PHYSIOLOGY OF FLOWERING IN THE GRAPEVINE — A REVIEW

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ABSTRACT

The vegetative and reproductive anatomy of the grapevine is discussed with emphasis on recent interpretations based on scanning electron microscopy. The terminology of flowering in *Vitis* is defined and a developmental code, comprising Stages 0 to 11, is proposed for the events leading to formation of flowers. In brief, flowering in the grapevine involves three main steps: 1) Formation of Anlagen or uncommitted primordia (Stages 0 to 1); 2) Differentiation of Anlagen to form inflorescence primordia (Stages 2 to 7); and 3) Differentiation of flowers (Stages 8 to 11). The literature on the factors that influence flowering in grapes is reviewed under three major headings: 1) Biochemical changes in apices during inflorescence primordia formation; 2) Effects of environmental factors, including temperature, light intensity, photoperiod, water stress and mineral nutrition and 3) Role of phytohormones.

Anlagen develop into inflorescences, tendrils or shoots depending on the environment and hormonal factors. A hypothetical scheme for the hormonal control of Anlagen, tendril and inflorescence formation is proposed. It is suggested that flowering in grapes is controlled by the gibberellin:cytokinin balance. Formation of the inflorescence axis (the Anlage) is gibberellin controlled, but subsequent differentiation into flowers is regulated by cytokinin.

The origin and development of inflorescences and flowers in the grapevine have been studied for more than a century (84) but research on the control of flowering has a short history. In 1971 Pratt (118) published an authoritative review on the reproductive anatomy of cultivated grapes, but she did not discuss the early stages of inflorescence inception or the factors which affect inflorescence formation. Subsequently, Buttrose (33) reviewed only the influence of climate on flowering. The present review is concerned with the mechanisms by which apices are transformed from the vegetative to the reproductive mode of development.

VEGETATIVE AND REPRODUCTIVE ANATOMY OF THE GRAPEVINE

The grapevine is a complex plant and opinions differ as to the naming of its parts. An abridged description of grapevine anatomy will be given here, the objects of which are to introduce the terminology and to set the scene for later discussion of the physiology of flowering.

Origin of the prompt bud and lateral shoot: The first bud which arises in the axil of the leaf subtended by a current season's shoot is known as the "prompt bud" (prompt bourgeon) (Fig. 1A). This bud has the characteristic of growing out in the same season as its formation, and the lateral shoot so formed is known in French as the "entre coeur" (124). Pratt (119) called this outgrowth the "summer lateral." The lateral shoot seldom bears inflorescences but there are varietal differences in this regard (124). Huglin (57) showed that the growth of the lateral shoot is inhibited by the apex of the main or primary shoot. If this correlative inhibition is removed, the lateral shoot develops into a vigorous fertile shoot (13).

The latent bud: The first leaf of the lateral shoot is reduced to a bract or prophyll (35). The bud which develops in the axil of the bract is the "'latent bud" (bourgeon latent, Fig. 1 B). The latent bud grows slowly within the bract. Depending on the cultivar the latent bud produces 6 to 10 leaf primordia and up to three inflorescence primordia before becoming dormant during the winter. Although the latent bud is established as an appendage of the lateral shoot, its association with the primary shoot is very close. The xylem vessels of the young latent bud lead directly to the primary shoot, but not directly to the xylem of the lateral shoot (84,119). Prillieux (120) was the first to discover that the latent bud of the vine does not adjoin the main shoot but arises as the axillary bud of the first

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bract-like leaf of the lateral shoot. This relationship was confirmed by Müller-Thurgau (94) and has been discussed by several more recent authors (25, 35,84,146).

Secondary and tertiary latent buds: The apex of the primary latent bud produces two or more bracts before producing leaf primordia. The buds in the axils of these two bracts are the secondary and tertiary latent buds. These buds exhibit limited growth and they produce mainly leaf primordia. The secondary buds may form inflorescence primordia in some cultivars. The tertiary buds usually remain vegetative (119).
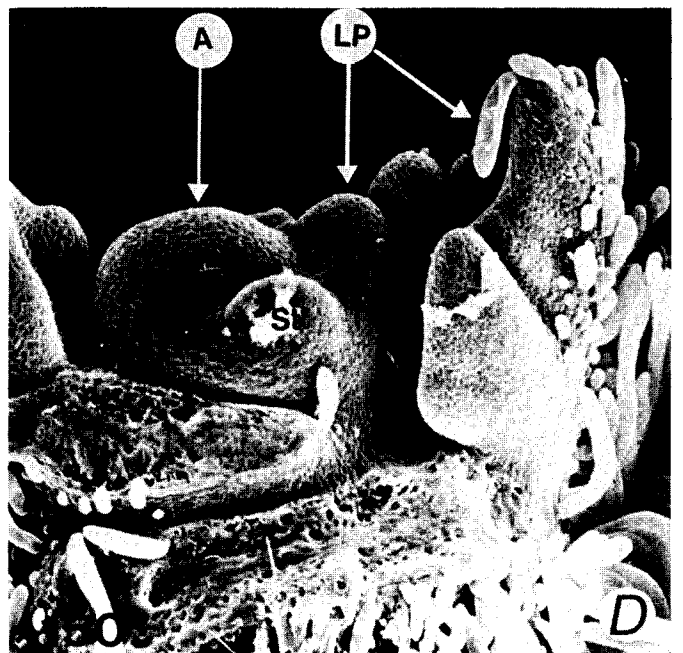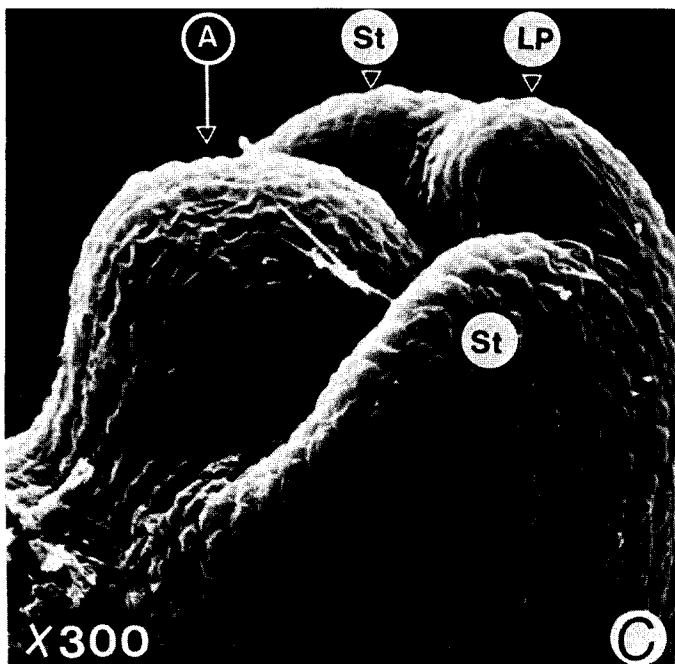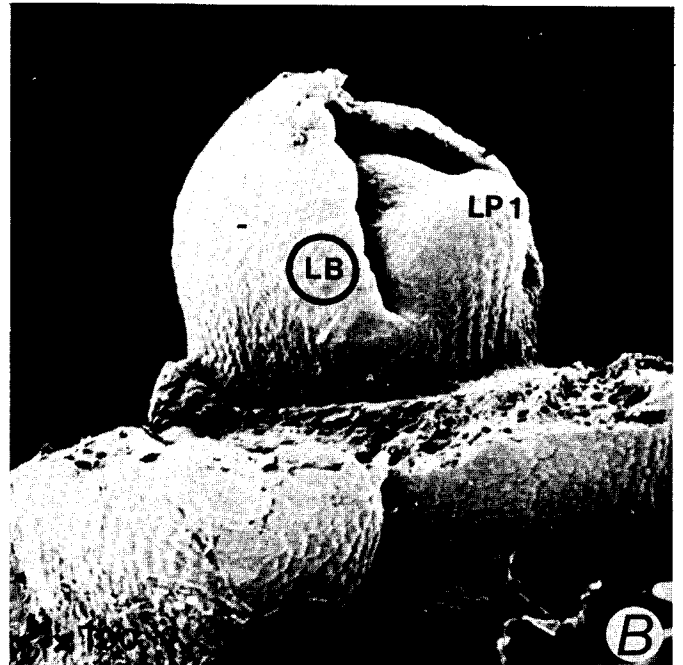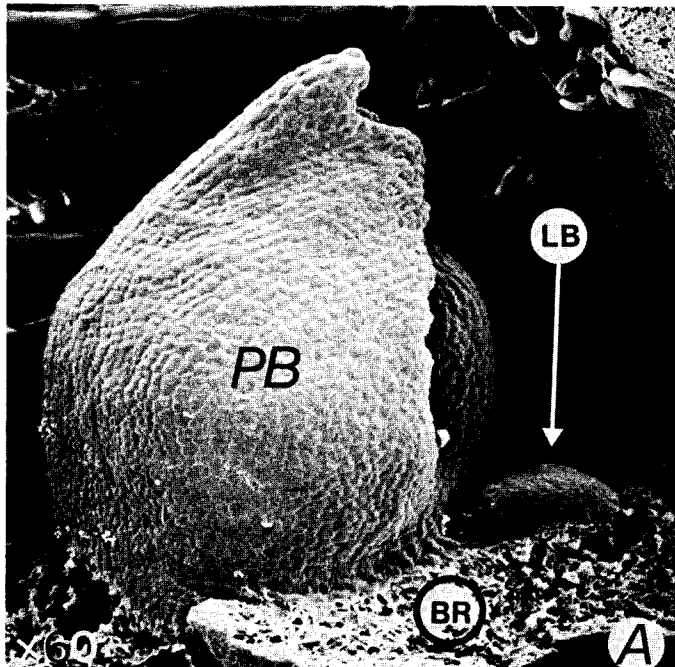

Fig. 1. Formation of the latent bud, leaf primordia and stipule primordia in the grapevine.

A. The prompt bud (PB) is covered by a bract (BR). The latent bud (LB) is formed in the axil of the first bract. In this preparation the first bract has been removed to expose the latent bud initial (LB). Note the prominent scar of the bract (BR).

B. A later stage in formation of the latent bud (the prompt bud has been removed). The latent bud (LB) has formed a bract and the first leaf primordium (LP$_1$).

C. Differentiation of the first leaf primordium (LP). Stipule primordia (St) are formed from the flanks of the leaf primordium. (A) - latent bud apex.

D. A latent bud in which three leaf primordia have been formed (LP$_1$, LP$_2$ and LP$_3$). Note the incipient stipules (St) on either side of the newly-formed leaf primordium (LP).

The primary, secondary and tertiary buds, which are enclosed by the bract of the lateral shoot and by the two basal bracts of the prompt bud, constitute the winter bud or "eye (l'oeil) of the dormant cane (mature grape shoot). The "eye" is a compound or mixed bud and it consists of several buds, the one located in the axil of the other.

Flowering in the grapevine: The formation of inflorescences and flowers in the grapevine involves three well-defined stages (8,18,19,25,26,36,84,114, 147).

Formation of Anlagen (singular - Anlage): Anlagen are club-shaped meristematic protuberances which arise from the apices of latent buds. Anlagen are "uncommitted primordia" which may be directed to form inflorescence primordia, tendril primordia or shoot primordia. Formation of Anlagen is also referred to in this review as inflorescence initiation or inflorescence axis formation (Fig. 2).

Formation of inflorescence primordia: Anlagen which have been directed to develop as inflorescences undergo repeated branching to form a conical structure composed of many rounded branch primordia (Fig. 3).

Formation of flowers: Differentiation of inflorescence primordia to form the individual flowers (Fig. 4).

The first two stages are completed during the current season. The final stage, flower formation, occurs shortly before and during bud burst in the next spring.

Fully mature latent buds containing one or more inflorescence primordia are called fruitful or fertile buds. This condition of latent buds is known as fruitfulness or fertility. Unfruitful, infertile or vegetative buds are latent buds which contain tendril primordia in the place of inflorescence primordia. Tendrils, which arise in a specific sequence in leaf-opposed positions, are produced by the apices of primary shoots, lateral shoots and primordial shoots of latent buds. The lateral meristems which give rise to tendrils are also called Anlagen regardless of the kind of shoot from which they originate. Anlagen which arise directly on main and lateral shoots of the current season do not usually form inflorescences and they remain as tendrils, but most Anlagen which are initiated in latent buds give rise to inflorescences. It has been demonstrated that Anlagen and young tendrils on main and lateral shoots have the potential to form inflorescences (148,149,150), but this potential is seldom expressed due to correlative inhibition by the respective shoot apices. Correlative inhibition is under the control of hormones (115). When Anlagen and their derivatives, the young tendrils, are freed from correlative inhibition by treatment with exogenous growth substances, or by excision and cultivation *in vitro* with the appropriate plant hormones, they give rise to inflorescences (148, 149,150).

DEVELOPMENTAL STAGES IN THE FLOWERING OF GRAPEVINES

An accurate description of the morphological changes in buds during the formation of inflorescences and flowers is an obvious prerequisite to the understanding of physiological controls in the flowering process.

Hitherto, much of the information on the formation of inflorescences and flowers in the grapevine has been accumulated from studies of median longitudinal sections of paraffin-embedded latent buds (19,35). With a complex three dimensional structure such as a latent bud the use of sections for the study of dynamic aspects of growth and development is laborious and prone to error. The interpretation of sections cut from blocks with differing orientations is often exceedingly difficult. For example, only sections which bisect the latent bud apex along the plane of leaf and inflorescence primordium formation will show some parts of both leaf and inflorescence primordia (35,182). The scanning electron microscope (SEM) is an ideal instrument for investigations of bud structure because it enables observations to be made at high magnification and with great depth of focus.

The complex morphology of the grapevine shoot system and the origin of inflorescences have been clarified recently by scanning electron microscopy (147). As an extension of this work, and as an aid to description, a developmental or phenological code for flowering in the grapevine has been constructed (146) in which the various stages (0 to 11) are related to changes in the shape of organs or to the addition of new structures (Figs. 2-4). Similar developmental codes have been proposed for flowering in *Xanthium* (127) and roses (56), using information from light microscopy, and for wheat (106) using SEM.

In the proposed code for flowering in the grapevine the latent bud apex is considered to be in a vegetative condition until the initiation of the first Anlage. Accordingly, apices containing leaf primordia only are referred to as Stage 0. The cleavage of the apex to form the Anlage is designated Stage 1. Later stages are numbered serially according to the extent of the ramification of the Anlagen (Figs. 2, 3, 4). Reference will be made to this code in the following discussion on development of primordia in latent buds.

FORMATION OF PRIMORDIA IN THE PRIMARY LATENT BUD

Leaf primordia: The formation of a leaf primordium at the flank of the latent bud apex is the first step in the development of the bud (Fig. 1B, C). Leaf primordia arise from the apical meristem in acropetal succession and with distichous phyllotaxy (35,147) (Fig. 1D). Associated with each leaf primordium are two ovoid stipular scales (Fig. 1C, D). These scales are located one on either side of the leaf primordium. Initially the scales are as big as leaf primordia but they soon stop growing and become sclerified (19,146,182). The leaf primordia are pointed structures which become lobed at their bases and rapidly assume a leaf-like appearance (Fig. 1D). The first two or three leaf primordia grow rapidly and envelop the subsequent primordia. The later-formed leaf primordia are relatively slow growing and they remain small (146). Hairs
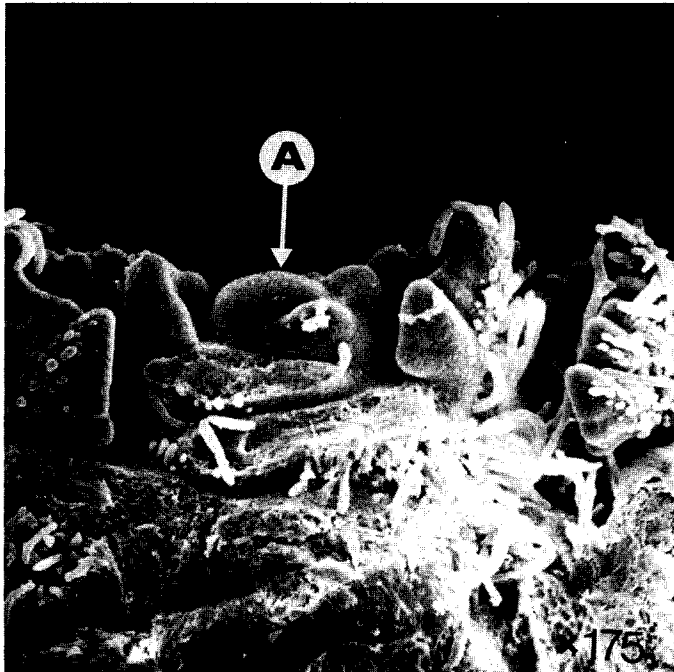
Stage 0. Depending on the cultivar, the apex (A) of a young latent bud forms a specific number of leaf primordia (5 in Shiraz) before the formation of the first Anlage.
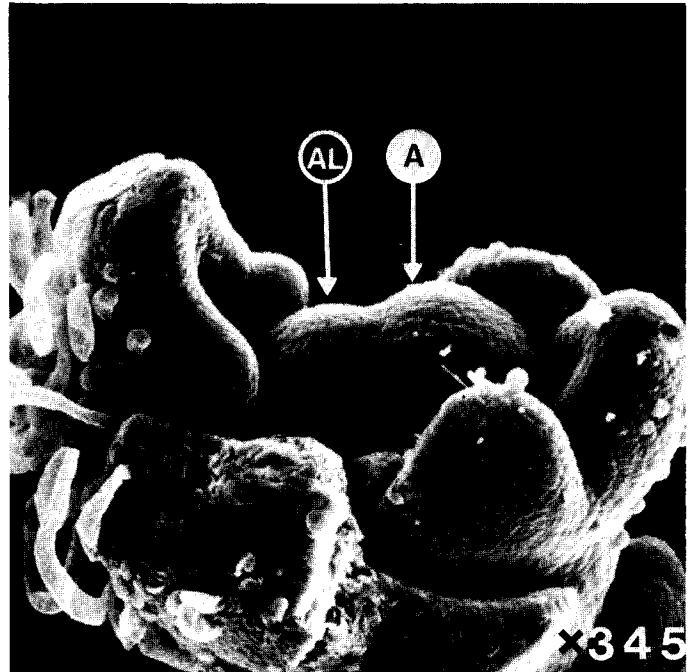
Stage 1. Bisection of the apex (A) to form the Anlage (AL). The Anlage is opposite the youngest leaf primordium.
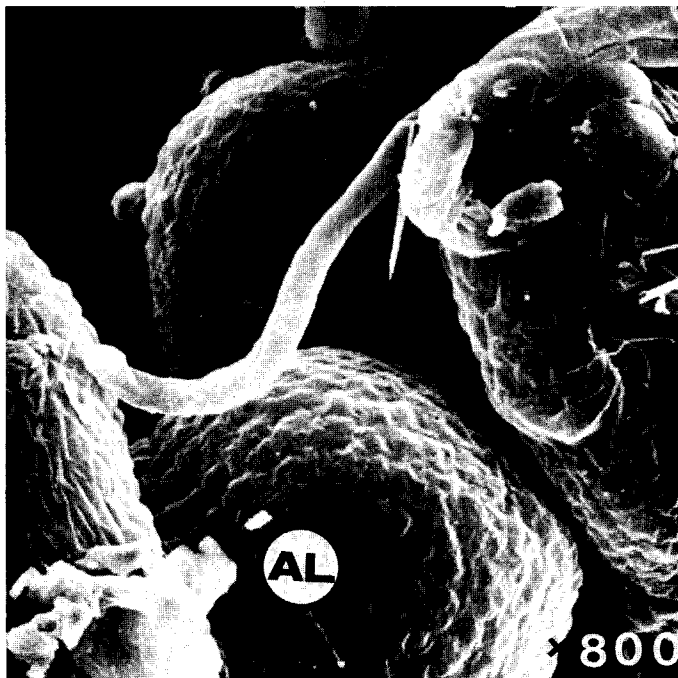
Stage 2. The Anlage (AL) has separated from the apex and is developing into blunt broad obovate structure.
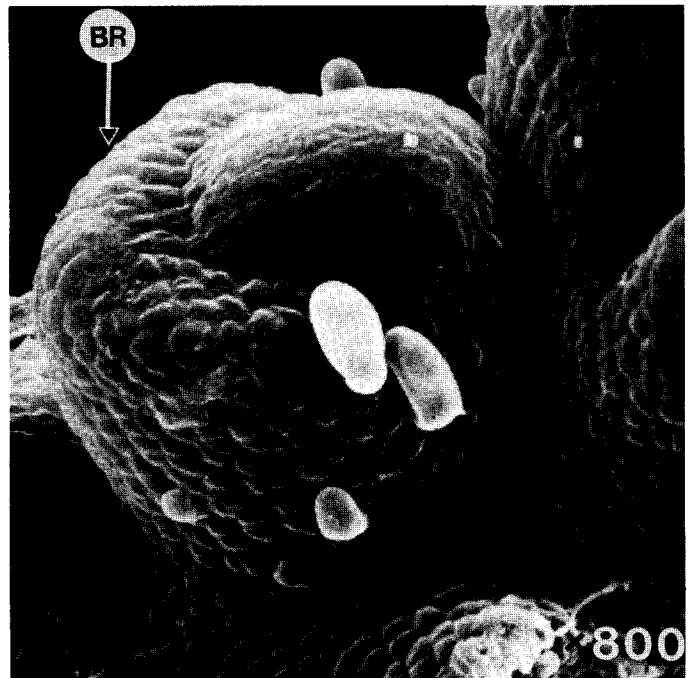
Stage 3. Formation of a bract primordium (BR) from the abaxial flank of the Anlage.

Fig. 2. Developmental stages in the flowering of Vitis vinifera L. — formation of Anlagen.
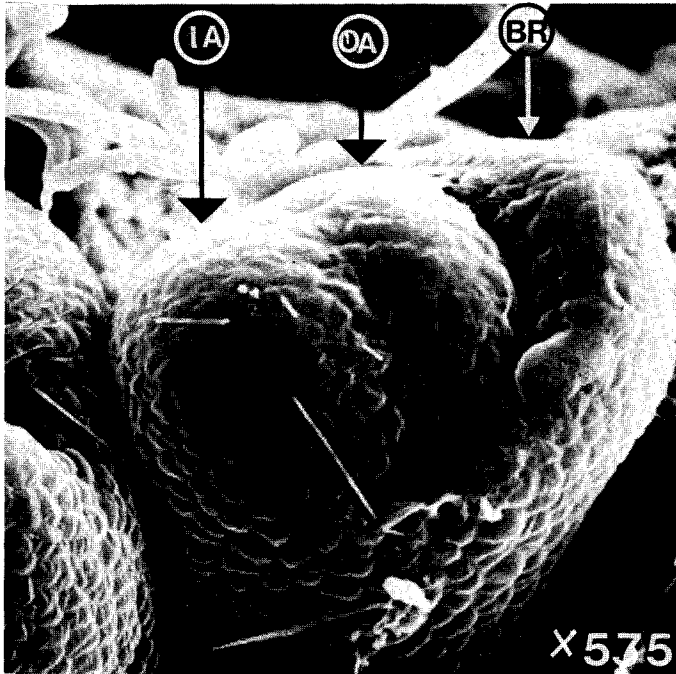
Stage 4. Division of the Anlage to form an inner arm (IA) and an outer arm (OA). The inner arm becomes the main axis of the inflorescence and the outer arm becomes the proximal branch of the inflorescence.
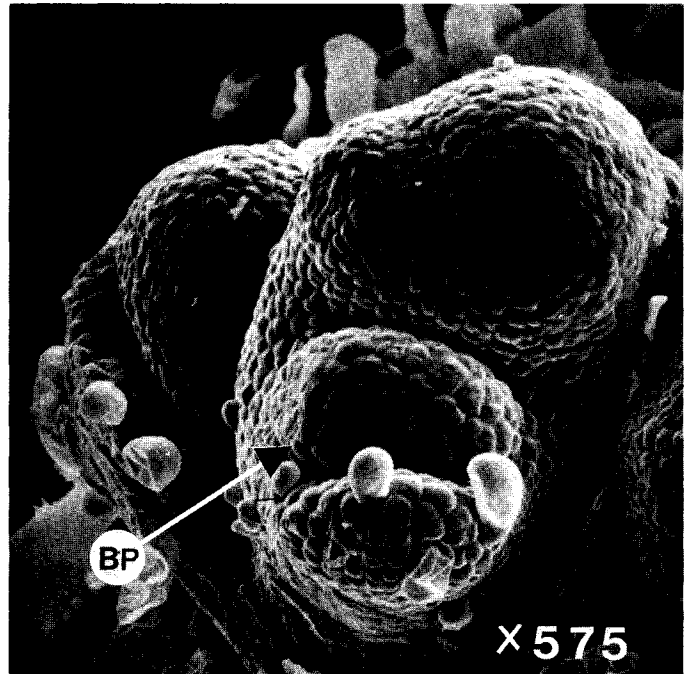
Stage 5. Growth of the main axis (inner arm) to give rise to the first branch primordium (BP).
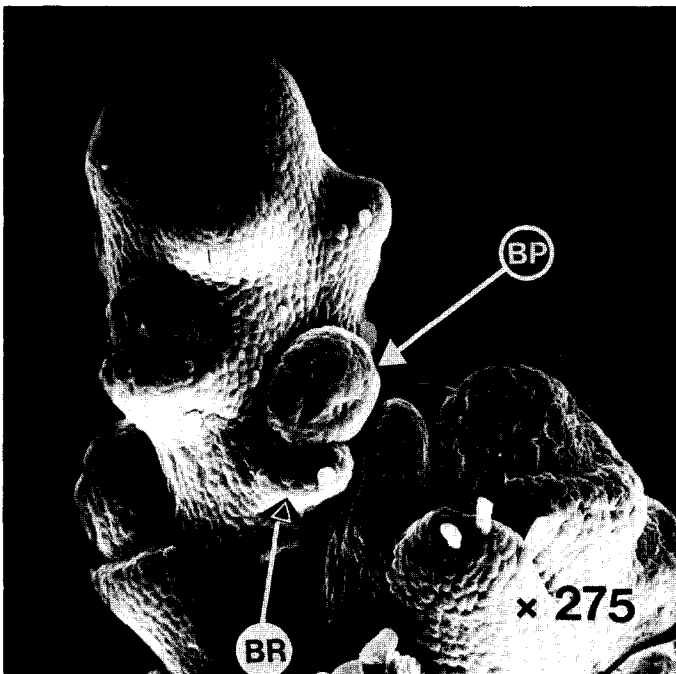
Stage 6. Growth of the main axis of the inflorescence primordium to form several branch primordia (BP) and bract primordia (BR).
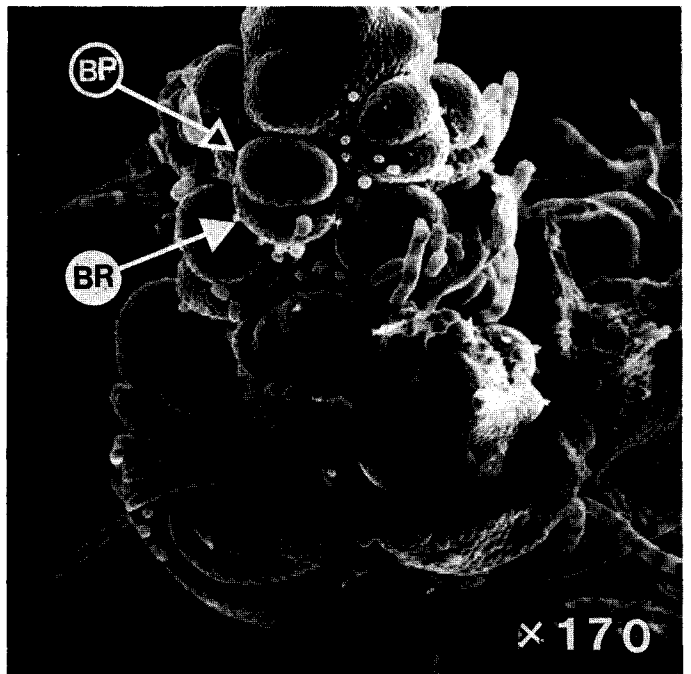
Stage 7. Differentiation of the branch primordium at bud burst and formation of the flower initials (FI). Note group of five flower initials.

Fig. 3. Developmental stages in the flowering of *Vitis vinifera* L. — formation of inflorescence primordia.
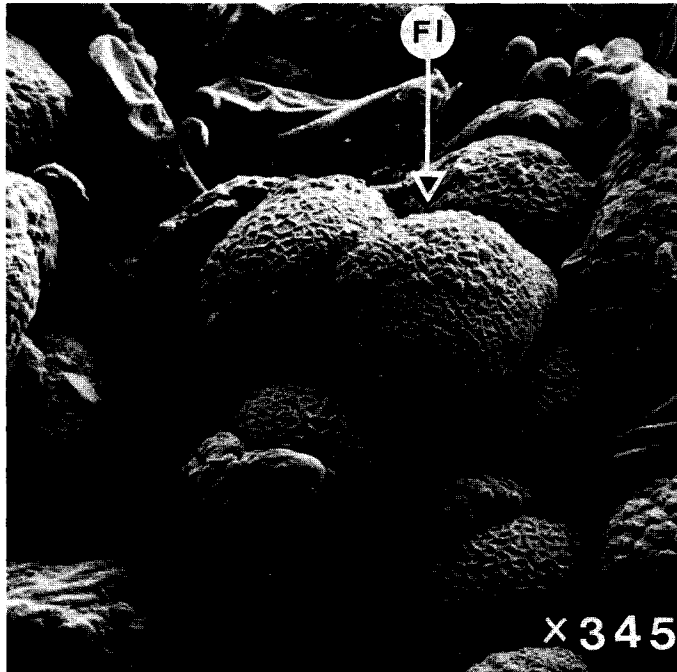
Stage 8. A fully developed inflorescence primordium in a mature dormant latent bud. Note the branch primordia (BP) and bracts (BR).
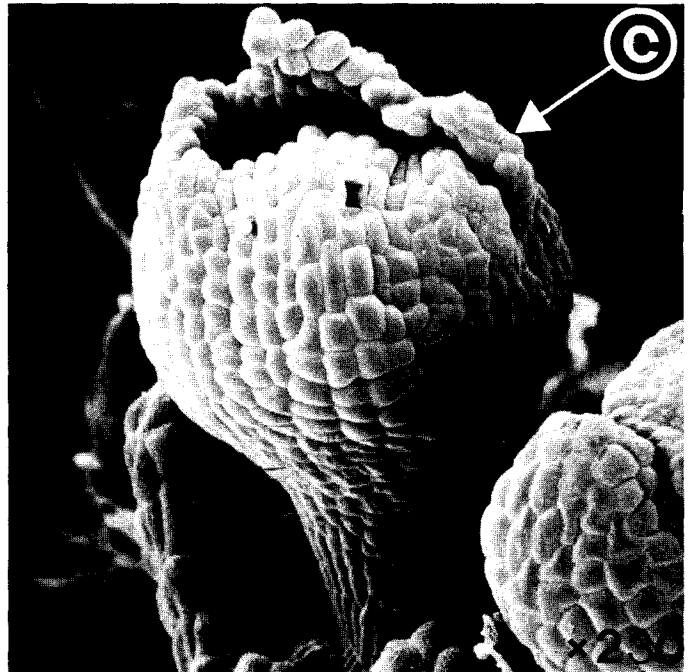
Stage 9. Development of calyx (C) in a flower initial (after bud burst). The calyx forms an incomplete cove over the developing flower.
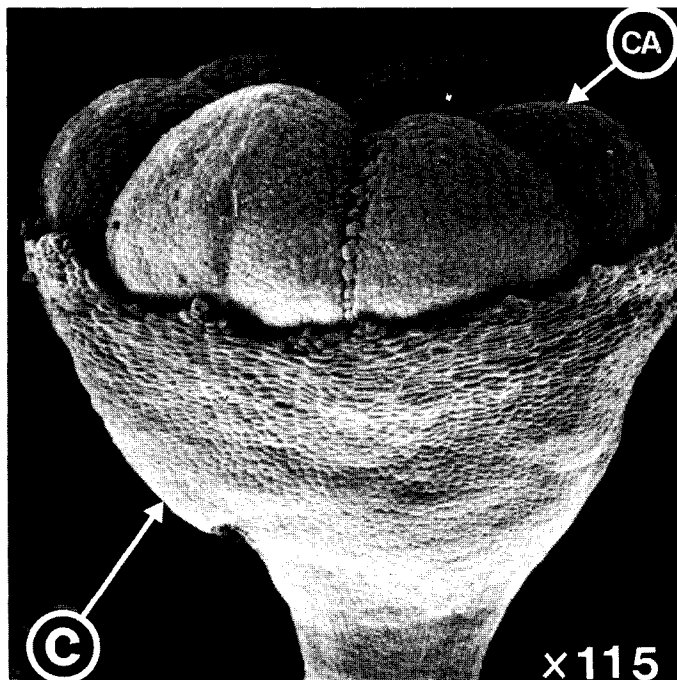
Stage 10. The calyptra (CA) lobes are visible through the top of the calyx (C).

Stage 11. A fully formed grape flower just before anthesis. There is full development of flower parts, viz., calyx, calypta, stamens and pistils.

Fig. 4. Developmental stages in the flowering of Vitis vinifera L. — formation of flowers.

develop from the upper epidermal cells of leaf and scale primordia and produce a tomentum which encases each whorl of leaves (146). The basal leaf primordia, stipular scales and tomentum all provide a protective cover for the meristematic apex of the latent bud.

In the cultivar Sultana, growth of latent buds ceases after the formation of 12 to 13 leaf primordia (19) but some cultivars of central European origin, for example, Gutedal (186), Riesling and Auxerrois (57), and Freismer (84), produce less than 12 leaf primordia before the latent bud becomes dormant.

Anlagen: Depending on the cultivar the latent bud apex produces three to eight leaf primordia (Fig. 2, Stage 0) and then divides into two almost equal parts (8,118,146). The part opposite the youngest leaf primordium is the Anlage (Fig. 2, Stage 1). The formation of Anlagen from the apex is the earliest indication of reproductive growth in the grapevine and the formation of the Anlage can be regarded as the stage of initiation of the inflorescence axis (84,147).

Initially, the two parts of the divided apex (Anlage and latent bud apex) have a similar appearance (Fig. 2, Stage 1) but they soon acquire different conformations. Anlagen develop as broad, blunt, obovate structures and they lack stipular scales (Fig. 2, Stage 2). Leaf primordia are narrow pointed structures and arise from the flank of the apex (Fig. 2, Stages 0, 1).

Bracts and arms: The further development of the Anlagen starts with the formation of a bract (182). Bracts originate as depressions in the distal ends of the Anlagen. Later, these depressions appear to move to the periphery and to form a collar-like structure (Fig. 2, Stage 3). The Anlage then divide into two unequal parts, called arms. The larger adaxial part (nearer to the apex) is the inner arm, and the smaller abaxial part adjoining the bract is the outer arm (Fig. 3, Stage 4) (118,147).

Inflorescence primordia formation: Inflorescence primordia are formed by extensive branching of the Anlage (Fig. 3, Stage 5). The inner arm divides and produces several globular branch primordia (133) which give rise to the main body of the inflorescence. Branching of the outer arm is less extensive and it develops into the lowest branch of the inflorescence (84). The branch primordia of the inner and outer arms give rise to branch primordia of the second and third order, each of which is subtended by a bract (Fig. 3, Stages 6 and 7). The degree of branching of the inner arm gradually decreases in an acropetal direction and this gives the inflorescence primordium a conical shape (Fig. 3, Stage 7). The appearance of a fully-developed inflorescence primordium is rather like a bunch of grapes in which each berry-like branch primordium is a protuberance of undifferentiated meristematic tissue (Fig. 3, Stage 7). After the formation of one to three inflorescence primordia (depending on the cultivar), the latent bud enters into dormancy (118).

DIFFERENTIATION OF FLOWERS

According to most reports the differentiation of flowers from inflorescence primordia begins after the dormant latent buds are activated in the spring (2,19,128,144,182). However, Alleweldt and Balkema (8) and Alleweldt and Ilter (10) have suggested that a large proportion of floral meristems may form sepals in the late summer preceding the season of flowering, but such early differentiation of flowers has not been confirmed in more recent studies with the scanning electron microscope (133,146).

Each branch primordium of inflorescence primordium divides many times and ultimately it produces the flower initials (Fig. 4, Stage 8). There are differences among cultivars in regard to the differentiation of flowers. In Muscat of Alexandria and Thompson Seedless the flower primordia are formed in groups of three (88,113), but in Shiraz the flowers occur in groups of five (146; Fig. 4, Stage 8).

Initiation and development of flowers parts was said by Barnard and Thomas (19) and by Snyder (144) to occur simultaneously in all parts of the inflorescence primordium but later workers have disputed that development of flower parts is synchronous (2, 121,182). It is now clear that sepals, petals (calyptrae), stamens and pistils develop one after the other (118,146) (Fig. 4, Stages 9,10,11).

The appearance of the calyx as a continuous ring of tissue on the rim of the presumptive flower primordium marks the beginning of the formation of flower parts [see Pratt, (118) for review]. The calyx ring does not coalesce at its tip but forms an incomplete cover over the petals (Fig. 4, Stage 9). The petals and stamens arise as five papillae soon after the formation of sepals. The petals do not coalesce at their margins or at their tips as suggested by Snyder (144), but special cells are formed at the margins which interlock with similar cells on the margins of the adjacent petals to form a calyptra (Fig. 4, Stage 10) (146,147). At anthesis in a mature flower (Fig. 4, Stage 11) the petals become free first at their proximal ends (bases) and then separate and curve upwards and outwards to release the stamens (118). In cytokinin-treated flowers the petals open from the top or remain closed as in cleistogamous flowers (149). Petals and stamens are persistent in cytokinin-treated flowers, but are deciduous in normally-pollinated flowers.

BIOCHEMICAL CHANGES IN APICES DURING THE FORMATION OF INFLORESCENCE PRIMORDIA

Treatment of grape leaves with uracil increases inflorescence primordia formation (66) and the yield of grapes (15,63,64). This response was associated with an increase in nucleic acids and protein synthesis in leaves. The relationship of nucleic acid changes in leaves to the yield of grapes is difficult to interpret, but the nucleic acid status of latent buds *in situ* seems to be related to fruitfulness. The DNA content is high during Anlage formation (Stage 1) and during the early stages of inflorescence primordium formation (Stages 2 to 6) but the RNA content increases before Anlage formation (Stage 0) and again during Stages 2 to 6 (122).

Addition of nitrogen enhances the synthesis of protein and nucleic acids and this has been related to an

increase in inflorescence formation (1,40). When radioactive P was applied to five grape cultivars at Stage 0, most of the absorbed ^{32}P was found subsequently in the nucleic acids fraction of latent buds during Stages 1 to 8 (158). The size of nuclei in the inflorescence primordia increases considerably during flower differentiation at bud burst (84). The nucleic acids content, RNA/DNA ratio and catalase and peroxidase activity are all higher in young shoots (at bud burst) bearing inflorescences than in vegetative shoots (160).

Histochemical changes in the apices of latent buds during the formation of Anlagen, inflorescence primordia and tendril primordia were studied in the cultivar Shiraz by Srinivasan and Mullins (151). Peripheral zone cells of latent bud apices showed mitotic activity and enzyme activity but the central zone cells are quiescent. Fluorimetric determination of the DNA content of nuclei in latent bud apices revealed an increase in the proportion of 2C nuclei during Anlage formation and differentiation. This condition was particularly evident in vines grown with 30°C day to 25°C night temperatures, a regime which is favorable for inflorescence primordia formation. In vines grown at 18°C day to 13°C night, a temperature unfavorable for inflorescences formation, nuclei with the 4C level occurred with a high frequency in Anlagen. The predominance of nuclei with the 2C-DNA level indicates a rapid mitotic turnover, but predominance of 4C-nuclei is the sign of slow mitotic activity. Carolus (35) also found that a high proportion of cells involved in Anlage formation are mitotically active. In fully matured inflorescence or tendril primordia cells contain mostly 4C nuclei. This indicates, perhaps, that mitotic activity is slowing down in latent buds in anticipation of organic dormancy (146).

Histochemical tests on cryostat sections of latent bud apices and Anlagen showed intense activity of acid phosphatase and peroxidase in the peripheral zone cells, i.e., the cells involved in the formation of Anlagen and inflorescence primordia. Accumulation of starch was found in the diaphragm cells and in older leaf primordia of latent buds (152) during Stages 3 to 6.

ENVIRONMENTAL FACTORS IN FLOWERING

Temperature: There are many reports of a requirement for high temperatures for inflorescence primordium formation in grapes (6,16,33,57,146). A close positive correlation (r = 0.82) was established between mean air temperature and the percentage of fruiting shoots (45). In particular, there is a positive relationship between temperature from the middle of June to the middle of July (northern hemisphere) and the number of inflorescences appearing on the shoot in the following season (6). Specifically, high temperatures during Stages 5 to 7 of latent bud development are closely correlated with subsequent fruitfulness of latent buds (16).

It is very difficult to demonstrate a specific effect of temperature on flower formation under vineyard conditions. To circumvent this difficulty Buttrose (28,29,30) used grapevines grown in small containers in controlled environments. After three months of growth, the latent buds were dissected under a stereomicroscope, and the size and weight of inflorescence primordia were measured. With the relatively fruitful cultivar, Muscat of Alexandria, the number of inflorescence primordia recognizable at three months varied from zero at 20°C to a maximum of 1.6 in vines grown at temperatures close to 35°C (28).

A pulse of only four hours per day (or night) of high temperature (30°C) was sufficient to induce a maximum number of inflorescence primordia (Stage 7). The critical period for susceptibility to the high temperature response is the three weeks before the formation of Anlagen by the apices of latent buds (29,31).

Substantial differences have been found in temperature requirements for inflorescence primordium formation among cultivars of different geographic origin (23,32,69,70,71,162). Riesling and Shiraz initiate inflorescences with temperatures as low as 20°C but Muscat of Alexandria requires a temperature of 25°C (32,113). Cabernet Sauvignon requires a lower temperature summation for flowering than Bolgar or Rkatsiteli (23). A high temperature pulse is essential for the initiation of the second and third inflorescence in many cultivars, including cool climate cultivars. Sultana and Ohanez are less fruitful than most other cultivars and are more responsive to changes in temperature (32). American cultivars (interspecific hybrids) such as Delaware produce inflorescences at lower temperatures (21° to 22°C) than do vinifera cultivars (27° to 28°C for Muscat of Alexandria).

Light intensity: Effects of light intensity on the fruitfulness of grapevine buds are independent of the temperature regime (31). The effect of light intensity on inflorescence formation has been studied in the vineyard in relation to the hours of sunshine (16) or to shading treatments (87). Most studies have indicated that shading reduces fruitfulness (6,12,16,24,44,55,87). A mean of 10 h sunshine per day during inflorescence formation is needed for an acceptable level of fertility in Sultana vines (16). Shading for four weeks during late spring reduced the fruitfulness of latent buds to a greater extent than shading treatments applied earlier or later during the season. In New Zealand, shoots covered with Hesian shades (26% full sunlight) during December to May (summer and autumn) produced fewer bunches than unshaded shoots (55). However, growth cabinet studies showed that illumination equivalent to one quarter of full sunlight (39 klx) is sufficient to obtain maximum fruitfulness in container-grown plants of Muscat of Alexandria (33). In vines grown in controlled environments, the number and size of inflorescence primordia increases with increase in light intensity (28).

Vertically-trained shoots are more fruitful than horizontally-trained shoots (29,86). Direct exposure of latent buds to high intensity light improves the fruitfulness of buds but no effects of the quality of light on

inflorescence formation have been detected (85).

It is often observed that buds situated inside the canopy of field-grown vines are less fruitful than those at the exterior where buds are more strongly illuminated (90). The use of trellises and split canopies, as in the Geneva Double Curtain system of training, gives improved fruitfulness of buds and an overall increase in productivity of 50 to 90% (90,129).

As with the effect of temperature, responses of vines to differing light intensities vary with the cultivar. Sultana, Ohanez, and Shiraz were fruitful only at light intensities higher than 19.5 klx but Muscat of Alexandria and Rhine Riesling were fruitful with an illumination of 19.5 klx (32).

Photoperiod: Photoperiod does not affect inflorescence induction in grapes (3,4,6,7), but there is evidence in some cultivars that the numbers of inflorescence primordia per bud are greater under long days than with short days (29,33).

The fruitfulness of vines grown in growth cabinets was promoted by increasing the time of exposure to high intensity light (3600 ft - C). These results could not be explained by the increase in quantity of light for use in photosynthesis (33). If the high light intensity was supplied for more than 12 hours per day, the formation of inflorescence primordia appeared to depend on the hours of illumination rather than on total incident energy (33). In contrast, the accumulation of dry matter was related to total incident light energy and not to the number of hours of illumination (27). Therefore, the mechanism leading to inflorescence primordium formation is not closely related to the mechanism of dry weight accumulation (i.e., photosynthesis) despite its requirements for high energy light.

American species, including *Vitis labrusca,* are more sensitive to day length than *Vitis vinifera* L. (70,71,162). Delaware vines (*Vitis vinifera* × *Vitis labrusca*) grown in long days formed nearly three times as many inflorescences as those grown in short days irrespective of the temperature regime. The fertility of Muscat of Alexandria was affected by temperature rather than by day length (32,162).

To sum up the influence of temperature and light on fruitfulness, Buttrose (33) considers that temperature is a dominant factor for inflorescence primordia formation, but according to Rives (124) light intensity is the limiting factor. However, it appears that a combination of exposure to high temperature and high light intensity is necessary for *maximum* fruitfulness of latent buds.

Water stress: Persistent water stress depresses the fruitfulness of latent buds (181), a fact which explains why rainfed vines usually bear fewer fruitful buds than irrigated vines (58). Soil moisture is one of the chief factors influencing inflorescence development in grapes (9), and studies with vines grown in controlled environment have shown that the number and size of inflorescence primordia are reduced by water stress (34). However, there are also reports that water stress increases the fruitfulness of buds (142). May (85)

suggests that the reduced foliage density of water-stressed vines improves the illumination within the canopy and results in improved fertility of basal buds, a factor which leads to increased fruitfulness of the vine as a whole.

Vine growth is sensitive to water stress (170) and there is a reduction in both bud fruitfulness and dry weight of shoots (34). This suggests that water stress may affect fruitfulness indirectly through a decline in photosynthesis (82). Moreover, water stress causes a decrease in cytokinin in xylem sap (79) and an increase in the abscisic acid levels in leaves and stems (47,82). The importance of cytokinin in grape flowering is well established and will be discussed later in this review.

The paucity of information on the relationship between water stress and fruitfulness of latent buds is due primarily to the difficulty of separating the specific effects of water stress from those of temperature and light intensity.

Mineral nutrition: Most studies on the mineral nutrition of grapes have been concerned with berry development and wine quality, and there are few reports on the effects of mineral nutrition on flower formation.

An adequate supply of nitrogen is necessary for inflorescence primordium formation and for the differentiation of flowers (7). Size of inflorescence primordia is generally little affected by N nutrition (159), but an increase in the number of inflorescence primordia following N application is found when the initial N status of the vine is low (17). However, under special circumstances, application of nitrogen can result in a reduction in fruitfulness. A survey of the petiole nutrient status in 30 tropical vineyards showed a significant negative correlation (r = − 0.946) between petiole-N and fruitfulness (100). There is also evidence that the N reserves of the cane are preferentially utilized for the growth of the shoot rather than added nitrogen, even when excess N fertilizer is supplied (111). This could explain why soil applied nitrogen seldom has an effect on inflorescence primordium formation in grapes.

Optimum phosphorus (P) nutrition promoted bud fruitfulness by increasing the vine vigor (68), and phosphate deficiency is detrimental to inflorescence formation (60). Low N, high P and water stress are the factors associated with high fertility in Sultana vines (17). Under tropical conditions, petiole P content is positively correlated with the yield of grapes (100). Studies with radioactive P indicated a preferential accumulation of P in actively growing shoot tips and in young buds which subsequently became fruitful (53,72,123). P is present in xylem sap in mineral form at the beginning of the so-called "bleeding period" but it is mostly in an organic form during flower differentiation and bud burst (67).

There have been several suggestions for a role for potassium (K) in inflorescence formation in the grapevine (145,156). Soil application of K in K-deficient vineyards in Michigan and in the Niagara Peninsula caused a marked increase in the fruitfulness

of latent buds of Concord (75). Similar effects of K-nutrition were found in Sultana vines in California (41). Potassium application increased the fruitfulness and yield by 45% in the first year and 156% in the second year. This large increase in yield may have been related to the larger size of inflorescence primordia which are produced by latent buds of K-fertilized vines (159). Potassium is implicated in enzyme activation and carbohydrate mobilization in grapes (21). The positive response of vines to potassium may be related to the fact that grapevines utilize the soil-applied K for growth and bud development rather than the K stored in the cane (111).

The growing of vine seedlings in mineral nutrient solutions induces precocious flowering (59,125,171) and hydroponic culture forms part of a breeding strategy for the grapevine proposed by Bouquet (22). Optimum levels of N, P and K are associated with maximum cytokinin production by grape roots (65).

PHYTOHORMONES AND FLOWERING

External factors are thought to exert their influence on flowering by modifying the internal chemical makeup of the plant, particularly the balance of endogenous hormones (161). Proof of this supposition requires establishment of the necessary relationship between levels of endogenous hormones and the flowering response. Information on endogenous hormones in relation to flowering is especially difficult to obtain in the grapevine. The organs of interest, the Anlagen, are very small and inaccessible, and the extraction and estimation of auxins, gibberellins and cytokinins from latent buds presents many technical problems. Therefore, most conclusions on the role of phytohormones in flowering in grapes have been based on inferences from the effects of exogenous hormones and growth regulators.

Gibberellins: Gibberellins are the only chemicals known, so far, that are capable of inducing flower formation in numerous plants under strictly non-inductive conditions (185). However, gibberellins are inhibitory to flower formation in the grapevine (5), and other fruit trees (62). Endogenous gibberellins have been detected in xylem sap of grapevines (101,109,134) and tentatively identified as GA_1, GA_3, GA_5 and GA_9 (51,134). Lilov and Christov (77) found no close relationship between endogenous gibberellin levels and grape flower formation. Nevertheless, grapevines are very sensitive to exogenous gibberellins (143,173,176). Application of GA_3 or GA_{4+7} at concentrations as low as 3 μmol/L induced bursting of latent buds in the current season and inhibited the formation of inflorescence from Anlagen (150). However, gibberellins seem to favor initiation of Anlagen (150). In GA_3-treated plants Anlagen were formed precociously at normal node positions and at nodes more proximally situated than is normal (146). These GA-induced Anlagen did not develop into inflorescences but gave rise to tendrils (150).

Grape tendrils usually contain a higher gibberellin content than other organs (92). The elongation of tendrils results from the activity of a subapical meristem

(167), and applications of gibberellin, a stimulator of subapical meristem activity, greatly promote the growth of tendrils (146,173). Exogenous gibberellin also brings about the transformation of inflorescences into tendrils or tendril-like structures (92,98).

Growth retardants: Complete or partial cessation of vegetative growth favors flower initiation in many orchard species (62). Accordingly, chlormequat, a growth retardant, was used by several workers to reduce the vegetative growth and to induce flowering in grapes (42,49,81,117,164,172,174). Besides promoting inflorescence primordium formation in latent buds, chlormequat induced "secondary inflorescences" by transforming the tendrils of the primary shoot and lateral shoots into inflorescences (42,78,150,163). Pinching and chlormequat treatment had additive effects in promoting the flowering of lateral shoots of Muscat of Alexandria (169).

The promotive effect of chlormequat on flowering in grapes was attributed by some workers to early shoot maturation (73,83,113). Chlormequat not only retards vegetative growth and induces flowering but also increases fruit-set and chlorophyll content of leaves (43). The wide spectrum of effects caused by chlormequat led Coombe (43) to suggest that this chemical could induce "far-reaching alterations in cell metabolism" in the grapevine.

Anlagen formation and tendril elongation are inhibited by chlormequat (150), an inhibitor of gibberellin biosynthesis (74). Chlormequat induced the formation of inflorescences in place of tendrils (150), a response which may be related to effects of this growth retardant on cytokinin synthesis (135). It is probable that chlormequat exerts a dual role in the control of flowering in grapes, viz., inhibition of gibberellin biosynthesis and the elevation of cytokinin levels. Validation of this hypothesis awaits the availability of precise information on changes in the endogenous levels of cytokinins and gibberellins in response to chlormequat treatment.

Applications of chlormequat stimulate biosynthesis of cytokinins not only in grapes (135,136,139) but also in *Vicia faba* (48) and in apical buds of *Chenopodium rubrum* during floral induction (165). Chlormequat induced swelling of roots (136) and doubled the volume of bleeding sap (xylem sap) of grapevines (107). Besides an increase in total cytokinin content of xylem sap (176) and leaves (11), an unusual cytokinin was also found in xylem sap of chlormequat-treated vines (107).

Chlormequat did not induce precocious flowering in grape seedlings, but mixtures of the cytokinin, PBA, and chlormequat induced precocious flowering and fruiting in Muscat Hamburg seedlings (153). Another growth retardant, Amo-1618 [Ammonium (5-hydroxycarvacryl) trimethyl chloride piperidine carboxylate] also induced precocious flowering in Muscat Hamburg seedlings when applied with PBA (153).

Daminozide and the growth inhibitors, maleic hydrazide, ethephon and morphactin, do not promote flowering in the grapevine *in vivo* (172,174) or *in vitro* (146).

Cytokinins: The role of cytokinin in the control of flowering in plants is not well understood and only a few species have been made to flower by treatment with exogenous cytokinin (185). Zeatin, zeatin riboside, and zeatin ribotide are found in the xylem sap of grapevines before bud burst and during the period of active shoot growth (80,110,137,138,140). Root apices are a major source of cytokinins in plants (139), but the relationship between cytokinin and flowering in grapes has only recently been the subject of investigation.

From studies of bud anatomy by scanning electron microscopy, it is now clear that Anlagen which undergo extensive branching give rise to inflorescences and that Anlagen which produce only two or three branches grow into tendrils (149). It follows that the control of inflorescence formation in grapes hinges upon the control of branching of Anlagen or their derivatives, the tendrils. Isolated tendrils were induced to branch profusely and they grew into inflorescences when cultured *in vitro* with benzyladenine (BA), 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA) or zeatin riboside (148). In intact vines, and in partially defoliated vines, repeated applications of PBA to shoot apices, caused inflorescences to be formed in place of tendrils and this effect of cytokinin was found in 12 cultivars of *Vitis vinifera,* in six other *Vitis* species, and in *Muscadinia rotundifolia* (149,154). Moreover, these cytokinin-induced inflorescences produced ripe fruits which contained viable seeds. Tendrils of cytokinin-treated plants were transformed into inflorescences regardless of the sex of the vine — male, female or hermaphrodite (154). Male vines were more susceptible to cytokinin-directed conversion of tendrils into inflorescences than female — or hermaphrodite vines. Mixtures of cytokinin (BA) and chlormequat induced inflorescence formation in vines grown under non-inductive temperatures (18°C to 21°C) (150).

The ability of cytokinin to transform tendrils into inflorescences is not restricted to cultivars but is found also in seedlings. Repeated treatment of shoot apices and young tendrils of grape seedlings with cytokinins (PBA or BA), induced the first-formed tendrils to initiate flowers within four weeks of germination. Seedlings of Cabernet Sauvignon and Muscat Hamburg were induced to flower and to develop bunches of grapes with viable seeds with cytokinin (PBA) within eight months of germination (153).

The differentiation of grape flowers from inflorescence primordia is also a cytokinin-requiring process (97,98,116) and there is evidence that cytokinins produced in roots are involved in flower development. Inflorescence primordia in latent buds of hardwood cuttings fail to develop and atrophy if bud burst precedes the emergence of adventitious roots but normal inflorescences are retained when cuttings are made to form roots in advance of bud burst (95). In rootless cuttings exogenous cytokinins are needed for normal development of inflorescences. Grape leaves are strong sinks for root-produced cytokinin (54). In cuttings, removal at bud burst of the young leaves formed basal to

the inflorescence seems to enhance the sink-capacity of inflorescence for cytokinin and inflorescences develop in normal manner (97).

Exogenous cytokinins (PBA, BA, zeatin and dihydrozeatin) induced pistil development in male vines of several Vitis species and interspecific hybrids (46,50,52,93,102,103,104,105). Cytokinins are thought to promote pistil development in male vines by counteracting the effects of a postulated inhibitor (104).

Cytokinins are implicated in the control of many aspects of grape reproduction including inflorescence formation (148,149,150,153); differentiation of flowers (97,98); pistil development (102); fruitset (178); fruit development (175) and somatic embryogenesis in unfertilized ovules *in vitro* (99,152). These findings, together with the knowledge of high cytokinin activity in xylem sap during bud burst and flowering and high cytokinin activity in fruits (38,110), strongly suggest that endogenous cytokinin is a primary regulator of reproductive growth in the grapevine.

The mechanism by which cytokinins control flower formation is unknown. Cytokinin is regarded as a mitotic component of the floral stimulus in *Sinapis alba* (20) and was also shown to be involved in photoperiodic induction in *Pharbitis nil* by promoting the translocation of the floral stimulus (112). Cytokinins increase the permeability of cell membranes (130,168), and exogenous cytokinins are strong mobilizers of photosynthates in grapes (131,132,177). Sachs and Hackett (126) have provided evidence that flower formation in Bougainvillea is related to the gibberellin: cytokinin balance and that these hormones control flowering by affecting the distribution of metabolites. It needs to be stressed, however, that the relationship of the various biochemical functions of cytokinins to cytokinin-directed flower formation is not yet clear. Other classes of plant hormones are also known to promote mitosis, membrane permeability and the distribution of metabolites, but the compounds concerned have not been closely linked with the control of flowering.

DEVELOPMENTAL PATHWAYS OF THE GRAPEVINE ANLAGE

As will be clear from the foregoing sections of this review, the Anlagen and tendril primordia (branched Anlagen) possess a remarkable morphogenetic plasticity. Tendril primordia and tendrils give rise to inflorescences when treated with cytokinin (148,149,150,153) and inflorescences are converted into tendrils or tendril-like organs by gibberellin (92,98). In addition, inflorescences have been induced to form from tendrils with cytokinin treatment both *in vitro* (96) and *in vivo*.

Although Anlagen normally give rise either to inflorescences or to tendrils, they may also be made to grow into shoots. Exposure to low temperatures (< 21°C) tends to induce shoot formation (147). Treatment of apices with high concentrations (> 1 mM) of exogenous cytokinins and/or chlormequat caused several An-

lagen to form shoots, both in cultivars and in seedlings (150,155). Excised tendrils grew into inflorescences when cultured *in vitro* with PBA or BA (5-10 μmol/L), but if these tendril-derived inflorescences were treated subsequently with a high concentration of PBA (> 20 μmol/L) they, too, grew into shoots (146).

The available information on the developmental pathways of Anlagen have been combined and presented in diagrammatic form (Fig. 5). For purposes of description, Anlagen which have produced a bract and two branches (inner arm and outer arm) are referred to as tendril primordia (Fig. 3, Stage 4).
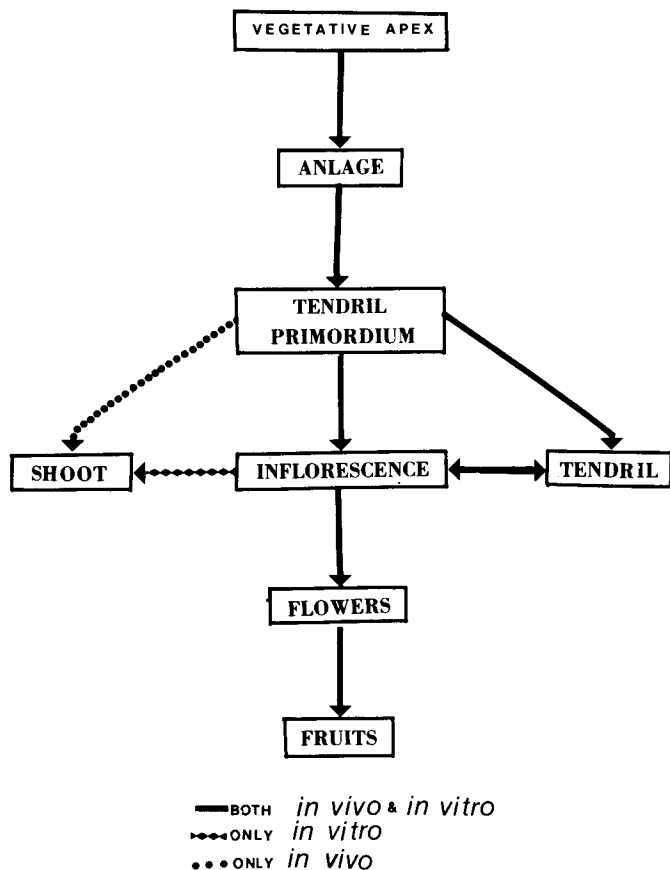

Fig. 5. Developmental pathways of the Anlage.

THE PHYSIOLOGICAL BASIS OF FLOWER FORMATION IN THE GRAPEVINE

Flowering in plants is believed by many workers to be induced by a single substance, "florigen" (180,185), but others have suggested that the floral stimulus consists of two complementary components (37,39). According to Thimann (166), flowering is merely a developmental process under the control of the interplay of hormones, and Zeevaart (184) has proposed that the requirement for a specific balance of hormones for flower formation is more readily applicable to woody perennials than to herbaceous annual plants.

In summarizing 40 years of research on the "hormonal concept of flowering" Chailakhyan (39) concluded that the main function of gibberellins is to control the formation and growth of floral stems or in-

florescence axes. The responses of grapevines to exogenous gibberellins and chlormequat are consistent with this view. Gibberellins are involved in the initiation of Anlagen (formation of inflorescence axes) and are necessary for the growth of inflorescence axes, i.e., the extension growth of the two-branched Anlagen (Stage 4).

According to Carr (37) two different inductive stimuli could be involved in the flowering of plants. One is the "primary induction" which is "contagiously propagated" and the other is a "secondary induction" which occurs locally. Initiation of Anlagen and tendril formation could be regarded as the "primary induction", a condition which is permanent in grape cultivars which are perpetuated by vegetative propagation. The "secondary stimulus", which is required annually for the direction of Anlagen into the pathway of inflorescence development, is probably cytokinin.

In nature, grapevines produce numerous Anlagen but most grow into tendrils and only a few Anlagen give rise to inflorescences. This suggests that gibberellin is readily available for initiation of Anlagen and for elongation of tendrils and that inflorescence formation is normally limited by the cytokinin supply (149,154). The finding that repeated applications of cytokinin are required for transformation of tendrils into inflorescences suggests that a continuous influx of endogenous cytokinins into Anlagen is needed for flower formation in the grapevine. A hypothetical scheme for the hormonal control of Anlage, tendril and inflorescence formation in the grapevine is presented in Fig. 6. The postulated inhibitors in this scheme are endogenous compounds which mimic the effects of the synthetic growth retardant, chlormequat, i.e., inhibition of gibberellin biosynthesis and promotion of cytokinin biosynthesis.
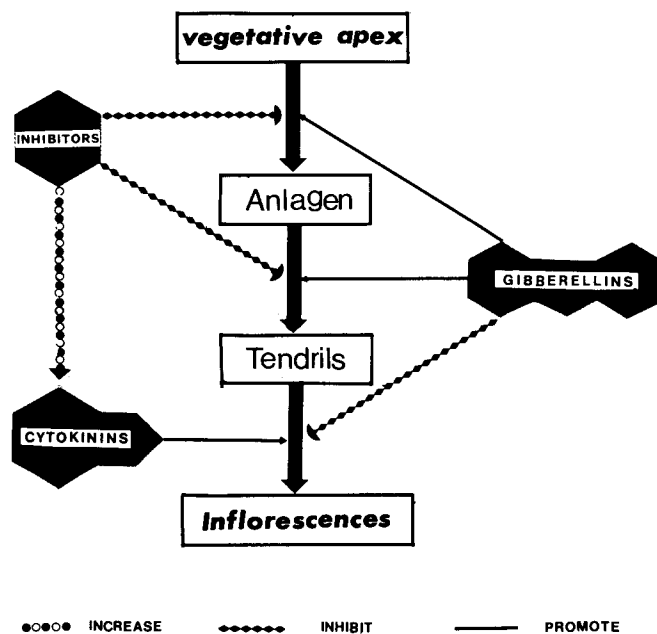

Fig. 6. A hypothetical scheme for the hormonal control of Anlage, tendril and inflorescence formation in the grapevine (*Vitis vinifera* L.).

CONCLUSION

The external factors which promote flowering in grapes, such as short term exposure to high temperature, high light intensity and optimum levels of soil moisture and macronutrients, are also factors which promote cytokinin biosynthesis in plants (14,65,91, 141,179,183). Conversely, factors which depress flower formation, such as low light intensity, low temperature and water stress, have an inhibitory effect on endogenous cytokinin production (61,79). Moreover, exogenous cytokinins promote flower formation in grapevines grown at non-inductive low temperatures, and plants grown in the dark (146). It is clear that cytokinin is of central importance in the control of flowering in the grapevine.

LITERATURE CITED

1. Abesatze, G. E., V. S. Khachidze, and L. A. Mosashvili. Effect of mineral fertilizers on nucleic acid content in vine shoots. Agrokhimiya No. 4:91-3 (1976).

2. Agaoglu, Y. S. A study of the differentiation and the development of floral parts in grapes (Vitis vinifera L.). Vitis 10:20-6 (1971).

3. Alleweldt, G. Die Wirkung der Gibberellinsäure auf einjahrige Reben bei verschiedener Photoperiode. Vitis 2:23-33 (1959).

4. Alleweldt, G. Die Beziehungen zwischen photoperiodischer Reaktion and Gibberellinsäure-Empfindlichkeit bei Reben. Z. Pflanzenzuecht. 43:63-83 (1960).

5. Alleweldt, G. Weitere Untersuchungen uber die sortenspezifische Gibberellinreaktion der Reben. Z. Pflanzenzuecht. 45:178-93 (1961).

6. Alleweldt, G. Einfluss von Klimafactoren auf die Zahl der Infloreszenzen bei Reben. Wein-Wiss. 18:61-70 (1963).

7. Alleweldt, G. Die Beeinflussing der Ertragsbildung bei Reben durch Tageslänge und Temperatur. Kali-Briefe, Fachgebiet 5, Folge 1:1-12 (1964).

8. Alleweldt, G., and G. H. Balkema. Über die Anlage von Infloreszenz- und Blütenprimordien in den Winter-Knospen der Rebe. Z. Acker Pflanzenbau. 123:59-74 (1965).

9. Alleweldt, G., and W. Hofäcker. Influence of environmental factors on bud burst flowering fertility and shoot growth of vines. Vitis 14:103-15 (1975).

10. Alleweldt, G., and A. Ilter. Über die apikale Dominanz bei Reben. Vitis 8:94-104 (1969).

11. Andanova, T. A., and D. T. Lilov. Changes of the free cytokinins in the shoots and leaves in plant differing in their growth. C.R. Acad. Bulg. Sci. 30:905-8 (1977).

12. Angyan, F., P. M. Kopcsai, and D. Polyak. Bud testing in commercial vineyards. Kertgazdasag 8:47-60 (1976).

13. Antcliff, A. J., and W. J. Webster. Studies in the Sultana vine. 1. Fruit bud distribution and bud burst with reference to forecasting potential crop. Aust. J. Agric. Res. 6:565-88 (1955).

14. Atkin, R. K., G. E. Barton, and D. K. Robinson. Effect of root-growing temperature on growth substances in xylem exudate of Zea mays. J. Exp. Bot. 79:259-66 (1973).

15. Balasubramanyam, V. R., and S. D. Khanduja. Effect of foliar sprays of uracil, xanthine and caffeine on the nucleic acid and protein content of leaves and fruiting of Thompson Seedless grapes. Vitis 12:100-4 (1974).

16. Baldwin, J. G. The relation between weather and fruitfulness of the Sultana vine. Aust. J. Agric. Res. 15:920-8 (1964).

17. Baldwin, J. G. The effect of some cultural practices on nitrogen and fruitfulness in the Sultana vine. Am. J. Enol. Vitic. 17:58-62 (1966).

18. Barnard, C. Fruit bud studies. I. The Sultana: An analysis of the distribution and behaviour of the buds of the Sultana vine, together with an account of the differentiation and development of the fruit bud. J. Coun. Sci. Ind. Res. Aust. 5:47-52 (1932).

19. Barnard, C., and J. C. Thomas. Fruit bud studies. II. The Sultana: Differentiation and development of the fruit buds. J. Coun. Sci. Ind. Res. Aust. 6:285-94 (1933).

20. Bernier, G., J. M. Kinet, A. Jacqmard, A. Havelange, and M. Bodson. Cytokinin as a possible component of the floral stimulus in Sinapis alba. Plant Physiol. 60:282-5 (1977).

21. Bouard, J. Influence of manuring on some morphological and biochemical characteristics of canes of Vitis vinifera L. Variety Ugni-Blanc. Potash Rev. 29:1-10 (1968).

22. Bouquet, A. Amélioration génétique de la Vigne: Essai de définition d'un schéma de sélection applicable a la création de nouvelles variétiés. Ann. Amélior. Plant. 27:75-86 (1977).

23. Braikov, D. The effect of temperature on the duration of organogenic phases in grapevines. Gradinar. Lozar. Nauka 12:108-14 (1975).

24. Braikov, D., B. Tsankov, and A. Pandeliev. The effect of light regime on the structure and photosynthetic activity of the leaves and the degree of differentiation of winter buds in grapevines: Differentiation of winter buds in the cv. Bolgar. Gradinar. Lozar. Nauka 14:116-22 (1977).

25. Bugnon, F. Recherches sur la ramnification des Ampelidacees. Imprimerie Beningaud et Privat, Dijon, France (1953).

26. Bugnon, F., and R. Bessis. Biologie de la Vigne. p. 160. Acquisitions Recentes et Problemes Actuals. Masson, Paris (1968).

27. Buttrose, M. S. Some effects of light intensity and temperature on dry weight and shoot growth of grapevine. Ann. Bot. 32:753-65 (1968).

28. Buttrose, M. S. Fruitfulness in grapevines: effects of light intensity and temperature. Bot. Gaz. 130:166-73 (1969).

29. Buttrose, M. S. Fruitfulness in grapevines: effects of changes in temperature and light regimes. Bot. Gaz. 130:173-9 (1969).

30. Buttrose, M. S. Vegetative growth of grapevine varieties under controlled temperature and light intensity. Vitis 8:280-5 (1969).

31. Buttrose, M. S. Fruitfulness in grapevines: development of leaf primordia in buds in relation to bud fruitfulness. Bot. Gaz. 131:78-83 (1970).

32. Buttrose, M. S. Fruitfulness in grapevines: the response of different cultivars to light, temperature and day length. Vitis 9:121-5 (1970).

33. Buttrose, M. S. Climatic factors and fruitfulness in grapevines. Hortic. Abstr. 44:319-25 (1974).

34. Buttrose, M. S. Fruitfulness in grapevines: effect of water stress. Vitis 12:299-305 (1974).

35. Carolus, M. Recherches sur l'organogenese et l'evolution morphologique du bourgeon latent de la vigne (Vitis vinifera L. var. Merlot). Theses 3° cycle, Bordeaux, p. 147 (1970).

36. Carolus, M. Description des Stades du development des primordia inflorescentiels durant l'organogenese des bourgeons latents de la Vigne (Vitis vinifera L. var. Merlot). Conn. Vigne Vin. 2:163-73 (1971).

37. Carr, D. J. The relationship between florigen and the flower hormones. Ann. N.Y. Acad. Sci. 144:305-12 (1967).

38. Chacko, E. K., T. Saidha, R. Doreswamy, Y. N. Reddy, and R. R. Kohli. Studies on the cytokinin in fruits. I. Occurrence and levels of cytokinin-like substances in grape berries at different developmental stages. Vitis 15:221-6 (1976).

39. Chailakhyan, M. K. Hormonal regulators of plant flowering. In: Plant Growth Regulation. P. E. Pilet, ed. Proc. 9th Int. Conf. Plant Growth Substances. p. 258-72. Springer-Verlag, Berlin (1977).

40. Cheban, A. I. Content and accumulation of nucleic acids in buds of wintering grape eyes at various stages of their seasonal development. Sov. Plant Physiol. 15:276-81 (1968).

41. Christensen, P. Long-term responses of Thompson Seedless vines to K fertilizer treatment. Am. J. Enol. Vitic. 26:179-83 (1975).

42. Coombe, B. G. Effect of growth retardants on *Vitis vinifera*. Vitis 6:278-87 (1967).

43. Coombe, B. G. Fruit set in grape vines: the mechanism of the CCC effect. J. Hortic. Sci. 45:415-25 (1970).

44. Dikan, A. P. The effect of total solar radiation on reproductive organ formation in grapevines. Fiziol. Biokhim. Kul't. Rast. 8:643-8 (1976).

45. Dimitrieva, K. V. The effect of meteorological conditions on fruitfulness in vines. Met. Klim. Gidrol., mezved. nauc. Sborn. No. 5, p. 150-3 (1970).

46. Doazan, J. P., and V. Cueller. Artificial modification of sex expression in the genus *Vitis*. Ann. Amelior. Plant. 20:79-86 (1970).

47. Düring, H., and G. Alleweldt. Der Jahresgang Abscisinsäure in vegetativen Organen von Reben. Vitis 12:26-32 (1973).

48. El-Antably, H. M. M. Studies on the physiology of shedding of buds, flowers and fruits in *Vicia faba*. II. Effect of cycocel (CCC) and the role of endogenous gibberellins and cytokinins. Z. Pflanzenphysiol. 80:29-35 (1976).

49. Forlani, M., and V. Coppola. The effect of 2-chloroethyl trimethyl ammonium chloride on grapevines of the cultivar I.M.6.0.13. Vignevini 5:29-32 (1978).

50. Gargiulo, A. A. Changes of sex in the vine. The transformation of male sex flowers into hermaphrodite ones by means of the application of synthetic kinin. Vitis 7:294-8 (1968).

51. Golinka, P. I. Gibberellin in grapevine sap. Fiziol. Biochim. Kul't. Rast. 5:321-4 (1973).

52. Hashizume, T., and M. Iizuka. Induction of female organs in male flowers of *Vitis* sp. by zeatin and dihydrozeatin. Phytochemistry 10:2653-5 (1971).

53. Hiroyasu, T., and H. Terani. Nutritional and physiological studies of grapevine. VI. Translocation and distribution of ^{32}P and ^{45}Ca. J. Jpn. Soc. Hortic. Sci. 32:85-90 (1963).

54. Hoad, G. V., B. R. Loveys, and K. G. M. Skene. The effect of fruit removal on cytokinins and gibberellin-like substances in grape leaves. Planta 136:25-30 (1977).

55. Hopping, M. E. Effect of light intensity during cane development on subsequent bud break and yield of Palomino grapevines. N.Z. J. Exp. Agric. 5:287-90 (1977).

56. Horridge, J. S., and K. E. Cockshull. Flower initiation and development in the glasshouse rose. Sci. Hortic. 2:273-84 (1974).

57. Huglin, P. Recherches sur les bourgeons de la vigne: Initiation florale et developpement vegetatif. Ann. Amelior. Plant. 8:113-272 (1958).

58. Huglin, P. Causes determinant les alterations de la floraison de la vigne. Ann. Amelior. Plant. 10:351-8 (1960).

59. Huglin, P., and B. Julliard. Sur l'obtention de sémis de vignes trés vigoreux á mise fruits rapide et ses répercussions sur l'amélioration génétique de la vigne. Ann. Amelior. Plant. 14:220-44 (1964).

60. Isoda, R. The effects of N, P, K and Ca fertilizer applications on the growth and yield of grapevines planted in granitic soil and diluvial soil derived from granite. J. Jpn. Soc. Hortic. Sci. 33:221-6 (1964).

61. Itai, C., and Y. Vaadia. Kinetin-like activity in root exudate of water stressed sunflower plants. Physiol. Plant. 18:941-4 (1965).

62. Jackson, D. I., and G. B. Sweet. Flower initiation in temperate woody plants. Hortic. Abstr. Flower initiation in temperate woody plants. Hortic. Abstr. 42:9-24 (1972).

63. Jako, N. The effect of treatment with adenine and uracil on the ribose and deoxyribose contents of vine leaves. Mitt. Klosterneuburg 18:411-5 (1968).

64. Jako, N. Einfluss der Behandlung mit Adenin, Uracil und TIBA auf den Geihalt an Nukleinsäuren sowie an frein aminosäuren der Rebenblätter. Mitt. Klosterneuburg 20:25-32 (1970).

65. Jako, N. The relationship between nitrogen phosphorus, potassium and magnesium nutrition and growth of grapevines and cytokinin production by the roots. Szoleszet es Borazet 1:35-47 (1976).

66. Kessler, B. R., R. Bak, and A. Cohen. Flowering in fruit trees and annual plants as affected by purines, pyrimidines and triiodobenzoic acid. Plant. Physiol. 34:605-8 (1959).

67. Kirikoi Ya, T. The effect of mineral fertilizers on changes in the composition of grapevine sap. Hortic. Abstr. 43:6748 (1973).

68. Kobayashi, A. S. Effect of potassium and phosphoric acid on the growth, yield and fruit quality of grapes. I. On the effect of the application level on non-fruiting and fruiting vines in sand culture. II. On the effect of time and level of application in sand culture. Mem. Res. Inst. Food Sci., Kyoto Univ. 23:28-46 (1961).

69. Kobayashi, A., S. Okamoto, H. Yukinaga, and S. Nakanishi. Studies on the thermal conditions of grapes. I. Effects of night temperatures on the growth and respiration of several fruit trees including grapes. Bull. Res. Inst. Food Sci., Kyoto Univ. 24:20-8 (1960).

70. Kobayashi, A., H. Yukinaga, and N. Nii. Studies on the thermal conditions of grapes. IV. Effect of day and night temperatures on the growth of Delaware. J. Jpn. Soc. Hortic. Sci. 34:77-84 (1965).

71. Kobayashi, A., A. Sugiura, H. Watanabe, and H. Yamamura. On the effects of day length on the growth and flower bud formation of grapes. Mem. Res. Inst. Food Sci., Kyoto Univ. 27:15-27 (1966).

72. Kozma, H. A., and D. Polyak. The rate of transport on different sides of vine shoots. Kortész Szöléz Fölskola Közl. 29:149-59 (1965).

73. Kurteeva, A. Ya, and Z. V. Kolesnik. Studies on the effect of some inhibitors on grapevine shoot growth. Primen. Fiziol. Akt. Veshchestva Sadovod. 2:123-7 (1974).

74. Lang, A. Gibberellins structure and metabolism. Annu. Rev. Plant Physiol. 21:537-70 (1970).

75. Larsen, R. P. Effect of potassium and magnesium fertilizers on the nutritional status and yield of a Concord grape vineyard. Q. Bull. Mich. State Univ. Agric. Exp. Stn. 45:376-86 (1963).

76. Lilov, D., and T. Andanova. Cytokinins, growth, flower and fruit formation in *Vitis vinifera*. Vitis 15:160-70 (1976).

77. Lilov, D., and Ch. Christov. Content of free gibberellins in the inflorescences and clusters of vines of different flower and flower formation. C.R. Acad. Bulg. Sci. 30:247-50 (1977).

78. Lilov, D., I. Ivanova, and D. Prodanski. Studies on the possible use of CCC for controlling inflorescence conversion in the grape cultivar Bolgar. Lazarstvo i vinarstvo. 23:5-8 (1974).

79. Livne, A., and Y. Vaadia. Water deficits and hormone relations. In: Water Deficits and Plant Growth. Vol. III. p. 241-75. T. T. Kozlowski, ed. Academic Press, New York, NY (1972).

80. Loeffler, J. E., and J. Van Overbeek. Kinin activity in coconut milk. In: Regulateurs Naturels de la Croissance Vegetale. p. 77-82. C.N.R.S., Paris (1964).

81. Loreti, S., and S. Natali. Effect of (2-chloroeythl) trimethylammonium chloride on growth and fruiting of Ciliegiolo grape variety. Am. J. Enol. Vitic. 25:21-3 (1974).

82. Loveys, B. R., and P. E. Kriedeman. Rapid changes in abscisic acid-like inhibitors following alterations in vine leaf water potential. Physiol. Plant. 28:476-9 (1973).

83. Mako, S. The effect of CCC treatment on grapevine shoots. Novenyvedelem 10:145-50 (1974).

84. May, P. Über die knospen — und infloreszenzentwicklung der Rebe. Wein-Wiss. 19:457-85 (1964).

85. May, P. Reducing inflorescence formation by shading individual Sultana buds. Aust. J. Biol. Sci. 18:463-73 (1965).

86. May, P. The effect of direction of shoot growth on fruitfulness and yield in the Sultana vines. Aust. J. Agric. Res. 17:479-90 (1966).

87. May, P., and A. J. Antcliff. The effect of shading on fruitfulness and yield in the Sultana. J. Hortic. Sci. 38:85-94 (1963).

88. May, P., and A. J. Antcliff. The fruitfulness of grape buds. I. Measuring bud fruitfulness on forced single-node cuttings. Ann. Amelior. Plant. 23:1-12 (1973).

89. May, P., N. J. Shaulis, and A. J. Antcliff. The effect of controlled defoliation in the Sultana vine. Am. J. Enol. Vitic. 20:237-50 (1969).

90. May, P., P. R. Clingeleffer, and C. J. Brien. Sultana (*Vitis vinifera* L.) canes and their exposure to light. Vitis 14:278-88 (1976).

91. Menary, R. C., and J. Van Staden. Effect of phosphorus nutrition and cytokinins on flowering in the tomato, *Lycopersicon esculentum* Mill. Aust. J. Plant Physiol. 3:201-5 (1976).

92. Monankov, M. K. The role of gibberellins in the morphogenesis of *Vitis vinifera* tendrils. Bot. Zh. (Leningrad) 61:69-77 (1976).

93. Moore, J. N. Cytokinin-induced sex conversion in male clones of *Vitis* species. J. Am. Soc. Hortic. Sci. 95:387-93 (1970).

94. Müller-Thurgau, H. Die geizen des weinstockes und deren bedeutung. Der Weinbau 9:9-10 (1883).

95. Mullins, M. G. Test-plants for investigations of the physiology of fruiting in *Vitis vinifera* L. Nature (London) 209:419-20 (1966).

96. Mullins, M. G. Regulation of fruitset in the grapevine. Aust. J. Biol. Sci. 20:1141-7 (1967).

97. Mullins, M. G. Morphogenetic effects of roots and of some synthetic cytokinins in *Vitis vinifera* L. J. Exp. Bot. 18:206-14 (1967).

98. Mullins, M. G. Regulation of inflorescence growth in cuttings of the grapevine (*Vitis vinifera* L.). J. Exp. Bot. 19:532-43 (1968).

99. Mullins, M. G., and C. Srinivasan. Somatic embryos and plantlets from an ancient clone of the grapevine (cv. Cabernet Sauvignon) by apomixis *in vitro*. J. Exp. Bot. 27:1022-30 (1976).

100. Muthukrishnan, C. R., and C. Srinivasan. Correlation between yield, quality, and petiole nutrients in grapes. Vitis 12:277-85 (1974).

101. Nakamura, R., and H. Arima. The effects of root temperature on the growth regulating substances in root exudates of Delaware vines. Sci. Rep. Fac. Agric., Okayama Univ. No. 34:47-56 (1969).

102. Negi, S. S., and H. P. Olmo. Sex-conversion in a male *Vitis vinifera* L. by a kinin. Science (NY) 152:1624-5 (1966).

103. Negi, S. S., and H. P. Olmo. Studies on sex conversion in male *Vitis vinifera* L. (Sylvestris). Vitis 9:89-96 (1970).

104. Negi, S. S., and H. P. Olmo. Conversion and determination of sex in *Vitis vinifera* L. Vitis 9:265-79 (1971).

105. Negi, S. S., and H. P. Olmo. Certain embryological and biochemical aspects of cytokinin SD 8339 in converting sex of a male *Vitis vinifera* (Sylvestris). Am. J. Bot. 59:851-7 (1972).

106. Nerson, H., M. Sibony, and M. J. Pinthus. A scale for the assessment of developmental stages of the wheat (*Triticum aestivum* L.) spike. Ann. Bot. 45:203-4 (1980).

107. Niimi, Y. Physiological effects of CCC on the growth of grapevines. J. Jpn. Soc. Hortic. Sci. 48:153-61 (1979).

108. Niimi, Y., M. Ohkawa, and H. Torikato. Cytokinin activities in grape berries. J. Jpn. Soc. Hortic. Sci. 46:297-302 (1977).

109. Niimi, Y., and H. Torikato. Changes in endogenous plant hormones in the xylem sap of grapevines during development. J. Jpn. Soc. Hortic. Sci. 47:181-7 (1978).

110. Nitsch, J. P., and C. Nitsch. Presence de phytokinines et autres substances de croissance dans la seve d'*Acer saccharum* et de *Vitis vinifera*. Bull. Soc. Bot. Fr. 112:11-9 (1965).

111. Obbink, J. G., D. McE. Alexander, and J. V. Possingham. Use of nitrogen and potassium reserves during growth of grape vine cuttings. Vitis 12:207-13 (1973).

112. Ogawa, Y., and R. W. King. Establishment of photoperiodic sensitivity by bezyladenine and a brief red irradiation in dark grown seedlings of *Pharbitis nil* chois. Plant Cell Physiol. 20:115-22 (1979).

113. Okamoto, G., Y. Konishi, and K. Shimamura. The retardation of lateral shoots growth and stimulation of inflorescence primordia development by spraying with CCC after harvest in Neo Muscat grapevines in a heated plastic glasshouse. Sci. Rept. Fac. Agric., Okayama Univ. 50:21-6 (1977).

114. Perold, A. I. A Treatise on Viticulture. p. 696. Macmillan, New York, NY (1927).

115. Phillips, I. D. J. Apical dominance. Annu. Rev. Plant Physiol. 26:341-67 (1975).

116. Pool, R. M. Effect of cytokinin *in vitro* development of Concord flowers. Am. J. Enol. Vitic. 26:43-6 (1975).

117. Pouget, R., and P. Casteran. Methode d'amelioration du pourcentage de de-bourrement sur les longs bois de taille de la Vigne. Conn. Vigne Vin. 4:393-403 (1971).

118. Pratt, C. Reproductive anatomy of grapes — A review. Am. J. Enol. Vitic. 22:92-109 (1971).

119. Pratt, C. Vegetative anatomy of cultivated grapes — A review. Am. J. Enol. Vitic. 25:131-50 (1974).

120. Prillieux, E. Considerations sur la nature vigne. Bull. Soc. Bot. Fr. 3:645-53 (1856).

121. Rao, V. N. M., and S. K. Mukherjee. Studies on pruning of grape. III. Fruit bud formation in Pusa Seedless grapes (*Vitis vinifera* L.) under Delhi conditions. Vitis 9:52-9 (1970).

122. Rao, V. N. M., and C. Srinivasan. Nucleic acid composition in the developing buds and petioles of grapes. Vitis 10:210-4 (1971).

123. Rao, V. N. M., S. Venkatachalam, C. P. Natarajan, and C. Srinivasan. Uptake and movement of phosphorus (^{32}P) in grapes. Vitis 10:103-6 (1971).

124. Rives, M. L'initiation florale chez la vigne. Conn. Vigne Vin. 2:127-46 (1972).

125. Roos, T. J. A shortening of the generation interval in the grape and the importance thereof in the breeding program. Dec. Fruit Grower. 18:11-13 (1968).

126. Sachs, R. M., and W. P. Hackett. Chemical control of flowering. Acta. Hortic. 68:29-49 (1976).

127. Salisbury, F. B. The dual role of auxin in flowering. Pl. Physiol. 30:327-34 (1955).

128. Sartorius, O. Zur Entwicklung und physiologie der Rebblüte. Angew. Bot. 8:29-62 (1926).

129. Shaulis, N. J., H. Amberg, and D. Crowe. Response of Concord grapes to light exposure and Geneva Double Curtain training. Proc. Am. Soc. Hortic. Sci. 89:268-80 (1966).

130. Schaeffer, G. W., and F. T. Sharp, Jr. Cytokinin Function: Kinetics of Methyl-[14]C Metabolism in Dormant and BA-activated Axillary Buds. R. L. Bieleski, A. R. Ferguson, and M. M. Cressell, eds. Bull. 12. p. 765-71. The Royal Society of New Zealand, Wellington (1974).

131. Shindy, W. W., and R. J. Weaver. Export of photosynthate affected when leaves are pretreated with growth substances. Nature (London) 227:301-2 (1970).

132. Shindy, W. W., W. M. Kliewer, and R. J. Weaver. Benzyladenine-induced movement of ^{14}C labelled photosynthate into roots of Vitis vinifera. Plant Physiol. 51:345-9 (1973).

133. Scholefield, P. B., and R. C. Ward. Scanning electron microscopy of the developmental stages of the Sultana inflorescence. Vitis 14:14-19 (1975).

134. Skene, K. G. M. Gibberellin-like substances in root exudate of Vitis vinifera. Planta 74:250-62 (1967).

135. Skene, K. G. M. Increase in the levels of cytokinins in bleeding sap of Vitis vinifera L. after CCC treatment. Science 159:1477-8 (1968).

136. Skene, K. G. M. The relationship between the effects of CCC on root growth and cytokinin levels in the bleeding sap of Vitis vinifera L. J. Exp. Bot. 21:418-31 (1970).

137. Skene, K. G. M. Cytokinins in xylem sap of grapevine canes: changes in activity during cold-storage. Planta 104:89-92 (1972).

138. Skene, K. G. M. The effect of ringing on cytokinin activity in shoots of the grapevine. J. Exp. Bot. 23:768-74 (1972).

139. Skene, K. G. M. Cytokinin production by roots as a factor in the control of plant growth. In: The Development and Function of Roots. J. G. Torrey and D. T. Clarkson, eds. p. 36-96. Academic Press, London (1975).

140. Skene, K. G. M., and A. J. Antcliff. A comparative study of cytokinin levels in bleeding sap of Vitis vinifera L. and the two rootstalks, Salt Creek and 1613. J. Exp. Bot. 23:283-93 (1972).

141. Skene, K. G. M., and G. H. Kerridge. Effect of root temperature on cytokinin activity in root exudate of Vitis vinifera L. Plant Physiol. 42:1131-9 (1967).

142. Smart, R. E., C. R. Turkington, and C. J. Evans. Grapevine response to furrow and trickle irrigation. Am. J. Enol. Vitic. 25:62-6 (1974).

143. Snir, I., and B. Kessler. Relationship between the cellular content of DNA in plants and their sensitivity to gibberellin. Plant Sci. Lett. 5:163-70 (1975).

144. Snyder, J. C. Flower bud formation in the Concord grape. Bot. Gaz. 94:771-9 (1933).

145. Srinivasan, C. Effect of certain fertilizer treatments with reference to the time of application in grapes (Vitis vinifera L.) var. Anab-e-Shahi. M.S. diss., Univ. Madras (1968).

146. Srinivasan, C. Control of inflorescence formation in the grapevine (Vitis vinifera L.). Ph.D. thesis, Univ. Sydney (1978).

147. Srinivasan, C., and M. G. Mullins. Reproductive anatomy of the grapevine (Vitis vinifera L.): Origin and development of the Anlage and its derivatives. Ann. Bot. 38:1079-84 (1976).

148. Srinivasan, C., and M. G. Mullins. Control of flowering in the grapevine (Vitis vinifera L.): Formation of inflorescences in vitro by isolated tendrils. Plant Physiol. 61:127-30 (1978).

149. Srinivasan, C., and M. G. Mullins. Flowering in Vitis: Conversion of tendrils into inflorescences and bunches of grapes. Planta 145:187-92 (1979).

150. Srinivasan, C., and M. G. Mullins. Effects of temperature and growth regulators on formation of Anlagen, tendrils and inflorescences in Vitis vinifera L. Ann. Bot. 45:439-46 (1980).

151. Srinivasan, C., and M. G. Mullins. Flowering in the grapevine (Vitis vinifera L.): Histochemical changes in apices during the formation of the Anlage and its derivatives. Z. Pflanzenphysiol. 97:299-308 (1980).

152. Srinivasan, C., and M. G. Mullins. High frequency somatic embryo production from unfertilized ovules of grapes. Sci. Hortic. 13:245-52 (1980).

153. Srinivasan, C., and M. G. Mullins. Induction of precocious flowering in grapevine seedlings by growth regulators. Ann.

Amelior. Plant. (in press) (1980).

154. Srinivasan, C., and M. G. Mullins. Flowering in Vitis: Effects of genotype on cytokinin — induced conversion of tendrils into inflorescences. Vitis (in press) (1980).

155. Srinivasan, C., and M. G. Mullins. Modification of leaf formation by cytokinin and chlormequat (CCC) in Vitis. Ann. Bot. (in press) (1980).

156. Srinivasan, C., and C. R. Muthukrishnan. Effect of potassium on the development of buds in grapes var. Anab-e-Shahi. Madras Agric. J. 57:700-3 (1970).

157. Srinivasan, C., and C. R. Muthukrishnan. Efficiency of time of application of nutrients on the development of the grape bud. Viticulture in Tropics. Proc. Int. Symp. Tropical and Subtropical Hortic. p. 129-35 (1972).

158. Srinivasan, C., C. R. Muthukrishnan, A. Shanmugam, and C. V. Chellam. Phosphorus metabolism in grape buds during floral initiation. Experientia 30:762-3 (1974).

159. Srinivasan, C., C. R. Muthukrishnan, and K. T. Shivashankara. Influence of the nutrients on the size of cluster primordia in grape buds (Vitis vinifera L.). Potash Review (Vitic.) 29:1-4 (1972).

160. Srinivasan, C., and V. N. M. Rao. Nucleic acids content and oxidative enzyme activity in the young shoots of grapevine. Indian J. Exp. Biol. 9:405-6 (1972).

161. Steward, F. C. Multiple interactions between factors that control cells and development. In: Perspectives in Experimental Biology. N. Sutherland, ed. Vol. 2. p. 9-24. (1976).

162. Sugiura, A., N. Utsunomiya, and A. Kobayashi. Effects of day-length and temperature on growth and bunch differentiation of grapevines. Jpn. J. Hortic. Sci. 43:387-92 (1975).

163. Sugiura, A., N. Utsunomiya, and A. Kobayashi. Induction of inflorescence by CCC application on primary shoots of grapevines. Vitis 15:88-95 (1976).

164. Sultanova, Z. K. Some data on the effect of retardants on grapevines in Kazakhstan. T. Tselinogr. S'kh. 12:168-72 (1975).

165. Teltscherova, L. Changes in the level of endogenous cytokinins in apical buds of Chenopodium rubrum L. Biol. Plant. 12:134-8 (1970).

166. Thimann, K. V. Fifty years of plant hormone research. Plant Physiol. 54:450-3 (1974).

167. Tucker, S. C., and L. L. Hoefert. Ontogeny of the tendril in Vitis vinifera. Am. J. Bot. 55:1110-9 (1968).

168. Usciati, M., M. Codaccioni, P. Mazliak, and V. Guern. Lipogenesis modifications induced by application of 6-benzylamino-purine to inhibited axillary buds of Cicer arietinum L. plants. Plant Sci. Lett. 2:295-301 (1974).

169. Utsunomiya, N., A. Sugiura, and T. Tomana. Effects of CCC and pinching treatment on the inflorescence induction and development on lateral shoots in grapevine. J. Jap. Soc. Hortic. Sci. 47:151-7 (1978).

170. Vaadia, Y., and A. N. Kasimatis. Vineyard irrigation trials. Am. J. Enol. Vitic. 12:88-98 (1961).

171. Wagner, R. Amelioration genetique de la vigne. Ann. Amelior. Plant. 25:151-75 (1975).

172. Weaver, R. J. Effect of growth retardant sprays on fruitfulness and cluster development of Thompson Seedless grapes. Am. J. Enol. Vitic. 26:47-9 (1975).

173. Weaver, R. J., and S. B. McCune. Response of certain varieties of Vitis vinifera to gibberellin. Hilgardia 28:297-350 (1959).

174. Weaver, R. J., and R. M. Pool. Effect of ethephon and a morphactin on growth and fruiting of 'Thompson Seedless' and 'Carignane' grapes. Am. J. Enol. Vitic. 22:234-9 (1971).

175. Weaver, R. J., and J. Van Overbeek. Kinins stimulate grape growth. Calif. Agric. 17:12-3 (1963).

176. Weaver, R. J., G. Alleweldt, and R. M. Pool. Absorption

and translocation of gibberellic acid in the grapevine. Vitis 5:446-54 (1966).

177. Weaver, R. J., W. W. Shindy, and W. M. Kliewer. Effect of growth regulators on movement of photosynthetic products into fruits of "Black Corinth" grapes. Plant. Physiol. 44:183-8 (1969).

178. Weaver, R. J., J. Van Overbeek, and R. M. Pool. Induction of fruit set in *Vitis vinifera* L. by a kinin. Nature (London) 206:952-3 (1965).

179. Wareing, P. F., and A. G. Thompson. Rapid effects of red lights on hormone levels. In: Light and Plant Development. H. Smith, ed. p. 285-94. Butterworth, London (1976).

180. Wellensiek, S. J. Principles of flower formation. Acta Hortic. 68:17-27 (1977).

181. Winkler, A. J. General Viticulture. p. 633. Univ. Calif. Press, Berkeley, CA (1965).

182. Winkler, A. J., and E. M. Shemsettin. Fruit bud and flower formation in the Sultanina grape. Hilgardia 10:589-600 (1937).

183. Yoshida, R., and T. Oritani. Studies on nitrogen metabolism in crop plants. 13. Effects of nitrogen top dressing on cytokinin content in the root exudate of rice plant. Proc. Crop Sci. Soc. Japan. 43:47-50 (1974).

184. Zeevaart, J. A. D. Physiology of flower formation. Annu. Rev. Plant Physiol. 27:321-48 (1976).

185. Zeevaart, J. A. D. Phytohormones and flower formation. In: Phytohormones and Related Compounds — A Comprehensive Treatise. Vol. 2. p. 291-328. D. S. Letham, P. B. Goodwin and T. J. V. Higgins, eds. Elsevier, North Holland (1978).

186. Zimmermann, J. Sprosshistologie und Holzreife bei Rebe. Mitt. Klosterneuburg 4:101-9 (1954).