followed by inflorescence formation, and several other authors have suggested that redistribution of metabolites is involved in the regulation of flowering (22, 23).

The effects of exogenous gibberellin and cytokinin on flowering in the grapevine are unequivocal and indicate that flowering is regulated by a gibberellin: cytokinin interaction. The origin and action of endogenous gibberellin and cytokinin in flowering in the intact plant is less clear. The xylem sap of the grapevine contains gibberellin (25) and has a high cytokinin activity during bud burst (18, 26). There is a specific effect of roots on inflorescence development and it is likely that the root system is an important source of the growth substances which are involved in the control of flowering. This has yet to be proved, however, and it is noteworthy that recent work by Wang and Wareing (34) has called into question the view that correlative phenomena such as apical dominance are regulated by cytokinin which originates in roots.

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INFLORESCENCE FORMATION IN THE GRAPEVINE

Three-step process

- (i) FORMATION OF ANLAGEN (Sing. ANLAGE) BY APICES OF LATENT BUDS ON GROWING SHOOTS OF CURRENT SEASON
 - Anlagen are "uncommitted" primordia, lateral outgrowths of apical meristem
 - Formation of Anlagen is progressive along cane — commences midsummer
- (ii) ANLAGEN develop either as INFLORESCENCE PRIMORDIA or as TENDRIL PRIMORDIA.
 - Latent bud enters ORGANIC DORMANCY
 - No further differentiation of inflorescence until spring
- (iii) AT TIME OF BUDBURST IN SPRING
 - Tendril primordia → Tendrils
 - Inflorescence primordia → flower primordia → fully differentiated flowers

SPECIAL FEATURES OF THE GRAPEVINE

- Anlagen
- Homology of tendrils/inflorescence
- PRIMORDIA which give rise to inflorescence axis and flowers arise at different times

ORIGIN OF THE INFLORESCENCE

