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Finally, proof that leaf floral induction with photoperiodic dependent flowering does not occur in the grapevine plant (*Vitis vinifera* L. 'Chardonnay')

P. W. Noyce

School of Environmental and Life Sciences, University of Newcastle, Callaghan, New South Wales, Australia

Summary

This research study, for the first time, presents unequivocal evidence that leaf derived photoperiodic dependent flowering (PDF) does not occur in the grapevine. The morphological changes in the shoot apical meristem (SAM), at the beginning of the flowering pathway in angiosperms, are described as starting in two possible ways: with floral induction stimulus from the leaf or a non-leaf stimulus directly on the SAM. Floral induction, floral evocation, floral initiation then floral development are defined and discussed, together with the current concepts and classification of plant PDF. The only two research papers in the English language literature suggesting grapevine PDF absence are described, with a detailed analysis in the discussion that raises doubt as to the validity of the data and subsequent conclusions. To examine the hypothesis that "leaf derived PDF does not occur in the grapevine" *Vitis vinifera* L. 'Chardonnay' grapevines were completely defoliated from budburst as leaves appeared, and compared to a control group (where all leaves were left) by microscopic dissection of compound latent buds at 31, 54 and 76 days post budburst. Latent bud inflorescence primordia developmental stages, at each of the sample dates, were the same in treatment and control groups. The experimental data supports the hypothesis, indicating that leaves, with floral induction, are not needed to initiate the grapevine flowering pathway. The initial stimulus to commence grapevine flowering is directly on the compound latent bud vegetative SAM with no leaf involvement. Further research investigating the presence or absence of floral evocation in grapevines, and its timing if the event occurs, is now enabled.

Key words: grapevine; inflorescence primordia; floral induction; floral initiation; floral evocation; photoperiodic dependent flowering.

Introduction

In flowering plants (angiosperms) the morphological changes of the flowering pathway commence in the vegetative shoot apical meristem (SAM), with a "floral induction" signal from the leaf or a non-leaf stimulus directly on the SAM. Both stimuli can induce "floral evocation". This is followed by "floral initiation", "floral development" and eventually anthesis (the mechanical process of flowering when mature flowers are ready for pollination), leading to the eventual formation of fruit (Baurle and Dean 2006, Ha 2014).

Floral induction: Photoperiod is the relative length of the day and night in a daily cycle of 24 h (Garner and Allard 1920). "Floral induction" was defined by Evans (1971) as the physiologic reactions, specific to the leaves of flowering plants, to a leaf photoperiod stimulus resulting in the production of a mobile signalling molecule which is transported to the SAM to induce floral evocation (see definition below). The leaf reactions to day-night length leading to flowering are well-researched and well known processes, originally named "photoperiodism" by Garner and Allard (1920). These authors described photoperiodism as a plant's ability to flower in response to changes in the photoperiod effect on the leaf. Since then research has shown that photoperiodism controls many more plant physiological events than just flowering-such as germination, tuberization, budbreak, stem elongation, leaf growth, anthocyanin pigment, sex expression, and dormancy onset (Vergara 1978, Thomas and Vince-Prue 1997, Thomas 2006). The photoperiod stimulus may be *via* the leaf or directly on the relevant organ. So the process concerned with flowering commencing with floral induction is perhaps best named "leaf photoperiodism" or "photoperiodic dependent flowering" (PDF).

Floral induction mechanisms include the detection of the light signal in the leaves, the entrainment of leaf circadian rhythms, and the production of a mobile signal in the leaf which is transmitted throughout the plant (Thomas and Vince-Prue 1997, Jackson 2009, Matsoukas *et al.* 2012). A protein, commonly termed "FT protein", has been shown to be produced in leaf phloem companion cells and is a mobile signal transporter from the leaves, *via* phloem, to the SAM where it triggers floral evocation in diverse species, including annual plants, biennial plants and woody and herbaceous perennial plants (Corbesier *et al.* 2007, Lagercrantz 2009, Andres and Coupland 2012, Jaeger *et al.* 2013, Matsoukas 2015, Shim *et al.* 2017). The molecular mechanisms and control of floral evocation by floral induction is best understood in the annual plant model species *Arabidopsis thaliana* (Tan and Swain 2006, Wong *et al.* 2009, Khan *et al.* 2014, Shim *et al.* 2017). Accumulating evidence suggests this is a highly conserved mechanism that evolved during the evolution

Correspondence to: P. W. Noyce, School of Environmental and Life Sciences, University of Newcastle, Callaghan, New South Wales 2308, Australia. E-mail: pwnoyce@gmail.com

of flowering plants, and likely occurs in all angiosperms undergoing floral induction (Paton *et al.* 2008).

Floral induction has been shown to commonly occur in annual plants and herbaceous perennials but has rarely been demonstrated conclusively in woody perennials (Wilkie *et al.* 2008), apart from several blueberry tree species (*Vaccinium darrowii* and *Vaccinium corymbosum*) (Spann *et al.* 2003) and avocado trees (*Persea Americana* Mill., 'Fuerte') (Buttrose and Alexander 1978). This may be because floral induction actually does not occur in woody perennials or because of the difficulties in conducting PDF research in these plants. Such plants, compared to annuals, have long juvenile periods, lifespans over many years, long flowering pathways (often over more than one season and year) and relatively large size. These constraints compound the difficulty of experiments in space-limited glasshouses or environmentally controlled growth chambers, the apparatus most commonly used for investigation of PDF (Thomas and Vince-Prue 1997).

Floral evocation: was first defined by Evans (1969, 1971) as: The initial biochemical events in the SAM in response to the arrival of a stimulus from the leaf (produced by floral induction) which commit the plant to subsequent formation of flowers. Since then the definition of floral evocation has been broadened to be: The irreversible biochemical and cellular changes in the SAM induced by non-leaf derived stimuli directly on the SAM (where evocation is the first event in flowering) or by mobile signal stimuli from leaf floral induction (where floral induction is the first event in flowering) (Bernier 1988, Noyce *et al.* 2016a). At evocation there is an irreversible SAM change from a vegetative phase, where leaves form and the meristem grows, to a reproductive phase where flowers are eventually formed (Huijser and Schmid 2011). The SAM has become competent to flower. The reproductive phase is divided into an inflorescence meristem mode, with the ability to eventually produce flowers as well as leaf primordia, then a floral meristem mode when stage 1 flower primordia actually appear on the inflorescence meristem (Batey and Tooke 2002). The change from a vegetative to an inflorescence mode is induced by exogenous environmental signals such as temperature and photoperiodism, both sensed in the leaf (as in annual plants), or by environmental and endogenous developmental signals operating directly on the SAM (as in woody perennial plants). Colsanti and Sundaresan (1996) summarise these phases as the **V** to **I** to **F** progression (**V**egetative to **I**nflorescence to **F**loral meristem) but comment that "in some plants the vegetative meristem is converted directly into a floral meristem".

Floral initiation: is defined as the first appearance of differentiated cells in the SAM (Tan and Swain 2006). It can be viewed as the first external morphological result of floral evocation (May 2000). In the angiosperm flowering model annual plant *Arabidopsis thaliana* this is the appearance of a sepal primordium bract (a flower primordium) on the inflorescence meristem flanks (Smyth *et al.* 1990). In the grapevine floral initiation is the appearance of stage 1 inflorescence primordia (IP), not flower primordia, on the vegetative meristem early in season 1 (see grapevine growth cycle below). In *Vitis vinifera* L. 'Chardonnay' floral initiation in the primary bud of the compound latent bud on node four is four to five weeks post budburst (Noyce *et al.* 2016a). Floral initiation then progresses acropetally to the shoot tip with two to three days between initiation in successive nodes (Barnard and Thomas 1932, Swanepol and Archer 1988).The grapevine IP then pass through growth stages, with increasing size and complexity, throughout season 1 and winter dormancy, into early season 2 the next year. Flower primordia then develop on the inflorescence branches (*i.e.* the formation of a floral meristem) with full mature flowers present after several weeks (Noyce *et al.* 2016a).

Floral development: refers to the entire pathway of development to a full mature flower, once floral evocation has occurred. In the grapevine this progresses over twelve months (Figure).

The grapevine annual growth cycle: The grapevine is a deciduous woody perennial plant with repeatable developmental stages and events throughout each year during a long lifetime. The beginning for a grapevine growth cycle description, in temperate climates, is at spring budburst on a shoot on a vegetative propagated adult vine (Mullins *et al.* 1992). This is the start of season 1. The growth cycle of season 1 continues into the next year, with season 1 finishing at the end of that next year's winter, at dormancy end (Figure). Season 2 then commences with the next spring budburst.

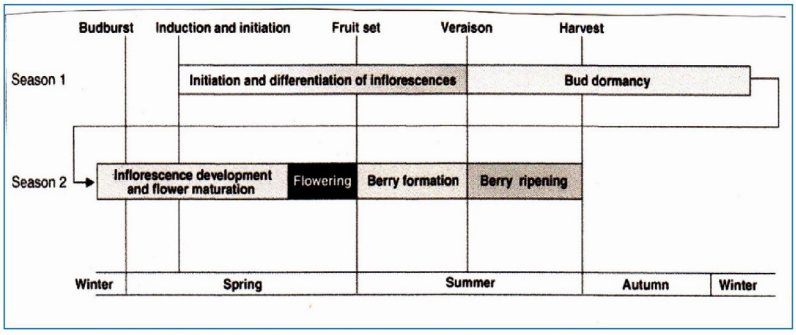

Figure: The grapevine annual growth cycle. A full season begins in spring (September in the southern hemisphere) and finishes at winter end of the next year (August in the southern hemisphere). The SAM reproductive cycle commences at evocation (called induction in this Figure) followed by initiation, leading eventually to the process of flowering. The cycle starts in the spring of season 1 and extends to the end of spring in season 2: a period of 12 to 14 months (from Carmo Vasconcelos *et al.* 2009, © 2009 American Society for Enology and Viticulture. AJEV **60**, 411-434).

The grapevine reproductive or flowering cycle: The formation of inflorescences and eventually flowers in the grapevine latent bud involves four progressive well-defined growth phases that occur over two consecutive growing seasons, commonly named season 1 and season 2 (Figure). The first three phases are all completed during the first or current season (season 1) in the compound latent bud SAM: Phase one is postulated to be floral evocation (Buttrose 1969b) although it is still not known if this event occurs in the grapevine. Phase two is IP initiation and phase three is IP differentiation (the progression of stage 4 IP to stage 5a IP (Noyce *et al.* 2016a) followed by development and growth of the primordia to IP stage 7 by the end of season 1. The fourth phase takes place in the second or next season (season 2), before budburst in the compound latent bud, and then on the post budburst inflorescence (Noyce *et al.* 2015, 2016a). This phase includes the further growth of differentiated IP and tendril primordia, then growth of flowers on the IP branches.

Photoperiodic dependent flowering classification: Plants showing a PDF response were first classified by Garner and Allard (1920, 1923) into three categories, based on the day length in a plants photoperiod. Subsequent research showed that a plant's leaf actually measured the length of night or darkness and it is the dark period that is crucial in flowering initiation (Hamner 1940, Thomas and Vince-Prue 1997). The three categories include: (i) Short day plants (SDP) show floral initiation when day length falls below a specific duration, called the critical day length (CDL)-thus in the photoperiod the day is short and night length long. (ii) Long day plants (LDP) show floral initiation when the day length exceeds the CDL, with the photoperiod having a long day and short night. The terms "short" and "long" are relative, merely indicating that one plant (SDP) will flower when day length is decreasing and falls below its CDL. Another plant species (LDP) will flower when day length is increasing and falls above its CDL (Kobayashi and Weigel 2007). The CDL varies greatly between species and often between cultivars within species, with each plant having a unique CDL. Within a species CDL can vary with environmental conditions, including latitude and changing temperature, and with plant age (Thomas and Vince-Prue 1997, Tsuchiya and Ishiguri 1981, Jackson 2009). (iii) Day neutral plants (DNP) are insensitive to day length with no PDF-flowering can occur in day lengths from five to 24 h. DNP are the most common angiosperm flowering pattern (Hillman 1962).

For convenience in many experiments photoperiod is set up for long day conditions as 16 h of light and 8 h of dark, while short days have 8 h of light and 16 h of dark. Many plants flower in these conditions but others have a very different CDL and fail to respond (Salisbury 1982, Thomas and Vince-Prue 1997). A photoperiodic induction cycle is the plant's specific and appropriate photoperiod under which flowering is initiated (Salisbury 1961). Plants require one or more inductive cycles for flowering and the critical number (CN) is the minimum number of inductive cycles to induce flowering. Most plants have a CN of one to ten with rare species well above.

A night break in PDF experiments is a flash of low intensity light during the dark period, usually in the middle. A night break prevents flowering in SDP and promotes flowering in LDP (Jackson 2009, Lagercrantz 2009).

Obligate or qualitative SDP and LDP only flower in short or long days respectively-the specific day length is an absolute requirement. A facultative or quantitative SDP and LDP will eventually flower regardless of day length but flowering is accelerated by short days and long days respectively (Thomas and Vince-Prue 1997).

Since the 1920s research has shown that the variation in PDF is quite complex and diverse, with at least four more categories described: Long-short day plants (LSDP) are short day plants that flower only after a sequence of long days followed by short days-i.e. in late summer and autumn when days are shortening. Short-long day plants (SLDP) are long day plants that flower only after short days followed by long days, *i.e.* in early spring when days are lengthening (Thomas and Vince-Prue 1997). Intermediate day plants are a rare modification of short day plants: Flowering is initiated when the length of darkness is a specific definite length (not greater or lesser than a critical night length)-flowering will not occur if night length is longer or shorter (Runkle *et al.* 2001). Ambiphotoperiodic plants, also rare, flower with two different induction cycles where the CDL is a precise length, usually between 12 and 14 h. Floral initiation occurs when the CDL is shorter or is longer than this length (Williams 1994).

Photoperiodic dependent flowering in the grapevine: PDF is said to be absent in the grapevine, which is classified as a day neutral plant. Research papers in the German language literature, by Alleweldt, from 1959 to 1964, are frequently quoted as the authority for citing the lack of PDF in grapevines (Buttrose 1974, Srinivasan and Mullins 1981, Carmona *et al.* 2007). These prior authors comment that Alleweldt's reports were based on field and growth cabinet work that was suggestive of lack of PDF in grapevines. In the English literature two papers, published during the 1960s, reported experimental evidence supporting this claim, with no research since:

May (1965) shaded individual 'Sultana' cultivar latent buds only, in the field in Southern Australia, early in the season during the time of floral initiation and subsequent IP differentiation, for four consecutive seasons (1957-1960). The response was measured by compound latent bud dissections at dormancy. The primary experimental aim was to examine the effect of irradiance reduction on floral initiation and IP differentiation. However the results also suggested a lack of PDF or as May (1965) stated "did not provide evidence that, in the sultana, floral initiation is connected with the phytochrome system which controls photoperiodic determination of flowering in many plants". In these experiments concerning PDF leaves were not shaded, yet compound latent bud shading, including heavy (this was not quantified), complete bud darkness, and red and blue light bud shading only, significantly reduced the number of bud IP. May (1965) did not further comment but the inference of these results is that, for floral initiation to occur, light stimulus must be directly on the latent bud with

no or little effect from light irradiance on the leaf. Buttrose (1969c), using environmentally controlled growth cabinets in Southern Australia, carried out a series of experiments to provide information on "whether day-length had any effect on fruitfulness of grapevines".

The eight treatments in growth cabinets, on 'Muscat Gordo Blanco' grapevine cultivars, over 13 weeks from bud burst, included: (i) continuous low intensity light of 900 foot candles (f.c.), 2400 f.c. and 3600 f.c. Horticultural irradiance is usually expressed as photosynthetically active radiation (PAR), best measured as instantaneous incident quanta in micro-moles per square metre per second ($\mu mol \cdot m^{-2} \cdot s^{-1}$) (McCree 1972). So these f.c. intensities are equivalent to 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 480 $\mu mol \cdot m^{-2} \cdot s^{-1}$and 720 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Thimijan and Heins 1983); (ii) varying night lengths (8, 12 and 16 h); and (iii) interrupting night/darkness by a light pulse (a 16 h night with 1 h light in middle and a 12 night with 4 h light in middle). Buttrose (1969c) did not explain the purpose of the individual treatments but the light regimes shown in the Table appear to describe simulations of SDP (12 and 16 h darkness), two night breaks on SDP which would inhibit flowering (breaks of 1 h and 4 h), LDP (8 h darkness) and DNP (continuous light). All treatments showed IP at latent bud dissection after 13 weeks, and the mean number of IP in all treatments were not statistically different (by Fisher's Least Significance Difference test (LSD) with $P < 0.05$). The only comments from Buttrose (1969c) concerning PDF from the results in this report were published in a later review paper (Buttrose 1974) - "the number of bunch primordia recognizable after a three month growing period was not photoperiodically controlled". No further analysis, explanation or discussion concerning PDF was given, in either paper. However the results can be interpreted as showing flowering in all simulations (SDP, SDP with night breaks, LDP, and DNP) which is not possible- hence the assumption is that the samples likely did not exhibit PDF and were DNP.

The results reported in May (1965) and Buttrose (1969c) are suggestive of lack of PDF, but there are concerns that raise doubt as to the validity of the data and subsequent conclusions. These are outlined in detail in the discussion.

As part of a project examining grapevine floral evocation (does this event occur and if so, when?) it was essential to know if the grapevine flowering pathway began with floral induction in the leaf, with FT protein moving to the SAM or by environmental and endogenous stimuli directly on the SAM (where evocation is the first event in flowering). Also in the present scientific environment, where all reports are closely scrutinised and experiments duplicated by independent researchers, it is past time that prior research and conclusions are tested and the question of whether grapevine PDF did occur finally settled.

The question of PDF could be simply resolved by comparing two treatment groups in the field, so avoiding complex growth cabinet manipulations. One treatment would involve complete absence of grapevine leaves (the site of PDF commencement) compared to a control where all leaves were left on the grapevine. The hypothesis that "PDF does not occur in the grapevine" would be satisfied if the same IP development was seen in both groups, confirming that leaves were not necessary for floral initiation and thus for the beginning of the floral pathway. To test this hypothesis, this study compares the basal IP developmental stage and full content of the latent bud growing point between two grapevine groups in the field-one fully defoliated from budburst, and a control group.

Table

Comparison of inflorescence primordia (IP) development in treatment and control 'Chardonnay' grapevines in the spring post budburst period

Components		Treatments[2]		Significance[1]
		C	T	
31 d post bud burst	IP stage[3]	0[a]	0[a]	ns
	Full content of bud growing point[4]	L-SAM-L[4]	L-SAM-L	
54 d post bud burst	IP stage	1[b]	2[b]	ns
	Full content of bud growing point	L-SAM-L-1	L-SAM-L-2	
76 d post bud burst	Basal IP stage	5b[c]	6[c]	ns
	Full content of bud growing point	5b-L-SAM-L-3[4]	6-L-SAM-L-4	

[1] Each developmental stage was the median count of 15 basal IP collected from the primary latent bud, on the sample date. Median counts with different letter superscripts separated within rows are significantly different ($p \leq 0.05$) by Fishers exact test (Chi-square analysis). ns = not significant, * = significant.

[2] Treatments: C = control-no defoliation from budburst, T = treatment- complete defoliation for 57 d post budburst. Leaves were removed as soon as separated from the tip mass.

[3] The developmental stage on the basal IP of the primary latent bud from node 4, as described in Noyce *et al.* 2015.

[4] Representative configurations of all IP stages present on the latent bud growing point at the sample date. The growing point on a horizontal plane is the SAM with IP and leaf primordia at each side of the SAM and small stipule primordia (often called scales) are at the SAM flanks, as described, with figures, in Noyce *et al.* 2015. SAM = shoot apical meristem, L = leaf primordium, number (e.g. 6) = IP developmental stage. For example: 5b-L-SAM-L-3 means IP Stage 5b at one end then a leaf primordium then the shoot apical meristem then a leaf primordium then IP Stage 3 at the other end.

Material and Methods

Plant material: Whole grapevines were selected from *Vitis vinifera* L. 'Chardonnay' in a 2.5 ha vineyard in Wollombi, New South Wales, Australia. The vineyard is located in the Hunter Valley wine region, which is classified as a warm climate viticulture area (lat: 32°56'18.54"S; long: 151°8'26.21"E). The grapevines were eight years old and grown on their own roots using vertical shoot positioning, drip irrigation, and winter spur-pruning. Identical management inputs were applied to all grapevines throughout the season.

Sampling procedures: One hundred grapevines were chosen by stratified random sampling; the selection was based on the average, plus or minus 5 %, of the trunk circumferences measured at the level of the irrigation drip line of 200 grapevines sampled randomly throughout the whole vineyard block. Using a random number allocation program (S-plus 8.5 for Windows; Tibco Software Inc., Palo Alto, CA), 30 of the 100 grapevines were selected, with 15 replicates randomly allocated to a control group and 15 replicates to a treatment group.

A shoot was selected from each grapevine in the control and treatment groups, at the sampling date, by using stratified random sampling as follows: shoots to be selected were within the average measurements (± 5 %) of (1) an internode distance between nodes 4 and 5 and between nodes 10 and 11, and (2) a shoot circumference of the internode between nodes 4 and 5.

On each shoot the compound latent bud on node 4 was removed, as the buds on this node are commonly reported as the most fruitful (Winkler and Shemsettin 1937, Sommer *et al.* 2000, Sánchez and Dokoozlian 2005). The compound latent buds sampled from shoots were stored in a 10 % neutral buffered formalin solution (ProSciTech Pty Ltd., Kirwan, Queensland, Australia), which acts as a preservative and fixative.

The compound latent buds were dissected, and within the primary bud, the basal IP developmental stage and full content of the bud growing point recorded. The basal IP is the first initiated, and consequently the largest primordium on the growing point (Noyce *et al.* 2016a).

Treatments and sampling times: A compound latent bud was considered to indicate budburst when a green tip of the first leaf tissue was visible through the open woolly bud. This corresponds to stage 4 of the modified E-L system of grapevine growth stages (Coombe 1995). The time of budburst, for the 100 grapevines selected, was defined as the date when 50-60 % of the buds in this selection showed The modified E-L system stage 4 (Antcliffe and May 1961, Martin and Dunn 2000).

From budburst, for 57 days, treatment vines were completely defoliated as soon as new leaves appeared from the tip mass on the shoots, no matter how small and even if unfolded. The tip mass consists of a SAM, leaf and tendril primordia and young unexpanded leaves and tendrils, all in close apposition, with no clear space between the shoot stem and leaf petioles or tendrils when examined in the field with a magnifying loupe at 10x magnification (Mullins *et al.* 1992, Noyce *et al.* 2016a). Control vines were untouched. Samples were collected at 31, 54 and 76 d post budburst.

Sample analyses: All latent bud samples were dissected with a dissecting light microscope (Nikon Stereoscopic Zoom Microscope SMZ745; Coherent Scientific, Hilton, South Australia). Within the primary latent bud the basal IP development stage was determined and the full content of the bud growing point recorded.

Methods used for IP sample analyses, including compound latent bud dissection techniques, IP staging descriptions, and definition and description of the compound latent bud, bud growing point and basal IP, were as described in Noyce *et al.* 2015, 2016a.

Statistical analysis: The measure of the central tendency of the IP developmental stage at each sample date was derived by taking the median count of samples (the basal IP in the primary latent bud of node 4) from 15 grapevine shoots, each from different grapevines. The measure of variability was examined by box plots of the 15 sample stages at each sample date. This median count of IP development at each sample date was compared between controls and treatments by using Fisher's exact test (Chi-square analysis). To comply with the assumptions of Chi-square analysis some categorical rows were combined to produce meaningful contingency tables. A 5 % probability was considered significant.

Results

Budburst was on 8 September. At 31 d post budburst the treatment and control growing point both contained a SAM with leaf primordia (Table) and no IP. At 54 d post budburst both growing points contained a single IP, with leaf primordia. Statistical analysis showed no difference in the IP developmental stage in both groups at 54 d post budburst (Table). At 76 d post budburst both growing points contained two IP, with leaf primordia. Statistical analysis showed no difference in the basal IP developmental stage in both groups at 76 d post budburst (Table).

Discussion

The data in two English language research papers, described in the introduction (May 1965, Buttrose 1969c), is suggestive of lack of grapevine PDF. However when examined, with current knowledge of IP development and current plant PDF classification, it is difficult to state that this absence has been shown unequivocally.

The experiments of May (1965) suggest a lack or "reduction" of PDF but in a situation of complete bud darkness an absence of floral initiation would be expected, not a reduction as reported in the paper.

In the experiments of Buttrose (1969c) there are three areas of uncertainty: (i) The mean IP number for treatments was low with only one treatment (continuous light at 2,400 f.c. intensity) greater than one (at 1.23) and four treatment IP means were less than the LSD of 0.20. At latent bud

dissections the IP, named "bunch primordia", were identified as "many lobed" and "massive" (Buttrose 1969a). These IP, at 13 weeks post budburst, would be at least 5a or stage 6 (Noyce *et al.* 2016a) and thus readily identified (Noyce *et al.* 2015). Each treatment mean was from 20 replicates and by 13 weeks post budburst at least two or three IP would be present on each primary latent bud growing point, with at least one or two at stage 5a (Noyce *et al.* 2016a). Hence greater numbers of IP would be expected in the dissections reported in Buttrose (1969c). There was no description of the bud dissection techniques, or photographs, so we cannot be confident as to exactly what structures the dissectors were reporting when compiling their numbers.

(ii) As described above other uncommon PDF categories are now recognised apart from the SDP, LDP and DNP simulated by Buttrose (1969c) in his experiments. Perhaps the treatment grapevines did exhibit PDF but were one of a plant type not tested. (iii) Approximately 88 million years ago, during the Eocene Epoch, the species *Vitis vinifera* L. is thought to have evolved in northern Eurasia, within the southern Caucasus region, between the Red Sea and Caspian Sea (Hardie 2000, Bessis 2007). Domestication of the wild grapevine, with human cultivation, likely originated in this region between the fourth and seventh millennia BC (Zohary 2004, Terral *et al.* 2010, Myles *et al.* 2011). The southern Caucasus region has always experienced relatively high global solar irradiance levels (Mammadov 2013, Time series of solar radiation data from the NASA-SSE database 2017) with current PAR at full midday sun of up to 2,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. It is likely that the grapevine flowering process thus evolved under high light intensity. Cooley *et al.* (2012), in a project in Southern Australia, examining temperature and light effects on grapevine flowering, comment that *Vitis vinif*era L. plants evolved in an area of high solar radiance and thus "evolved to only flower when the light intensities are in full sunlight (800 µmol upwards)". The maximum light intensity applied by Buttrose (1969c) in the experiments was 3600 f.c. (equivalent to 720 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Thimijan and Heins 1983), likely not sufficient to induce grapevine flowering if grapevines have a PDF classification other than the SDP, LDP or DNP simulated.

In the study reported here, post budburst in the control group, stage 1 IP were present at 54 days, and basal IP stage 5b at 76 d. Floral evocation (followed by floral initiation) in this group, was either due to a mobile signal stimulus from leaf floral induction or by non-leaf derived stimuli directly on the SAM. Post budburst in the treatment group, stage 2 IP were present at 54 d, and basal IP stage 6 at 76 d, so floral evocation and floral initiation must have occurred. As no leaves were present at any time in this treatment group the only signal for evocation could be a non-leaf derived stimulus, directly on the SAM. The conclusion is that the experimental data supported the hypothesis and that leaf floral induction is not necessary for floral evocation (or floral initiation if evocation is not a physiological event in grapevines) in *Vitis vinifera* L. 'Chardonnay'. It is not unreasonable to propose that grapevine PDF does not occur.

The question arises as to whether the complete defoliation treatment affected the results, due to lack of photo-assimilate carbohydrate. Lebon *et al.* (2008) emphasise that the annual cycle of grapevine carbohydrate physiology can be separated in two phases defined by the net movement of carbohydrate into and out of reserve storage. Phase one starts at the end of dormancy when starch is mobilised from reserve stores and moves out to support the annual organs during early growth in spring before there is net photo-assimilate export from leaves. Phase two coincides with net leaf photo-assimilate export, towards the end of the process of flowering, and supports continued growth of the current season and the replenishment of reserves. Many studies between 1945 to 2009 have supported this concept, that initial spring growth of vegetative sinks in overwintering latent buds depends not on photo-assimilate, but on carbohydrate reserves (non-structural carbohydrates stored mostly as starch in roots) for at least four to five weeks post-budburst, and probably longer (Winkler and Williams 1945, Scholefield *et al.* 1978, Yang and Hori 1979, 1980, Yang *et al.* 1980, Goffinet 2004, Zapata *et al.* 2004, Greer and Sicard 2009). Similar studies are lacking for reproductive sinks, but the same conclusion can be presumed. These studies also show that reserves are not completely depleted when photo-assimilate once again begins to be deposited back into these stores, in phase two.

Defoliation will give smaller IP and less numbers of IP initiated, if carbohydrate stores are fully depleted, as shown by Noyce *et al.* (2016b), where the physiological causes of defoliation effects on reproductive parameters were explored. In these experiments defoliation treatments were very extreme (complete defoliation from early in season 1 to completely deplete stores by the next spring) to achieve meaningful statistical differences between treatments. The stores in the treatment group of the study reported here were not fully depleted, even at 54 and 76 d post budburst. This is shown by the finding of the same basal IP developmental stage and IP stages on the growing point, in both control and treatment groups. It can be concluded that the defoliation treatment had no effect on the results other than removing a possible site of photoperiodic flowering.

Conclusion

In this study, for the first time, unequivocal evidence that PDF in grapevines does not occur, has been presented. Compound latent bud IP developmental stages post budburst for 76 d were the same in grapevines having no leaves and grapevines with full leaf complement from budburst, indicating that leaves, with floral induction, are not needed to initiate the grapevine flowering pathway. The stimulus for floral evocation is directly on the vegetative SAM. Further physiological studies investigating the presence or absence of floral evocation in grapevines, and its timing if the event occurs, have been facilitated.

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