High Metabolizable Energy (HME) ryegrass - towards proof of concept

+ 2021 field trial update

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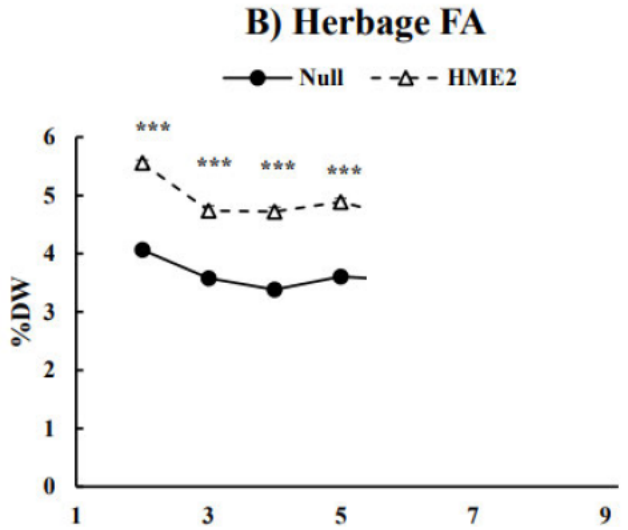
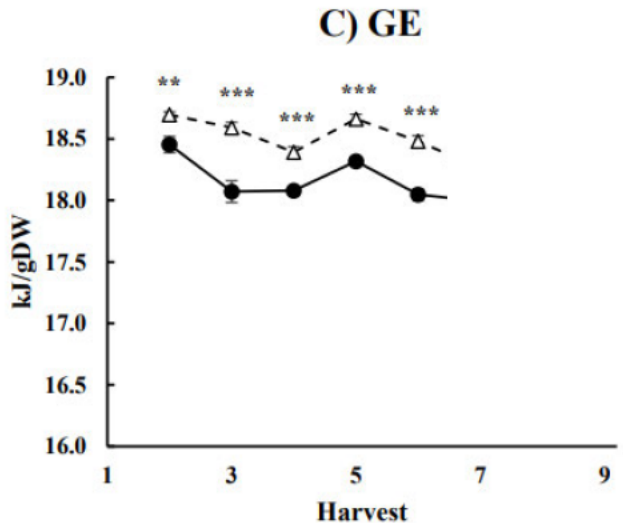
	Primary traits		Secondary traits				
	Leaf Fats	Gross energy	SLA	Photosynthesis	Yield	Methane (IV)	s9(2)(i)
'Impact' Spaced pots (indoors)	✓	✓	✓	✓	✓	✓	n/a
'Elite' Spaced pots (indoors)	✓	✓	✓	✗	✓	n/a	n/a
'Impact' Swards (indoors)	✓	✓	✗	✗	✗	n/a	✓
'Elite' Swards (indoors)	✓	✓	✗	✗	✗	n/a	?
'Elite' Field trials	✓	✓	?	?	?	n/a	n/a

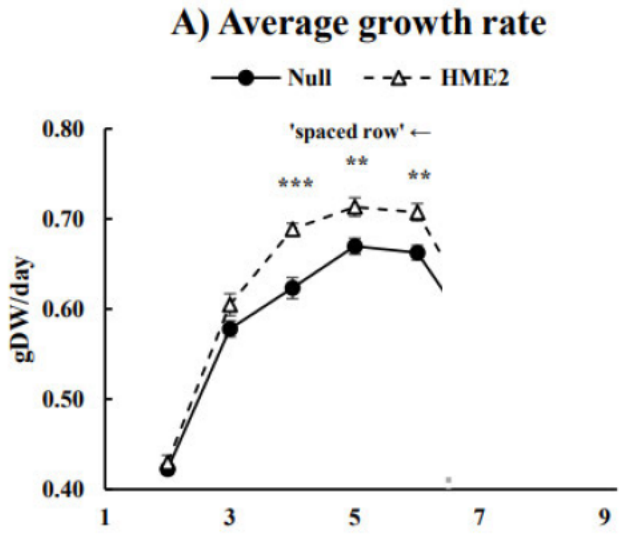
	Primary traits		Secondary traits				
	Leaf Fats	Gross energy	SLA	Photosynthesis	Yield	Methane (IV)	s9(2)(i)
'Impact' Spaced pots (indoors)	✓ 1,2,3,5	✓ 5	✓ 1,2,5	✓ 1,2,5	✓ 1,2,5	✓ 2	n/a
'Elite' Spaced pots (indoors)	✓ 2,4	✓ 4	✓ 4	✗ 4	✓ 4	n/a	n/a
'Impact' Swards (indoors)	✓ 5	✓ 5	✗ 5	✗ 5	✗ 5	n/a	✓ 6
'Elite' Swards (indoors)	✓ 4	✓ 4	✗ 4	✗ 4	✗ 4	n/a	?
'Elite' Field trials	✓ 4	✓ 4	?	?	?	n/a	n/a

[1]Beechey-Gradwell *et al.* (2020) *J Exp. Bot.*; [2]Cooney *et al.* (2021) *Front. Plant Sci.*; [3]Winichayakul *et al* (2020) *J. Dairy Sci.*
[4]Beechey-Gradwell *et al.* (2021) *Field Crops Res. In press*; [5]Beechey-Gradwell (2021) *J. NZ Grasslands*. In press; [6]s9(2)(i)

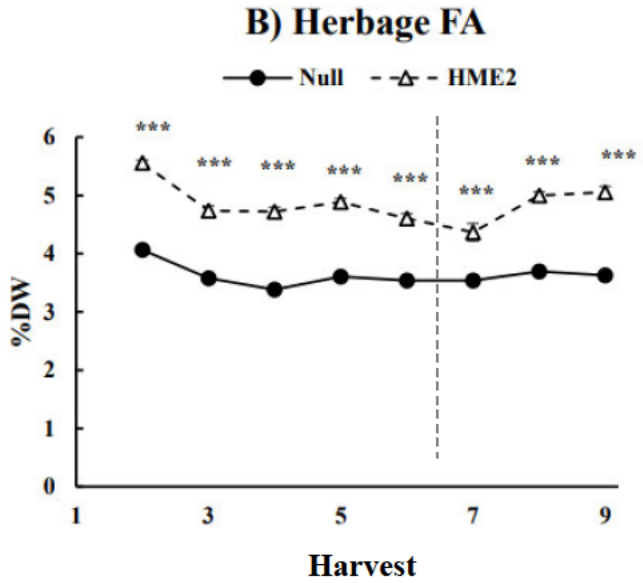
HME Ryegrass Growth Room Trial 2020

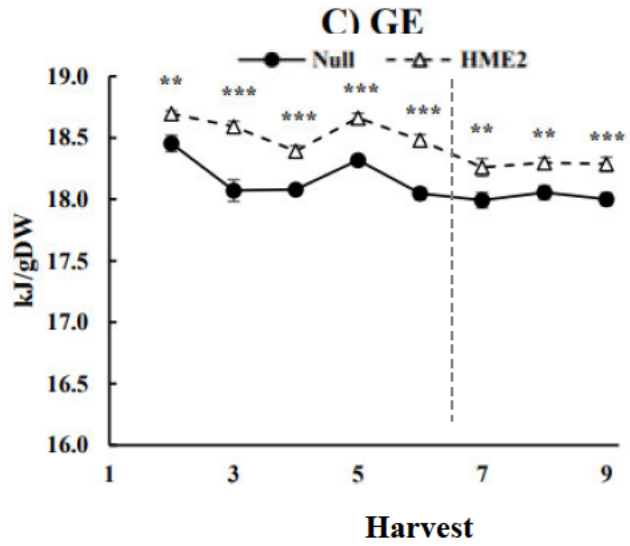


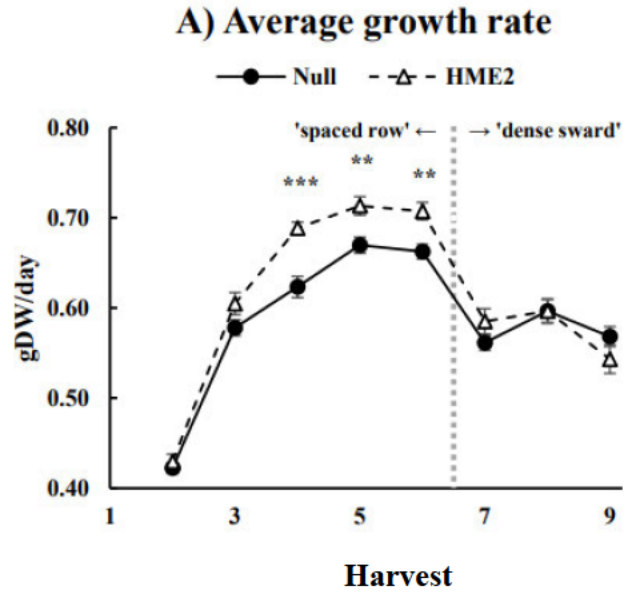




Source: Beechey Gradwell *et al. Field Crops Research.* In press.







Source: Beechey Gradwell *et al. Field Crops Research.* In press.

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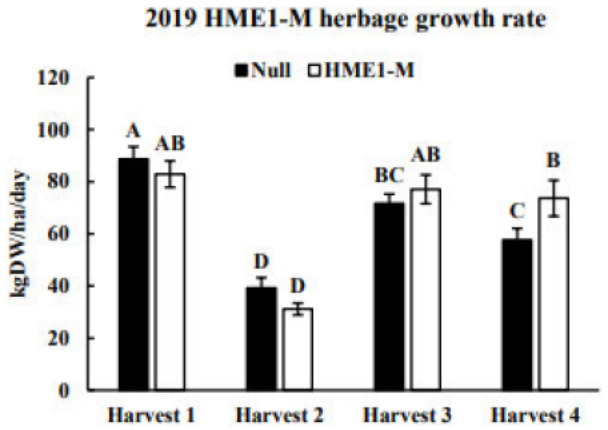
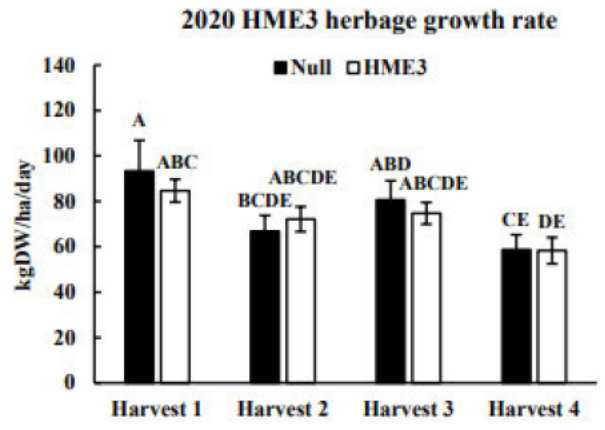
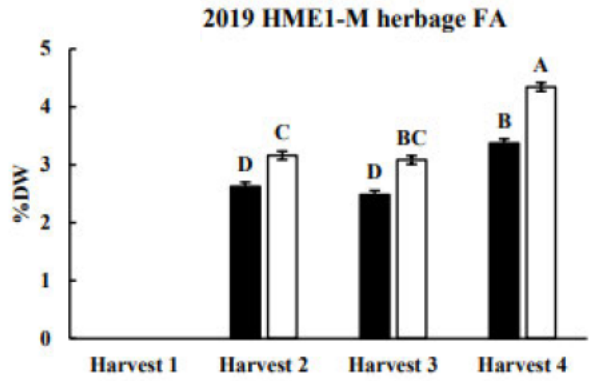
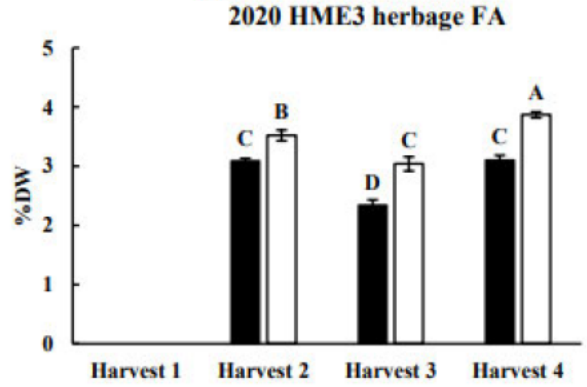
HME Ryegrass 2019-2020 Field Trials

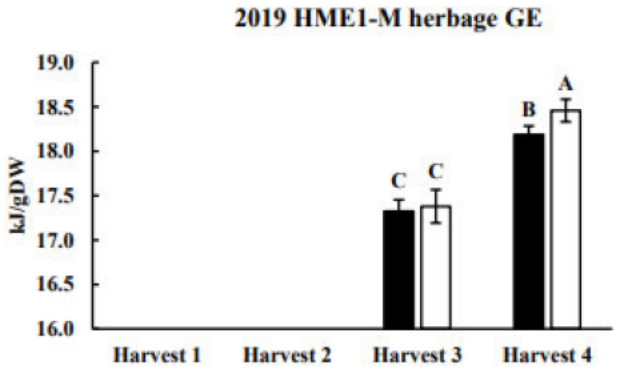
Midwest, USA

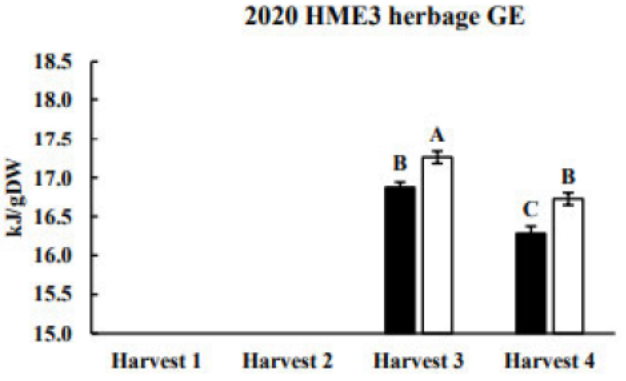












Source: Beechey Gradwell *et al*. *Field Crops Research.* In press.

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HME Ryegrass 2019-2020 Field Trials

In the field HME delivers:

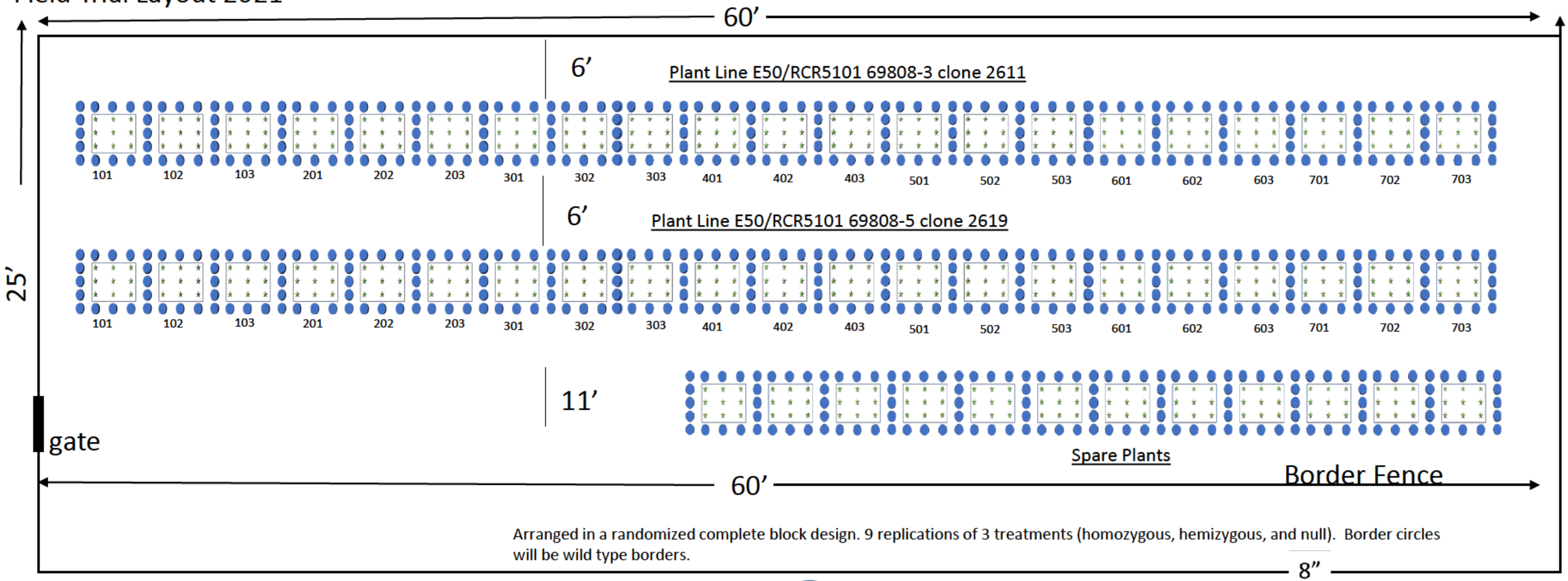
- 25-34% higher fatty acids (~1% DW)
- +0.3-0.5 MJ/kg DW gross energy

‘it has been estimated that about 0.05 MJ/kg DM per decade increases in perennial ryegrass ME concentration have been achieved through traditional genetic selection for improved dry matter digestibility’

Ludemann *et al.* 2015

2021 field trial update

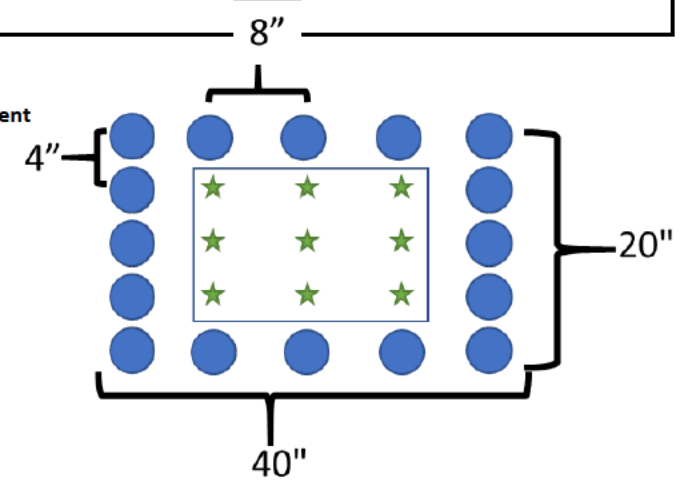
Field Trial Layout 2021



Arranged in a randomized complete block design. 9 replications of 3 treatments (homozygous, hemizygous, and null). Border circles will be wild type borders.

= WT

= null or transgenic in a mini-sward arrangement

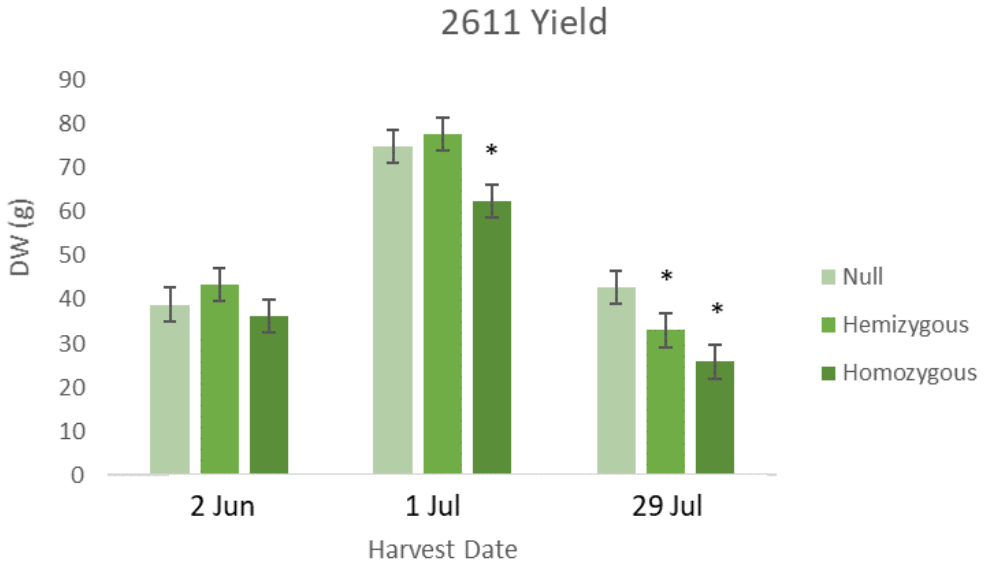


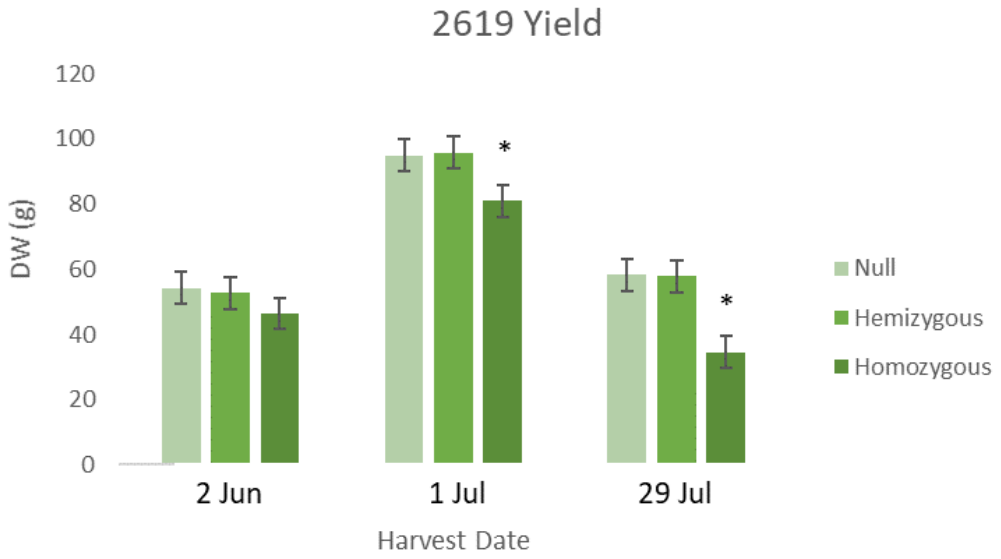
Line	Null GH FA (%DW)	Hemi GH FA (%DW)	Homo GH FA (%DW)
E50/RCR5101 2611	4.4	5.6	6.8
E50/RCR5101 2619	4.4	5.4	6.6

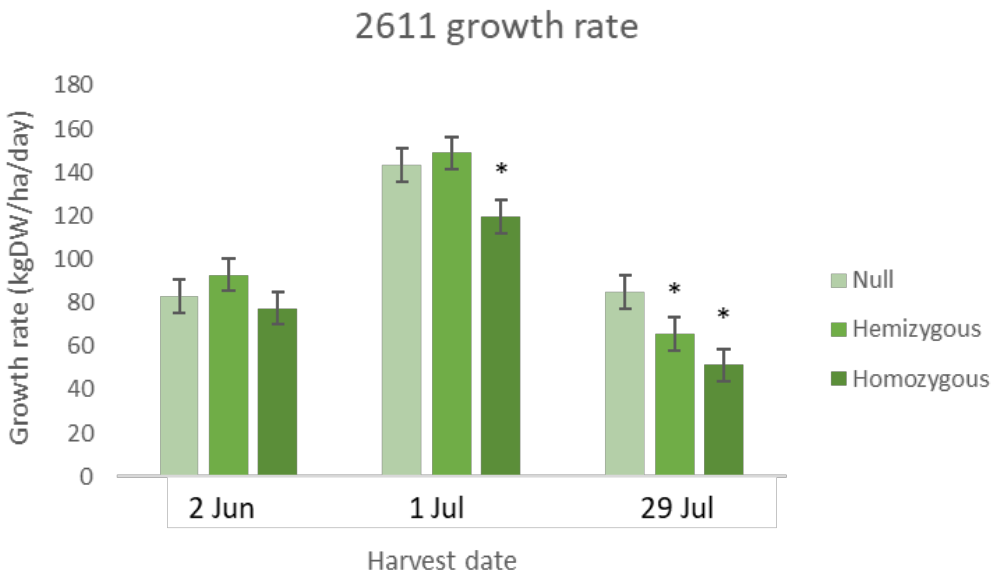
+ 0.5 kJ gDW^{-1} GE

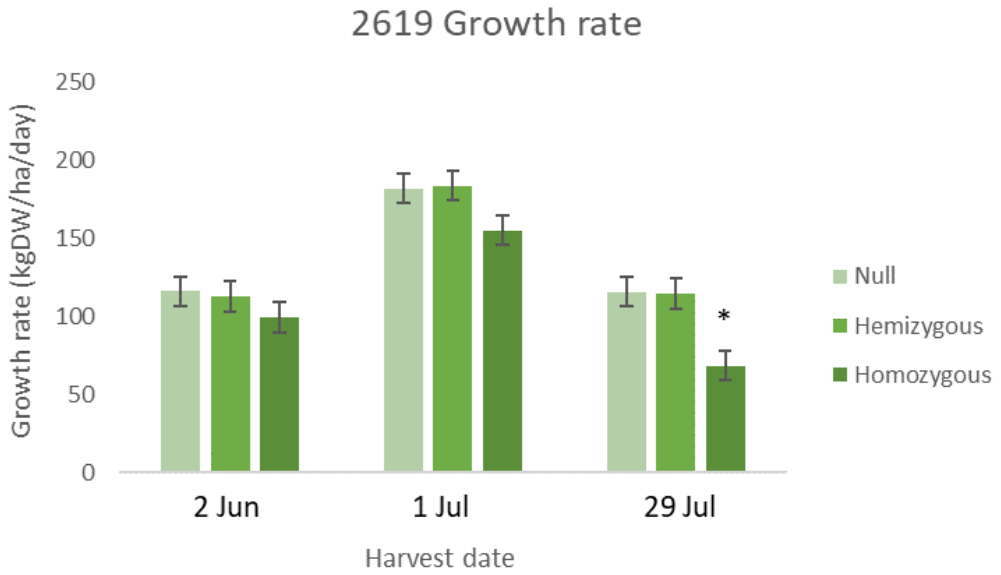
GE increase tbc. Estimated up to 1 MJ $kgDW^{-1}$

2021 harvest data to date



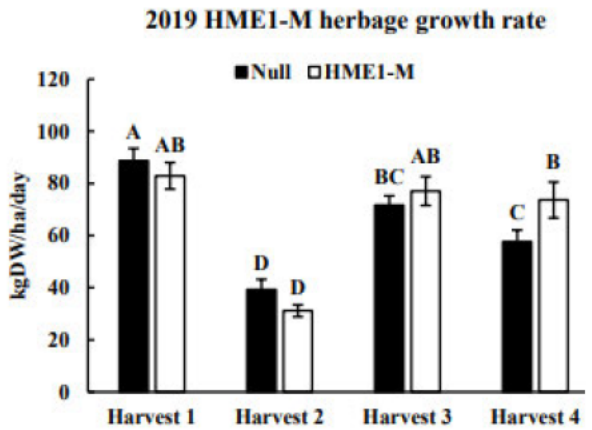


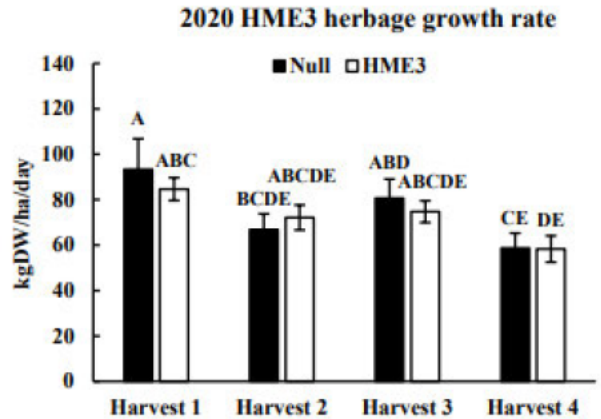




Important considerations

- Gene disruption at insertion site
- s9(2)(b)(ii) climate – comparable to 2019 and 2020
- Border competition – investigating this….
- FA levels





Novelty weather data 1 Jun – 31 Jul

Year	Ave max temp (°C)	Ave Daily Temp (°C)	Solar Radiation (MJ/day)	Days max temp exceeded 32 °C
2019	28.5	23	20.57	10
2020	28.9	23.6	20.81	1
2021	28.3	22.9	19.38	8

Important considerations

Border has now been removed for Plant Line E50/RCR5101 69808-5 clone 2619

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Science requirements for Australian market entry

Value proposition

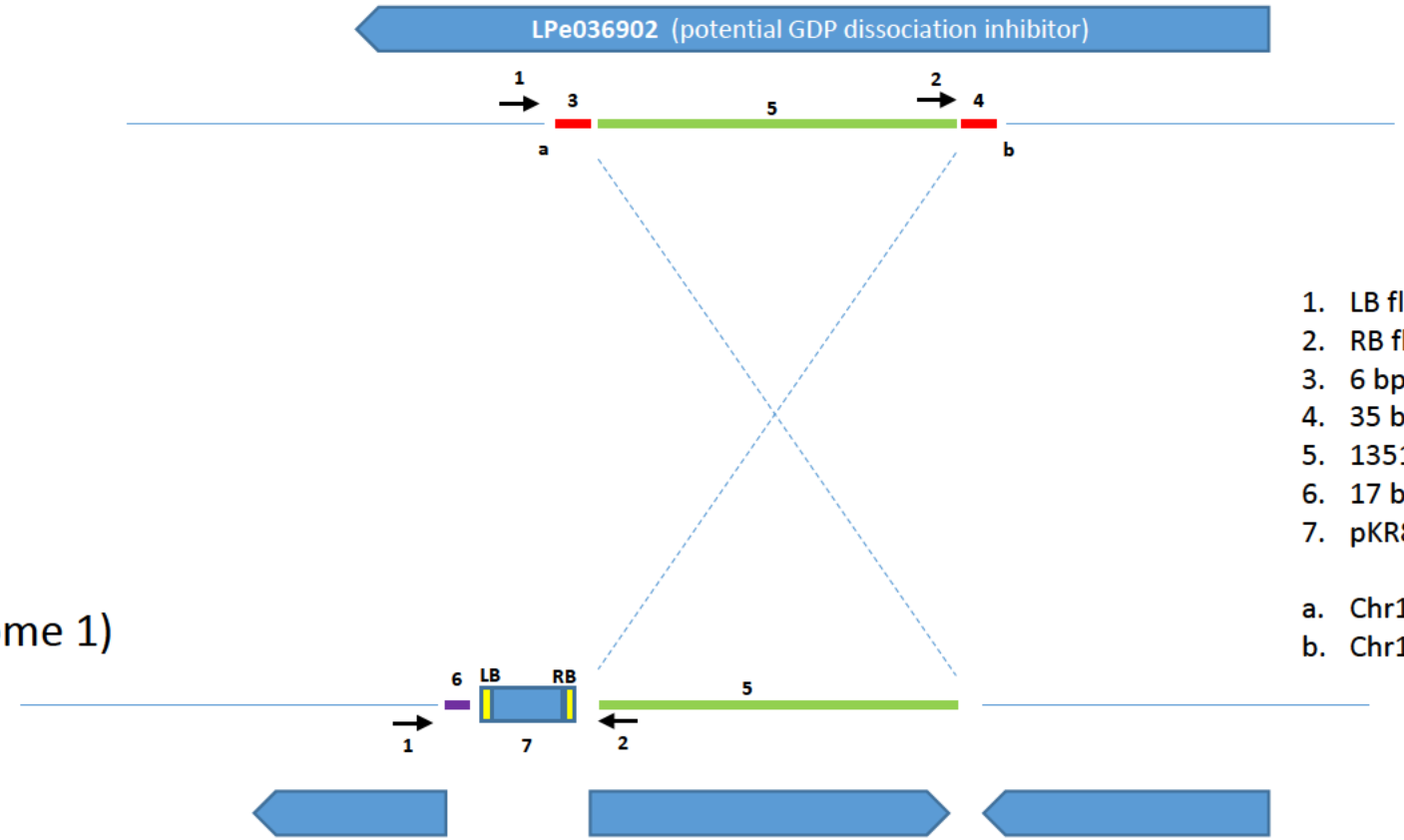
- Proof of concept:
  - Grows in Australia
  - Digestible energy
- Market development:
  - Animal feeding trials (e.g. live weight gain)

Regulatory access

- Case for safety (in seeds and storage)
- Molecular analysis – genetic stability and segregation
- Composition analysis
- Permission to operate (public and industry)

Appendix 1: 5101 insertion site

Chromosome 1



1. LB flank
2. RB flank
3. 6 bp deletion in RCR5101
4. 35 bp deletion in RCR5101
5. 1351 bp inverted sequence at RCR5101 RB
6. 17 bp filler sequence
7. pKR88 HME T-DNA

a. Chr1, 117,266,097
b. Chr1, 117,264,733

RCR5101 loci (Chromosome 1)

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Appendix 2: Leaf sugar vs. photosynthesis

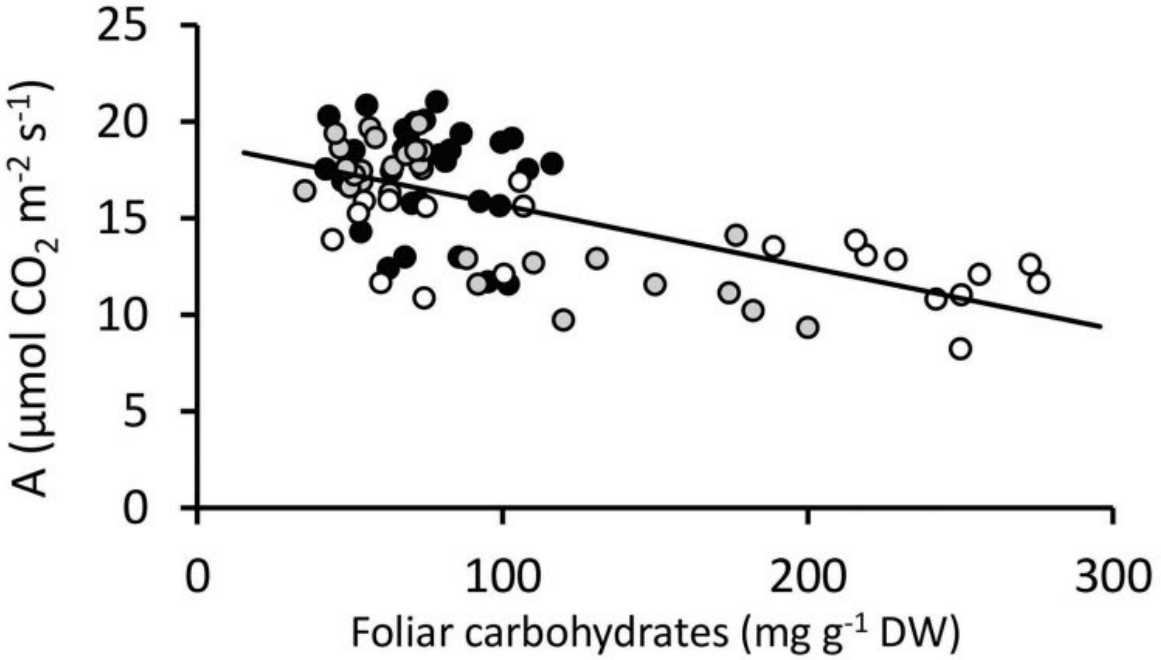

FIGURE 5 | Photosynthesis vs. foliar carbohydrates for DGAT + CO and NT *Lolium perenne*. Lines from each genetic background are shaded together irrespective of DGAT + CO or NT; NT1 and DGAT + CO1-2 (●), NT2 and DGAT + CO3-4 (●) and NT3 and DGAT + CO5 (○). Trendline represents NT2 and NT3 derived lines. Photosynthesis measured at 600 µmol photons m^{-2} s^{-1}.

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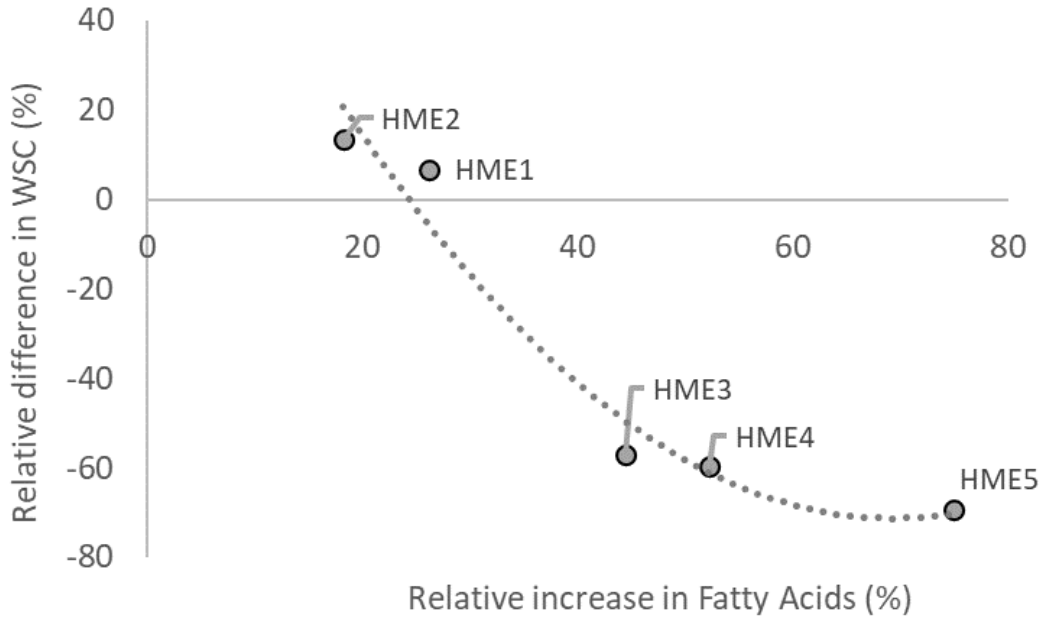
Appendix 3: Why does HME expression increase carbon assimilation?

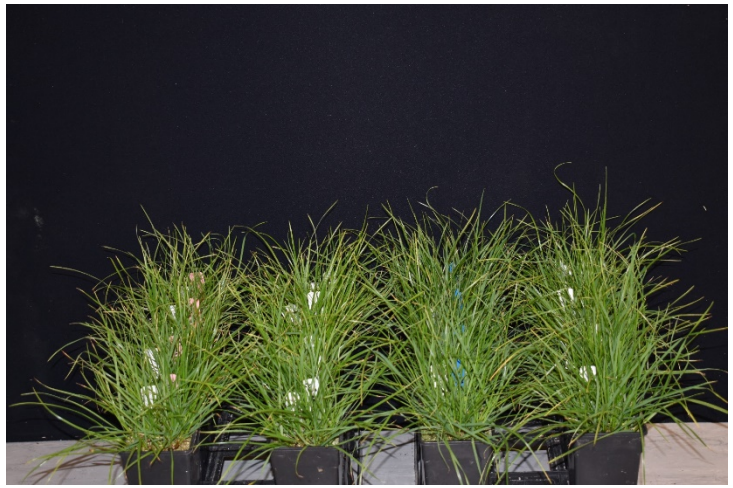


Relative increase in leaf fatty acids multiple HME lines compared to relative difference in water soluble carbohydrates

Increased fatty acids correspond to decreased leaf sugar

Source: Cooney *et al.* 2021. *Front. Plant Sci*. Vol 12

Appendix 4: Carbon assimilation Spaced vs. sward



		Spaced Pot			Sward	
	Genotype	Value	% change for HME	Genotype	Value	% change for HME
Noon leaf WSC (mg/g DW)	**WT**	240 (±9)		**WT**	107 (± 5)	
	HME	74 (±7)	-69%	**HME**	82 (± 4)	-23%
Net Photosynthesis (µmol m^{-2} s^{-1})	**WT**	11.9 (± 0.5)		**WT**	16.4 (± 0.7)	
	HME	14.4 (± 0.6)	20%	**HME**	18.3 (± 0.3)	11%
SLA (cm^2 g^{-1})	**WT**	213 (± 8)		**WT**	319 (± 7)	
	HME	342 (±9)	61%	**HME**	364 (± 6)	14%

Source: Cooney *et al.* 2021. *Front. Plant Sci*. Vol 12 & Beechey-Gradwell *et al.* 2021. *Journal of NZ Grasslands. In Press*