REGULATION OF FRUIT SET IN THE GRAPE VINE

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Summary

Defoliation reduces fruit set in the grape vine, but the mechanism of this effect is the subject of controversy. Effects of leaf removal on set have been attributed either to reduction in supply of organic nutrients to the developing bunch or to reduction in supply of fruit-setting factors. In the experiments reported here, small immature fruits developed on defoliated and decapitated vine cuttings, on cuttings in which leaves, apices, and roots were removed as they appeared, and on inflorescences which were cultured in the light *in vitro* on a medium devoid of exogenous growth substances. These results indicate that fruit set is regulated by supply of organic nutrients rather than by specific hormonal stimuli originating from organs external to the developing bunch.

I. INTRODUCTION

The early stages of fruit development in the grape vine (*Vitis vinifera* L.) involve rapid growth of the ovary wall and prevention of abscission layer formation in the pedicel. In horticultural literature these processes are usually referred to collectively as "fruit set".

In cinctured shoots of seeded grape vine cultivars, Coombe (1962) has shown that fruit set is enhanced by the presence of mature leaves, but reduced by immature leaves and shoot apices. Supply of organic nutrients to developing ovaries was suggested as a controlling factor in fruit set, and effects of immature leaves and shoot apices were attributed to their ability to procure nutrients at expense of the developing bunch. These conclusions are supported by results of autoradiographic studies of assimilate movement during fruit development by Hale and Weaver (1962). However, other work by Weaver, McCune, and Hale (1962) discounts nutrient availability as a controlling factor in fruit set. In defoliation experiments with cinctured shoots of the seedless cultivar Black Corinth (syn. Currant) they found that fruit set was nil if no leaves were retained, but that presence of only 1 sq in of leaf lamina was sufficient to enable set of 50% of berries. Weaver, McCune, and Hale concluded that carbohydrate production alone was unlikely to be a cause of set, and they postulated that leaves produce a fruit-setting factor.

The object of experiments to be reported here was to attempt to resolve these conflicting theories of regulation of fruit set in the grape vine. On the one hand, if fruit set is regulated by supply of organic nutrients, growth of fertilized flowers into fruits could be expected to occur in the absence of leaves, provided that nutrient supply is non-limiting. On the other hand, if the early stages of fruit development are controlled by specific factors produced by leaves, absence of leaves would prevent further development of fertilized flowers even under conditions of favourable nutrition. These hypotheses were tested by investigations of fruit set in specially propagated vine cuttings, and by culture of vine inflorescences *in vitro*.

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II. Materials and Methods

(a) *Production of Experimental Cuttings*

Dormant canes of the cultivar Cabernet Sauvignon were placed in plastic bags and stored under refrigeration (4°C) until required. Canes of uniform diameter were made into cuttings with varying numbers of nodes (1–6). The uppermost bud on each cutting was retained and all others were cut off. Cuttings were made so that their uppermost buds all originated from the same region of parent canes, i.e. nodes 7, 8, or 9 from the point of insertion of the cane on the grape vine. Cuttings were weighed, numbered, and planted in pots containing a mixture of perlite and John Innes No. 1 compost. Plants were grown in a glasshouse under natural illumination during spring and summer (max. 30°C, min. 18°C).

The inflorescences of woody vine cuttings usually stop growing soon after bud burst and they shrivel and die. Atrophy of inflorescences is prevented if expanding leaves are removed from the base of the new shoot, and partial defoliation allows production of small bunches of grapes by single-node cuttings (Mullins 1966). In the present experiments manipulation of growth was carried a stage further. Instead of removing only the basal leaves, all organs except a single inflorescence were dissected out of the expanding buds of vine cuttings, and experimental plants consisted of a single inflorescence borne on a woody cane. Dormant vine canes are a rich source of reserve carbohydrates, and much of the new growth during establishment of cuttings is made at the expense of reserve materials (Buttrose 1966).

In the present experiments leaves, apices, and second inflorescences were excised soon after bud burst (Plate 2, Fig. 1). Any lateral or stipulary buds which grew on the new stem, proximal to the base of the rachis, were removed as they appeared. Inflorescences were pruned to 200 flowers following elongation of rachises and emergence of flowers from their enclosing bracts.

(b) *Effects of the Presence of Roots on Fruit Set*

This was investigated with defoliated and decapitated cuttings grown in aerated solution cultures (half-strength standard Hoagland solution). The bases of treated cuttings were scraped with a razor-blade at frequent intervals to prevent the establishment of a root system, and retention of fruits by treated cuttings was compared with that of plants in which roots were allowed to develop normally. Establishment of cuttings in aerated solution cultures was difficult because of contamination of the solutions by substances which exuded from the bases of cuttings and from decaying bark tissues. Solutions were changed on alternate days, but cuttings grew less vigorously than those propagated in soil mixtures.

(c) *Culture of Vine Inflorescences* in vitro

Inflorescences were excised from single-node cuttings at the stage shown in Plate 2, Figures 1 and 2, and surface-sterilized by shaking with a 3·5% solution of sodium hypochlorite for 35 min. Inflorescences were then washed four times with sterile glass-distilled water, dried between sterile filter papers, and planted into

150-ml Erlenmyer flasks containing 50 ml of agar medium. The composition of the medium was according to White (1954), but without addition of auxin. Cultures were grown either in a controlled-environment chamber with fluorescent illumination of 2000 f.c. for 12 hr, or in the dark and at a constant temperature of 23°C. Experiments reported here were with single passages. Attempts to transfer explants to fresh media were usually unsuccessful. Developing inflorescences became very fragile, and the slightest damage during transfer caused explants to turn brown and die. When anthers were produced by developing inflorescences the sides of the culture flasks were tapped gently with a glass rod to vibrate the explant and assist in the dissemination of pollen.

III. Results

(a) *Inflorescence Growth and Fruit Set in Defoliated and Decapitated Vine Cuttings*

In experimental cuttings which retained their bunches, the course of inflorescence growth was similar to that of normally grown grape vines. Elongation of the rachis was accompanied by swelling of flowers and expansion of pedicels. Flowers emerged from their enclosing bracts and became separated shortly before anthesis. Few inflorescences survived up to anthesis when the original fresh weight of cuttings was less than 8 g. Included in this category were most of the single-node cuttings. In cuttings of two and three nodes there were portions of the inflorescence which failed to elongate, and flowers remained enclosed by bracts. These areas of attenuated development were found at the tips of inflorescences and at the base of the rachis. Before pruning of inflorescences to 200 flowers it was noted that cuttings of five and six nodes produced inflorescences with similar numbers of flowers to those of established vines of Cabernet Sauvignon, e.g. 400—1000 flowers. An unusual feature of the inflorescences of defoliated and decapitated cuttings was the development of a dark pigmentation by the hypoclade (Plate 1, Figs. 1 and 2). These areas of dark pigmentation have not been observed in the inflorescences of field vines.

Fruit set in defoliated and decapitated cuttings occurred in the fortnight following full bloom, and fruitlets present at the end of this period were usually retained, i.e. a situation similar to that in field vines. However, numerous, very small fruitlets were retained by the experimental cuttings—fruitlets which normally absciss under field conditions. There was only slight growth of the pericarp in these fruitlets and lack of seed development, and they were discarded for recording purposes. Within each inflorescence many fruitlets continued to grow for 6–8 weeks after full bloom. In all cases, growth of these seeded fruits ceased before there were any visible signs of berry maturation, i.e. change in colour or softening of the berry.

Fruits were harvested when no increase in diameter was observed over a 4-week period, and included in the harvest were all seeded fruits of diameter greater than 3 mm. It was found that the greater the original fresh weight of the cutting the greater was the number of fruits retained and the greater was the final size of the fruit (Plate 1, Figs. 1–3; Table 1).

(*b*) *Fruit Set in the Absence of Leaves, Apices, and Roots*

Removal of emerging roots had no detectable effects on inflorescence growth in decapitated and defoliated cuttings, and similar numbers of flowers were produced by both treated plants and by controls. As in previous experiments inflorescences were pruned to 200 flowers shortly before anthesis. Fruit set was very variable in cuttings grown in aerated solution cultures, and attempts to derive quantitative

TABLE 1

FRUIT PRODUCTION BY DEFOLIATED AND DECAPITATED CUTTINGS

Original number of cuttings per treatment = 40. Inflorescences were pruned to 200 flowers

	Number of Nodes				
	2	3	4	5	6
Mean fresh weight of cuttings at planting (g)	13·0	17·8	23·2	26·4	34·3
Number of cuttings with inflorescences surviving at anthesis	6	14	13	16	18
Mean number of fruits harvested	41±11	53±7	48±7	69±7	94±6
Mean diameter of the five largest fruits (mm)	4·1±0·5	4·0±0·3	5·0±0·3	5·2±0·3	6·2±0·3

estimates of effects of numbers of roots on numbers of fruits retained were unsuccessful. However, numerous immature seeded fruits were produced by both rooted cuttings and by cuttings which were prevented from establishing a root system. Hence, it appears that lack of a root system does not preclude fruit set in the grape vine (Table 2).

TABLE 2

EFFECT OF ROOT REMOVAL ON FRUITING OF DECAPITATED AND DEFOLIATED CUTTINGS

Cuttings were of 4 nodes. Original number of cuttings per treatment = 80

	Roots Present	Roots Removed
No. of cuttings with inflorescences surviving at anthesis	29	37
No. of cuttings producing seeded fruits greater than 3 mm diameter	18	24
Mean fresh weight of fruits harvested (g)	0·93±0·14	0·73±0·17

(*c*) *Fruit Set* in vitro

Aseptic culture of vine inflorescences was made difficult by high rates of infection of explants. Approximately 65% of cultures were lost because of infections within 4 or 5 days from inoculation. There were also latent infections, and inflorescences frequently succumbed to fungi after 2 or 3 weeks in culture. All forms of infection were difficult to control, and probably resulted from presence of spores in portions of the inflorescence which were inaccessible to the sterilizing agent.

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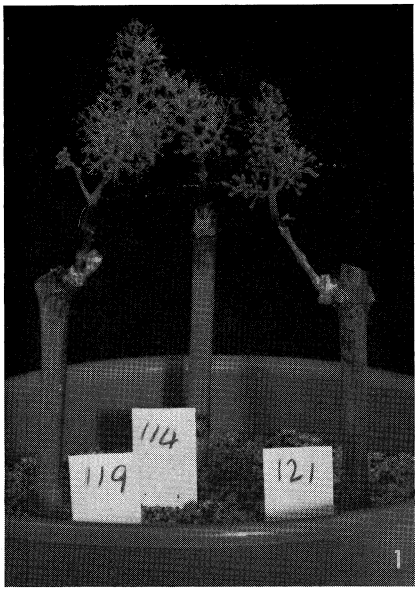

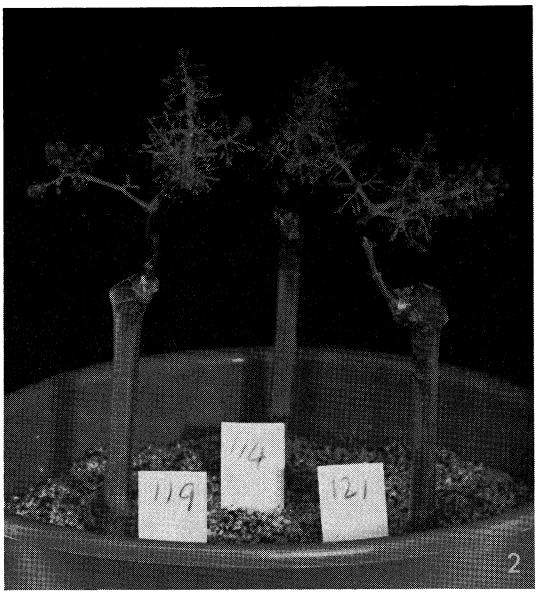

Figs. 1 and 2.—Two-node cuttings of Cabernet Sauvignon photographed at anthesis (Fig. 1) and 6 weeks later (Fig. 2).

Fig. 3.—Cuttings of Cabernet Sauvignon composed of two, three, four, and six nodes respectively. Inflorescences were pruned to 200 flowers before anthesis. Photographed 8 weeks after anthesis.

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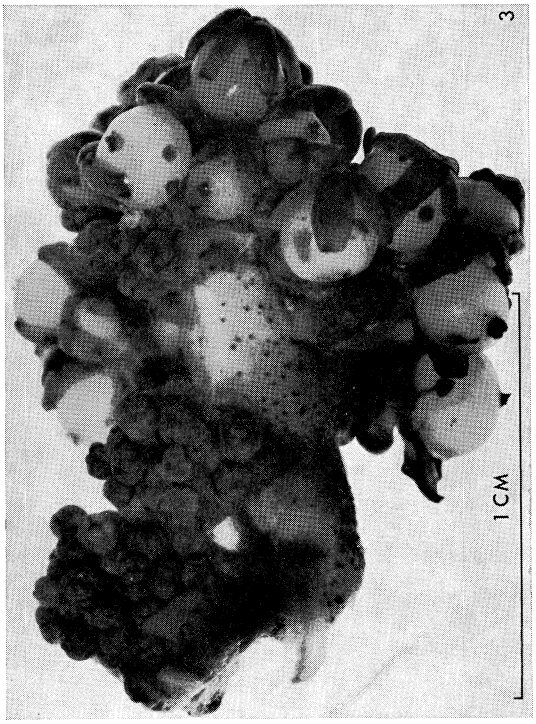

Fig. 1.—Cutting of Cabernet Sauvignon showing stage of growth when inflorescences were excised for culture *in vitro*. (Decapitation and defoliation treatments were applied at a similar stage of growth for production of fruiting cuttings.)

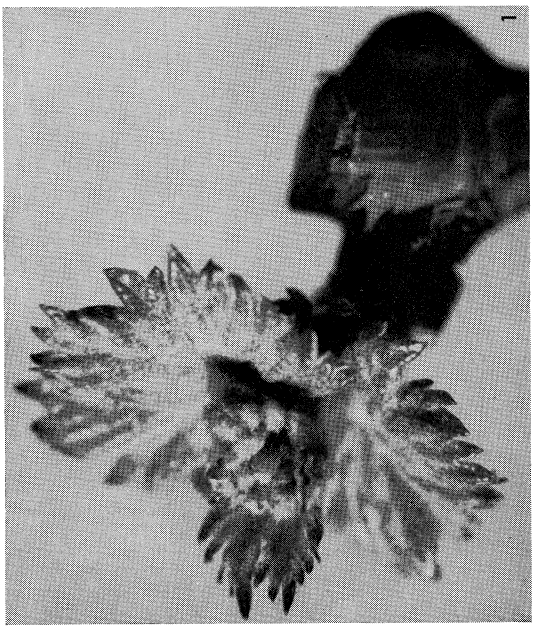

Fig. 2.—Excised inflorescence.

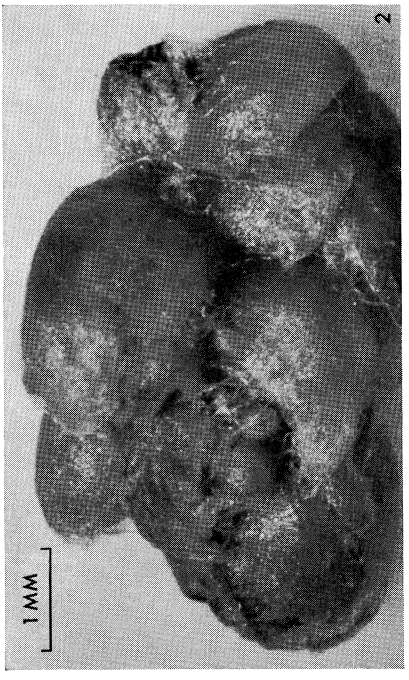

Fig. 3.—Fruiting explant. Photographed 4 months after planting of immature inflorescence in the culture medium.

Explants grown in the dark produced a mass of callus tissue and a few roots, but there was no further development of flowers. Growth and differentiation of flowers and production of fruits occurred only when explants were illuminated. Here, the first sign of growth in sterile cultures occurred after 2 weeks when the rachis began to elongate and to produce callus tissue at its surface. This callus tissue later grew to ensheath the entire rachis (Plate 2, Fig. 3). Elongation of the rachis was accompanied by swelling of flowers, and the enclosing bracts folded back and atrophied. There was little or no elongation of pedicels, and developing flowers remained in compact groups. After 2 months in culture, explants appeared to separate into two distinct populations with respect to their future growth and development. Most inflorescences turned red in colour and stopped growing, but a few explants remained green and they subsequently flowered and produced a number of small fruits (Plate 2, Fig. 3). Of 160 successfully established explants, growth of flowers into fruits occurred with only six inflorescences. However, the course of flower development in these explants resembled that of normally grown grape vines. The flowers continued to swell, and calyptrae dehisced to expose the anthers. Anther size was greatly reduced as compared with that of established vines and the filaments failed to elongate. Swelling of the pericarp was observed approximately 3 weeks after the appearance of anthers, and fruitlets continued to grow for another month. After this no further increase in fruitlet size was observed. The experiment was terminated after explants had been in the culture flasks for 4 months. The largest fruits produced *in vitro* were 3 mm in diameter, and microscopic examination of seeds revealed evidence of embryo development.

IV. Discussion

Small, immature, seeded fruits were produced by defoliated and decapitated cuttings (Plate 1, Figs. 1–3), by cuttings in which leaves, apices, and roots were removed as they appeared (Table 2), and by a few inflorescences which were cultured *in vitro* on a medium containing only sucrose, mineral salts, glycine, and vitamins (Plate 2, Fig. 3). In the experiments with cuttings, supplies of organic nutrients were provided by the reserves of the parent stem, and the larger the cutting the greater were the numbers and sizes of fruits produced (Table 1).

Presence of leaves is evidently not a prerequisite for fruit set, and results do not support the hypothesis of Weaver, McCune, and Hale (1962) that fruit set in vines is regulated by specific factors produced in leaves. Similarly, it seems unlikely that the early stages of fruit development are regulated directly by factors originating in either apices or elongating roots. Fruit set does not appear to be initiated or triggered by receipt of hormonal stimuli from organs external to the developing bunch. Results of the present experiments suggest that fertilized flowers possess the potential for fruit growth, and that realization of this potential is related to availability of organic nutrients. Further support for this theory is provided by re-examination of the findings of Weaver, McCune, and Hale (1962). No fruits were set when all leaves were removed from cinctured shoots of Black Corinth vines, and they found that presence of only 1 sq in of leaf lamina enabled set of 50% of berries. In the case of defoliated cinctured shoots, lack of set may be attributed to lack of assimilates

because presence of the cincture would prevent translocation of organic nutrients to the developing bunch from other regions of the vine. In addition, there would be little or no reserve starch within the shoot distal to the cincture, the shoots having been cinctured before the onset of starch accumulation. The fact that a very small area of leaf had such a marked effect on fruit set may have been due to a high photosynthetic rate. A positive correlation between photosynthetic rate and sink size has been observed in a number of plants (e.g. Nösberger and Humphries 1965).

Inflorescences and developing fruits possess a degree of self-sufficiency with respect to growth substance requirements, and this attribute appears to be related to light-dependent processes. Isolated inflorescences failed to develop when grown in the dark on a medium devoid of exogenous growth substances, but inflorescences grown on a similar medium in the light produced flowers and fruits (Plate 2, Fig. 3). Moreover, production of fruits was found only with explants which retained a green coloration.

In the Calimyrna fig, Crane (1965) has shown that applications of auxin, gibberellin, and cytokinin all produce parthenocarpic fruits of similar gross morphology, and he suggests that endogenous hormones regulate fruit growth by effects on the mobilization and translocation of nutrients. Fruits are able to mobilize both organic and inorganic nutrients (Leonard 1962), and effects of growth substances on the translocation of nutrients are thought to be of importance in many aspects of plant development (Mothes, Engelbrecht, and Kulajewa 1959; Booth *et al.* 1962; Seth and Wareing 1964; Nanda and Purohit 1965). Crane postulates that the hormones originating in seeds regulate fruit growth by attracting metabolites from other regions of the plant. A similar theory has been proposed by Coombe (1965) who suggests that treatments which increase fruit set in vines do so by enhancing the ability of developing ovaries to attract organic nutrients. These theories are supported by evidence of correlative effects on fruit set, for example, stimulation of fruit set following removal of shoot tips and immature leaves (Coombe 1962), and production of small fruits by defoliated and decapitated vine cuttings. Accordingly, an important factor in regulation of fruit set in the intact plant would appear to be the relative rates of growth substance production by inflorescences, expanding leaves, apices, and perhaps roots, because these relationships would determine the relative abilities of organs to attract metabolites.

In their early work on regulation of abscission, Addicott, Lynch, and Carns (1955) suggested that abscission-layer formation is an auxin-controlled process, and dependent upon the maintenance of a positive concentration gradient from the proximal to the distal side of the abscisson zone. More recently, Addicott and his coworkers have isolated abscission-accelerating substances or abscisins from cotton fruits (Addicott *et al.* 1964), and Chaterjee and Leopold (1964) have shown that gibberellin and cytokinin are also implicated in abscission regulation. It now seems clear that factors other than auxin are involved, but the importance of these factors in control of fruitlet abscission in the grape vine has yet to be decided. Applications of exogenous auxins to fruiting vines often produce effects which are consistent with the auxin-gradient theory of abscission regulation; for example, small undeveloped berries which normally absciss are retained if bunches are treated with

p-chlorophenoxyacetic acid (Coombe 1953). Results of the present experiment are also consistent with the auxin-gradient theory. In the experimental cuttings it is likely that removal of apices and leaves reduced the supply of auxin to the proximal side of the pedicel base, and that production of auxin by berries alone was sufficient to prevent abscission layer formation. Such an effect would account for the retention of numerous undeveloped berries (Plate 1, Fig. 2). The failure of these fruits to grow, although attached to the plant, suggests that abscission layer formation and fruit growth are independently controlled.

V. Acknowledgment

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