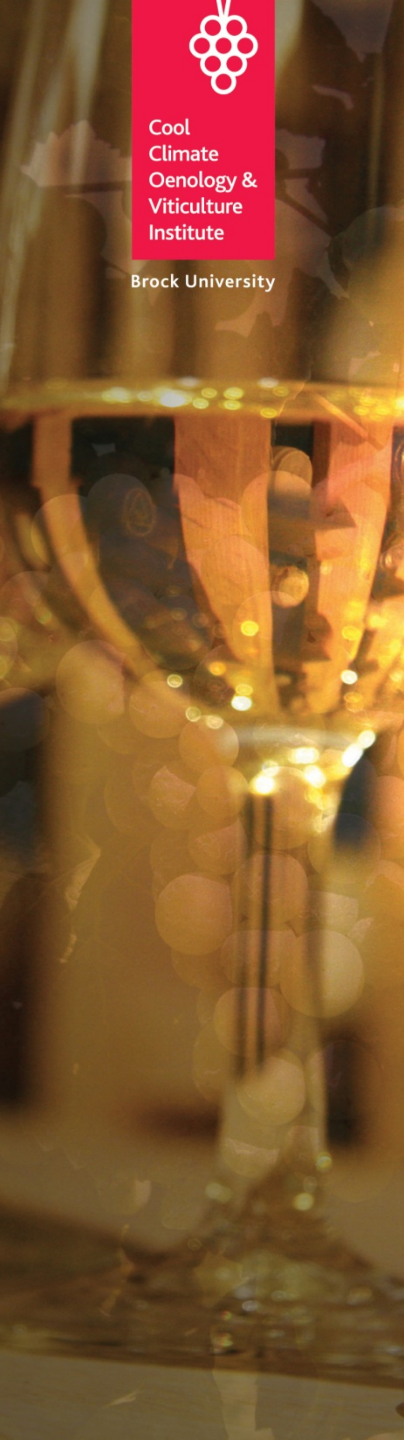

Brock University
Environmental Sustainability Research Centre
Cool Climate Oenology & Viticulture Institute
Brock University




# Proselytizing pyrazines

## - How to avoid and remediate greenness in wine -

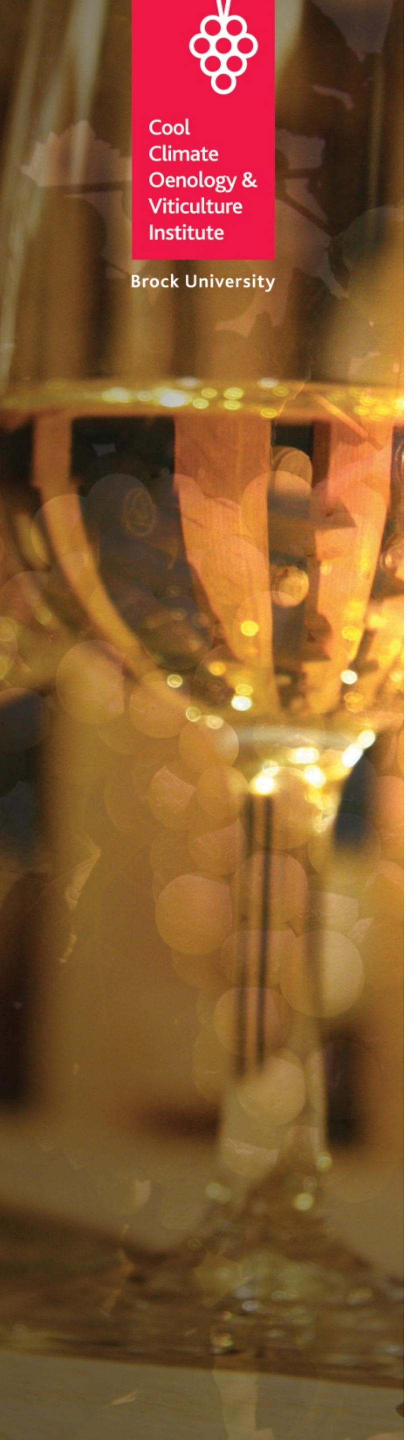
---

Gary J. Pickering, PhD

Professor, Cool Climate Oenology & Viticulture Institute, Environmental Sustainability Research Centre, Department of Biological Sciences, Brock University, Canada

Adjunct Professor, National Wine & Grape Industry Centre, Charles Sturt University, and University of Sunshine Coast, Australia

Scientific Director, Ontario Grapevine & Wine Research Network



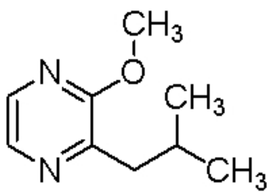

# Acknowledgements


- Dr David Adams, Dr Ai-Lin Beh, Amy Blake, Dr Andreea Botezatu, Ryan Brewster, Dr Ian Brindle, Neil Carter, X Chen, Dr Christoph Hoffmann, Dr Thomas Hudlicky, Eric Hulmes, Dr Debbie Inglis, Anna Karthik, Dr Belinda Kemp, Kevin Ker, Dr Susanne Koegel, Dr George Kotseridis, James Lin, Dr Wendy McFadden-Smith, Dr Andy Reynolds, Dr Roland Riesen, Gavin Robertson, Dr Mark Sears, Dr George Soleas, Mason Spink, Terrance van Rooyen, Lynda Van Zuiden, Tony Wang, Shufen Xu, Fei Yang, Dr Paul Zelisko

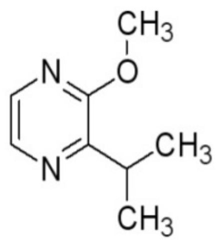
- Ontario Research Fund, Ontario Grape & Wine Research Inc., Natural Sciences and Engineering Research Council of Canada, Faculty of Mathematics and Science Brock University

# What are alkyl-methoxypyrazines (MPs) ?

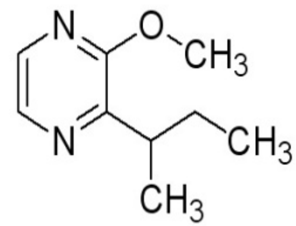
- An important and potent class of fruit- , microbial- and insect-derived odorants associated with juice and wine quality



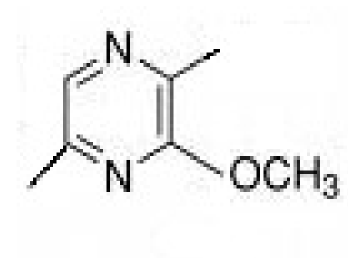
3-isobutyl-2-MP (IBMP)



3-isopropyl-2-MP (IPMP)

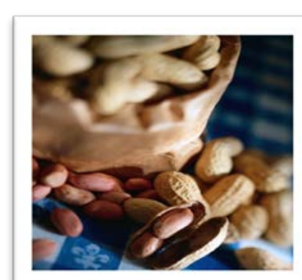


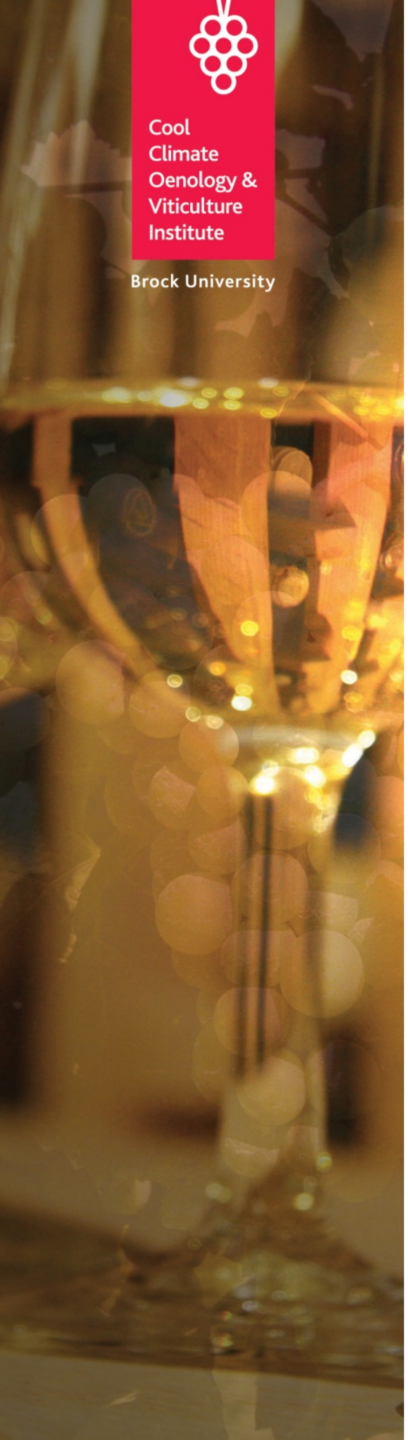
3-secbutyl-2-MP (SBMP)



2,5 dimethyl -3 – MP (DMMP)

- Elicit green and vegetative aroma and flavour

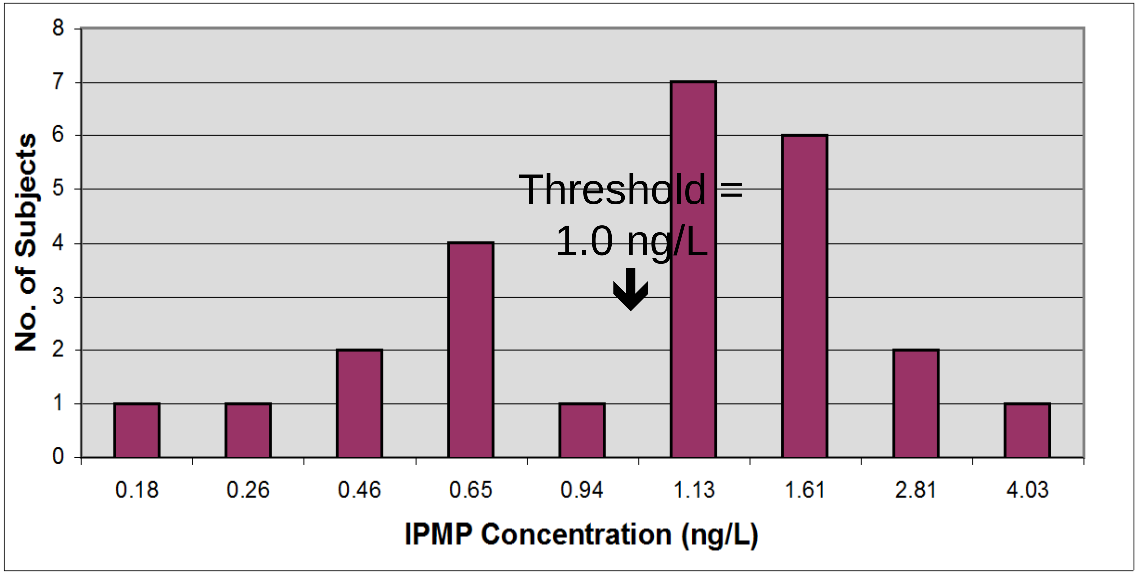




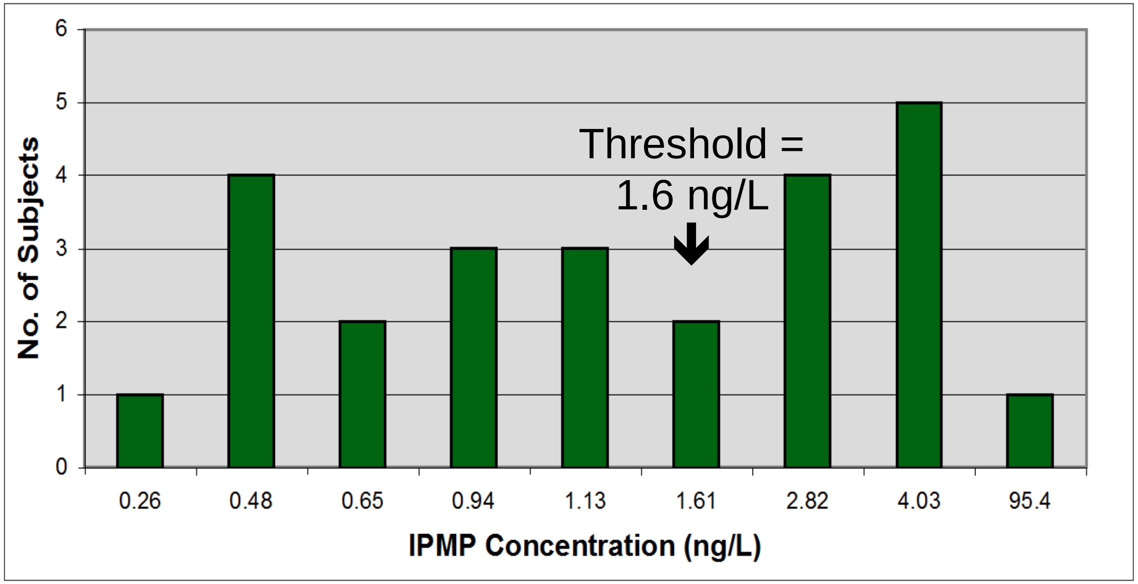

# MPs in wine: significance

- DMMP and IBMP most prevalent (Botezatu et al., 2016)
- IBMP most studied til recently: contributes **typicity:**
  - Sauvignon blanc (Parr et al., 2007)
  - Carmenere (Belancic and Agosin, 2007)
  - Cabernet franc, Merlot, Cabernet sauvignon (low levels) (Roujou de Boubee et al., 2000)
- Elevated MP concs:
  - Dominant, unpleasant in wine (Allen et al., 1994)
  - Associated with low wine quality (Roujou de Boubee, ‘00)
- Very, very low sensory threshold –
  - IBMP, SBMP: 1-16 ng/L (reviewed in Sidhu et al., ‘15)
  - DMMP: 31 ng/L (Botezatu et al., 2012)
  - IPMP: down to 320 pg/L (Pickering et al., 2007)
- Note variation due to matrix and individual sensitivity …

# Distribution of individual IPMP thresholds in wine




**Red blend**



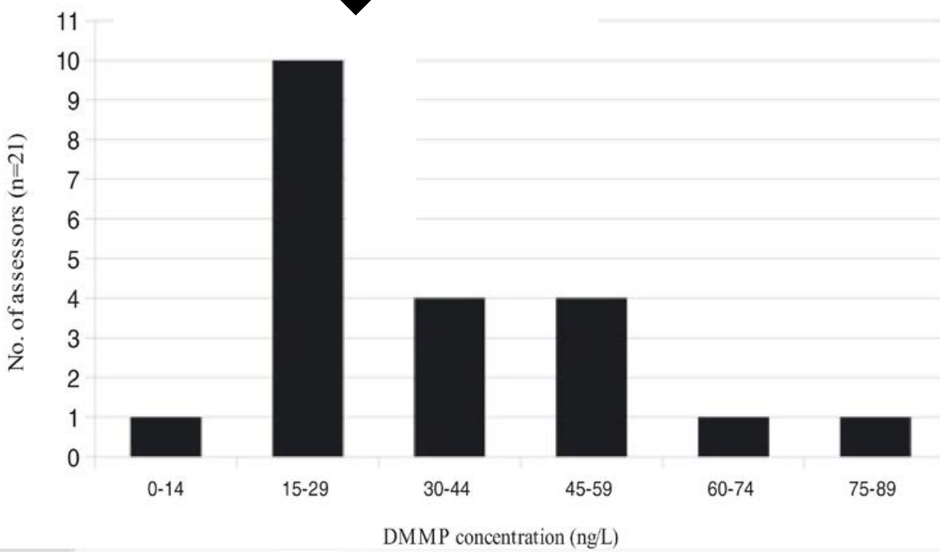

**Gewürz-traminer**

From:

Pickering et al (2007) *J. Food Sci*

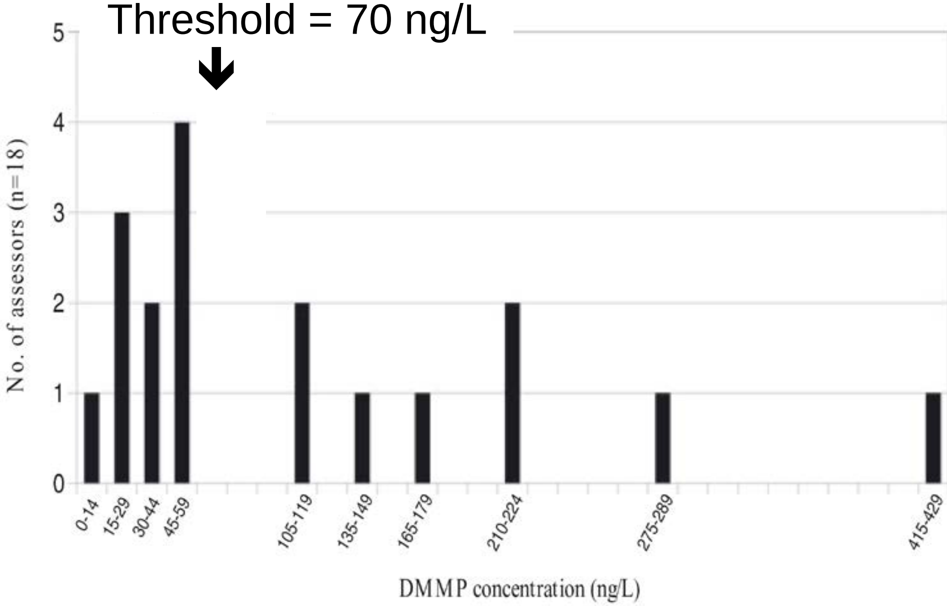
# Mode of evaluation matters

Threshold = 31 ng/L




## Orthonasal

Threshold = 70 ng/L



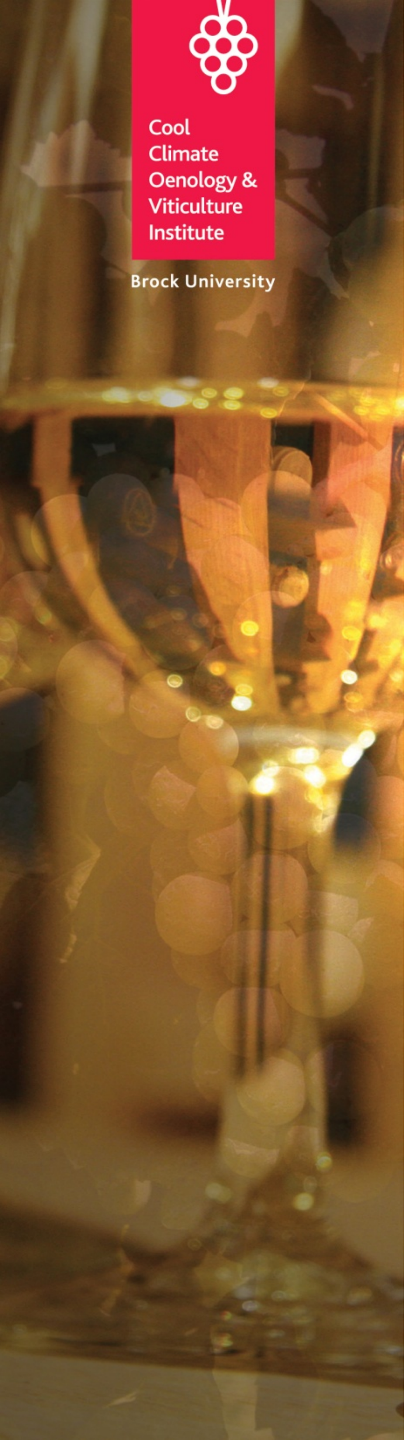

## Retronasal

From: Botezatu & Pickering *et al* (2012) *J. Food Sci*

Cool Climate Oenology & Viticulture Institute
Brock University

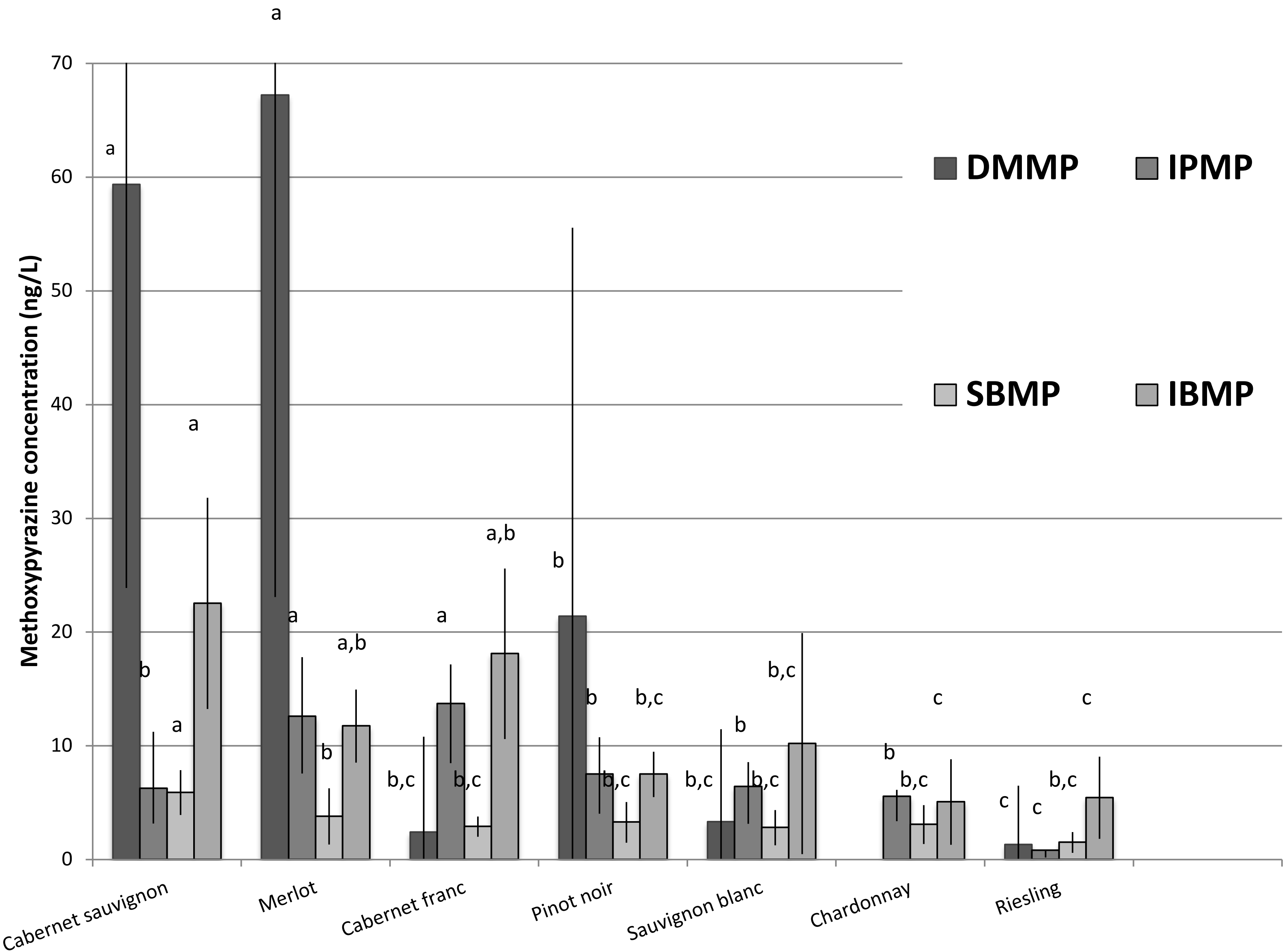
# Sources of MPs in wine

- Grape-derived
- Adulteration
- Coccinellidae (ladybeetles)

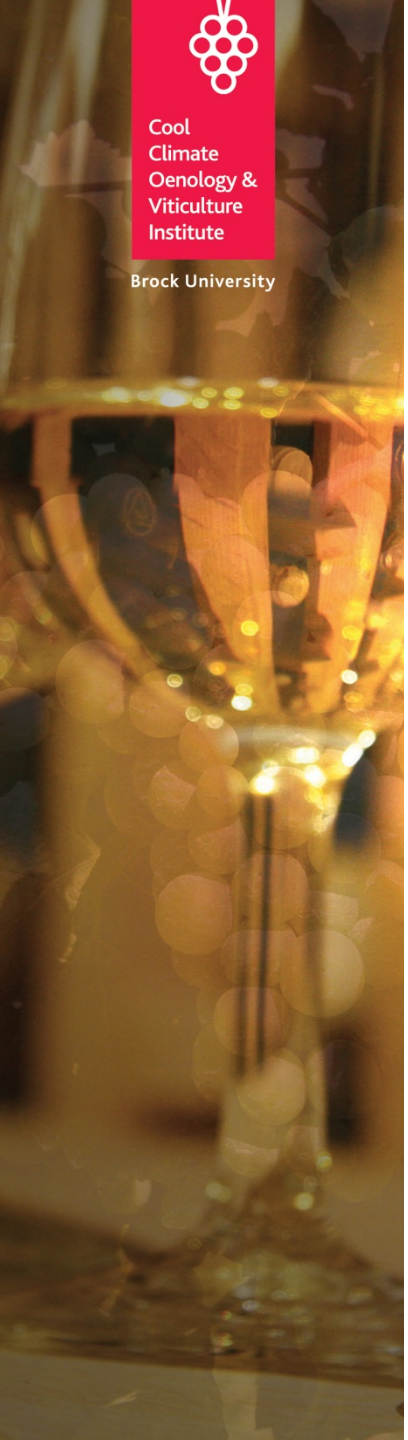



# Sources of MPs in wine

- Grape-derived
  - Sauv blanc, Cab sauv, Cab franc, Merlot, Pinot noir, Carmenere & others (Botezatu et al., 2016)
  - Concentration in the berry varies. Most located in stems, skins & seeds (Hashizume & Samuta, ’96)
  - Mediated by climate, light exposure & ripeness
    - ➢ Higher concs in cooler climate, but some divergent findings (Scheiner et al., ‘13; Botezatu et al., ‘16)
    - ➢ ↓ during grape ripening (Ryona et al., 2007)
    - ➢ Climate variability may be increasing MP loads
    - ➢ Reviewed in Sidhu et al (2015)



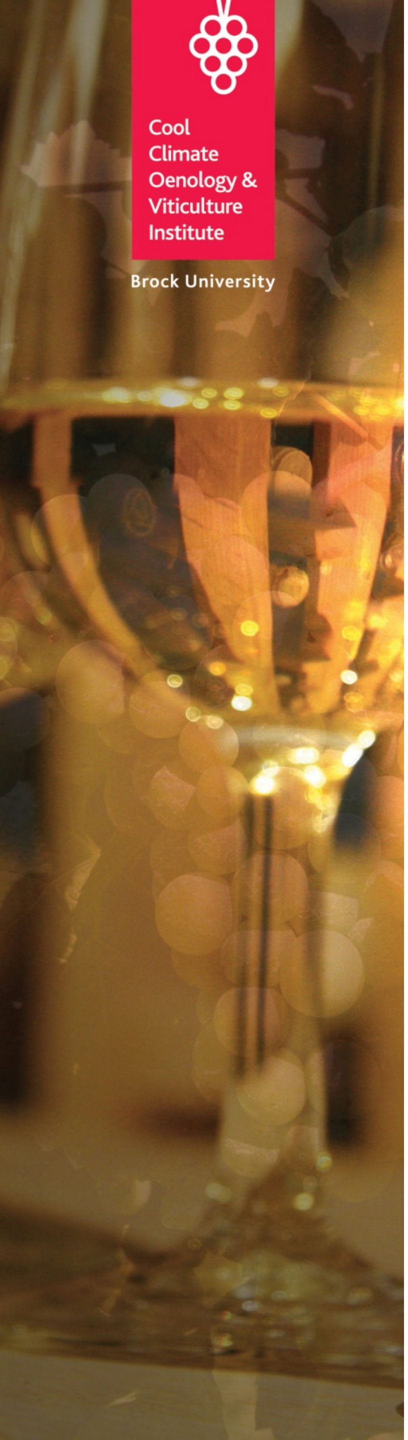

Dimethyl methoxypyrazine (DMMP), isopropyl methoxypyrazine (IBMP), secbutyl methoxypyrazine (SBMP) and isobutyl methoxypyrazine (IBMP) concs vary with variety (n=187) (From: Botezatu et al (2016) *J Food, Ag. Env*)




# Sources of MPs in wine

- Grape-derived

- Adulteration
  - (illegal) fortification of wine
  - South African Sauvignon blanc (Fridjhon, 2003; Morris, 2004; Galpin, 2006)




# Sources of MPs in wine

- Grape-derived

- Adulteration

- Coccinellidae (ladybeetles)
  - *Harmonia axyridis* (‘MALB’) and *Coccinella septempunctata* (‘C7’)

# Multicoloured Asian Lady Beetle

- "MALB"; *Harmonia axyridis;* "Halloween beetle"; "Harlequin ladybird'"; "Japanese LB"
  - Introduced as bio-control tool
  - Migrates into vineyards in Fall …

Problem: Incorporation of MALB or C7 with grapes at harvest (> approx 1 beetle/vine)

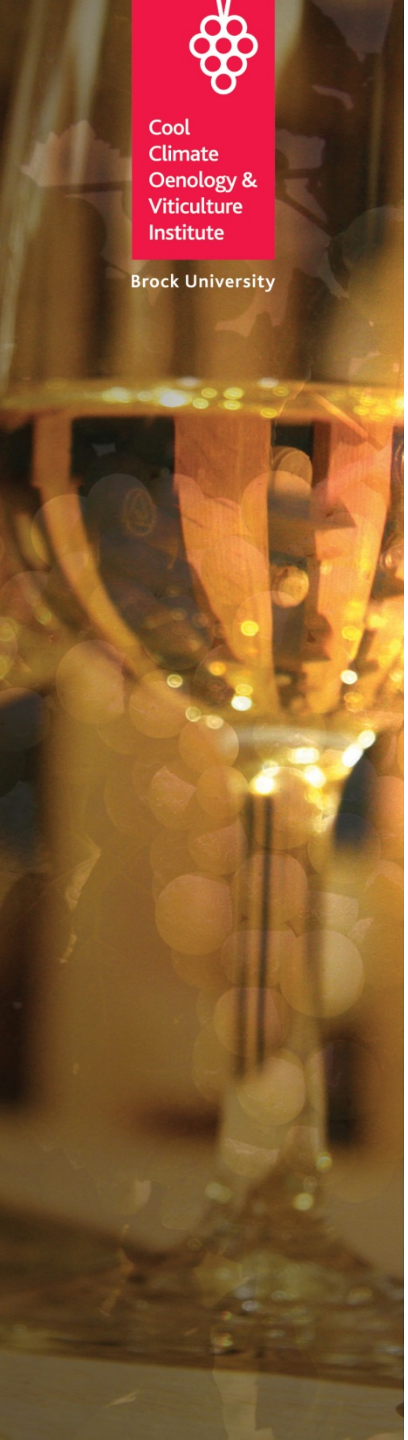
- Atypical peanut, green pepper, vegetal aroma + flavour = 'Ladybug taint'
- MPs (IPMP) from haemolymph are responsible (Pickering et al., '04, 05, 08, '10; Kogel et al, '14; + others)
- Known problem in France, Germany, USA, Canada. Present in UK & many other wine regions
  - Global warming





*Harmonia axyridis*

K Ker & R Brewster




# MPs in wine (grape): prevention ?

- No. Intrinsic to many varieties.

- Can minimize through:

  - light exposure

    - light exposure pre-veraison (Roujou de Boubee et al. ’02; Scheiner et al., ’10; Suklje et al., 2013)

      - Leaf removal

  - ripeness

    - ↓ during grape ripening (Ryona et al., 2007)

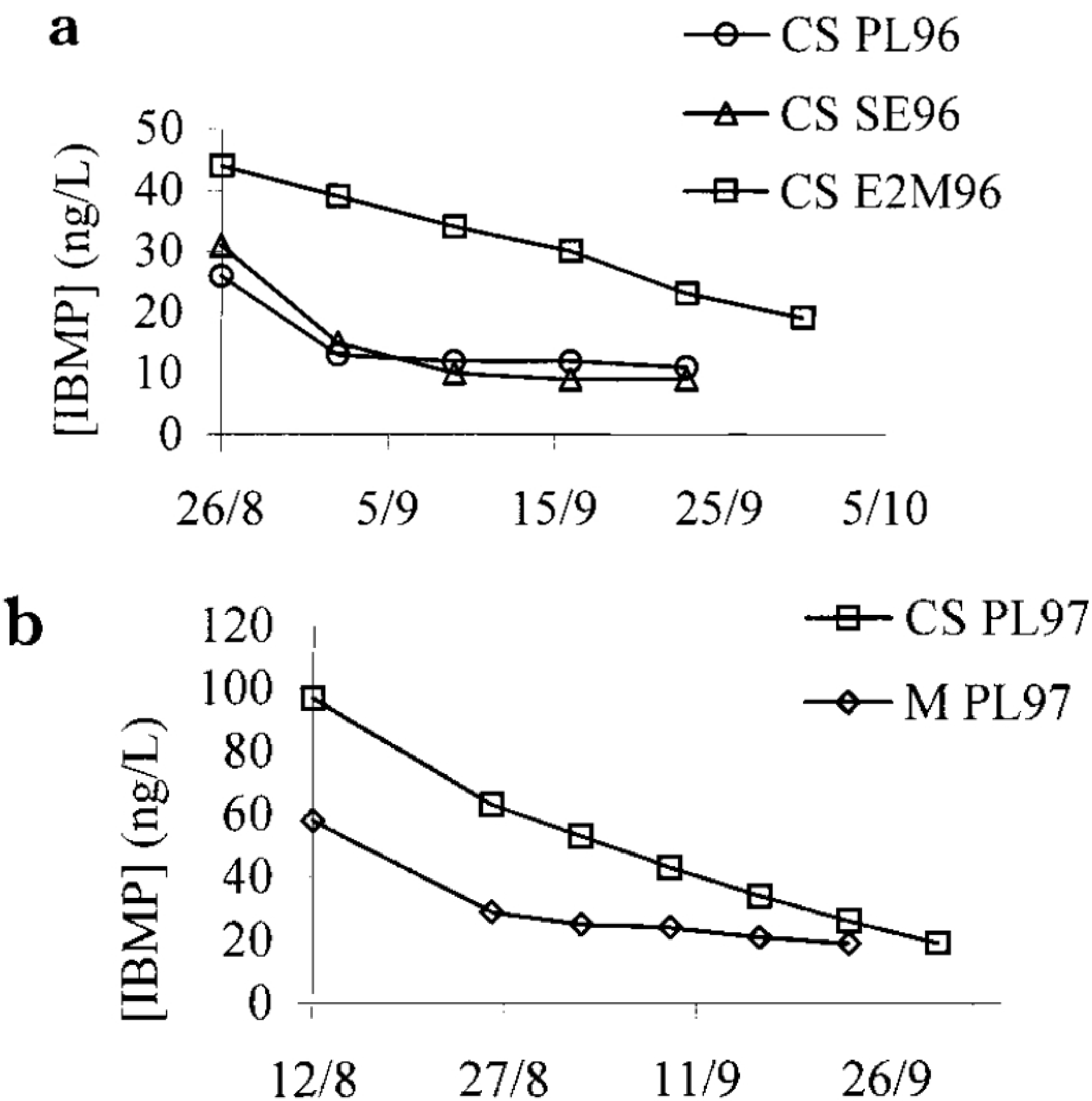



**Figure 2.** Variations in the IBMP concentration of grapes during ripening in three different environmental conditions in 1996 [(a) Pessac-Léognan (PL), Saint Emilion (SE), and Entre-Deux-Mers (E2M)] and in one Pessac-Léognan vineyard in 1997 (b). CS, Cabernet Sauvignon; M, Merlot.

# Effect of leaf and lateral shoot removal in the bunch zone pre-veraison on IBMP in Sauvignon blanc

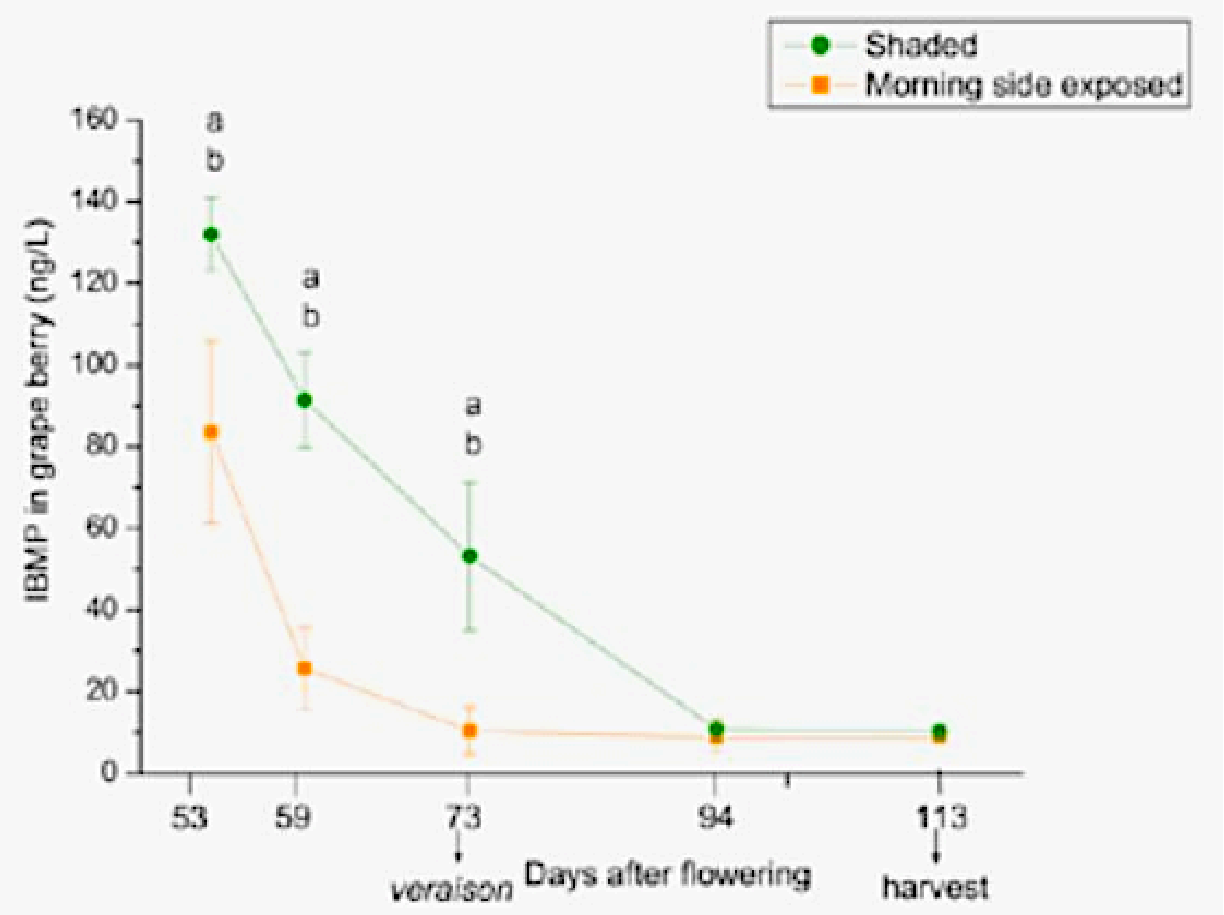



FIGURE 6. The concentration of IBMP (ng/L) in Sauvignon blanc grape berries from 53 days after flowering (12 January 2012) to harvest, 113 day after flowering (13 March 2012) for the shaded and the morning side exposed treatments.

Suklje et al. (2013)

# MPs in wine (ladybugs): prevention ?

Insecticide sprays in vineyard main management tool

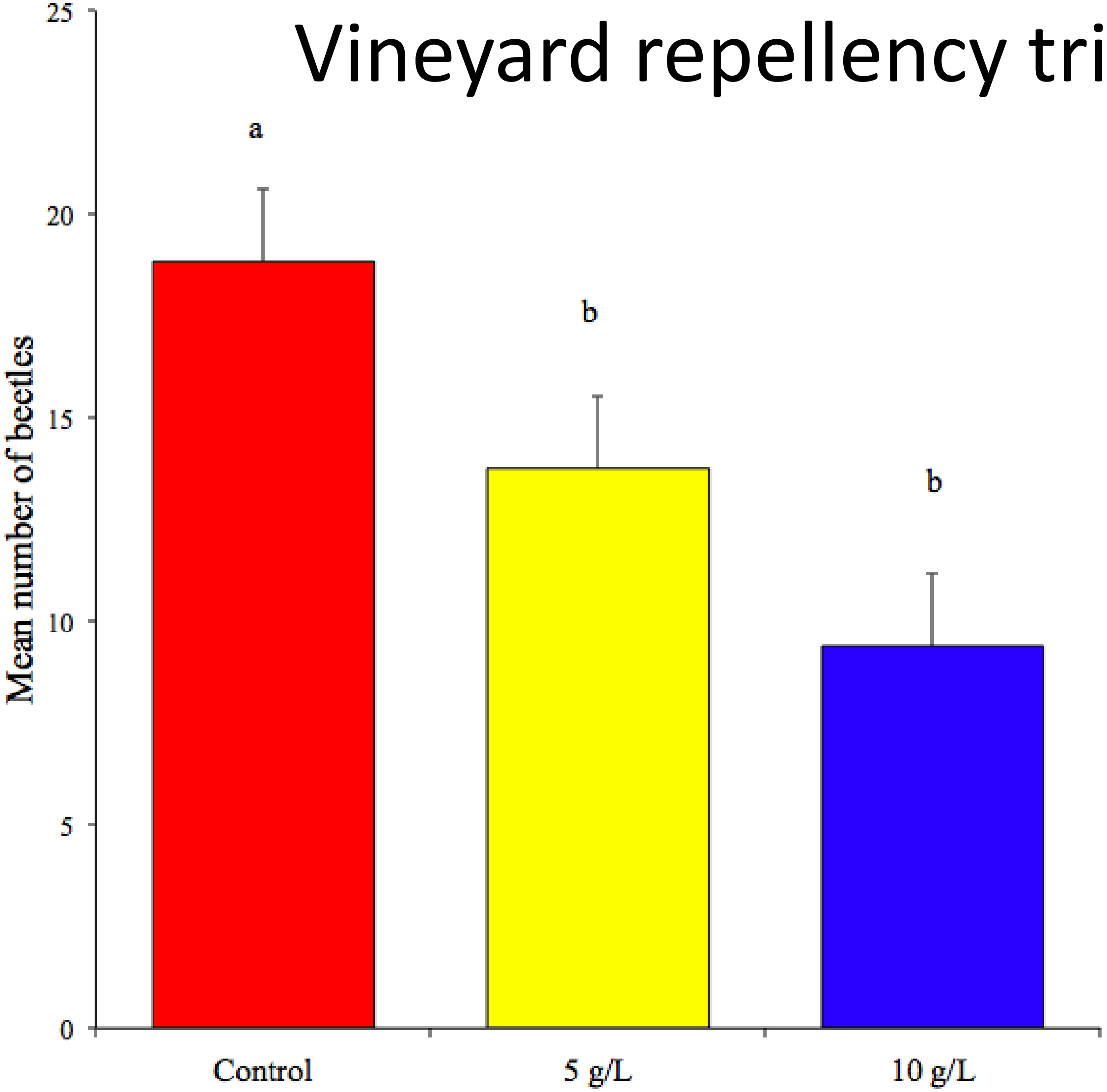
> Ontario - Cypermethrin, Malathion

> Potential concerns:

- Unsafe levels of residues from injudicious use

- Pre-harvest intervals limit efficacy

# Vineyard repellency trials - KMS



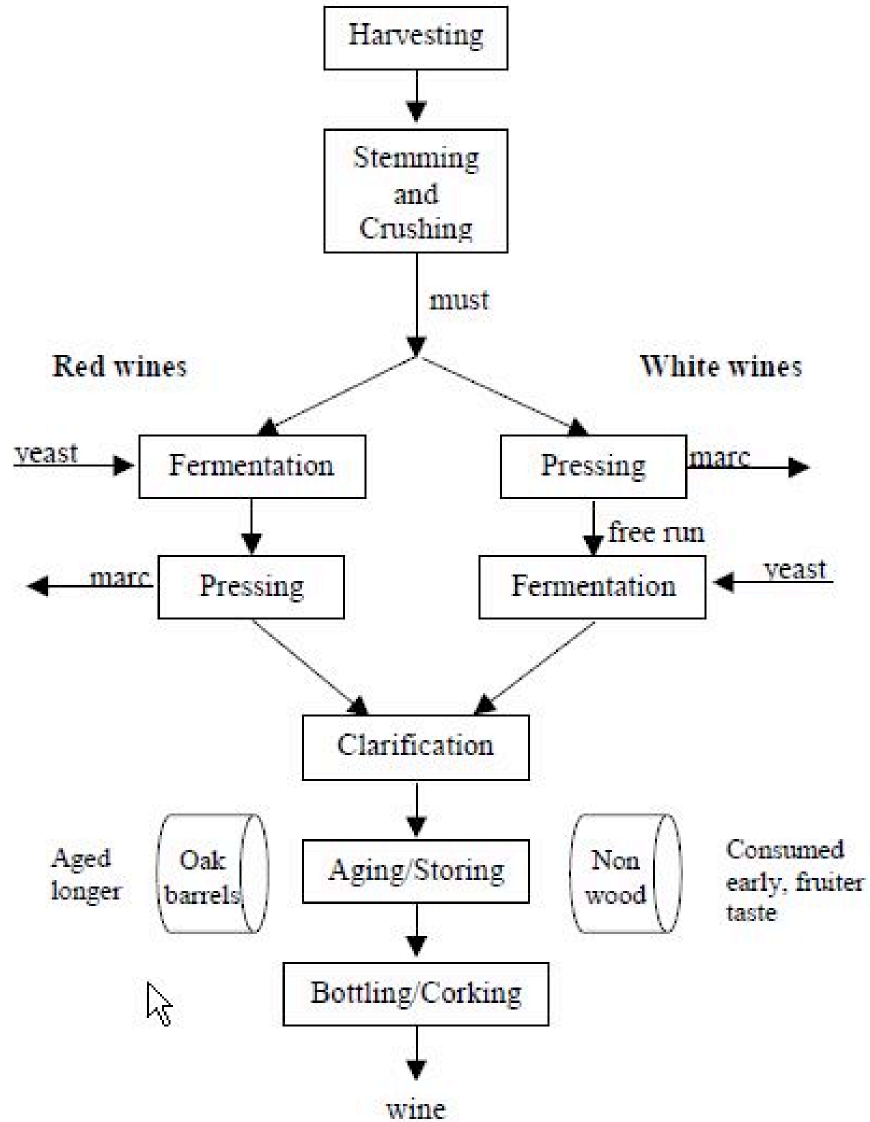

Mean number of MALB present on grape vines (Gewürztraminer) 24 h after application of potassium metabisulphite (at 5, or 10 g/L). Treatments were replicated five times (*N*=20). Means followed by the same letter are not significantly different (Tukey's $HSD_{0.05}$). Adapted from Glemser *et al* (2012).

# MPs in wine (ladybugs): prevention ?

1. Water soak
   - anecdotally successful (USA)
   - dilution, quality & VQA issues

2. Shaker tables

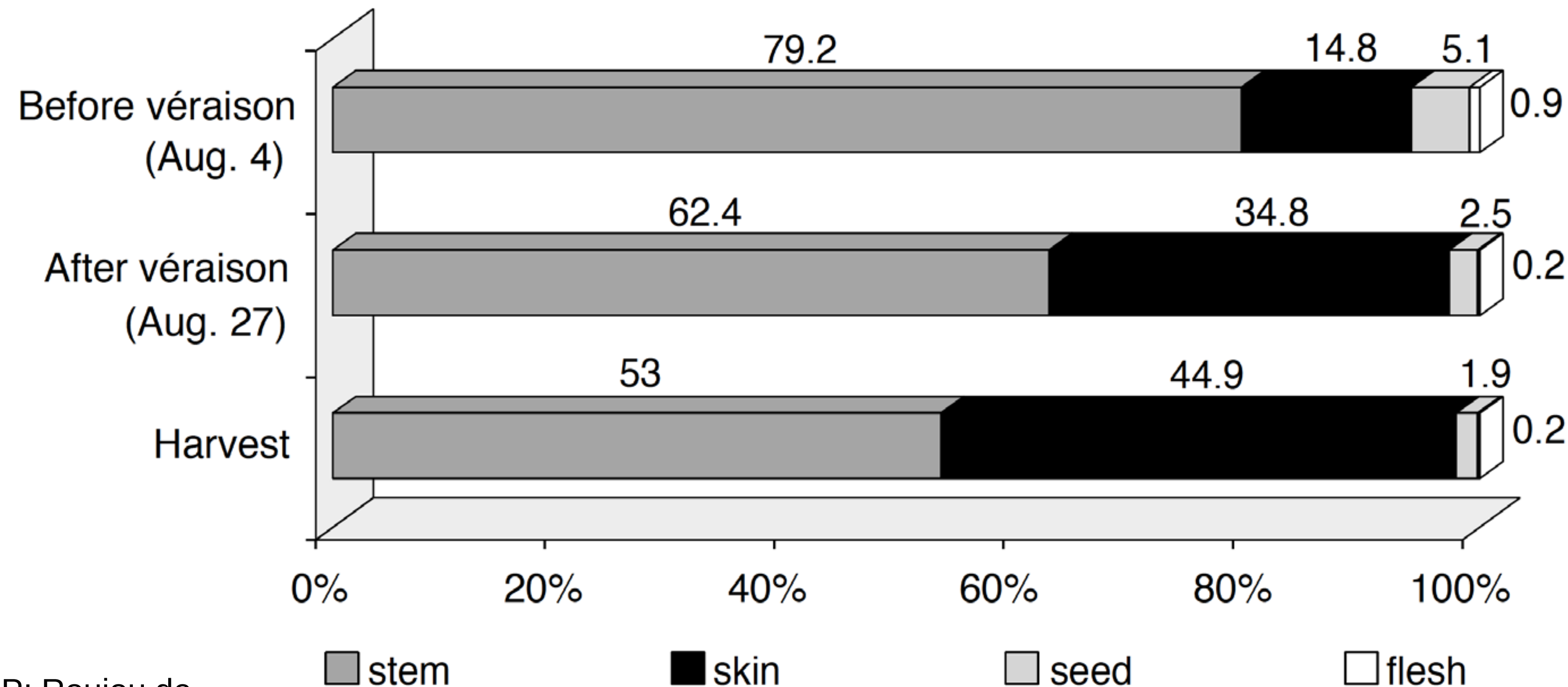
# MPs in wine: remediation



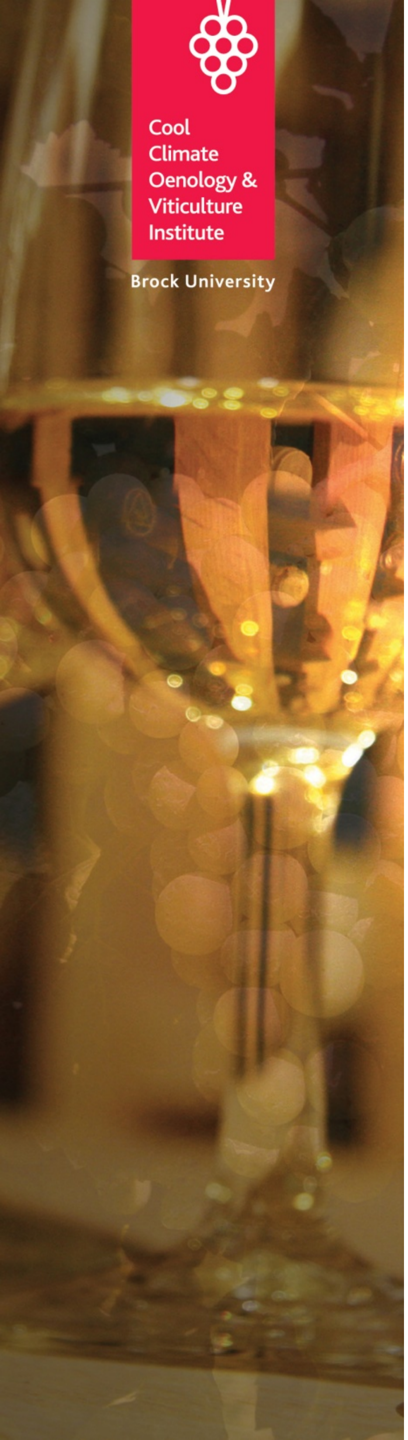

University of California, 2005

# Crush/de-stem, clarify ?

- Very important to de-stem
  (Hashizume & Umeda, 1996; Roujou de Boubee et al., '02)



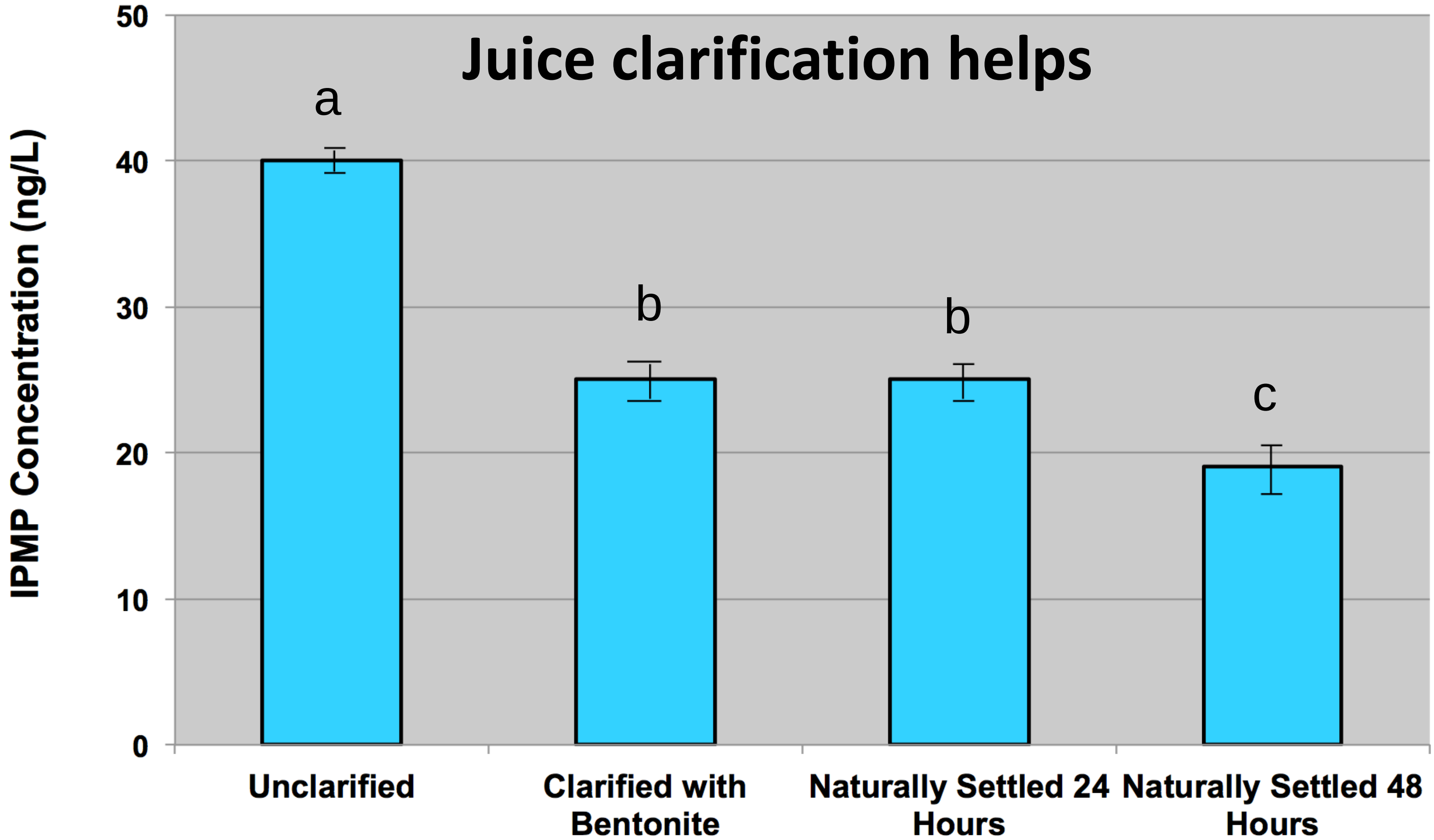

IBMP; Roujou de Boubee et al., '02)




# Crush/de-stem, clarify ?

- Very important to de-stem
  (Hashizume & Umeda, 1996; Roujou de Boubee et al., ‘02)

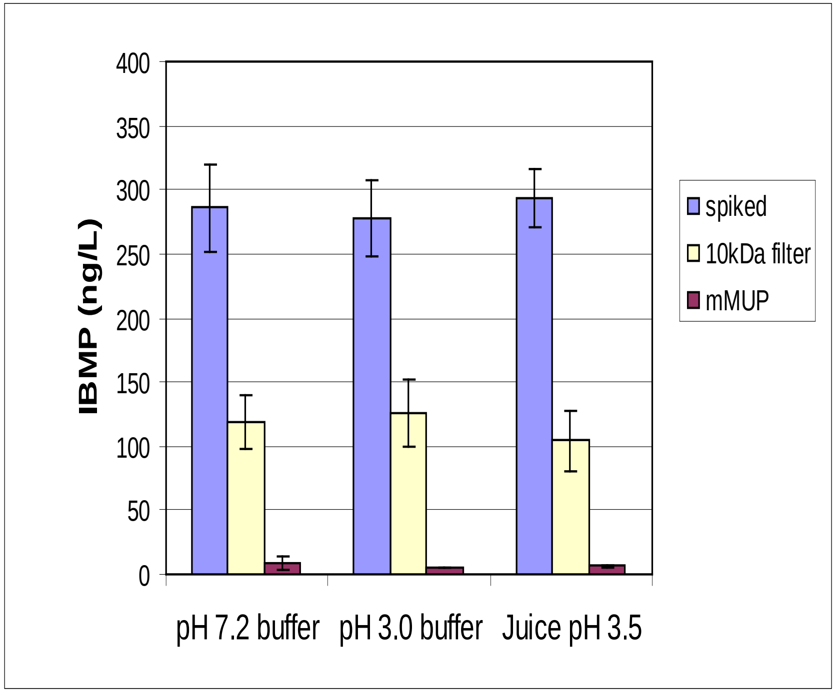
- Minimise skin contact if possible
  - MP levels 2-3x higher in juice after crushing compared to fermented wine (Sidhu et al., 2015)
  - Alcohol not critical to MP extraction as most MPs extracted during first 24 hrs (Sala et al., 2004; Sidhu et al., 2015)

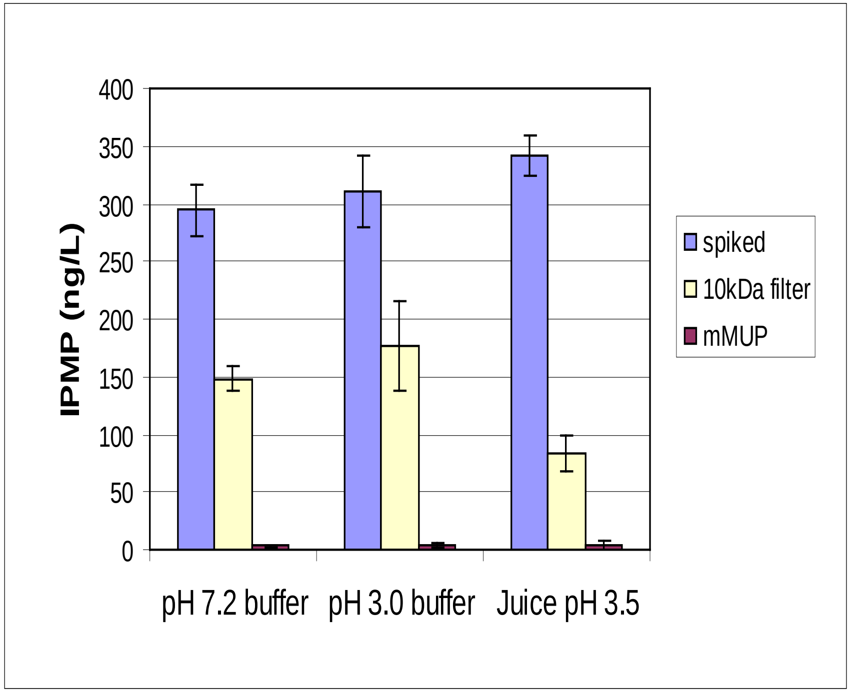



Mean IPMP concentrations in Chardonnay juice after various clarification treatments

(from Kotseridis et al '08 *J Chrom A*, 1190. NB: high turbidity juice (1280 NTU))

# Reduction of methoxypyrazines by mMUP (Odorant Binding Protein) & filtering




IBMP reduced from 300ng/L to less than 5ng/L (LOQ) in Chardonnay juice

IPMP reduced from 300ng/L to less than 2ng/L (LOQ) in Chardonnay juice

*Not shown: bentonite fining gave 95% reduction*

Inglis et al. (2010)

# Thermovinification?

**Table 1.** Concentrations of 2-isopropyl- (IPMP), 2-*sec*-butyl- (SBMP), 2-isobutyl- (IBMP) and 2,5-dimethyl- (DMMP) 3-methoxypyrazine in Pinot noir wines infested with varying numbers of *Harmonia axyridis* (Ha) beetles. One treatment was must heated (3 h, 65 °C) (MH) prior to fermentation (6 days) and one was not (control)

| Treatment | Ha beetles $kg^{-1}$ grapes | IPMP ($ng\ L^{-1}$) | SBMP ($ng\ L^{-1}$) | IBMP ($ng\ L^{-1}$) | DMMP ($ng\ L^{-1}$) |
|---|---|---|---|---|---|
| Control | 0 | $9.1 \pm 1.3$ | $5.4 \pm 0.3$ | $14.0 \pm 1.1$ | $51.0 \pm 0.1$ |
| | 1 | $14.9 \pm 1.0$ | $4.9 \pm 0.6$ | $13.7 \pm 0.2$ | $58.2 \pm 2.0$ |
| | 10 | $38.9 \pm 5.4$ | $18.0 \pm 0.4$ | $14.3 \pm 4.3$ | $54.5 \pm 5.6$ |
| Must heated | 0 | $11.0 \pm 1.4$ | $5.3 \pm 1.1$ | $11.2 \pm 1.8$ | $47.7 \pm 5.9$ |
| | 1 | $11.4 \pm 6.0$ | $3.7 \pm 0.5$ | $14.2 \pm 0.0$ | $46.1 \pm 9.6$ |
| | 10 | $28.4 \pm 4.6$ | $7.2 \pm 1.7$ | $12.2 \pm 1.1$ | $46.2 \pm 2.4$ |
| Δ(MH – control) (0 beetles $kg^{-1}$) | | 1.8 (20%) | −0.1 (2%) | −2.8 (20%) | −3.3 (6%) |
| Δ(MH – control) (1 beetle $kg^{-1}$) | | −3.5 (23%) | −1.2 (24%) | −0.5 (4%) | −12.1 (21%) |
| Δ(MH – control) (10 beetles $kg^{-1}$) | | −10.5 (27%) | −10.7 (59%) | −2.0 (14%) | − 8.3 (15%) |

Data shown are mean values of duplicate analytical replicates +/− standard deviations.

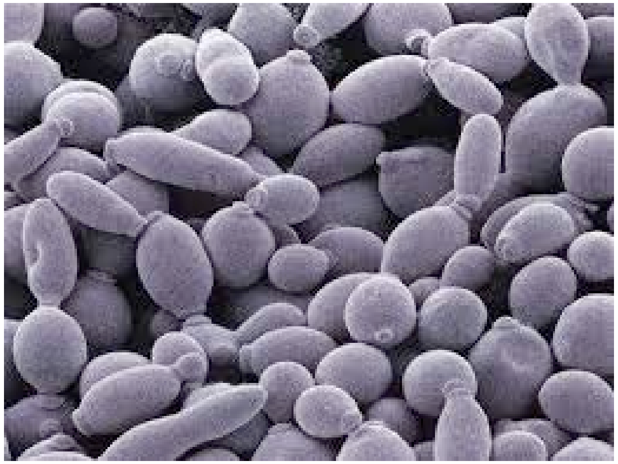
Kogel et al. (2015). *J Sci Food Agric 95*: 509–514

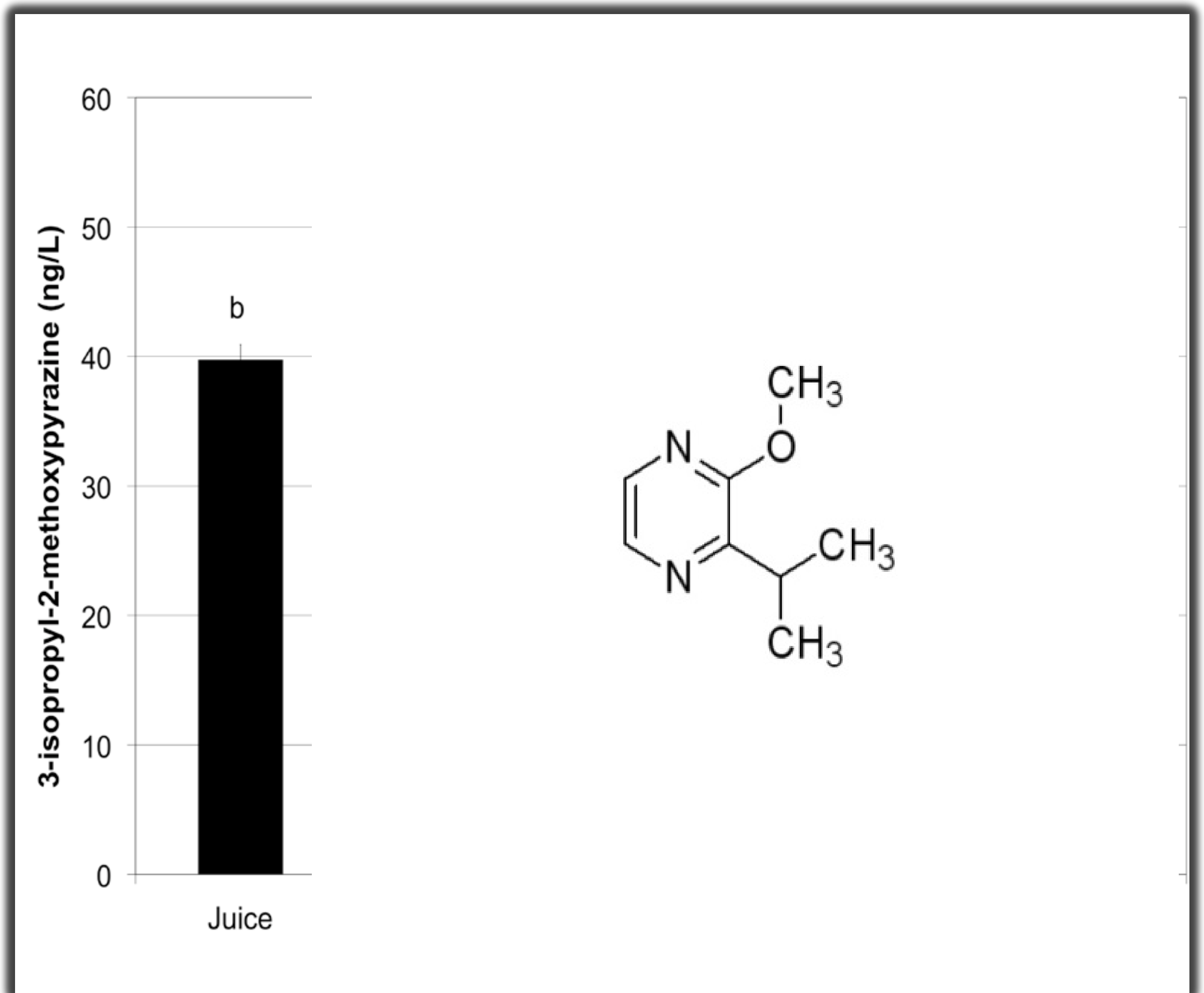
- 29-67% reduction for IBMP in red wine (Roujou de Boubee et al. 2004)

Cool Climate Oenology & Viticulture Institute

Brock University

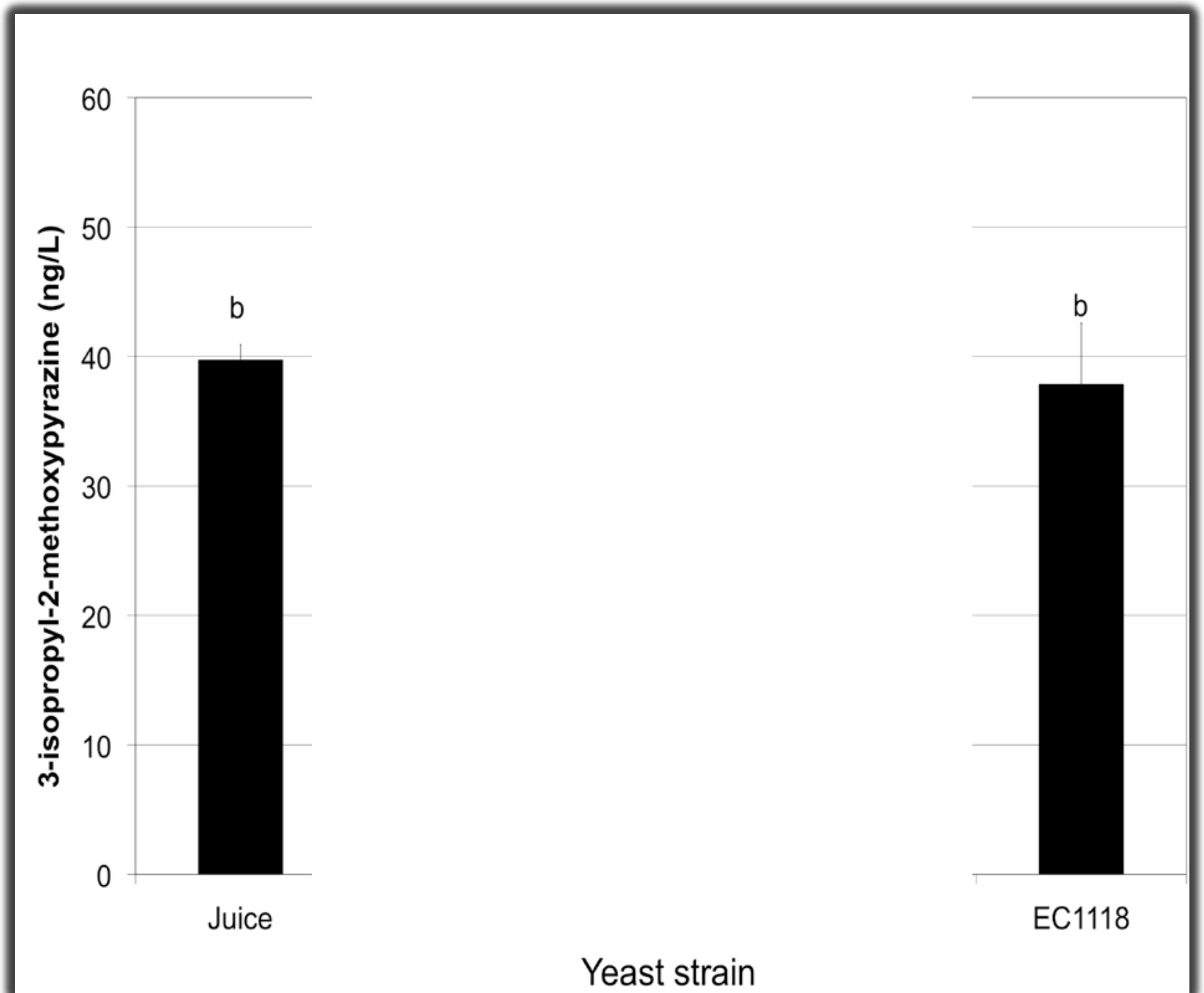
# Yeast Strain ?





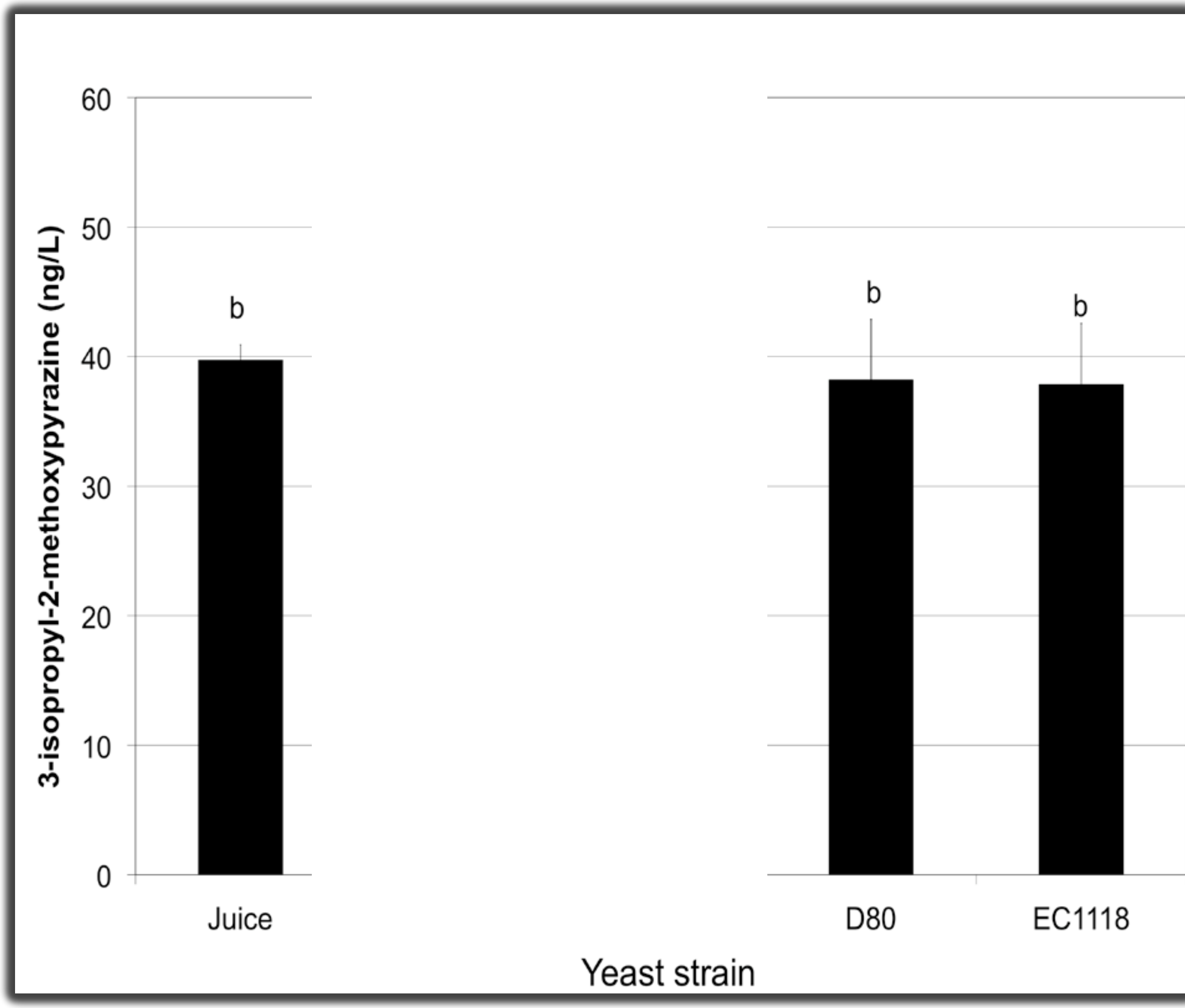

IPMP conc for Cab Sauv wine made from juice spiked with 30 ng/L IPMP & fermented with 4 different yeast strains

(adapted from Pickering et al., ’08 *Aust J Grape & Wine Res*, 14)



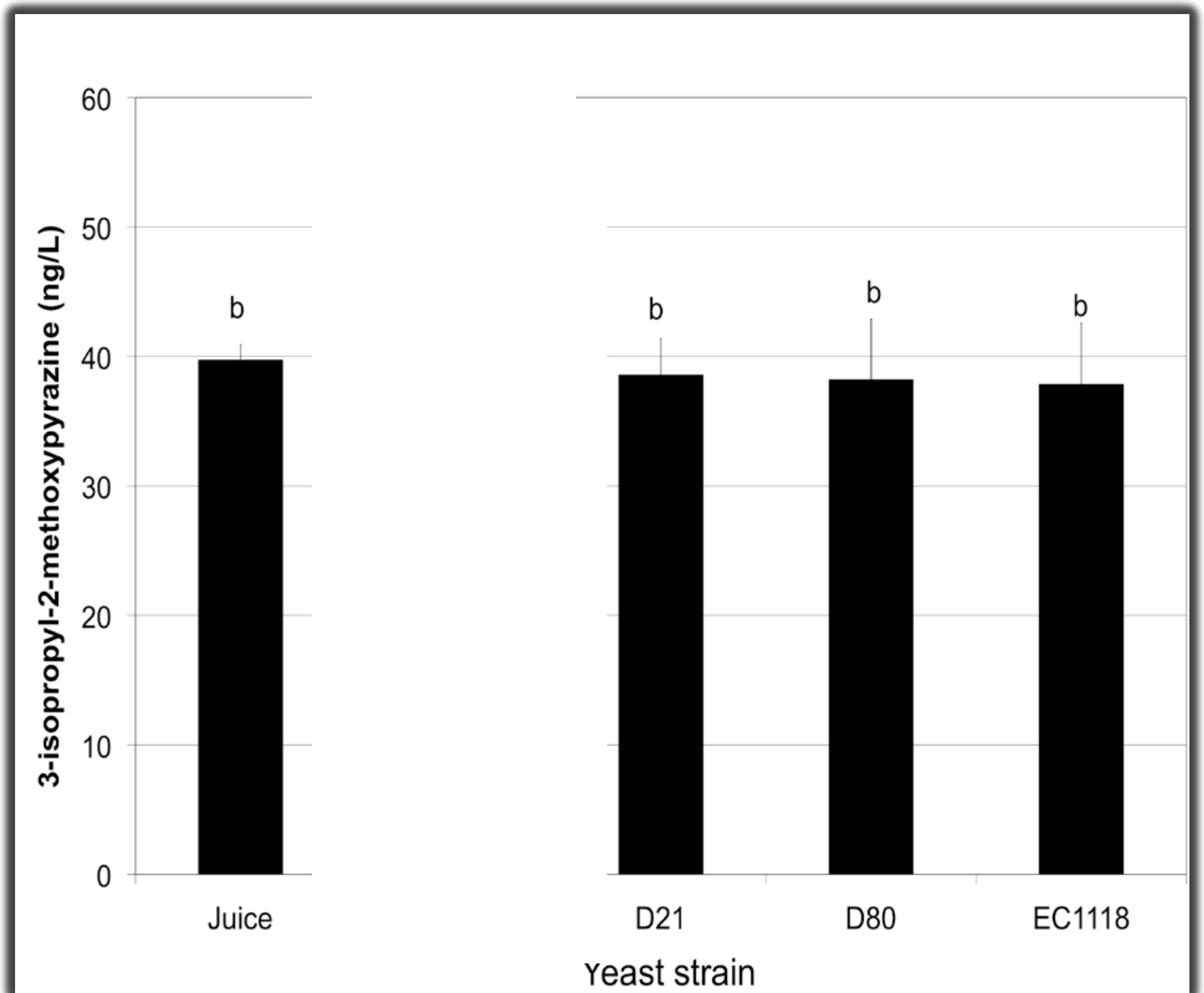

IPMP conc for Cab Sauv wine made from juice spiked with 30 ng/L IPMP & fermented with 4 different yeast strains

(adapted from Pickering et al., ’08 *Aust J Grape & Wine Res*, 14)



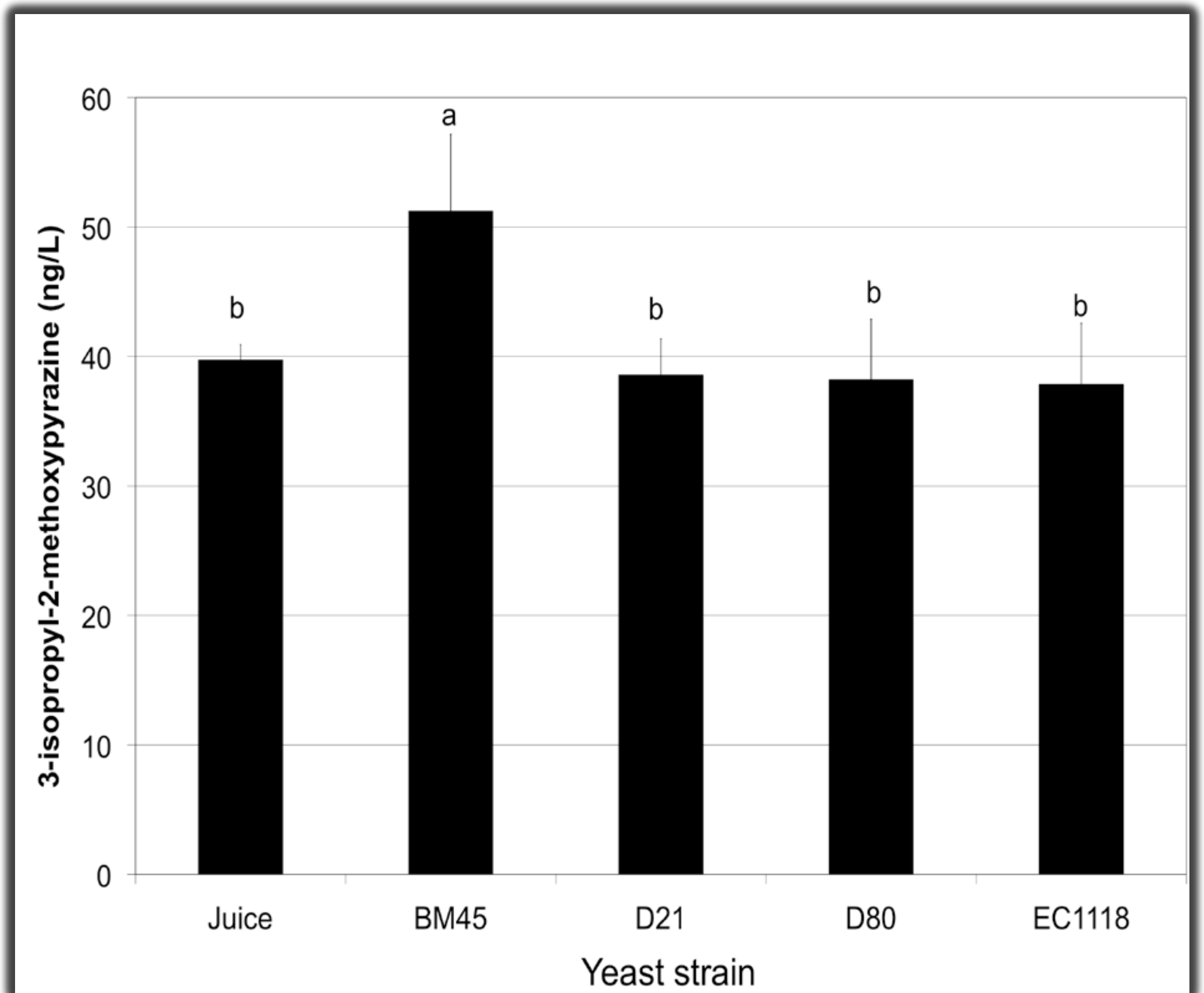

IPMP conc for Cab Sauv wine made from juice spiked with 30 ng/L IPMP & fermented with 4 different yeast strains

(adapted from Pickering et al., ' 08 *Aust J Grape & Wine Res*, 14)




IPMP conc for Cab Sauv wine made from juice spiked with 30 ng/L IPMP & fermented with 4 different yeast strains

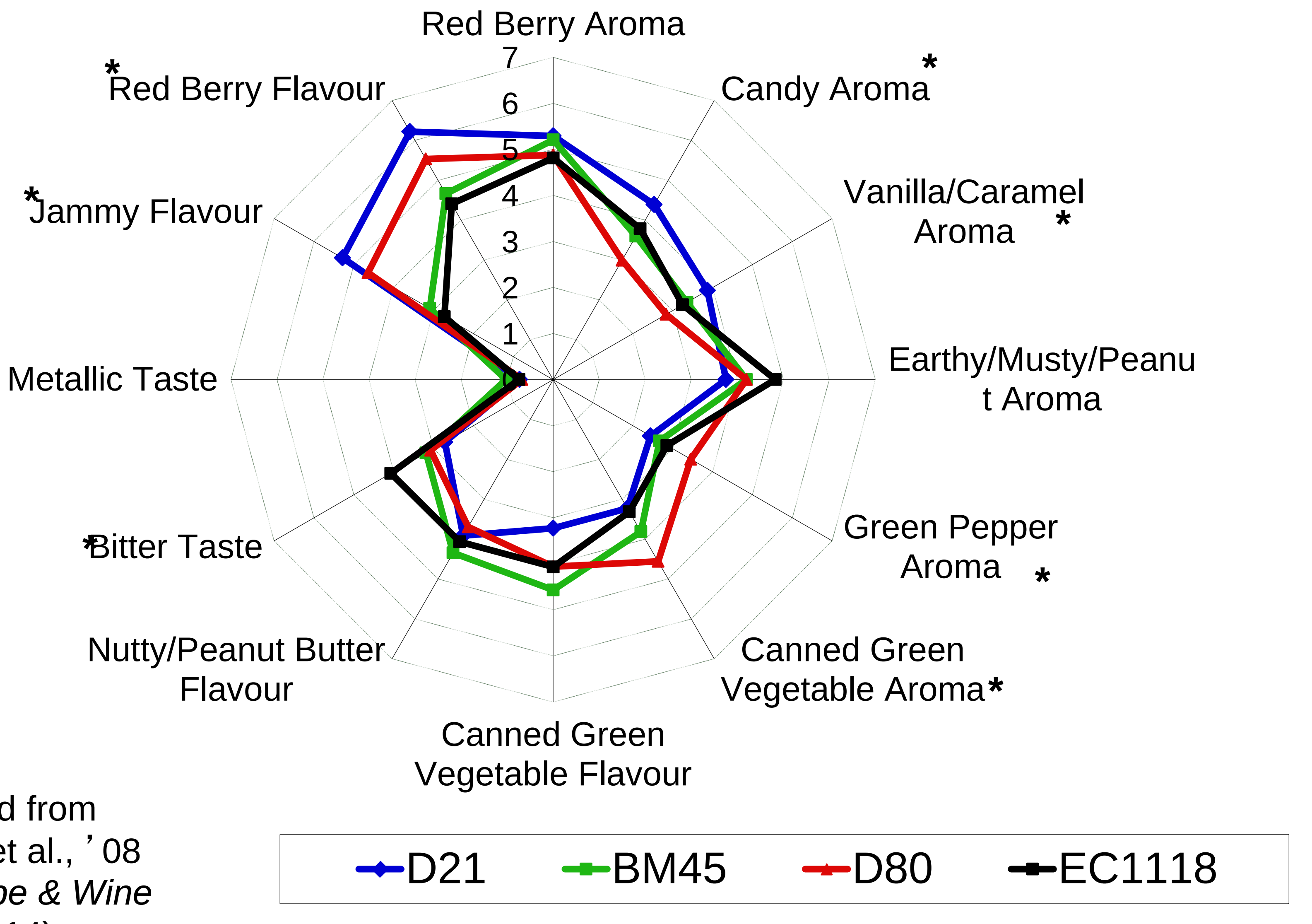
(adapted from Pickering et al., ’08 *Aust J Grape & Wine Res*, 14)



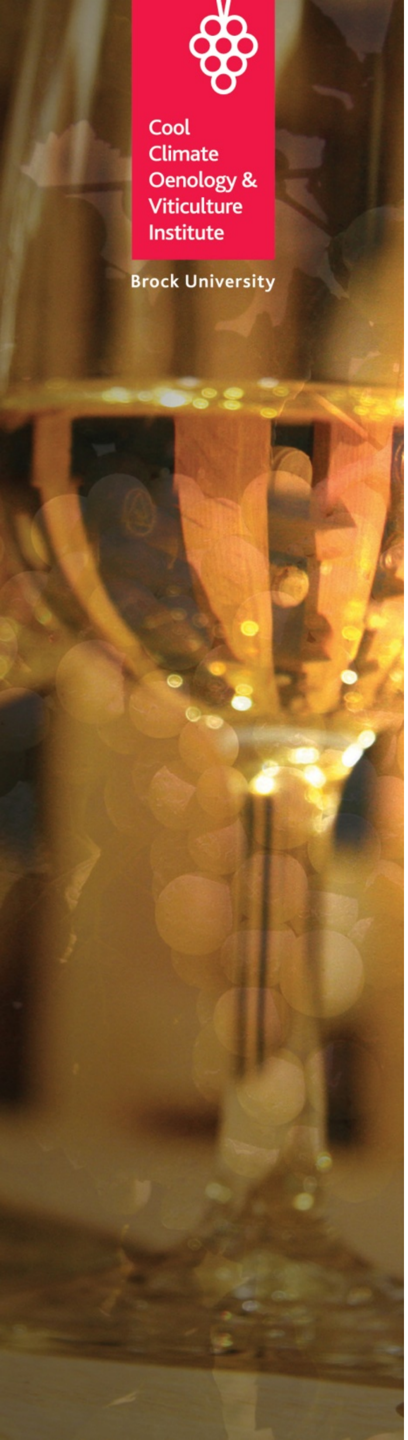

IPMP conc for Cab Sauv wine made from juice spiked with 30 ng/L IPMP & fermented with 4 different yeast strains

(adapted from Pickering et al., ’08 *Aust J Grape & Wine Res*, 14)

# Sensory intensity scores for Cab Sauv wine made from juice spiked with 30 ng/L IPMP




(adapted from Pickering et al., ’08 *Aust J Grape & Wine Res*, 14)

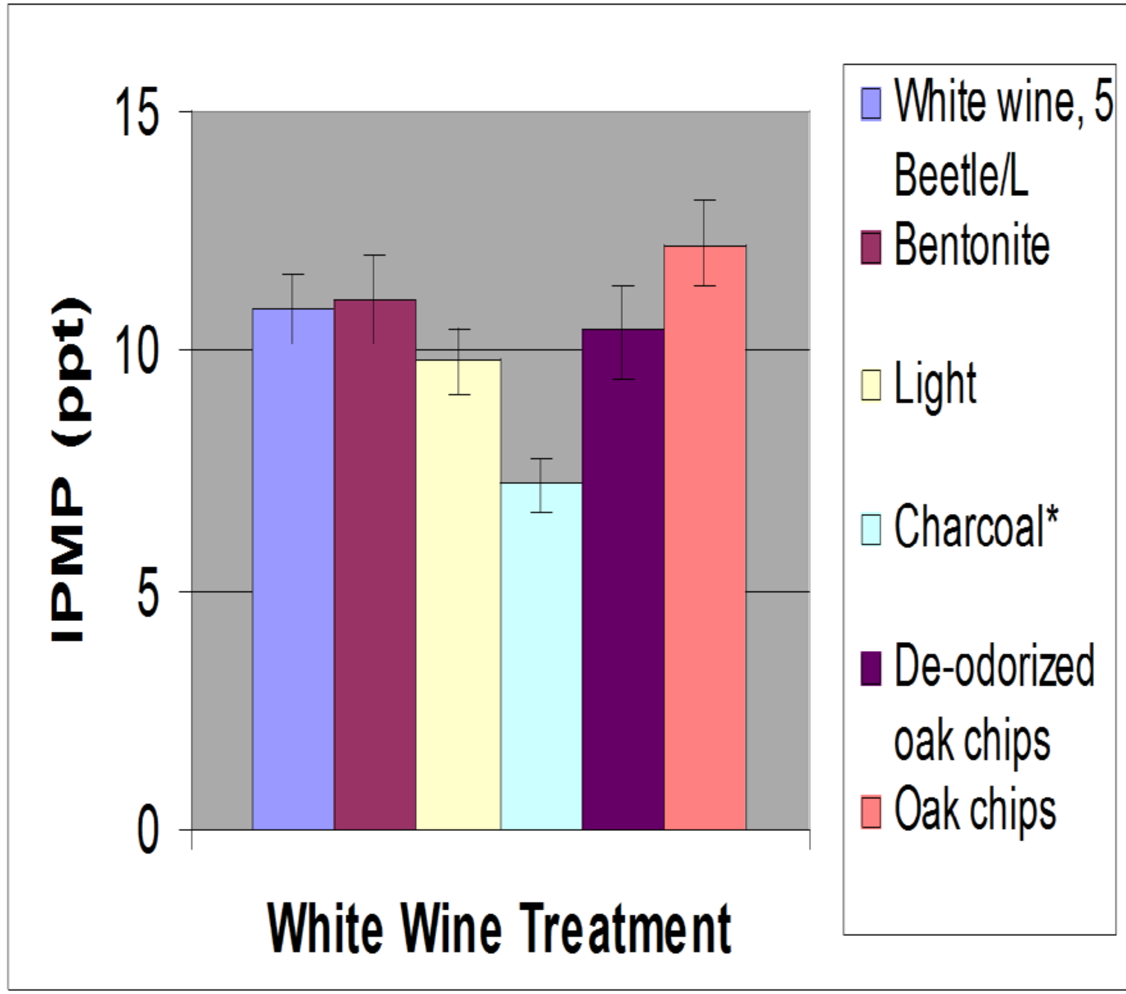



# After alcoholic fermentation?

- Malolactic fermentation doesn't affect MP levels (Sala et al., 2004)
- Anecdotal (but no/limited peer-reviewed) evidence that micro-oxygenation reduces green/vegetal aroma (? possibly changes in thiols or hexanol)
- Fining ? ….

- Only activated charcoal decreased IPMP

- Similar for red wine



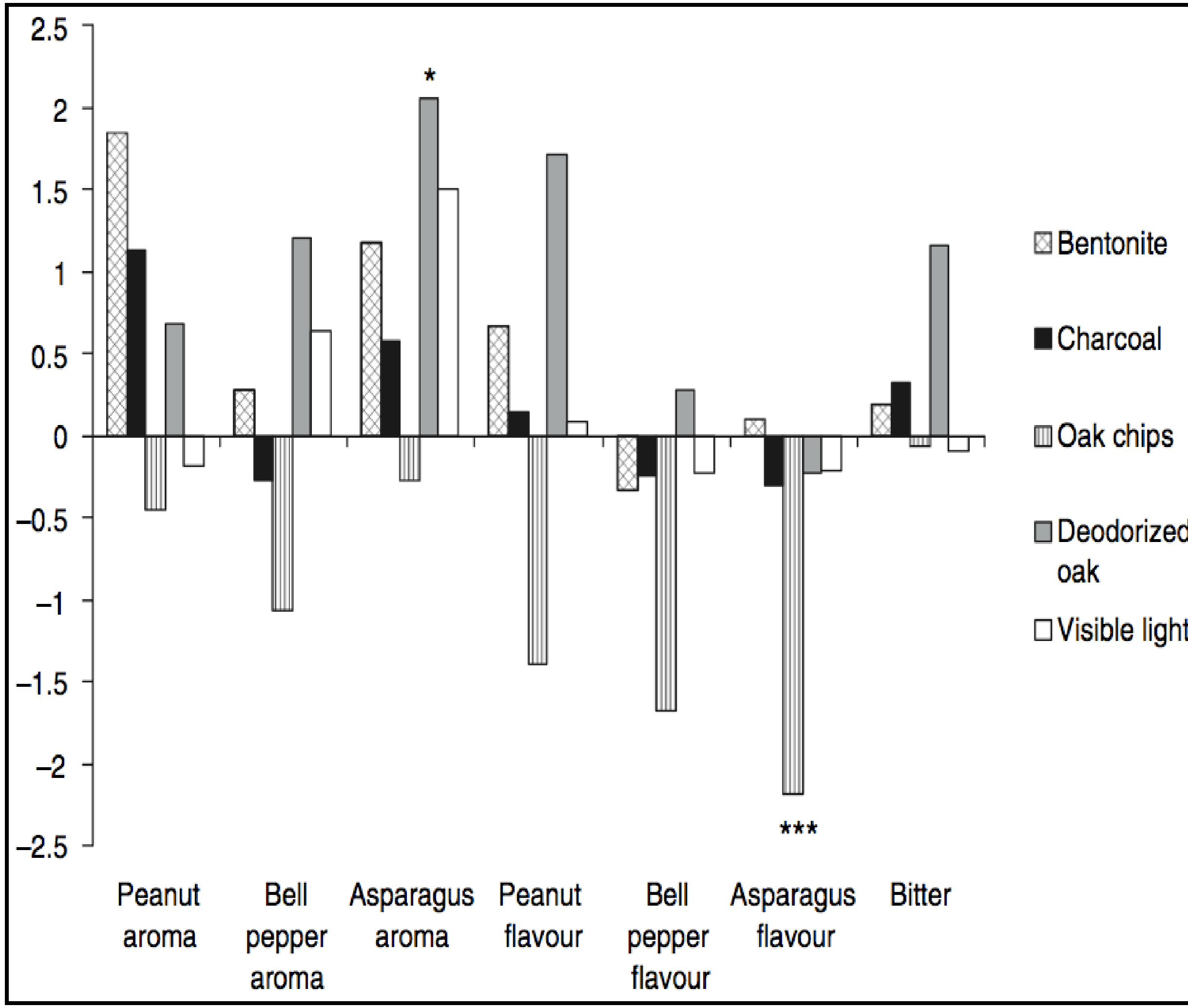

- However ….

(From: Pickering et al., ’06, *Inter J Food Sci Tech*, 41)

• MP-associated attributes only consistently reduced in oaked wine

• Similar result in red wine

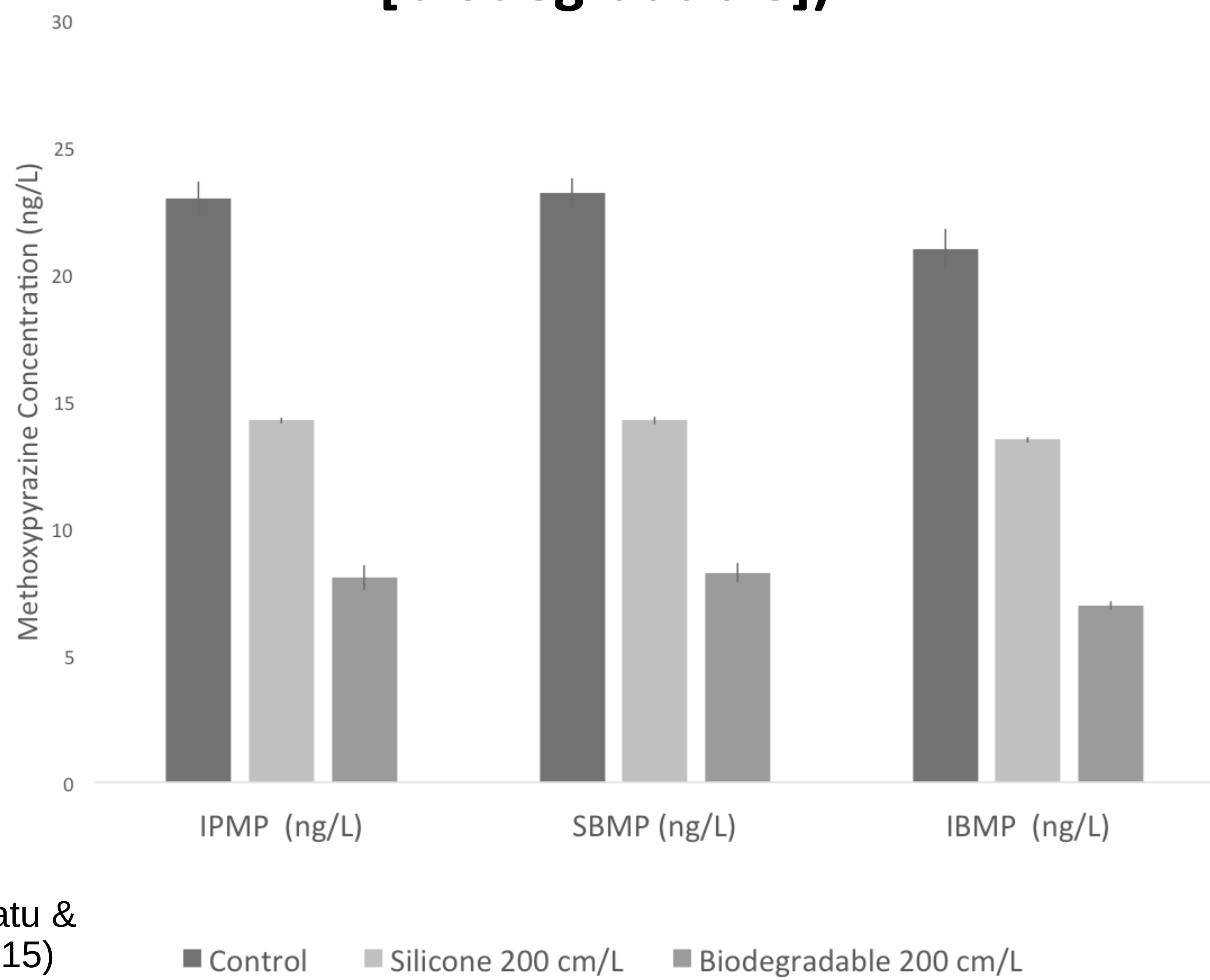
  ➢ masking effect



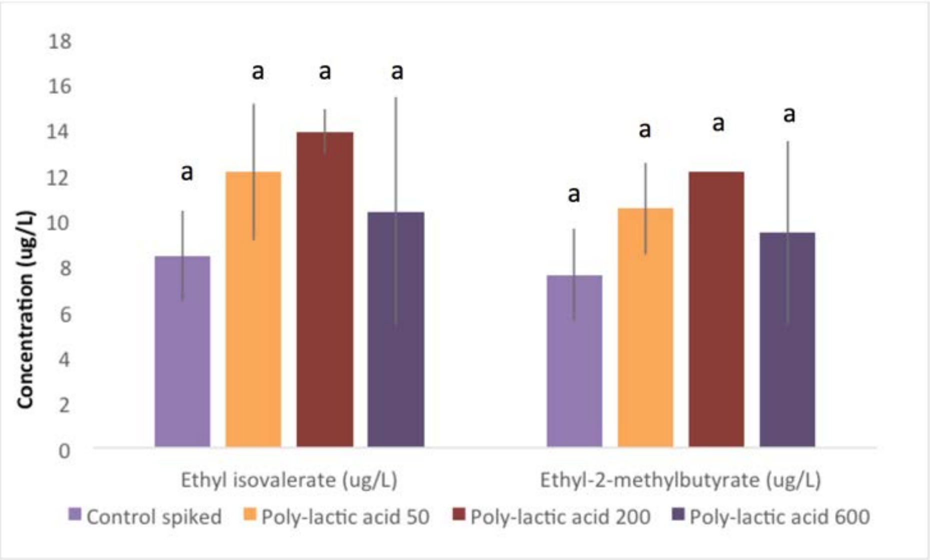


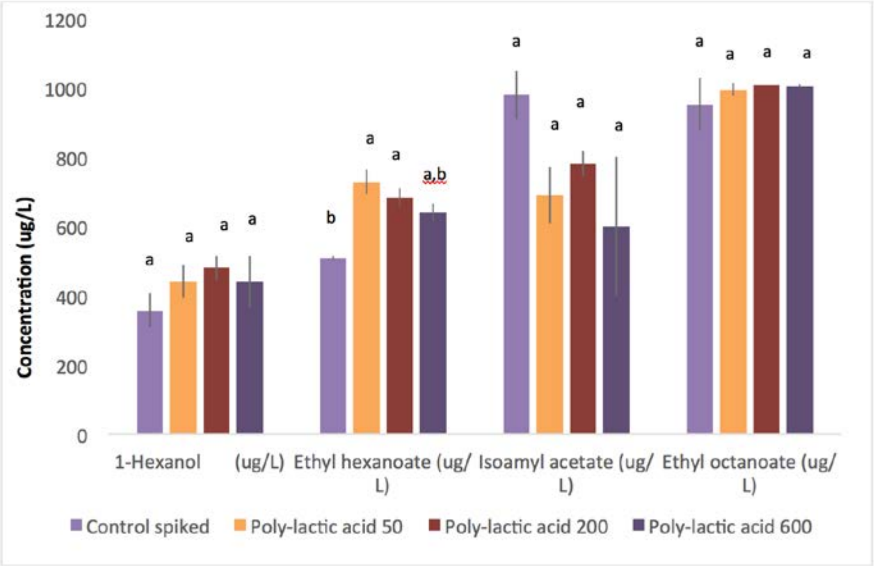

(From: Pickering et al., ’06, *Inter J Food Sci Tech*, 41)

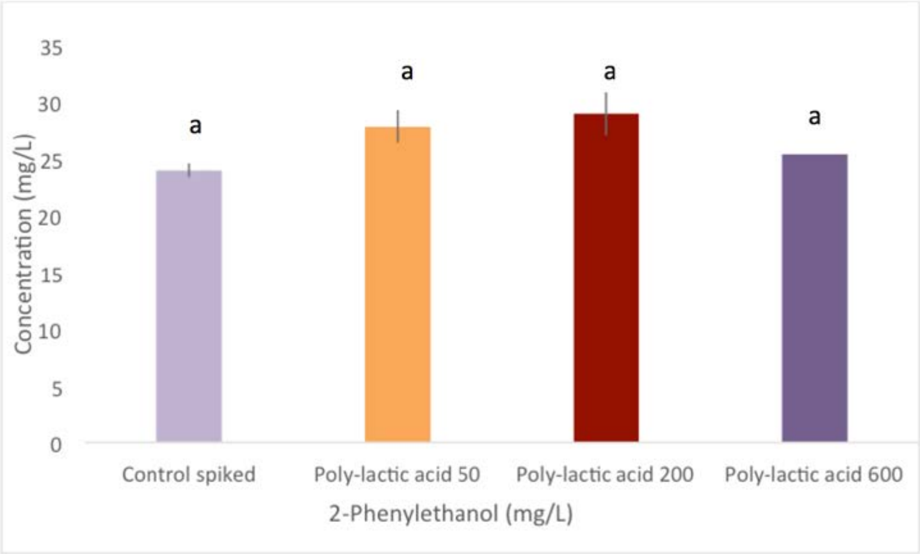
# Reduction of MPs in red wine after selected polymer treatments (silicone and polylactic acid [biodegradable])

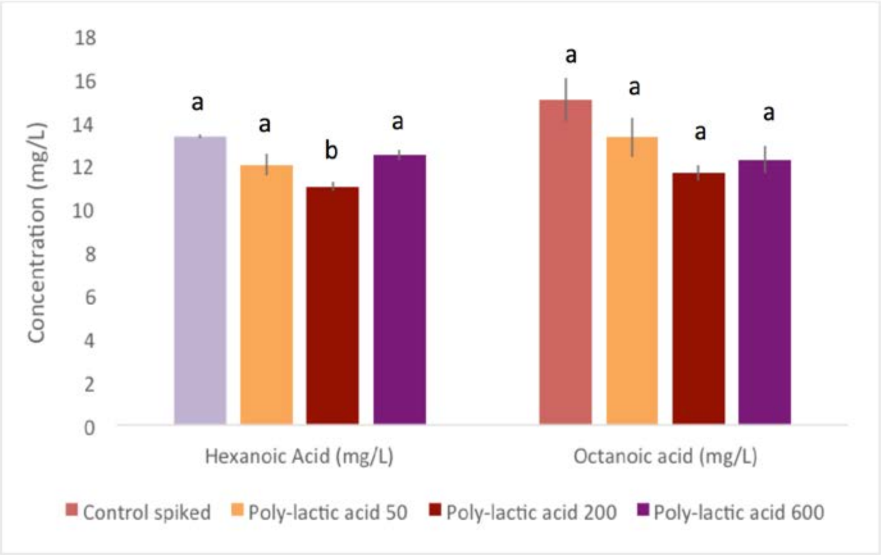



(from: Botezatu & Pickering, 2015)



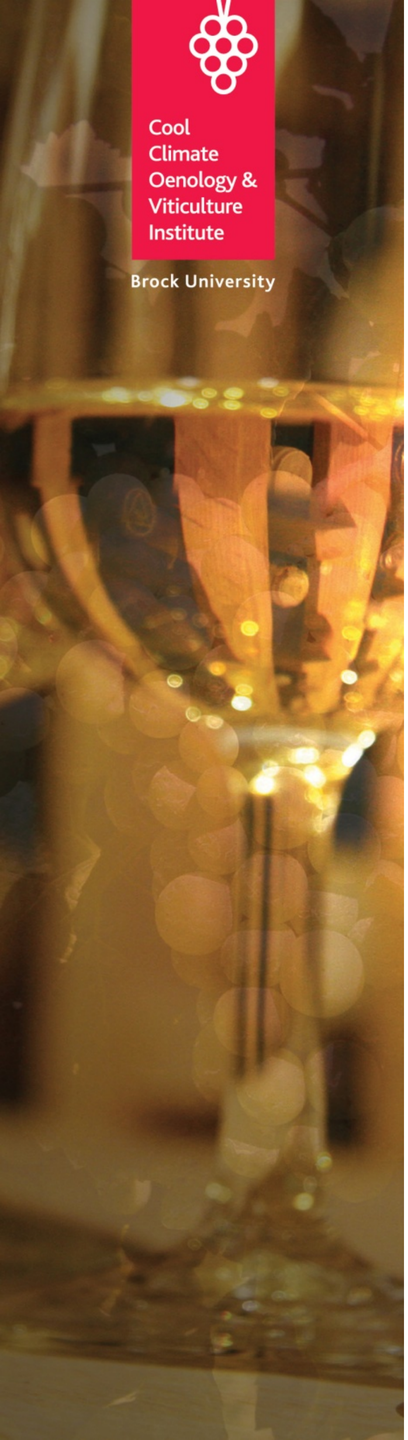



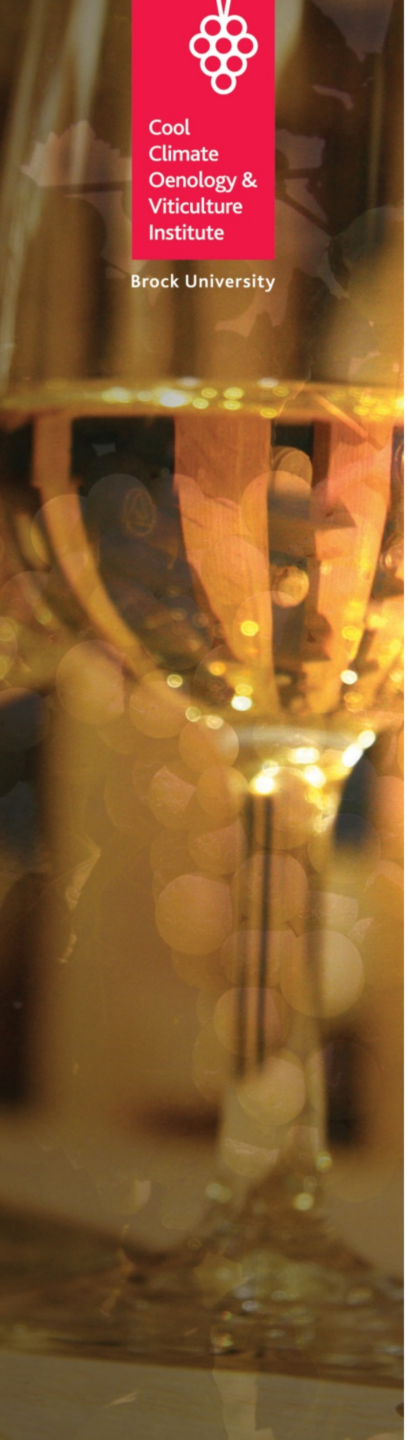

# Impact of polylactic acid by surface area on non-target VOCs

Botezatu, Kemp & Pickering (2016). *Molecules*, 21, 1238

# Packaging and ageing?




- Ageing
  - Maga (1990): stable in dark (up to 1 yr)
  - Blake et al. (2009, 2010): IPMP & IBMP decreased by approx 30% (18 months)
    - Binding with polyphenols possible mechanism (Sidhu et al., 2015)
- Package/closure (Blake et al., 2009)
  - Tetrapak showed significant and rapid decrease in MPs compared to bottle
  - Bottles sealed with synthetic corks showed greater decrease than those closed with natural cork or screw-cap
- Storage conditions
  - Exposure to light reduced MPs, especially in clear bottles (up to 57% for IBMP; Maga,1990)




# Conclusions

- MPs very potent grape and insect-derived juice & wine odorants = greenness
- Viticultural interventions that mediate concs mainly associated with level of bunch-exposure & ripeness
- De-stem and minimise skin contact where possible
- Juice clarification prior to fermentation advantageous
- Thermovinification can reduce levels
- Yeast strain matters
- MPs resilient to most fining agents. Oak helps.
  - Polylactic acid and silicone polymers promising
  - Odorant-binding protein (high specificity) v. promising
- Closure & packaging type can affect MP composition

- Prevention best.