

Identification of gibberellins and involvement in hop flowering

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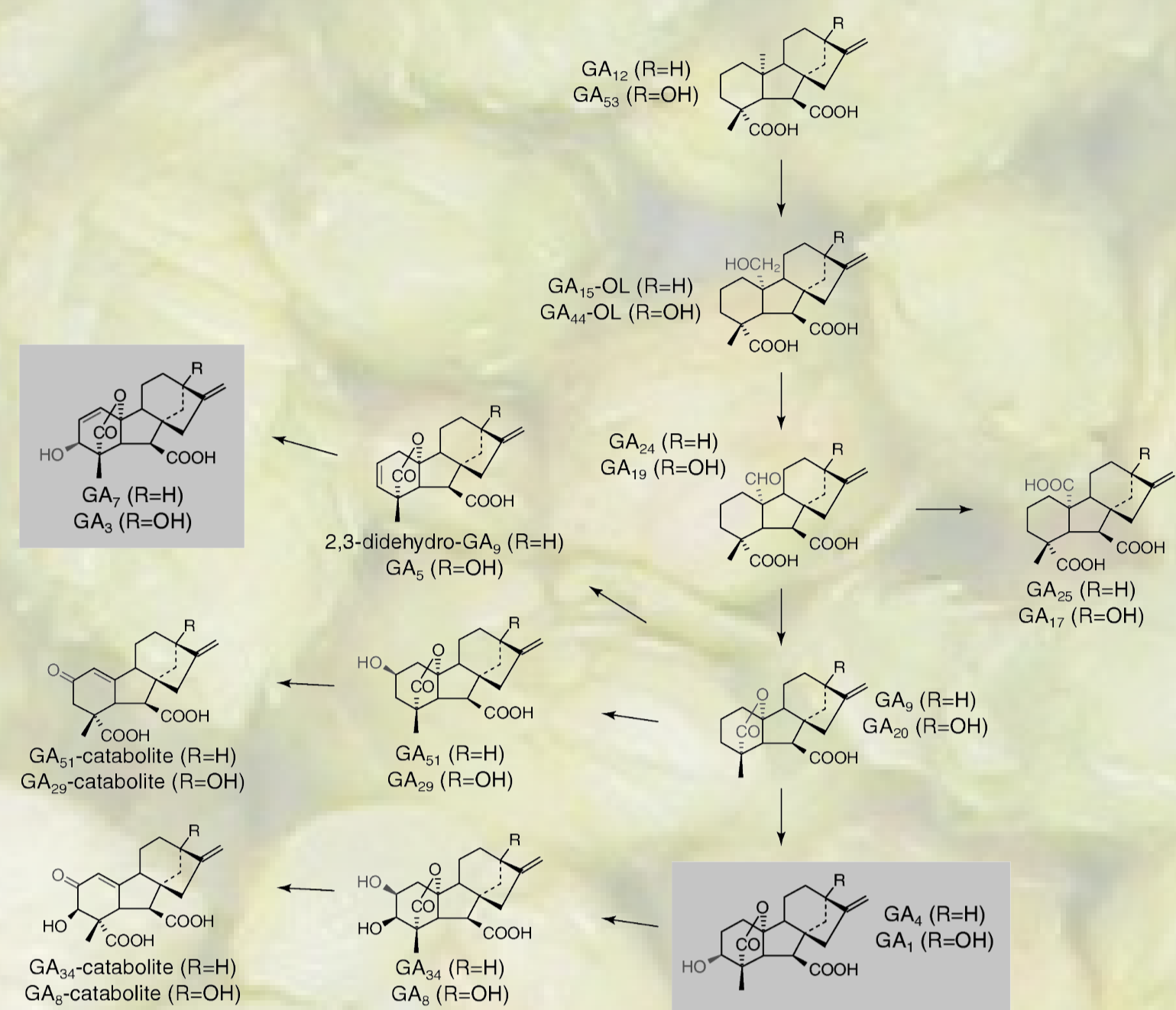
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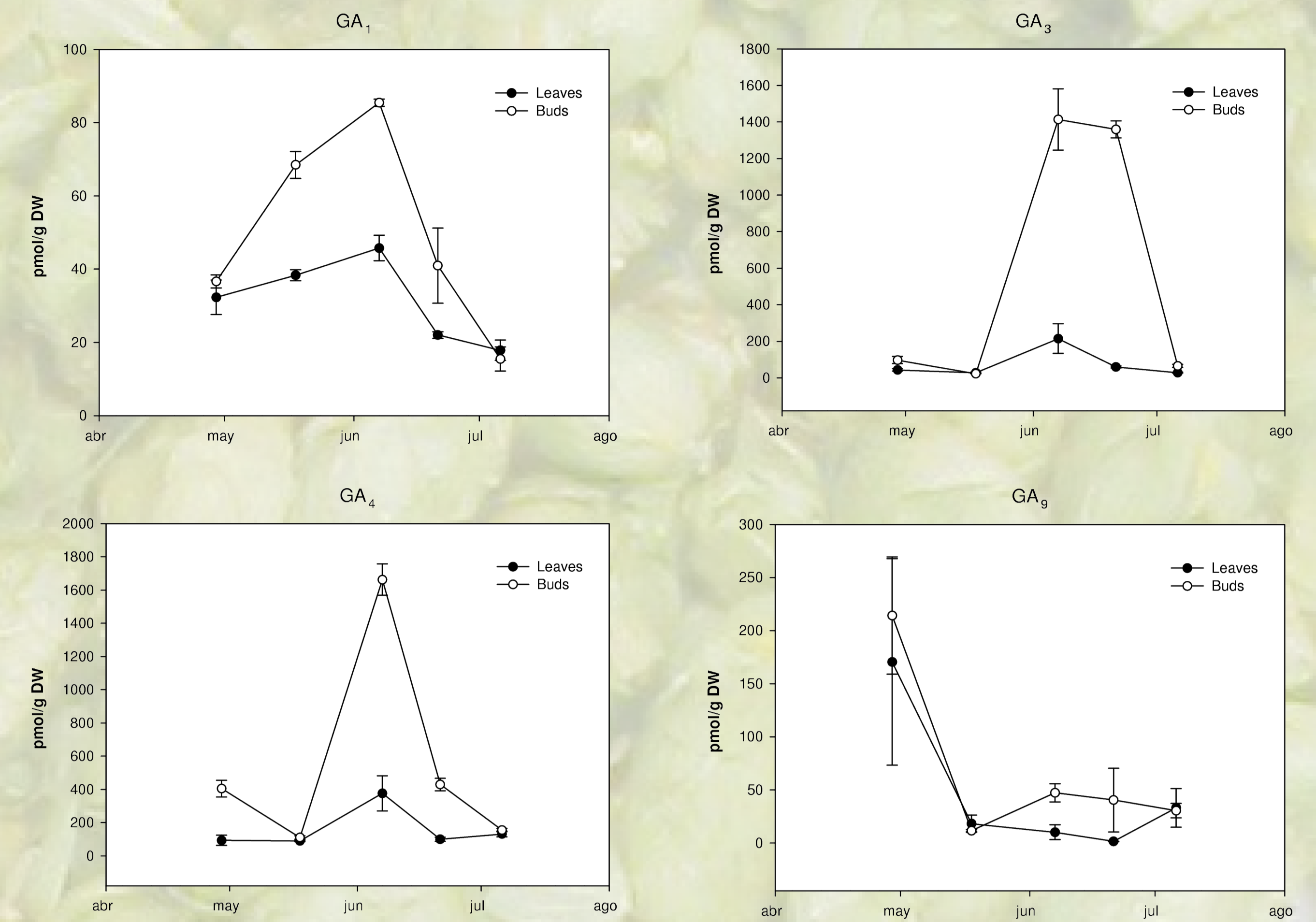
INTRODUCTION

Among all plant growth regulators studied so far, gibberellins (GAs) play a main role in internode spacing, induction and promotion of flowering in many plants, and are able to modify the flower sex expression in some plant species. Exogenous application of GAs has been assayed in hops [1] causing a delay in flowering time, even though an increase in the number of flowers per plant has been observed.

In this poster we present the identification of GAs in *Humulus lupulus* L. and its involvement in vegetative growth and early floral development.



RESULTS



Figures show the evolution in time of the different measured gibberellins. The two first points (April and May) correspond to vegetative growth of the main shoot and the three following (June and July) are data corresponding to floral development in lateral branches.

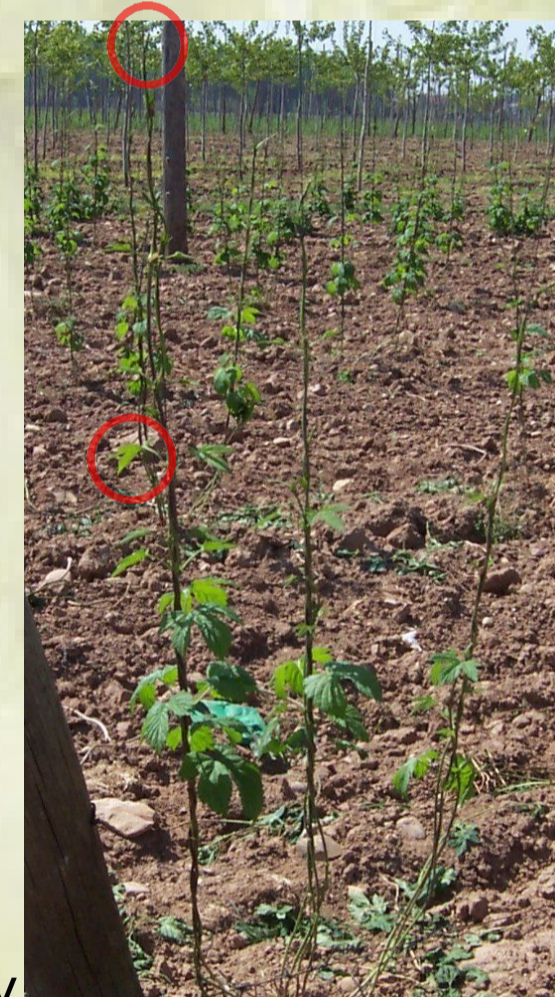
EXPERIMENTAL

Quantitative analyses of endogenous GAs (GA₁, GA₃, GA₄ and GA₉) have been carried out in the Nugget variety growing in the fields of S.A.E. de Fomento del Lúpulo placed in Villanueva de Carrizo (León, Spain) in the following times and organs:

Vegetative growth:



29th April



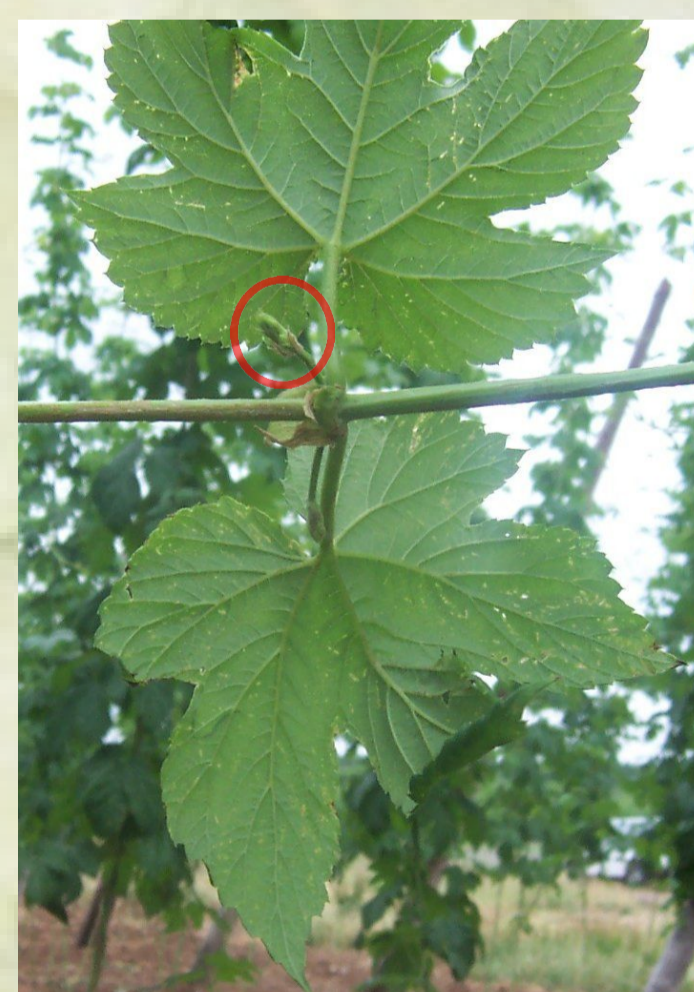
18th May

Photographs from first and second collections: apex and first pair of fully expanded leaves were analysed. At first collection, plants were only 20 cm high and at second collection they measured about two metres.

Floral development:



7th June



21st June

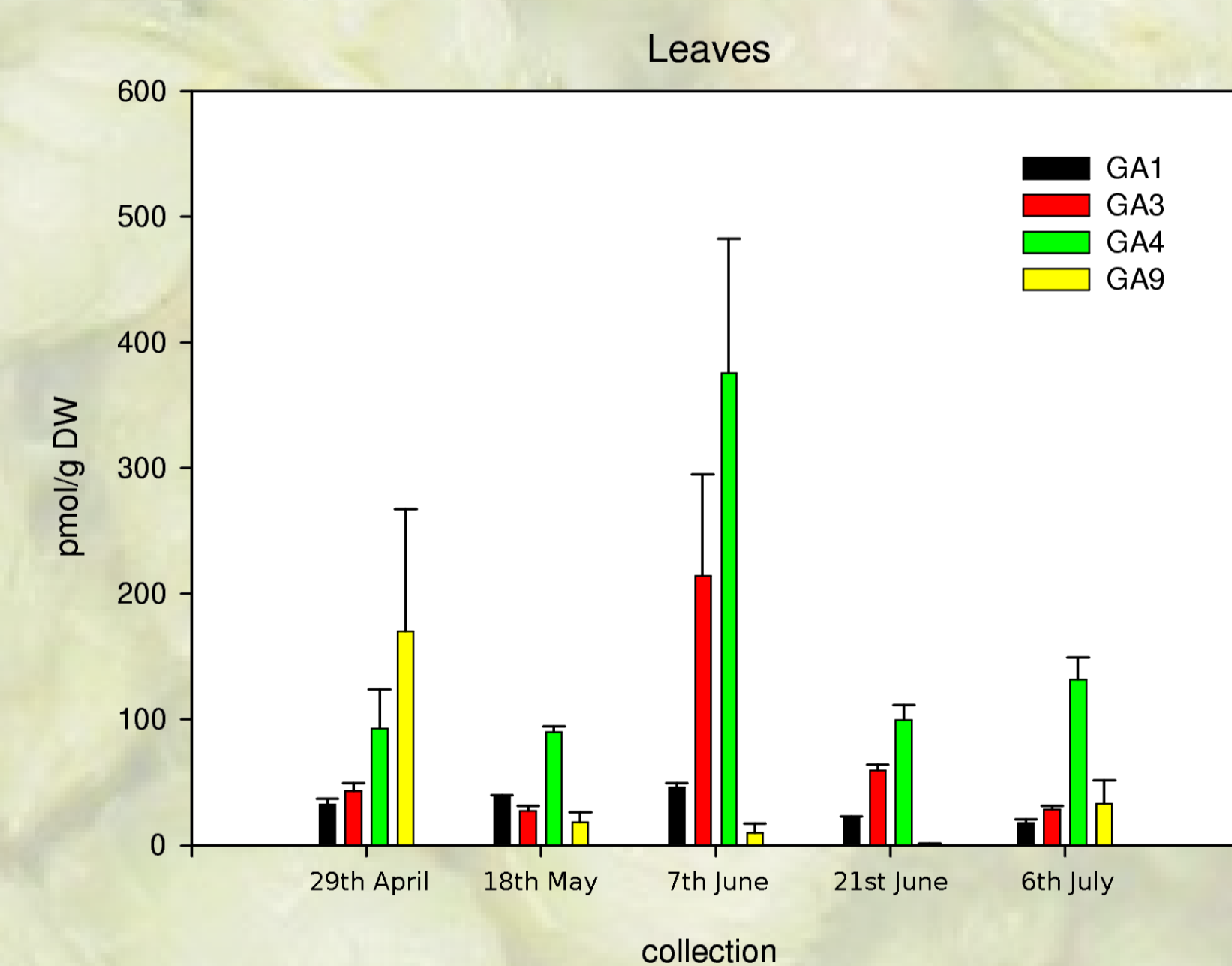
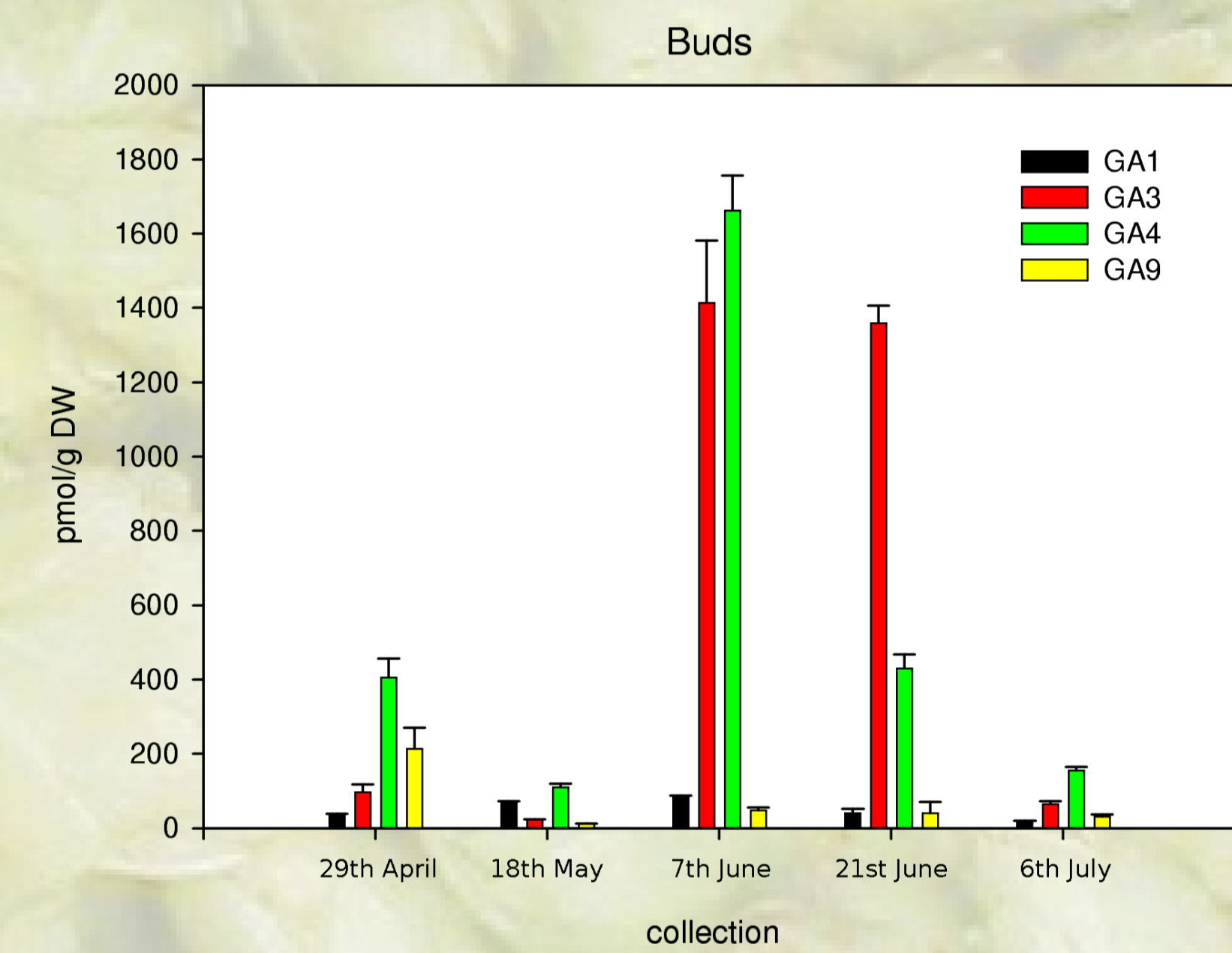


6th July

Photographs from third, fourth and fifth collections: buds and first pair of fully expanded leaves from lateral branches were analysed. At third collection, there were no visible floral development although branches were fully grown.

Analysis steps: (modified from [2])

- Overnight extraction of LN powdered samples in 80% methanol with addition of deuterated GAs for final quantification.
- Evaporation of methanol and extraction of GAs from the acidified aqueous extract with ethyl acetate (1:2) x3. Ethyl acetate layers are pooled and evaporated to dryness.
- Sample is resuspended in 80% methanol and a solid phase extraction (Sep-Pack C18) is performed.
- Further purification is achieved using a SiO₂ column.
- Phytohormones are separated by RP-HPLC and fractionated. Retention time is verified running templates of IAA and tritiated GAs, kinetin is used as internal standard in every sample.
- Fractions are brought to dryness, methylated with ethereal diazomethane and trimethylsilylated using BSTFA with 1% TCMS (v/v).
- Quantification is performed by GC-MS.



CONCLUSION

- Relationship of GAs with vegetative growth has been proved in hops: they play an important role in the elongation process.
- Levels of GAs decrease dramatically during early floral development.

Future prospects:

- Analyses of other phytohormone groups (cytokinins, indol-3-acetic acid and abscisic acid), which have an important role in plant development as well.
- Establishing the influence of exogenous application of growth regulators in biosynthesis of alpha acids, beta acids and xanthohumol.
- Having such a complete hormonal characterization, endogenous phytohormone balances could be used as precocious production markers in hops.

REFERENCES

- [1] Thomas, G. G. & Schwabe, W. W. 1969: Factors controlling flowering in the hop (*Humulus lupulus* L.). *Ann. Bot.* **33**: 781–793.
 [2] Fernández, H.; Doumas, P.; Bonnet-Masimbert, M. 1997: Quantification of GA₁, GA₃, GA₄, GA₇, GA₈, GA₉, GA₁₉ and GA₂₀; and GA₂₀ metabolism in dormant and non-dormant beechnuts. *Plant Growth Regulation.* **22**: 29–35.