

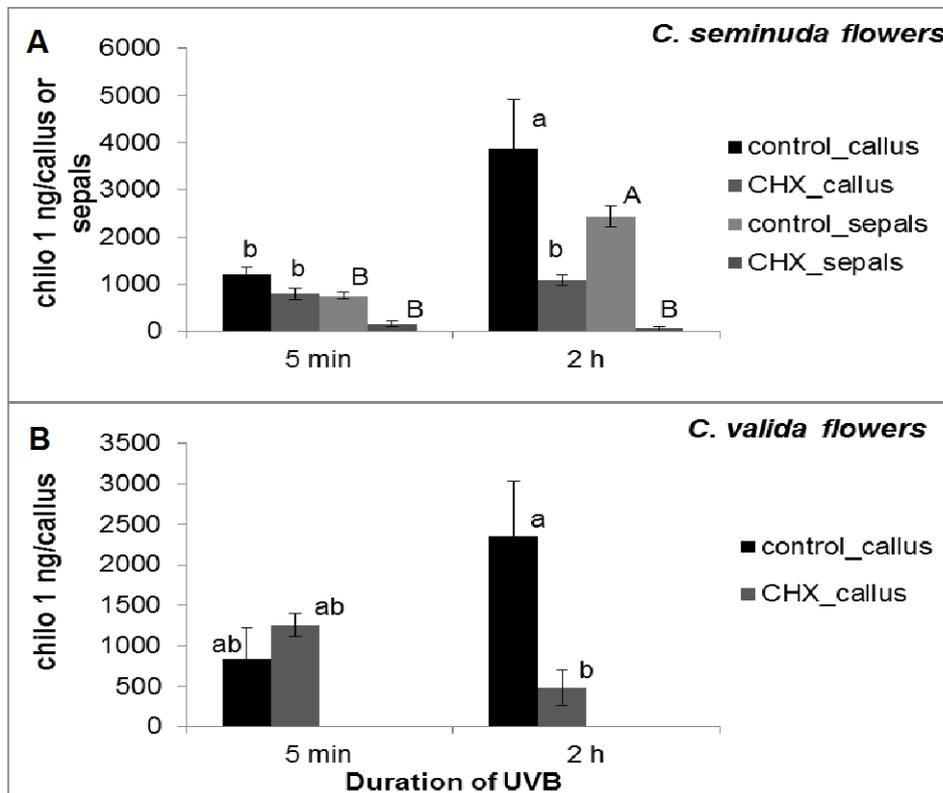
## SUPPLEMENTARY DATA

FIG. S1. A photograph showing the bud developmental series as classified in Table S1.



Key with codes used in the paper in parentheses: vy=very young bud (vyb), y=young bud (yb), m=mature bud (mb), vm=very mature bud (vmb); f=flower (different codes depending on source and treatment)

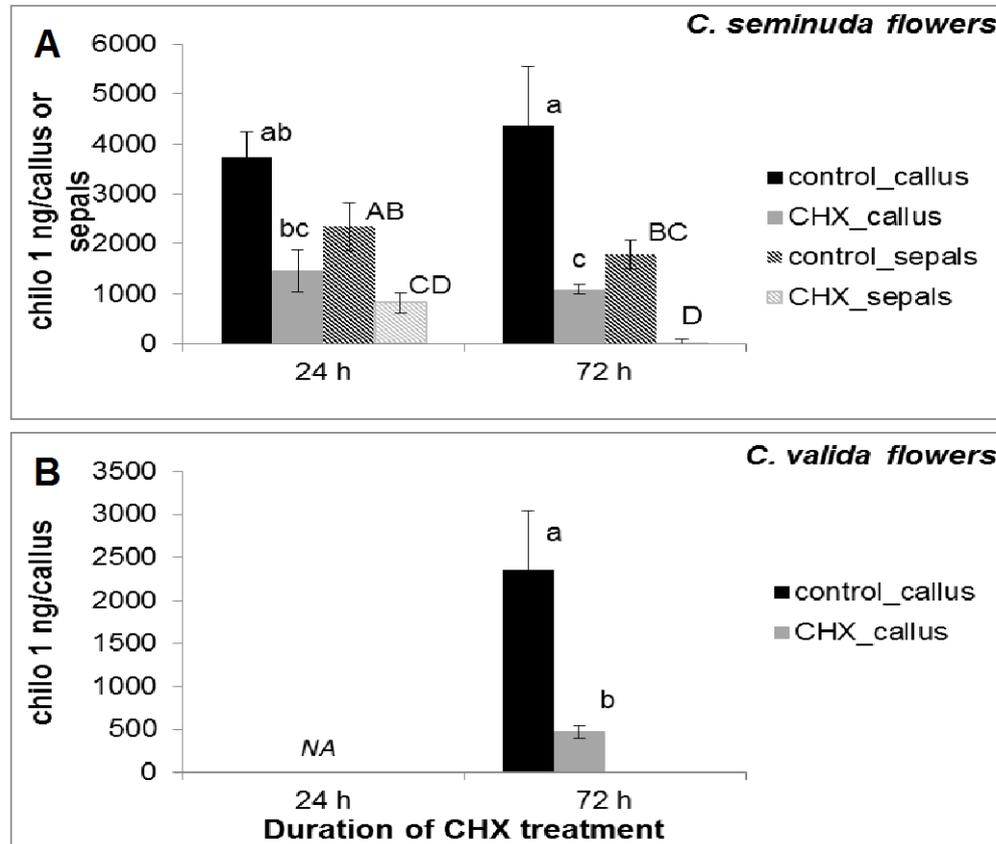
FIG. S2. Mean chiloglottone 1 amounts (ng/callus or ng/sepals) within the chiloglottone producing tissues of *Chiloglottis seminuda* and *C. valida* incubated with cycloheximide for 72 h followed by either a 5 min or a 2 h UV-B exposure.



Flowers were sourced from the field and held in a growth chamber in the absence of UV light. Cycloheximide (CHX) treatments consisted of incubation with 100  $\mu$ M CHX in water containing 0.07% (V/V) ethanol for 72 h, prior to UV-B exposure for the specified time at an irradiance of 51  $\mu$ W/cm<sup>2</sup>/nm at 300 nm. The controls consisted of water containing 0.07% (V/V) ethanol, but no CHX. The start point for incubation was timed such that all flowers were sampled 5 days after chiloglottone depletion had commenced. Note that in *C. valida* the 24 h incubation was not tested and the control refers to water only with no ethanol.

Chiloglottone1 amounts represent the mean of 4 or more biological replicates for each of floral tissues indicated. Error bars represent standard error of the mean based on pooled estimate of error variance. Labelled columns not connected by the same letter are significantly different at  $\alpha = 0.05$  based on a Tukey-Kramer HSD test. ANOVA (A) callus,  $F_{4,21} = 8.72$ ,  $P < 0.0003$ , sepals  $F_{4,22} = 17.7$ ,  $P < 0.0001$ ; (B)  $F_{3,22} = 4.17$ ,  $P < 0.0176$ .

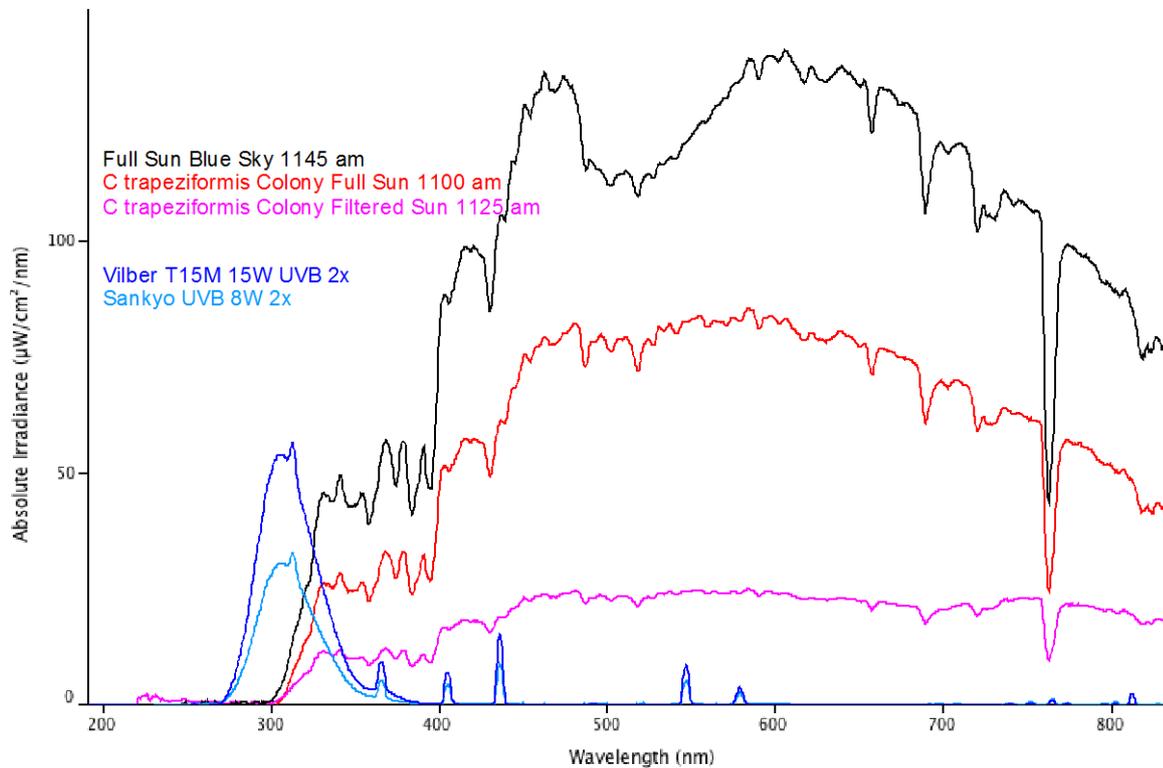
FIG. S3. Mean chiloglottone 1 amounts (ng/callus or ng/sepals) within the chiloglottone producing tissues of *Chiloglottis seminuda* and *C. valida* incubated with cycloheximide for 24 and 72 h, followed by a 2 h UV-B exposure.



Buds and mature flowers were sourced from the field and held in a growth chamber in the absence of UV light. Cycloheximide (CHX) treatments consisted of incubation with 100  $\mu$ M CHX in water containing 0.07% (V/V) ethanol for the specified period, prior to 2 h UV-B exposure at an irradiance of 51  $\mu$ W/cm<sup>2</sup>/nm at 300 nm. The controls consisted of water containing 0.07% (V/V) ethanol, but no CHX. The start point for incubation was timed such that all flowers were sampled 5 days after chiloglottone depletion had commenced. Note that in *C. valida* the 24 h incubation was not tested and the control refers to water only with no ethanol.

Chiloglottone1 amounts represent the mean of 4 or more biological replicates for each of floral tissues indicated. Error bars represent standard error of the mean based on pooled estimate of error variance. Labelled columns not connected by the same letter are significantly different at  $\alpha = 0.05$  based on a Tukey-Kramer HSD test. ANOVA (A) callus  $F_{5,20} = 5.49$ ,  $P < 0.002$ ; sepals  $F_{5,23} = 21.3$ ,  $P < 0.0001$ ; (B)  $F_{1,14} = 9.8$ ,  $P < 0.0074$ .

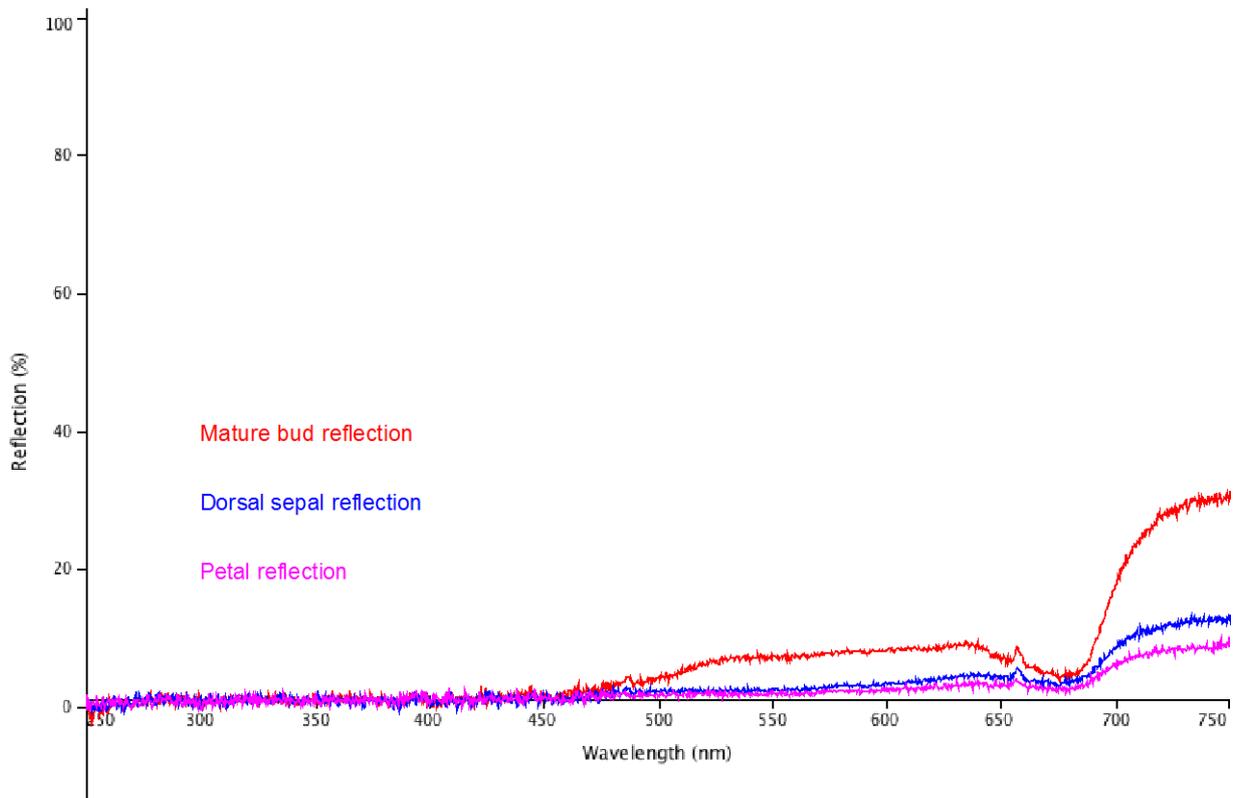
FIG. S4. An overlay of outdoors irradiance measurements made on a single day during the flowering season of *Chiloglottis trapeziformis* compared with the irradiance spectra of the UV-B lights within the light box used in the laboratory experiments.



The outdoors measurements were taken in proximity to the orchid colony within the Australian National Botanic gardens on a clear sunny day mid flowering season (15<sup>th</sup> Sept 2012) between 11 and 11.45 am. The measurements taken in the field included: full sun with probe position in open space and facing unobstructed blue sky (black line), full sun with probe positioned within the colony and facing the unobstructed sky within the forest canopy (red line), filtered sun with the probe position within the colony and facing the forest canopy (pink line). Measurements taken in the light box are shown for two lighting configurations: 16W (2x Sankyo Denki G8T5E UV-B 8W lamps, Hitachi, Japan) and 30W (2x Vilber T15M UV-B 15W lamps, Vilber Lourmat, Germany).

The irradiance measurements were taken with a UV/VIS preconfigured JAZ-EL200 portable spectrometer (Ocean Optics, Florida, USA). One end of a 400 µm fibre (QP400-1-UV-VIS) was attached to the spectrometer. At the other end a Cosine Corrector (CC-3-UV-S) was attached for taking light readings sampled across 180°. Just prior to use, the spectrometer with attached fibre and probe was calibrated for absolute irradiance measurements against a radiometrically calibrated Deuterium Tungsten-Halogen light source (DH-2000, 220-1050 nm), following the manufacturers instructions.

FIG. S5. An overlay of the reflectance spectra from a *Chiloglottis trapeziformis* mature bud, showing reflectance of the outer whole bud, and dissected dorsal sepal and petal. Note that the flower parts produce very limited reflectance in the visible range (400 to 750 nm) and no detectable reflectance in the UV range (250 to 400 nm).



The reflectance measurements were taken with a UV/VIS preconfigured JAZ-EL200 portable spectrometer (Ocean Optics, Florida, USA) using a 400  $\mu\text{m}$  fibre reflection probe (R400-7-UV-VIS) consisting of a bundle of 7 optical fibres. The six illumination fibres surrounding the central read fibre were connected to a Deuterium Tungsten-Halogen light source (DH-2000, 220-1050 nm), while the read fibre was connected to the spectrometer. Reference and reflection measurements were taken in the dark with the probe held at 45°, using a Reflection probe holder (RPH-1). The reference spectrum was taken with the WS-1 Diffuse Reflectance Standard. The integration time was set to 170 ms, with each reflection spectrum the average of 5 scans.

TABLE S1. Flower and bud classifications used in the study.

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Code	Classification and description.
<i>Bud stages</i>	
vyb	<i>Very young bud.</i> Small and very tightly closed with both stalk and bud green in color.
yb	<i>Young bud.</i> Tightly closed with purple color developing on the stalk.
mb	<i>Mature bud.</i> Larger bud with purple color now developed on the stalk and bud, but sepals and petals remain tightly closed.
vmb	<i>Very mature bud.</i> Sepals and petals have begun to separate and it is evident the bud is about to open.
<i>Manually opened buds. See above for description.</i>	
mo-vyb	Manually opened very young bud.
mo-yb	Manually opened young bud.
mo-mb	Manually opened mature bud.
mo-vmb	Manually opened very mature bud.
<i>Flower stages and treatments</i>	
gdf	<i>Depleted flower initially opening in growth cabinet.</i> Flowers that had opened in the growth cabinet (with zero chiloglottone 1) were exposed to the sun for 6 h and then returned to growth cabinet for depletion to mean chiloglottone amounts of approximately zero (as assessed by the analysis of a subsample of flowers with successful depletion over 3 days in these experiments). [Figures 2, 3]
nsf	<i>No sunlight flower.</i> Flowers had opened in the growth cabinet (with zero chiloglottone 1) and were used 2 to 3 days after opening. Flowers had never been exposed to sunlight or UV-B prior to use. [Figures 2, 3].
fdf	<i>Depleted flower initially opening in the field.</i> Flowers that had opened in the field and were then maintained in the growth cabinet for depletion to mean chiloglottone amounts of approximately zero (as assessed by the analysis of a subsample of flowers for the given experiment with depletion typically over 5 or more days in these experiments). [Figure 4]
pdf	<i>Partially depleted flower initially opening in the field.</i> Flowers that had opened in the field and were then maintained in the growth cabinet for partial depletion to low, but not zero, chiloglottone amounts (depletion over less than 5 days) [Figure 5].

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