Australian Government

Department of Health

Office of the Gene Technology Regulator

The Biology of *Carthamus tinctorius* L. (safflower)

Photo: GRDC.

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This document provides an overview of baseline biological information relevant to risk assessment of genetically modified forms of the species that may be released into the Australian environment.

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PREAMBLE

This document describes the biology of *Carthamus tinctorius* L., with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origins of cultivated *C. tinctorius*, general descriptions of its morphology, reproductive biology, biochemistry, and biotic and abiotic interactions. This document also addresses the potential for gene transfer to occur to closely related species. The purpose of this document is to provide baseline information about the parent organism for use in risk assessments of genetically modified (GM) *C. tinctorius* that may be released into the Australian environment.

The common names for *C. tinctorius* vary with country, region, language and use, but it will be referred to as safflower or *C. tinctorius* in this document.

Safflower is a branching, spiny, thistle-like herbaceous annual plant. Originally, safflower was grown for its floral pigments for use as red (carthamin) and yellow dyes and for medicinal purposes. Now, it is mainly cultivated in hot dry climates as an oilseed and to a lesser extent for meal, birdseed and seed for small animals such as mice and guinea pigs. Safflower is one of humanity's oldest crops, and yet it remains a minor crop compared to other oilseeds.

Section 1 TAXONOMY

Cultivated safflower (*Carthamus tinctorius* L.) is an annual oilseed crop that is a member of the family Asteraceae (Compositae), tribe Cardueae (thistles) and subtribe Centaureinae (Berville et al. 2005). Asteraceae is recognised as the largest family of flowering plants and contains more than 1500 genera and 22,000 species ranging from annual herbs to woody shrubs. *C. tinctorius* is the only species in this genus cultivated for human use (Griffee 2001).

The taxonomy of *Carthamus* has changed substantially as data for this group has been obtained and interpreted (McPherson et al. 2004; Sehgal & Raina 2011). There have been as few as four species in the genus (with related species in a separate genus) to as many as 25 species and subspecies divided in up to five sections. The sections were based on five chromosome groups identified by Ashri and Knowles (1960) n=10, 11, 12, 22 and 32. Safflower belongs to a *Carduncellus-Carthamus* complex and morphological and cytological characters have not been sufficient to delimit the species into discrete sections and genera. Depending on the taxonomist and the emphasis on particular morphological characters, species have been moved between the genera *Carthamus* and *Carduncellus* (McPherson et al. 2004). Determining species relationships is made more difficult by the low levels of genetic variation that occurs when clear morphological differences are present (Mayerhofer et al. 2011).

The classification scheme followed in this document is that of (Lopez Gonzalez 1990) (Table 1), which recognises 16 species within *Carthamus* and another closely related species, *Femeniasia balearica.* The species have been further divided into three sections based on chromosome numbers, the Section *Carthamus* (n=12), Section *Odonthagnathis* (n=10 or 11), Section *Atractylis* (n=22 or 32) and two species of uncertain placement.

C. oxyacantha and *C. persicus* were thought to be the parent species of *C. tinctorius* (Ashri & Knowles 1960). More recent genetic analysis and geographic evidence indicate that *C. palaestinus* is the wild progenitor of safflower and originated in the Middle East, near Israel and is fully cross-compatible with safflower (Pearl et al. 2014).

Table 1 Taxonomic groups of *Carthamus sensu* (Lopez Gonzalez 1990)

Section	Species	Chromosome number	Recorded as present in Australia?
Carthamus L.	*C. tinctorius* L.	2n=2x=24, n=12	Yes
	C. oxyacanthus Bieb.	2n=2x=24, n=12	No
	C. palaestinus Eig	2n=2x=24, n=12	No
	C. persicus Willd. (basionym *C. flavescens* auct.)	2n=2x=24, n=12	No
	C. curdicus Hanelt.	2n=2x=24, n=12	No
	C. gypsicola Ilj.	2n=2x=24, n=12	No
Odonthagnathis (DC.) Henelt	*C. divaricatus* Beguinot & Vacc.	2n=2x=22, n=11	No
	C. leucocaulos Sm.	2n=2x=20, n=10	Yes
	C. glaucus Bieb.	2n=2x=20, n=10	Yes*
	C. tenuis (Boiww. & Bl.) Bornm.	2n=2x=20, n=10	No
	C. dentatus (Forssk.) Vahl	2n=2x=20, n=10	Yes
	C. boissierei Haláacsy	2n=2x=20, n=10	No
Atractylis Reichemb.	*C. lanatus* L.	2n=4x=44, n=22	Yes
	C. creticus L. (syn *C. baeticus* (Boiss & Reuter) Nyman)	2n=6x=64, n=32	No
	C. turkestanicus Popov	2n=6x=64, n=32	No
Uncertain placement	*C .nitidus* Boiss.	2n=2x=24, n=12	No
	Femeniasia balearica Susanna	2n=2x=24, n=12	No

** Some uncertainty, see Section 8.2.*

Section 2 ORIGIN AND CULTIVATION

2.1 Centre of diversity and domestication

Safflower is a very ancient crop that is believed to have a single origin of domestication from approximately 4000 years ago in the Fertile Crescent region. This region ranges from southern Israel to Western Iraq (Chapman et al. 2010). Safflower has been grown for centuries in India, China and North Africa. Over 800,000 tons are grown worldwide in over 20 countries; the main producers being India, United States, Mexico and Argentina. Other countries that produce safflower include Australia, Canada, Russia, China, Spain, Italy and Turkey (Gilbert et al. 2008). India produces about half of the world's safflower crop each year (430,000 tons). Worldwide safflower is a minor crop compared to other oilseeds.

Seven "centres of similarity" were identified by Knowles (1969) namely the Far-East, India, the Middle-East, Egypt, Sudan, Ethiopia and Europe. Ashri (1971) added more centres but these were not centres of diversity or origin, but of very similar safflower types. Considerable genetic diversity exists across different genotypes. When 60 representative genotypes from India and other countries were examined it was observed that plant height, seed yield, branching height and seed weight accounted for 80% of the diversity (Patel et al. 1998). Patel et al (1980) identified 14 clusters of genetic diversity, but distribution into clusters was random showing that geographic isolation is not the only factor causing genetic diversity. Up to ten centres of similarity throughout the world were identified based on morphology. Nuclear microsatellite analysis of accessions suggests the presence of five genetic clusters, one in each of the following regions: Europe; Turkey-Iran-Iraq-Afghanistan; Israel-Jordan-Syria; Egypt-Ethiopia; and Far East-India-Pakistan (Chapman et al. 2010).

The different species of *Carthamus* are all believed to have one common ancestor, probably from Iraq & north-western Iran. With the exception of cultivated safflower, the species are all spiny weeds that grow in the wild. There appear to be three wild species that are very closely related. *Carthamus flavescens* (= *C. persicus*) is usually found in wheat fields in Lebanon, Syria, and Turkey. *Carthamus oxyacantha* is a very serious weed in the area from western Iraq to north-western India and northward into the southern parts of some former republics of the USSR. *Carthamus palaestinus* is found in the desert regions of Iraq, Israel, and Jordan. These species readily cross with *C. tinctorius* to produce fertile progeny. It is thought that early in its evolution, safflower spread to Egypt, Ethiopia, South Asia, and the Far East, where distinct types have evolved (see review by Smith (1996) and references therein).

Domestication of safflower has resulted in traits such as reduced shattering, smooth seeds, reduced duration of the early vegetative growth stage, restriction of branching to the upper part of the stem, and reduced seed dormancy (Berville et al. 2005). Breeding programs have resulted in the release of cultivars with higher oil content and/or increased disease resistance in recent years (GRDC 2010).

2.2 Commercial uses

Historically safflower was grown for the flowers or floral pigments that were used in making red (carthamin), orange and yellow dyes for colouring fabrics until cheaper aniline dyes became available in the early 19th century (Dajue & Mündel 1996).

Prior to the 1960s in the United States, the oil from seeds was used mostly as a base for paints and in Australia was introduced in the 1950's due to shortages in drying oils for the paint and resin industries (Smith 1996). It is still used in paints and varnishes today because of its non-yellowing characteristic.

In Australia the primary use is as an oilseed (Gilbert et al. 2008), but it also used as birdseed. Whole safflower seeds are used in the birdseed industry, mainly for wild birds, especially for members of the parrot family and pigeons (Dajue & Mündel 1996). In Canada, most safflower produced is for the birdseed market (Mündel et al. 2004).

Worldwide the primary use for safflower is edible seed oil for use in cooking, salad oils and margarine. The meal left over after extraction of oils from seeds can be used as a stockfeed for cattle and other livestock. The meal is unsuitable for monogastric animals such as swine and poultry, due to hulls not being removed resulting in a high fibre content (30–40%) (Dajue & Mündel 1996).

Cultivated varieties of safflower range in seed oil content from 20–45% of the whole seed (Dajue & Mündel 1996). There are two groups of safflower cultivars differing in seed oil composition, characterised by high linoleic acid (70–75% of total fatty acids) and high oleic acid (70–75%) (Singh & Nimbkar 2006). Commercial safflower cultivars grown in Australia are either those high in the monounsaturated fatty acid, oleic acid or those high in the

polyunsaturated fatty acid, linoleic acid. The safflower varieties that are high in oleic oil are used as heat stable cooking oil, cosmetics and infant food formulations. The linoleic oil varieties contain nearly 75% linoleic acid which is used for edible oil products such as salad oils and soft margarines (GRDC 2010). Public awareness about the health benefits of certain fatty acids has already made safflower an important crop for the vegetable oil market (Dajue & Mündel 1996).

2.2.1 Livestock feed

The use of safflower seed or seed meal as livestock feed is limited by several factors. Unless the seed or seed meal has been dehulled, the high fibre content present palatability and digestibility problems, particularly for ruminants and poultry. Most commercial safflower meal includes hulls, and therefore has very high fibre content. Compared to other oilseed meal, the quality of safflower protein is low due to its deficiency in lysine, methionine and isoleucine, the sulphur containing amino acids. Additionally, the protein fraction of the meal contains two phenolic glucosides, the bitter-flavoured matairesinol-β-glucoside and the purgative 2-hydroxyarctiin-β-glucoside.

A summary of a recent review of the use of safflower seed and seed meal as livestock feed is presented below. Unless otherwise cited, the information below is from the website *feedipedia* (Heuzé et al. 2012).

RUMINANTS

Generally whole safflower seeds and hulled seed meal are less palatable than other common oilseeds. Whole seeds can be feed to beef cattle but due to the high fibre content their use should be limited or animal performance will suffer. The incorporation of hulls can lead to a reduction in feed efficiency (because of low digestibility) unless the diet is supplemented with adequate energy and protein.

Palatability of the hulled seed meal is variable, sometimes presenting a problem for beef cattle but apparently not for dairy cattle or rams. In young sheep, supplementing poor quality diets with safflower meal resulted in increased weight gain and wool growth compared to a barley/urea supplement. Research has shown safflower meal to be a valuable ingredient for dairy cows, with no noticeable effect on flavour or odour of milk produced. However, an oxidised flavour may develop in milk, if lactating dairy cows are fed more than 2–3 kg/day of high linoleic acid safflower (Mündel et al. 2004). Replacing cottonseed meal with safflower meal may increase milk fat content in Friesian cows and buffaloes.

Safflower could be used as forage, but there are much more productive crop/pasture options. Use of safflower as a forage could occur where seed may be of inferior quality, such as after an early frost or drought. Hay from safflower cut after flowering is likely best suited to sheep and goats, as they are not irritated by the spines; but cattle consuming the spines are more susceptible to mouth ulcerations (Mündel et al. 2004).

PIGS

Unhulled safflower seed is not usable by pigs due to the high fibre content. Safflower meal is not very suitable feed for pigs; protein quality is low due to its deficiency in essential amino acids. It is not recommended to feed safflower meal to weanling pigs. Dehulled safflower meal is suitable for grower-finisher pigs, but only if supplemented with lysine. Up to 12% safflower meal can be included in the diet of growing pigs provided additional lysine is included. Pregnant sows may have up to 15% dehulled safflower meal in their diets, but this should be reduced to much lower levels when lactating.

POULTRY AND OTHER BIRDS

Generally, due to the high fibre content, whole seed and unhulled seed meal are of low value. Partial or total dehulling can enable the use of safflower products in poultry but incurs the additional expense of the dehulling process.

Whole safflower seeds are used as feed for broiler chickens at levels of up to 20% with no effect on performance and carcass traits. In contrast, levels of up to 10% tended to lower performance of layers (not significantly) and can increase linoleic acid in the yolk. Safflower seeds can be safely included in broiler and layer diets at a 10% level.

The use of dehulled safflower seed meal for poultry needs to include supplementation with some amino acids (lysine and methionine). For example, performance in broilers on a diet of 22% safflower meal (supplemented with lysine) was almost halved without supplement. Layers would require supplemental lysine and methionine.

Safflower seeds are used as birdseed especially for members of the parrot family and pigeons.

OTHER ANIMALS

Safflower seed or seed meal can be included in the diet of rabbits, gerbils, hamsters and chinchillas.

2.2.2 Medicinal uses

Safflower seeds, oils and flowers have a wide range of medicinal uses in many countries. Safflower has been used in China since the 2nd century B.C. almost exclusively for medicinal purposes (Dajue & Mündel 1996). The flowers are used as tonics for a range of conditions such as dilation of arteries, reduction of hypertension and increased blood flow.

Seed decoctions are used as laxatives, for urinary tract infections and to reduce rheumatic pain (Mündel et al. 2004). A tea made from flowers has been developed in China and India and marketed as herbal health teas. Women in India and Afghanistan have used teas made from foliage to prevent abortion and infertility (Emongor 2010). It was expected that use of both seeds and flowers may increase profits for farmers and increase production areas in India (Singh & Nimbkar 2006).

The oil is used in Iran to treat liver and heart ailments and in India to treat sores and rheumatism. It has also been used to treat cerebral thrombosis and has lowered blood pressure in over 90% of patients (Dajue & Mündel 1996; Emongor 2010). Safflower decoctions have been used to successfully treat male sterility (Qin Yuehao (1990) as cited in Dajue & Mündel 1996). The oil of a GM safflower has been approved by the US-FDA for use as a dietary supplement (Nykiforuk et al. 2012).

2.2.3 Industrial applications

Over the last decade, there has been increased demand for vegetable oils in food, feed and bio-based industrial materials. Vegetable oils consist of triacylglycerides (TAGs). The energy density of TAGs has made vegetable oils an attractive source of biodiesel, produced by transesterification of TAG fatty acids. Monounsaturated fatty acids such as oleic acid are highly heat stable and biodegradable and are well suited to use in the oleochemical industry (bio-based plastics, foams, and fluids) and could replace petroleum based sources in the manufacture of a number of industrial products such as lubricants and hydraulic fluids (GRDC 2010). Other minor industrial uses for safflower oil include cosmetics, soaps, and infant formula.

2.3 Cultivation in Australia

2.3.1 Commercial propagation

Safflower is an annual oilseed crop that is propagated by seed. It is either self- or insect-pollinated, with little or no pollination by wind. Outcrossing rates between adjacent plants can be quite high, approaching 100% in some varieties (see Section 9.1). Long distance outcrossing between safflower plants has been reported to occur at a rate of 0.01% at a distance of 100 m or not at all when plots were separated by 300 m (McPherson et al. 2009a). The OECD Seed Scheme for Varietal Certification, which applies in Australia and many other countries, requires that crops of certified safflower seed be grown with an exclusion distance of 200 m from other safflower crops, and that basic safflower seed (the source for certified seed) be grown with an exclusion distance of 400 m (OECD 2013). The Association of Official Seed Certifying Agencies (AOSED) which administers standards for certified seed production in the USA requires and isolation distance of 1,320 ft (403 m) for all classes of safflower seed (AOSCA 2012).

2.3.2 Scale of cultivation

Safflower has been grown in Australia since the 1950's. It was introduced in response to shortages in drying oil in the paint and resin industries. Production expanded to 48,000 ha by 1968. Safflower was initially mainly grown in Queensland until its decline in 1970s due to droughts and a severe disease outbreak of *Alternaria* (a fungal pathogen). Following the abolishment of quotas on the use of vegetable oils for margarine production in 1976, safflower production increased again peaking at 74,688 ha in 1979 when record prices were paid for safflower (GRDC 2010). This represents less than 0.5% of total cropping area in Australia. Production did decline again, due to a combination of volatile market prices and competition from other oilseed crops such as cotton, canola and sunflower which developed in the 1960's and 1970's (Jochinke et al. 2008; Wachsmann et al. 2008).

In Australia safflower production has shifted from northern NSW and southern Qld to include the higher rainfall (>450 mm) cereal growing regions of southern NSW, VIC and SA (GRDC 2010). This shift in production coincided with the introduction of two disease resistant cultivars released in the late 1980's (Jochinke et al. 2008). In 1987 CSIRO released the varieties Sironaria (resistant to *Alternaria* and moderate resistance to *Phytophthora*) and Sirothoria (resistant to *Phytophthora* and susceptible to *Alternaria*) (GRDC 2010). *Phytophthora* is a mould, a fungal-like pathogen. The Australian industry was based primarily on Sironaria which has high linoleic acid content and is also suitable for birdseed markets. Additional cultivars were introduced in the 1990's such as S555 (high linoleic oil) and S517 (high oleic oil) (Jochinke et al. 2008). Other cultivars have been imported in recent years but production is still limited to about 10,000 ha (ABARES 2014).

Between 1970 and 2005, average safflower production in Australia has ranged from 1.9–57.7 kt from an area of 3.6–74.7 kha (FAO 2008) per annum. Safflower yields are variable, ranging from 0.2–4.5 t/ha, and is dependent on many factors such as planting date (winter vs. spring), sowing rates, cultivars and water availability (Wachsmann et al. 2008).

2.3.3 Cultivation practices

Traditionally, safflower was grown in hot arid dry regions, but it is a highly adaptable plant. In the Americas, commercial production extends from southern Canada south into Argentina (Dajue & Mündel 1996).

In Australia, safflower is an annual plant with a long growing season. It is generally sown in June or early July in northern and central NSW and during July in southern NSW, VIC and SA.

Provided there is water available, sowing could occur as late as September and early October in parts of VIC and SA. However, yield is related to sowing time and is most reliable when the crop is sown in late June or early July (GRDC 2010). Sowing date has been shown to affect seed oil content (Mirshekari et al. 2013). Safflower may be sown later than other winter crops, which allows it to be used for weed management or as an option when earlier planted winter crops have failed to establish (GRDC 2010).

Sowing rates depend on region and moisture availability with typical rates of 12–15 kg/ha in northern NSW to 18–24 kg/ha in Victoria and SA, seeding rates would be lower in drier conditions (9 kg/ha in northern NSW) and as high as 25–31 kg/ha under irrigation. Typical plant stands would be 20–25 plants m^{-2} in the north and 30–40 plants m^{-2} in the southern regions (GRDC 2010).

Ideally sowing should be into moist soil, typically between 2 and 5 cm depth but this will vary with soil type and conditions. Delayed emergence and reduced early vigour can occur due to deeper sowing, leaving plants susceptible to pest, disease and competition from weeds (Mikkelsen et al. 2008). Safflower is normally planted with standard cereal sowing equipment in rows 18–36 cm apart. Narrower rows help suppress weeds, while wider spacing allows for better airflow for disease control (GRDC 2010).

Seedlings emerge 1–3 weeks after sowing. Emergence takes longer under cooler temperatures, which also increases the risk of insect damage and disease. Plants spend 2–3 weeks in the rosette stage while growing leaves and are susceptible to frosts below -7 °C. The rosette stage is followed by stem elongation, branching and flowering stages. After flowering the time to maturity is about four weeks. The time from sowing to harvest is around 26–31 weeks, but varies with variety, location, sowing time and growing conditions. Timing of flowering is influenced more by day length than sowing date. In Australia, flowering of winter sown safflower generally coincides with wheat harvest (GRDC 2010).

It has a deep root system, which makes it ideal for rain-fed cropping systems (Singh & Nimbkar 2006). Well-drained, deep, fertile, sandy loam soils provide maximum safflower yields (GRDC 2010). In Australia, due to its deep tap root, safflower is often used on problem soils to break up hard pans and improve water and air infiltration in the subsoil (GRDC 2010).

Safflower does have high water requirements but does not tolerate waterlogging well. Due to the large tap root, which can elongate up to three metres, the plant can extract water into deeper layers of soil than many other crop plants (Dajue & Mündel 1996; GRDC 2010) and thus is considered quite drought tolerant. Irrigation can extend the growing season by 2 weeks whereas maturity is reached earlier (hastened) by drought, salinity, increased temperatures or day length. Safflower is considered to have moderate to high tolerance to salinity, being similar to barley or cotton (GRDC 2010).

Safflower is moderately frost tolerant, during the rosette stage, but is susceptible to frost damage from the stem elongation stage to maturity. It is also relatively resistant to hail or wind damage (Mündel et al. 2004). Although safflower can access nutrients from deeper than cereal crops, fertilisers tend to increase yields and oil levels, especially in irrigated or higher rainfall areas. Fertiliser application rates are dependent on expected yields based on available soil moisture (or irrigation). One tonne of safflower seed removes 25 kg nitrogen, 4.3 kg phosphorous and 4 kg sulphur from the soil. Most soils (with the possible exception of sandy soils) contain adequate levels of potassium and sulphur (GRDC 2010).

Safflower is a poor competitor with weeds and weed management is essential when growing this crop. Cultivation could be used to control weeds when the safflower plants are 7 to 15 cm tall. As a minor crop, the number of herbicides available for use in Australia is limited (GRDC 2010), but some pre-emergent herbicides, as well as in-crop grass and broadleaf selective products are registered for use in safflower (see Section 7.1).

HARVEST

Safflower sown in winter is usually ready for harvest 4 to 6 weeks after wheat. Harvest of safflower generally begins in late December in northern NSW and continues into March in the south east of SA. In Australia, it is recommended that seed moisture at the time of harvest should be less than 8% to avoid overheating and mould formation during processing and storage. It is also recommended that harvest occur as soon as possible, as rain can stain the seed reducing its value (GRDC 2010). In parts of Canada, seed is harvested at 12–15% moisture and then dried by aeration (Mündel et al. 2004).

Safflower is generally harvested without swathing. The same machinery used for cereals can be used for safflower but ground speeds are slower to reduce seed loss. Periodic cleaning of equipment to remove bristles from radiators and hot engine components may be necessary to minimise the risk of fire (GRDC 2010). In addition, harvesting in cooler or more humid parts of the day is recommended both to reduce the risk of fire and to increase seed cleanliness (Jochinke et al. 2008). In Australia, seed loss during harvest (direct heading) is about 3 to 4% (GRDC 2010).

2.4 Crop Improvement

Safflower produces some of the healthiest oils for human consumption and despite favourable agronomic traits such as drought resistance and adaption to arid regions; it still remains a minor crop worldwide. In the past this has been due to its low oil content and yield relative to other oilseed crops such as canola and cotton and susceptibility to diseases and insect pests. Hence, the major breeding objectives have been to improve seed yield, seed oil content and quantity and disease resistance

The primary end uses of safflower seed oil are for the edible and industrial oil markets and to a lesser extent the bird seed market (Knowles 1989). Modern plant breeding has been used to develop cultivars with different fatty acid oil profiles, quantity and quality. This includes speciality oils thought to have beneficial health effects such as oils with high γ-linoleic acid (GLA) and increased tocopherol content (Nykiforuk et al. 2012; Velasco et al. 2005). Safflower oil also has potential in the biofuel industry (Patrascoiu et al. 2013) and as a platform for production of pharmaceuticals in GM safflower seed (Mündel et al. 2004; Nykiforuk et al. 2012) (see section 2.4.2).

2.4.1 Breeding

OIL CONTENT

The primary objective of safflower breeding programs over the years has been to increase oil content. Prior to 1942, seed of commercial cultivars had less than 28% oil per whole seed. Current varieties grown in Australia have up to 42% oil content (GRDC 2010). Breeding programs in the United States have successfully developed cultivars with oil content of 45–55% (see review by Sehgal & Raina 2011).

Selection from local varieties is the most common breeding method used for safflower cultivar development in India and several germplasm lines with desired traits have been developed (Singh & Nimbkar 2006). This germplasm can then be used for breeding in other countries, through selection and/or hybridisation with local lines. In the 20th century, safflower cultivars were developed in the United States, Canada and Argentina using introduced germplasm from India, Russia and Turkey (Singh & Nimbkar 2006).

Seed yield and oil content are the most complex traits in safflower and selection for them is hampered by large genetic-environment interaction. Seed yield is positively correlated, but seed weight negatively correlated, with oil content. The proportion of hull content is positively associated with seed weight but negatively associated with oil content. The thick pericarp keeps oil production low, so a reduction of the pericarp will increase oil content. Selection for

high oil content can be performed using the thumbnail method, due to seeds with high oil content having thin hulls and are easily pressed using thumbnail pressure (Singh & Nimbkar 2006).

HYBRID SAFFLOWER

Dominant and recessive genetic male sterility (GMS), cytoplasmic male sterility (CMS) and thermo sensitive genetic male sterility (TGMS) systems for producing hybrid safflower plants have been developed (Meena et al. 2012; Singh et al. 2008).

GMS safflower lines (both spiny and non-spiny flowered lines), which exhibit an increase of 20 to 25% in seed and oil yield are available in India. Similarly CMS and TGMS lines are commercially available in India (Meena et al. 2012). Average yield and oil content of CMS hybrid lines were greater than the open pollinated lines in trials run across sites in California, Arizona, North Dakota, Canada, Pakistan, Mexico and Spain (Dajue & Mündel 1996). In Australia, comparison of 4 California derived - CMS lines with open pollinated lines was inconclusive with regard to yield (Wachsmann et al. 2003). Despite development of systems to produce hybrid safflower and testing of hybrids, globally speaking commercial production of hybrid safflower is considered largely elusive (Mundel 2008).

MODIFIED FATTY ACID COMPOSITION

Safflower is an oilseed crop that is primarily grown for its high quality edible oil. Safflower seeds contain the fatty acids, palmitic acid, stearic acid, linoleic acid and oleic acid. Safflower lines have been developed with the following modified fatty acid compositions; increased palmitic acid, increased stearic acid, high to very high linoleic acid, high to very high oleic acid with reduced saturated fatty acids (palmitic and stearic acids) (Hamdan et al. 2008; Singh & Nimbkar 2006).

Oleic acid and linoleic acid are the two major fatty acids in safflower seed oil accounting for 90% of fatty acids present. Cultivated safflower seed oil traditionally had a high linoleic acid content of about 70% but breeding since the 1940s has changed the ratio of oleic and linoleic acids to produce high linoleic (70–90%) and high oleic acid (75–85%) cultivars.

Breeding for modified fatty acid composition using a few genes has been successful. The allele *ol* has been bred into cultivars in the United States to produce the two types; high oleic and high linoleic cultivars (Knowles 1989). Vegetable oils high in oleic acid have nutritional value and industrial applications. The normal oleic acid amount in safflower is 10–15% with a natural mutant (*ol*) accumulating up to 70%. The *ol* allele has now been incorporated into safflower breeding programs worldwide and has resulted in the release of numerous high oleic acid safflower varieties including Saffola 317 (S-317) (Cao et al. 2013).

Safflower varieties introduced to Australia from the United States have included the high oleic oil variety Saffola 517 and the linoleic oil variety Saffola 555 (GRDC 2010). High oleic acid cultivars have been developed by conventional breeding and by genetic modification (see below). Non-food applications or potential industrial uses of HO vegetable oils with high oxidative stability include uses in biodiesel, lubricants, and hydraulic oils, all products that require high oxidative stability (Vanhercke et al. 2013).

NON-SPINY VARIETIES

Safflower cultivars are generally spiny but in some countries, especially where hand picking of seeds is practiced, production is dominated by non-spiny cultivars e.g. China and India. Non-spiny varieties introduced and developed in India in the past, such as CO-1 and JS-1, had poor yields. More recent non-spiny cultivars introduced in India, NARI-6 and NARI-NH-1 have comparable yields to spiny cultivars and have increased tolerance to foliar and wilt diseases (Singh & Nimbkar 2006).

DISEASE RESISTANCE

Disease incidence is relatively low in safflower due to safflower being a rain-fed crop. However, under favourable conditions, outbreaks can devastate safflower crops as seen with *Alternaria* outbreak in India in 1997 (Singh & Nimbkar 2006). In Australia, the fact that safflower is a minor crop is an important contributor to reduced disease incidence. Low production levels, the long time between successive plantings of safflower in the crop rotation, and the distance between safflower fields would all contribute to low levels of inoculum.

To make safflower more competitive as an oilseed crop, cultivars with increased disease resistance to foliar diseases were developed. The most devastating worldwide are *Alternaria* leaf blight and *Fusarium* wilt (both are fungal pathogens), which can cause up to 50% losses (Sehgal & Raina 2011). Breeding safflower for disease resistance is the simplest method for controlling disease in the crop. Resistance to *Alternaria* and *Fusarium* are known to be due to single dominant genes. Germplasm line VFR-was developed with resistance to multiple diseases including the fungal pathogens: *Verticillium* wilt, *Fusarium* wilt and *Rhizoctonia* root rot (Singh & Nimbkar 2006).

The first commercial oilseed safflower variety grown in Australia in the 1950s was Gila, from Arizona. Gila was the main cultivar grown in most countries for three decades (1960-1990s). However, its susceptibility to diseases and its low oil yield and improved yields observed in new cultivars resulted in its decline (Mundel 2008). In the 1970s and 1980s, Gila suffered severe losses due to *Alternaria* disease. This led to the development of disease resistant varieties by CSIRO namely Sironaria and Sirothora in 1987. Sironaria is resistant to *Alternaria* leaf blight and moderately resistant to *Phythopthora* root-rot while Sirothora is susceptible to *Alternaria* and resistant to *Phythopthora* (GRDC 2010). Sironaria has lower oil content than newer varieties grown worldwide. Little research on breeding and developing new varieties has been done in Australia since 1987. In Australia, Sironaria is the most commonly grown cultivar followed by Saffola (S555 and S517) and Gila (Jochinke et al. 2008).

MOLECULAR BREEDING

Traditional breeding methods have contributed much to crop improvement of safflower in particular with the development of disease resistant cultivars, spineless cultivars and high oil content varieties but these methods do have several limitations. Breeding programs have been hampered by limited information on genetic variability in *C. tinctorius* and lack of genomics tools for trait breeding (Mayerhofer et al. 2010). In this respect, molecular tools such as linkage maps, gene identification, genetic engineering and genetic/genome information will be important in order to improve productivity/yield and develop resistance to other stresses such as drought, salinity and insect pests.

Gene discovery, development of techniques for comparison of DNA, and linkage maps are needed to understand relationships within and between *C. tinctorius* and its wild relatives. Such comparisons would help to identify homologous genes/alleles in wild species or homeologous loci within polyploidy taxa for trait improvement (Sehgal & Raina 2011). Identifying genes important to certain traits will help to identify functional markers within the genes and these markers will allow high throughput selection for such traits as yield and flowering time (Mayerhofer et al. 2010; Sehgal & Raina 2011).

2.4.2 Genetic modification

Efficient transformation and stable integration of transgenes in safflower using an Agrobacterium-mediated approach has been developed, see for example (Belide et al. 2011).

GENETICALLY MODIFIED SAFFLOWER

Gamma linoleic acid (GLA) is an important essential fatty acid synthesised from linoleic acid by delta-6-desaturase in the endoplasmic reticulum. High GLA lines have been developed that

are stable and heritable across generations and show no penalty in oil content, viability or fitness. The US-FDA approved the use of GLA derived from GM safflower, as a dietary supplement called SONOVA™ 400. Clinical trials have shown GLA is effective in treatment of eczema, viral infections and some types of cancer (Nykiforuk et al. 2012).

METABOLIC ENGINEERING

In the oil seed industry there is a growing trend towards developing oils that are nutritionally beneficial. These oils would be low in SFAs, high in MUFAs such as oleic acid and have functional stability without the need for hydrogenation (Liu et al. 2002). Many such oilseed crops have been developed, some through genetic engineering e.g. omega-9- canola, omega-9-sunflower, HO rapeseed, HO peanut, HS soybean, HO and HS sunflower, and HO cotton.

The high level of oleic acid (75–85%) found in some safflower cultivars is ideal for food use but not ideal for industrial uses because of the very high level purity required. Potential industrial uses of HO vegetable oils with high oxidative stability include uses in biodiesel, lubricants, hydraulic oils and oleochemical applicants.

MOLECULAR PHARMING

Safflower has been developed as a host platform for the production of proteins such as pharmaceuticals and industrial enzymes in GM seed (Mayerhofer et al. 2010); (Mündel et al. 2004). A Canadian-based company, SemBioSys Genetics Inc., genetically modified safflower to accumulate human insulin in the mature seeds. The insulin was readily purified along with the oil-bodies fraction of the seed (Mündel et al. 2004). This system was used for the transgenic expression and isolation of Apolipprotein A1 Milano and high levels of gamma-linoleic acid (over 70% (v/v) from seed oil (Nykiforuk et al. 2012).

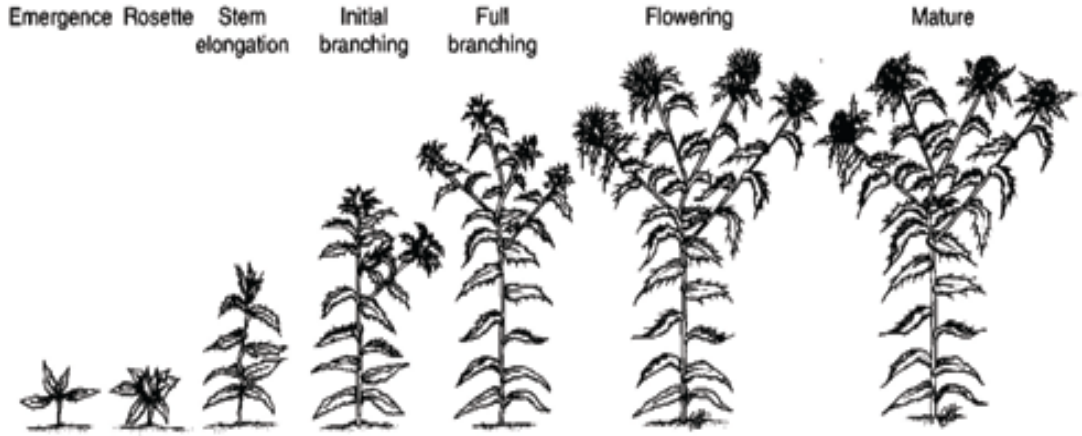
Section 3 MORPHOLOGY

3.1 Plant morphology

Safflower is an erect, thistle-like plant that grows from 30 to 150 cm in height and from sowing to harvest can take 26 to 31 weeks depending on variety, management and growing conditions. Safflower emerges 1 to 3 weeks after sowing and the first leaves emerge forming a rosette. The rosette stage is slow and can last several weeks. As temperature and day length increase the stem begins to elongate and branch. Lateral branches develop on stems that are about 20 to 40 cm high and these lateral branches may branch to produce secondary and tertiary branches. The more branches that grow the higher the yield as each branch ends in a flower head.

Leaves are arranged on both sides of the stem. Leaf size varies with variety and position on the plant; leaves are typically 2.5–5 cm wide and 10–15 cm long. Upper leaves often develop hard spines, while those lower on the stem are usually spineless. These spines make the crop difficult to walk through but act as a deterrent to larger animals such as pigs and kangaroos (GRDC 2010). As plants mature they become stiff and woody and resistant to some stresses such as hail or wind. The period from flowering to maturity takes around four weeks. Plants produce a strong taproot that, in the right soils, can elongate up to three meters, with numerous thin horizontal roots. This deep root system allows the plant to extract water and nutrients from deeper layers of soil than many other crop plants (Dajue & Mündel 1996; GRDC 2010).

Figure 1 Development of a safflower plant (Kaffka and Kearney, 1998; as cited in GRDC, 2010).



3.2 Reproductive morphology

Safflower flowers are typically brilliant orange, yellow or red, or more rarely white. The inflorescence is of the composite type characteristic of the family Asteraceae, with each plant producing 3-50 or more flowering heads called capitula on the ends of the branches. Each head contains between 20 and 180 individual florets (GRDC 2010).

Figure 2 Safflower flowering head. Photo: GRDC.

Section 4 DEVELOPMENT

4.1 Reproduction

Safflower reproduces by seed and is not known to reproduce vegetatively (USDA-APHIS 2008). The flowering period in safflower generally lasts from 10 days to a month. Capitula on the primary branches flower first, followed by those on secondary and tertiary branches. Flowering of the individual florets in each capitulum starts at the margin of the head and proceeds inward over 3–5 days. It may take from 10 to 45 days for all flowers on a plant to reach anthesis (Dajue & Mündel 1996).

4.2 Pollination and pollen dispersal

4.2.1 Pollination

Safflower is primarily self-pollinating and cross-pollination rates or outcrossing rates are thought to be on average around 10% (Knowles 1969) (see Section 9.1). Self-pollination is predominant because the style and stigma grow through the surrounding anther column; after elongation, the stigma is usually covered with pollen from the same floret (Claassen 1950). Individual safflower florets are largely self-pollinating, as safflower florets produce pollen that will outcompete with adjacent florets. However, an un-pollinated elongated stigma can remain receptive for several days, and outcrossing rates and seed set can be increased by insect pollinators (Claassen 1950; Dajue & Mündel 1996; GRDC 2010). Outcrossing rates vary depending mainly on insect pollinators but also on variety, pollen source size and environment. Intra- and interspecific cross-pollination are considered in greater detail in Section 9.1.

4.2.2 Pollen movement

WIND

Safflower pollen is yellow and relatively large with a mean diameter of 53–56 µm (USDA-APHIS 2008) and it is not transferred significantly by wind (Claassen 1950; Dajue & Mündel 1996). Claassen (1950) examined outcrossing rates for safflower plants grown either with or without insect exclusion cages. Depending on the cultivar, uncaged plants had outcrossing rates averaging 8.2–35% (range 6.3–58%), whereas the caged plants averaged 0.4–1.2% outcrossing (range 0–3.2%). The author acknowledged that the outcrossing observed in the caged plants could have been due to wind or to insect pollination of a few stigmas that had grown through the cage. In a glasshouse study, which excluded insects, no outcrossing was detected among the safflower plants (Claassen 1950).

In the same study, pollen traps were placed at heights of 46, 76 and 122 cm above ground level while the safflower plants were in full flower. Safflower pollen was only detected at 46 cm, which was below the level of some of the flowers (Claassen 1950). The height of the safflower plants was not given. Based on the assumption that some flowers were at or near the 46 cm height, there was no wind-dispersed pollen detected at distances of about 30 and 76 cm from the flowers (i.e. on the traps located 76 and 122 cm above ground). The results of these studies suggest that wind does not facilitate significant outcrossing or transport of safflower pollen and outcrossing is primarily due to insect-mediated pollen movement.

INSECT POLLINATORS

Safflower florets are largely self-pollinating but outcrossing rates and seed set can be increased by insect pollinators (Claassen 1950; Dajue & Mündel 1996; GRDC 2010). Cross pollination is thought to occur in safflower at approximately 10% but this is highly variable and honey bees, bumblebees, beetles and other insects can increase the level of cross pollination (Emongor 2010). Honey bees are the primary insect pollinators of safflower but other insects such as other species of bees and non-hymenopterous insects do forage in safflower (AOSCA 2012). In

studies in the United States, 80–90% of insects observed visiting safflower plants were honey bees and over 80% of observations occurred between 8 am and noon (Boch 1961; Levin & Butler 1966). Bumblebees (*Bombus* spp.) play a role in the transfer of pollen in the Northern Hemisphere where they represent less than 10% of insect pollinators in safflower, but since bumblebees only occur in Tasmania (Cresswell 1999; Cresswell 2000), bumblebees do not play a major role in pollination of safflower in Australia.

In Australia, the most important insect pollinator in safflower are honey bees (*Apis mellifera*) which visit the flowers for both pollen and nectar, yet it has been suggested the presence of honeybees is unlikely to increase yield by more than 5% (GRDC 2010). Langridge and Goodman (1980) examined insect visitors to the safflower variety Gila grown in Australia and found 75% of insect visitors were honey bees followed by a native species of halicitidae (21%), hoverflies (4%) and diptera species. Hoverflies and diptera species were not significant pollinators and other hymenopterous species are most effective in mediating cross pollination.

POLLINATOR BEHAVIOUR

Honey bees captured in Californian safflower fields were marked and recaptured upon returning to their hives located up to 4 miles (6.4 km) away. About 97–99% of bees returned to hives located 1.2–2 miles (1.9–3.2 km) away, the other 1–3% returned to hives 3–4 miles (4.8–6.4 km) away (Gary et al. 1977). The average foraging distance of honey bees from the colony is only a few hundred meters in agricultural areas and typically they do not move beyond one mile (1.6 km). However there is evidence of long distance foraging with honeybees traveling up to 6.4 km between apiaries and safflower fields (Gary et al. 1977).

Safflower ranks highly among the commercial crops for honey bee preference. Chaney (1985, (as cited in Van Deynze et al. 2005) found honey bee pollen collectors bypass cotton and fly five miles (8 km) to safflower while nectar collectors forage in nearby cotton. Bees attracted to pollen sources may travel longer distances than bees attracted to a nectar source (Gary et al. 1977). Nectar gatherers were observed to be the predominant visitors in Australia on "Gila" safflower fields but many were well dusted with pollen (Langridge & Goodman 1980). The distance of pollen dispersal or movement is dependent on pollinator behaviour but also on plant density and sparse areas of plants receive fewer pollinator visits (Kunin 1997). Long distance bee foraging has been documented with one bee of 2000 marked bees found 7.1 km from the hive on safflower (Gary et al. 1977). Foraging distances of pollen-collecting honey bees is longer in simple sparse landscapes than complex landscapes with ample vegetation (AOSCA 2012).

Studies of the foraging habits of honey bees on safflower fields in India observed honey bees made foraging trips that lasted 15 mins, visiting 5 to 8 flowers per trip with an average of 15 sec to two minutes spent per flower (Pandey & Kumari 2008). In a study of safflower fields (variety Gila) in Australia, honey bees were observed to visit on average 9 flowers per head, usually visit one head per plant and spend 12.2 sec per plant. One bee visited 54 plants in 15 min while another visited 48 plants in under 8 min (Langridge & Goodman 1980).

POLLEN VIABILITY

The likelihood of successful pollination or cross-pollination is both dependent on pollen dispersal and on how long the pollen grain remains viable. In general, pollen viability is dependent on a number of factors including temperature and humidity.

There is limited information on safflower pollen viability. Safflower is usually grown in dry conditions, where pollen is expected to desiccate rapidly (USDA-APHIS 2004). Safflower anthers contain 150–300 pollen grains and pollen can be shed for 10–45 days (Pandey & Kumari 2008). Safflower pollen has a short life, with no experimental evidence showing viability beyond the day pollen is released. However, anecdotally, breeders have reported

viability extending into the second day after release (Knowles 1980). The stigma is receptive for about two days after its exertion from the corolla tube (Knowles 1980).

4.3 Fruit/seed development and seed dispersal

4.3.1 Fruit/seed development

Each safflower head or capitulum usually produces 15 to 60 seeds. Safflower seeds are contained within a thick hull, this type of fruit is known as an achene, which mature 4 to 5 weeks after flowering (Dajue & Mündel 1996; Singh & Nimbkar 2006). The seeds are usually white but can be striped also and relatively large, 6–7 mm long with an average weight of 40 mg or 0.030–0.045 g (25,000 seeds/kg) (GRDC 2010). The white hulled varieties are used for the birdseed and pet food market; seed with brown stipes or with mould or staining are not acceptable (Mündel et al. 2004). Seeds are typically smooth but some varieties have tufts of hairs (pappus) on the ends, which is not desirable in commercial cultivars (Dajue & Mündel 1996). Therefore, most seeds of cultivated safflower lack a pappus or, if present, it is reduced (Berville et al. 2005).

4.3.2 Seed dispersal

WIND

Safflower seed is not appreciably dispersed by wind. During domestication of safflower, traits that increased seed recovery at harvest were selected, and as a result cultivated safflower is highly shatter resistant compared to its wild relatives (Berville et al. 2005; McPherson et al. 2009b). Safflower does not lodge readily but branches/flower heads could be dispersed by very strong winds, particularly if the plants or stems were weakened due to pathogen infections, or damaged through the activity of birds or other animals (GRDC 2010; McPherson et al. 2009b).

WATER

No data is known on seed transport rates by water of safflower seed. It is likely that seed could be carried by heavy rains and flooding either shortly after planting or at harvest. If there were heavy rainfalls, transported seed is likely to germinate because safflower seed has little or no dormancy. However, safflower is very sensitive to excess moisture/water either as heavy rainfalls, standing water (waterlogging) or humidity. This is due to the increased chance of disease (e.g. *Phythophtora*) under these conditions and can lead to substantial yield losses (Nimbkar 2008); (GRDC 2010).

HUMANS

Spillage during movement of seed on equipment for planting, harvest or post-harvest storage/shipping provides the greatest potential for dispersal of safflower seed. Seed could be spilled during transport but may also be dispersed if inadvertently transported on the machinery (e.g. on muddy wheels). It is also possible for small amounts of seed to be transported on or in clothing (e.g. pockets and cuffs) or boots (especially muddy boots) of workers.

ANIMALS

Primary loss of crop seeds is due to predation by insects, birds, mammals, pathogen attack and loss at harvest. Predation can result in large seed losses from the seed bank for crop seeds lost during harvest. Safflower seeds are a food source for a range of species including mammals, birds and invertebrates. Secondary seed dispersal may occur also and some seeds may be transported intact by ants, dung beetles or scatter-hoarding rodents (Vander Wall et al. 2005). Safflower seeds are firmly held within the seed heads and are highly shatter resistant, therefore limiting access by rodents. Post-harvest dispersal of seeds by small mammals, i.e. rodents, is most likely with predation of seeds present on the soil surface. Safflower seed may be dispersed (scattering) and hoarded by rodents.

For some larger animals such as cattle, foraging or grazing is minimal due to the spiny nature of mature safflower plants (Cummings et al. 2008), but sheep and goats are not irritated by the spines. Feral pigs or boars are very destructive and difficult to exclude from fields. Native animals may also feed on safflower. However, pests such as pigs and kangaroos are deterred from grazing safflower by its spines and unpalatability (GRDC 2010). The viability of safflower seed after passing through the digestive gut of animals is poorly understood.

Safflower dispersal by birds is most likely as some safflower seed varieties are sold as birdseed. Small birds can feed on ripening safflower seed and larger birds such as cockatoos can chew safflower plants at the base in order to access seeds (GRDC 2010). Safflower seed dispersal by several bird species (blackbirds, mallard ducks, pigeons and pheasants) was examined and it was observed that viable seed did not pass through the digestive tract but did remain viable in the oesophagus/crop and gizzard regions for several hours. A few seeds were also transported externally on soil attached to feet or legs of pheasants and pigeons (Cummings et al. 2008). Seeds did not attach to plumage possibly due to the fact that safflower seeds are smooth. The researchers also mentioned other bird species that hoard or cache seeds such as ravens, jays and crows as potential transport vectors of safflower seeds.

There is limited information on predation by Australian bird species, such as cockatoos and galahs. These can be present in larger numbers but their ability to disperse viable safflower seed is unknown.

Figure 3 Safflower seed. Photo: N. Wachsmann, GRDC (2010).

4.4 Seed dormancy and germination

4.4.1 Dormancy and germination

Safflower seed has been selected for reduced dormancy during domestication (Berville et al. 2005; McPherson et al. 2009b). Seeds of modern cultivars generally lack dormancy and can germinate in the head if rainfall occurs at harvest time (Berville et al. 2005; Dajue & Mündel 1996). Dajue et al. 1993 (as cited in Dajue & Mündel, 1996) examined germination of freshly harvested seed from 1973 accessions from over 50 countries. The seed was germinated at 20ºC.

The average time to achieve at least 60% germination was 60 hours for approximately 99% of the accessions. The remaining 1% required more than 120 hr to reach at least 60% germination. The little dormancy found in safflower appears to be cultivar dependent and is lost during storage, e.g. 24 weeks storage at room temperature (Kotecha & Zimmerman 1978).

Safflower is ideally sown into moist soil at a depth of 2 to 3.5 cm; deeper sowing increases susceptibility of the seed to *Pythium* (GRDC 2010). Germination can occur at temperatures as low as 2 to 5°C and takes between 3 and 8 days, depending on temperature (Dajue & Mündel 1996; Emongor 2010). However, germination is poor when soil temperatures are below 5°C. Safflower seedlings are frost resistant to about -7°C. Sowing depth, light, temperature and moisture will all influence germination (McPherson et al. 2009b). Timing of emergence also depends on temperature but generally plants emerge 1–3 weeks after sowing (GRDC 2010).

4.4.2 Seed banks/Persistence

Dormancy can affect the persistence of seeds in soil, but as discussed above, safflower generally has no or very little long-term seed dormancy which limits its persistence in seed banks.

In Australia, safflower seed loss during harvest is about 3–4% (GRDC 2010). Similarly, harvest losses in California were estimated at 3–4%, or 192–384 seeds m^{-2} on yields of 2200 to 3400 kg/ha (Knowles & Miller 1965 as cited in McPherson et al. 2009b). In one study conducted over 6 sites in Alberta, Canada, seed losses ranged from 230–1070 seeds m^{-2} with 80–520 viable seeds m^{-2}, representing a range of 26 to 84% viable seed depending on the site (McPherson et al. 2009b). It is not unusual that a large portion of seed lost during harvest is non-viable. Combine settings (e.g. sieve size, wind speed) are normally such that low weight and small sized seed are dispersed during harvest. Such seed is usually immature and is unlikely to be viable. However, these levels are relatively high and represent up to 5 times the recommended seeding rate for that region. The researchers did state that similar pre-harvest and harvest losses are found in wheat fields. Despite these large losses, safflower volunteers, emerging in spring ranged from 3–11 seedlings m^{-2}. Volunteers did not survive in fields under chemical fallow. In only three of ten cereal fields surveyed, a few volunteers (0.05–0.33 plants m^{-2}) survived the first year and generated viable seeds (1–4 seeds per plant). However, volunteer populations did not persist beyond two years (McPherson et al. 2009b).

Seed viability of safflower on soil surface and buried at two different depths was also examined (McPherson et al. 2009b). Viability of the seed was evaluated after burial in artificial seed banks or spreading the seed on the surface. Seeds did not persist beyond two years at the soil surface and beyond one year if buried at 2 cm or 15 cm. Thus, the authors recommended tillage to reduce persistence of the seed bank because the buried seed lost viability faster than the seed on the soil surface (McPherson et al. 2009b).

4.5 Vegetative growth

Safflower does not spread vegetatively and propagates only through seed germination (USDA-APHIS 2008). After seed germination, safflower goes through a slow growing period called the rosette stage, during which several leaves are produced near the ground and taproots begin to develop but no stem is formed (Figures 1 and 4) (Dajue & Mündel 1996). This stage generally lasts between 25 and 30 days, but the duration varies with variety and growing conditions and can be as long as several months. The rosette stage occurs in winter and is longer in southern than northern growing regions of Australia (GRDC 2010).

The rosette stage is followed by rapid stem elongation and extensive branching (Figure 5), the degree of which depends on both variety and environment (Dajue & Mündel 1996; Singh & Nimbkar 2006). The number of branches is an important determinant of yield as each branch ends in a flower head (GRDC 2010).

Figure 4 Rosette stage of safflower. Photo: N. Wachsmann; GRDC (2010).

Figure 5 Stem elongation stage of safflower. Photo: N. Wachsmann; GRDC (2010).

Section 5 BIOCHEMISTRY

Safflower plants have many uses, the seed oil is used as an edible oil and in industrial applications, the whole seed is used for the birdseed market, dehulled seed meal is used as feed for livestock and floral extracts have food and medicinal uses. Safflower plants contain many compounds including phenolic compounds, flavonoids, alkaloids and aromatic glucosides (Zhou et al. 2014) . Many of these compounds are beneficial with antioxidant properties. Some compounds from safflower have reputed beneficial effects and this is reflected in the medicinal use of safflower plant, in China for treatment of a broad range of ailments including hypertension, coronary heart ailments, rheumatism and male and female fertility problems (Chengaiah et al. 2010). However, there is a lack of quality randomised control trials on safflower. There have been some reports of adverse effects of safflower use, primarily examining the effects of whole safflower flower extracts.

5.1 Toxins

5.1.1 Seeds

Safflower seed oil is generally not known to be toxic and has a long history of safe use. Safflower oil is used in clinical trials as a placebo and is well tolerated. Some of the toxic or anti-nutritive compounds present in low amounts in seeds include lignans, tannins, cyanidin and oxalates (Ingale & Shrivastava 2011; Kuehnl et al. 2013). The main lignin compounds present in safflower seeds are trachelogenin, arctigenin and matairesinol and while having anti-nutritive properties these lignans may also have beneficial anti-inflammatory effects (Kuehnl et al. 2013).

Safflower meal, the by-product after oil extraction, can be used as a feed but there are natural toxins present. The chemical composition of two Indian hybrid safflower varieties was analysed and anti-nutritive or toxic compounds identified included hydrogen cyanide (3.5 mg/100 g), tannins (0.5 g/100 g) and oxalates (0.8 g/100 g). In animal feeding studies of these safflower seeds, the toxic compounds were present in such low amounts that they were non-toxic to rats. The safflower seeds showed comparable nutritive value to other oilseed crops (Ingale & Shrivastava 2011). Fatty acid composition of safflower seed in presented in Table 3. High fibre content of the safflower seed or seed meal is the main factor limiting its use in livestock feed (see Section 2.2.1).

5.1.2 Flowers

Safflower petal extracts have been used in Chinese herbal medicine for centuries. The effect of safflower flowers or extracts from flowers has been examined and both harmful and beneficial effects have been reported.

The effect of safflower aqueous floral extract on mouse spermatogenesis was reported in a trial where mice were given doses of 200 mg/kg of extract for 35 days, resulting in damage to testicular tissue (Mirhoseini et al. 2012). However, other studies have examined the effect of safflower extract (dried safflower petal aqueous extract) in infertile rats and observed a positive effect with spermatogenesis and sperm count increased and researchers suggested safflower could improve fertility (Bahmanpour et al. 2012). Iranian researchers examined the potential teratogenic and cytotoxic effects of safflower extract in pregnant mice. The water extract of safflower was administered at 1.6 and 2 mg/kg/day to pregnant mice and elicited embryo abortion at lower doses and appeared to have negative effects on the mouse central nervous system (Nobakht et al. 2000) .

Histological, ultra-structural and biochemical studies on the kidneys of mice treated with whole safflower methanol extracts revealed toxic effects. Exposure at doses of 1.4 and 2.8 mg/kg had harmful effects on the renal tissue of mice and therefore researchers recommended popular consumption of this plant should be reconsidered (Monfared 2013). Previous work by the same researchers showed toxic impacts of safflower methanol extracts on mice embryo development and organogenesis.

The above consider the effects of whole safflower flower extracts, but it is not clear what compound or active ingredient may be causing adverse effects. Hydroxysafflor yellow A (HSYA) is thought to be one of the main active ingredients or components of floral pigments in safflower. Purified HSYA can cause slight nephrotoxicity in rats but not in mice (Liu et al. 2004). In another study, HSYA had a neuroprotective effect at doses as low as 6.0 mg/kg in rats (Zhu et al. 2003).

In a 90-day sub-chronic toxicity study using HSYA at 20, 60 or 180 mg/kg/day, researchers observed prolonged blood coagulation time at 60 and 180 mg/kg/day. HSYA at180 mg/kg also increased the liver index without an obvious pathological change in liver histological analysis. There was no other organ injury found in this study (Liu et al. 2004). In a similar study,

researchers observed histopathological kidney and liver abnormalities in sub-chronic toxicity studies of safflower floral extracts (Mohseni et al. 2011). Extracts did not harm the acute toxicity system but HSYA did induce slight haematological, biochemical and pathological changes.

5.2 Allergens

Safflower oil is non-allergenic and suitable for use in injectable medications and cosmetics (Smith 1996). Nonetheless, a recent study of adverse drug reactions reported in a hospital in China observed that some of these cases were due to safflower injections used as a traditional Chinese medicine. The manifestations included drug rash, shock, chest tightness and renal insufficiency (Shen & Chen 2012). However, the adverse reactions may be due to other components of the injection. Zhang et al (2009) observed anaphylaxis induced by safflower injection of guinea pigs but safflower specific IgG antibody was not found in human blood samples and researchers indicated anaphylaxis may be due to liposoluble ingredients of the injection (Zhang et al. 2009). In a review of randomised clinical trials (RCTs) assessing the neuroprotective properties of safflower yellow as a treatment for ischemic stroke, of 39 RCTs only 7 RCTs were of an acceptable standard and skin rash was reported as an adverse reaction in one of the RCTs while it was unclear if any adverse reactions were observed in 4 of the RCTs (Fan et al. 2014).

Safflower flowers are used as a flavouring and food additive in Iran and India. In China, they have been used almost exclusively for medicinal purposes since 2nd century B.C. and are still widely used as a traditional medicine known as Hong Hua (Zhou et al. 2014). There is a safflower flower industry in some countries such as Japan and the United States (California). Rare cases of allergic reactions to safflower plants have been reported (Compes et al. 2006). An IgE-mediated immunological mechanism was responsible for occupational asthma in a single patient in response to dried safflowers.

5.3 Beneficial phytochemicals

Plants and seeds may contain many phytochemicals such as flavonoids, alkaloids, polyphenols, anthocyanins, phenols, terpenoids, glycosides, sterols/oils. Many of these phytochemicals have beneficial attributes such as antioxidant activity, anti-inflammatory and neuro-protective properties.

5.3.1 Compositional analysis of safflower seed

Cultivated varieties of safflower can range in seed oil content from 20–45% (Dajue & Mündel 1996), with many modern cultivars containing about 30-40% oil, as well as 20% protein and 35% fibre (Sehgal & Raina 2011). Safflower seeds are also rich in minerals (Zn, Cu, Mn, Fe), vitamins (thiamine, β-carotene) and tocopherols (α, β and γ). The leaves and shoots are rich in vitamin A, phosphorus, iron and Ca, and young shoots are sold as a vegetable in India and other countries. While the primary use of safflower is its seed oil, the flowers are also used in many countries for food flavouring and as medicines in China, India and Iran (see review by Sehgal & Raina 2011). The fatty acid composition of different seed oil crops is listed in Table 2.

Table 2 Fatty acid composition (g/100 g) in two safflower lines compared to two other common oil seed crops.

FATTY ACID PATTERN (WT %)	SAFFLOWER[a]	SAFFLOWER[b]	SOYBEAN	RAPESEED
MYRISTIC ACID (C14;0)	0.15			
PALMITIC ACID (C16:0)	6.69	11.07 ± 0.10	16.29 ± 0.54	8.23 ± 1.01
PALMITOLEIC ACID (C16:1)	0.13	–	–	0.32 ± 0.08
STEARIC ACID (C18:0)	2.06	4.37 ± 0.22	6.66 ± 0	2.92 ± 0.79
OLEIC ACID (C18:1)	12.71	12.76 ± 0.24	22.70 ± 0.07	53.84 ± 0.97
LINOLEIC ACID (C18:2)	77.74	69.65 ± 1.15	44.13 ± 0.60	23.38 ± 0.53
LINOLENIC ACID (C18:3)	0.08	0.49 ± 0.05	8.97 ± 0.52	9.82 ± 0.87
ARACHIDIC ACID (C20:0)	0.27	0.78 ± 0.09	0.62 ± 0.11	0.99 ± 0.03
EICOSANOIC ACID (C20:1)	0.13			
BEHENIC ACID (C22:0)		0.59 ± 0.09	0.63 ± 0.02	0.52 ± 0.10
LIGNOCERIC ACID (C24:0)		0.29 ± 0.13	–	–

[a] *= high linoleic acid safflower cultivar (Cosge et al. 2007)*
[b] *= medium linoleic acid safflower cultivar, soybean and rapeseed (Patrascoiu et al. 2013).*

5.3.2 Beneficial phytochemicals- Fatty acids

Safflower can have a high content of linoleic acid of up to 90% of total seed oil fatty acids. Linoleic acid is an essential omega-6-PUFA required in the human diet. Gamma-linoleic acid (GLA) is an important essential omega-6-PUFA synthesised from LA. Other safflower varieties contain high levels of oleic acid (OA), an omega-9-MUFA. Monounsaturated fatty acids such as oleic acid tend to lower blood levels of low density lipoproteins ("bad" cholesterol) without affecting high density lipoproteins ("good" cholesterol).

While some fatty acids, such as linoleic acid, are associated with lowering blood cholesterol, recent research has shown that this does not always translate to the expected benefits such as reduced risk of cardio vascular disease. Recent literature suggests the clinical benefits of n-6-PUFAs, the most abundant being LA, are not established and that omega-3-PUFAs may instead be responsible for any clinical benefits (Chilton et al. 2014; Ramsden et al. 2013); (Ramsden et al. 2011).

5.3.3 Beneficial phytochemicals -Antioxidants

Safflower has been used as a medicine for centuries especially in China. The safflower petals are used usually in the form of an aqueous concoction. Floral extracts are used in the form of infusions for circulatory system related diseases. In addition, extracts have been used to treat several chronic conditions including – hypertension, coronary heart ailments, rheumatism, male and female infertility problems. One of the active ingredients from the petals is thought to be carthamin or hydroxysafflor yellow A (HSYA) a glucoside. Its active ingredients possess many reported biological activities including modulating the immune system, anticoagulation and anti-thrombosis, antioxidant, and anti-fatigue (Chengaiah et al. 2010).

Other researchers have reported anti-tumour activity and cardio-protective and neuro-protective properties (Zhou et al. 2014). Safflower extract may also have anti-diabetic properties (Asgary et al. 2012). In contrast, some researchers have observed no changes in these parameters, but have observed a level of toxicity when other parameters are examined such as embryo development or liver and kidney indices (Mohseni et al. 2011)(see Section 5.1.2).

Tocopherols are naturally occurring antioxidants in vegetable oils and have a role in reducing cardiovascular disease (ODS 2007). There are four natural tocopherol isomers (all found in safflower) with differing antioxidant activities. In safflower, α-tocopherol (> 95% of total tocopherols) is the main tocopherol in seed (Velasco et al. 2005). The four tocopherol isomers together with four corresponding tocotrienols make up the eight vitamers that constitute

vitamin E (Chester et al. 2001). The term vitamin E is used as a generic descriptor for tocopherol and tocotrienol derivatives exhibiting α-tocopherol activity (IUPAC-IUB 1982).

5.3.4 Beneficial phytochemicals -Flavanoids

Safflower florets contain yellow and red quinochalcone natural dyes specifically safflower yellow A and B, safflomim C, precarthamin, and carthamin. These chalcones are the main constituents of a number of glycosylated flavonoids present in safflower petals that are known to have antioxidant activity (Salem et al. 2014). Variation in environment and region can affect composition of the natural dyes/pigments and their antioxidant ability (Salem et al. 2014). In addition to the glucosylquinochalcones, safflower petals also contain flavonoid glycosides. These naturally occurring flavonoids are polyphenolics with antioxidant activities (Lim et al. 2007).

Antioxidant activity is thought to be due to the presence of α and β unsaturated keto groups in the chalcone structure that act as metal chelators, and can play a role in bioavailability and toxicity of metals. It has been suggested that safflower dyes should be used as food additives /natural food colorants. HSYA can have a neuro-protective effect at doses as low as 6.0 mg/kg in rats (Zhu et al. 2003). Kinobeon A, an antioxidant isolated from cultured safflower cells was compared to two natural antioxidants, lignin and quercitin and found to exhibit stronger anti-oxidative effects and may be useful as a cryo-protective agent (Kanehira et al. 2003). Nicotiflorin, an antioxidant isolated from safflower has been shown to have a neuro-protective effect on memory/dementia in rats (Huang et al. 2007).

Section 6 ABIOTIC INTERACTIONS

6.1 Abiotic stresses

6.1.1 Nutrient stress

Safflower can be grown in a range of soil types but prefers alkaline soils that are well drained. Fertile deep black or grey self-mulching or cracking soils that allow full development of the root system are ideal, but alluvial and loam soils are also suitable.

Safflower has similar nutrient requirements to cereals, requiring similar amounts of nitrogen (25 kg/t seed) but more phosphorous (4.3 kg/t seed) and sulphur (4 kg/t seed). Surface applied fertilisers are not always effective while foliar fertilisers may be more suitable in allowing nutrients to be absorbed directly by leaves. The deep taproot of safflower can extract nutrients such as nitrates from deep in the soil that are beyond the reach of most other crops (GRDC 2010). Nitrogen is generally the most limiting nutrient to safflower production, the application rate depending on soil moisture (Mündel et al. 2004).

On certain soil types in Northern NSW and South Australia, safflower does respond to manganese, iron and/or zinc. These micronutrients are best applied 6 weeks after sowing as a foliar application (GRDC 2010).

6.1.2 Temperature stress

Seedlings will emerge at soil temperatures above 4°C, but 15°C is considered optimal. The rosette stage of young safflower plants is resistant to cold and frosts as low as -7°C, as the growing point is protected by leaves. During the stem elongation phase, even a light -4°C frost can cause substantial damage to the stem and growing point (GRDC 2010), and a frost just after flowering can dramatically lower yields and oil levels or kill the seed completely (Dajue & Mündel 1996).

Safflower needs long days to flower, so flowering and seed growth occur in late spring and summer. Safflower can tolerate the hot dry conditions at this time of year as long as adequate

water is supplied; hotter/drier conditions can hasten plant development. In Australia, mean daily temperatures above 26°C during flowering and seed growth can depress yield and oil content (GRDC 2010). Pollination and seed set are reduced by high temperatures (>32°C) during pollen shedding (Dajue & Mündel 1996). In the US, research has shown that safflower can tolerate up to 46°C but that yields tend to be highest when temperatures during flowering remain below 32°C (GRDC 2010).

6.1.3 Water stress

DROUGHT

Safflower is considered a moderately drought resistant crop due to its ability to access deep water due to its taproot system; it can access a larger area to retrieve water compared to other crops. It actually has a relatively high water requirement, preforming best (yields approaching 4t/ha) in regions receiving more than 450 mm annually. However, yields exceeding 1 t/ha can be expected on clay soils that are wet to 1 m depth at sowing, providing at least 50 mm post-sowing rainfall is received (GRDC 2010).

RAINFALL AND WATERLOGGING

Despite a relatively high water requirement, safflower is not tolerant of waterlogging, especially when air temperatures exceed 20°C. Waterlogging for more than 48 hours can starve roots of oxygen and kill crops and such conditions favour the development of *Phytophthora* root rot. Older crops are more susceptible to waterlogging than younger crops. Pollination can be inhibited (Dajue & Mündel 1996), diseases encouraged, seeds discoloured and sprouting can occur due to heavy rains and high humidity during flowering and seed maturation (GRDC 2010); (Nimbkar 2008).

6.1.4 Other stresses

HERBICIDE RESISTANCE

Safflower has limited tolerance to herbicides. Plants are easily controlled by cultivation and a wide range of hormone and other herbicides (GRDC 2010).

TOLERANCE TO WIND AND HAIL

Hail can severely damage young/succulent plants, but as they mature, plants become stiff and woody and therefore develop more tolerance. Safflower resists lodging and mature plants are not prone to shattering (GRDC 2010).

SALINITY STRESS

The salinity stress of safflower is considered moderate to high, being similar to that of barley or cotton. It is more tolerant of sodium than calcium or magnesium salts, with the later growth stages more tolerant than seedlings. Tolerance is cultivar dependent, with little information available on the Australian safflower cultivars (GRDC 2010).

Section 7 BIOTIC INTERACTIONS

7.1 Weeds

Weeds that compete with safflower include grass and broadleaf weeds. Control of weeds in safflower is essential for optimum yields. Safflower is a poor competitor with weeds, due to slow growth at the rosette stage early in the season (GRDC 2010). Later in the season many weeds can outgrow safflower in height and the resulting shading can reduce crop yields significantly (Dajue & Mündel 1996).

Safflower can be sown later than other winter crops which enables more time for control of weeds prior to sowing. Harrowing when the safflower plants are 7 to 15 cm tall can give

satisfactory control of small, later germinating weeds, but it is not clear if this approach is regularly used in Australia. Safflower is tolerant of some herbicides, but and as a minor crop in Australia fewer herbicides are registered for use. Several pre-emergent herbicides are registered for control of broadleaf and grass weeds such as ethyl dipropylthiocarbamate, trifluralin and pendimethalin. Post-emergent herbicides, dicloflop-methyl and propaquizafop are used for control of grass weeds while methosulfuron is used for control of broadleaf weeds (GRDC 2010).

7.2 Pests and diseases

Safflower is usually grown as a rain-fed crop which means the incidence of diseases and pests are relatively low. However, safflower has developed from wild species growing in arid desert environments and is particularly susceptible to foliar diseases (favoured by moist environments), root-rot organisms (favoured by irrigation) and a large number of insects (especially in regions where it evolved) (Dajue & Mündel 1996). If grown under irrigation, humid conditions and waterlogging, favour the development of disease (GRDC 2010).

7.2.1 Pests

INSECTS

In Australia, the main insect pests of safflower are aphids (Plum, Green peach, leaf curl), cutworms (*Agrotis* spp.), native budworm or heliothis (*Helicoverpa* spp.), rutherglen bugs (*Nysius vinitor*), red-legged earth mites (*Halotydeaes destructor*) and blue oat mite (*Penthaleus major*) all of which can be readily controlled with insecticides and some with biological controls (GRDC 2010). Aphids are a major pest in many countries (e.g. Spain, India) (Dajue & Mündel 1996) and infestations have caused losses of up to 74% (Nimbkar 2008).

Other pests known to infest safflower crops in Australia include thrips, Lucerne flea, black field crickets, grasshoppers, locusts, wireworms, false wireworms, jassids and myrids (GRDC 2010).

Safflower often requires less pest management than other crops. In Australia, growers have found large numbers of beneficial insects such as ladybirds and spiders, in safflower fields (GRDC 2010).

Safflower fly (*Acanthiophilus helianthi* Rossi) is one of the main limiting factors on production of the crop in several countries. The safflower fly is confined to Africa, Asia and Europe so is not a major pest in Australia. Resistance to safflower fly has been found in wild accessions of *C. oxyacanthus* and may be used in breeding programs to develop fly-resistant safflower cultivars (Sabzailian et al. 2010). Additional insect pests observed in safflower include aphids, cutworms (*Agrotis* spp.), heliothis (*Helicoverpa spp.*)

VERTEBRATES

Pests such as pigs and kangaroos are deterred from grazing safflower by its spines and unpalatability. Bird damage can be an issue especially near timbered areas which harbor birds (GRDC 2010).

7.2.2 Diseases

Under irrigation conditions, diseases are much more prevalent than if rain-fed (Nimbkar 2008). Safflower is susceptible to many diseases caused by fungi, bacteria and viruses and some of these can cause considerable damage/devastation under the right conditions (Singh & Nimbkar 2006). Outbreaks can devastate safflower crops as seen with *Alternaria* outbreak in India in 1997 (Singh & Nimbkar 2006). In Australia, the fact that safflower is a minor crop is an important contributor to reduced disease incidence. Low production levels, the long time

between successive plantings of safflower in the crop rotation, and the distance between safflower fields would all contribute to low levels of inoculum.

At present there are no fungicides registered for disease control in safflower in Australia (GRDC 2010)[1]. Control of disease in Australia relies on using appropriate crop rotations, selecting resistant varieties, using clean seed, controlling volunteer and weed hosts, sound irrigation practices and selecting appropriate soils. Safflower diseases can be hosted on stubble, volunteer plants, other *Carthamus* species such as saffron thistle and some broadleaf crops (GRDC 2010).

The three main diseases of safflower in Australia are the fungal diseases Alternaria blight (*Alternaria carthami*), Phytophthora root rot (*Phytophthora cryptogea*) and rust (*Puccinia carthami*). Other less prevalent diseases in Australia include seedling damping off, grey mould, charcoal rot, leaf spot and sclerotinia (GRDC 2010).

Section 8 WEEDINESS

8.1 Weediness status on a global scale

As with all crops cultivated and harvested at the field scale, some seed may escape harvest and remain in the soil until the following season when it germinates either before or following seeding of the succeeding crop. In some instances the volunteers may provide competition to the seeded crop and warrant chemical and/or mechanical control. Volunteers can also be expected away from the planting site (e.g. along roadsides and around storage facilities) as a result of transportation of seed out of fields (e.g. in farm equipment) and spillage during transport.

Safflower lacks characteristics that are common to weeds, such as very high seed output, high seed dispersal, long-distance seed dispersal and seed shattering, persistent seed banks, and rapid growth to flowering. During the early stages of growth, safflower is slow growing and a poor competitor with fast growing weeds (Dajue & Mündel 1996). However, it is considered a minor weed of agricultural and natural ecosystems in Australia (Groves et al. 2003). Primarily it is an agricultural or ruderal weed found in disturbed land use areas such as debris, roadside or disused fields (Groves et al. 2003).

Lack of seed dormancy in safflower (see Section 4.4.1) reduces the weediness potential and volunteers after harvest are uncommon (USDA-APHIS 2008). However, some feral populations of safflower have become established in agro-ecosystems in the United States in several states (California, Iowa, Illinois, Kansa, New Mexico, Ohio, and Utah) (Berville et al. 2005). There is little information on how long these populations persist, but anecdotal reports suggest safflower does not become established outside of agricultural areas (Berville et al. 2005).

In Canada, there are no cases of *Carthamus* species volunteering as weeds. Safflower has no weedy tendencies and is considered non-invasive in habitats in Canada. In 2005, safflower volunteers were not detected in the three large scale weed surveys of cultivated land in Canada (Leeson et al. 2005). Growers have suggested safflower does not persist or escape Canadian agricultural systems extensively and are not concerned with volunteers (McPherson et al. 2009a). However, safflower is a minor crop in Canada grown on about 1000 ha by a small number of producers whose fields may not have been covered by the survey. In contrast, over 80,000 ha per annum are grown in the U.S.A.

[1] The Australian Oilseeds Federation and NSW-DPI both indicated that as of Nov 2014, there were no permits from APVMA for use of fungicides on safflower.

Studies over several years in Canada (see Section 4.4.2) suggest that safflower seed and volunteers would not persist beyond 2 years. Safflower is a poor competitor with weeds so safflower volunteer survival and fecundity was expected to be low in competitive following crops such as barley or wheat. Safflower is also not tolerant of many herbicides, so those commonly used on following crops or along roadsides are likely to further reduce safflower volunteers (McPherson et al. 2009b). This is likely the case in Australia (see Section 8.3)

8.2 Weediness status in Australia

In 2000/2001 a rating system was applied to weeds of natural and agricultural ecosystems in Australia (Groves et al. 2003). The weeds or naturalised non-native flora of Australia, were categorised on a scale from 0 (indicated naturalised but the population no longer exists or removed) to 5 (indicating naturalised and a major problem at four or more locations within a State or Territory). Safflower was classified as a category 1 weed of agricultural ecosystems and as a category 3 weed of natural ecosystems in Australia (Groves et al. 2003). Wheat, which is grown in rotation with safflower, is a category 2 weed in natural ecosystems and a category 3 weed in agricultural ecosystems (Groves et al. 2003).

There are no studies on percentages of safflower volunteers in crops in Australia. In Australia, like Canada, safflower is still a minor crop with less than 10,000 ha grown annually (ABARES 2014). In Canada volunteer densities were low at 3–11 plants m^{-2} (McPherson et al. 2009a). However, Canada and Australia may not be directly comparable. For example, the ecology of the related safflower weedy species, *C. lanatus* was compared in France and Australia. Australia had much larger soil seedbanks than France and that was thought to be due to greater seed production per plant in Australia and due to the different types of herbivores present in Australia compared to Europe (Grace et al. 2002).

There are four related species which have naturalised in Australia: *C. lanatus*, *C. leucocaulos*, *C. dentatus* and *C. glaucus* (Atlas of Living Australia). Both *C. lanatus* and *C. leucocaulos* have been declared noxious weeds in some states or territories (Weeds Australia). There are doubts about the existence of *C. glaucus* in Australia; the two specimens that formed the basis of the record of this species in the 1986 Flora of SA have now been re-determined as *C. leucocaulos*, and the same may have happened in other States (personal communication Micheala Heinson, PIRSA, SA government).

8.3 Weediness in agricultural ecosystems

Safflower is unlikely to become a weed under most agricultural conditions and Australia is the only country that currently classifies cultivated safflower as a weed. It is considered a category 1 weed of agricultural ecosystems in Australia, specifically in Queensland, South Australia and the Northern Territory. A category 1 weed denotes it is naturalised and may be a minor problem but not considered important enough to warrant control (Groves et al. 2003). In New South Wales, Tasmania, Western Australia and Victoria, safflower is not considered an agricultural weed because it is not considered to be a problem (Groves et al. 2003).

Safflower seed may be inadvertently dispersed into neighbouring fields or non-agricultural areas by water, wind, animals and insects (see Section 4.3.2). It is also deliberately and inadvertently spread by humans during transport and on farming equipment. If dispersed seed were to germinate it is unlikely to persist as safflower is a poor competitor and is easily controlled by standard agricultural practices and standard road side weed control measures. Overseas data suggests safflower plants/populations are unlikely to persist in an agricultural setting (see Section 8.1 above).

In an agricultural setting, safflower may impact follow on crops. As noted previously, safflower can extract water from deep in the soil profile at a greater depth than many other crops due to its deep tap root system and as such is effective at lowering the water table where drainage is required. Some growers use safflower to dry soil profiles (e.g. after irrigated cotton)

to reduce waterlogging in subsequent crops. However, this can have impacts on subsequent crop yields, as it takes time for the water profile to replenish (GRDC 2010).

8.4 Weediness in natural ecosystems

In Australia, safflower is classified as a category 3 weed in natural ecosystems, meaning it is naturalised and known to be a minor problem warranting control at 4 or more locations within a state or territory. However, (Groves et al. 2003) emphasises that safflower is primarily an agricultural or ruderal weed. Anecdotal evidence from weed risk experts in the different states in Australia indicate that *C. tinctorius* is not a significant weed in natural ecosystems in Australia (personnel communication with Stephen Johnson, Department of Primary Industries NSW).

8.5 Control measures

Safflower is a poor competitor with weeds so safflower volunteer survival and fecundity is expected to be low in competitive following crops such as barley or wheat. Safflower is also not tolerant of many herbicides, so those commonly used on following crops are likely to further reduce safflower volunteers (McPherson et al. 2009b); (Dajue & Mündel 1996).

Tillage following harvest is also recommended as a means to reduce persistence of the safflower seed, because seed on the soil surface remained viable longer than seed buried to depths of 2 or 15 cm (see Section 4.4.2).

Section 9 POTENTIAL FOR VERTICAL GENE TRANSFER

9.1 Intraspecific crossing

Vertical gene transfer is the transfer of genetic information from an individual organism to its progeny. In flowering plants vertical gene transfer mainly occurs via pollen dispersal and cross pollination between related sexually compatible plants. Intraspecific crossing refers to fertilisation between *C. tinctorius* safflower plants. Outcrossing in safflower is mainly insect-mediated with wind-mediated outcrossing playing a minor role (see Section 4.2.2). Honeybees and bumble bees are the main pollinators of safflower. Bumble bees only occur in Tasmania so would not contribute to outcrossing in the safflower growing regions of the Australian mainland.

There is no information on intraspecific crossing of safflower in Australia. Worldwide, studies show that outcrossing rates appear to be quite variable (Table 3) and may depend on a number of factors, such as pollen source size and shape, environmental climatic conditions, insect numbers and type and variety/cultivar. Summaries of these studies are provided below.

One of the earliest studies to examine outcrossing in a number of safflower cultivars, using corolla colour as a marker was conducted in the United States (Claassen 1950). Outcrossing levels between rows 1 -1.5 m apart, ranged from 0 to over 50% for some cultivars, but most had rates of less than 10%. Individual plants varied considerably with outcrossing frequencies ranging from 0 – 100% at the 1m spacing (Claassen 1950). In inbred varieties selected for high yield and high oil content, the average outcrossing between rows was less than 5%. Researchers also measured outcrossing rates in different regions within Nebraska, but didn't find any significant difference. These results indicate outcrossing rates were more dependent on variety than topography however other studies have not supported this.

In another early study conducted in India also using corolla colour as a marker, cross-pollination rates ranged from 1–28%, with an average of 10%, between safflower plants in close proximity (exact distance not given). At a distance of 13.7 m, the average outcrossing rate ranged from 0.8–5.9% (average 1.9%) (Kadam & Patankar 1942).

Table 3 Outcrossing rates in safflower

Study	Outcrossing range % (average %)	Distance
(Kadam & Patankar 1942) India	1–28 (10)	Close proximity
	0.8–5.9 (1.9)	13.7 m
(Claassen 1950) United States	8.3–100 (34.2)	1 m
	0–26 (14.9) low outcrossing lines	1 m
	31.8–93.6 (57.3) high outcrossing lines	1 m
(Rudolphi et al. 2008) Germany	6–33 (9.7–18)	Close proximity
	0–11.5 (6.5)	At least 5 m
(McPherson et al. 2009a) Canada & Chile	0.48–1.7	0.3–3 m
	0–0.86	~10m
	0–0.26	~ 20m
	0–0.10	~ 30m
	0.03–0.16	~ 40m
	0.0024–0.04	~50m
	0.01	~100m
	nil	~300m
(Cresswell 2010)	0.005–0.05 (mathematical model)	Field to field
(Velasco et al. 2012) Spain	0.5–35.9 (10.3)	1–1.5 m
(Nabloussi et al. 2013) Morocco	8–53 (26.6)	1–1.5 m

More recently, a small study in Germany found the level of outcrossing between plots of safflower ranged from 0–33%, with averages of 6.5–18% depending on the location of the sampled plant (Rudolphi et al. 2008). Outcrossing rates were also measured between plants grown together in the same plot and dropped from 63% in 2004 to 30% in 2005. The large variation between the two years of the study may have been due to different environmental conditions (Rudolphi et al. 2008).

A study in Spain examined outcrossing from a high oleic content cultivar (CR-6) to a low oleic content cultivar (Rancho) separated by 1 to 1.5 m. The CR-6 plants were surrounded by Rancho plants and high oleic acid was used as a biochemical marker to estimate outcrossing. The experimental crops were grown at three different times, winter sowing in 2009, winter sowing in 2010 and spring sowing in 2010. Average outcrossing rates of 5.7%, 12.1% and 13.2% were observed, respectively. Higher frequencies were detected at the single-plant level (35.9%) and at the single-head level (58.3%) (Velasco et al. 2012).

Nabloussi et al (2013) used the same cultivars and field layout as Velasco et al. 2012 (above) to determine outcrossing rates under Moroccan conditions. The average outcrossing rate at 1–1.5

m was 26% with a range of 8.3–53% at the plant level. This rate was approximately twice that reported by Velasco et al (2012). As this and the Velasco study used the same cultivars and field layout, these studies demonstrate the influence of the environment and possibly pollinators on outcrossing rates.

The frequency of natural outcrossing from GM safflower to non-GM safflower was measured under field conditions in three different environments. Outcrossing experiments were conducted in the province of Santiago, Chile (2002) and the Canadian provinces of British Columbia (2002) and Alberta (2004) (McPherson et al. 2009a). The GM safflower contained the *pat* gene which confers tolerance to the herbicide glufosinate and this trait was used to confirm outcrossing to the non-GM safflower. The three trial sites varied in design layout including the distance from the GM-safflower to the first rows of non-GM safflower (0.3–3.0 m), distance over which outcrossing was measured, and size of the GM pollen source (99 –900 m^2) (McPherson et al. 2009a).

The highest rate of outcrossing of 1.67% was detected at the British Columbia site at a distance of 3 m, which was the nearest distance measured. Outcrossing was observed at each distance sampled at this site (from 3–101 m), except for a single measurement at 300 m where no outcrossing was detected. At the site in Santiago, outcrossing was observed at nearly every distance (0.7– 60.5 m) with the highest outcrossing rate of 0.48% again observed in samples taken at the closest distance of 0.7 m. No outcrossing was detected at most distances measured at the Alberta site (from 0.3–49.5 m), the highest outcrossing rate observed was 0.62% at 0.3 m (McPherson et al. 2009a). Highest levels of outcrossing occurred closest to the pollen source and declined over distance for all three sites.

Outcrossing frequencies were as heterogeneous between the three sites as they were between blocks (replicates). Researchers indicated this variation may be due to non-random movement of pollen by insects, as wind is not a significant factor in safflower outcrossing (Claassen 1950; McPherson et al. 2009a). In addition, the size of the pollen source may be a factor. The area of the British Columbia pollen source was about 9 times larger (900 m^2) than either of the other two sites (99 and 110 m^2) and outcrossing close to the pollen source at this site was four times greater. The larger site also demonstrated a slower decline in outcrossing with distance (McPherson et al. 2009a). Other differences in site design may have affected outcrossing rates. The Alberta site had a barren zone between the GM and non-GM safflower and this may have affected insect-mediated cross pollination. Differing insect populations at the sites has been proposed as a possible cause for the lack of outcrossing observed at the Alberta site (McPherson et al. 2009a).

McPherson et al (2009a) also considered directionality at the three sites and noted that there were predominately westerly winds during flowering. However, greater outcrossing was not found on the leeward side of the trial sites, which supports Claassen's (1950) findings that wind-mediated pollination plays a minor role, if any, in outcrossing of safflower.

Outcrossing rates in the McPherson et al (2009a) study over 0.3–3m ranged from 0–1.7% and this is at least an order of magnitude lower than the other studies for distances of 1–1.5 m (see Table 3). One reason for this is environmental differences which can influence outcrossing rates e.g. Velasco et al (2012) and Nabloussi et al (2013) used the same cultivars and field designs in different countries (Spain vs Morocco) but had a 2-fold difference in outcrossing rates. The different outcrossing rates would be influenced by the cultivars studied, e.g. Claassen's work (1950) demonstrated huge variability in outcrossing (14.9% and 57.3% in low and high outcrossing lines, respectively). In addition, outcrossing would be influenced by the type and number of pollinators at the trial site.

McPherson et al (2009a) did point out that this work cannot predict maximum distances of pollen movement by pollinators due to long distance foraging by bees, pollen can potentially be dispersed by bees foraging over a range of kilometres. In addition, the researchers found

that the outcrossing rate in safflower was spatially heterogenous as was the case observed by (Nabloussi et al. 2013), indicating that bee and other insect visitations occur in a random and unbalanced way. Cross pollination of safflower plants is predominantly insect-mediated, wind can only facilitate pollen movement over short distances (< 1 m) between plants grown close together (Claassen 1950). There is evidence of long-distance insect-mediated pollen transfer in other predominantly self-pollinated crops, such as cotton and oilseed rape, due to the long-distance foraging capability of honey bees and bumble bees (AOSCA 2012).

Bumblebees have been suggested as being more effective at field-to-field pollination of safflower than honeybees. Using a mathematical model of field-to-field gene flow due to insect pollination, the maximum level of bee-mediated gene flow between large fields was estimated at 0.005–0.05% (Cresswell 2010). The highest value occurred when it was assumed that fields were pollinated exclusively by bumble bees. Values for the model were determined using observations of honey bee and bumble bee behaviour on a 40 ha field of safflower in Canada. Bees made long foraging bouts within the field, making between field pollinations rare. This factor, as well as safflower's high capacity for self-pollination, resulted in the very low estimates of pollinator mediated gene flow between fields (Cresswell 2010). In Australia outcrossing rates over long distances may therefore be reduced due to the lack of bumblebees. The predominant insect pollinator of safflower is the honeybee (see Section 4.2) and long distance bee foraging has been documented in safflower (Gary et al. 1977).

It has also been suggested from safflower growers observations that safflower varieties grown in Australia have less than 10% outcrossing rates unless hives are brought in for the purpose of cross-pollination (GRDC 2010).

9.2 Natural interspecific crossing

Hybridisation between safflower and wild *Carthamus* species has probably played a role in the evolution of *C. tinctorius* in the Mediterranean and Asia where they are sympatric (McPherson et al. 2004). The information below includes literature regarding natural hybrid formation between *C. tinctorius* and species within the sections of this genus (see Table 1). Although Section 9.3 provides details related to artificial hybridisation, the results from artificial crosses are also mentioned here as indicative of the possibility of natural hybridisation.

SECTION CARTHAMUS (N=12)

Natural hybrids have been identified between *C. tinctorius* and *C. oxyacanthus* and *C. palaestinus*, which are all members of the *Carthamus* section (see Table 1) (Ashri & Knowles 1960). *C. oxyacanthus* and *C. tintorius* have a relatively high rate of natural hybridising when grown side by side and the F1 plants showed hybrid vigour. In contrast, Mayerhofer et al (2011) indicated that hybrids between *C. tinctorius* and either *C. oxyacanthus* or *C. palaestinus* did not demonstrate any hybrid vigour, increased fitness or weediness.

A review by Knowles & Ashri (1995) indicates that *C. flavescens* (= *C. persicus*), *C. oxyacantha* and *C. palaestinus* can easily be artificially crossed with *C. tinctorius* and occasionally will form natural hybrids. Hybrids of *C. tinctorius* and *C. oxyacanthus* have been documented in greenhouses and in the field in Pakistan and India where they are sympatric (McPherson et al. 2004). *C. oxycanthus* is rated as one of the top ten weeds in Pakistan. Hybrids of safflower and *C. paleastinus* have been found in Israel where the two species are sympatric (Ashri & Rudich 1965; Knowles & Ashri 1995).

The possibility of artificial or natural hybrids occurring between *C. tinctorius* and *C. gypsicolus* or *C. curdicus* have not been determined so far (Knowles & Ashri 1995).

Although safflower can naturally hybridise or be artificially crossed and produce a fertile hybrid with some of the other *Carthamus* species, *C. tintorius* is the only species within the

Section *Carthamus* which is present in Australia. Thus there is no potential for natural interspecific crosses between *C. tinctorius* and other members of this section in Australia.

SECTION ODONTHAGNATHIS (N=10, 11)

A few species from this section are present in Australia. Naturalised populations of wild safflower species, specifically, *C. leucocaulos, C. dentatus* and *C. glaucus*, have been reported in most states and territories in Australia (Groves et al. 2003) but few studies have examined interspecific crosses. *C. glaucus* may not be present in Australia as previous samples have been re-classified as *C. leucocaulos* (see Section 8.2). *C. leucocaulos* is a noxious weed in Australia and California (Mayerhofer et al. 2011). There are no reports of species within this section crossing with *C. tinctorius* under natural conditions. However, hybrids with some of these species have resulted from artificial crossing and tended to be sterile or have limited fertility.

Artificial crosses between *C. tinctorius* and other members of the species with n=10, are reported to be difficult to make and the F1 hybrids are highly sterile (Knowles & Ashri 1995). *C. tinctorius* has been artificially crossed with *C. glaucus* but all F1 hybrids were sterile (Ashri & Knowles 1960). Mayerhofer et al (2011) crossed *C. tinctorius* with *C. leucocaulos* and found that the crosses resulted in sterile offspring. The potential for artificial or natural crossing between *C. tinctorius* and *C. dentatus* or *C. boissierei* (both n=10) have not been determined.

C. tinctorius has been artificially crossed with *C. divaricatus* (the only n=11 member of this section) to produce self-sterile F1 hybrids which show some female fertility in backcrosses with *C. tinctorius* (Knowles & Ashri 1995). *C. divaricatus* is not present in Australia.

SECTION ATRACTYLIS (N=22, 32)

Naturalised populations *C. lanatus* (n=22) have been reported in many states and territories in Australia (Groves et al. 2003). *C. lanatus* is a noxious weed in Australia and California (Mayerhofer et al. 2011). *C. tinctorius* has been artificially crossed with *C. lanatus* (see Section 9.3), but the probability of a natural fertile hybrid occurring is highly unlikely.

There is no potential for natural interspecific crosses between *C. tinctorius* and *C. creticus* or *C. turkestanicus* as the latter two are not known to occur in Australia.

SPECIES OF UNCERTAIN PLACEMENT (N=12)

There is no potential for natural interspecific crosses between *C. tinctorius* and *C. nitidus* or *Femeniasia balearica* as the latter two are not known to occur in Australia.

9.3 Crossing under experimental conditions

SECTION CARTHAMUS (N=12)

Most *Carthamus* species with n=12 chromosomes, including *C. tinctorius, C. oxyacanthus* and *C. palaestinus*, can be intercrossed using emasculation and hand pollination to produce fertile progeny (Ashri & Knowles 1960; Mayerhofer et al. 2011). As discussed in Section 9.2, natural hybrids of these species have also been identified. The success rate of these interspecific hybridisations under artificial conditions was 30% with *C. palaestinus* and 56% with *C. oxyacanthus*. In comparison, *C. tinctorius x C. tinctorius* control crosses occurred at a rate of 40% (Mayerhofer et al. 2011).

Crosses between *C. tinctorius* and *C. flavescens* (= *C. persicus*) produced fertile F1 and F2 progeny (Berville et al. 2005). The possibility of artificial or natural hybrids occurring between *C. tinctorius* and *C. gypsicolus* or *C. curdicus* have not been determined so far (Knowles & Ashri 1995).

SECTION ODONTHAGNATHIS (N=10, 11)

Safflower has also been crossed with 4 species outside the section Carthamus, to produce viable hybrids. *C. tinctorius* has been artificially crossed with *C. divaricatus* (n=11) and

produced self-sterile F1 hybrids which show some female fertility in backcrosses with *C. tinctorius* (Knowles & Ashri 1995). However, backcrossing these hybrids with *C. tinctorius* results in offspring with low fertility (Estilai & Knowles 1976).

Ashri and Knowles (1960) crossed *C. tinctorius* with *C. tenuis* and *C. glaucus* and obtained sterile hybrids in both cases. Mayerhofer et al (2011) crossed *C. tinctorius* with *C. leucocaulos* and *C. glaucus* and found that the cross with *C. leucocaulos* resulted in sterile offspring (seed was produced but would not germinate). The cross with *C. glaucus* produced fertile F1 plants. However, the authors noted that there was some uncertainty about the identity of the *C. glaucus* seeds used (Mayerhofer et al. 2011). Different regional variants of *C. glaucus* behave differently in interspecific crosses, therefore it is possible that some subspecies or varieties may produce viable hybrids with *C. tinctorius* (McPherson et al. 2004). The F1 hybrids generated did not demonstrate any hybrid vigour or increased fitness or weediness (Mayerhofer et al. 2011).

The potential for artificial or natural crossing between *C. tinctorius* and *C. dentatus* or *C. boissierei* (both n=10) have not been determined. However, cytogenetic analysis of the interspecific hybrids within this section showed a high frequency of chromosome pairing at meiosis, indicating the close relationship among them (see review by Kumar 1991). In contrast, analysis of crosses between *C. leucocaulos* or *C. tenius* (both n = 10) with *C. tinctorius* (n = 12) showed very low chromosome pairing at meiosis, poor pollen stainability and a failure of the hybrids to produce seeds. A review by McPherson et al. (2004) of the potential for safflower to hybridise with other Carthamus species indicated that crosses between species with n = 10 and *C. tinctorius* produced sterile hybrids. Similarly, Knowles (1980) indicated that most n = 10 species will cross *C. tinctorius* , but the hybrids are highly sterile. Thus, it is highly likely that crosses between *C. tinctorius* and *C. dentatus* or *C. boissierei* will also have very low levels of chromosome pairing at meiosis and generate sterile offspring.

SECTION ATRACTYLIS (N=22, 32)

Successful crosses between *C. tinctorius* and *C. lanatus* (n=22) have been achieved, especially with *C. tinctorius* as the female parent, but all resulting F1 plants are sterile (Ashri & Knowles 1960; Heaton & Klisiewicz 1981; Mayerhofer et al. 2011). Fertile hybrid plants could only be achieved by treating rescued embryos with colchicine (Heaton & Klisiewicz 1981). F1 hybrids did not demonstrate any hybrid vigour or increased fitness or weediness (Mayerhofer et al. 2011).

Crosses between *C. tinctorius* and two other members of section Atractylis, *C. creticus* or *C. turkestanicus* (both n=32) produced viable fertile offspring (Berville et al. 2005); (McPherson et al. 2004) but with very low success rates (< 2% and 0.3%, respectively) (Mayerhofer et al. 2011).

SPECIES OF UNCERTAIN PLACEMENT (N=12)

C. nitidus (n=12) has been artificially crossed with *C. tinctorius* with the F1 hybrid showing low meiotic pairing and is sterile (Knowles & Ashri 1995). Attempts to cross *C. nitidus* with other *Carthamus* species produced viable but sterile hybrids (Knowles 1989; Knowles & Schank 1964). There is no information on the potential for crossing between *C. tinctorius* and *Femeniasia balearica.*

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