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Secondary and tertiary budbreak release is enhanced by extended dormancy chilling in 'Shiraz' grapevines

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Summary

Limited information exists regarding the dormancy of secondary and tertiary buds within the compound bud of grapevines. We were interested to evaluate how extended chilling duration would affect the budbreak percentage of all three bud orders within the compound bud. Dormant potted 'Shiraz' grapevines, with thirty retained buds per vine, were placed in a cool room at 4 °C over a 20-month period to extend dormancy. Vines were then systematically removed over five dates and placed in a controlled growth environment to assess budbreak percentages and cane tissue concentrations of abscisic acid and cytokinins. Budbreak was hastened by at least 13 d with vines receiving any extra chilling compared to no initial chilling. Furthermore, the firstly observed correlative inhibition of basal buds was apparently removed with chilling. Removal of correlative inhibition within the compound bud with increased chilling duration was also observed with the increases of secondary (doubles) and tertiary (triples) buds breaking simultaneously with the primary bud at each node. This resulted in 91 % of the nodes having two developing shoots and 56 % of the nodes presenting three developing shoots by the end of the experiment. Furthermore, a sigmoidal relationship between percent secondary buds and chilling duration was observed. Possible phytohormone connections were observed with the decline of ABA as secondary and tertiary buds broke at 414 d of chilling and depletion of the CKs, which coincided with the greatest rate of primary bud break after 242 d of chilling. Other possible involvement of ABA and Cis-type cytokinin interactions with secondary and tertiary bud dormancy is discussed. The results suggest future investigations into secondary and tertiary bud release within the grapevine using similar techniques may help to better understand the biochemical mechanisms associated with dormancy.

K e y w o r d s : budbreak; chilling; correlative inhibition; dormancy; abscisic acid; cytokinins.

Introduction

The overwintering buds of grapevines are compound structures that contain several orders of shoot primordia. The primordia on the main bud axis is usually the largest and is commonly referred to as the 'primary' bud. Axillary to this primary bud are often two smaller shoot primordia, one on each side, referred to as 'secondary' and 'tertiary' buds. Generally, the primary bud develops into the new shoot in spring, with the secondary and tertiary buds not normally developing unless the primary bud suffers mechanical damage or is affected by primary bud necrosis (Dry and Coombe 2004). On occasions, the primary along with a secondary bud break at the same time with this event termed 'doubles' within the industry. No data on the relative occurrence of these 'doubles' has been published. However, New Zealand vineyards have seen incidences of up to 15 % of nodes containing double shoots in 'Sauvignon blanc', 'Pinot gris' and 'Pinot noir' varieties (V. Raw, pers. comm.). This increase in shoot number versus the desired count nodes retained results in either increased management costs with shoot thinning or a dense canopy that will have increased disease pressure in the current harvest and could furthermore reduce inflorescence formation for the following season (Dry 2000).

Grapevines require a certain period of chilling to terminate the state of dormancy and allow normal bud-break with dormancy progressing through three stages; paradormancy, endodormancy and ecodormancy (Lavee and May 1997). The specific biochemical mechanisms controlling dormancy have been widely studied but the complex nature of these processes means that they are still not fully understood. One of the major mechanisms studied widely has been the role of plant hormones in the regulation of bud dormancy. Abscisic acid (ABA) is considered to be a key hormone enforcing endodormancy (Düring and Bachmann 1975, Noriega and Pérez 2017, Vergara *et al.* 2017). It is now generally accepted that the balance of auxin and cytokinin (CK), along with strigolactones, is important with the dormancy breaking of axillary buds in plants (Yuan *et al.* 2015). Similarly, increases in CK and auxin have been associated

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with release of winter dormancy (Noriega and Pérez 2017). However, it is implicit that during endodormancy, the bud is under a growth inhibition until a certain number of chilling hours is reached and then moves into ecodormancy where bud growth can occur under favorable climatic conditions. No evidence has been found to conclude if secondary and tertiary buds are also under this chilling growth inhibition. However, it is assumed that chilling growth inhibition was comparative to correlative inhibition, where distal shoot growth inhibited proximal budbreak on the same cane (Keller 2015). The present study was undertaken to investigate if chilling duration increases the release of secondary and/or tertiary buds from dormancy. To explore the potential for hormone-meditated dormancy release, both a dormancy enforcer, ABA, and dormancy releasing hormone group, the CKs, were quantified in nearby cane tissues in correspondence with chilling treatments.

Material and Methods

Twenty-five potted (36 L), 3-year-old own-rooted grapevines, 'Shiraz', were grown to three vertically trained shoots in the 2006/07 growing season. In July 2006, these dormant vines were pruned to 10 buds per shoot leaving 30 buds per vine. Twenty vines were then placed in a cool room at 4 °C. Vines were watered every month to stop the soil from drying out.

Initially, five vines were placed in an environmentally controlled growth chamber (TH6000, Thermoline Scientific, Smithfield, NSW, Australia) to stimulate bud-break. The growth chamber was set at 25 °C/15 °C day/night temperatures with a relative humidity of 80 % to produce a consistent environment for the vines to break dormancy. Every four to six months, another five potted vines were taken out of the cool room and placed in the growth chamber, at the same environmental conditions, to stimulate bud-break.

Time of budbreak was determined as the date on which the first green leaf on the vine became visible through the bud scales (Eichhorn-Lorenz [E-L] stage 4; Pearce and Coombe 2005). Percent bud-break was measured with the number of single, double or triple buds counted per node. A destructive sample of cane combined from all internodes along the three canes was collected. Apart from the initial sampling date where the phenology of buds along the canes ranged from E-L stage 4 to 7, destructive sampling with all other dates was conducted uniquely at E-L stage 5 (Fig. 1). All samples, at time of collection, were washed with detergent, rinsed with distilled water and immediately frozen and stored at -80 °C. Samples were freeze dried and then ground to 0.12 mm using an ultra-centrifugal mill (Retsch ZM100, Haan, Germany). Concentrations of abscisic acid (ABA), trans-zeatin riboside ([9R]Z), cis-zeatin riboside (cis[9R]Z), dihydrozeatin riboside ([9R]DZ), isopentenyl adenosine ([9R]iP), trans-zeatin nucleotide ([9R-MP]Z), cis-zeatin nucleotide (cis[9R-MP]Z), dihydrozeatin nucleotide ([9R-MP]DZ), and isopentenyl nucleotide ([9R-MP]iP) were determined according to the method of Farrow and Emery (2012).

Fig. 1: Simultaneous bud-break of the primary, secondary and tertiary buds after extended dormancy of 601 d at 4 °C.

Budbreak data were subjected to Kruskal-Wallis with Wilcoxon rank post-hoc testing, using R V3.6.0 (The R Foundation). Hormone data were subjected to one-way ANOVA with Tukey-Kramer HSD post-hoc testing, using Minitab 19 (Minitab Inc., State College, PA, USA, version 19.1).

Results and Discussion

Time to budbreak was significantly affected by chilling duration at 4 °C (Tab. 1). Initially, with no cool room chilling, the time taken for the first bud on a vine to break occurred at an average of 22 d after being placed in the growth chamber. This time to budbreak declined over the next two sampling days until a fastest rate at 4 d to bud break, after 242 d of chilling was reached. Interestingly, the time to bud break more than doubled when exposed to 414 and 601 chilling days (8 and 9 d to bud break, respectively). In general, the shortening of days to bud break (that is faster budbreak) agreed with modelling of budbreak in warm climates where a longer chilling period resulted in a shorter forcing phase (Fila *et al.* 2012, Prats-Llinàs *et al.* 2019).

Table 1

Effect of chilling duration on initial time to observe vine bud break and percentage bud break per node

Days in cool room	Days to break-bud	Percent bud-break
0	21.6 ± 0.4a	64.1 ± 8.5c
109	7.2 ± 0.2d	90.0 ± 3.2b
242	4.0 ± 0.0e	95.6 ± 1.7b
414	8.0 ± 0.0c	100 ± 0.0a
601	9.0 ± 0.0b	99.4 ± 0.6ab

Data are means ± SE. Kruskal-Wallis, Wilcoxon rank post-hoc testing, ($P < 0.05$) ($n = 5$). Values within a column with different letters signify significance.

Percent primary bud-break per node was significantly increased when the vines were exposed to chilling, with nearly 100 % of nodes breaking bud from 414 d onwards (Tab. 1). Initial budburst percentage in primary buds (64 %)

indicated that the vines had apparently experienced significant chilling prior to the vines being transferred into the cool room. Assessment of a local weather station indicated that the vines probably experienced approximately 500 hours of chilling hours below 7 °C between leaf fall (1st June 2006) and the initial sampling date. This conforms to significant chilling hours were, most likely to overcome endodormancy (Anzanello *et al.* 2018). Thus, the lower % budbreak at the initial sampling date may well have been the result of correlative inhibition, or paradormancy, rather than linked to release from endodormancy. That the distal buds on the cane were advanced in phenology, compared to the basal buds which broke at the initial sampling date, also supported this. All further sampling dates showed no difference in bud phenology along the cane with simultaneous budbreak observed. Correlative inhibition is commonly seen with cane pruned vines and is more prevalent in warm climates (Keller 2015). Application of bud-breaking compounds, such as hydrogen cyanamide, are frequently used to improve this uneven budbreak and enable profitable yields in grapevines grown in warm climates (Khalil-Ur-Rehman *et al.* 2019, Lavee and May 1997). The present study indicates chilling could be used to determine the likelihood of correlative inhibition. Modeling could provide greater certainty to growers about seasons to intervene to maintain crop production and quality levels, especially in temperate environments where short/warmer winters may result in lower % budbreak while budgeting for increased shoot removal or disease management costs in longer/colder winters.

No secondary and/or tertiary buds were observed at the initial date of budbreak. However, as duration of chilling lengthened, an increase in the proportion of 'double' (we assumed to be the primary and secondary bud) and 'triples' occurred (Fig. 2). Destructive removal of the primary bud is the only proven mechanism where correlative inhibition of the secondary bud is overcome (Keller 2015). To our knowledge, this is the first study demonstrating that the mechanisms controlling correlative inhibition of the secondary bud (and tertiary bud) are also removed by chilling duration, without removal of the primary bud. Thus, the duration of chilling appears to be a major factor in the

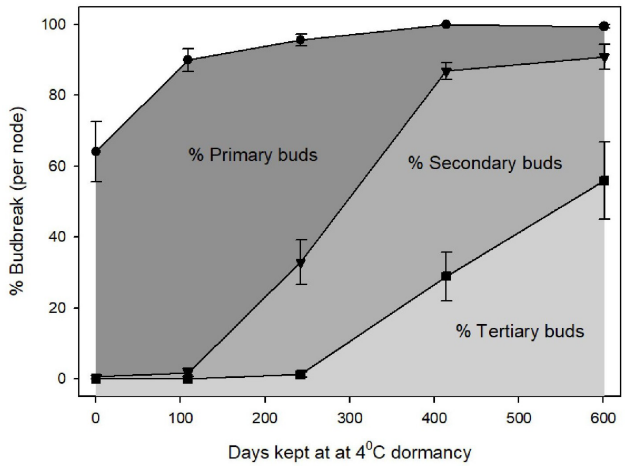

Fig. 2: Percentage of primary, secondary and tertiary bud break per node with duration of chilling. Error bars ± SE mean (n = 5).

occurrence of double shoots in the vineyard. Although not separated between the proportion of latent buds and 'doubles' in budbreak (although both occurrences were mentioned), Bernizzoni *et al.* (2009) reported significantly higher number of shoots per count node, when vines were spur compared to cane pruned. This suggested removal of the correlative inhibition from distal to basal nodes through spur pruning (Keller 2015) may also reduce the correlative inhibition within the bud, resulting in greater occurrence of doubles. This suggested spur pruning in cooler climates will most likely result in more doubles and thus additional management may be required to reduce the numbers of double and triple shoots.

Percent budbreak of the primary bud (in single node cuttings) is generally accepted to follow a sigmoidal function, with chilling duration and release from endodormancy (Anzanello *et al.* 2018, Mohamed *et al.* 2010). Interestingly, the percent of secondary buds also increased, following a sigmoid curve with chilling duration, in the present study (Fig. 2). The increase in % of tertiary buds also seemed to follow similar kinetics although restricted to the linear portion of the sigmoid curve. This perhaps indicated that the secondary (and possibly tertiary buds) were under similar biochemical mechanisms related to bud chilling release from endodormancy. Investigating the biochemical changes induced by duration of chilling, and not associated with the removal the primary bud, to stimulate growth of the secondary bud may provide a more controlled approach to understand dormancy in future studies.

Samples for hormone analysis were collected after budbreak and show potential correlation with different aspects of dormancy release. ABA, which is known as a signal of dormancy maintenance, was consistently low at 414 d of chilling treatment, upon which the ABA concentrations were subsequently reduced by half (Tab. 2). Interestingly, this pattern corresponded with the first timepoint at which secondary and tertiary buds were undergoing significant bud-break (80 % and 25 %, respectively). Such a decline in ABA concentration within the cane tissue conforms with the idea that reduced phloem-delivered ABA was related to the release of dormancy (Holalu and Finlayson 2017, Nguyen and Emery 2017). Because of sampling design and harvest strategies, it was not possible to obtain hormone measurements directly from individual buds but future studies may test these dynamics.

CK data were analyzed according to the different biosynthetic pathways which produce opposite isomers of CK products (cis and trans), which are thought to have different functions in plants (Gajdošová *et al.* 2011). Trans-isomer, dihydro-types and iP-type CKs are all thought to come from the *de novo* adenylate pathway and have high activity rates for rapid growth functions. On the other hand, cis-isomer CK forms are believed to have lower activity and serve a more growth maintenance function (Gajdošová *et al.* 2011). Consistent and clear trends were observed in concentrations of all types of CKs, in the cane tissues of this study. However, these patterns appeared to be counter-intuitive for the proposed function of CKs as dormancy releasing and growth promotive signals. Total CKs showed a biphasic pattern, starting

Table 2

Effect of chilling duration on ABA and Cytokinin concentrations (pmol·g^{-1}) in cane tissue after budbreak

Days in cool room	ABA	[9R]Z + [9R-MP]Z	cis[9R]Z + cis[9R-MP]Z	[9R]DZ + [9R-MP]DZ	[9R]iP + [9R-MP]iP	Total CK
0	19357a	311ab	5.9b	5.1ab	76bc	398bc
109	16894a	414a	6.2ab	4.7b	105b	529ab
242	17182a	233b	5.3b	4.1b	56c	299c
414	9634b	407ab	7.6a	6.4a	167a	588a
601	12665ab	275b	7.7a	5.5ab	106bc	394bc

Data are means. One-way ANOVA Tukey-HSD post-hoc testing, ($P<0.05$) ($n=5$). Values within a column with different letters signify significance.

at high concentration and then significantly decreased at day 242, followed by a subsequent increase again by days 414 and 601. This pattern was strikingly similar to that seen in number of days to budbreak (Tab. 1) and suggestive of some mechanistic relationship. The same pattern of high/low/high was observed in all groups of the CKs, including: the cis-isomers, trans-isomers, dihydro-forms and iP-forms. That each pathway and group predictably followed each other suggested a functional role. Representing the *de novo* adenylate pathway, the trans-zeatin forms, which also represent to the greatest potential activity, were in the greatest abundance (between 35 to 66-fold higher than cis-isomer counterparts), suggesting they may be important for rapid growth such as is needed by breaking buds. However, we had hypothesized that dormancy release measures, such as days to bud break, would be mirrored by increases in CKs as they are known to be dormancy releasers and growth promotors. This was based on the work showing CKs strongly promote lateral bud outgrowth (Emery *et al.* 1998, Mader *et al.* 2003). However, the results of the present study may reflect differences between grapevine buds and lateral buds of other species thus far studied. That the opposite effect was observed, possibly reflected the complex relationship between cane and bud tissues and source-sink relationships connected by the phloem more generally. Further study of these relationships is essential to further comprehend the bud burst processes. In particular, some seed dormancy systems show that cis-ZR is capable of enforcing dormancy (Goggin *et al.* 2010). It is worth noting that the cis-type CK did show a clear statistically significant trend, whereby concentrations were significantly higher in the later sampling dates and represented a possible inverse relationship with ABA concentrations. Cis isomers of Zeatin are thought to be an active form of CK specifically related to dormancy control (Mader *et al.* 2003, Schäfer *et al.* 2015) and known for growth maintenance. This suggests Cis type CKs and ABA interactions are possibly involved in correlative inhibition of secondary and tertiary buds but needs further investigation.

Conclusions

Correlative inhibition, either from the apical to the basal buds down the cane or from the primary to the secondary/tertiary buds within the compound bud, is reduced with the duration of chilling. This resulted in an increase in the percentage of secondary and tertiary buds breaking with longer dormancy. ABA and cis-type CK may play a biochemical role in regulating this dormancy. The sigmoidal relationship with percent secondary bud occurrence and duration of chilling suggested future studies investigate secondary bud growth by adopting the present approach, and not by removal of the primary bud, to help better understand the biochemical mechanisms associated with dormancy in more intact bud systems.

Finally, increased winter temperature variation is predicted to occur under climate change. In temperate climates, this could lead to correlative inhibition down the cane in warm winters or increases of double bud occurrence in cold winters. Innovative modelling of chilling duration could allow for intervention strategies to maintain crop production levels in short/warmer winters or budget for increased shoot removal or disease management costs in longer/colder winters.

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References

Anzanello, R.; Fialho, F. B.; Santos, H. P. dos.; 2018: Chilling requirements and dormancy evolution in grapevine buds. Ciênc. Agrotec. **42**, 364-371. DOI: https://dx.doi.org/10.1590/1413-70542018424014618

Bernizzoni, F.; Gatti, M.; Civardi, S.; Poni, S.; 2009: Long-term performance of Barbera grown under different training systems and within-row vine spacings. Am. J. Enol. Vitic. **60**, 339-348 (https://www.ajevonline.org/content/60/3/339).

Dry, P. R.; 2000: Canopy management for fruitfulness. Aust. J. Grape Wine Res. **6**, 109-115. DOI: https://doi.org/10.1111/j.1755-0238.2000.tb00168.x

Dry, P. R.; Coombe, B. G.; 2004: Viticulture Volume 1 - Resources, 2nd ed. Winetitles, Adelaide (https://winetitles.com.au/product/viticulture-volume-1-resources-2nd-edition/).

Düring, H.; Bachmann, O.; 1975: Abscisic acid analysis in *Vitis vinifera* in the period of endogenous bud dormancy by high pressure liquid chromatography. Physiol. Plant. **34**, 201-203. DOI: https://doi.org/10.1111/j.1399-3054.1975.tb03821.x

Emery, R. J. N.; Longnecker, N. E.; Atkins, C. A.; 1998: Branch development in *Lupinus angustifolius* L. II. Relationship with endogenous ABA, IAA and cytokinins in axillary and main stem buds. J. Exp. Bot. **49**, 555-562. DOI: https://doi.org/10.1093/jxb/49.320.555

Farrow, S. C.; Emery, R. N.; 2012: Concurrent profiling of indole-3-acetic acid, abscisic acid, and cytokinins and structurally related purines by high-performance-liquid-chromatography tandem electrospray mass spectrometry. Plant Methods **8**, 42. DOI: https://doi.org/10.1186/1746-4811-8-42

Fila, G.; Lena, B. D.; Gardiman, M.; Storchi, P.; Tomasi, D.; Silvestroni, O.; Pitacco, A.; 2012: Calibration and validation of grapevine budburst models using growth-room experiments as data source. Agric. For. Meteorol. **160**, 69-79. DOI: https://doi.org/10.1016/j.agrformet.2012.03.003

Gajdošová, S.; Spíchal, L.; Kamínek, M.; Hoyerová, K.; Novák, O.; Dobrev, P. I.; Galuszka, P.; Klíma, P.; Gaudinová, A.; Žižková, E.; Hanuš, J.; Dančák, M.; Trávníček, B.; Pešek, B.; Krupička, M.; Vaňková, R.; Strnad, M.; Motyka, V.; 2011: Distribution, biological activities, metabolism, and the conceivable function of cis-zeatin-type cytokinins in plants. J. Exp. Bot. **62**, 2827-2840. DOI: https://doi.org/10.1093/jxb/erq457

Goggin, D. E.; Emery, R. J. N.; Powles, S. B.; Steadman, K. J.; 2010: Initial characterisation of low and high seed dormancy populations of *Lolium rigidum* produced by repeated selection. J. Plant Physiol. **167**, 1282-1288. DOI: https://doi.org/10.1016/j.jplph.2010.04.004

Holalu, S. V.; Finlayson, S. A.; 2017: The ratio of red light to far red light alters Arabidopsis axillary bud growth and abscisic acid signalling before stem auxin changes. J. Exp. Bot. **68**, 943-952. DOI: https://doi.org/10.1093/jxb/erw479

Keller, M.; 2015: The Science of Grapevines: Anatomy and Physiology. Academic Press, London, UK/San Diego, USA. DOI: https://doi.org/10.1016/B978-0-12-419987-3.00001-7

Khalil-Ur-Rehman, M.; Wang, W.; Dong, Y.; Faheem, M.; Xu, Y.; Gao, Z.; Guo Shen, Z.; Tao, J.; 2019: Comparative transcriptomic and proteomic analysis to deeply investigate the role of hydrogen cyanamide in grape bud dormancy. Int. J. Mol. Sci. **20**, 3528. DOI: https://doi.org/10.3390/ijms20143528

Lavee, S.; May, P.; 1997: Dormancy of grapevine buds - facts and speculation. Aust. J. Grape. Wine. Res. **3**, 31-46. DOI: https://doi.org/10.1111/j.1755-0238.1997.tb00114.x

Mader, J. C.; Emery, R. J. N.; Turnbull, C. G. N.; 2003: Spatial and temporal changes in multiple hormone groups during lateral bud release shortly following apex decapitation of chickpea (*Cicer arietinum*) seedlings. Physiol. Plant. **119**, 295-308. DOI: https://doi.org/10.1034/j.1399-3054.2003.00179.x

Mohamed, H. B.; Vadel, A. M.; Geuns, J. M. C.; Khemira, H.; 2010: Biochemical changes in dormant grapevine shoot tissues in response to chilling: Possible role in dormancy release. Sci. Hortic. **124**, 440-447. DOI: https://doi.org/10.1016/j.scienta.2010.01.029

Nguyen, T. Q.; Emery, R. J. N.; 2017: Is ABA the earliest upstream inhibitor of apical dominance? J. Exp. Bot. **68**, 881-884. DOI: https://doi.org/10.1093/jxb/erx028

Noriega, X.; Pérez, F. J.; 2017: ABA biosynthesis genes are down-regulated while auxin and cytokinin biosynthesis genes are up-regulated during the release of grapevine buds from endodormancy. J. Plant Growth Regul. **36**, 814–823. DOI: https://doi.org/10.1007/s00344-017-9685-7

Pearce, I.; Coombe, B. G.; 2005: Grapevine phenology, 150-166. In: P. R. Dry, B. G. Coombe (Eds): Viticulture Volume 1 - Resources, 2nd ed. Winetitles, Adelaide. (https://winetitles.com.au/product/viticulture-volume-1-resources-2nd-edition/)

Prats-Llinàs, M. T.; DeJong, T. M.; Jarvis-Shean, K. S.; Girona, J.; Marsal, J.; 2019: Performance of a chill overlap model for predicting budbreak in Chardonnay grapevines over a broad range of growing conditions. Am. J. Enol. Vitic. **70**, 50-59. DOI: https://doi.org/10.5344/ajev.2018.18008

Schäfer, M.; Brütting, C.; Meza-Canales, I. D.; Grosskinsky, D. K.; Vankova, R.; Baldwin, I. T.; Meldau S.; 2015: The role of *cis* -zeatin-type cytokinins in plant growth regulation and mediating responses to environmental interactions. J. Exp. Bot. **66**, 4873-4884. DOI: https://doi.org/10.1093/jxb/erv214

Vergara, R.; Noriega, X.; Aravena, K.; Prieto, H.; Pérez, F. J.; 2017: ABA represses the expression of cell cycle genes and may modulate the development of endodormancy in grapevine buds. Front. Plant Sci. **8**, 812. DOI: https://doi.org/10.3389/fpls.2017.00812

Yuan, C.; Xi, L.; Kou, Y.; Zhao, Y.; Zhao, L.; 2015: Current perspectives on shoot branching regulation. Front. Agric. Sci. Eng. **2**, 38-52. DOI: https://doi.org/10.15302/J-FASE-2015053

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