The value of Indian mustard in cereal and legume crop sequences in northwest NSW

By

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Declaration of Originality

This thesis reports the original work of the author, except as otherwise stated. It has not been submitted previously for a degree at this or any other university.

Graeme Rapp

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Abbreviations:

Allyl isothiocyanate:	AITC
Bureau of Meteorology	BoM
Canola:	Canola (*Brassica napus or B.napus*)
Chickpeas:	C
Days after planting	Dap
Gas Chromatography (Flame Ionization Detection)	GC-FID
Glucosinolates:	GLS
Harvest Index:	HI
Isothiocyanate:	ITC
Mustard Seed Meal Powder:	MSMP
Mustard:	Indian mustard (*Brassica juncea or B.juncea*)
Nitrogen Use Efficiency:	NUE
Normalized Difference Vegetation Index:	NDVI
Poly Aromatic Hydrocarbons:	PAH
Thousand Kernel Weight:	TKW
Trademark	TM
Treatment:	Trt.
Water Use Efficiency:	WUE
Wheat:	W

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Abstract

Mustard (*Brassica juncea*) could be well suited to the northwest cropping region of NSW, where winter cereals and legumes are produced under environmental conditions often characterized by high temperatures, low rainfall and low humidity with cold and frosty nights during the winter growing season. Mustard is a hardy plant that is resistant to many of the diseases suffered by canola with the exception of powdery mildew and Beet Western Yellow Mosaic virus (*Polerovirus*: Beet western yellows virus). It is grown as an annual crop and offers greater flexibility compared to oil producing trees. The economic value of mustard is currently limited by the small size of the domestic condiment market but the species produces several products with industrial uses. These include oil, glucosinolates and limited above ground biomass that can be used for the manufacture of pharmaceuticals, veterinary products, agricultural chemicals and food additives. For example, sinigrin is currently used as a natural anti-fungal compound added to food, biofuels, automotive and machine lubricants and the bio-chemical industry, including the production of bio-plastics and organic fertilisers. Furthermore, Glucosinolates when hydrolysed from Isothiocynate have a break crop effect on the soil and under the right conditions of temperature and soil moisture may reduce soil borne diseases and weeds, thus enhancing the health of arable land for the following cereal crops and potentially increase productivity of subsequent crops.

This study examined the potential economic benefits of growing mustard in northwest NSW. The research focused primarily on the impact of mustard in crop sequences with wheat and chickpeas; the two most important grain crops in this region. The impact on the yield and market quality of all three crops was assessed and the economic consequences for biodiesel and other industrial products examined. Therefore the hypotheses tested in this study were (i) that mustard can yield well under a harsh environment in northern NSW and yield can be influenced by the sequence of crops grown, (ii) that mustard can produce a range of potentially marketable products, such as oil and glucosinolates, which could also be affected by the crop sequence and (iii) that mustard can have an impact on the other crops in the sequence.

Crop sequence experiments were established at Narrabri between 2013 and 2015 to examine the impact of mustard in crop sequence with wheat and chickpea. The environmental conditions were dry and warm with 156 mm and 246 mm rainfall received during the growing seasons in 2014 and 2015, respectively.

A supplementary irrigation of 35mm was applied in 2014. Rainfall was well below the long-term May - November average of 339 mm for Narrabri. Average minimum and maximum temperatures of 7.6/22.7°C and 8.9/23.3 °C were recorded for 2014 and 2015, respectively. These were close to the long-term average of 8.0/22.8°C.

Environmental conditions, soil moisture, crop growth and development characteristics, grain yield and grain protein content were assessed each year. In addition, mustard oil yield, chemical constituents and glucosinolate (GLS) concentration were assessed to determine the economic potential of mustard in crop sequence.

The impact of different crop sequence treatments on mustard grain yield, protein content, oil yield and glucosinolate concentration were determined. However, in 2014 the mustard treatment was impacted by a late and virulent powdery mildew infection which compromised the yield and oil assessments. The previous crop had little impact on the yield of wheat, mustard or chickpea, although differences in seed characteristics and quality were noted. However, when the whole crop sequence was examined a range of differences were observed. The Wheat-Chickpea-Wheat sequence increased wheat yield by 12.6% and the Wheat-Chickpea-Chickpea increased chickpea grain protein percentage by 9.9% over Wheat-Wheat-Chickpea. In 2015, the wheat yield in the Wheat-Mustard-Wheat-Wheat sequence was 10% higher than continuous wheat, possibly due to the break crop effect of mustard and utilization of nitrogen mineralised from decaying mustard plant material. However, the Wheat-Mustard-Wheat-Wheat sequence produced 5% lower grain protein than continuous wheat. Powdery mildew, in the mustard, was controlled through the growing season in 2015 by spraying Prosaro twice; at the vegetative and flowering stages. High mustard grain yield and high grain protein concentration were observed in the Wheat-Chickpea-Chickpea-Mustard crop sequence, which increased yield by 60% and grain protein by 17.6% compared to continuous mustard. The highest seed oil yield was produced in the continuous mustard sequence and this was 45% higher than the Wheat-Chickpea-Chickpea-Mustard sequence. However, if high glucosinolate are the intended production target, then the crop sequences Wheat-Chickpea-Mustard-Mustard and Wheat-Wheat-Mustard-Mustard produced 11.9% more glucosinolates than Wheat-Chickpea-Chickpea-Mustard. Grain yield was inversely proportional to oil and

glucosinolate yield. The chickpea treatment in 2015 was impacted by a late stunt virus infection which influenced yield and grain protein assessments.

Even though these crop sequences indicate different treatments optimise the production of different commodities, such as mustard oil and glucosinolates, ultimately seed yield is the most critical factor in the production of these commodities because the Wheat-Chickpea-Chickpea-Mustard treatment produce 60% more seed yield than the continuous mustard sequence which was higher in oil and glucosinolate yields.

Mustard used significantly more soil moisture than wheat or chickpea, however the levels of soil sulphur and phosphorous after harvest were much higher after mustard. This was offset by generally lower levels of soil N and soil carbon compared to wheat and chickpea. However, the Wheat-Chickpea-Chickpea-Mustard sequence used more of the available phosphorous, nitrogen and sulphur than other mustard crop sequences and made better use of the higher residual soil moisture retained in the soil after chickpea. This produced lower biomass and higher yield (2 t/ha) indicating higher harvest index. However, the primary economic and environmental benefit to the grain-grower is the enhanced yield of cereal crops following a crop sequence with mustard. This research indicates that mustard production can be successfully expanded in a northern farming crop sequence. Mustard provides a break crop, suppression of disease, weeds and microfauna under the right conditions of soil moisture and temperature, making income from a fallow using the seed, oil and glucosinolates to produce high value products and cleaning the soil allowing the possibility of improved yields from following crops on enhanced arable land. All these can be achieved under harsh environmental conditions.

Introduction:

The agricultural region of northwest NSW is characterised by hot, dry summers, cool winters and warm dry spring and autumn periods. The long term average minimum/maximum temperatures are 8.0/23.0 ^{o}C and the long term average growing season, April to October, rainfall is 339mm over 50 to 70 days of rainfall per year (BoM).

Figure 1: Map of the northwestern cropping area of NSW

Northwest NSW has predominantly fertile grey vertosol soils suited to extensive winter cropping. Figure 1 shows the extent of the cropping area in north western NSW and Table 1 summarises the areas sown to winter crops in NSW annually and the total tonnage recovered between 2013 and 2017.

Table 1: Winter crops sown and harvested in NSW; 2013 to 2017 (ABARES).

		2013–14	2014–15	2015–16	2016–17
Winter crops					
Wheat					
Area	'000 ha	3,269	3,166	3,410	3,500
Production	kt	6,596	6,654	7,500	11,375
Barley					
Area	'000 ha	715	882	900	870
Production	kt	1,486	1,869	1,890	2,697
Canola					
Area	'000 ha	673	699	560	510
Production	kt	922	1,014	833	842
Chickpeas					
Area	'000 ha	220	209	291	300
Production	kt	251	282	439	495
Faba beans					
Area	'000 ha	29	33	50	30
Production	kt	71	77	129	78
Field peas					
Area	'000 ha	50	51	48	50
Production	kt	53	66	73	85
Lentils					
Area	'000 ha	1	1	3	1
Production	kt	1	1	2	0
Lupins					
Area	'000 ha	57	56	62	51
Production	kt	57	66	76	66
Oats					
Area	'000 ha	268	362	300	322
Production	kt	283	350	360	477
Triticale					
Area	'000 ha	28	29	50	50
Production	kt	55	67	105	148

Note: Mustard figures will be embedded in the canola figures. Mustard is juncea canola or canola quality mustard.

Table 1 indicates that little mustard is grown in NSW. Approximately 3,000 tons of mustard is produced annually for the condiment market (Haskins et al 2009). Most farmers do not anticipate economic returns from mustard production for food. However, mustard does have advantages and provides potential benefits in crop sequences with other crops (Burton et al 2008). These benefits include cost savings associated with the break crop properties of mustard and potential new markets for industrial products from oil, meal and glucosinolates.

Mustard (*Brassica juncea*) in the northern wheat belt of NSW is grown on a small scale compared to canola (*Brassica napus*). Canola breeders have developed canola quality mustard with the yield, oil and meal consistent with canola quality specifications (Burton et al 2008; Haskins et al 2009). However, there is a potential trade-off between the high quality of these mustards and adaptation to the hot, dry conditions of

northwest NSW. The future of mustard in this region is therefore dependent upon the development of new markets for products. These markets may be industrial; not food based and the production of these types of mustards should not impinge upon the condiment and food oil markets.

Knowledge of how mustards fit into the northwest farming system is limited. This study examines the potential economic benefits of growing mustard in northwest NSW with a focus on the impact of mustard in crop sequences with wheat and chickpeas; the two most important grain crops in this region. The impact on the yield and market quality of all three crops was assessed and the economic consequences discussed including the potential of mustard for biodiesel and other industrial production.

To determine the economic value of growing mustard in northwest NSW, different rotation treatments of wheat, mustard and chickpeas were established and the environment in each year of assessment recorded. The impact of these treatments on yield, protein content and other important traits was assessed in all three crops. Thus the following hypotheses were tested in this study: (i) that mustard can yield well under a harsh environment in northern NSW and yield can be influenced by the sequence of crops grown, (ii) that mustard can produce a range of potentially marketable products, such as oil and glucosinolates, which could also be affected by the crop sequence and (iii) that mustard can have an impact on the other crops in the sequence.

Chapter 1: Literature review

Mustard History

Mustard is a member of the *Cruciferae* family, which includes broccoli, cabbage, brussels sprouts, rapeseed, field rape, canola, Ethiopian mustard and black mustard. Indian mustard (*Brassica juncea*) is a close relative to canola (*B.napus*) and Ethiopian mustard (*B. campestrus*).

Indian mustard is a cross between field mustard (*B. rapa*) and black mustard (*B. nigra*) (Chen et al 2013). It is believed to have been agriculturally and economically important as an oilseed crop since ancient times and was probably part of the spice trade between Europe, the Middle East, India, China and other Asian destinations (Chen et al 2013). It is thought to have originated from a number of locations in West Asia, possibly around Afghanistan, Iran and Iraq, although the exact location is unknown. There are accounts of the plains of Iran being covered in a mass of yellow in early texts (Chen et al 2013).

Chen et al (2013) identified two distinct groups of accessions across the Middle East, India and China. While the Middle East is regarded as the primary origin, India and China are regarded as secondary sites or centres of diversity and the likely origin of modern mustards. Group1accessions came from central and western India and eastern China, whereas group 2 accessions originated in central and western China and northern and eastern India. According to Chen et al (2013), the Chinese accessions have greater allelic diversity and more unique alleles than the Indian accessions. On at least two independent occasions mustard was transported along the Silk Road to India and China by traders, thus establishing these secondary sites, according to the famous Russian botanist and germplasm collector, Nicolai Vavilov (Chen et al 2013). Once established, these accessions led to local and regional adaption and independent centres of diversity. Accessions from group 2 are the source material for modern cultivars in Europe, USA and Australia. They are generally a mixture of black/dark brown seeded types and yellow seeded types. It is significant that the brown seeded autumn sown varieties originated in India and the yellow seeded spring sown varieties in China. These group 2 materials show more diverse agro-ecological adaption than group 1.

Brassica Genetic Makeup

According to Chen et al (2011), there are three ancestral species of *Brassica*; *Brassica nigra*, *Brassica rapa*, and *Brassica oleracea*. *B.juncea* is one side of the tri-genomic bridge species (Figure 2) and is derived from a cross between *B.rapa* and *B.nigra*, whereas canola (*B.napus*) has one parent in common with *B. juncea*: *B.rapa*. From the three ancestral, diploid species; *B.rapa* (AA), *B.nigra* (BB) and *B.oleracea* (CC), three allotetraploid species developed; these are *B.juncea* (AABB), *B.napus* (AACC) and *B.carinata* (BBCC). These six economically significant forms are the basis of the *Brassica* family. This structure has been confirmed by DNA and proteins studies (Chen et al 2011).

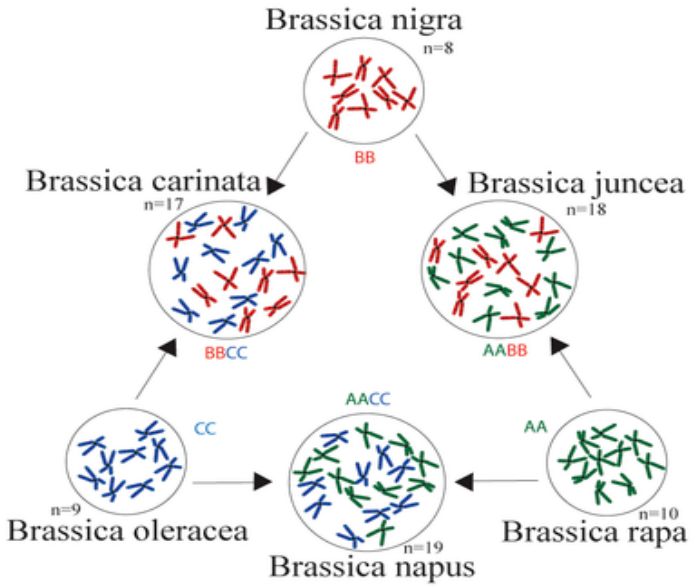


Figure 2: The "Triangle of U" author Woo Jang-choon (Korean name) or Nagaharu U (Japanese form) 1935 - genetic relationships between the six species of the genus Brassica.

Chen et al (2011) reported that different colours represent chromosomes from each of the genomes A, B and C. The tri-genomic bridges in *Brassica* provide new genetic resources, enabling *Brassica* breeders to improve yield and broaden adaptation of *Brassica* crops to meet human food and industrial uses, particularly in those areas affected by climate change. These new polyploids, either allotetraploids or allohexaploids, were then stabilised by diploidisation to create the basis of modern mustard varieties (Chen et al 2011). This diversity is vital to the development of improved cultivars given the challenges of climate change and increasing population pressure and the need to develop new markets for food and fuel.

Canola in Australia

Canola is by far the most widespread *Brassica* species cultivated in Australia. It is generally suited to the cooler wetter areas of Australia (Gunasekera et al 2006) and is predominantly grown in the southern cropping areas. However, in more recent years attempts have been made to establish canola in the northern wheat belt of NSW where cereal root diseases, severe frost and generally drier conditions prevail (Robertson et al 2004; Schwinghamer et al 2010). This region is characterised by a sub-tropical climate with summer dominant rainfall and winter crops are generally sown on stored soil moisture.

The northwest region of NSW is generally regarded as too hot and dry for canola cultivation and for this reason, mustard may provide an effective oilseed alternative in rotation with winter cereals and summer sorghum and cotton.

The environment is defined by interactions between temperature, rainfall/irrigation, humidity, radiation, soil type and soil/water borne contaminants and these interactions influence plant growth. Mustard can grow in drier, hotter and more variable circumstances than canola, including contaminated soils, making it well adapted to adverse agronomic conditions (Gunasekera et al 2006). Hunt and Norton (2011) verified that mustard grew better and yielded more than canola in hotter, drier climates.

Grain oil concentrations of mustard vary depending on the time of sowing and the amount of rain/irrigation post-anthesis (Hocking et al 1997; Burton et al 2008; Browne et al 2012). The quantity and quality of seed oil is most important for the production of biodiesel and other industrial products (Gunasekera et al 2006). These production areas are geographically and climatically diverse and range from southern NSW, across the Wimmera and Mallee regions of Victoria to southern Western Australia. These are quite geographically and climatically diverse regions of Australia that are characterized by a Mediterranean climate.

Mustard provides many benefits when incorporated into a winter/summer crop sequence. Gunasekera et al (2006) investigated the effects of mustard and canola genotypes and environment and their effects on crop growth and seed yield in low rainfall environments. Mustards produced higher dry matter than canola under stressful conditions. However, high biomass production did not translate into higher grain yield because the yield structure of canola (higher harvest index) is different to mustard: canola has fewer pods with more seeds/pod than mustard (Gunasekera et al 2006). The ability of mustard to cope with stressful environments

gives it an above average phenotypic stability and a greater tolerance to low rainfall, high temperature and later sowing.

Seed oil concentration is inversely proportional to seed protein concentration in both mustard and canola**.** According to the literature an increase in seed yield leads to an increased oil concentration, but a decrease in protein concentration (Gunasekera et al 2006). Differences in oil concentrations and protein content between mustard and canola have been observed in drier environments where mustard outperforms canola (Gunasekera et al 2006).

Canola Sowing Window

The sowing window for canola in northwest NSW is between 30th April and 15th May. The same planting window has been used for mustard. Therefore mustard flowering occurs between the end of June and the end of July, which includes the coldest time of the year. For example, on the northwest plains of NSW, winters can be cold, with overnight temperatures as low as -5.6ºC (Weatherzone). The temperatures recorded at the local airport are approximately a metre above the ground, so ground and field temperatures maybe lower: well explained on the BoM website. At these temperatures mustard is less likely to drop flowers as readily as canola as mustard tends to tolerate stressful conditions better than canola (Gunasekera et al 2006).

Osmotic Adjustment

Mustard is known for its drought tolerance and Hocking et al (1997) suggests that the mechanism for drought tolerance is osmotic adjustment. In canola, this is conditioned in the vegetative stage whereas tolerance is manifested in both the vegetative and reproductive stages in mustard (Hocking et al 1997). Both temperature and moisture affect the quantity and quality of the oil (Gunasekera et al 2006). However, not all mustard genotypes undergo osmotic adjustment and grain yield may drop by as much as 40% under rain fed conditions compared to yield reductions of 0 – 10% in those genotypes with high osmotic adjustment (Niknam et al 2003).

Generally, the stressed mustard plant copes through osmotic adjustment among other strategies. Osmotic adjustment is often assessed at the reproductive stage of development as this is critical for grain yield (Niknam, et al 2003) and is the point where stress reduces the number of seeds per pod and pods per plant (Kumar et al 1994).

Osmotic adjustment has not been observed in either mustard or canola during pod fill (Ma et al. 2006). At this stage the plant goes into survival mode to ensure enough seed is produced at the expense of biomass. The higher rate of biomass accumulation in mustard compared to canola is a function of higher photosynthetic rate and better WUE and reflects the two periods of osmotic adjustment in mustard; however harvest index is lower compared to canola (Gunasekera et al 2006).

Proline is implicated in osmotic adjustment in mustard and canola. These stress-induced changes in the osmotic adjustment of expanded leaves are due to the accumulation of nitrate (42–47%), soluble sugars (31–38%), and proline (11–14%) (Ma et al 2006). According to Ma et al (2006), K+ accumulation was significant (23–27%) as was proline (17–22%) in expanding leaves, whereas nitrate and soluble sugars were less important. Na+, Cl−, water-soluble Mg2+ and Ca2+ ions have little influence on osmotic adjustment. Proline was barely detected in well-watered plants, but sharply increased in the leaves of water stressed plants in direct proportion to the magnitude of osmotic adjustment (Ma et al. 2004). Cell proline levels increase in response to moisture stress, high salt conditions, heavy metal contamination, disease and insect attack. Proline content tends to be greater in rain-fed crops at both the flowering and pod-filling stages compared to irrigated crops (Paul et al 1993). Both Paul et al (1993) and Ma et al (2004) concluded that osmotic adjustment was closely correlated with the accumulation of K+ ($r = 0.91$), soluble sugars ($r = 0.90$) and proline ($r = 0.96$), whereas other solutes (Na+, NH4+, Cl–, NO3–, Mg2+ and Ca2+) made little or no contribution to osmotic adjustment. Leaf proline concentration could therefore be a good indicator of osmotic adjustment in *Brassica* oilseeds (Ma et al, 2006). During osmosis, the proline levels in cells increases and the amount of water passing through the cell wall decreases, leading to a lower water requirement and therefore greater drought-tolerance in mustard compared to canola and chinese cabbage (Alam et al. 2013). Hence, proline in water-stressed conditions acts as water-stress adjuster in mustard plants (Ma et al. 2004). In mustard and canola, proline metabolism is genotype dependent (Phutela et al. 2000) and proline levels can therefore be used for mustard plant selection under dry conditions (Ma et al. 2006). Nitrogen differentially regulates proline production and ethylene formation to alleviate the adverse effect of salinity on photosynthesis in mustard (Iqbal et al 2015). An increase in moisture stress and increasing proline levels in mustard changes the fatty acid (FA) composition of the oil and the erucic acid metabolic pathway (that is, erucic acid increases with stress and

oleic acid decreases). Stress also increases protein content but not sugar levels (Bouchereau, et al 1996). Increasing proline can lead to an increase in the accumulation of phenolics and glucosinolates (Bouchereau, et al 1996).

Mustard Time of Sowing

The time of sowing influences seed and oil yield potential. When time of sowing is delayed oil quality and quantity and seed yield are reduced. Mustard sown outside the optimum planting time suffers a decrease in oil concentration of 1.1% and a reduction in grain yield of 309kg/ha for every two weeks delay in planting (Hocking et al 2001). This reduction is exacerbated if the crop is grown in a low rainfall area.

If time of sowing is delayed then flowering and grain fill occur during warmer, suboptimal periods in the growth cycle. This post-anthesis period is very important as it influences both oil quantity and quality. Early flowering cultivars can extend the post-anthesis period as every10 day increase in the post-anthesis period increases the oil concentration by 1.2% (Si et al 2004). Oil and seed yield increase with higher post-anthesis rainfall and lower temperatures, optimum temperatures 20^{o}C daytime and 15^{o}C night time (Angadi et al 2000) with some estimates suggesting that increases of 0.7% in oil yield and 116kg/ha in grain yield are possible for every 10mm increase in rainfall (Si et al 2004). In addition, oil yield is reduced by 0.68% and seed yield by 289kg/ha for every 1^{o}C increase in temperature at 28^{o}C/23^{o}C day/night temperature loosing as much as 52% oil at 38^{o}C (Si et al 2004; Angadi et al 2000). Early sowing and early flowering cultivars with high grain yield and high oil yield potential and tolerance to both high and low temperature and good water use efficiency would be profitable (Walton et al 2004).

Delayed sowing also reduces oil quality. Wilkes et al. (2013) reported lower oleic acid and higher linoleic acid in late sown materials; a fatty acid constitution that affects the oxidative stability and kinetic viscosity of biodiesel.

In northwest NSW, delaying sowing by one day after 15th May delays flowering and maturity by approximately 0.5 day. This results in a decline in yield, oil concentration and biomass (Robertson et al 2004).

Photoperiodism and vernalisation

The photoperiod responses of mustard and canola have been studied recently and canola grain and oil yields can be improved by sowing earlier (Kirkegaard et al. 2016; Robertson et al 2016; Lilley et al. 2015).

This in part is highlighted by findings of Wilkes et al (2013).

Photoperiodism is the physiological response of plants to the length of daylight hours. This is not well understood in mustard. In northwest NSW, photoperiod is likely to influence yield as the crop is sown with 11 to 12 hours of daylight. The closer the crop is sown to the 21st March, the more rapidly it will approach flowering and possibly lengthen the post-anthesis period, potentially improving grain and oil yield in the absence of frost. This may explain, in part, why late sown mustard crops do not produce higher grain yield or oil concentration than earlier sown crops. Late sown crops are harvested during the hotter part of the year, around early to mid-November, when temperatures can be very high (Wilkes et al. 2013). To gain the most benefit from photoperiodism, plants would need to be exposed to 12 to 14 hours of daylight (Burton et al 2008).

In addition to photoperiod, grain yield can also be influenced by vernalisation (Myers et al 1982). However, in mustard vernalisation has less effect on flowering date and yield than photoperiod (Burton, et al. 2008). Robertson et al (2002) found that all genotypes of canola and Indian mustard reduced the time to bud stage in response to vernalisation and responded to photoperiod from 10.8 to 16.3 hours of daylight post emergence. The vernalisation and photoperiod affects occur in the early vegetative stage between the summer solstice and the end of May, where daylight hours range from 14 to 10.8 hours.

With increasing day-length the plant speeds up development through the vegetative stage, making more efficient use of nitrogen and thus reaches flowering earlier, resulting in a longer pod filling period in the cooler part of the season (Kirkegaard et al 2016; Robertson et al 2016; Lilley et al. 2015). Sowing date is critical for optimal flowering time to avoid frost damage and not limit yield potential. Since optimal flowering time is crucial in any environment, then the variety needs to be matched with sowing date (Robertson et al 2004). If mustard flowering occurs in June then the longer pod filling period may lead to an increase in erucic acid levels (Wilkes et al. 2013).

There is a relationship between stem swelling, photoperiod and growth hormones in mustard and the growth rates of genotypes differ for stem swelling and levels of endogenous gibberellin and cytokinin under different photoperiods (Xu et al 2008). According to Xu et al (2008), longer photoperiod tended to promote stem elongation, increase photosynthetic activity and increase the levels of endogenous gibberellin and

cytokinins. These results indicate that stem growth and swelling is a physiological process under hormonal control and that photoperiod possibly exerts its influence by altering the balance of endogenous phytohormones (Xu et al. 2008).

Harvest

Windrowing is the conventional way to harvest canola and mustard; where the crop is cut while still green and allowed to dry in the field before machine harvesting. This harvest method was adopted to reduce shattering; however it can be difficult to pick up the crop for subsequent threshing. Mustard is less prone to shattering than canola and can be direct harvested without windrowing (Gan et al 2008).

While direct harvesting is more efficient (Gan et al 2008) it is vital that the crop matures evenly to reduce the proporation of green seeds (AOF).

Syngenta Reglone (the active ingredient is 200g/lt Diquat) is the only product permitted as a pre-harvest desiccant for mustard and canola in all states of Australia. Reglone is sprayed when 70% of the pods are yellow and the seeds are brownish/bluish and pliable. The crop is then ready for direct harvest four to seven days post spraying. To improve the effectiveness of Reglone a wetter BS1000 is used at 160ml/100lt of water (Syngenta). Reglone promotes even crop ripening and control of weeds, but is expensive. However, Reglone is used primarily on canola and if suitable for mustard would further reduce shattering and improve the utility of mustard for direct harvest (Haskins et al 2009).

Once the seed is harvested it can be used to produce oil and meal and the biomass can be removed for the development of a number of high and low value products including biodiesel (Kirkegaard et al 2000).

Seed Size

The smaller seed size of mustard compared to canola is a market disadvantage and reflects the relatively lower investment in mustard genetic improvement (Tahira et al 2014) and affects seed quality, vegetative growth, grain yield, oil content and quality, marketing options and harvest efficiency all of which can be improved through directed selection (Robertson et al 2004; Gugel et al 2006). Genetic variation determines the boundaries of seed size, whereas plant nutrition determines the expression of genetic potential. Seed size could also be related to the physical position of the developing seed in the pod (Ambika et al 2014). Distal seed will tend to be smaller than those seeds at the point of attachment to the stem or in the centre of the

pod. The seed coat and embryonic axis develop first within the pod and the seed subsequently accumulates assimilate reserves (Ambika et al 2014). Seed size affects germination, emergence and many other agronomic factors, including both grain and oil yield. In general, larger seeds have better field performance than smaller seed (Ambika et al 2014) leading to better establishment and early vegetative growth and better yield components including thousand kernel weight, germination percentage, seedling vigor and oil yields (Vijaya et al 2013). Mustards produce approximately 13 seeds per pod compared to 18 seeds per pod in canola Gunasekera et al (2006). Thus unless the season is severe for canola, mustard is unlikely to out yield canola with current varieties.

However, recently released canola quality mustard varieties (Haskins et al 2009), sold as canola used in hotter, drier areas may alter the balance between the two crops in northern NSW.

Mustard seed soaked pre-sowing in 20mM proline for 8 hours significantly increased plant growth, photosynthetic rate and the activities of antioxidant enzymes, compared with untreated seedlings (Wani et al. 2012). Thus higher proline may potentially improve mustard adaptation if combined with optimization of photoperiod responses and improved nitrogen use efficiency.

Seed Colour

Seed colour is an important market consideration and yellow seeded varieties tend to produce more oil than black/brown seeded varieties (Rahman et al 2011).

According to Rahman et al (2011), there are naturally occurring yellow seeded mutants of *Brassica rapa, B. juncea* and *B. carinata* species. Yellow seeded *Brassica* have higher oil content and protein percentage but lower fibre content. There are two duplicate genes responsible for seed colour in *B. juncea.* These were identified by segregation analysis as two unlinked loci with duplicate gene action (Mahmood et al 2005). However, quantitative trait loci (QTL) analysis showed the influence of three QTLs for seed colour: SC-B4, SC-A10 and SC-A6. However, the effects of QTL SC-A10 alone were not significant for seed colour (Mahmood et al 2005). Many different molecular markers for seed colour genes in *B. rapa, B. juncea and B. napus* have been developed for use in marker-assisted selection in plant-breeding programs (Rahman et al 2011).

Yellow seeded mustard arose from mutation and the colour of the embryo and the transparent nature of

the testa (or seed coat) give the seed its distinct yellow colour (Yan et al 2010).

Oil quantity and quality and implications for biodiesel production

Seed oil concentrations and quality can vary depending on the time of sowing and the amount of rain/irrigation post-anthesis (Hocking et al 1997).

Wilkes et al (2013) observed genotype by environment interaction for oil quality and found that erucic acid levels were higher in cooler growing conditions and that oleic and linoleic acids were inversely correlated with erucic acid content. Biodiesel produced from two genotypes evaluated in these experiments met most Australian standard requirements for biodiesel with the exception of oxidation stability and kinematic viscosity (Wilkes et al 2013). High concentrations of polyunsaturated fatty acids reduced biodiesel quality, and hence there is a need to develop mustard genotypes with higher levels of oleic acid (a monounsaturated fatty acid) to increase oxidation stability. However, high erucic acid levels and excessive glycerol accumulation during biodiesel production resulted in poor kinematic viscosity (Wilkes et al. 2013).

When linoleic acid (double bonded) is lower and oleic acid (single bonded) higher, the quality of the biodiesel fuel is improved through positive effects on gel, cloud points and iodine levels. These affects can be expressed as oxidative stability and kinematic viscosity (Wilkes et al. 2013). However, the reported development of a mustard variety using genetic engineering with up to 73% oleic acid could be a breakthrough for biodiesel quality and processing (Stoutjesdijk et al 2000).

Biofumigation

A major agronomic benefit of mustard in crop sequences is the break crop effect. Break crops that do not host the pathogens found on other crops in the sequence reduce disease load (Kirkegaard et al 2000). However, biofumigation can also reduce pathogens. Biofumigation refers to the active suppression of soil-borne pathogens and pests by biocidal compounds released by *Brassica* crops when glucosinolates (Allyl ITC) in their residues as it decays in the soil (Kirkegaard et al 2000; Watt et al 2006; Angus et al 2015). Biofumigation is thought to add additional benefits which have been difficult to demonstrate in the field and depend on adequate levels of soil moisture and optimal soil temperature. Low soil moisture is a major inhibitor to good biofumigation (Kirkegaard et al 1999; Okunade et al. 2015). The potential of disease-suppressive crops such as mustard warrant further investigation to determine not only the benefits of mustard

as a break crop but also their biofumigation effects For example, using mustard as a green manure crop may potentially increase farm incomes and save the cost of expensive chemicals for fumigating infected soil (Larkin and Halloran 2014).

Mustard in rotation can therefore improve arable land by suppressing weeds and diseases. The food versus fuel argument is based on the premise that arable farmland should be used for food production only as arable land is a limited resource (Atabani et al. 2013). However, the food producing capacity of the farming system in northwest NSW could be increased through the introduction of mustard, as the yield of wheat and legumes could potentially be increased. The glucosinolate is stored predominantly in the cells of roots, stems, shoots and seed of the mustard plant. Higher yielding and genetically improved mustard varieties have higher levels of 2-phenylethyl glucosinolate in the tap and lateral roots (>2mm diameter) whereas the finer roots (<2mm diameter) have higher levels of indolyl glucosinolate (Kirkegaard et al 1999).

The mustard plant exudes the Isothiocyanate into the surrounding soil thus suppressing C3 and C4 pathway plants, nematodes, soil borne diseases and plant borne diseases such as crown rot (Kirkegaard et al 1997; Kirkegaard et al 2004; Angus et al. 1994). The process of Isothiocyanate exudation into the surrounding soil is called bio-fumigation (Sarwar et al. 1998; Angus et al. 1994).

There is evidence that mustard can reduce weed infestation, disease and pest load in some rotations because the Isothiocyanate is released into the soil as the plant develops (Rathore et al 1998).

No adverse effects of glucosinolates on beneficial soil borne fungi, such as arbuscular mycorrhizal fungi, have been reported (Glenn et al 1985). However, the arbuscular mycorrhizal fungi are not able to colonise *Brassica* roots (Glenn et al 1988; Ryan et al 2002) because of glucosinolate concentration close to the roots.

Animal Feed

The resulting mustard meal post-oil extraction can be fed to animals as a source of high protein. In addition, glycerine, a by-product of biodiesel production, can be used to manufacture soap or as a feed additive: although the later requires further investigation. Mustard meal produced from the crushed seed has a protein content of between 23 - 26% (Wilkes et al. 2013). This makes it ideal as an animal feed. However, mustard meal should be fed advisedly because of the high glucosinolate levels in mustard meal. Animal feed

intake can decrease when feeding mustard meal due to the high level 3-butenyl glucosinolate content in the meal. This glucosinolate is bitter to the taste and can adversely affect animal wellbeing even causing death if fed in a high proportion of the feed ration. Zhou et al (2014) found that more than 20% mustard meal fed to pigs led to a decrease in feed intake, weight gain, carcass weight, loin depth and an increase in jowl fat unsaturation. In contrast there is a benefit to animal health if glucosinolates via mustard meal are included in animal diets at safe levels, as they may reduce parasites infestations in the rumen. However, this effect remains to be confirmed (Zasada, 2009).

Biodiesel

The yellow seeded variety Muscon 973 was developed in the late 1980's with high oleic acid (60-65%) specifically for the condiment market (Oram et al 1995). However, this food quality mustard can also be used for biodiesel production. High quality biodiesel can be produced from mustard seed with little or no proline when produced on an adequate supply of water, either through rainfall or irrigation (Ma et al. 2004). When mustard has high levels of proline and other phenolics, these antioxidants reduce the efficient oxidation of biodiesel (Nitièma-Yefanova et al 2015). Phenols (or phenolics) are chemical compounds with a hydroxyl group (—OH) bonded to an aromatic hydrocarbon group. These produce toxic compounds and that may promote poly aromatic hydrocarbons (PAH) during combustion in the engine (Nitièma-Yefanova et al 2015). It is therefore better to use ethanol derivatives as antioxidants in biodiesel as they form non-polluting compounds: even if the phenolics react with ethanol during the conversion of mustard oil into biodiesel. There is a need to minimize aerial pollutants and proline can be used as a marker because it is not present in plants that are well watered, but in plants that are drought stressed. Thus cultivars low in proline can be expected to contribute lower levels of phenolics in biodiesel.

Glycerine is a by-product of biodiesel production when biodiesel is produced using the Transesterification method for creating biodiesel. This by-product can be used to manufacture soaps and in animal feed rations as mentioned above. Glycerine may also be suitable for the manufacture of cosmetics (Kerdudo et al. 2015; Zhao et al 2015), food additives (Kuplennik et al 2015), medicinal compounds (Rahim et al 2015; Braithwaite et al. 2015) and chemical wetters (Alromeed et al. 2015). Gylcerine derived from

mustard oil, mustard meal and mustard biomass needs to be assessed for potential economic benefits other than soap.

Mustard Commodity Market

Unlike canola, the commodity market for mustard in Australia is small (Oram et al. 2005). Currently, there are attempts to establish a larger commodity market for mustard and firms such as Palos Verdes at Cowra, Yandilla at Wallendbeen, Australian Mustard Oil at Young and Botanical Innovations at Orange, NSW process mustard for the food additive market by removing glucosinolates from mustard meal (Oram et al. 2005). These firms process approximately 2,000 to 4,000 tons annually for the condiment market in a closed loop market where these firms purchase mustard ex-farm in a small niche market (Haskins et al 2009).

This literature review hints at the potential of Indian mustard for our rural communities, not only from an agricultural point of view in cleaning soil naturally, reducing chemical costs and improving yields when used in crop sequencing, but also the scope for industrial development in regional areas by manipulating the agronomic and plant characteristics to improve oil, glucosinolate and meal qualities for pharmaceuticals, veterinary products, stock feeds, biofuels, biolubricants and bioplastics; to name a few. This crop has great potential to withstand very stressful and harsh environmental conditions.

Chapter 2: Methods and Materials

Site Location and Map

The University of Sydney, Plant Breeding Institute, I. A. Watson Research Station is situated approximately 7.5km North of Narrabri on the Newell Highway; (Latitude: S30º 16' 13.3", Longitude: E149º 48' 14.7") bordered on the western side by the Newell Highway and to the east by Killarney Gap Rd. The site of the cropping sequence trial was situated in Field B3, southern most point of I. A. Watson Research Station, at the junction of the Newell Highway and Killarney Gap Rd.

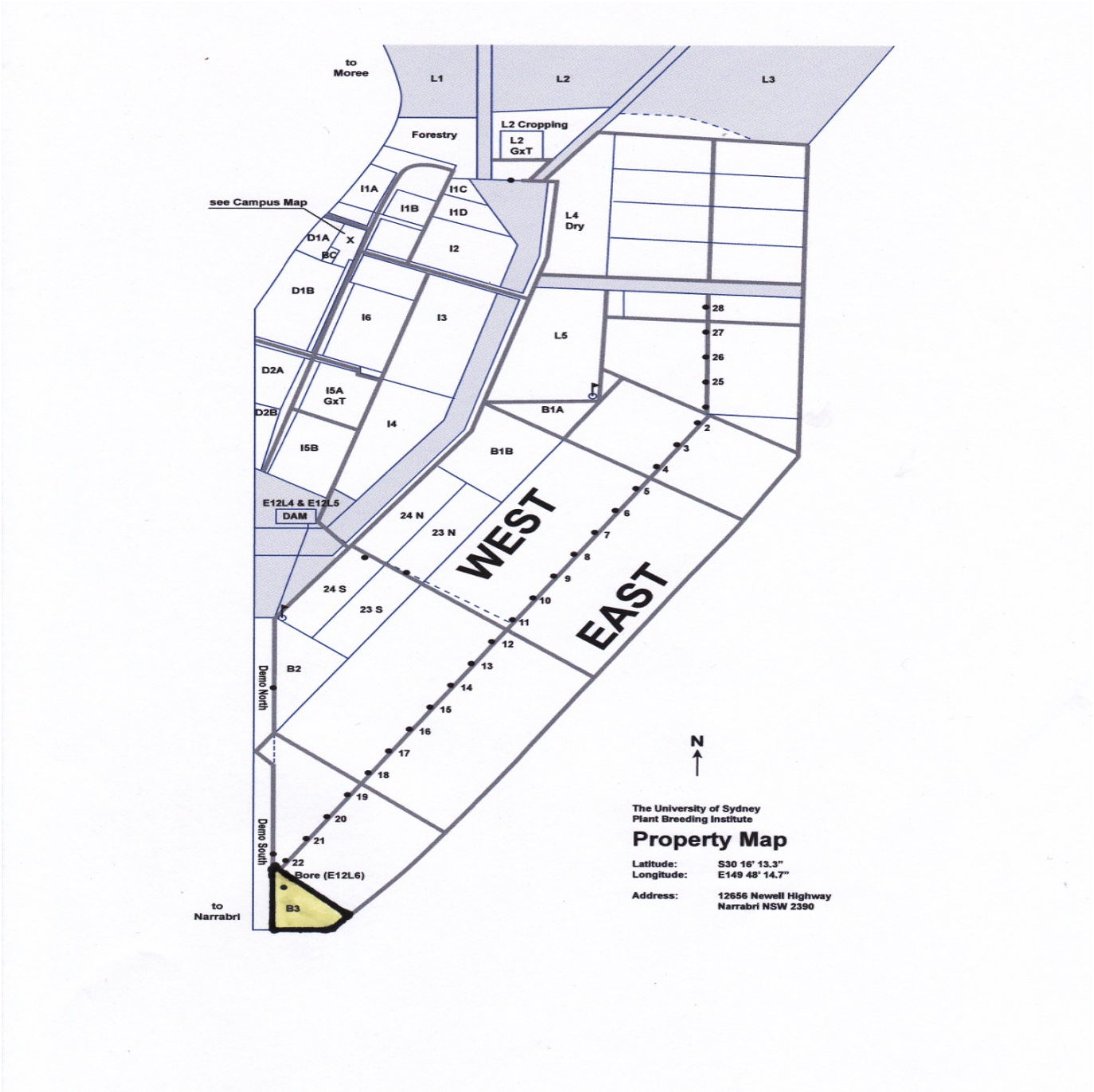


Figure 3: Map of the I A Watson Grains Research Centre and the trial site location: B3

Field B3 is a self-mulching, grey Vertosol of very fine or medium fine clay soil classified as 5YR 4/1 for colour. It is a cracking (shrinking-swelling type soil) clay Vertosol. According to the Australian Soil Classification System (Isbell 2002) it is defined as a smectite soil made up of 2 parts silica to 1 part alumina based on Illite clay. According to the Koppen-Geiger climate map (Peel et al, 2007), the climate is classified as temperate with a dry winter.

A crop sequence of wheat, mustard and a grain legume was established at this site in 2013. Prior to the establishment of these treatments, the site was sown to field peas in 2011 and LongReach Spitfire™ wheat in 2012. The rotation plan for 2013, 2014 and 2015 is presented in Figure 4. The crop sequences were designed to reflect a probable crop sequence that a grain grower in northwestern NSW might use when introducing mustard on farm.

In 2013, wheat, mustard and chickpea crops were sown on a 36m x 240m block running east to west to stabilise the site and establish the rotational areas. The site was squared using triangulation. Three 12m wide strips of 240m length were sown to Lincoln™ Wheat, Indian mustard (USyd #7) and HatTrick™ chickpeas. The chickpea strip was further divided into +/- chickpea rhizobium inoculum.

In 2014 and 2015, Wheat, Mustard and Chickpea treatments where established in plots sown perpendicular to the strips of each species established in 2013. The wheat variety Suntop™, released by Australian Grain Technologies (AGT) in 2013, replaced LongReach Lincoln™ in 2014 and 2015 and the mustard and chickpea cultivars remain the same. Three replications of each Wheat/Mustard/Chickpea crop sequence treatment were established in 2014 and 2015.

Pre-trial operations in 2014 and 2015

In 2014, the sowing seed rate for mustard was 4kg/ha giving a plant density of 19 plants/m^2. The wheat sowing rate was 40kg/ha giving a plant density of 93 plants/m^2 and the chickpea sowing rate was 40kg/ha giving a density of 20 plants/m^2; this was slightly below the recommended 30 chickpea plants/m^2. The sowing rate for mustard in 2015, was adjusted in line with standard canola rates to 2kg/ha and to reduce the number of plants affected by overcrowding, resulting in a plant density of 19 plants/m^2. Thus the same plant density for mustard in 2014 was achieved at half the sowing rate in 2015. Sowing rates for wheat and chickpea remained the same as for 2014.

Soil moisture was monitored in plots 4, 5, 6, 10, 11, and12 using a CPN 503 Hydroprobe (CPN International owned by Instrotek Inc., Concord, California, USA) and 6 neutron probe access tubes 1.5m deep with readings taken at 10cm, 20cm, 30cm, 40cm, 60cm, 80cm, 100cm, 120cm and 134cm (Figure 2). The neutron probe access tubes were inserted pre-sowing into the treatment plots. These access tubes were installed in April during dry conditions to avoid issues with wet, muddy soil after sowing. The neutron probe readings were taken every ten days to determine water usage over the season. The first measure was made prior to planting and the last post-harvest. The neutron probe emits fast neutrons reflected back at slow speeds to the neutron probe in proportion to the concentration of hydrogen ions found in the soil; found predominantly in water molecules. The neutron probe detecting these neutrons measures them as a count rate. The count rate was expressed as a ratio against the count rate measured in a drum of water. The count rate ratio was then converted to volumetric water content using equation:

$$\Theta = 1.6852 + 0.0017*\text{Counts}$$

Where Θ = volumetric water content, cm3 cm-3; Counts = neutron probe counts; 0.0017 and 1.6852 are the coefficients of the calibration equation. The coefficient of determination was 0.82.

There were three (3) neutron probe access tubes inserted into each Wheat/Wheat, Mustard/Mustard and Chickpea/Chickpea crop sequence in 2 replicates. A red cross (**X**) highlights the placement of neutron probe access tubes in Figure 4. The access tubes were inserted in the continuous wheat, mustard and chickpea treatments to estimate the general crop effect on soil moisture.

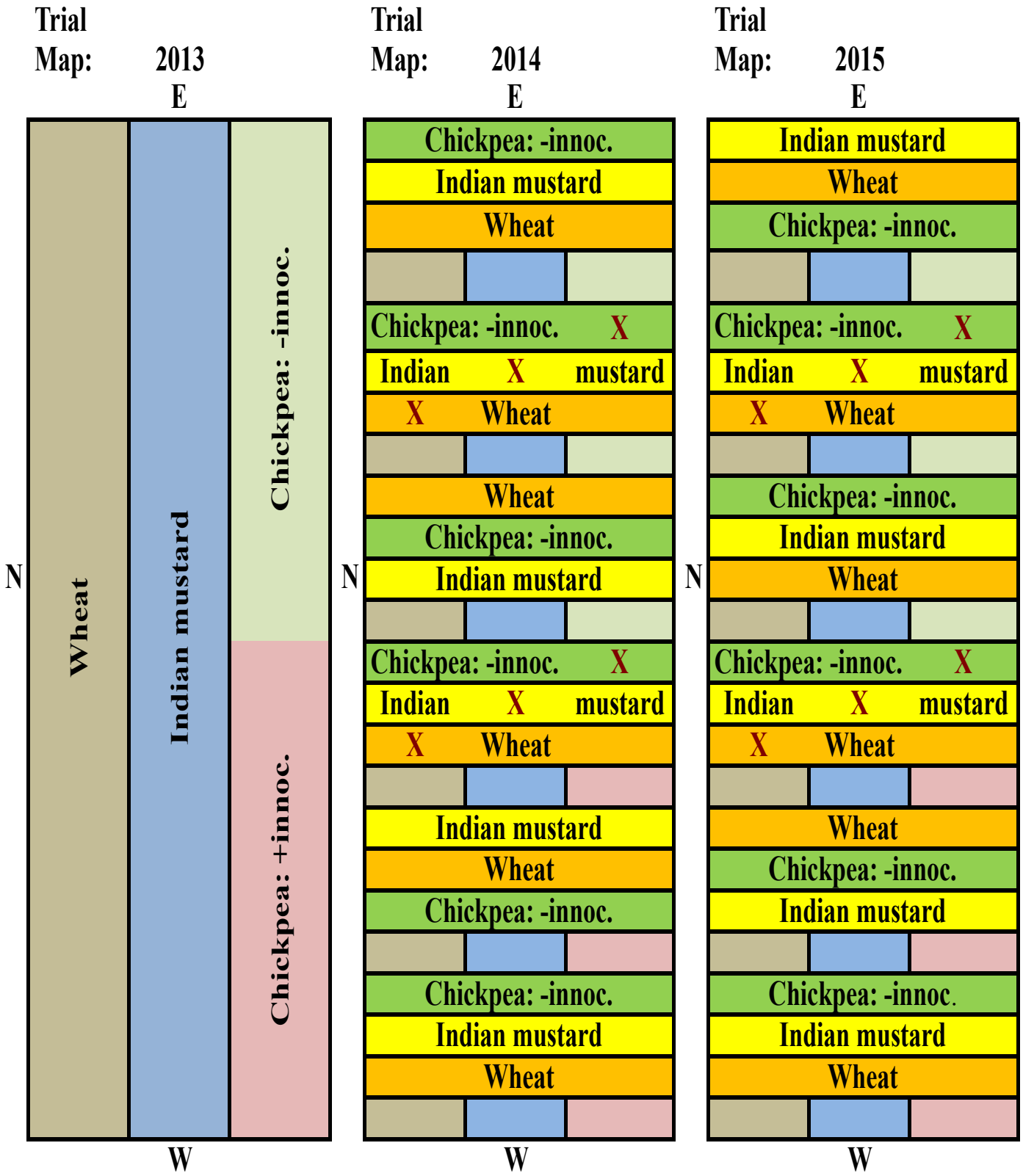


Figure 4: Crop sequential treatment layout with neutron probe access tube locations in 2014 and 2015. There are 3 strips including Wheat, Mustard, and Chickpeas in 2013. Each strip is 240m long by 12m wide running West to East on Field B3. In 2014-15, each strip of crop was 12m wide by 36m long.
Footnote: Beige, blue, pink and grey colour shows the position of the 2013 treatments under the 2014-15 crop sequences. No +/- chickpea inoculum in 2014-15 crop sequences. Neutron probe locations are indicated by a X.

Soil samples were taken to 150 mm depth using a hand corer across the whole site for PreDicta B (Gutteridge, et al 2008) testing to confirm nematode and root rot disease levels prior to sowing. These soil

samples were taken at random across the entire site in 2014 and a mixed representative sample sent to the South Australian Research and Development Institute (SARDI) for testing.

The PreDicta B service provided by SARDI included tests for cereal cyst nematode (*Heterodera avenae*), take-all (*Gaeumannomyces graminis var tritici (Ggt)* and *G.graminis var avenae (Gga*)), Rhizoctonia barepatch (*Rhizoctonia solani* AG8), crown rot (*Fusarium pseudograminearum* and *F.culmorum*), root lesion nematode (*Pratylenchus negectus* and *P. thornei*), stem nematode (*Ditylenchus dipsaci*), and blackspot of peas (*Mycosphaerella pinodes, Phoma medicaginis var pinodella* and *Phoma koolunga*). The PreDicta B evaluation for soil-borne biotic constraints was below the threshold values for nematodes and root rots in both years.

In early 2014, soil samples taken at depths of 0-15, 15-30, 30-60cm at two sites (east and west) where neutron probe access tubes were inserted and a full soil analysis was conducted including pH, chloride, phosphorus, carbon, organic matter, sulphur, nitrogen, electrical conductivity, magnesium, potassium, sodium, cation exchange capacity, colour, texture, copper, zinc, manganese, iron, boron, calcium, aluminium, and molybdenum. The soil samples were taken from 50mm diameter soil cores. In 2014, two soil core samples of 60cm depth were selected and sent for analysis at Agricultural Chemistry Pty Ltd, Ipswich, Qld according to the Australian Laboratory Handbook of Soil and Water Chemical Methods (Rayment and Higginson 1992). In late 2015, soil samples taken 0- 30cm depth were analysed for nitrogen including ammonia, pH, organic carbon, phosphorus, sulphur and soil moisture at the same laboratory. A summary of key nutrients for 2014 and 2015 can be found in Appendix tables 10 – 12. No nutrient analysis was conducted in 2013 as bulk crop was sown in strips to establish the rotations for 2014 and 2015.

In season measurements

Each crop was sown separately on dates matching their optimal planting window. Mustard was sown on 29th April, wheat on 17th May and chickpea on 22nd May in 2014. In 2015 the respective dates for each crop were the 28th April, 16th May and the 12th June.

Plots were sown using the USyd PBIN single cone 6 row seeder on a 2m (wheel centre to wheel centre) wheelbase with tyne spacings of 375mm/250mm/250mm/250mm/250mm/250mm/375mm using a planting depth of 5cm for mustard, chickpeas and wheat. At planting, Granulock Z at 40kg/ha (N 11%, P 21.8%, S 4.0%, Z 1.0% Incitec Pivot Ltd, Southbank, Victoria) was incorporated through the cone seeder for

all crops and treatments with an additional 50kg N/ha applied as a general pre-seeding application over the site in 2014. Two additional applications of Urea 50kg N/ha were applied to the wheat in the vegetative stage and mustard in the vegetative and early flowering stage in 15th June and 9th July, 2015.

Plots were harvested in both years in late October/early November using a KEW (Kingaroy Engineering Works, Kingaroy, Queensland) small plot harvester and the seed was bagged and weighed and subsequently converted to kilograms per hectare (kg/ha). The dimension of each harvested plot was 120m^2 (12m x 10m) after a 2m strip between plots/treatments was removed.

The only irrigation applied was 37 mm in mid-July in 2014, using a low-pressure irrigation boom while no irrigation was applied in 2015. In season rainfall was assessed using a rain gauge at the site.

Weeds were controlled in both years using a chipping hoe. The weeds present in the trial area were Phalaris species, bindweed, milk thistle, barely grass, summer grass, bladder kepnia and fumitory. These weeds occurred in the wheat treatments only. In both the chickpea and mustard treatments the weeds grew predominantly at the end of the plots. Fumitory was the most invasive weed and in 2014 was difficult to control. Once Fumitory was removed prior to seeding it didn't reoccur in 2015 to the same extent. Chickpeas out competed the weeds except where there were gaps in the plant canopy. Weeds were not present in the mustard treatments likely because of the biofumigation action of AITC, large biomass and moisture deprivation created by the high moisture extraction of the mustard plant. Soil cracking occurred earlier in the mustard treatments than either the wheat or the chickpea treatments.

In northwest NSW mustard can be infected by Beet Western Yellow Mosaic Virus, which is carried by green aphids; and powdery mildew (*Podosphaera xanthii*). A late powdery mildew infection was observed in 2014 and the height of the crop at this time prohibited the mechanical application of fungicide. In 2015, Bayer Prosaro 420 SC was applied at the rate of 450mL/ha on the 16th June (Das 49 mustard) and a second application of 375mL/ha on the 10th August (Das 104 mustard).

The wheat cultivar Suntop was resistant to stripe rust however two applications of Folicur 430SC – Bayer Crop Science, were applied at 290mL/ha on 15th July (Das 61 wheat) and the 16th August (Das 92 wheat) in 2014 as a precautionary measure. In 2015, Prosaro 420 SC was applied at 150 mL/ha on the 16th June (Das 49 mustard) and the 10th August (Das 104 mustard).

Ascochyta blight (*Ascochyta rabiei*) was controlled in the chickpea treatment in 2014 by applying Unite 720 @ 1lt/ha on 4th September. In 2015 Prosaro 420 SC was applied at 150 mL/ha on 10th August (Das 59 chickpea). A late September infection of stunt virus was noted in 2015 and this affected plant vigour and yield.

Germination and plant establishment were assessed using a one metre ruler to count the number of plants per metre and 3 readings per plot were taken. Germination percentage was recorded 2 to 3 weeks after germination and establishment counts approximately 6 weeks post germination.

The number of days until 50% of the plants in each plot were flowering was determined for wheat and mustard while days to first flower appearance was assessed for chickpea. The number of days to first pod was also recorded for chickpea.

Plant height was assessed from the ground to the top of each inflorescence at the end of the season at physiological maturity using an extended ruler.

Leaf photosynthesis and stomatal conductance were measured at the youngest fully expanded leaf on wheat, mustard and chickpeas plants in the vegetative, flowering and podset stages of plant growth. Measurements were conducted using a portable photosynthesis system LiCor 6400XT open gas exchange analyser (LI-COR Lincoln, Nebraska USA) where the sample leaf, while remaining attached to the plant, was placed in a chamber called an infra-red gas analyser (IRGA) to simultaneously measure CO2 exchange, chlorophyll fluorescence, light, temperature, pressure and humidity. This allowed accurate measurement of plant photosynthetic characteristics in the field. Three plants per plot were assessed between 10am and 2pm when plants are most active and the results subsequently averaged to obtain a value for each plot.

Normalized Difference Vegetation Index (NDVI) provided by Trimble Greenseeker Crop Sensing Systems was used to assess wheat, chickpeas and mustard to measure plant biomass and greenness, indicating crop nitrogen levels. Calibrating the NDVI on bare soil gave values below 0.1 and used to ensure the sensor was working correctly. In other words values showing greater than 0.1 on bare soil would indicate that the Greenseeker crop sensor was faulty where plant readings of 0.3 to 0.8 in the biomass shows medium to high levels of nitrogen. In 2014, two readings were taken on the 9th September and the 17th September. A total of seven readings were made in 2015 on 29th June, 20th July, 29th July, 30th July, 31st July, 20th August and the 29th September. These were also taken across a period of frost damage on 29th July and 31st July, 2015.

Biomass was assessed on each plot at anthesis from a 0.4 x 1m quadrat used to cut plants at ground level from each treatment with a hand sickle, plants samples separated into roots, stems, leaves, spikes/heads and flowers and then placed in a dehydrator for 4 days at 105^{o}C and total biomass recorded for each component; roots, stems, leaves, spikes/heads and flowers. Heads and flowers were combined as reproductive parts. These components were oven dried and weighed. Biomass was assessed on mustard only in 2014 and on all three crops in 2015.

A 1m ruler was used to determine the growth rate of each mustard plot. The plant height was recorded at the end of the vegetative stage, the end of flowering stage and again at maturity, one week prior to harvest. The growth rate was estimated at the end of these stages using height differences in each rotation treatment.

Disease was assessed throughout the season when relevant by determining the percentage of the leaf area affected.

Post-harvest measurements

The grain yield of each crop in each crop sequence treatment was determined post-harvest. A sample of mustard seed was taken from each treatment and the oil percentage determined using an Infrared NIR machine on a Canola standard. The canola calibration was available on the NIR and this gave a comparative oil level but not a total oil level.

A 6 kg sample of seed was subsequently cleaned and 5.5kg crushed to determine oil quantity by weight and volume. The levels of free fatty acids were assessed using gas chromatography at the School of Chemical and Biomolecular Engineering, University of Sydney following the protocol of Orsavova et al (2015).

Oil volume was confirmed using weight in grams and volume in milliliters of oil from seed crushed using a laboratory crusher (as above) at University of Sydney Plant Breeding Institute, Narrabri, NSW. This oil was quantified from each treatment in 2014 and 2015. Oil weight was converted from milligrams to millilitres using 1.13mg to 1.0ml as a correction factor to show that the weight of oil confirmed the volume of oil. Biodiesel amount produced per treatment was estimated by multiplying the total oil yield by 0.90. This was subsequently converted to litres of biodiesel/ha.

Wheat carbohydrates were assessed from ten flag leaf samples randomly selected per plot and freeze dried. The wheat samples were ground and weighed ready to extract metabolites. Standard curves for each

metabolite were used to identify metabolite patterns in each sample. Metabolites including amino acids were subsequently assayed on wheat flag-leaf samples from each treatment using liquid chromatography (as above) at the University of Sydney, Centre for Carbon, Water and Food at Cobbitty, NSW and following the protocol of Smith et al (2016). Wheat carbohydrate measurements were taken to examine treatment effects.

Glucosinolate and Sinigrin content of mustard seed was determined using reverse phase-high performance liquid chromatography according to the protocol of Herzallah and Holley (2012). Samples were boiled to prevent degradation by myrosinase prior to analysis. The lowest detection limit for sinigrin was set at 0.05 mg/kg. The analysis was conducted on a fee-for-service basis by Dr Ming Williams on behalf of Australian Agricultural Technologies, Wee Waa, NSW. The yield of sinigrin, a high value component of glucosinolate (Herzallah and Holley 2012), was estimated in mg/100g and converted to kg/ha.

Total biomass to determine harvest index was sampled one week prior to the grain harvest. The biomass cuts from a 0.4m^2 quadrat. Samples were bagged and labelled, total weights obtained. All seed heads were removed, threshed and the grain weighed. Harvest index was then determined as the ratio of grain to total biomass.

Chickpea samples were assessed for nitrogen content using the Kjeldahl method in both years. The nitrogen results were subsequently converted to protein percentage. Protein content of wheat and mustard grain was assessed using a Near Infra-Red (NIR) machine. Mustard protein and oil content were determined using NIR with a canola calibration. There was no mustard calibration for the NIR.

While moisture percentage was determined for wheat and mustard using NIR, the seed of each chickpea treatment was weighed, dried at 40^oC for 48 hours and dry weights subsequently recorded. Test weight was assessed for wheat only. Thousand kernel weights (TKW) were assessed on all mustard, wheat and chickpea treatments using a seed counting machine to count two hundred and fifty seeds. These seeds were weighed and multiplied by four.

Climatic Data

Weather data from the Bureau of Meteorology (BoM) weather station at Narrabri Airport was used to determine the growing conditions each year. There were 2 types of weather data collected, firstly basic weather data consisting of day, date, max/min. temperatures (^oC) and rainfall (mm). More detailed weather

data was also available consisting of wind direction, wind speed (k/h), wind gusts (k/h), dew point (^{o}C), relative humidity (%) and barometric pressure (hPa).

Statistical Analysis

All data was analysed using the REML linear mixed model function of GenStat (Version 16.2, VSN International Ltd, UK) and the significance of variance components were determine through calculation of Wald Statistics. Crops were analysed individually. The previous crop and crop sequence regime were considered a fixed effects and replications within crops as random effects. In 2013, the three crops were sown as unreplicated strips to stabilize the site for experiments in 2014 and 2015 and these data were not included in the analysis. The aim was to compare the impact of the previous crop and the specific crop sequences on crop yield and related characters. Fisher's protected least significant difference (LSD) test at $P \leq 0.05$ was used to examine differences among rotation treatment means. Relationships among traits were computed using the Pearson's simple correlation test (GenStat v 16.2).

Chapter 3: Results

Growing season conditions

The impacts of crop sequence with mustard, wheat and chickpea on each crop are presented in this section by year. The crop sequential effects on agronomic and post-harvest traits were significant for some variables in some treatments, although the season significantly impacted results. The experimental conditions were relatively dry with 156 mm and 246 mm rainfall received during the 2014 and 2015 growing seasons, respectively. The 2014 weather data was summarised in Figure 5. Rainfall was well below the long-term May - November average of 339 mm for Narrabri. Average minimum and maximum temperatures of 7.6/22.7°C and 8.9/23.3 °C were recorded for 2014 and 2015, respectively. These were close to the long-term average of 8.0/22.8ºC.

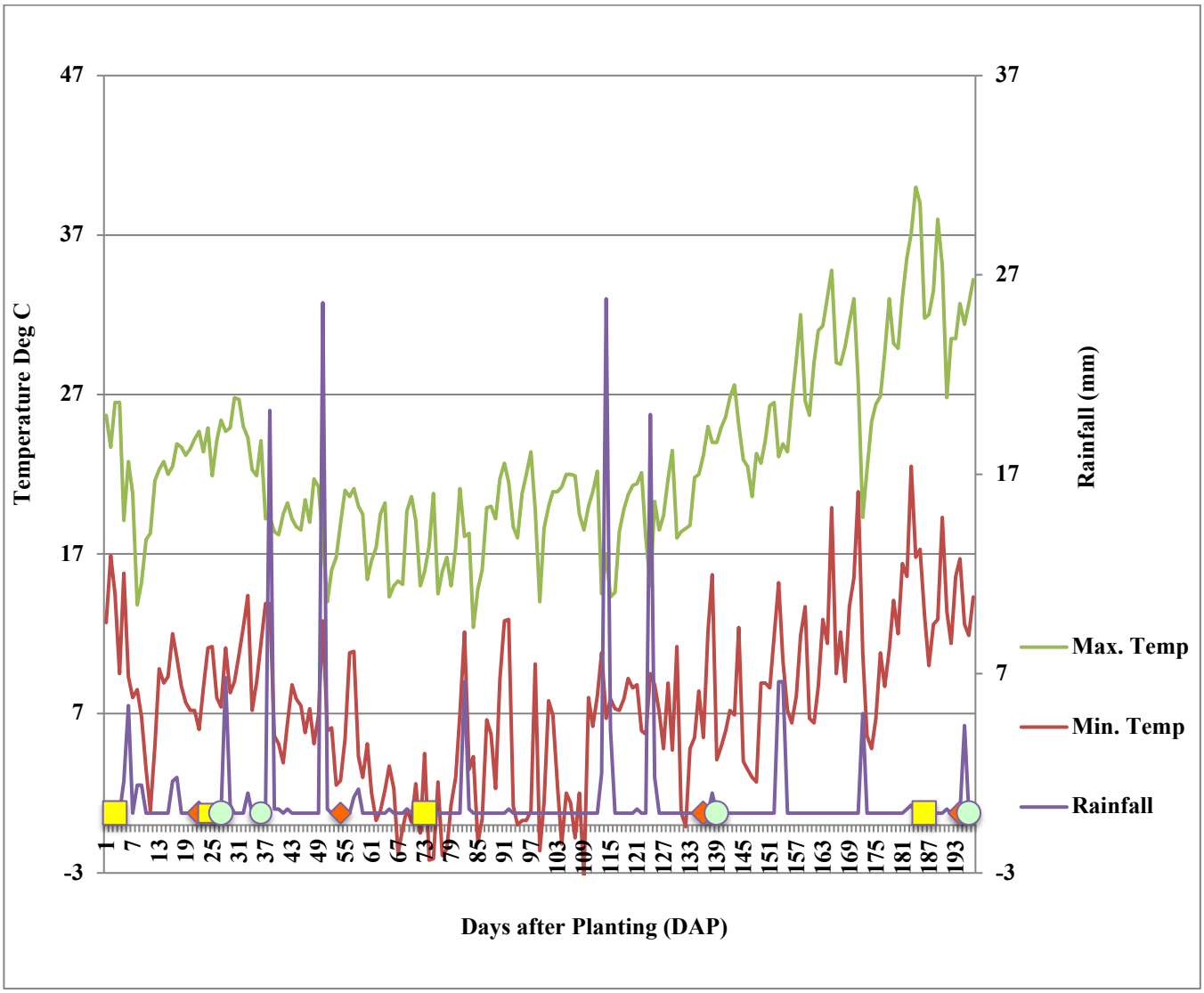


Figure 5: The average maximum and minimum temperatures and rainfall at the BoM weather station, Narrabri Airport; 2014 growing season.
Footnote: Yellow squares represent Indian mustard planting, germination, flowering and harvest in order. Wheat is similarly represented with orange circles and chickpea by light green circles.

The maximum and minimum temperatures and rainfall for the 2015 season are summarised in Figure 6.

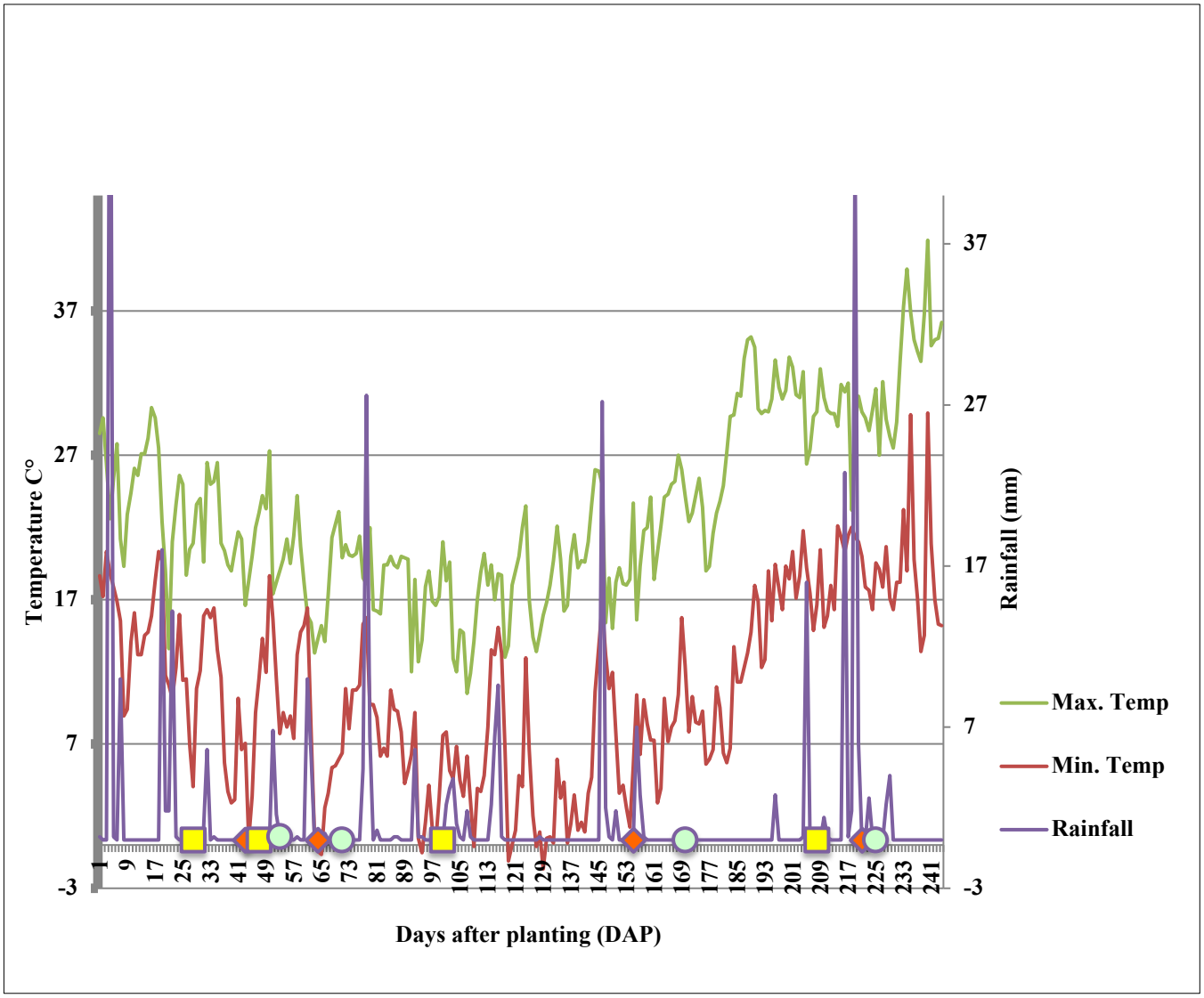
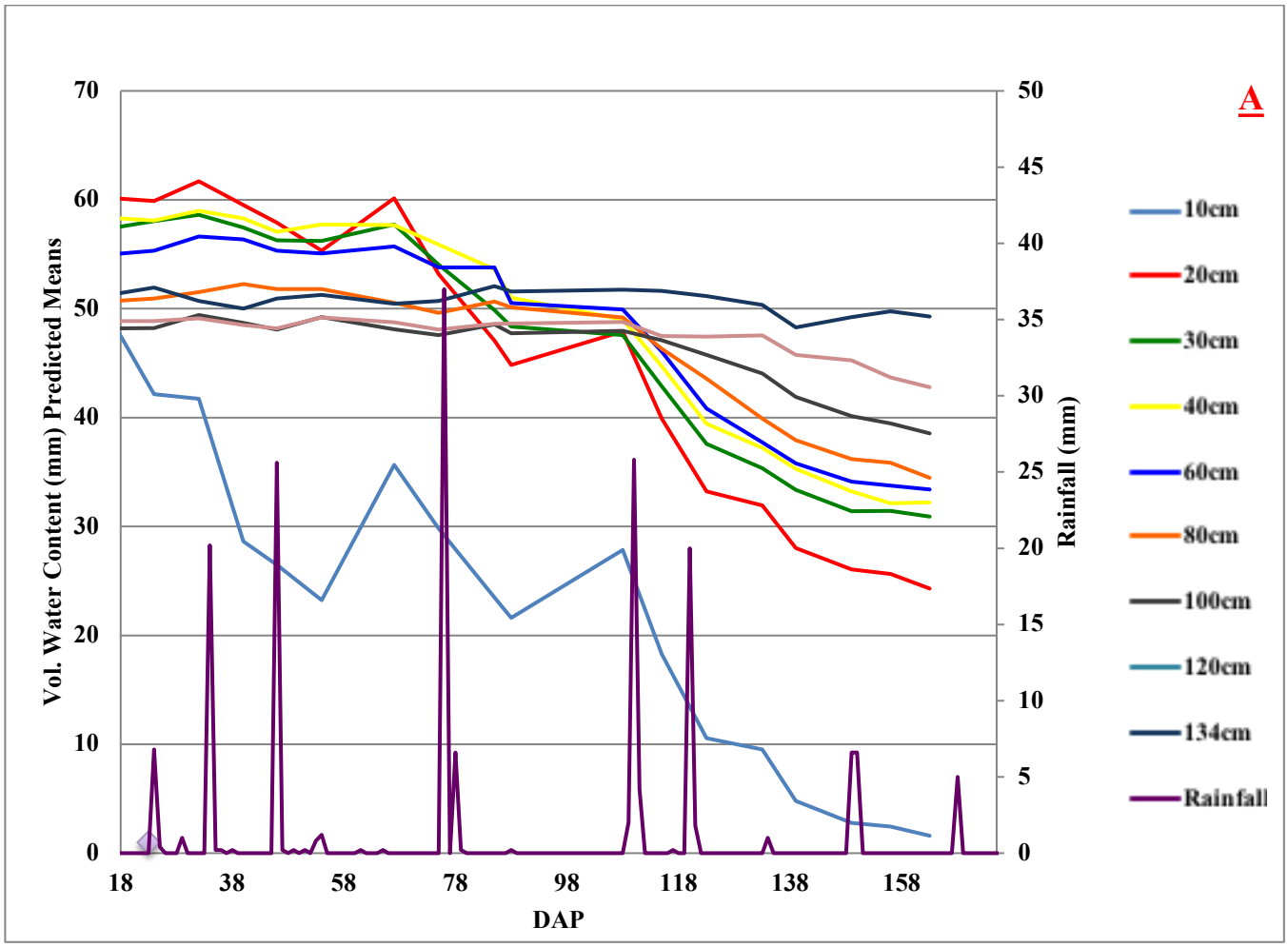
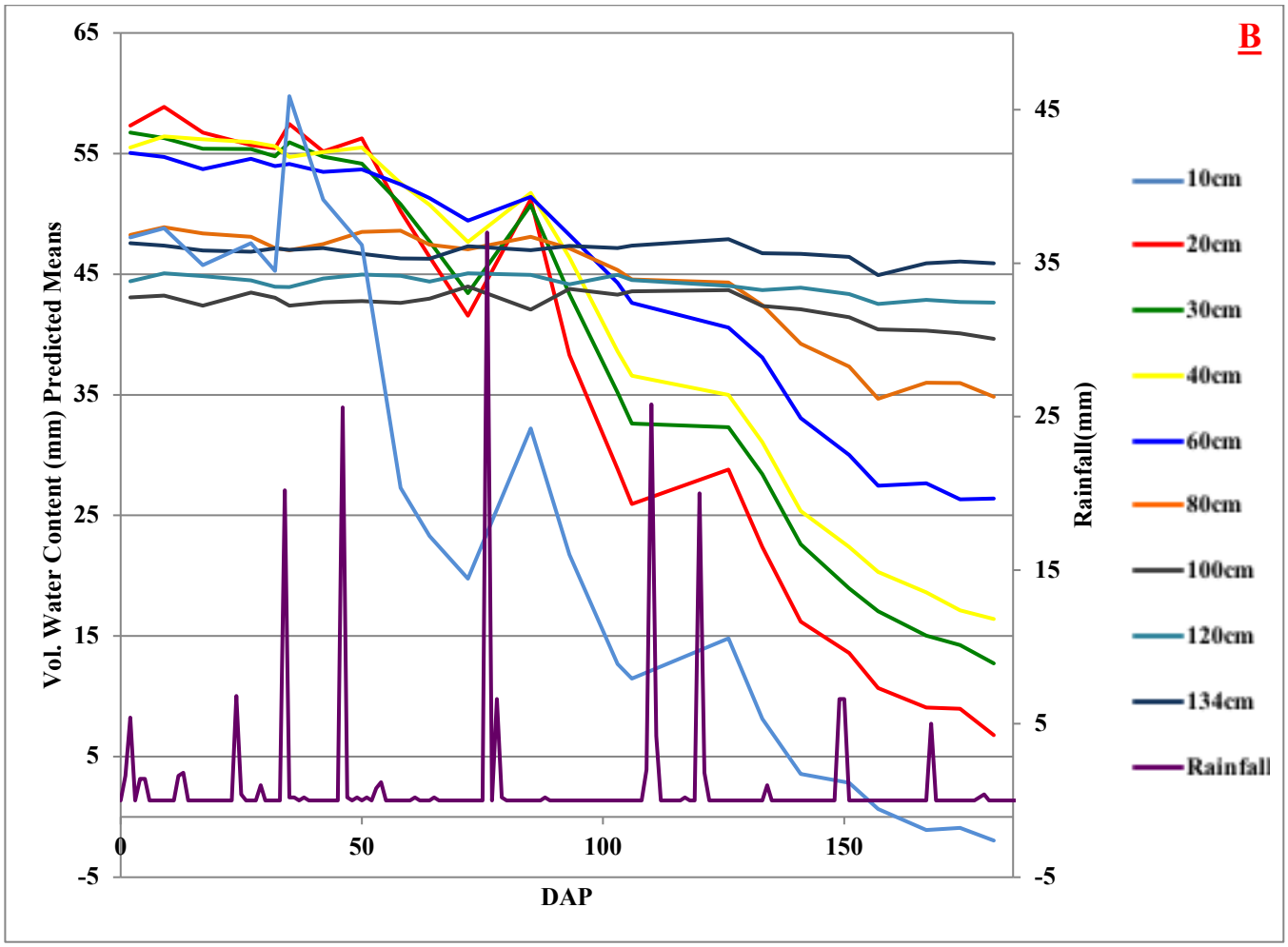


Figure 6: The average maximum and minimum temperatures and rainfall at the BoM weather station, Narrabri Airport; 2015 growing season.
Footnote: Yellow squares represent Indian mustard planting, germination, flowering and harvest in order. Wheat is similarly represented with orange circles and chickpea by light green circles**.**

Variation in growing season soil moisture in 2014 and 2015

Figure 7 A shows the distribution of rainfall over the season, including an irrigation at 76 days after sowing (das) of 37 mm in 2014 that boosted the amount of water received for the season from 156.2 mm to 193.2 mm. This was still drier than the 2015 season, which received 246 mm seasonal rainfall. The water use in the top 10cm layer of soil is greatest compared to the other depths, although plants drew moisture down to 134 cm. Wheat almost exhausted the supply of soil water in the top 10 cm.


A
Vol. Water Content (mm) Predicted Means
Rainfall (mm)
DAP
10cm
20cm
30cm
40cm
60cm
80cm
100cm
120cm
134cm
Rainfall


B
Vol. Water Content (mm) Predicted Means
Rainfall(mm)
DAP
10cm
20cm
30cm
40cm
60cm
80cm
100cm
120cm
134cm
Rainfall

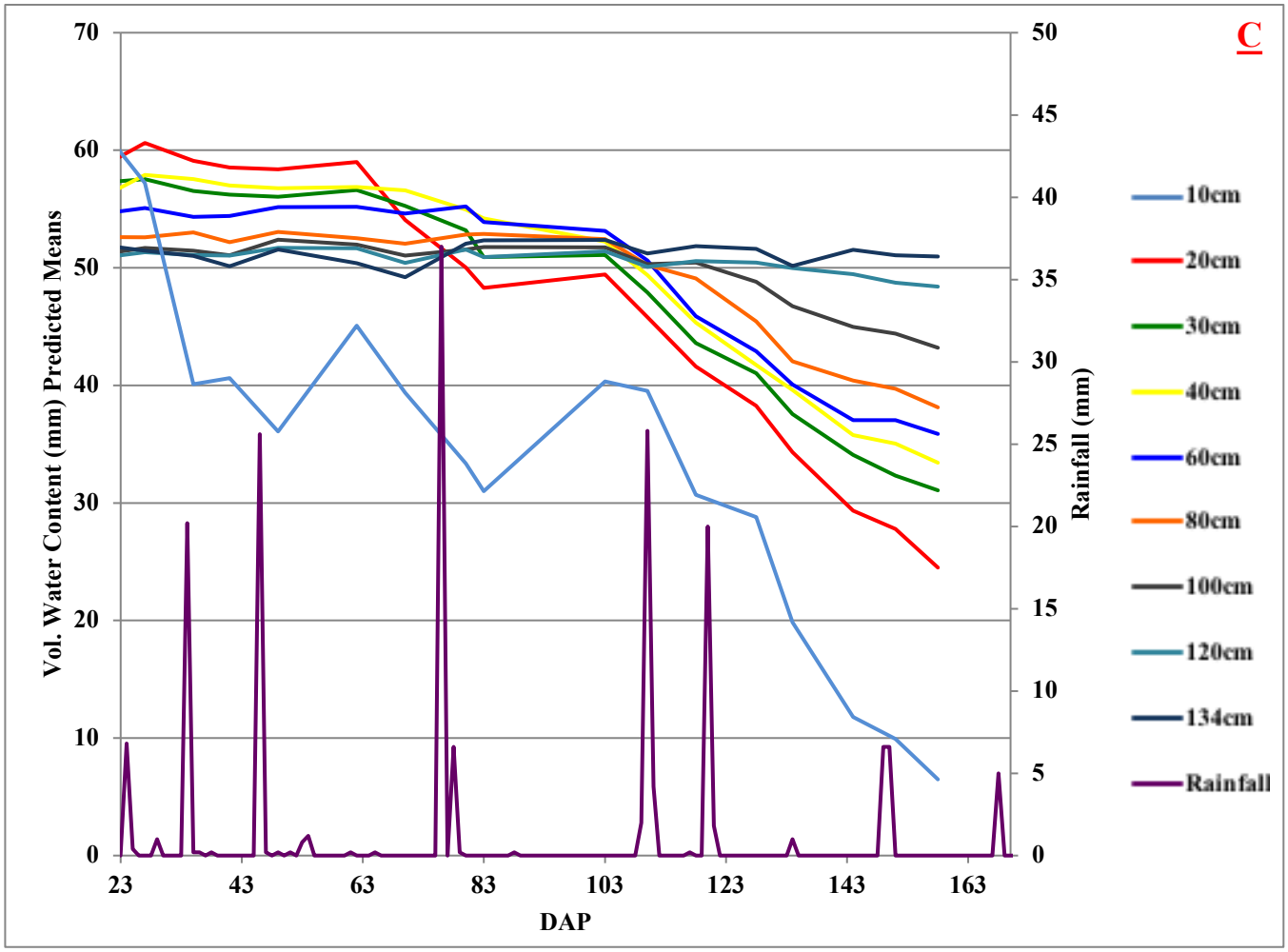
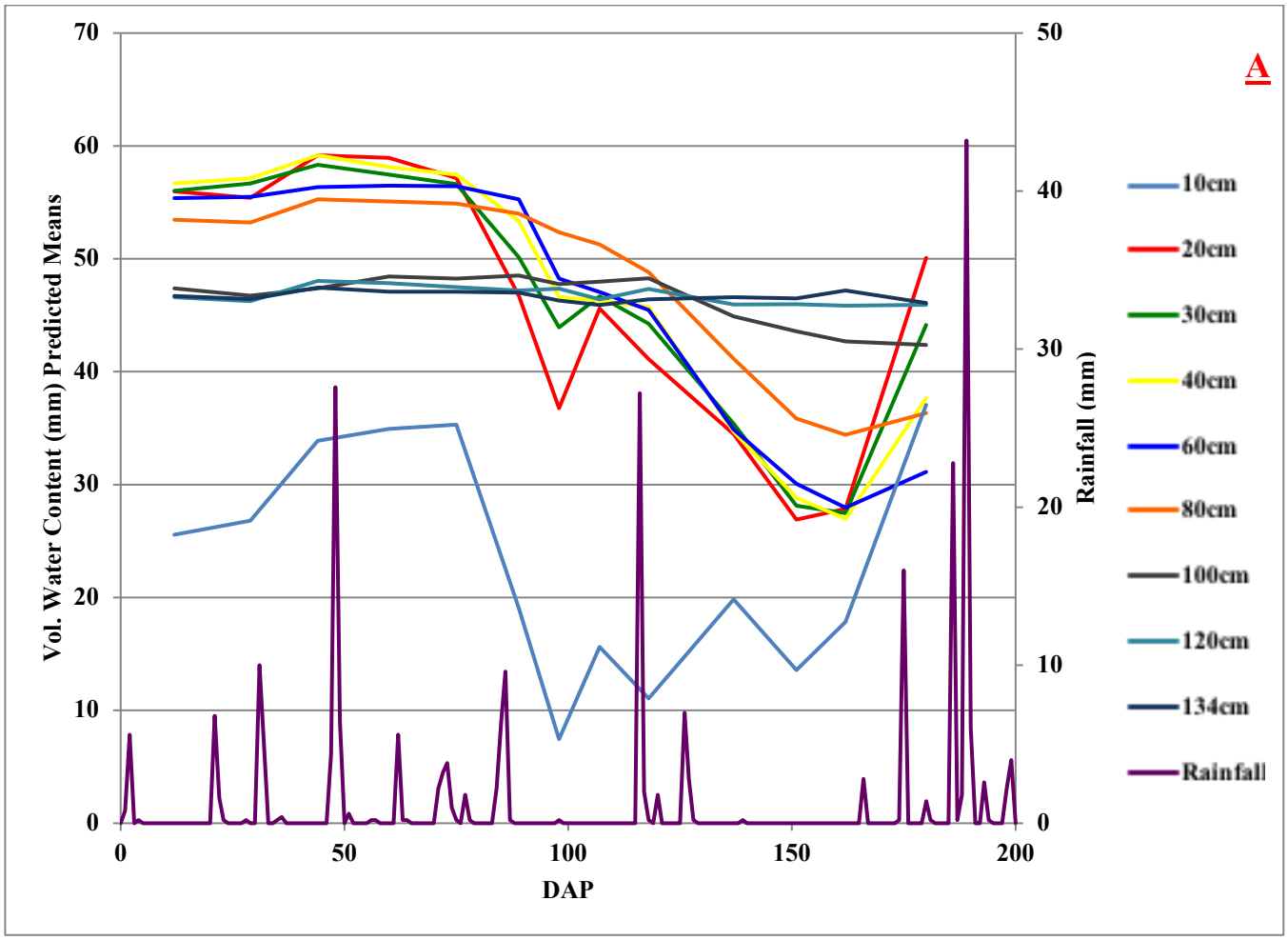
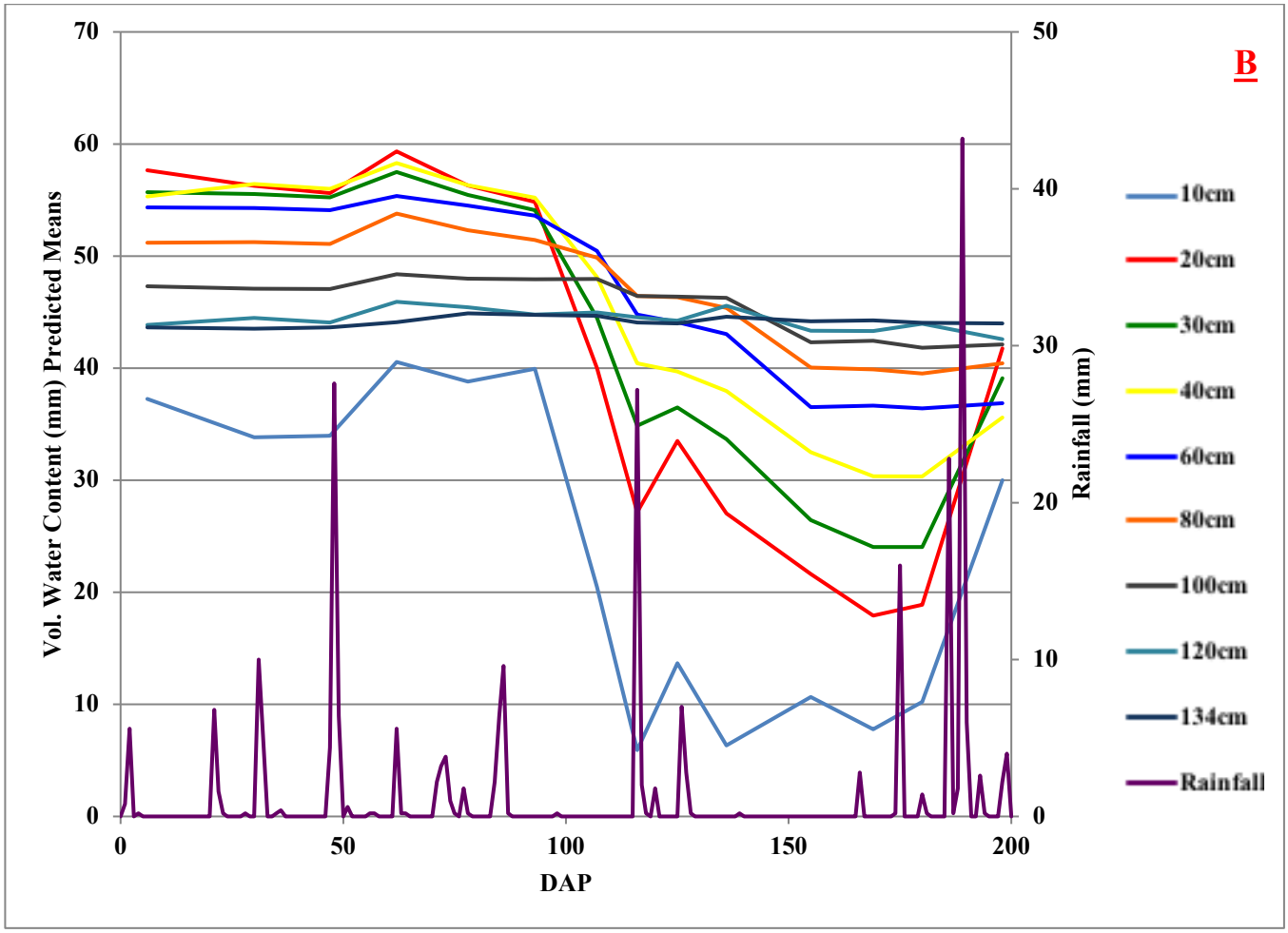


Figure 7: Comparison of soil moisture at various depths overlaid with rainfall and irrigation (mm) at day76 das mustard for (A) Wheat, (B) Mustard and (C) Chickpeas at Narrabri in 2014.

The water use pattern of mustard was different to wheat (Figure 7 B). Mustard exhausted the 10cm layer and drew more water from the lower layers to compensate, although 2014 was relatively dry due in part to less in season rainfall and to a high sowing rate of 4 kg/ha. Measurements in the top 10cm have more error because of evaporation and soil cracking due to evaporation. The neutron probe can have it's readings effected by air gaps in the cracking soil. This resulted in increased competition for moisture, light and nutrients. The water use of chickpea in the 10cm layer was greater compared to the other depths (Figure 7 C). However, chickpea used less water in the top 10cm layer than both wheat and mustard. It also used less moisture at depth than the other two species.

In 2015, an additional 52 mm of seasonal rainfall was recorded compared to 2014 (Figures 8 A, B and C). The experimental site received sufficient rainfall to negate the need for irrigation and water deficit was less than 2014. The greatest water up-take by all crops was in the top 10 cm of soil. Water up-take by the wheat treatments extended to 100 cm in this year.


A
Vol. Water Content (mm) Predicted Means
Rainfall (mm)
DAP
0
10
20
30
40
50
60
70
0
50
100
150
200
10cm
20cm
30cm
40cm
60cm
80cm
100cm
120cm
134cm
Rainfall


B
Vol. Water Content (mm) Predicted Means
Rainfall (mm)
DAP
0
10
20
30
40
50
60
70
0
50
100
150
200
10cm
20cm
30cm
40cm
60cm
80cm
100cm
120cm
134cm
Rainfall

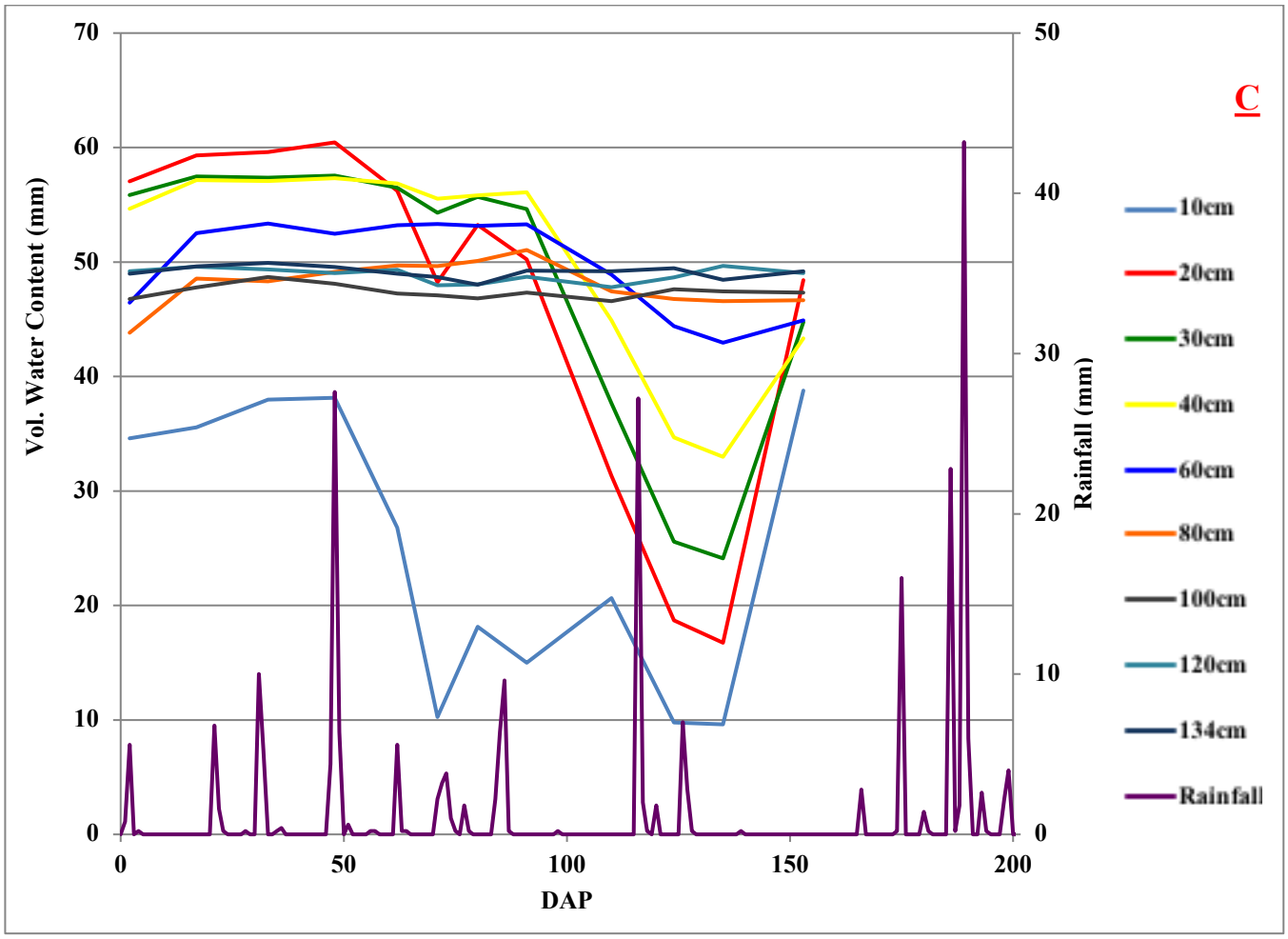


Figure 8: Comparison of soil moisture at various depths overlaid with rainfall (mm) for (A) wheat, (B) mustard and (C) chickpeas at Narrabri in 2015. Chickpea crop contracted stunt virus and died early; prior to harvest. Hence the gap between late season rains and crop harvest.

Mustard water up-take extended to 134 cm depth, although the greatest uptake remained in the top 10cm soil layer. The lower sowing rate in 2015 reduced competition for moisture, light and nutrients leading to reduced up-take from depth compared to 2014, although no irrigation was applied due to increased in-crop rainfall. Water up-take in the chickpea treatments extended to 80 cm in 2015 with more dramatic water-use in the top 10 cm layer.

Wheat extracted down the soil profile to 134 cm in both years and extracted less water than mustard but more water than chickpeas.

In both 2014 and 2015, neither soil profiles are full at the start. However, even after irrigation in 2014 the total soil water extracted was greater than in 2015. In 2015 the crop order was the same but the amount of water extracted by the three crops was lower.

Effects of crop sequences in 2014

Wheat response

1. *Impact of the previous crop*

The impact of the previous crop on wheat yield in 2014 was non-significant for yield and plant height (Table 2). However, days to heading, grain protein and thousand kernel weight were influenced by the previous crop. Wheat following wheat tended to be earlier heading with lower grain protein and kernel weight. In contrast, grain protein was higher following mustard and kernel weights were superior after chickpea.

Table 2. The impact of the previous crop on wheat traits in 2014

Previous crop	Yield kg/ha	Days to heading	% grain protein	Plant height (cm)	TKW
Wheat	3389 a	117.7 bc	13.4 a	101.9 a	29.8 a
Mustard	3243 a	118.2 ac	14.3 b	101.1 a	28.3 a
Chickpea	3709 a	118.5 a	13.5 a	100.6 a	32.2 b

Note: different letters in columns indicate significance at $P<0.05$. TKW is thousand kernel weight

2. *Impact of the crop sequence*

Significant impacts of crop sequence on some post-harvest traits such as yield, thousand kernel weights and grain protein were observed for wheat (Figures 9 A, B, C). However, no significant response to rotation was evident for flowering time, plant height, and biomass at maturity and NDVI assessments at different stages of development.

The Wald statistics calculated from the linear mixed model analysis showed that none of the traits assessed in season were significant. While no mean differences in growing season traits were observed, there were significant differences among post-harvest traits (Figures 9 A, B, C). The crop sequence that most impacted yield and thousand kernel weight for wheat was WCW, indicating that wheat following chickpea improved both productivity and grain size, while protein content was significantly higher in the WMW sequence. High grain protein was inversely proportional to yield, as expected. Yield was not significantly different among treatments although there were trends to higher yield in WCW compared to WWW (Appendix Table 15; Figure 9 B).

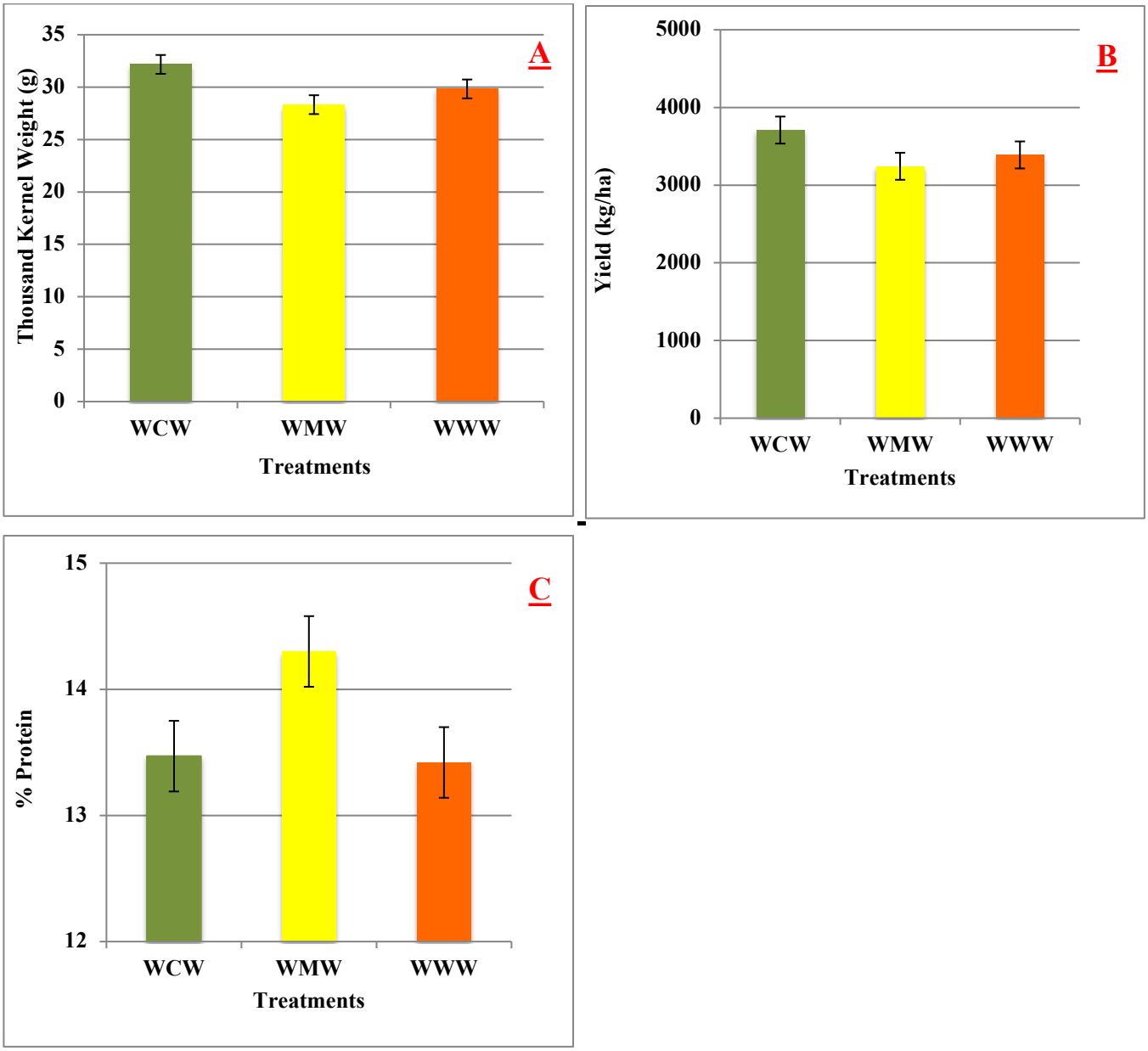


Figure 9: The response of wheat in six sequential treatments of (W) wheat, (C) chickpea and (M) mustard for (A) thousand kernel weight, (B) yield and (C) grain protein at Narrabri in 2014 with standard error bars.

Mustard response

1. *Impact of the previous crop*

   The previous crop had little effect on mustard yield, biomass, kernel weight or grain oil percentage in 2014 (Table 3). However, grain protein tended to be higher following chickpea and plants were considerably taller after wheat.

Table 3. The impact of the previous crop on mustard traits in 2014

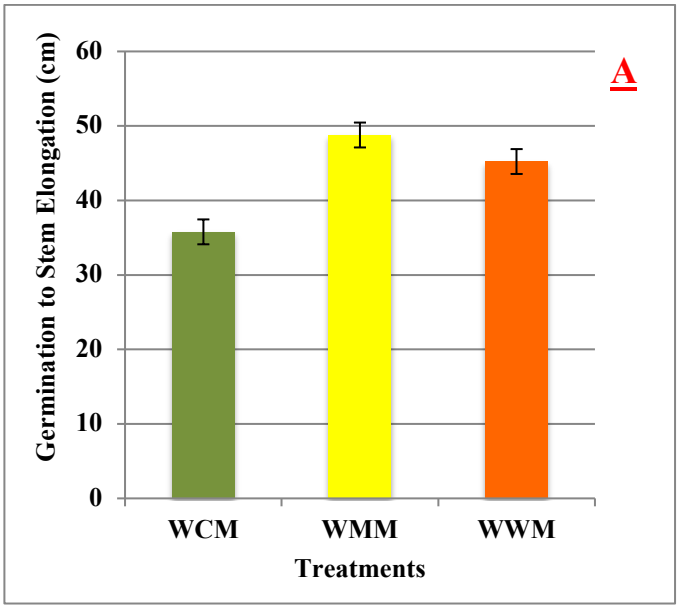
Previous crop	Yield kg/ha	TKW (g)	Grain oil %	Grain protein %	Biomass at anthesis kg/ha	Plant height (cm)
Wheat	555 a	3.67 a	35.7 a	23.6 b	6494 a	195.6 a
Mustard	472 a	3.83 a	35.7 a	23.6 b	7544 a	184.7 b
Chickpea	543 a	3.67 a	35.5 a	24.0 a	6892 a	182.9 b

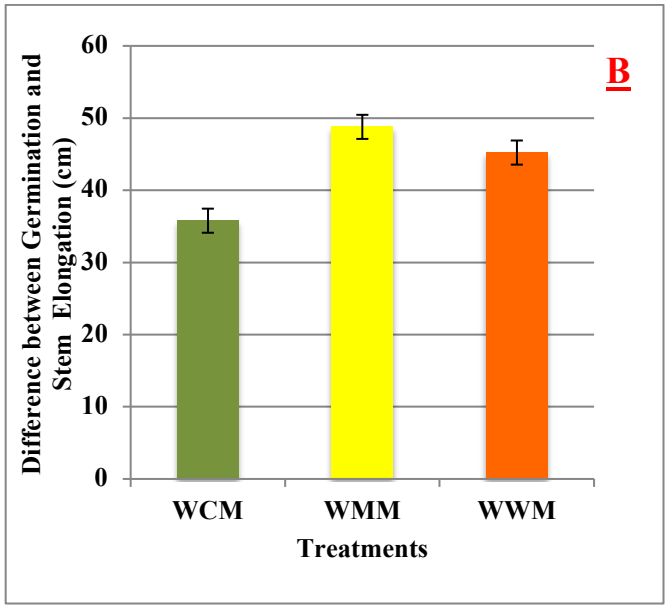
Note: different letters in columns indicate significance at P<0.05. TKW is thousand kernel weight

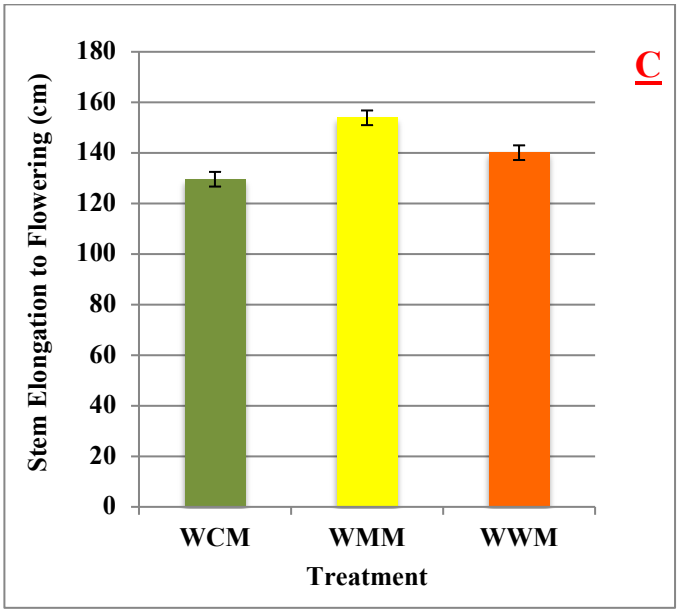
2. *Impact of the crop sequence*

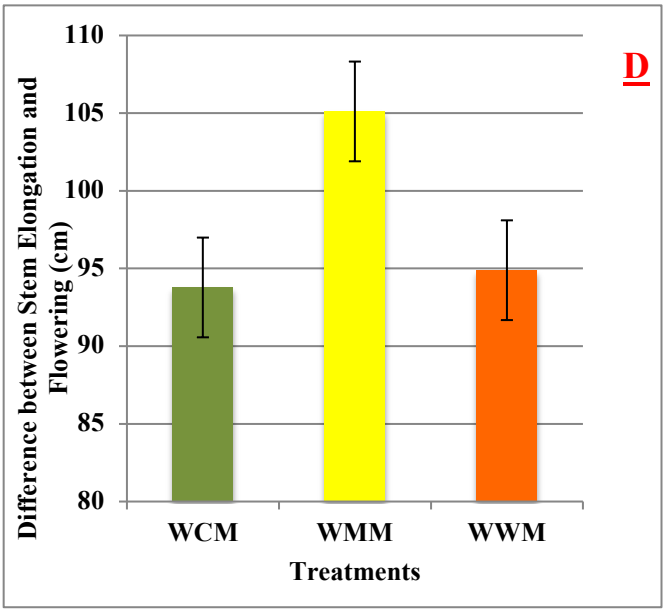
The 2014 mustard crop sequence was adversely affected by powdery mildew from the initiation of pod-fill onwards. However, differences among crop sequences were observed pre-anthesis and the WMM rotation was superior for growth rate between germination and flowering. These growth rates reduced between flowering and maturity for this crop sequence.

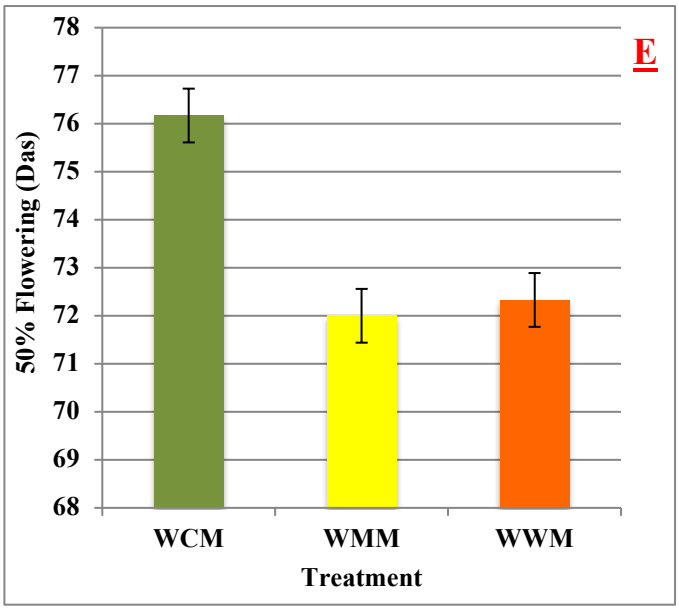
A number of the growing season traits were significantly impacted by crop sequence with the exception of germination, establishment and NDVI. Grain protein was significantly impacted by crop sequence with WCM producing slightly higher values. Crop sequence did not significantly impact yield. However, there is an impact on biodiesel production with WCM producing slightly more biodiesel than WWM and significantly more than WMM (Figure 10 J; Appendix Table 16). This trend in biodiesel production was reinforced in 2015 with the WCCM crop sequence producing more biodiesel (Appendix Table 14; Figure 15 L).

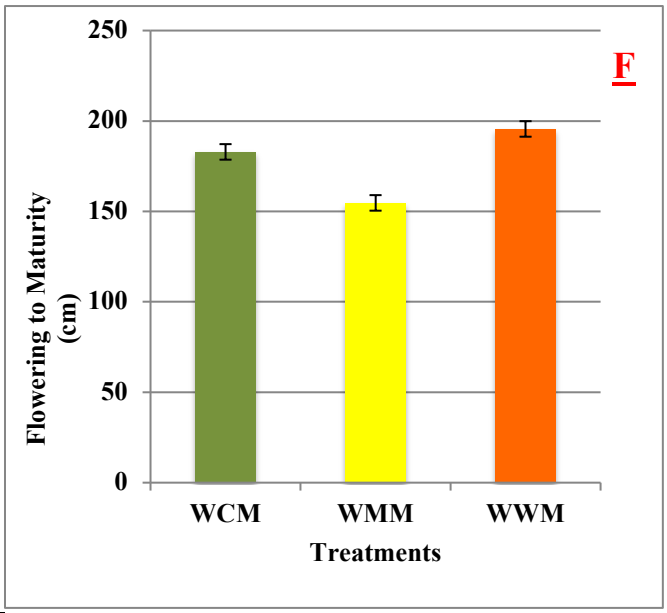


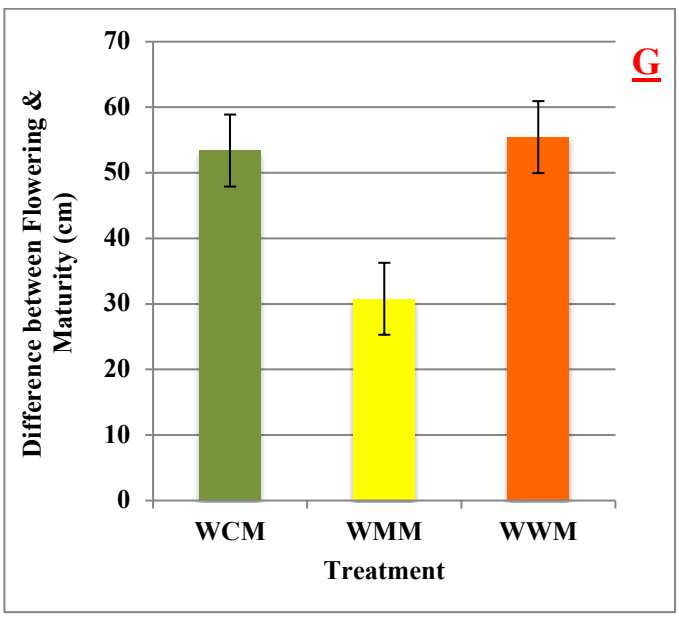


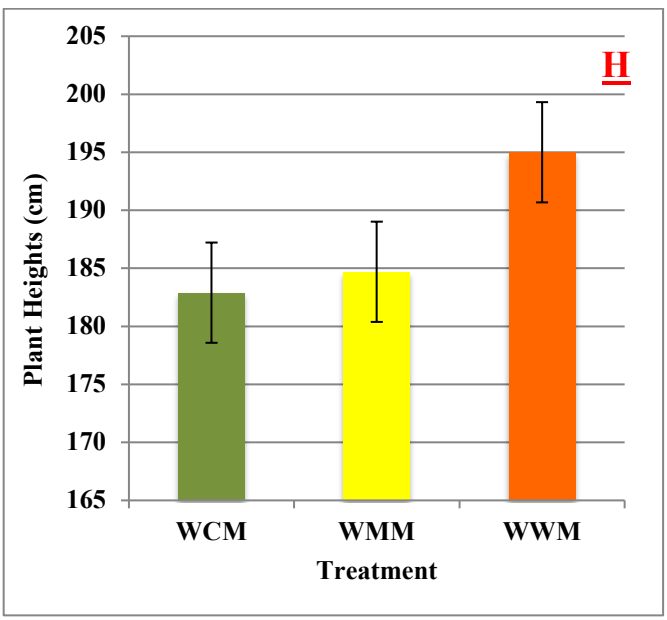

C
Stem Elongation to Flowering (cm)
180
160
140
120
100
80
60
40
20
0
WCM
WMM
WWM
Treatment


D
Difference between Stem Elongation and Flowering (cm)
110
105
100
95
90
85
80
WCM
WMM
WWM
Treatments


E
50% Flowering (Das)
78
77
76
75
74
73
72
71
70
69
68
WCM
WMM
WWM
Treatment


F
Flowering to Maturity (cm)
250
200
150
100
50
0
WCM
WMM
WWM
Treatments


G
Difference between Flowering & Maturity (cm)
70
60
50
40
30
20
10
0
WCM
WMM
WWM
Treatment


H
Plant Heights (cm)
205
200
195
190
185
180
175
170
165
WCM
WMM
WWM
Treatment

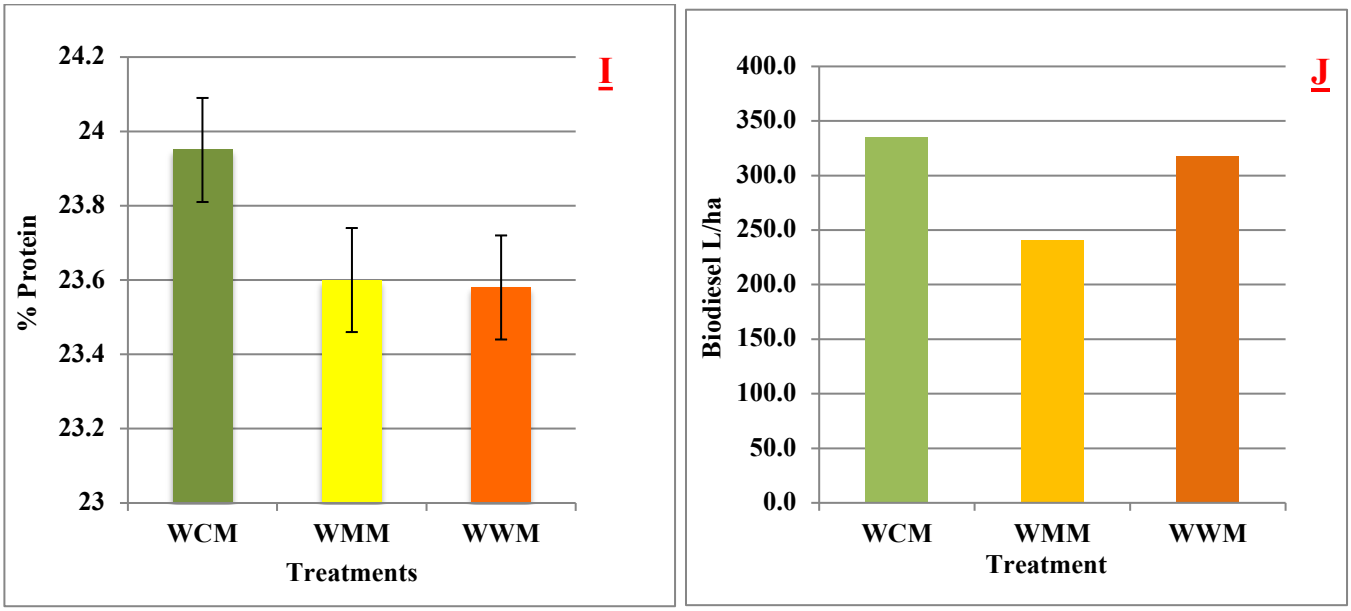


Figure 10: The response of mustard in six sequential treatments of (W) wheat, (C) chickpea and (M) mustard for (A) plant heights from germination to stem elongation, (B) plant height differences between germination and stem elongation, (C) plant heights from stem elongation to flowering, (D) plant height differences between stem elongation and flowering, (E) days to 50% flowering, (F) days flowering to maturity, (G) plant height differences between flowering and maturity, (H) plant height, (I) % protein and (J) biodiesel yield at Narrabri in 2014 with standard error bars.

Chickpea response

1. *Impact of the previous crop*

The previous crop had little impact on chickpea yield, kernel weight, plant height and flowering time (Table 4). However, grain protein was significantly higher when chickpea followed chickpea in rotation.

Table 4. The impact of the previous crop on chickpea traits in 2014

Previous crop	Yield kg/ha	TKW (g)	Plant height (cm)	Days to first flower	Grain protein %
Wheat	2014 a	218 a	71.8 a	112.0 a	21.5 b
Mustard	1969 a	215 a	68.0 a	111.5 a	22.4 ab
Chickpea	2062 a	214 a	66.3 a	111.0 a	23.7 a

Note: different letters in columns indicate significance at P<0.05. TKW is thousand kernel weight

2. *Impact of the crop sequence*

The chickpea crop sequences of 2014 were the highest yielding of the two years assessed. Grain protein was the only trait that was significantly affected. Protein percentage was significantly higher in crop sequence WCC compared to chickpea following either wheat or mustard (Figure 11 A) and plant height was reduced (Figure 11 B).

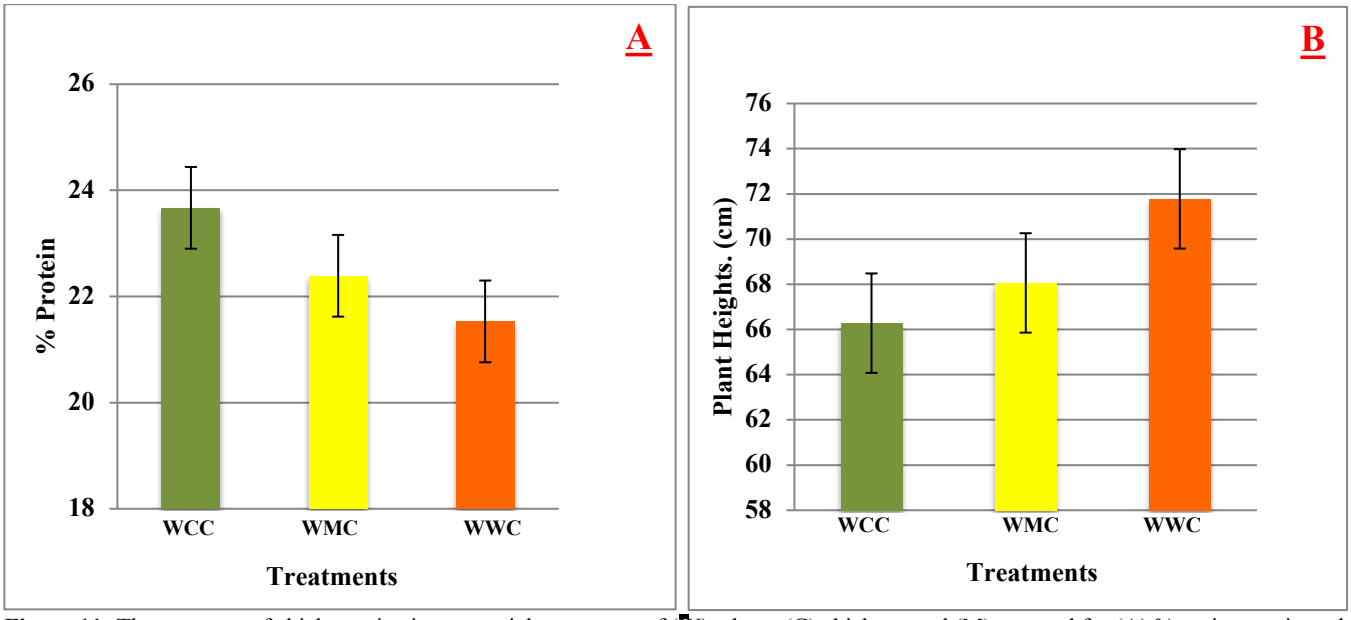


Figure 11: The response of chickpeas in six sequential treatments of (W) wheat, (C) chickpea and (M) mustard for (A) % grain protein and (B) plant height at Narrabri in 2014 with standard error bars.

Effects of crop sequence in 2015

The aim of this experiment was to examine the impact of the entire crop sequence on crop growth and quality. As such the impact of the previous crop in 2015 was limited to just two comparisons for each of wheat, mustard and chickpea. However, significant impacts of the crop sequences on a combination of growing season and post-harvest traits were observed in 2015 for all three crops (Appendix Table 18).

Wheat response

1. *Impact of the previous crop*

There was no significant impact of the previous crop on wheat yield, maturity date and biomass at harvest in 2015 (Table 5). However, days to heading were shorter and grain protein higher following mustard. The harvest index was higher when wheat was sown after wheat.

Table 5. The impact of the previous crop on wheat traits in 2015

Previous crop	Yield kg/ha	Days to heading	Days to maturity	Grain protein %	Biomass at harvest kg/ha	Harvest index
Wheat	3738 a	120.7 a	156.6 a	12.4 a	25989 a	0.34 a
Mustard	3651 a	118.6 b	155.8 a	13.6 b	27438 a	0.31 b

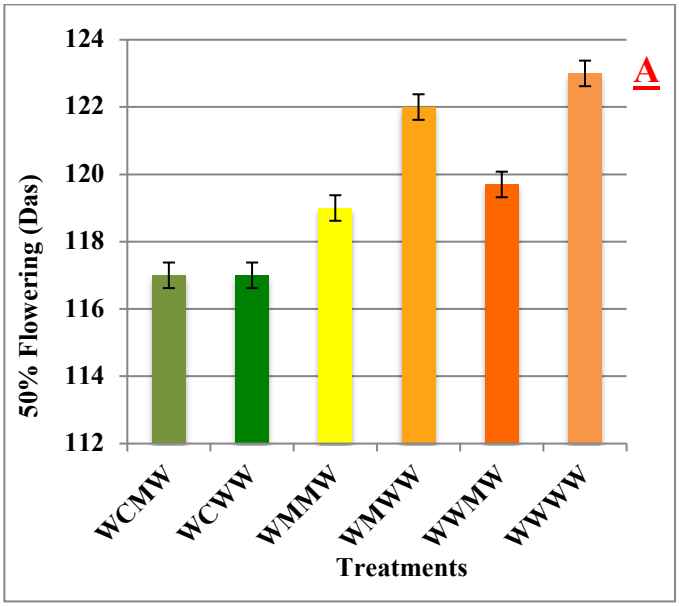
Note: different letters in columns indicate significance at P<0.05. TKW is thousand kernel weight

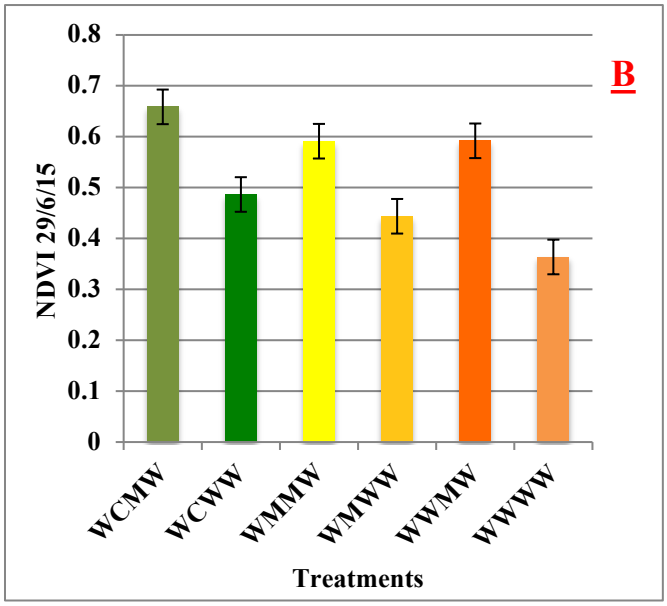
2. *Impact of the crop sequence*

Six crop sequences were established in 2015 and no significant wheat response to crop sequence was observed for germination, establishment and plant height. However, all other traits were significantly impacted by crop sequence including grain yield, NDVI, HI, grain protein % and test weight (Appendix Table 13; Figures 12 A-H; Figures 12 A-E). The crop sequence that most improved grain yield and thousand kernel weight was WMWW. While wheat following mustard improved both productivity and grain size, protein content was significantly higher in WCMW indicating the importance of chickpea in crop sequence. High grain protein was inversely proportional to yield as expected.

The trait means for each crop sequence treatment are given in Appendix Table 7. The crop sequence WCMW and WCWW had the shortest number of days to flowering and these were 6 days earlier than the WWWW baseline treatment (Figure 12 A). WCMW also had the highest NDVI readings (Figure 12 B-F), indicating a high level of greenness, possibly due to higher soil nitrogen content fixed by the chickpeas and mineralised by the mustard preceding the wheat.

The crop sequences WCMW, WCWW, WMMW and WWMW were early flowering and no clear effect of earliness on wheat yield or yield components was observed (Figure 12 A-F). However, the very early flowering WCMW and WCWW sequences did have a higher photosynthetic rate compared to other sequences (Figure 12 G).





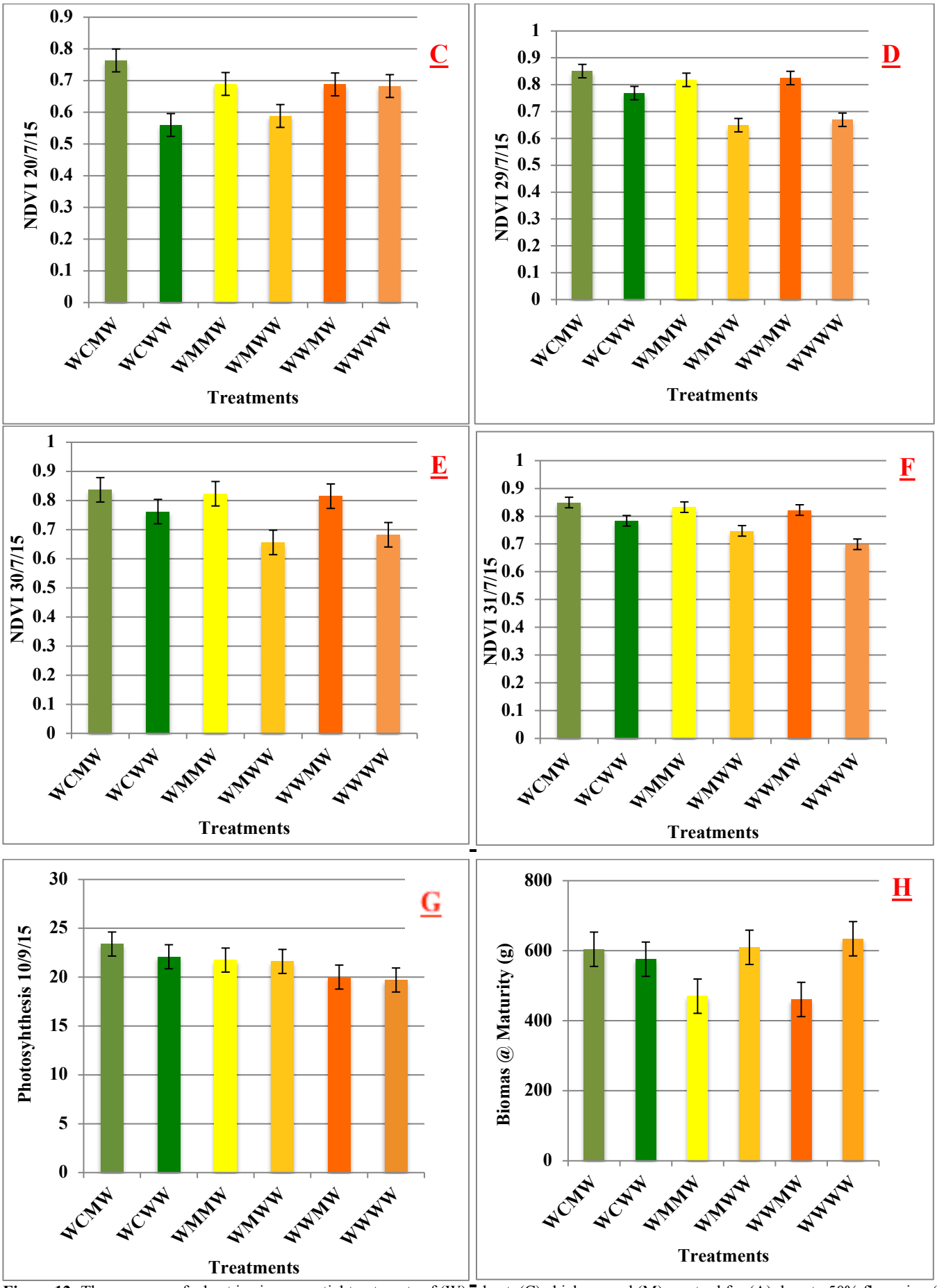
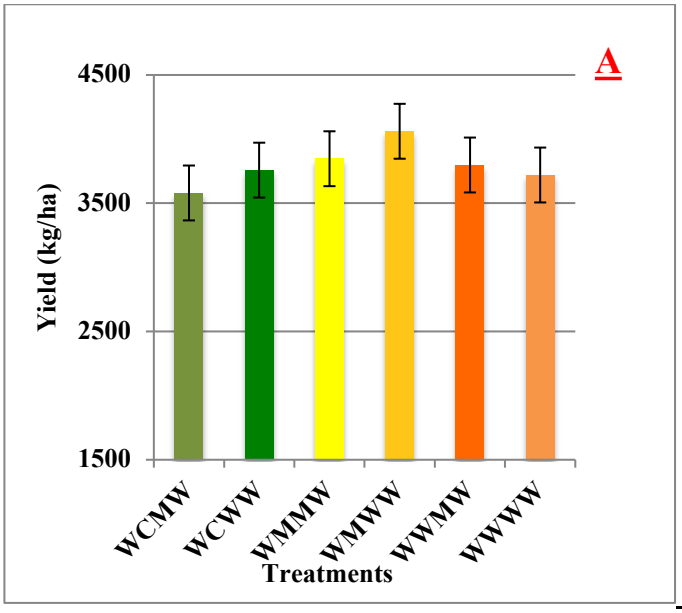
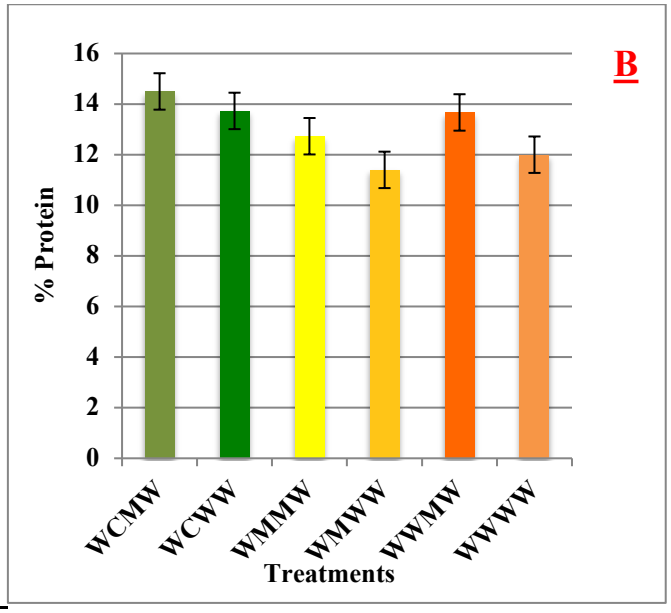
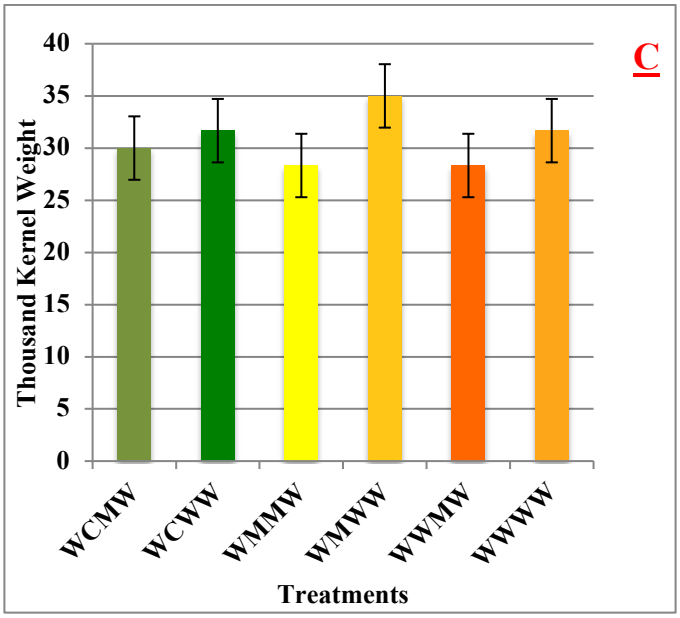


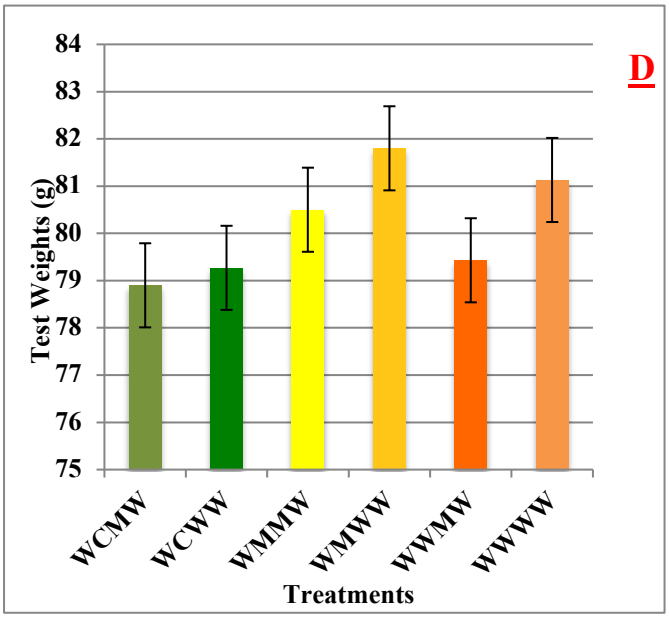
Figure 12: The response of wheat in six sequential treatments of (W) wheat, (C) chickpea and (M) mustard for (A) days to 50% flowering (B), NDVI 29/6/15, (C) NDVI 20/7/15, (D) NDVI 29/7/15, (E) NDVI 30/7/15, (F) NDVI 31/7/15, (G) photosynthesis 10/9/15, and (H) biomass at maturity at Narrabri in 2015 with standard error bars.

The differences among sequential treatments were evident in growing season and post-harvest traits. The grain Proline content in WCMW was significantly higher than other crop sequences and reflects the higher grain protein and lower grain yield in this treatment (Figure 13F).









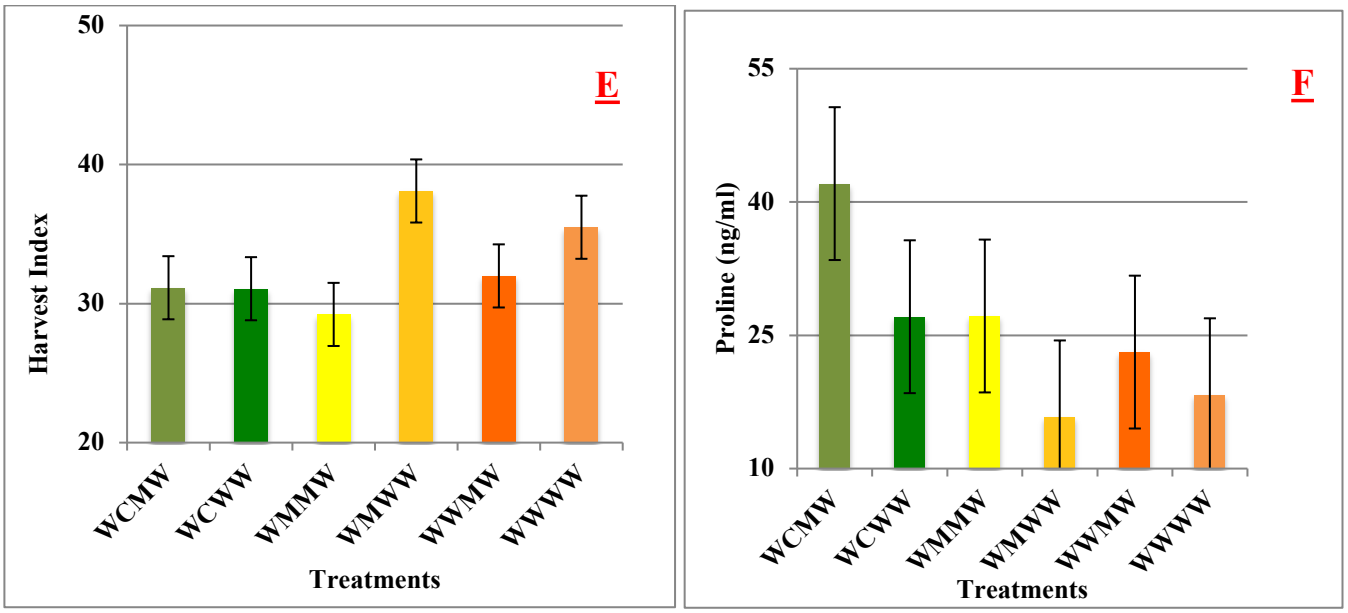


Figure 13: The response of wheat in six sequential treatments of (W) wheat, (C) chickpea and (M) mustard for (A) yield, (B) grain protein, (C) thousand kernel weight, (D) test weight, (E) harvest index, and (F) proline levels at Narrabri in 2015 with standard error bars.

Mustard response

1. *Impact of the previous crop*

The previous crop did not impact mustard yield, flowering time and fatty acid content including oleic acid in 2015 (Table 6). However, mustard sown after chickpea did mature earlier and had higher grain oil percentage (extracted using a press). Interestingly, grain protein was higher following mustard compared to chickpea in the previous year.

Table 6. The impact of the previous crop on mustard traits in 2015

Previous crop	Yield kg/ha	Days to flowering	Days to maturity	Grain protein %	Grain oil % (extracted)	Oleic acid
Mustard	1378 a	87.9 a	171.1 a	25.5 a	14.9 a	0.692 a
Chickpea	1834 a	88.0 a	168.2 b	23.6 b	19.8 b	0.694 a

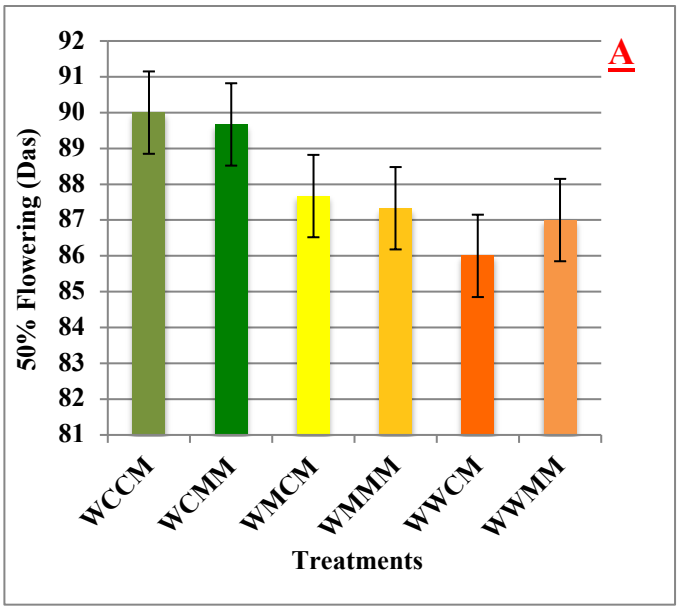
Note: different letters in columns indicate significance at P<0.05.

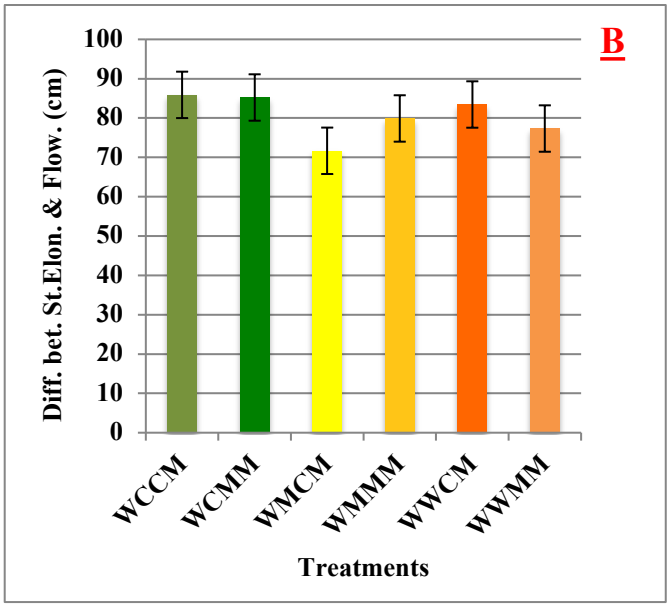
2. *Impact of the crop sequence*

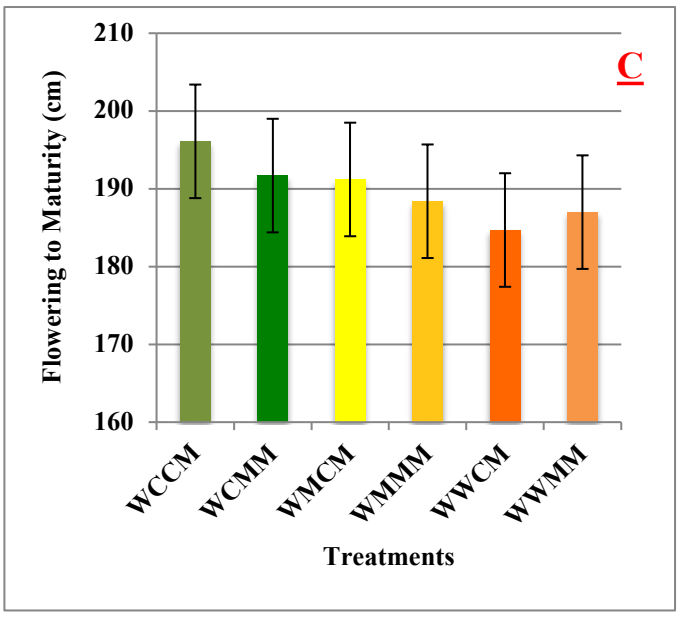
Plant development in WCCM was slow compared to other treatments and the plant height greater during early development as was eventual grain yield (Appendix Table 8). In contrast, WWCM flowered and matured 4 days earlier but produced lower grain yield and protein and slightly higher oil %. Mustard after

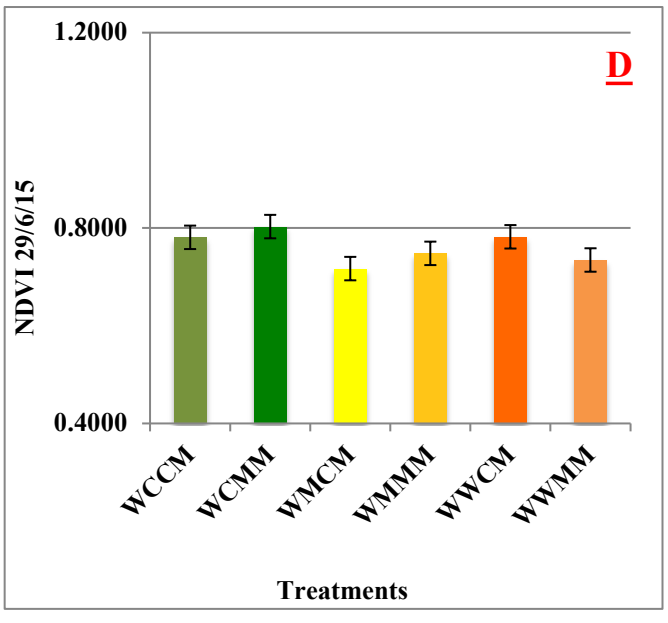
mustard was a poor rotation with WWMM, WMMM and WCMM producing the lowest yield.

Nevertheless, oil yield was highest in the continuous three-year mustard WMMM and WMCM (Appendix Table 14; Figure 14 E). However, WCCM and WWCM produced the highest number of litres of biodiesel per ha and these two crop sequences produced the highest mustard yield (Appendix Table 14; Figure 14 B and K). However, if sinigrin is the intended production target then the WCMM and WWMM crop sequences were superior based on higher glucosinolate concentrations (Appendix Table 14; Figure 14 I). As in the case of biodiesel, WCCM and WWCM produced the highest amount of sinigrin in kg/ha and these two crop sequences produced the highest mustard yield (Appendix Table 14; Figure 14 B and J). Oleic acid is the highest value fatty acid and this was unaffected by crop sequence.









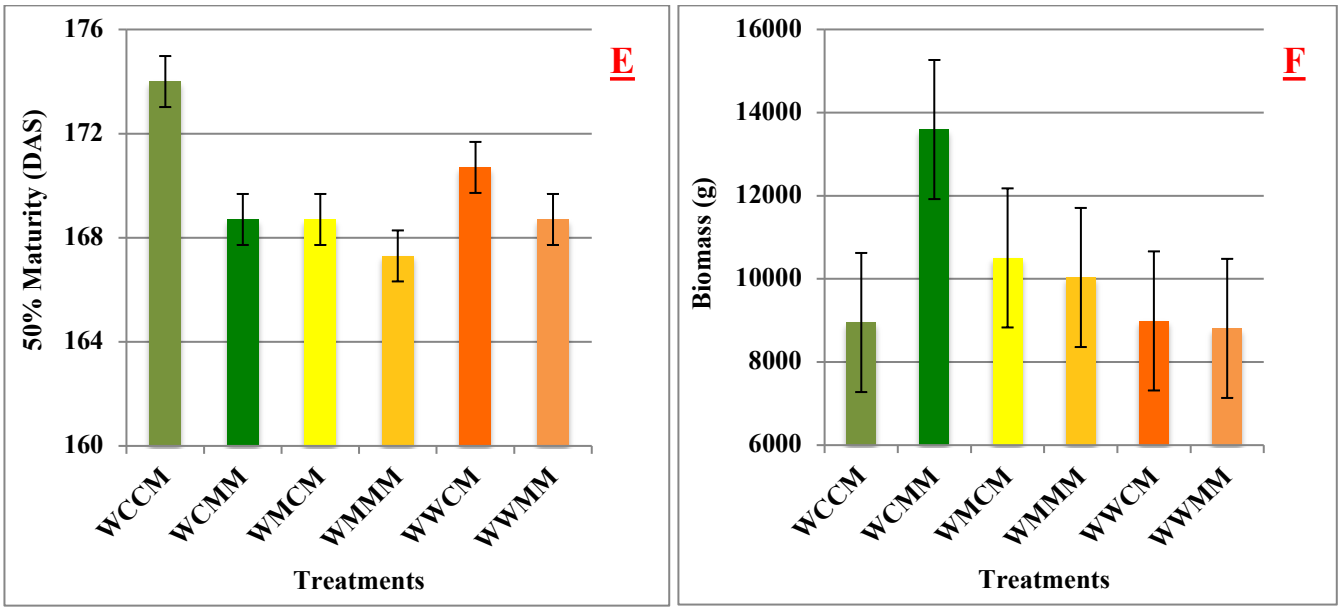


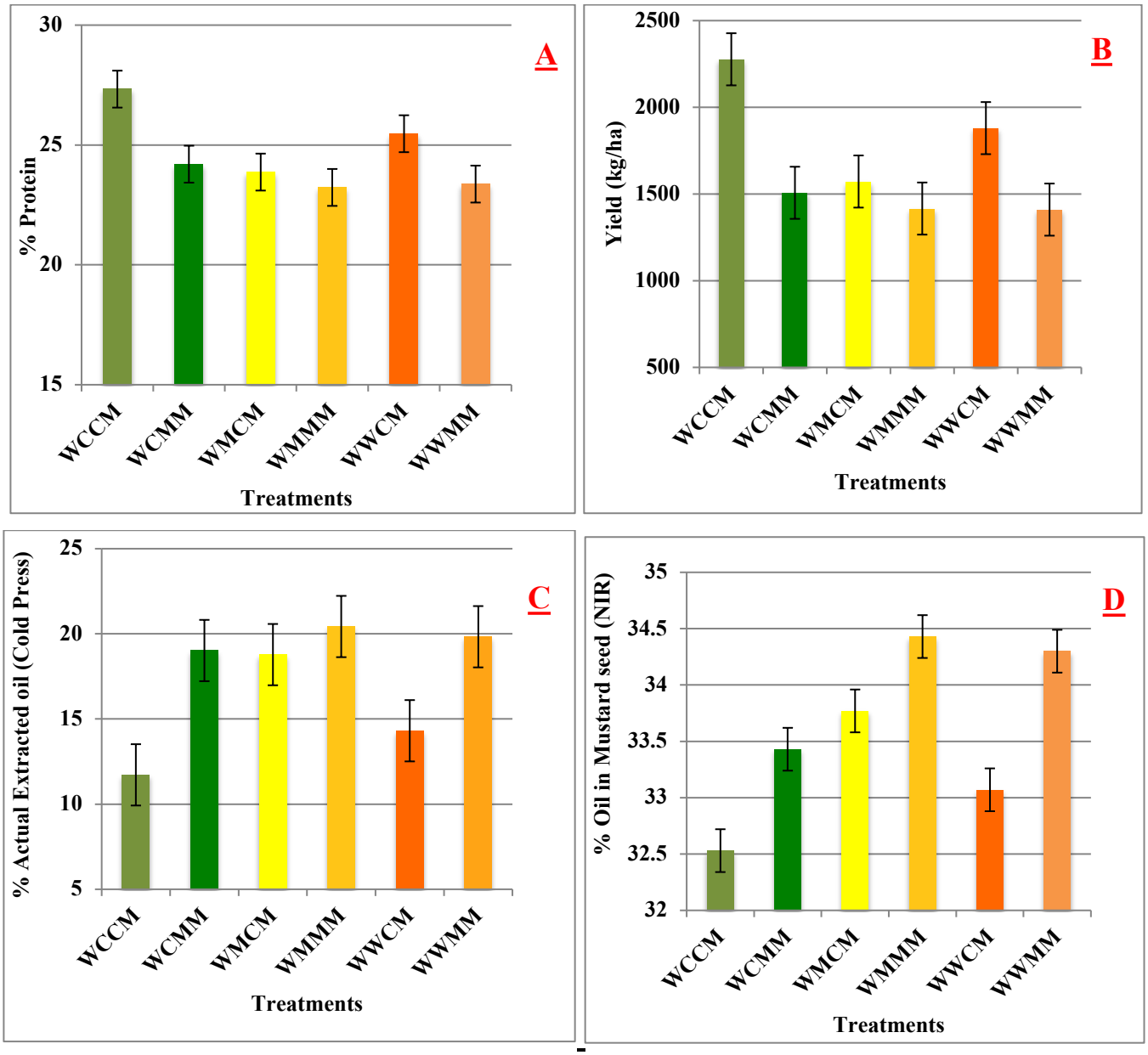
Figure 14: The response of mustard in six sequential treatments of (W) wheat, (C) chickpea and (M) mustard for (A) days to 50% flowering, (B) plant height stem elongation to flowering, (C) plant height from flowering to maturity, (D) NDVI 29/6/15, (E) days to 50% maturity, and (F) plant biomass at anthesis at Narrabri in 2015 with standard error bars.

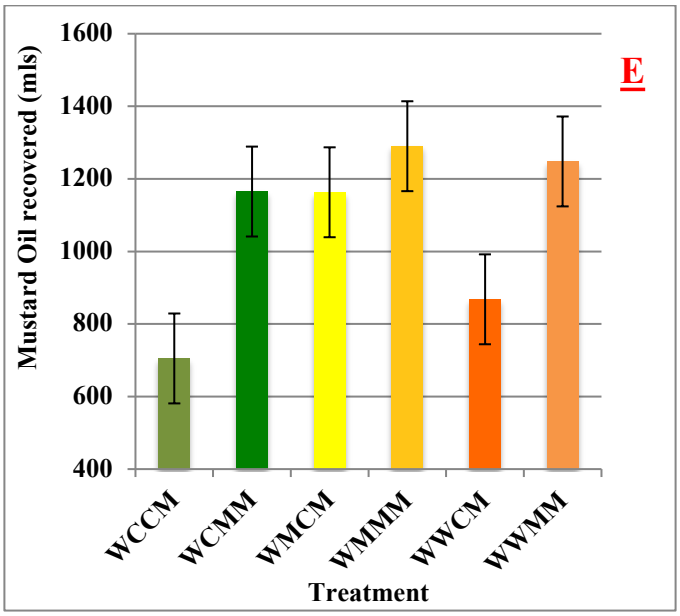
Grain protein and grain yield in mustard was highest in the WCCM rotation followed by WWCM (Appendix Table 14; Figure15 A and B). Most treatments produced the same level of linoleic acid with the exception of WMMM, which was significantly lower (Appendix Table 14; Figure 15 F). As linoleic acid levels are inversely proportional to the oleic acid levels in mustard oil, the rotation WMMM can be considered superior if oil quality is the production objective; however WCCM and WWCM are superior if quantity is the objective.

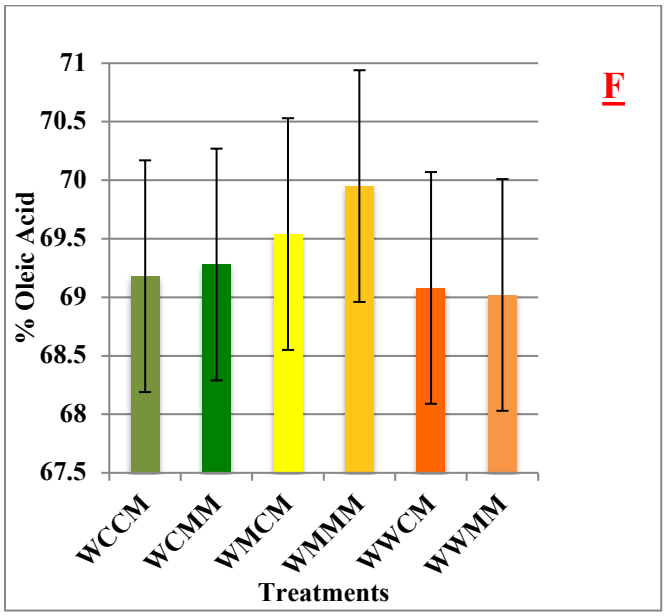
Oil yield was greatest in WMMM and WWMM (Appendix Table 14; Figures 15 C and D). It also appears that continuous mustard WMMM, although not significant, produced higher oleic acid levels than other rotations (Appendix Table 14; Figure 15 F). Oil yield for biodiesel production is heavily influenced by grain yield: a big consideration when trying to maximise biodiesel production from mustard (Figures 15 C, D, and E).

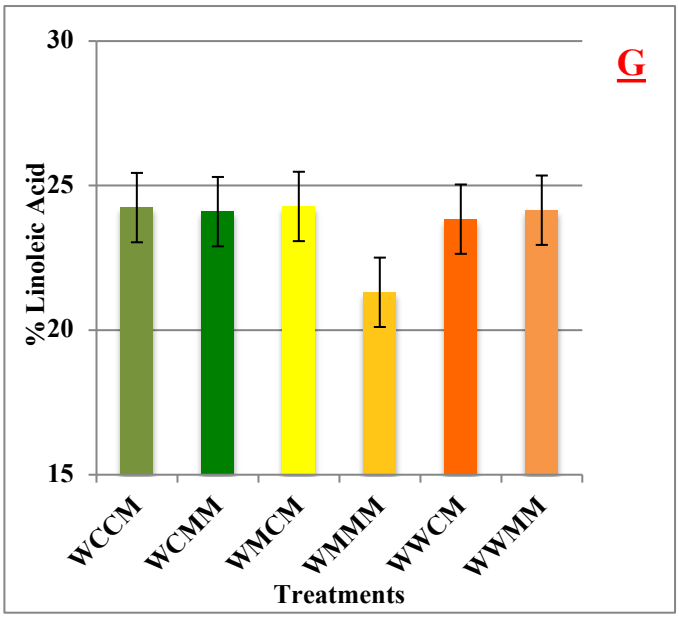
Using the conversion presented in the Materials and Methods section, oil yields were converted to litres of biodiesel per ha. For example, the grain yield of the rotation WCCM was 2277 kg/ha equates to 752.6 L/ha of biodiesel. The WMMM produced the greater quantity of mustard oil in L/ha but because it did not produce the yield of seed that WCCM produced it did not produce the quantity of biodiesel that WCCM produced (Appendix Table 14; Figure 15 L). Mustard oil quantity appears to be inversely proportional to mustard oil quality.

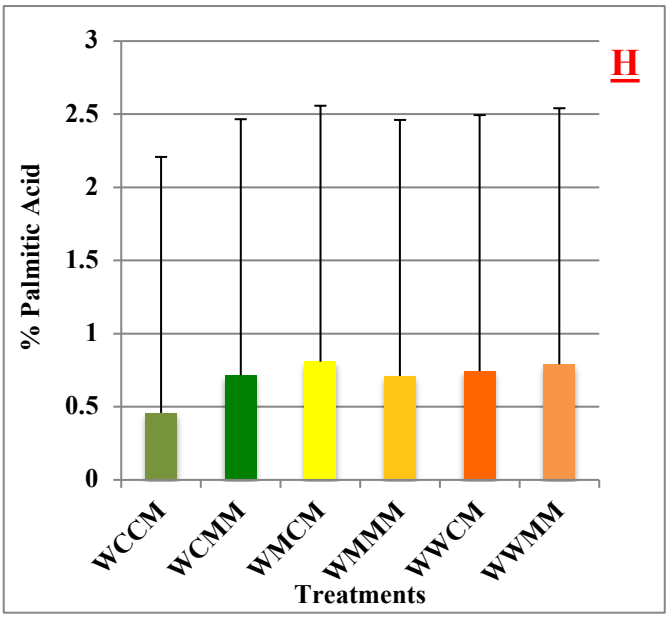
High glucosinolate and sinigrin concentrations were produced in the WCMM and WWMM rotations. Sinigrin is a high value constituent of glucosinolate and this was proportionally high in the same rotation treatments. For example, the rotation WCCM produced a yield 2277 kg/ha and 4116 mg/100 g of sinigrin. This gave a total of 93.7 kg/ha of sinigrin. High sinigrin concentrations were found in WCMM and WWMM. However these rotations also had the lowest grain yield and therefore low kg/ha of sinigrin. The high yielding rotations (WCCM and WWCM) therefore maximize sinigrin per ha (Appendix Table 14; Figure 15 K).

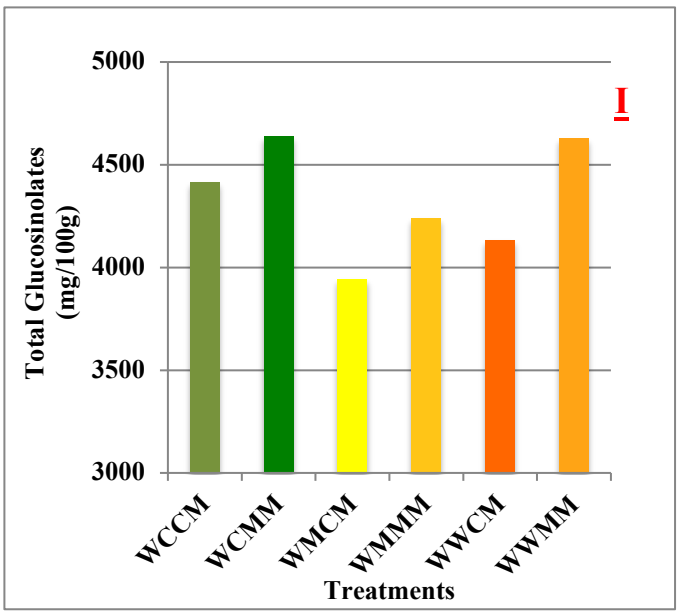


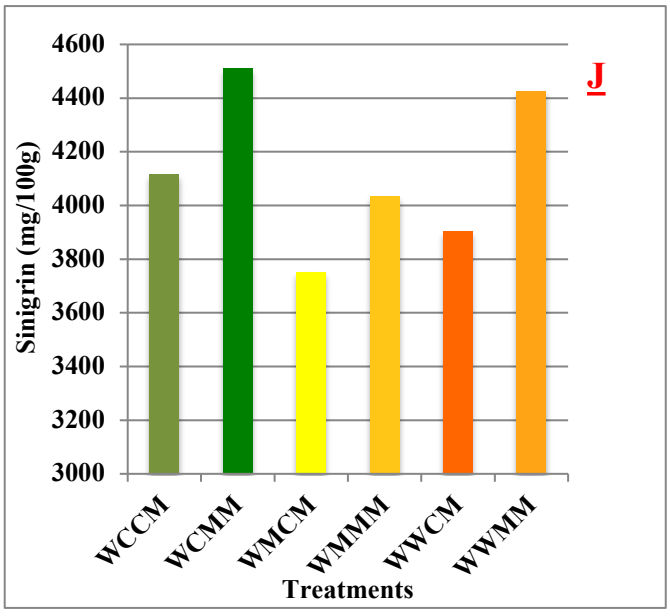

E
Mustard Oil recovered (mls)
1600
1400
1200
1000
800
600
400
WCCM
WCMM
WMCM
WMMM
WWCM
WWMM
Treatment

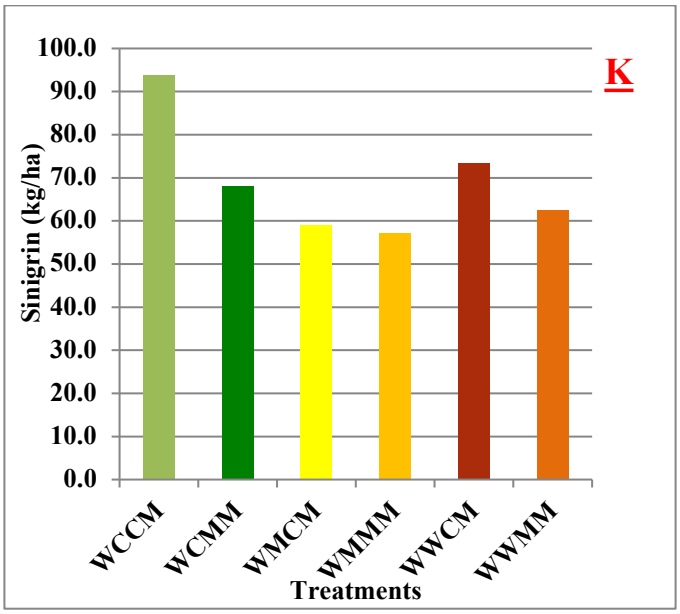

F
% Oleic Acid
71
70.5
70
69.5
69
68.5
68
67.5
WCCM
WCMM
WMCM
WMMM
WWCM
WWMM
Treatments


G
% Linoleic Acid
30
25
20
15
WCCM
WCMM
WMCM
WMMM
WWCM
WWMM
Treatments


H
% Palmitic Acid
3
2.5
2
1.5
1
0.5
0
WCCM
WCMM
WMCM
WMMM
WWCM
WWMM
Treatments


I
Total Glucosinolates (mg/100g)
5000
4500
4000
3500
3000
WCCM
WCMM
WMCM
WMMM
WWCM
WWMM
Treatments


J
Sinigrin (mg/100g)
4600
4400
4200
4000
3800
3600
3400
3200
3000
WCCM
WCMM
WMCM
WMMM
WWCM
WWMM
Treatments



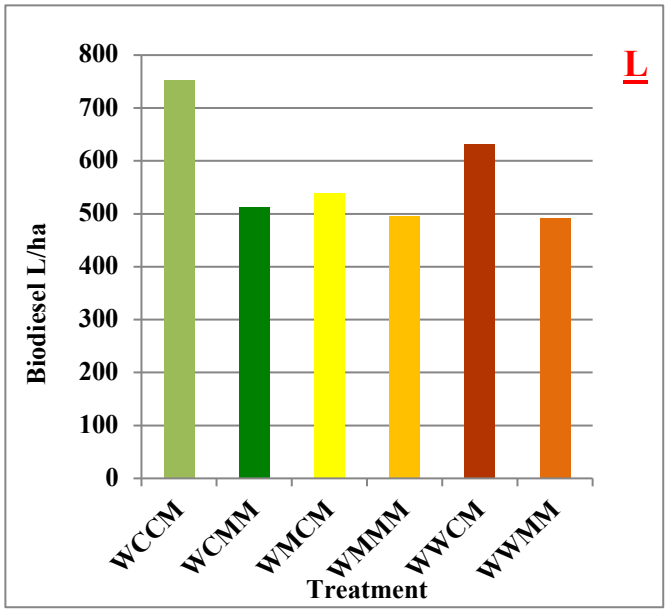


Figure 15: The response of mustard in six sequential treatments of (W) wheat, (C) chickpea and (M) mustard for (A) grain protein, (B) yield, (C) % actual extracted oil (Cold Press), (D) % oil in mustard seed (NIR), (E) mustard oil recovered mls, (F) oleic acid (G) linoleic acid, (H) palmitic acid, (I) total GLS, (J) sinigrin (mg/100g), (K) sinigrin (kg/ha) and (L) biodiesel (l/ha) at Narrabri in 2015 with standard error bars.

Chickpea response

1. *Impact of the previous crop*

There was no impact of the previous crop on any chickpea trait in 2015 with the exception of grain protein (Table 7). Grain protein was higher when chickpea was sown following chickpea.

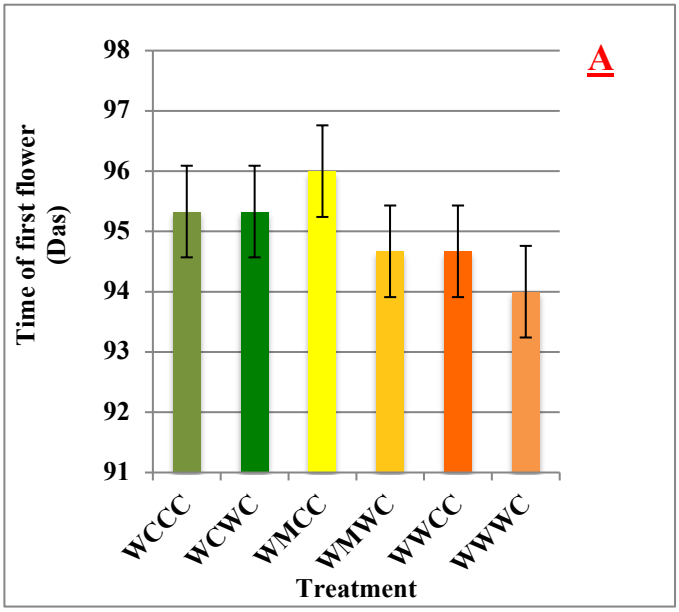
Table 7. The impact of the previous crop on chickpea traits in 2015

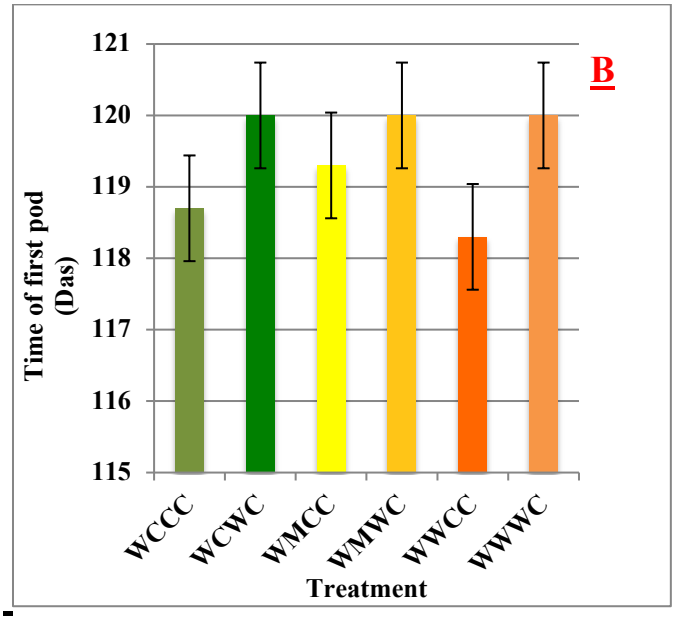
Previous crop	Yield kg/ha	Days to first flower	TKW (g)	Grain protein %	Harvest Index	Plant height (cm)
Wheat	635 a	94.7 a	161.7 a	20.9 a	0.41 a	59.4 a
Chickpea	597 a	95.3 a	174.4 a	23.4 b	0.38 a	61.0 a

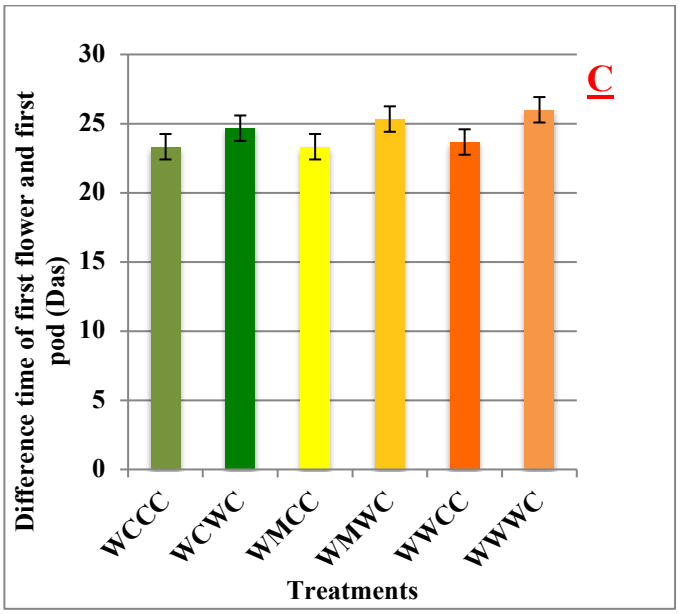
Note: different letters in columns indicate significance at P<0.05. TKW is thousand kernel weight

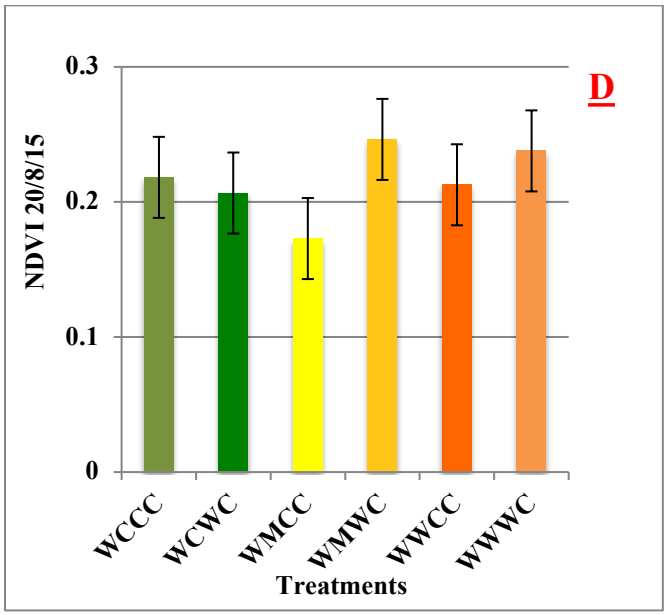
2. *Impact of the crop sequence*

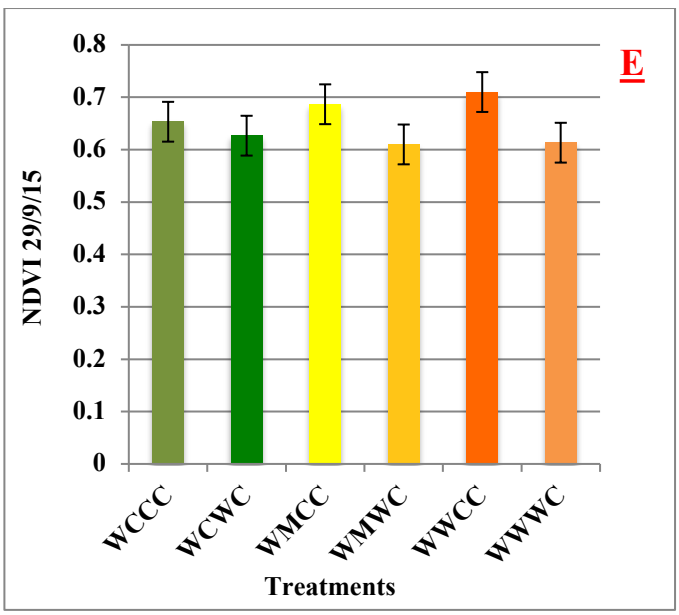
In 2015, the chickpea rotation was infected with stunt virus late in the season and this reduced overall yield. However, most traits showed a non-significant response to crop sequence with the exception of biomass at anthesis and maturity (Figure 16 G and H), grain protein percentage (Figure 16 L) and harvest index (Figure 16 K). The significance of all traits is presented in Appendix Table 9.

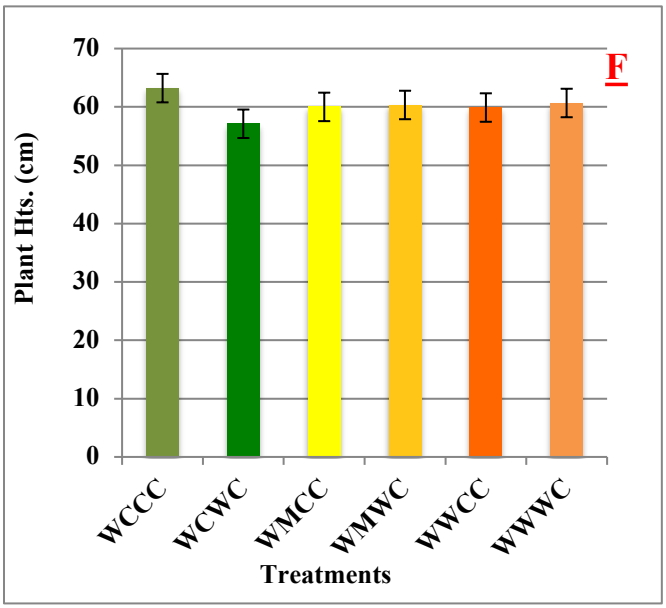

A
Time of first flower (Das)
98
97
96
95
94
93
92
91
WCCC
WCWC
WMCC
WMWC
WWCC
WWWC
Treatment


B
Time of first pod (Das)
121
120
119
118
117
116
115
WCCC
WCWC
WMCC
WMWC
WWCC
WWWC
Treatment


C
Difference time of first flower and first pod (Das)
30
25
20
15
10
5
0
WCCC
WCWC
WMCC
WMWC
WWCC
WWWC
Treatments


D
NDVI 20/8/15
0.3
0.2
0.1
0
WCCC
WCWC
WMCC
WMWC
WWCC
WWWC
Treatments


E
NDVI 29/9/15
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
WCCC
WCWC
WMCC
WMWC
WWCC
WWWC
Treatments


F
Plant Hts. (cm)
70
60
50
40
30
20
10
0
WCCC
WCWC
WMCC
WMWC
WWCC
WWWC
Treatments

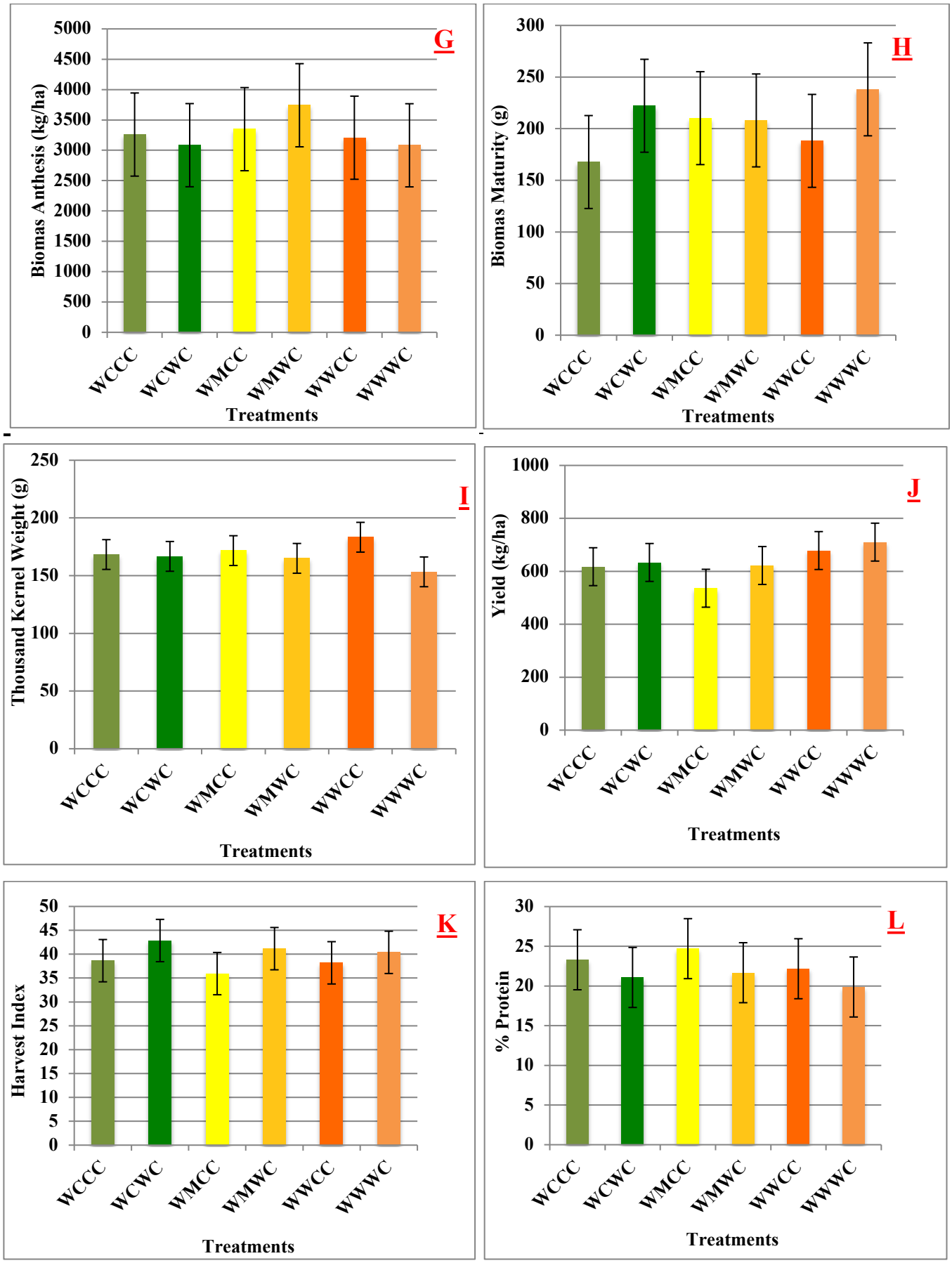


Figure 16: The response of chickpeas in six sequential treatments of (W) wheat, (C) chickpea and (M) mustard for (A) first flower, (B) first pod, (C) difference between first flower and first pod, (D) NDVI 20/8/15, (E) NDVI 29/9/15, (F) plant height, (G) biomass at anthesis, (H) biomass at maturity, (I) thousand kernel weight, (J) yield, (K) harvest index and (L) % protein at Narrabri in 2015 with standard error bars.

Impacts of crop sequences on soil moisture and nutrition

Soil moisture assessed after harvest in 2015

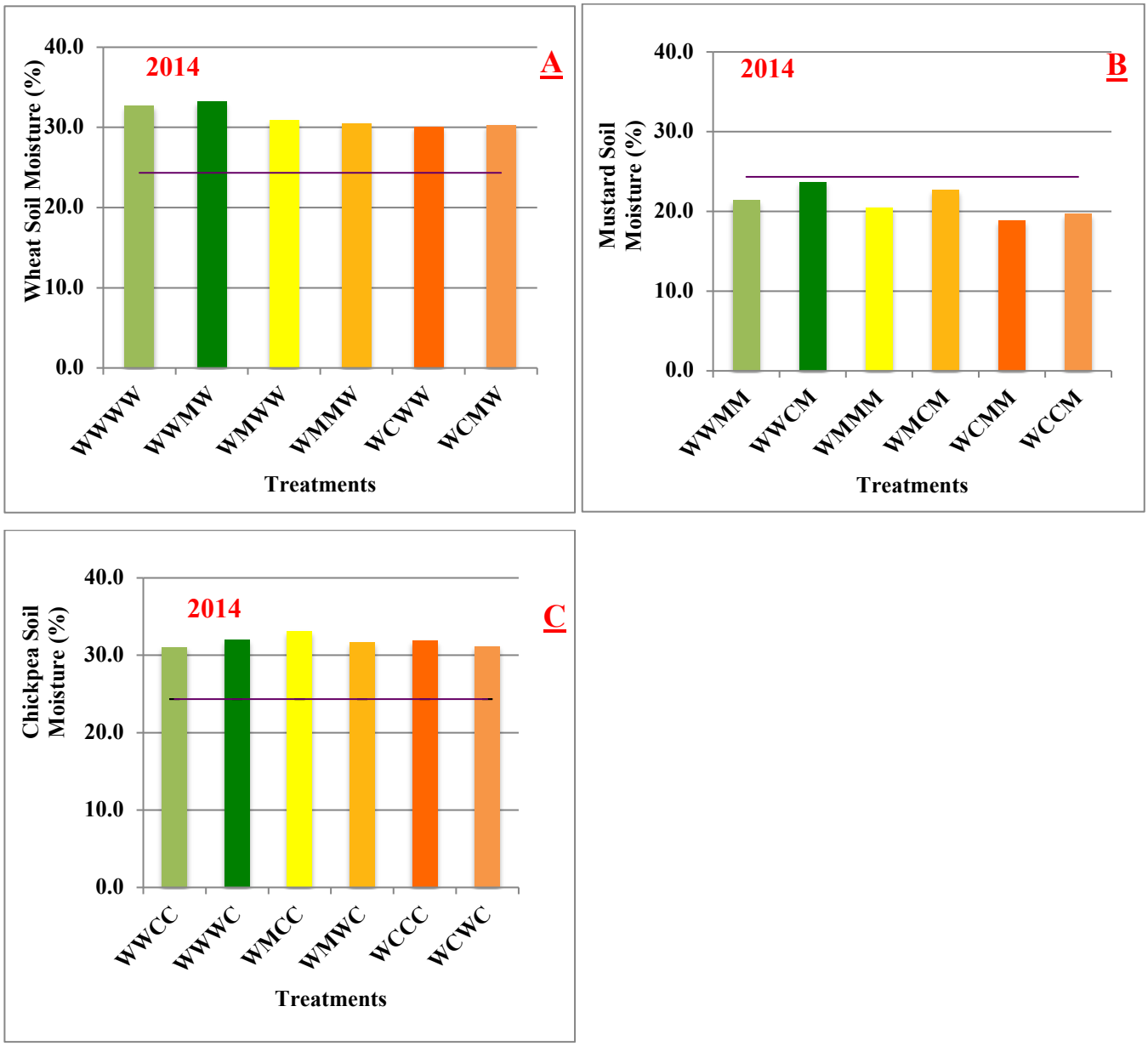


Figure 17: The response of six sequential treatments to residual soil moisture levels after harvest in 2015: (W) wheat, (M) mustard and (C) chickpea.
Footnote: the purple line denotes starting soil moisture prior to sowing in 2014.

Mustard used approximately 30% more soil moisture than either the wheat or chickpea treatments in all comparisions (Figure 17). Among wheat treatments in 2015, more moisture was left in the top 30 cm of soil in the WWWW and WWMW treatments than all other rotations. In the mustard treatments, WCMM and WCCM used more soil moisture than other treatments. However, in chickpeas the differences were less noticable. Nevertheless, the sequences WWCC and WCWC used the most moisture.

Residual soil Nitrogen assessed after harvest in 2015

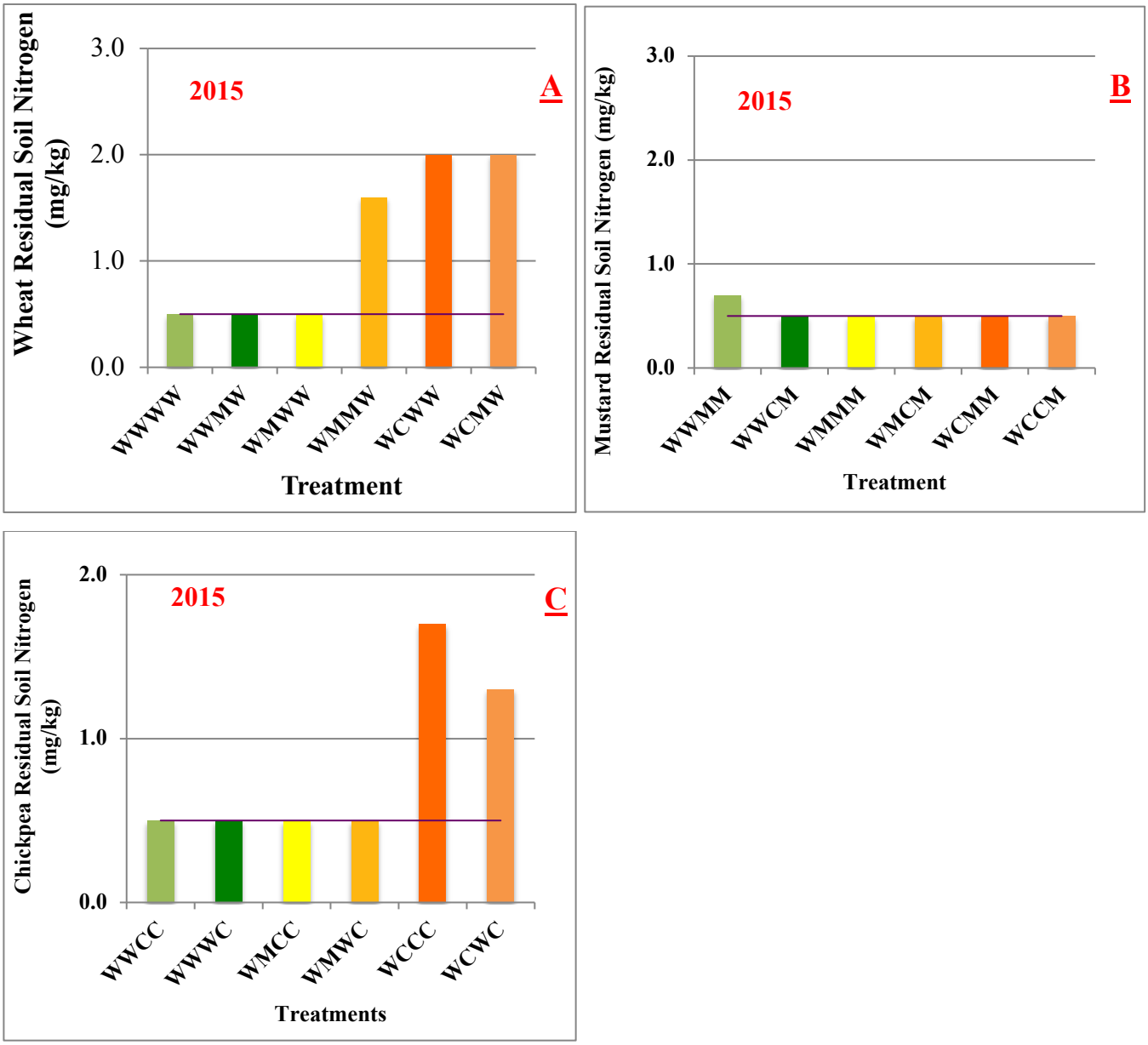


Figure 18: The response of six sequential treatments to residual nitrogen levels after harvest in 2015: (W) wheat, (M) mustard and (C) chickpea.
Footnote: the purple line denotes the final lowest residual nitrogen level at post harvest in 2015.

In the wheat sequence of 2015, the highest levels of residual nitrogen were observed in the WCWW and WCMW sequences (Figure 18 A). In the mustard treatments the residual nitrogen was very low and there was little difference among crop sequences (Figure 18 B). However, the WMMW (Figure 18 A) and WCCC sequences (Figure 18 C) had a similar level of residual nitrogen, although this was lower than WCWW and WCMW (Figure 18 A). Finally, residual nitrogen was lower in mustard in all sequences than either wheat or chickpea.

Residual % Organic Carbon assessed after harvest in 2015

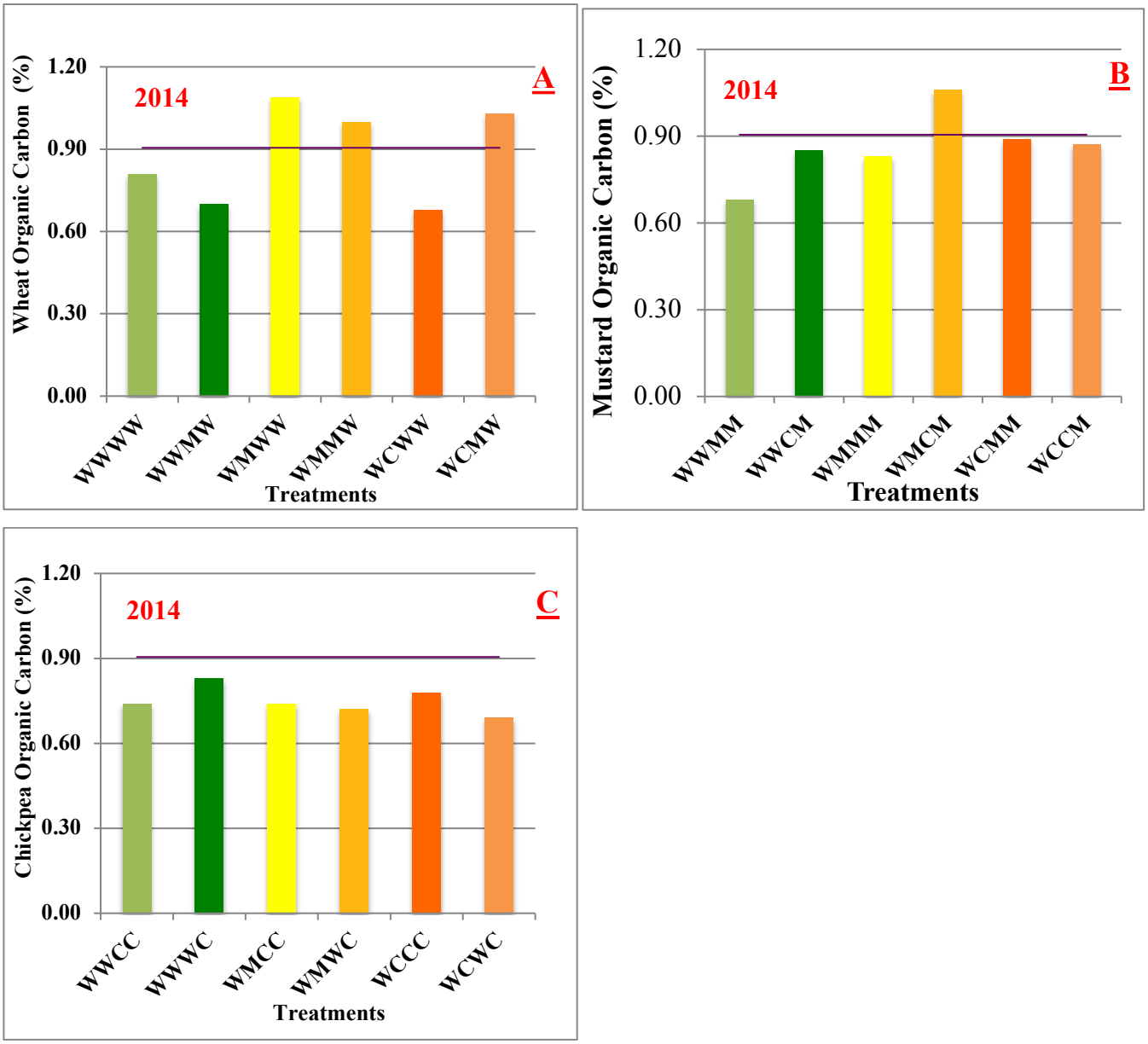


Figure 19: The response of six sequential treatments to residual % organic carbon levels after harvest in 2015: (W) wheat, (M) mustard and (C) chickpea.
Footnote: the purple line denotes starting organic carbon % prior to sowing in 2014.

In wheat treatments in 2015, the highest levels of observed organic carbon were found in the WMWW, WMMW and WCMW sequences (Figure 19 A). In mustard treatments, WMCM (Figure 19 B) produced the highest levels of carbon and the sequences WWCM, WMMM, WCMM and WCCM had organic carbon levels close to the 2014 levels. Chickpea treatments (Figure 19 C) had reduced organic carbon compared to 2014 levels with the highest values observed in WWWC and WCCC.

Residual Phorphorus assessed after harvest in 2015

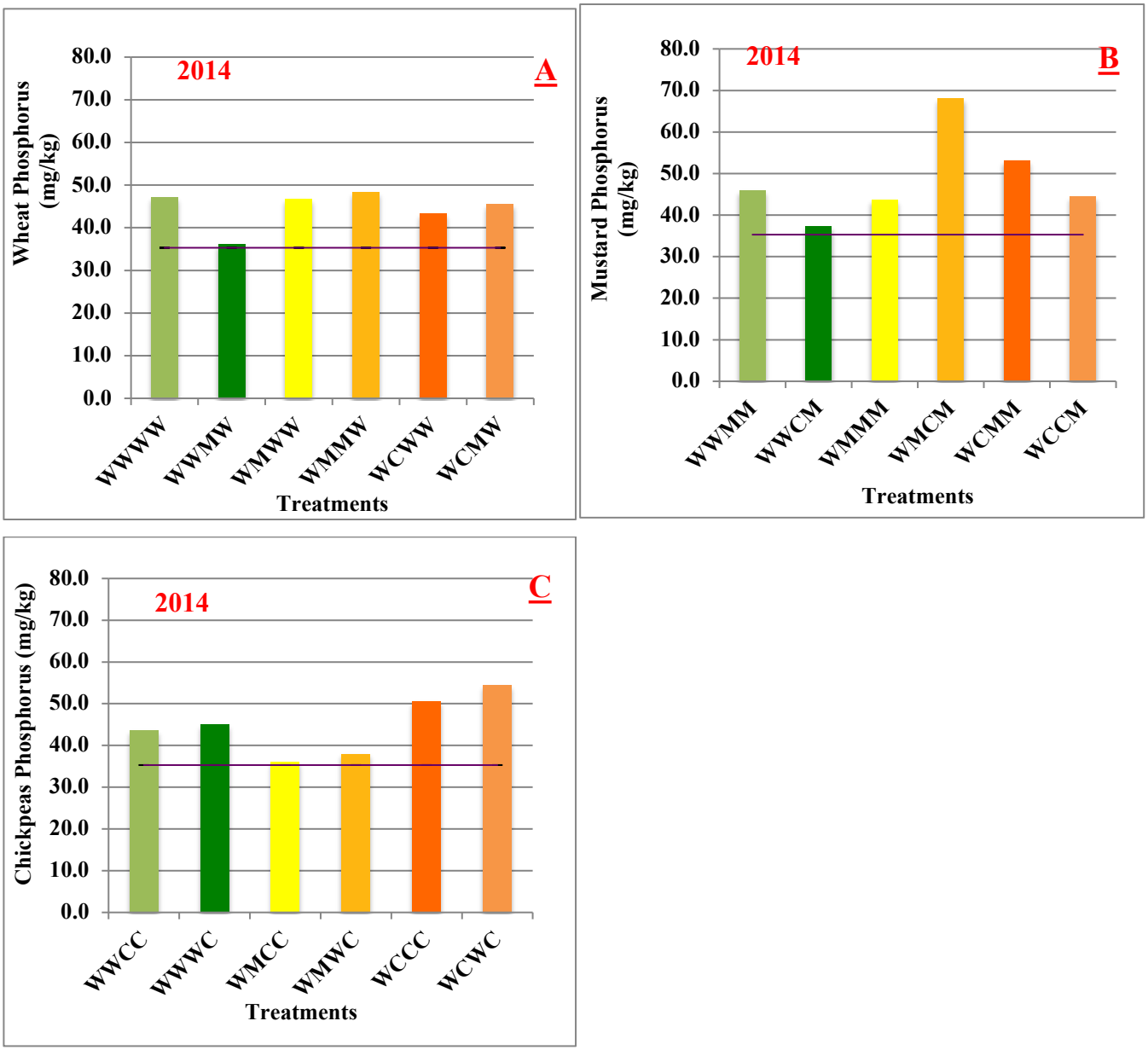


Figure 20: The response of six sequential treatments to residual phosphorus after harvest in 2015: (W) wheat, (M) mustard and (C) chickpea.
Footnote: the purple line denotes starting phophorus levels prior to sowing in 2014.

All crop sequences either left 2014 phosphorus levels unchanged or increased the levels of P. In wheat, five sequences (WWWW, WMWW, WMMW, WCWW, WCMW) finished with phosphorus levels between 40-50 mg/kg higher than at the start of the rotations(Figure 20 A). In mustard, all six sequences finished with higher phosphorus levels with WMCM producing close to double the levels of 2014 (Figure 20 B). The sequences WWMW, WWCM, WMCC (Figure 20) used more phosphorus and yet maintained levels close to 2014. In chickpeas, the sequences WCCC and WCWC produced phosphorus levels of over 50 mg/kg higher than the starting level, and WWCC and WWWC 40 mg/kg higher.

Residual Sulphur assessed after harvest in 2015

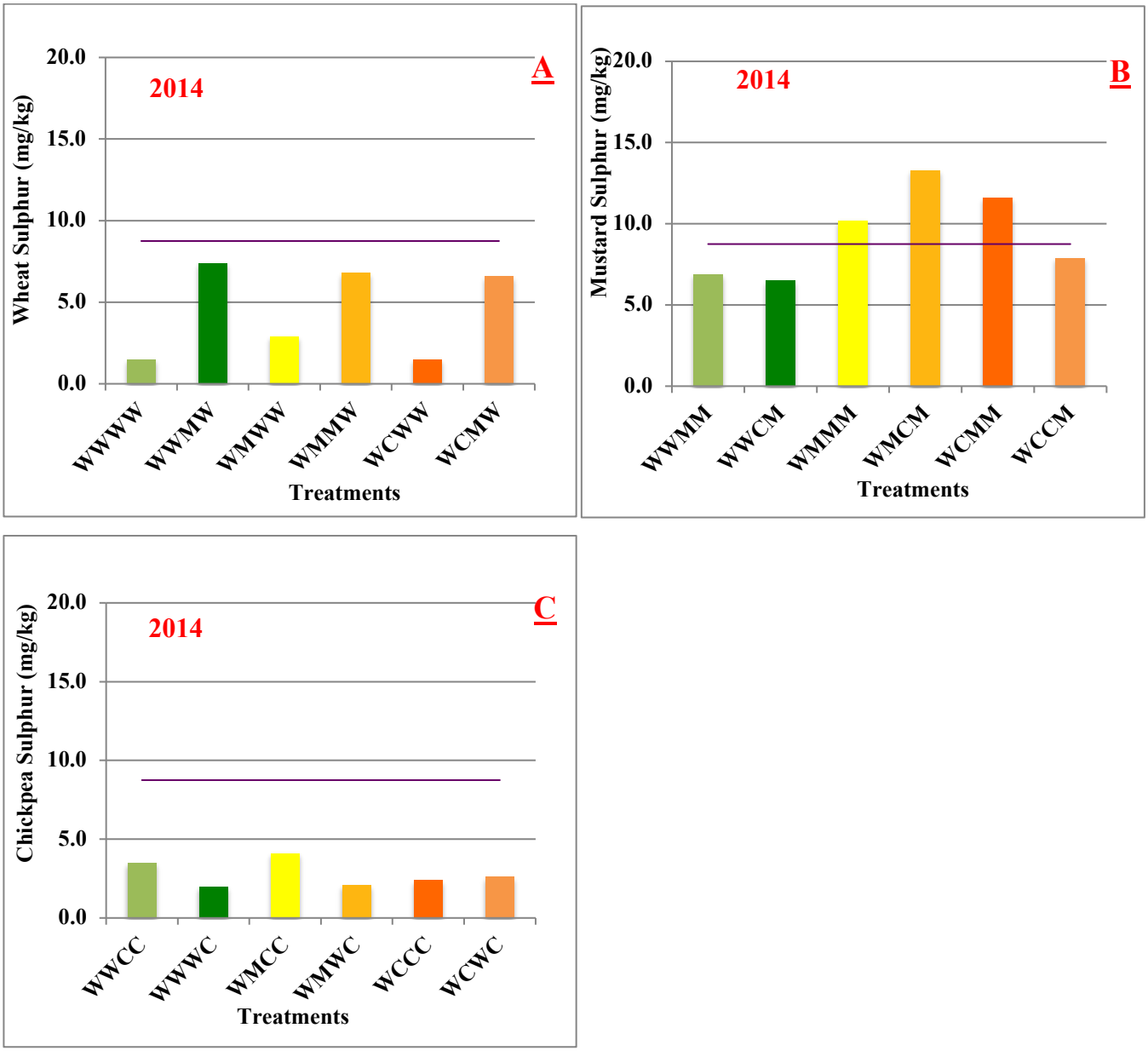


Figure 21: The response of six sequential treatments to residual sulphur after harvest in 2015: (W) wheat, (M) mustard and (C) chickpea.
Footnote: the purple line denotes starting phophorus levels prior to sowing in 2014.

Sulphur levels are higher in all mustard treatments compared to wheat. The highest mustard levels were observed in WMMM, WMCM (highest) and WCMM (Figure 21 A & B). However the sulphur levels were very low in the chickpea treatments; the sequences WWCC and WMCC produced the highest sulphur levels but these were below 5 mg/kg. The wheat and chickpea treatments used a large amount of sulphur through the 2014-15 seasons. The mustard treatment in the sequence WCCM used more sulphur than other mustard sequences but this was less than either wheat or chickpea (Figure 21 A & C).

Chapter 4: Discussion

Investment in mustard genetic improvement has been at a much lower level than canola over the past forty to fifty years. However, a small cadre of researchers has generated new information on the agronomic benefits of mustard in rotation, its genetic improvement and the development and deployment of genetically modified mustard with higher oleic fatty acid content in the oil (Green et al 2008).

Nevertheless, gaps in knowledge of the agronomic management of mustard still exist including its rotational benefits and role in the broader farm enterprise. The crop sequences used in the current study were designed to explore these issues in a brassica/cereal/pulse crop sequence on a grey vertosol soil, typical of northwest NSW. A typical crop sequence in this region is wheat and barley followed by a pulse such as chickpea or faba bean (GRDC-PBA). Mustard replaced barley in the experimental design used in this study. Mustard is well suited to conditions in the region (Oram et al 1995) and its impact on both wheat and chickpea; two very important grain crops, were not previously well defined. The choice of mustard as a rotational crop was based on a number of potential economic benefits including soil fumigation properties, oil that can be used in food and industrial processes and meal that can be fed to animals (Seymour et al. 2012; Kirkegaard et al 2000; Jham et al. 2009; Zhou et al. 2014). The experiment was conducted over three years to examine the impact of crop sequence on all elements of the farming system including wheat yield and quality, chickpea grain yield and mustard grain yield, oil yield, protein content, fatty acid composition, amino acid levels and glucosinolate concentration. Treatments were irrigated as required to produce an average target yield of 2 t/ha in the mustard treatment. Therefore, the experiments were largely independent of seasonal rainfall fluctuations.

The impact of the previous crop on wheat, mustard and chickpea productivity and quality

The lack of a significant effect of the previous crop on the yield of wheat, mustard and chickpea was probably influenced by the diseases apparent in the mustard and chickpea treatments in 2014 and 2015, respectively. The increased error from the heterogeneous nature of these infections negated and statistical significance in these years. In both cases, the infection occurred late in the season thus most in-season data were largely unaffected. It was hypothesised that wheat following mustard would have higher yield compared to other treatments and this was not observed. However, mustard did increase wheat protein in both years while

chickpea as the previous crop increased kernel weight. The increase in kernel weight probably diluted grain protein as the ratio of endosperm to aleurone would have changed.

Chickpea sown previously had the largest impact on mustard traits with increased grain oil percentage and protein observed. This was clearly influenced by residual nitrogen remaining in the soil post the chickpea treatment. Wheat and mustard sown previously did not influence chickpea characteristics at all. The greatest impact on chickpea was observed following chickpea with the increased grain protein clearly attributable to the residual nitrogen.

Crop sequences impacts on soil moisture and nutrition

The different crop sequences did impact soil moisture and nutrition with significant differences noted in available soil moisture post-harvest and changes in nitrogen, phosphorous and sulphur. Mustard tended to use 30% more water than either wheat or chickpea and mustard treatments tended to increase soil phosphorous. Chickpea used the least soil moisture in season. However, by far the largest increase in soil phosphorus was observed when mustard followed chickpea. The highest levels of organic carbon were found in wheat and the lowest in chickpea, possibly because the rate of decomposition in chickpea stubble was greater than wheat. Nitrogen levels were observed to be higher in wheat and chickpea and lower in mustards. The results show that sequences that produced higher yielding mustard tended to use more soil moisture, sulphur, nitrogen and phosphorus. This increased yield was at the expense of biomass suggesting higher harvest index. Possibly, the lack of surplus nutrients reduced overall biomass production and more nutrients were translocated to the developing seed.

The highest yielding mustard sequence (Wheat-Chickpea-Chickpea-Mustard), utilized the most water, nitrogen and organic carbon supplied by the preceding chickpea. The chickpea clearly fixed nitrogen and the mineralised chickpea residue would have added a small amount of additional phosphorus to the soil. The mustard in this sequence grew the most through the vegetative stage and flowered and matured later, but had lower biomass than other sequences.

Mustard response to crop sequences

While powdery mildew impacted yield in the 2014 mustard treatments and reduced differences among treatments, the higher protein observed on Wheat-Chickpea-Mustard was clearly an impact of extra soil

nitrogen following rotation with chickpea. The mustard genotype was mixed for grain colour and was divided into black and yellow seed based on an undocumented test looking at weight differences between 100 black Indian mustard seeds and 100 yellow Indian mustard seeds from Indian mustard variety BJ#7. The black seed had lower thousand kernel weight and reduced oil content compared to the larger yellow seed. There are similar observations on the influence of seed size and colour made by Rahman et al (2011). This author also reported that yellow seeded mutants of mustard were higher in oil and protein but lower in fibre because the yellow varieties of Indian mustard have a clear seed coat (or testa) over the embryo.

However, the crop sequence Wheat-Chickpea-Mustard in 2014 produced both high yield and high protein while grain weight and oil content were not impacted by this rotation. The percentage content of oil in mustard seed was not significantly different in any of the treatments. However, grain yield was important in determining the production biodiesel in L/ha, The higher yielding Wheat-Chickpea-Mustard crop sequence produced the most biodiesel and the low yielding Wheat-Mustard-Mustard treatment the least. A similar trend was observed in 2015, where mustard following chickpea produced the most biodiesel.

Powdery mildew was controlled in 2015 and the Wheat-Chickpea-Chickpea-Mustard treatment produced higher yield and protein than Wheat-Mustard-Mustard-Mustard. The higher yield is the result of slightly higher thousand kernel weight in Wheat-Chickpea-Chickpea-Mustard and Wheat-Wheat-Chickpea-Mustard**,** both treatments that benefited from the extra available soil nitrogen and soil moisture following chickpea. However, the thousand kernel weight was not significantly different across treatments. Mustard oil production was reduced by 45% and sinigrin dropped by 11.9% in Wheat-Chickpea-Chickpea-Mustard. It is worth noting that both Wheat-Chickpea-Mustard-Mustard and Wheat-Wheat-Mustard-Mustard have low yields, and high in glucosinolate compared to Wheat-Chickpea-Chickpea-Mustard. The extra nitrogen from the chickpea crop sequence increased yield and diluted other chemical constituents in the grain such as glucosinolate. The high oil content in Wheat-Mustard-Mustard-Mustard and Wheat-Mustard-Chickpea-Mustard compared to Wheat-Chickpea-Chickpea-Mustard is again a function of increased yield which reduced oil content, a correlation often reported in the literature (Wilkes et al, 2013). However, while the Wheat-Mustard-Mustard-Mustard and Wheat-Wheat-Mustard-Mustard rotations had higher mustard oil concentrations, the Wheat-Chickpea-Chickpea-Mustard and Wheat-Wheat-Chickpea-Mustard crop sequences

produced larger amounts of biodiesel because higher grain yield more than compensated for lower grain oil content. The current study shows the variation in oil content between Wheat-Chickpea-Chickpea-Mustard and Wheat-Mustard-Mustard-Mustard is less than 2%. Therefore mustard oil content has little effect on biodiesel quantity and any differences in oleic acid constitution will have very little impact on mustard oil quality. Perhaps it is worth noting that high oil and glucosinolate content is a result of soil moisture and nitrogen stress. Therefore, grain yield alone should be the selection target for plant breeders if oil and glucosinolates are the target market. The high grain yield and higher protein observed was the result of a good nitrogen and soil moisture base following chickpea. It is possible that the Wheat-Chickpea-Chickpea-Mustard treatment built up more than 100 kg/ha of extra nitrogen by the second year, compared to cop sequences without chickpea, based on published rates of nitrogen fixation and usage in chickpea (Herridge et al 2011; Ryan et al 2006).

Time of sowing was critical to achieving high mustard grain and oil yields with late sowing reducing both (Wilkes et al 2013). In the current study, the sowing time was optimised for mustard to minimise the impact of environment on yield and oil content. These sowing times, where based on documented work in Wilkes et al (2013). When mustard was sown outside the optimum planting time there was a decrease in oil concentration of 1.1% for every two week delay in planting and a grain yield reduction of 309kg/ha (Hocking et al 2001). These reductions are exacerbated if the crop is grown under low rainfall conditions. If time of sowing is delayed, then flowering and pod fill occur later, thus exposing the crop to temperature and moisture stress during grain fill. Optimising sowing date may unlock future benefits for Indian mustard (Kirkegaard et al 2016).

Early flowering cultivars can extend the post-anthesis grain-filling period and for every 10-day increase in the grain-filling period, oil concentration increases by 1.2% (Si et al 2004). Both oil concentration and seed yield also increase with higher post-anthesis rainfall and lower temperatures. Some estimates suggest that 0.7% more oil and 116kg/ha more grain yield occurs for every 10mm rainfall received and oil concentration reduces by 0.68% and grain yield by 289kg/ha for every 1^{o}C increase in temperature (Si et al 2004). However, these environmental constraints are influenced by genotype and varieties with an optimised phenology for northwest NSW will reduce these impacts. While only one genotype of mustard was assessed in

this study, others have observed significant genotype x environment interaction for yield, oil content and oil quality (Wilkes et al 2013). Walton et al (2004) suggests that high-yielding longer season cultivars that flower early, have tolerance to high and low temperatures and water-use-efficiency, produce high oil yield and have high glucosinolate concentration will be of great value to this region. However, such cultivars have yet to be developed.

Delayed sowing not only impacts yield and oil content but also reduces oil quality. Wilkes et al (2013) reported lower oleic acid and higher linoleic acid in late sown materials; a fatty acid constitution that affects the oxidative stability and kinetic viscosity of biodiesel. However, the Wheat-Mustard-Mustard-Mustard treatment produced high oil content (34.4%) and although non-significant, this treatment tends to have the highest oleic acid and the lowest linoleic acid level of all treatments. Evidence suggests that low phosphorus and high nitrogen increase oil content (Pinkerton et al 1991). In this treatment there were high levels of Colwell P (56–106 kg/ha at 0-15cm) and low levels of N (52-75 kg/ha at 0-15cm) and this may have influenced the result. However, it could also be interpreted that oil and glucosinolate production in this treatment are related to higher moisture stress (Antonious et al 2009). This observation was supported by the Wheat-Chickpea-Chickpea-Mustard treatment, where oil production was 45% lower and glucosinolate production 11.9% lower than other treatments with low protein and grain yield. Wheat-Mustard-Mustard-Mustard produced more oil because of a shorter vegetative stage, earlier flowering, earlier maturity, low protein and low grain yield. This sequence also had low sinigrin and used more soil moisture and nitrogen.

Total glucosinolates were significantly higher in Wheat-Chickpea-Mustard-Mustard and Wheat-Wheat-Mustard-Mustard and sinigrin levels significantly higher in Wheat-Chickpea-Mustard-Mustard only. Sinigrin concentration was not significantly different in any of the other treatments, including Wheat-Wheat-Mustard-Mustard. These results indicate that glucosinolate and oil production are increased when the plant is under nitrogen and moisture stress. The crop sequence Wheat-Chickpea-Mustard-Mustard was later flowering with larger biomass, low protein, low yield and average oil quantity but was high in sinigrin. This sequence used more soil moisture and nitrogen. The low yield may have resulted from late season heat and moisture stress thus reducing overall yield. Similarly, the lower yield of Wheat-Wheat-Mustard-Mustard was a

function of later flowering. However the lower grain yield and average oil yield was offset by higher grain sinigrin

The water use efficiency of mustard in the two years was influenced by differences in the sowing rate. The sowing rate was altered from 4 kg/ha in 2014 to 2 kg/ha in 2015 to reduce excessive biomass production thus minimizing the impacts of powdery mildew. As a result more water was used by mustard in all treatments in 2014 compared to 2015. However, no effect of crop sequence treatment was observed for water use efficiency in either year.

Wheat response to crop sequences

Wheat following chickpeas (Wheat-Wheat-Chickpea-Wheat) produced the highest wheat yield in all the crop sequences assessed. This is not surprising, as nitrogen fixation from the preceding legume crop will have provided more available nitrogen in the wheat phase of rotation. This observation agrees with many previous findings on the benefits of legumes (Evans et al 2001; Armstrong et al 1997). The thousand kernel weight was higher in Wheat-Wheat-Chickpea-Wheat contributing to the higher yield in Wheat-Wheat-Chickpea-Wheat as reported by Wang et al (2012). The Wheat-Wheat-Chickpea-Wheat crop sequence produced 8% more grain than the Wheat-Wheat-Wheat-Wheat crop sequence indicating the benefits of nitrogen fixation. Chickpea rhizobia in root nodules convert nitrogen gas from the atmosphere into ammonia (NH_3); the plant assimilates nitrogen into amino acids and proteins and exudes nitrogen rich compounds (Ndegwa et al 2008; Norlin et al 2002). Ammonia uptake is soil pH and temperature dependant (Wang, et al, 2012) and water soluble at low temperatures and low pH, greater plant availability; conversely at high temperatures, high pH becomes a gas and soil structure offset nitrogen contributions; less plant available. The equilibrium between ammonia and ammonium is dependent on soil pH. At pH 9.25 the ratio of ammonia: ammonium is 1:1. However, if the pH drops to 8.25 the ratio becomes 1:10 and at pH 7.25 it drops to 1:100 (Ndegwa et al 2008; Norlin et al 2002). Some nitrogen losses occur due to high summer temperatures prior to autumn planting; not quantified in this current study. Although nitrogen fixation is beyond the scope of the current study, nitrogen is released into the soil by crop mineralisation, ammonification and nitrification. Nitrogen gain in the soil is usually due to nitrate sharing and residues (Clark et al 2007). Looking at the results of soil samples collected from Field B3 nitrogen levels, while not included in the results, varied from east to

west in the 2014 experimental area; explained by chickpea treatments in 2013 with 30% increase in nitrate in the soil following chickpea. These results are indicative of extra nitrogen from legume fixation and mineralised from plant decomposition. Leaching and volatilisation throughout the summer due to soil structure and high temperatures offset nitrogen contributions.

Wheat following mustard (Wheat-Wheat-Mustard-Wheat**)** produced higher protein concentration in 2014 and generally lower yield than the optimum rotation with chickpea. This likely reflects the lower water available at depth following mustard. This would overcome any disease suppression or crop sequence affect in drier years as observed by Smith et al (2004). While mustard is unable to fix nitrogen, it can contribute to nitrogen supply by nitrogen mineralisation from decaying plant material and this is dependent on high temperature and adequate moisture. Mustard contains high levels of glucosinolates and active myrosinase, an enzyme involved in the mustard defence mechanism against herbivores (Antonious et al 2009). This leads to the generation of more Allyl Isothiocyanates compared to canola, which contains a low level of glucosinolates and inactive myrosinase (Kirkegaard et al 1999). Unlike chickpea, mustard mineralises plant materials to ammonium (NH_4) (Reardon et al 2013; Snyder et al 2010). Ammonium is rapidly converted to nitrates in alkaline soils (Angus et al 2015; Rochester et al 2001), which readily dissolve in soil water to provide available nitrogen for the plant. This process may explain the protein advantage of 7% observed in Wheat-Wheat-Mustard-Wheat compared to Wheat-Wheat-Wheat-Wheat**.**

The 10% increase in wheat yield in the Wheat-Mustard-Wheat-Wheat rotation in 2015 compared to Wheat-Wheat-Wheat-Wheat and relatively low protein and proline levels suggests that the effects of mustard in crop sequences extend over more than one year. It is not surprising that proline levels are lower as proline is an amino acid. Amino acids are the building blocks of protein and lower proline will have contributed to a reduced total protein (Clemente et al., 2007).

The lower yield and higher protein of the Wheat-Chickpea-Mustard-Wheat crop sequence was difficult to explain as the residual nitrogen from the chickpea phase would have most likely been used by the following mustard. The lower nitrogen possibly reduced overall yield and seed size thereby increasing grain protein. The previous mustard treatment in 2014 took longer to flower and was higher in protein and oil than other mustard treatments. However, this mustard treatment was adversely affected by a virulent strain of powdery mildew at

pod fill and it is unknown how this affected the treatment. Hypothetically, the Wheat-Chickpea-Mustard sequence should have produced a higher yield due to a longer vegetative period compared to other sequences (e.g. Wheat-Mustard-Mustard or Wheat-Wheat-Mustard).

Wheat-Mustard-Wheat-Wheat had a long flowering and maturity time, equivalent to the continuous wheat rotation, low NDVI readings but above average biomass at maturity and high yield. The high yield was likely a function of larger grain size in this rotation. Yield was inversely proportional to protein as was observed in 2014.

Chickpea response to crop sequences

The impact of crop sequence on chickpeas was less significant than either wheat or mustard. The Wheat-Wheat-Chickpea-Chickpea crop sequence produced the highest protein (23.7%) and highest yield in 2014, although the yield effect was not significant. Interestingly, this treatment also produced the shortest plants. The extra nitrogen from two years of chickpea produced high harvest indices and therefore the highest yield and grain protein.

However, in 2015, chickpea yield was reduced by the arrival of late season stunt virus. This may have influenced the lack of treatment consistency across years. Thus Wheat-Wheat-Wheat-Chickpea produced significantly higher yield than other treatments in this year. In general, the impact of rotation on chickpea yield and quality was much reduced compared to mustard and wheat where effects were more significant. When wheat and mustard followed chickpea their yield and protein levels were generally higher.

Future climate change and the potential of mustard

Mustard withstands hot, dry, and frosty conditions by using osmotic adjustment to increase proline levels in the vegetative and reproductive stages (Tumdam et al, 2012). Mustard is an annual crop and thus provides more flexibility and less fixed costs compared to perennial crops such as *Pongamia pinnata* (Odeh et al 2011). Although mustard may have a place in a changing and drier climate, it does use more soil moisture than either wheat or chickpea (Figures 7&8). Therefore, the crop sequence needs to be carefully planned to manage soil moisture and the productivity of subsequent crops. Some predictions suggest that the climate in northwest NSW will be hotter and drier in future (Hunt et al 2009) and mustard may have a role in mitigating these effects in future cropping systems**.** However, even mustard will be impacted by more extreme climate

predictions. Modelling evidence indicates that mustard yield reductions in eastern India could be as high as 67% (Boomiraj et al 2010).

The CSIRO Mark 2 coupled global climatic model for a 10,000 year simulation and was used to investigate the occurrence of dry episodes for the northeast, southeast and southwest of the Australian continent. No predictability associated with the initiation, duration or termination of individual dry episodes was found (Hunt 2009). This is typical of the Australian environment and extended dry periods, poor predictability and highly variable climatic conditions define Australian farming. The stress tolerance associated with mustard and the scope to genetically improve this tolerance make this species an option for future farming systems in Australia should markets for mustard products be found or developed. Proline varied among the agronomic treatments in the current study and this amino acid could be a future target for selection to improve the stress tolerance of this species. Nevertheless, while mustard has stress tolerant characteristics it will be vital to grow mustard in optimized rotations to obtain full value from this crop.

The benefits of growing mustard in a crop sequence

The positive economic benefits of growing wheat after mustard are generally related to the break crop affect and a degree of active disease suppression attributed to mustard's glucosinolate properties (Handiseni et al, 2012). The data suggests no impact of mustard on wheat yields in 2014 or 2015 and this may have occurred due to a lack of soil moisture. There may be some nitrogen benefit from mineralization effects, however as discussed earlier, these are generally minimal and difficult to measure. Nevertheless, even though the endemic northern cropping root diseases of crown rot and root lesion nematode were at low levels at this site (based on Predicta B testing) there was a wheat yield advantage (not significant) of up to 10% following mustard in the crop sequence.

Potential products from mustard seed and biomass

There must be a value attributable to mustard products if it is to become a viable rotation crop. There are three components related to value: high grain and protein yields, high oil yields with high oleic acid content and high glucosinolate yields, particularly sinigrin, currently. High percentage protein and high grain yield are inversely proportional to oil content (Angus et al 1994) and glucosinolate yield. Therefore, depending on the objective of production, it may be economically desirable to compromise on some

components to maximize others and different rotations can be used to target the desired product (Gunasekera et al. 2006).

There are a number of reasons why high grain and/or protein levels are desirable. The most common uses for mustard grain are as condiments or additives for food preparation and/or animal feed preparations (which require higher protein). The mustard food condiment market in Australia is relatively small and requires approximately 2,000 to 4,000 tonnes annually with Yandilla Mustard Oil Enterprises (Biomass Producer) and Australian Mustard Oil the major manufacturers. The oil is removed and the resultant meal is fed to animals. The seed meal thus produced has a protein content of between 23 and 26% (Wilkes et al. 2013). However, mustard seed meal should be fed carefully to animals and as a low proportion of the ration because glucosinolate levels in the meal are generally high. The recommended rate is 10% of the total feed ration (Zhou et al. 2014). Animal feed intake will decrease when feeding mustard seed meal above 10% of the ration. This is because 3-Butenyl glucosinolate, known for its extreme bitterness, reduces food intake and adversely impacts animal health. While the animal feed market is large, it is not very lucrative as many low value grain options are available. (Zhou et al. 2014) Therefore, meal will only ever be a small portion of the economic value of mustard production.

The most significant economic benefit from growing mustard is the oil content and oil fatty acid constitution, especially oleic acid. The higher oleic acid provides flexibility and the oil can be used for food, biodiesel, bio-lubricants and bio-plastics.

Mustard can be used to make biodiesel and the current study suggests that different crop sequence treatments can influence the efficiency of biodiesel production. The Wheat-Mustard-Chickpea-Mustard and Wheat-Mustard-Mustard-Mustard treatments produced an estimated 264 and 265 l/ha of biodiesel, respectively. Biodiesel as B100 was worth $1.07/lt (Echotech Biodiesel) at the Terminal Gate Price effective for November 2, 2016. Biodiesel (B20) was worth $1.09c/lt and Fossil Diesel $1.0757c/lt (United Petroleum). The BioCube (BioCube) is a modular portable biodiesel production plant designed for use by farmers, universities, councils, cooperatives, fleet owners, mining companies, transportation companies and many more applications (BioCube). They are modular so any number of units can be used and the purchase costs recouped

in 18 months. These units are produced in Canada and biodiesel production is based on transesterification. The BioCube can be powered by biodiesel or electricity, depending on the intended use.

Using the BioCube method of producing biodiesel, the production costs fall to $.070/lt from waste vegetable oil in Canada, $0.65/lt from Indian mustard seed oil in Canada, and $0.74/lt from *Pongamia* oil in India. Fossil diesel prices in Australia range from a $1.20/litre to $2.50/litre depending on the location of purchase. At current exchange rates ($A1 = $C1.02), mustard is the cheapest feedstock from which to produce biodiesel in Australia. It also provides greater flexibility than used vegetable oil, which is a limited resource and *Pongamia,* which requires more fixed production costs.

Gucosinolates can be extracted using hot water or ammonium chloride (NH_4Cl) and marketed to offset the cost of biodiesel production (He et al 2004). For example, the aim maybe to enhance the glucosinolate potential of the mustard, therefore sacrificing grain, protein and oil yields. Once the seed is crushed and the oil removed the mustard meal becomes a by-product. In the current study a 12% increase in sinigrin was found in the Wheat-Chickpea-Mustard-Mustard rotation, which is equivalent of 102.7 kg/ha of sinigrin. The glucosinolate or sinigrin can easily be removed from the meal. The mustard meal is formed into a powder called mustard seed meal powder (MSMP). Mustard seed meal powder is a wide spectrum antimicrobial compound called Allyl isothiocyanate (AITC): a hydrolysed form of sinigrin, produced when tissue is crushed (Elfoul et al 2001). When the tissue is crushed the breakdown of cells releases the myrosinase enzyme which catalyses the release of Allyl isothiocyanate; increasing amounts of Allyl isothiocyanate are released with increasing humidity and temperature. Mustard seed meal powder provides a natural antimicrobial material to improve food shelf life. When maximising glucosinolate levels, there are 13 separate compounds that make up glucosinolates in mustard plants. According to (Pharmacompass), on the 16thAugust, 2016, sinigrin monohydrate was valued at $US83.00 or $A146.00/ 100mg, where $A1 = $US 0.76.

Biofumigation potential of mustards

Glucosinolates are found in the roots, stems, leaves, shoots and seeds. However, glucosinolate concentrations reduce as plants mature and reach their lowest levels after plant maturation (Kirkegaard et al 2000). However, the Isothiocyanates (ITC) found in mustard are more effective against fungal attack than are the Isothiocyanates found in canola because the canola Isothiocyanates are 2-phenylethyl Isothiocyanates

(PEITC - C_9H_9NS), while the ITC in mustard are 2-phenylethyl Isothiocyanates (PEITC - C_9H_9NS) and 2-propenyl Isothiocynates; which are possibly more potent (Watt et al 2006). Phenylethyl Isothiocyanates is a compound produced from gluconasturtiin (a compound found in glucosinolates) a product of myrosinase activity and is used in chemoprevention (or chemoprophylaxis) for cancer treatment (Deswal et al. 2005; Kumar et al 2009). The financial value of these compounds is approximately $A591.50/20mg.

It is important to understand that seed meal type glucosinolates and their hydrolysed products, such as Isothiocyanates are important in reducing soil pathogens and increasing overall soil health because of myrosinase activity in the generation of Allyl isothiocyanate. In practical terms, the biofumigation effect controlling nematodes and fungal diseases depends on the myrosinase activity and glucosinolate concentration of any meal added to the soil (Snyder et al 2010; Reardon et al. 2013). Myrosinase activity and glucosinolate level effect soil biofumigation and this is influenced by mustard genotype and the rotational history of the site.

There is a large array of diversified crude products that can be developed from Indian mustard including biofuels, fuel additives, lubricants, bio-chemicals and bio-plastics. In addition, glucosinolates obtained from seeds, meal, stems, leaves, shoots and roots also impact the development of pharmaceuticals, veterinary products, food additives, and pesticides. All these products and outcomes are influenced by both the mustard genotype and the farming system including crop rotation (Table 8).

Table 8. Summary of potential Indian mustard products and benefits

Agronomic benefits	Potential products
Active disease suppression	Fuel additives
Tolerates hot dry, environments	Lubricants for machinery
Land rehabilitation	Bio-chemicals
Break crop effect on diseases	Bioplastics-general and structural
	Medicinal oils
	Pharmaceutical products
	Veterinary products
	Food additives
	Pesticides
	Flours & powders
	High protein animal pellets
	Organic fertilisers
	Cogeneration
	Whole seed condiments

Chapter 5: Conclusion

Mustard appeared to have little or no effect on chickpea yield or quality (assessed as protein content). However, chickpea most impacted the following crop (both mustard and wheat) with higher grain yield, grain protein % grain oil content and even glucosinolate concentration. Higher mustard yield and protein in 2015 was a result of a double chickpea treatment in the preceding two years while higher oil content and glucosinolate concentration in the same year was observed following a single chickpea treatment in either 2013 or 2014.

Yield and protein % were inversely proportional to mustard oil concentration and glucosinolate concentration. However, if there is high yield and high protein then high yield will compensate for any reduction in the amount of glucosinolate and mustard oil as observed in the Wheat-Chickpea-Chickpea-Mustard crop sequence.

Crop sequences will vary depending on the mustard market objective with the Wheat-Mustard-Chickpea-Mustard and Wheat-Mustard-Mustard-Mustard sequences producing more biodiesel per ha and the Wheat-Chickpea-Mustard-Mustard and Wheat-Wheat-Mustard-Mustard more sinigrin. The highest mustard yield and protein was realized in the Wheat-Chickpea-Chickpea-Mustard rotation. The highest overall yield in wheat was observed in the Wheat-Mustard-Wheat-Wheat treatment, indicating the value of mustard to cereal crops in sequence. Crop sequences did not appear to impact chickpea productivity although yields following wheat tended to be higher.

Mustard contains GSLs 2-phenylethyl ITC, 2-propenyl ITC, 3-butenyl ITC, in greater concentration than canola (Zhou et al 2014) and benzyl ITC (Olivier et al 1999), while canola has a higher level of 2-phenylethyl ITC and 3-butenyl ITC. According to Kirkegaard et al (1999) Indian mustards with high GSLs concentrations have a higher level of 2-propenyl ITC, while the level of 2-phenylethyl ITC remains the same. This means that high GSL Indian mustard has greater myrosinase activity and improved stress tolerance conditioned by better osmotic adjustment. However, canola is more commonly found in southern Australia where the rainfall is more reliable and the temperatures milder. The superior stress tolerance of mustard, reduced shattering and more effective disease suppression properties than canola suggests that means mustard could provide grain growers with more flexibility in a changing climate. Nevertheless, mustard production is

limited by the lack of an established market, although canola quality mustard in the northern region is a substitute for canola. The development of products for industrial uses will be critical if mustard production and the additional rotational benefits of this crop are to be realised. The establishment of new markets and the development and/or improvement of the processes required to extract these products could lead to new industries and should be the focus of future research. Suitable mustard germplasm and effective agronomic practices, as the current work has shown, are available and can increase the high value elements of mustard.

Finally, mustard provides a great deal of benefit over canola in the cereal and legume crop sequences in hotter and drier climates through physiological attributes such as osmotic adjustment, photoperiodism, seed size and seed colour. The genetic attributes that need to be improved include seed size, osomotic adjustment, seed colour, yield, glucosinolate concentration, oil content and quality. The benefits that come from growing mustard include biological fumigation of the soil to remove weeds and soil and plant borne diseases thus increasing yields in subsequent years. Once the oil, meal and biomass are separated the glucosinolate can be removed for high value products, such as pharmaceuticals, and oil for biodiesel and low value products developed from the waste.

In conclusion, the hypotheses that (i) that mustard can grow well in northern NSW and can be influenced by the sequence of crops grown, (ii) that mustard can produce a range of potentially saleable products which could also be affected by the crop sequence and (iii) that mustard can have an impact on the other crops in the sequence have all been answered in the affirmative.

Chapter 6: Future Research

Development of an Industrial Market

Indian mustard is becoming part of the canola market for food when grown in the northwest of NSW in the form of juncea canola or canola quality Indian mustard. However there is currently no established market for Indian mustard as an industrial crop and the establishment of this will be vital to expanding the production of this crop (Haskins et al 2009). Market development required or high, medium and low value industrial products made from the Indian mustard. The range of industrial product possibilities is large and includes high value pharmaceuticals, medium value lubricants and low value fertiliser.

Particle Film Technology

Future research into particle film technology may be of benefit in a changing climate as a way of cooling plants down. Kaolin particle film technology might have application in reducing the impact of stress (Jifon et al 2003). These kaolin particle films, when applied at 60g/lt, increase leaf whiteness, reduce midday leaf temperature, reduce leaf to air vapour pressure deficits (VPD), increase stomatal conductance and net CO_2 assimilation rates, increase photosynthetic rate, increase WUE by increasing CO_2 assimilation rates (Jifon et al 2003) and improve carbon uptake (Saour et al 2003; Teerarat et al 2013). The results suggest kaolin particle film sprays could potentially increase grapefruit leaf carbon uptake efficiency under high radiation and temperature stress. Although not yet applied to mustard, most work has been conducted on tree crops such as citrus, pome and olive trees (Jifon et al 2003; Wand et al 2006) and increases in monounsaturated fatty acids and oleic/linoleic acid ratios in olive oil from kaolin treated olive trees have been noted with higher oxidative stability and improved shelf life. Furthermore, there is a suggestion that when used in olive trees, the oleic acid levels increased to 65% (Khaleghi et al 2015). The aim of particle film technology is to optimise photosynthetic and conductance rates to improve yields and oil quality through improved oleic acid profiles and glucosinolate production. Hence the need for more intensive photosynthetic readings on the Indian mustard crop through the vegetative, flowering and early pod stages.

Phytoremediation of contaminated soil and water

Mustard grows on a range of soil types from black alluvial soil of the northwest NSW and Queensland to the sandy soils of Western Australia. Mustard also grows on soils that have been chemically compromised

such as mine sites, polluted water from gas sites, heavy metal contaminated soils and soils contaminated with PAHs (Harris et al 2009). While mustard cannot remove all contaminants, it can remove arsenic, chromium, lead, strontium, nickel, and caesium. These contaminants are stored in the roots, shoots and stems (Mullainathan et al 2007; Batty et al 2008; Iqbal et al 2012; Adair et al 2014). When mustard is used for phytoremediation it is important to grow the crop without additional stresses and with adequate nutrition. This will allow the crop to take up larger quantities of heavy metal contaminants (Hamlin et al 2003; Adair et al 2014).

Genetic modification

Genetic modification (transformation) may provide some additional benefits. Examples of genetic modification include altering the fatty acid profile and developing a waxy or hairy leaf surface. Genetic modification of the fatty acid profile of Australian germplasm led to the development of mustard genotypes producing 73% oleic acid levels. This was achieved by silencing oleate desaturase genes in elite Australian lines (Stoutjesdijk et al 2000).

While leaf coatings are beneficial in reducing plant temperatures, leaf genetic modification that absorbs less heat either through waxy or hairy surfaces could also be of benefit (Callihan et al 2000). Genetic modification may also influence glucosinolate levels, increase seed size, pod size, seed number, and reduce plant height; depending on the end-use requirement.

Future adaption of mustard

As climate change influences Australian agriculture, the work of Gunasekera, Hocking, and Stapper show that mustard is already able to withstand more extreme climatic conditions.

While grain yield and oil yield and oil quality need to be improved, it is important that the ability to withstand harsh environmental conditions is maintained. The benefit of mustard includes its annual nature, which provides greater flexibility compared to perennial crops that require significant on-going investment and long lead times to income generation (Odeh et al 2011).

There are predictions that the climate in northwest NSW will be hotter and drier in the future (Hunt et al 2009). Mustard provides additional benefits in flexibility over more permanent sources of biofuel such as *Pongamia pinnata* (Odeh et al 2011). Nevertheless, the agronomic and economic implications of introducing

mustards in a cereal-based rotation in northwest NSW are not entirely clear and dependent on climate change, even though economic quantities of oil can be produced under limited moisture (Gunasekera et al 2009).

The genetic development of mustard should also address phenology to optimise sowing date and variety selection (Kirkegaard et al 2016).

Developing the disease-suppressive ability of mustard

Historically, mustard grown in a crop sequence provides an effective disease break (Kirkegaard et al 2000). Mustard can actively suppress soil-borne pathogens and pests by releasing biocidal compounds called glucosinolates (Allyl ITC) in the soil (Kirkegaard et al 2000; Watt et al 2006; Angus et al 2015). This active pathogen suppression by the Allyl ITC compounds is called biofumigation and is thought to add some additional impacts, which have been difficult to demonstrate in extensive agriculture because expression depends on adequate soil moisture and soil temperature (Kirkegaard et al 1999; Okunade et al. 2015). However, future research into the disease-suppressive nature of crops such as mustard may enhance the break crop benefit, especially in the extensive farming systems. For example, a mustard green manure crop may potentially increase farm incomes by suppressing pathogens and pests (Larkin and Halloran 2014). This could potentially save millions of dollars over a season.

Chapter 7: Bibliography

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Chapter 8. Appendix

2014 Tables

Table 9: Means for agronomic and post-harvest traits in wheat in 2014.

	Crop Sequence				
Traits	WCW	WMW	WWW	Mean	Lsd (5%)
50% Flowering (DAS)	118.5	118.2	117.7	118.1	0.9 ns
NDVI 9/9/14	0.655	0.553	0.547	0.585	0.412 ns
NDVI 17/9/14	0.750	0.740	0.726	0.739	0.125 ns
Plant Heights (cm)	100.6	101.1	101.9	101.2	1.8 ns
Biomas Maturity (g)	463.7	449.7	442.4	451.9	85.5 ns
TKW (g)	32.2 c	28.3 a	29.8 b	30.1	0.2
% Protein	13.47 a	14.3 b	13.42 a	13.73	0.613
Yield (kg/ha)	4361 b	3670 a	3873 a	3968	687.4

Note: Means in columns followed by the same letter are non-significant at P<0.05. ns indicates a non-significant LSD

Table 10: Means for agronomic and post-harvest traits in mustard in 2014.

	Crop Sequence				
Traits	WCM	WMM	WWM	Mean	Lsd (5%)
Plant Height A (cm)	35.8 a	48.8 c	45.2 b	43.3	3.6
Plant Height C (cm)	129.6 a	153.9 c	140.1 b	141.2	6.3
Plant Height E (cm)	182.9 a	184.7 a	195.6 b	187.7	9.2
Plant Height B (cm)	35.8 a	48.8 c	45.2 b	43.3	3.6
Plant Height D(cm)	93.8 a	105.1b	94.9 a	97.9	6.8
Plant Height F (cm)	53.4 b	30.8 a	55.4 c	46.5	11.7
50% Flowering (DAS)	76.2 b	72.0 a	72.3 a	73.5	1.2
Total Biomass Dry Wt. (g)	275.7	301.8	259.8	279.1	64.2 ns
NDVI 9/9/14	0.488	0.480	0.502	0.49	0.377 ns
NDVI 17/9/14	0.679	0.660	0.672	0.670	0.151 ns
Plant Heights (cm)	182.9 a	184.7 a	195 b	187.7	9.2
Biomass Maturity (g)	216.0	208.9	248.8	224.6	81.1 ns
TKW (g)	3.7	3.8	3.7	3.7	0.6 ns
% Protein	24.0	23.6	23.6	23.7	0.3 ns
% Mustard oil (NIR)	35.6	35.7	35.7	35.7	0.2 ns
Mustard Oil (mls)	1146	1050	1164	1120	156.2 ns
Biodiesel (l/ha)	334.9	240.5	318.0	297.8	
Yield (kg/ha)	925	662.5	875.8	821.1	297.1 ns

Note: Means in rows followed by the same letter are non-significant at P<0.05.

Table 11: Means for agronomic and post-harvest traits in chickpeas in 2014.

	Crop Sequence				
Traits	WCC	WMC	WWC	Mean	Lsd (5%)
1st Pod (DAS)	122.7	122.7	122.7	122.7	1.0 ns
DAS 1	10.67	11.17	11.67	11.17	2.19 ns
NDVI 9/9/14	0.593	0.653	0.687	0.644	0.315 ns
NDVI 17/9/14	0.753	0.755	0.743	0.75	0.088 ns
Biomas Maturity (g)	264.3 ab	205 a	281.6 ac	250.3	87.3
% Protein	23. 7 b	22.4 a	21.5 a	22.5	1.6
Plant Heights (cm)	66.28 a	68.06 a	71.78 b	68.7	4.8
TKW	21.4	21.5	21.8	21.6	1.2 ns
Yield (kg/ha)	2229	1582	2107	1972	675 ns

Note: Means in rows followed by the same letter are non-significant at P<0.05
Footnote:
Das 1 = Difference between 1st Flower & 1st Pod

2015 Tables

Table 12: Wald statistics for various wheat, mustard and chickpea traits assessed in crop sequence experiments in 2015 at Narrabri.

	Species		
Traits	Wheat	Mustard	Chickpeas
Germinations (Plants/m)	34.24		5.963
Establishment (Plants/m)	31.31	6.241	
50% Flowering (DAS)	119.6***	87.94*	
1st Flower(DAS)			95
1st Pod (DAS)			119.4
DAS 1 (DAS)			24.39
Plant Height A (cm)		58.46	
Plant Height B (cm)		58.46	
Plant Height C (cm)		139.2	
Plant Height D (cm)		80.74	
Plant Height E (cm)		189.9	
Plant Height F(cm)		50.65	
Plant Heights (cm)	107.3	189.9	60.2
50% Maturity Das	156.2	169.7***	
Biomas Anthesis (g)	8046	10146	3287
Total Biomas Dry Wt.(g)	22.65 **	405.8	2.96
Photosynthesis 8/07/15		22.71	
Conductance 8/07/15		0.8053	
Photosynthesis 4/08/15		13.85	
Conductance 4/08/15		0.6063	
Photosynthesis 8/09/15	17.22		
Conductance 8/09/15	0.2166		
Photosynthesis 10/09/15	21.43		
Conductance 10/09/15	0.3513		
NDVI 29/06/15	0.5121***	0.761	
NDVI 20/07/15	0.6587***	0.7586	
NDVI 29/07/15	0.7636***	0.7487	
NDVI 30/07/15	0.7625**	0.6894	
NDVI 31/07/15	0.789***	0.6016	
NDVI 20/08/15	0.5963	0.5259	0.2157
NDVI 29/09/15	0.7067	0.5711	0.65
TC/TN Ratio	0.8333	0.8333	0.8333
Yield (kg/ha)	3794	1677***	632.9
Harvest Index	32.84*	33.64	39.53

TKW (g)	30.83	3.722	168.1
% Protein	13.01**	24.58***	22.13
Test Wt (g)	80.17*		
% Moisture		12.26	5.499
Arginine (ng/mL)	15.63		
Isoleucine (ng/mL)	40.41		
Leucine (ng/mL)	129.3		
Methionine (ng/mL)	18.33		
Phenylalanine (ng/mL)	110.4		
Proline (ng/mL)	25.59		
Tryptophan (ng/mL)	16.45		
Tyrosine (ng/mL)	272.2		
Valine (ng/mL)	79.38		
Mustard Oil mls		1073**	
%Mustard Oil		33.59***	
%Linolenic Acid C18		3.758	
%Linoleic Acid C18		23.65	
%Oleic Acid C18		69.34	
%Stearic Acid C18		1.205	
%Stearic Acid C18		0.01615	
%Erucic Acid C22		2.024	
Total Glucosinolates (mg)		4332	
Sinigrin (mg)		4123	

Note: *, **, *** equals significance at P<0.05, P<0.01, P<0,001 respectively.

Table 13: Means of wheat traits for each crop sequence treatment in 2015.

	Crop Sequences							
Traits	WCMW	WCWW	WMMW	WMWW	WWMW	WWWW	Mean	Lsd (5%)
50% Flower (DAS)	117 a	117 a	119 b	122 c	119.7 b	123 d	119.6	0.8
Biomass Anthesis (g)	8151	8421	8245	7536	8398	7528	8046	1536 ns
NDVI 29/6/15	0.659 d	0.487 b	0.591 c	0.444 a	0.592 c	0.364 a	0.512	0.075
NDVI 20/7/15	0.764 c	0.56 a	0.689 b	0.588 a	0.688 b	0.683 b	0.659	0.079
NDVI 29/7/15	0.851 d	0.769 b	0.818 c	0.649 a	0.825 c	0.670 a	0.764	0.056
NDVI 30/7/15	0.837 d	0.762 b	0.823 c	0.656 a	0.815 c	0.682 a	0.763	0.093
NDVI 31/7/15	0.849 f	0.784 c	0.833 e	0.822 d	0.747 b	0.699 a	0.789	0.043
Photosyn. 8/9/15	17.7	17.4	15.9	16.3	18.1	18.1	17.2	5.4 ns
Conduct.8/9/15	0.236	0.181	0.212	0.181	0.277	0.211	0.217	0.12 ns
Photosyn.10/9/15	23.4 b	22.1 a	21.8 a	21.6 a	20.0 a	19.7 a	21.4	2.68
Conduct.10/9/15	0.353	0.379	0.319	0.401	0.310	0.346	0.351	0.17 ns
Plant Hts (cm)	107	107.3	106.8	108	107	107.9	107.3	3.5 ns
Maturity (Das)	156.3 a	155.7 a	154.7 a	157.3 b	156.3 a	156.7 a	156.2	2.3
Biomas Mat.(g)	604.2 c	575.8 b	470.2 a	609.7 c	460.8 a	634.2 d	559.1	108.5
Yield (kg/ha)	3580 a	3758 a	3847 a	4061 b	3798 a	3720 a	3794	468
% protein	14.5 c	13.7 b	12.7 a	11.4 a	13.7 b	12 a	13.0	1.6
TKW (g)	30 a	31.7 a	28.3 a	35 b	28.3 a	31.7 a	30.8	6.6
Test Wt.(g)	78.9 a	79.27 a	80.5 a	81.8 b	79.4 a	81.1 b	80.2	1.9
Harvest Index	31.14 a	31.07 a	29.22 a	38.1 c	31.99 a	35.49 b	32.84	4.95
Arginine (ng/ml)	18.52	15.83	15.88	14.38	14.0	15.17	15.63	5.79 ns
Isoleucine (ng/ml)	49.19	42.08	39.98	36.99	38.39	35.82	40.41	12.98 ns
Leucine (ng/ml)	147.8	132.4	134.3	120.3	124.5	116.8	129.3	33.96 ns
Methion.(ng/ml)	22.71	20.33	18.48	18.5	14.49	15.5	18.33	9.38 ns
Phenylal. (ng/ml)	130.3	113.1	117.4	102.9	98.5	100	110.4	38.01 ns
Proline (ng/ml)	42.07 c	27.09 b	27.17 b	15.82 a	23.11 a	18.31 a	25.59	8.97
Trypto. (ng/ml)	21.38	17.46	18.23	15.14	12.34	14.13	16.45	9.65 ns
Tyrosine (ng/ml)	327.8	288.6	275.5	255.1	240.5	245.5	272.2	111 ns
Valine (ng/ml)	92.09	81.07	80.43	72.26	77.0	73.44	79.38	15.57 ns

Note: Means in rows followed by the same letter are non-significant at $P<0.05$

Footnote:
Meth. = Methionine
Phenyl. = Phenylalanine
Tryp. = Tryptophan

Table 14: Means of mustard traits under different crop sequences in 2015.

	Crop Sequences							
Traits	WCCM	WCMM	WMCM	WMMM	WWCM	WWMM	Mean	Lsd (5%)
50%Flow. (DAS)	90 c	89.7 b	87.7 a	87.3 a	86 a	87 a	87.9	2.5
Plant Ht.B (cm)	61.7	55.3	61.3	56.9	57.6	58	58.5	9.1 ns
Plant Ht.D (cm)	85.9 c	85.2 c	71.7 a	79.9 a	83.4 b	77.3 a	80.7	11.1
Plant Ht.F (cm)	147.6 b	141.6 a	133 a	136.8 a	141 a	135.3 a	139.2	13
Plant Ht.E (cm)	196.1	191.7	191.2	188.4	184.7	187	189.9	15.9 ns
Plant Hts (cm)	196.1	189.2	191.2	188.4	184.7	189.4	189.9	16.2 ns
NDVI 29/6/15	0.781a	0.803 c	0.717 a	0.748 a	0.782 b	0.735 a	0.761	0.065
NDVI 31/7/15	0.729a	0.782 b	0.672 a	0.730 a	0.693 a	0.728 a	0.722	0.079
Photosyn.8/7/15	23.1	22.6	21.7	22.3	22.4	24.2	23.1	2.9 ns
Conduct. 8/7/15	0.829	0.910	0.688	0.862	0.609	0.934	0.805	0.397 ns
Photosyn.4/8/15	13.7	13.3	12.9	15.4	13.8	14.0	13.9	3.3 ns
50% Mat. (DAS)	174 c	168.7 a	168.7 a	167.3 a	170.7 b	168.7 a	169.7	2.1
Biom. Anth. (g)	8950 a	13592 b	10504 a	10033 a	8988 a	8808 a	10146	3644
Tot.Biom. D.Wt.(g)	358 a	543.7 b	420.2 a	401.3 a	359.5 a	352.3 a	405.8	145.8
Biomas Mat (g)	406.2	303.2	392.9	369.3	445.3	329.6	374.4	181.8 ns
TKW (g)	4	3.7	3.7	3.7	4	3.3	3.7	1.1 ns
Harvest Index	36.1	32.6	36.2	34.2	31.8	31.1	33.6	7.99 ns
% Protein	27.3 c	24.2 a	23.9 a	23.2 a	25.5 b	23.4 a	24.6	1.68
Yield (kg/ha)	2277 c	1507 a	1572 a	1416 a	1880 b	1410 a	1677	327.5
% Mustard oil	32.5 a	33.4 b	33.8 b	34.4 c	33.1 b	34.3 c	33.6	0.421
Must. Oil (mls)	705 a	1165 b	1163 b	1290 c	868 a	1248 c	1073	269.9
Must.Oil (L/ha)	258 a	283 b	294 c	294 c	262 a	283 b	279	13.95
Biodiesel (L/ha)	753.4	512.3	539.6	495.9	632.2	491.5	570.8	
% Oleic Acid	69.2	69.3	69.5	70.0	69.1	69.00	69.3	2.2 ns
% L/leic Acid	24.2 b	24.1 b	24.3 b	21.3 a	23.8 a	24.2 b	23.7	2. 7
% L/lenic Acid	4.2	4.1	3.3	2.9	4.1	4.0	3.8	2.2 ns
% Palm. Acid	0.46 a	0.72 b	0.81 c	0.71 b	0.74 b	0.79 b	0.705	0.18
% Stearic Acid	0.022	0.009	0.014	0.031	0.003	0.018	0.016	0.035 ns
% Erucic Acid	1.9	1.8	2.1	2.1	2.3	2.0	2.0	1.59 ns
Tot. GLS (mg/100g)	4415	4637	3943	4237	4133	4626	4332	
Sinig.(mg/100g)	4116	4511	3751	4033	3902	4427	4123	
Sinigrin (kg/ha)	93.7	68.0	59.0	57.1	73.4	62.4	68.9	

Note: Means in rows followed by the same letter are non-significant at P<0.05

Table 15: Means of agronomic and post-harvest traits in chickpeas in 2015.

	Crop Sequences							
Traits	WCCC	WCWC	WMCC	WMWC	WWCC	WWWC	Mean	Lsd (5%)
F1 DAS	95.3 a	95.3 a	96 b	94.7 a	94.7 a	94 a	95	1.7
P1 DAS	118.7 a	120 b	119.3 a	120 b	118.3 a	120 b	119.4	1.6
DAS 1 (DAS)	23.3	24.7	23.3	25.3	23.7	26 b	24.4	2.0 ns
BA (g)	3259	3084	3348	3742	3207	3082	3287	1495 ns
NDVI 20/8/15	0.218 a	0.210 a	0.173 a	0.246 b	0.213 a	0.238 a	0.216	0.070
NDVI 29/9/15	0.653 a	0.627 a	0.687 a	0.61 a	0.71 b	0.613 a	0.65	0.084
P.H. (cm)	63.2 b	57.1 a	60 a	60.3 a	59.9 a	60.7 a	60.2	5.3
B.M.	167.7	222.2	210.2	208	188.2	238.1	205.7	99.8 ns
% Protein	23.3	21.1	24.7	21.7	22.2	19.9	22.1	8.3 ns
TKW (g)	168.3 a	166.7 a	171.7 a	165 a	183.3 b	153.3 a	168.1	28.3
H.I.	38.64	42.86	35.93	41.16	38.19	40.38	39.53	9.66 ns
Yield kg/ha	617.4 a	633.4 a	536 a	621.9 a	678.4 a	710.2 b	632.9	156.1

Note: Means in rows followed by the same letter are non-significant at P<0.05

Footnote:
F1 = % 1st Flower

P1 = % 1st Pod

BA = Biomas Anthesis (g)

PH = Plant Heights (cm)

BM = Biomas Maturity (g)

H.I. = Harvest Index

W = Wheat

M = Mustard

C = Chickpea

DAS = Days after sowing

Soil Data 2014-15

Table 16: A comparison of soil moisture and soil nutrients for 2014-15 in wheat treatments

			Crop Sequences					
	Measurements		WWWW	WWMW	WMWW	WMMW	WCWW	WCMW
2015	Soil Moist	%	32.7	33.2	30.9	30.5	30.1	30.3
2014	Av. Soil Moist	%	24.34	24.34	24.34	24.34	24.34	24.34
2015	OC	%	0.81	0.70	1.09	1.00	0.68	1.03
2014	OC	%	0.9	0.9	0.9	0.9	0.9	0.9
2015	NO3-N	mg/kg	0.5	0.5	0.5	1.6	2.0	2.0
2015	NO3-N	base line	0.5	0.5	0.5	0.5	0.5	0.5
2015	Colwell P	mg/kg	47.0	36.0	46.7	48.3	43.3	45.4
2014	Colwell P	mg/kg	35.3	35.3	35.3	35.3	35.3	35.3
2015	SO4-S	mg/kg	1.5	7.4	2.9	6.8	1.5	6.6
2014	SO4-S	mg/kg	8.8	8.8	8.8	8.8	8.8	8.8

Note: Means in rows followed by the same letter are non-significant at P<0.05

Table 17: A comparison of soil moisture and soil nutrients for 2014-15 in mustard treatments

			Crop Sequences					
	Measurements		WWMM	WWCM	WMMM	WMCM	WCMM	WCCM
2015	Soil Moist	%	21.4	23.7	20.5	22.7	18.8	19.7
2014	Av. Soil Moist	%	24.34	24.34	24.34	24.34	24.34	24.34
2015	OC	%	0.68	0.85	0.83	1.06	0.89	0.87
2014	OC	%	0.9	0.9	0.9	0.9	0.9	0.9
2015	NO3-N	mg/kg	0.7	0.5	0.5	0.5	0.5	0.5
2015	NO3-N	base line	0.5	0.5	0.5	0.5	0.5	0.5
2015	Colwell P	mg/kg	45.8	37.3	43.6	68.0	53.0	44.5
2014	Colwell P	mg/kg	35.3	35.3	35.3	35.3	35.3	35.3
2015	SO4-S	mg/kg	6.9	6.5	10.2	13.3	11.6	7.9
2014	SO4-S	mg/kg	8.8	8.8	8.8	8.8	8.8	8.8

Note: Means in rows followed by the same letter are non-significant at P<0.05

Table 18: A comparison of soil moisture and soil nutrients for 2014-15 in chickpea treatments

			Crop Sequences					
	Measurements		WWCC	WWWC	WMCC	WMWC	WCCC	WCWC
2015	Soil Moist	%	31.1	32.0	33.1	31.7	31.9	31.2
2014	Av. Soil Moist	%	24.34	24.34	24.34	24.34	24.34	24.34
2015	OC	%	0.74	0.83	0.74	0.72	0.78	0.69
2014	OC	%	0.9	0.9	0.9	0.9	0.9	0.9
2015	NO3-N	mg/kg	0.5	0.5	0.5	0.5	1.7	1.3
2015	NO3-N	base line	0.5	0.5	0.5	0.5	0.5	0.5
2015	Colwell P	mg/kg	43.6	45.0	36.0	38.0	50.5	54.4
2014	Colwell P	mg/kg	35.3	35.3	35.3	35.3	35.3	35.3
2015	SO4-S	mg/kg	3.5	2.0	4.1	2.1	2.4	2.6
2014	SO4-S	mg/kg	8.8	8.8	8.8	8.8	8.8	8.8

Note: Means in rows followed by the same letter are non-significant at P<0.05