Chapter 1 Introduction

The genus *Echinochloa* (barnyard grass) includes two species listed in the top five worst weeds of agricultural crops, *Echinochloa crus-galli* (L.) Beauv. and *E. colona* (L.) Link (Holm *et al*. 1977). Commonly referred to as barnyard grasses, they have been reported to occur as common summer grass weeds in farming systems of the northern grain region (NGR) of Australia in northern New South Wales (NSW) and southern and central Queensland (Qld). It is often difficult to distinguish between the species due to a variability in form between plants of the same species, and a tendency of some of the species to intergrade (Michael 1983; Yabuno 1966). Additionally, confusion exists within the literature and amongst agronomists concerning the identity and ecological characteristics of some species. Therefore accurate identification of the species and sub-species present is the first step in developing and implementing suitable weed management strategies.

Rapid evolution of herbicide resistance amongst overseas species of *Echinochloa*, particularly *E. crus-galli* and *E. colona*, has raised concerns over achieving effective weed control in Australia. A 2001 survey in dryland cotton cropping systems of subtropical Australia indicated varied levels of control of barnyard grasses, with no farmers reporting good control in sorghum and only 38 percent reporting good control in cotton (Walker *et al*. 2005). In an associated project barnyard grasses were identified as having the highest risk of developing glyphosate resistance in no-till cropping systems of the NGR (Walker *et al*. 2002). Since that report, management of *E. colona* has become even more important and potentially difficult due to the confirmation of glyphosate resistant populations in Australia (Heap 2010). Given the inconsistency in control, a detailed knowledge of variability within and between populations and species of *Echinochloa* is necessary in directing appropriate management strategies.

The existence of morphologically intergrading types complicates species identification and diversity studies, yet little is known about the genetic structure of this genus in Australia. The development of genetic markers to measure genotypic variation has assisted in resolving taxonomic uncertainties and providing information on population diversity of many plant species.

This study aims to provide methods to assist in the correct identification of *Echinochloa* species including morphological and molecular techniques, and to determine the genetic diversity of *Echinochloa* at both geographic and field levels in the NGR, as well as in relation to herbicide susceptibility. This information will be useful when devising principles for effective and sustainable management of *Echinochloa* species.

The basic structure of the thesis is presented below; the findings of the research are discussed in each chapter and then brought together in a general conclusion where future research priorities are also identified.

The literature review (Chapter 2) contains information on *Echinochloa* with particular reference to *E. crus-galli* and *E. colona*. While much of the information available relates to research undertaken in Asia and the Americas, gaps in Australian research have been identified and form the basis for this study. The taxonomy of the genus and problems that exist with species identification are summarised, along with the application of molecular tools to establish genetic diversity and species identification. The review concentrates on molecular techniques used on *Echinochloa* species; restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) or microsatellites. The economic impacts of *Echinochloa* species on farming systems in Australia is examined in addition to the control methods currently being employed and the potential evolution of glyphosate resistant populations in the NGR.

Chapter 3 comprises a study into the morphology of *Echinochloa* species and the composition of populations based on field surveys undertaken across three major regional centres of the NGR. In addition, pot trials were established to ascertain if morphological characteristics were related to populations and/or regional centres and therefore genetically or environmentally determined.

In Chapter 4 I report on the genetic diversity existing in *Echinochloa* collected from three major centres across the NGR. Five microsatellites (SSRs) previously developed

for *Echinochloa* species were used to investigate the genetic diversity of the populations grown in the morphological trials in addition to 206 other plants sampled from across the NGR. In addition the SSRs were used to investigate the degree of heterozygosity in *E. crus-galli* and *E. colona* plants.

In Chapter 5 I examine potential variations in glyphosate efficacy on *Echinochloa* by subjecting 18 populations of each species to four different rates of glyphosate.

The findings of the research presented in the preceding chapters are then brought together in a concluding chapter (Chapter 6), where I investigate if any relationships exist between herbicide susceptibility, molecular and morphological characteristics of *E. colona* and *E. crus-galli*. In addition the contributions of this research to an integrated weed management plan for *Echinochloa* species in the NGR are presented, and directions for future research outlined.

Chapter 2 Literature Review

2.1 Introduction

Modern assessments of the grass family Poaceae recognise five major subfamilies that are widely accepted by taxonomists, with some authorities recognising a small sixth one (Chapman 1992, 1996). However, disagreements and uncertainties over classifications at the tribe, genus and species level exist (Chapman 1992, 1996), and this is certainly the case with the genus *Echinochloa*.

Echinochloa belongs to the subfamily Panicoideae A. Br., a collection of herbaceous grasses that are concentrated in the tropics and subtropics, with occasional outliers occurring in the temperate regions of the world (Chapman 1996; Gould 1968). A tropical to warm temperate genus, *Echinochloa* is comprised of approximately 50 to 60 species (Michael 1983, 1994), usually found in open habitats and associated with water and moist or marshy places (Watson and Dallwitz 1992). Species within this genus are commonly adventive and can also be found in disturbed ground and weedy places (Watson and Dallwitz 1992).

The genus contains both annual and perennial species with growth forms ranging from caespitose to decumbent (or floating) (Figure 2.1).

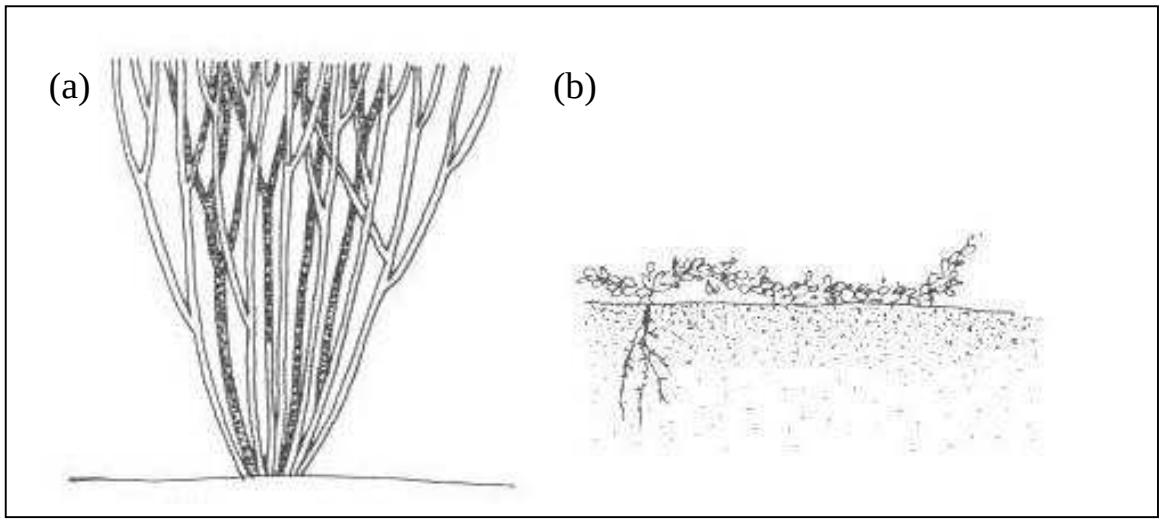


Figure 2.1: Plant growth forms (a) caespitose - growing in dense tufts and (b) decumbent - reclining on ground but with tips ascending (Source: Harris and Harris 1999).

The genus includes species cultivated for fodder such as *E. frumentacea* and *E. stagnina* (Watson and Dallwitz 1992); minor cereal crops including *E. utilis* (China and Japan), *E. colona* (Shama millet) and *E. pyramidalis* (Watson and Dallwitz 1992); important native pasture species that are more or less palatable to stock (Watson and Dallwitz 1992); and important components of tropical marshlands such as *E. polystachya* and *E. pyramidalis* (Barrett and Wilson 1981). In addition, several species including *E. crus-galli*, *E. colona*, *E. oryzoides*, *E. stagnina*, *E. crus-pavonis* and *E. pyramidalis* are considered significant weed species (Watson and Dallwitz 1992). All *Echinochloa* species have a C_4 photosynthetic cycle and show great competitive advantage when they grow with C_3 crops (Bouhache and Bayer 1993). Amongst the weedy species, two in particular are of major concern. *Echinochloa crus-galli* and *E. colona*, were ranked as the third and fourth most serious weeds in agricultural systems worldwide (Holm *et al.* 1977).

Herbicide resistance has evolved rapidly in *Echinochloa* species, particularly *E. crus-galli* and *E. colona* (Heap 2010). Other species with known resistance include *E. crus-pavonis* (Brazil), *E. erecta* (Italy), and *E. oryzicola* and *E. phyllopogon* (California) (Heap 2010). The evolution of herbicide resistance, combined with the confusion in the literature and amongst agronomists concerning the identity and ecological characteristics of taxa within this genus (Barrett and Seaman 1980), has raised concerns over achieving effective weed control (Rutledge *et al.* 2000).

Michael (1973), in his study of *Echinochloa* species in the Asian-Pacific region, verified that the taxonomy of this genus was confused, and stated that research can only be properly interpreted with correct species identification. It is often difficult to distinguish between the species, due to the variability in form within plants of the genus (Yabuno 1966) and a tendency of some of the species to intergrade (Michael 1983). Therefore, basic knowledge on the classification, morphology, physiology and ecology of specific weeds is needed for the development of optimal control methods (Yabuno 1983). Recent studies of genetic variation in weeds have provided new insights into the heterogeneity within weedy populations (Dekker 1997) and have shed some light on the taxonomic relations of *Echinochloa* species.

Currently, very little is known about the genetic variation and population structure of *Echinochloa* species in Australia, although numerous studies have been undertaken overseas investigating the genetic diversity of *E. crus-galli* ecotypes.

A molecular marker technique used by many authors to investigate genetic diversity was random amplified polymorphic DNA (RAPD). Tasrif *et al.* (2004) used RAPD-PCR markers to reveal relatively low genetic variation among 40 ecotypes of *E. crus-galli* var. *crus-galli* collected from Malaysian and Indonesian rice fields, but did establish that the Malaysian ecotypes were more variable than the Indonesian ecotypes. In Arkansas, Rutledge *et al.* (2000) used RAPD markers to determine the genetics of propanil resistant and susceptible populations of *E. crus-galli* populations. The analysis revealed two distinct clusters that may have represented different species, and established that several resistant populations from different regions were nearly genetically identical.

Other molecular techniques used in *Echinochloa* studies included: amplified fragment length polymorphisms (AFLPs) to investigate the origin and spread of herbicide-resistant late watergrass (*E. phyllopogon*) (Tsuji *et al.* 2003); restriction fragment length polymorphisms (RFLPs) to establish reliable practices in identification of *E. oryzicola* and *E. crus-galli* (Yasuda *et al.* 2002); and the development (Danquah *et al.* 2002a) and subsequent use of microsatellites (SSRs) to investigate genetic diversity of *Echinochloa* species in the Cote d'Ivoire (Danquah *et al.* 2002b).

2.2 *Echinochloa* species as problem weeds overseas and in Australia

2.2.1 Overseas

Holm *et al.* (1977) compiled a list of weeds considered to be problematic to the world's agriculturalists. The top 10 were distinguishable as they were cited more often and also rated as the greatest problems in the largest number of crops. *Echinochloa crus-galli* and *E. colona* were ranked third and fourth respectively on this list and were considered amongst the most serious weeds in agricultural systems worldwide (Holm *et al.* 1977).

Echinochloa crus-galli, a cosmopolitan weed in both the temperate and tropical regions, was reported as a problem in 36 different crops in at least 61 countries with the exception of Africa (Holm *et al.* 1977) (Figure 2.2).

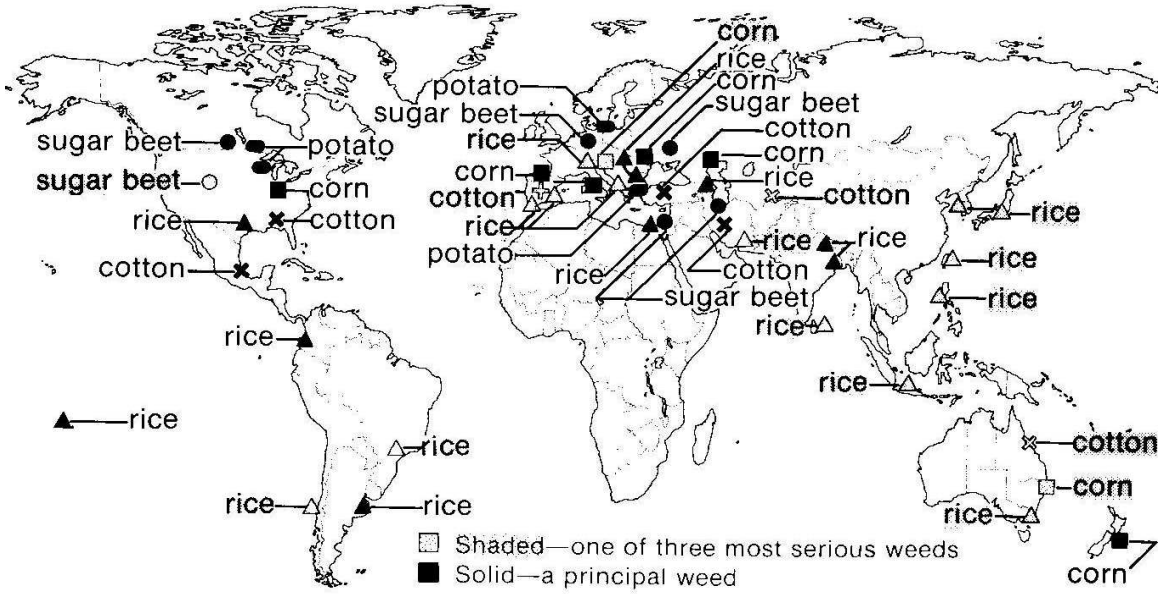


Figure 2.2: Distribution of *E. crus-galli*, indicating the major crops in which it occurs as a serious or principal weed (Source: Holm *et al.* 1977).

Echinochloa crus-galli was present in almost every area where rice (*Oryza sativa*) was grown, and was ranked as a very serious weed in this crop (Holm *et al.* 1977). It was particularly troublesome in crops in Australia, Brazil, Sri Lanka, Chile, Greece, Indonesia, Iran, Italy, Japan, Korea, Philippines, Portugal, Spain and Taiwan (Holm *et al.* 1977).

Echinochloa colona, a principal weed of rice, was reported as a problem in 35 crops from more than 60 countries, although it was seldom a problem in the Mediterranean areas of North Africa and Europe (Holm *et al.* 1977) (Figure 2.3).

Similar to *E. crus-galli*, *E. colona* was found growing almost everywhere that rice was grown and was rated as the second most important weed of this crop (Holm *et al.* 1977). While *E. colona* clustered along the equator and was the worst weed of upland

rice, *E. crus-galli* had a greater north-south range and was the most important weed in both lowland rice and in upland rain-fed rice (Holm *et al.* 1977).

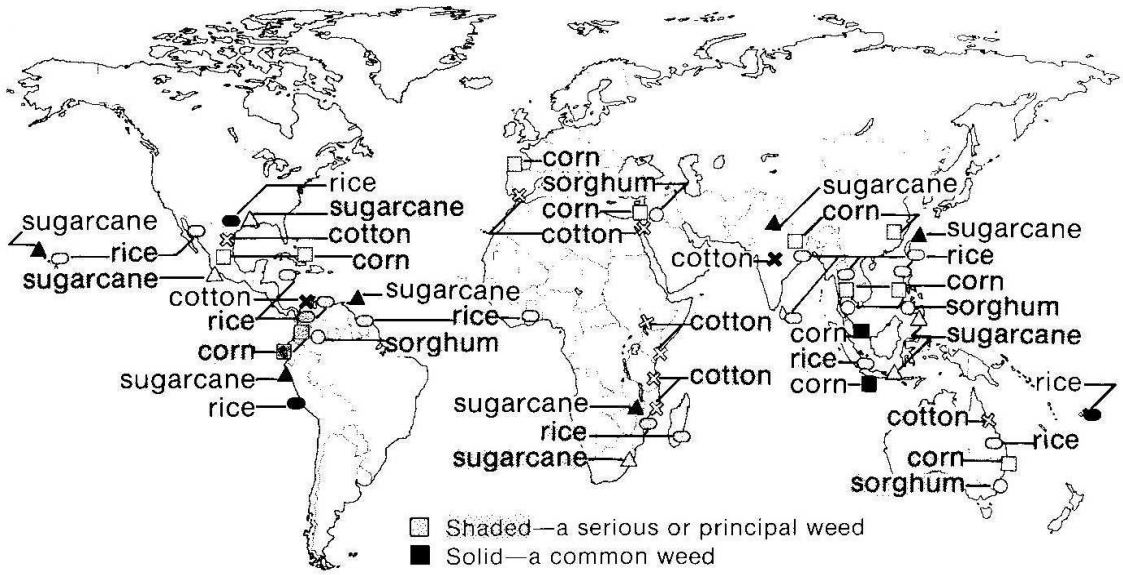


Figure 2.3: Distribution of *E. colona*, indicating the major crops in which it occurs as a serious, principal or common weed (Source: Holm *et al.* 1977).

While not considered a significant weed in Holm *et al.* (1977), *E. oryzoides* was considered by Michael (1983) to be the most widespread species apart from *E. crus-galli* to occur as a weed in rice-growing areas throughout the world. Other species within this genus also reported as serious weeds in rice fields included *E. phyllopogon* (Tabacchi *et al.* 2006; Tsuji *et al.* 2003), *E. oryzoides*, *E. oryzicola* and *E. hispidula* (Lopez-Martinez *et al.* 1999), and *E. frumentacea* and *E. picta* (Michael 1983).

In addition to rice, both *E. crus-galli* and *E. colona* were reported as serious weeds in other major crops including maize (*Zea mays*), cotton (*Gossypium* spp.) and sorghum (*Sorghum* spp.). After examining almost 1 000 reports on maize, Holm *et al.* (1977) established that *E. colona* was considered a serious weed in every country that reported it, while *E. crus-galli* was only considered serious in Australia and Yugoslavia. While not as widespread in cotton as in maize, both species were also considered to be serious weeds in cotton, with *E. crus-galli* amongst the three worst weeds of cotton in Australia, USSR and Spain. In sorghum, *E. colona* was mainly a

weed in Asia and Australia, whereas *E. crus-galli* was reported growing in crops some distance from the tropics (Holm *et al.* 1977).

2.2.2 Australia

Currently 20 species of *Echinochloa* have been recorded as occurring in Australia, including native and introduced species (Table 2.1).

Table 2.1: *Echinochloa* species known to occur in Australia listed by region of origin (Source: Hetherington 2003; Michael 1973, 1983, 1999, 2001; Michael and Vickery 1980; Sharp and Simon 2002).

Species	Region of Origin
*E. colona**	World tropics
*E. crus-galli**	Eurasia
E. esculenta (syn. *E. utilis*)*[#]	Asia
*E. frumentacea**	Asia
*E. oryzoides**	Asia
E. praestans	Asia
E. picta	Asia
*E. microstachya**	North America
*E. crus-pavonis**	South America
E. polystachya	South America
E. stagnina	Africa
E. pyramidalis	Africa
E. dietrichiana	Australia
E. elliptica	Australia
*E. inundata**	Australia
E. kimberleyensis	Australia
*E. lacunaria**	Australia
E. macrandra	Australia
E. telmatophila	Australia
E. turneriana	Australia

* Reported as weedy species in Australian cropping systems.

[#] Michael (1994) stated that this cultivated species has been known in recent years as *E. utilis* (Ohwi *et* Yabuno) but Scholz 1992 named this species *E. esculenta* and according to the International Rules of Botanical Nomenclature the latter has priority.

Echinochloa crus-galli and *E. colona* were serious weeds in Australian summer crops including rice, cotton, corn and sorghum (Holm *et al.* 1977). Along with other weedy species of the genus, they were of greatest significance in irrigated rice-growing areas (south-eastern Australia, the Burdekin Valley in Queensland, Northern Territory, north-west Western Australia), in coastal summer cropping areas (New South Wales and Queensland) and in irrigated crops in the river valleys west of the Great Dividing Range in central and northern New South Wales (Hetherington 2003; Michael 1973). *Echinochloa crus-galli* was introduced prior to the commencement of rice culture and is common in many parts of Australia (Vickery 1975) with several distinct biotypes occurring in rice fields in New South Wales, possibly indicating multiple introductions of genetic material (McIntyre and Barrett 1985). This species remains a serious weed in these fields and was particularly weedy in northern irrigation areas where sod and combine sowing were most widely employed (McIntyre and Barrett 1985).

Echinochloa crus-pavonis and *E. utilis* were reported in rice fields in New South Wales, and *E. microstachya* was reported as being abundant and growing vigorously in rice in southern New South Wales (Michael 1983). *Echinochloa microstachya* was first recorded in rice fields in the Murrumbidgee Irrigation Area (Vickery 1975), and was almost certainly the result of contaminants in seed imported from California, the major source of Australian rice seed at that time (McIntyre and Barrett 1985). Similarly, *E. oryzoides* and a long-awned form of *E. crus-galli* may also have been introduced via California (Michael 1981).

Echinochloa frumentacea, a cultivated derivative of *E. colona*, was reported as occasionally weedy in unirrigated areas of coastal Queensland and *E. picta*, a variable species abundant in tropical Asia, also occurred on Cape York Peninsula (Michael 1983). In a more recent study, Pratley and Broster (2004) identified at least five species of *Echinochloa* in the rice growing regions of southern New South Wales: these were *E. crus-galli*, *E. colona*, *E. oryzoides*, *E. inundata* and *E. esculenta.*

2.3 Economic impacts of *E. crus-galli* and *E. colona* in Australian cropping systems

In a 2001 study in the northern grain region, *Echinochloa* species were collectively identified as barnyard grasses and identified as major summer weeds in farming systems including dryland cotton, sorghum and fallows (Walker *et al.* 2005). Growers indicated varied levels of control of barnyard grasses, with no farmers reporting good control in sorghum and only 38 percent reporting good control in cotton. Agronomists and growers also rated barnyard grass as the sixth most common weed in crops and the second most common in fallow (Walker *et al.* 2002).

Earlier surveys also grouped *Echinochloa* species together as barnyard grass. Martin *et al.* (1988) conducted a survey of management practices on wheat farms in northern New South Wales between 1983 and 1985 and found that barnyard grass was the most troublesome summer grass weed with 28 percent of the growers listing it as a problem. A later survey in 1989, in wheat stubble and grain sorghum in northern New South Wales, found that barnyard grass was the most common weed in both fallow and grain sorghum (Felton *et al.* 1994). A comparison of the two studies showed that barnyard grasses had become more important weeds in 1990 (Felton *et al.* 1994). More recently, in a survey on the diversity of weeds across three regional centres in the northern grain region, growers and agronomists identified *Echinochloa* species as a major weed in crops and fallows. Sixty-five percent of the respondents in southern Queensland listed *Echinochloa* species as a main weed, compared to 25 percent from central Queensland and 26 percent from northern New South Wales (Osten *et al.* 2007). The majority of the respondents rated *Sonchus oleraceus* as the major weed in crops and fallows across all three regional centres.

Numerous surveys report on the economic impacts of weeds in cropping systems, without concentrating on the impact of individual weed species. A recent study by Walker *et al.* (2005) on economic impacts of weeds in dryland cotton systems calculated that annual on-farm financial costs, based on direct weed control costs and yield losses, ranged from $148 to $224/ha depending on the rotation. An earlier study by Charles (1991) found that annual weed control in irrigated cotton in New South

Wales cost the grower an average of $187/ha, the major components being herbicides ($76/ha) and hand chipping ($67/ha).

Osten *et al.* (2006) used Japanese millet (*E. esculenta*) to mimic *Echinochloa* species in crop row spacing studies in sorghum in central and southern Queensland. Results showed yield reductions ranging from 7 to 81 percent across the region depending on the region, the season and row spacing. In weed-free trials, the yield of sorghum ranged from 2.1 to 7.4 t/ha across the region, whereas in the weedy trials the yield was decreased to a range of 0.5 to 5.4 t/ha.

Echinochloa species were also major impediments to rice productivity in the Murrumbidgee Irrigation Area of New South Wales. A 2004 survey established that more than 90 percent of farms in the area were affected by *Echinochloa*, in addition to which, seeds were the main contaminant of rice delivered to silos (Pratley and Broster 2004). In order to calculate the cost of weeds on rice yield, Pratley and Broster (2004) assumed an equivalent of 10 percent of the rice yield was spent on herbicides, other control methods and dockages for barnyard grass contamination. This amounted to a loss equivalent to that of 80 000 to 100 000 tonnes of rice/annum for the industry.

2.4 Distribution, ecology and biology of *E. crus-galli* and *E. colona*

2.4.1 Echinochloa crus-galli

The most widespread and economically important members of the *Echinochloa* genus belong to the *E. crus-galli* complex (Barrett and Wilson 1981; Michael 1994), varieties of which are amongst the world's most serious agricultural weeds. This complex contains forms that are strikingly different in ecology, distribution, and weediness, resulting in problematic identification and classification (Barrett and Wilson 1981).

Echinochloa crus-galli has a variety of common names. Overseas, it is referred to as water grass, cock's foot, cockspur grass, barn grass, panicum, cock's foot panicum, and barnyard millet (Mitich 1990). In Australia, the preferred common name for *E.*

crus-galli is barnyard grass, but it is also referred to as barnyard millet, cocksfoot grass, cockshin grass, cockspur grass and cockspur panic (Shepherd *et al.* 2001).

2.4.1.1 Origin and distribution

While the geographical origin of *E. crus-galli* is not known with any certainty (Maun and Barrett 1986), it has been referred to by many authors as an Eurasian species (Barrett and Wilson 1981; Michael 1983; Mitich 1990). This broad classification encompasses other descriptions of its origin as a naturalised species from Europe (Spencer 1957), a native of Europe and India (Holm *et al.* 1977), and a native of Europe and Asia (Maun and Barrett 1986). Although considered a tropical plant, *E. crus-galli* has extended its range into the temperate zone (Good 1964 cited in Maun and Barrett 1986) and is now naturalised in many of the cooler parts of the world with a latitudinal range extending from 50°N to 40°S (Holm *et al.* 1977) (Figure 2.4).

Figure 2.4: Global distribution of *E. crus-galli* indicating where it has been reported as a weed and the number of crops it infests (Source: Holm *et al.* 1977).

There is no documented introduction of *E. crus-galli* into the United States (Mitich 1990), but it was introduced to the North American continent at an early date from Europe (Dore and McNeill 1980 cited in Maun and Barrett 1986), being recorded in California between 1825 and 1848 (Barrett and Seaman 1980). The introduction of *E.*

crus-galli could be associated with European colonisation and agricultural settlement as plants of the *Echinochloa* genus were common weeds of wet, disturbed ground and other irrigated crops in California before the commencement of rice culture (Barrett and Seaman 1980). In contrast, species that were common as rice weeds in Asia may have originally been introduced to Californian rice fields as seed contaminants in shipments of imported rice seed from those regions (Barrett and Seaman 1980).

Echinochloa crus-galli is now widespread in North America, ranging from southern Canada to Mexico (Barrett and Wilson 1981). It is distributed across the prairies, along roadsides and in cultivated fields (Maun and Barrett 1986), occurring as a weed of disturbed, moist, waste ground and as a serious weed of irrigated crops (Barrett and Wilson 1981). Additionally, Maun and Barrett (1986) reported that *E. crus-galli* extended to 53°N in Canada and was found in all provinces with the exception of the Northern Territories.

In New Zealand *E. crus-galli* is a common weed in waste places and in cultivated land on the North Island and northern half of the South Island (Lambrechtsen 1992). Similarly in Australia, *E. crus-galli* is a cosmopolitan weed of settled areas in the temperate regions and beyond (Lamp *et al.* 2001). Information gathered from herbaria records suggests that *E. crus-galli* occurs in all Australian states but is predominant in the south-eastern states (Figure 2.5).

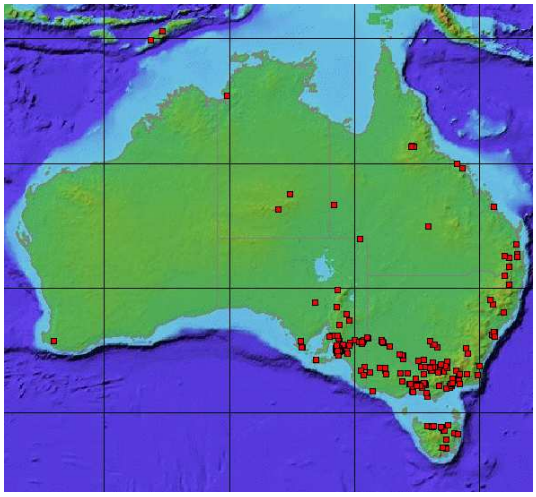


Figure 2.5: The distribution of *E. crus-galli* in Australia according to specimens lodged with Australian herbaria (Source: Australia's Virtual Herbarium 2010).

Echinochloa crus-galli is known to exist as a number of races or ecotypes scattered over the world, some of which may have been distinct species (Yabuno 1966). Holm *et al.* (1977) stated that four definite varieties were described in Japan and five in the United States, but failed to name the varieties. Earlier reports recognised three wild varieties of *E. crus-galli* in Japan; *E. crus-galli* var. *caudata*, var. *formosensis* and var. *praticola* (Yabuno 1966) and three varieties in North America; *E. crus-galli* var. *crus-galli*, var. *frumentacea* and var. *oryzicola* (Gould *et al.*1972). The latter has since been identified as a tetraploid crop-mimic and is probably best treated as a separate species (either *E. oryzicola* or *E. phyllopogon*) (Maun and Barrett 1986).

Michael (1994) considered that the *E. crus-galli* complex in the Asian–Pacific region included two subspecies each with two varieties: namely the more temperate *E. crus-galli* spp. *crus-galli* (var. *crus-galli* and var. *praticola*) and the more tropical *E. crus-galli* spp. *hispidula* (var. *hispidula* and var. *austro-japonensis*). Similarly, Yabuno (1983) stated that in East Asia *E. crus-galli* included three varieties: var. *crus-galli*, var. *praticola* and var. *formosensis*.

Echinochloa crus-galli var. *crus-galli* in its many forms occurred in the cool and warm-temperate parts of the Asian-Pacific region (Michael 1994). *Echinochloa crus-galli* var. *praticola* was abundant in upland or drier situations in eastern Asia (Michael 1983; Yabuno 1966), occurred as a common roadside weed in far east Asia (Yabuno 1966), but was uncommon in Australia (Michael 1983). In the sub-tropical and more tropical areas of Asia and in East Africa, *E. crus-galli* var. *hispidula* (Michael 2001) or var. *austro-japonensis* (Michael 1983) replaced var. *crus-galli*. *Echinochloa crus-galli* var. *hispidula* was abundant in the Pacific Islands and also occurred in coastal northern Queensland (Michael 1983), whereas var. *austro-japonensis* extended from southern Japan to south-east Asia, New Guinea and the Pacific Islands (Michael 2001).

2.4.1.2 Historical uses and benefits

Historic information relating to the use of *E. crus-galli* is limited. Some varieties were cultivated as cereals in the tropics and sub-tropics, whereas others were grown for

coarse fodder and hay (Holm *et al.* 1977). King (1966) stated that it was 'historically' used as a famine food by the Chinese, but did not clarify in what historic era. *E. crus-galli* var. *frumentacea* was advertised in North America as a "billion dollar grass" and recommended for forage as green feed, silage or hay (Wilkinson and Jacques 1972). In Australia, Wheeler and Hedges (1972) considered that this variety might be satisfactory to use in drought mitigation programs where large numbers of sheep were kept in small areas, but that it was not promising as a forage crop in cool environments.

2.4.1.3 Ecology and biology

Habitat

In collating data from Canadian herbaria sheets, Maun and Barrett (1986) established that *E. crus-galli* was a weed of warm regions, found predominantly along roadsides, in ditches, along mud banks and wet shores of lakes and sloughs, on river banks, in waste places and dumps, railway embankments, gardens, gravel pits and field crops.

Echinochloa crus-galli grew on a wide range of soil types and textures ranging from sandy loam to medium heavy soils (Holm *et al.* 1977) and, in Australia and New Zealand, favoured moist, rich, loamy-alluvial soils (Lambrechtsen 1992; Lamp *et al.* 2001). Taxa in the *E. crus-galli* complex were amongst the most abundant and widely distributed rice weeds in California and grew in areas where the principal soils were heavy clay and clay adobes with impervious subsoils (Barrett and Seaman 1980). *Echinochloa crus-galli* preferred moist soil with a high nitrogen content (Chin 2003; Holm *et al.* 1977) and plants responded to nitrogen, phosphorus and potassium in that order (Holm *et al.* 1977). Soils with a relatively high water holding capacity and high fertility provided an ideal substrate (Maun and Barrett 1986) for *E. crus-galli*, but it was also capable of thriving on drier soils where it produced smaller plants with lower seed yields and reduced panicle and tiller numbers (Holm *et al.* 1977). It was normally found growing only at low and medium altitudes (Holm *et al.* 1977).

Plant growth

The success of *E. crus-galli* as a weed may be attributed to many factors including the production of large numbers of small, easily dispersed seeds, possession of seed dormancy, rapid development and ability to flower under a wide range of photoperiods (Maun and Barrett 1986). Holm *et al.* (1977) considered photoperiod one of the most important factors governing the distribution and competitive ability of *E. crus-galli*. Able to grow and flower in photoperiods ranging from 8 to 16 hours, *E. crus-galli* could produce late seed flushes in short hour days because the plant sacrificed vegetative growth for quick flowering (Holm *et al.* 1977). However, *E. crus-galli* was susceptible to shading and not a serious problem when it emerged after the establishment of a tall, vigorous crop. Maun and Barrett (1986) believed that the phenotypic plasticity prevailing in this complex was due to its habitat adaptation.

A study undertaken in Canada by Maun and Barrett (1986) established that a frost-free period of 160 to 200 days per year, an average July temperature of 16 to 25°C and abundant moisture were required for plant growth and seed dispersal. The same study found that the increase in plant height of *E. crus-galli* was directly related to temperature, with plant growth slow in spring with low temperatures but rapid in the heat of summer.

Flowering and seed production

Echinochloa crus-galli reproduces and spreads by seeds with flowering recorded as occurring year round in the Phillippines, in the irrigated Gezira cotton scheme in Sudan (Holm *et al.* 1977) and in areas of Vietnam (Chin 2003). The plants are self-compatible and highly autogamous, but occasional out-crossing can occur when mediated by wind (Maun and Barrett 1986). This results in high levels of homozygosity within populations and low levels of heterozygosity. Barrett and Seaman (1980) reported on the breeding systems of *E. crus-galli* var. *crus-galli* and var. *oryzicola*, tested to determine their capacity for autogamous seed production. Isolated plants were grown in a pollinator-free glasshouse and undisturbed flowers produced seed, suggesting that they were either self-compatible and autogamous or capable of apomixis.

No authenticated hybrids between *E. crus-galli* and other taxa in North America have been documented (Maun and Barrett 1986) but Yabuno (1966) artificially hybridised *E. crus-galli* with *E. oryzicola* and *E. oryzoides*. In both cases the F_1 hybrids were sterile.

Much is known about the seeds of *E. crus-galli* as they have been the subject of several studies around the world. Seed output is considered a highly plastic character and responsive to local growing conditions, particularly nutrient availability, day-length and plant density (Maun and Barrett 1986).

Capable of rapid growth in late spring and summer, Canadian studies found that *E. crus-galli* was able to set seed after a few weeks growth and could mature several generations a year (Maun and Barrett 1986). Anthesis of individual spikelets occurred within a single day, but the flowering period of inflorescences can last for several days with spikelets at the top of the inflorescence entering anthesis before those at the base (Maun and Barrett 1986). Keeley and Thullen (1989) compared seed production of *E. crus-galli* in Californian fields seeded between March and October. Seedlings emerged in March when soil temperatures reached 17°C at a depth of 5 cm. These plants produced on average 5 900 seeds/plant compared with the larger plants of the April to June plantings that produced the greatest number of seeds (15 000 to 25 000 seeds/plant). While the July and August plantings were the first to flower and produce seeds, their production was lower, July (9 800 seeds/plant) and August (9 700 seeds/plant). The September plantings had the lowest seed production with an average of 37 seeds/plant. This meant that seed production would be expected from seedlings emerging in March and April in early June, and seedlings emerging in September could produce seeds before killing frosts. Barrett and Wilson (1983) also observed that under field conditions in Californian rice fields, *E. crus-galli* began germinating in March-April.

Norris *et al*. (2001) estimated that *E. crus-galli* could produce over 400 000 seeds/plant in the absence of interspecific competition, decreasing to about 10 000 seeds/plant when weed density exceeded 50 plants/m of row. A Malaysian study compared the seed production of similar infestations of two varieties of *E. crus-galli*

(Bakar and Azmi 2003). *E. crus-galli* var. *formosensis* produced 12 000 to 37 000 seeds in a single life cycle of three to four months compared with 11 000 to 48 000 seeds by *E. crus-galli* var. *crus-galli* within the same period. Holm *et al.* (1977) reported that single plants in the United States produced 5 000 to 7 000 seeds, and that such production could result in a yield of 1 100 kg of weed seeds/ha in a weedy field.

Seed dispersal

Seed is primarily dispersed by water (Halvorson and Guertin 2003). Spread within and between agricultural landscapes can be facilitated by irrigation systems and overbank flooding from sumps adjacent to riparian areas. Seeds of *E. crus-galli* exhibited great buoyancy when tested by Barrett and Wilson (1983), with approximately half the seeds remaining afloat after four to five days in water. It was also established that the seeds weighed on average 1.7 mg and that the buoyancy and hence dispersal by water, is probably influenced by the light weight of the seeds (Barrett and Wilson 1983). *Echinochloa crus-galli* is also human-dispersed as a common contaminant of crop seeds (Maun and Barrett 1986), on machinery and via stock movement.

Seed dormancy and longevity

According to Martinkova *et al.* (2006) only a small fraction of the seeds produced by plants in fields of the European temperate zone were capable of germination at dispersal or following a short period of storage. The dormancy in seeds that remained on the soil surface or were incorporated into the soil by tillage was mostly terminated by stratification. Seed stored in a controlled environment room at 25°C and 40 percent relative humidity lost dormancy within two years after dispersal whereas buried seed passed through annual dormancy/nondormancy cycles.

Barrett and Wilson (1983) found freshly collected seeds of *E. crus-galli* exhibited innate dormancy, the duration of which varied considerably. Depending on photoperiod, seed dormancy has been recorded as ranging from 0 to 48 months, while seed viability has been reported as 100 percent viable after 6 to 8 years dry storage (Maun and Barrett 1986). The duration of dormancy and storage viability are characteristics peculiar to ecotypes around the world (Holm *et al.* 1977).

In 1957 Dawson and Bruns (1975) buried seeds at different depths in irrigated and non-irrigated fields in North America to investigate seed longevity. Thirteen years after burial, 3 percent of the seeds exhumed from 20 cm germinated while none of the seeds buried at 2.5 cm or 10 cm germinated. Regardless of the field and depth of burial, seeds exhumed after 15 years were not viable. Seeds from the original seed lot that had been kept in dry storage at room temperature for 15 years had an 82 percent germination success rate.

In Malaysian studies, seed viability varied considerably according to soil moisture content (Bakar and Azmi 2003). While the duration of the seed burial was unclear, viability ranged from 27 to 36 percent under rain-fed soil conditions and 12 to 16 percent viability in inundated fields. However, the potential of the seed bank to augment existing populations of *E. crus-galli* was fairly low with only 2.3 to 3.9 percent of the total seed rain emerging as seedlings per season.

Germination and emergence patterns

Holm *et al.* (1977) stated that the optimum temperature for germination was in the range 32 to 37°C, with germination falling off sharply below 10°C and above 40°C.

A European study undertaken by Martinkova *et al*. (2006) found the optimum temperatures for germination in *E. crus-galli* were between 27°C and 31°C. The constraint on optimum germination temperature decreased with increased seed age and the range of adequate temperatures increased with seed age. Therefore, seedling emergence may vary according to whether the seed population originated from the previous year or from older soil seed banks.

Compact soil also favoured germination and emergence with germination 30 percent better in compact soil than in loose soil in the USA (Holm *et al*. 1977). Optimum moisture for germination in Japan varied with soil characteristics but was usually 70 to 90 percent maximum water-holding capacity (Holm *et al.* 1977). At this moisture content, maximum depth of seedling emergence was 10 cm, whereas in water saturated soil it was 3 cm. A study by Barrett and Wilson (1983) indicated that seed burial caused a reduction in the rate of emergence of seedlings from saturated soils.

Over 80 percent of the seeds on the surface germinated compared with less than 5 percent of the seeds buried at 1 cm. Seeds recovered from all burial depths were resown on the soil surface and produced over 80 percent germination indicating that burial in saturated soil resulted in the enforced dormancy of the seeds. This study also revealed significant variations among populations of *E. crus-galli* var. *crus-galli* and var. *oryzicola* in levels of germination. *Echinochloa crus-galli* var. *oryzicola* populations gave uniformly high germinations after nine months in dry storage, whereas var. *crus-galli* populations were highly variable in their behaviour with most populations failing to germinate. However after 18 months of dry storage, germination levels were uniformly high for both varieties.

2.4.2 Echinochloa colona

This species is also referred to in the literature as *E. colonum* and there are conflicting reports from taxonomists regarding the correct name for the species (Michael 1983; Ward 2005). This thesis will comply with current Australian authors and refer to the species as *E. colona*, after the universally accepted combination *E. colona* (L.) Link.

The preferred common name for this species in Australia and overseas is awnless barnyard grass, but it is also referred to as barnyard grass, river grass, swamp grass, jungle rice, tiger millet and zebra grass (Hazlewood and Johnson n.d.; Shepherd *et al.* 2001).

2.4.2.1 Origin and distribution

Echinochloa colona, a native of India, is well known as a weed of rice and is widespread throughout the tropics and sub-tropics to warm-temperate areas (Michael 2001). An important rainy season annual, it was widely distributed in India, commonly occurring in cultivated fields and waste places (Kapoor and Ramakrishnan 1974).

Now considered a serious weed in world agriculture, *E. colona* has a range extending from latitude 45°N to 40°S (Holm *et al.* 1977), and is particularly troublesome in southern Asia, the warmer regions of Australia and the Pacific Islands, the northern

part of South America and the Caribbean (Holm *et al.* 1977) (Figure 2.6). In Central America and the Caribbean, *E. colona* was widely distributed and ranked as the main grass weed in rain fed and irrigated rice (Valverde *et al.* 2000).

Figure 2.6: The distribution of *E. colona* indicating where it has been reported as a weed (Source: Holm *et al.* 1977).

Information gathered from herbaria records suggests that *E. colona* occurs in all Australian states with the exception of Tasmania and is prevalent in the northern and eastern areas (Figure 2.7).

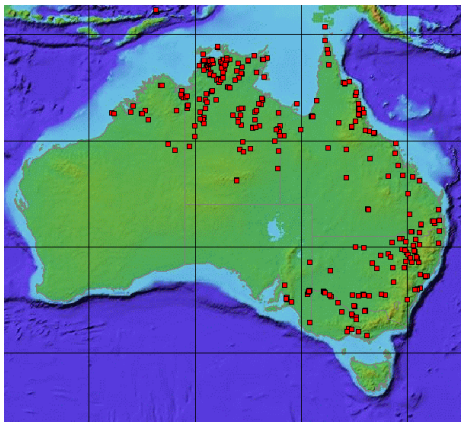


Figure 2.7: The distribution of *E. colona* in Australia according to specimens lodged with Australian herbaria (Source: Australia's Virtual Herbarium 2010).

2.4.2.2 Historical uses and benefits

Echinochloa colona has a long history of being used as a food source with grains being found in the intestines of Egyptian mummies (Ward 2005). Sharp and Simon (2002) state that *colona* is Latin for colonist and that *E. colona* was planted as a cereal. It continued to be used as a food source in India (Ward 2005) and in tropical Asia and Africa, where it was cultivated for the seeds that were made into flour (Holm *et al.* 1977). The grass is now best known as secondary forage for cattle (Ramakrishnan 1960; Ward 2005), although Tothill and Hacker (1996) regarded it as having little economic value for grazing.

2.4.2.3 Ecology and biology

Habitat

Echinochloa colona occurs in a wide variety of habitats ranging from wetland to dryland, and from sites at very low altitudes to sites located 2 000 m above sea level (Chun and Moody 1987). The species grows as a weed in cultivated grounds, grasslands, waterlogged soils such as along the margin of ponds and drainage channels, ditches and puddles (Ramakrishnan 1960). In Australia it is common along stream banks, levees, irrigation channels, around waterholes, in gilgai country (Hazlewood and Johnson n.d.) and in cultivation on periodically wet, rich soils (Tothill and Hacker 1996).

Echinochloa colona is found growing on a wide range of well-drained soils, including light (sandy), medium (loamy) and heavy (clay), and can grow in soils ranging from acidic to alkaline (Plants for a Future 2004). Few studies on ecotypical variation have been conducted on *E. colona*. One by Ramakrishnan (1960) reported the existence of two distinct ecotypes in India related to the moisture in the substratum. The first (tall form) grew in waterlogged soils while the second (short form) was restricted to well aerated moist soils. Both ecotypes preferred non-calcareous substratum. However, the tall form only grew in calcium-poor soils, while the short form occasionally grew in soils richer in exchangeable calcium. Further studies undertaken by Kapoor and

Ramakrishnan (1974) indicated the existence of at least three sub-ecotypes within the short form, each adapted to a narrow range of calcium concentration in the soil.

Plant growth

The prostrate growth habit of *E. colona* in its early seedling stages, the ability to root at the nodes to gain space, and the ability to assume an erect posture when light is limited makes it a very competitive weed for most crops (Holm *et al.* 1977). Although an annual, it may be vegetatively propagated, as roots are struck wherever nodes come in contact with the soil and give rise to new shoots at these regions (Holm *et al.* 1977; Ramakrishnan 1960). When separated, these new shoots can give rise to independent plants (Holm *et al.* 1977; Ramakrishnan 1960).

Chun and Moody (1987) reported significant variations in growth characteristics of *E. colona* including plant height, accumulative tiller length, leaf area, and dry matter among 12 ecotypes from different regions throughout the Philippines. Days to panicle emergence were correlated to the latitude at which the ecotypes were originally growing, with plants from higher latitudes producing panicles at least 10 days before those from lower latitudes.

Flowering and seed production

There is limited information available relating to flowering and seed production of *E. colona*. The flowers are hermaphroditic and wind pollinated (Plants for a Future 2004) with plants capable of producing thousands of seeds (Holm *et al.* 1977). Ramakrishnan (1960) compared the seed production of two forms of *E. colona* found in India. The average seed output of plants found in nature was compared to plants grown in a neutral substrate from garden beds (cultures). The tall form produced an average of 2 835 seeds/plant in nature compared to 20 264 in cultures while the short form had an average output of 541 seeds/plant in nature compared to 1 387 in cultures. The reproductive capacity of *E. colona* was substantial with 96 percent of the seeds of both forms and situations germinating.

Seed dispersal

Echinochloa colona seeds can be dispersed after ingestion by animals, including cattle and ducks which have been observed eating the seeds (Halvorson and Guertin 2003). The seeds can also be spread by farm machinery, in crop seed or transplants, in irrigation canals, and in mud adhering to animals and humans (Halvorson and Guertin 2003; Holm *et al.* 1977). In Australia, it is suspected that wild ducks may have been important in the initial distribution of the weed (Holm *et al.* 1977).

Seed dormancy and longevity

Some strains of *E. colona* were reported to have a short period of dormancy following harvest, but dormancy disappeared in less than eight weeks of dry storage (Holm *et al.* 1977). Ramakrishnan (1960) measured a dormancy period of about two months for the plants included in his study, and found that percentage germination increased with dry storage with a maximum 96 percent germination obtained in the next rainy season.

A study undertaken by Walker *et al.* (2006) in southern Queensland found that seed persistence of *E. colona* increased markedly when seed was buried at greater depths. After two years of burial in non-disturbed soil, 10 to 21 percent of seeds persisted at a depth of 10 cm, while only 1 to 2 percent remained in the top 2 cm.

Germination and emergence patterns

In their native India, *E. colona* seeds began to germinate just after the first rain at the beginning of July (Ramakrishnan 1960). The flowering phase of the plants commenced by the middle of August and continued until the end of September. By the middle of October, almost all the plants had completed their life-cycle and died after shedding the grains (Ramakrishnan 1960). In Australia the species grows rapidly during the spring to autumn period, with flowering occurring during summer and autumn, particularly in response to rain (Hazlewood and Johnson n.d.). A study undertaken in southern Queensland indicated that emergence of the buried seeds was highly dependent on available soil moisture (Wu *et al.* 2004). Germination flushes of 3.6 to 7.2 percent occurred between October and January after rainfall events of 90 to 141 mm, whereas less than 1 percent of buried seeds germinated in February and

March after 76 mm of rainfall. In most crops, *E. colona* has the ability to germinate any time during the growing season with light being required for best germination (Holm *et al.* 1977). In India plants were not found growing out of their normal growing season, and this was attributed to a poorer germination in drier soils and to the inability of seedlings to withstand low winter temperatures (Ramakrishnan 1960).

2.5 Taxonomy of *Echinochloa* species

2.5.1 Historical background

Carolus Linnaeus (1707-1778) named barnyard grass *Panicum crus-galli* in volume 56 of his *Species Plantarum* in 1753 (Mitich 1990). *Panicum*, a genus in which he placed many grasses, is a word meaning miller in Latin and is related to *panis*, Latin for bread (Mitich 1990). Linnaeus, in his original recognition of awnless barnyard grass as a member of the neuter genus *Panicum*, chose *colonum* as the epithet (Ward 2005). Certain species were removed from *Panicum* as deserving of independent rank, and *Echinochloa* was among the first of these distinctive groups to be given generic standing by Beauvois in 1812 (Mitich 1990; Ward 2005).

Echinochloa is a composite word, *Echino* from the Latin and Greek means 'prickly' or 'spiny' and *chloa* is Greek for grass or young herbage (Mitich 1990). Alternately, Sharp and Simon (2002) stated that the name derived from the Greek *echinos* (hedgehog) and *chloe* (grass) alluding to the echinate inflorescence branches. This new genus contained barnyard grass or cockspur grass and was given the name *E. crus-galli* (L.) Beauv. (Ward 2005). Names such as cockspur grass probably preceded Linnaeus and likely influenced the plant's botanical names, as there is a cockspur resemblance in the awns of the seed (Mitich 1990; Spencer 1957). The name *crus-galli* is made up of two Latin words; *crus* meaning shank, the place where the spur grows, and *galli*, which is a form of the word *gallus*, meaning cock (Sharp and Simon 2002, Spencer 1957).

In 1812 Beauvois also formed a second generic segregate, *Oplismenus* (Ward 2005). Kunth then transferred *Panicum colonum*, to form *O. colonus* (L.) HBK. in 1816, and

Link published the now universally accepted combination *E. colona* (L.) Link in 1833 (Ward 2005).

Although the genus *Echinochloa* was not universally accepted at first, *Echinochloa crus-galli* was recognized as a synonym for *Panicum crus-galli* in the Brewer *et al.* (1876 cited in Mitich 1990) publication on plants of California.

2.5.2 General description

The genus *Echinochloa* contains both annual and perennial species, usually with weak, succulent culms and broad flat blades. The inflorescences can be contracted or moderately open panicles, with few to numerous, simple or rebranched, densely flowered branches (Gould 1968). Spikelets are subsessile, in irregular fascicles or regular rows, disarticulating below the glumes (Gould 1968) and are all alike in sexuality (Watson and Dallwitz 1992). The basic chromosome number of the genus is $x = 9$ (Gould 1968) with 2n = 27, 36, 42, 48, 54, 72, and 108 (Watson and Dallwitz 1992). The chromosomes are 'small' with a mean diploid 2c DNA value of 2.7 pg (Watson and Dallwitz 1992).

2.5.2.1 Echinochloa crus-galli

The polymorphic nature of this complex has resulted in the naming of countless intraspecific taxa (Barrett and Seaman 1980). *Echinochloa crus-galli* is an extremely variable species, which has frequently been split into various varieties and forms in several countries particularly Australia, Japan and the United States (Holm *et al.* 1977; Lamp *et al.* 2001). In fact, the considerable intraspecific variations displayed by this species in regard to morphological traits is considered more than many known weed species (Bakar and Azmi 2003). The existence of several ecological and physiological ecotypes within the species has been identified, although the correlation between most of these and the species' morphological variation has not been established (Michael 1993).

In general, *E. crus-galli* is a robust, tufted, annual growing up to 1 to 2 m tall with glabrous culms that can be erect and ascending (Chin 2003) or often branching and rooting from a decumbent base (Holm *et al.* 1977; Maun and Barrett 1986) (Figure 2.8).

Figure 2.8: Line sketches displaying the seedling and mature habits of *E. crus-galli* (L.) Beauv. (Source: Holm *et al.* 1977).

The leaves are linear with a broad base and an acute tip, between 5 to 50 cm long and 5 to 20 mm wide (Holm *et al.* 1977). The inflorescence of *E. crus-galli* consists of an erect or nodding apical panicle that is green to purplish and from 5 to 20 cm long (Holm *et al.* 1977). The spikelets are crowded on the rachis, about 3 to 4 mm long

(excluding awns) and are elliptical and pointed in shape (Holm *et al.* 1977). When awns are present they are generally up to 10 mm long but sometimes can be as long as 30 mm (Chin 2003). The caryopsis, usually 2.5 to 3 mm long, can be ovate to obtuse, tan to brown, with longitudinal ridges on the convex surface (Michael 1983).

Of the varieties described by Michael (1994), *E. crus-galli* ssp. *crus-galli* had broadly ovate spikelets, with the two varieties var. *crus-galli* and var. *praticola* differing in size, branching of the inflorescences and bristliness of the spikelets. The spikelets (excluding awns) of var. *crus-galli* were between 3 to 5 mm long whereas those in var. *praticola* were 3 mm or less in length. While the distinction between these two varieties can sometimes be blurred, especially in Japan, Michael (2001) firmly believed that in the Asian-Pacific region there was a strong case for distinguishing between them. The more tropical *E. crus-galli* ssp. *hispidula* had ovate or elliptical spikelets (Michael 1994). The spikelets (excluding awns) of var. *hispidula* were 3 to 5 mm long whereas those in var. *austro-japonensis* were 3 mm or less.

It may or may not be appropriate to consider forms of *E. crus-galli* as varieties on the basis of absence of awns (as in var. *mutica*), presence of short awns (as in var. *breviaristata*) or long awns (as in var. *longiseta*) (Michael 1973). Awning, at least in some forms of *E. crus-galli*, may be greatly affected by environmental conditions (Michael 1994, 2001).

A closely related species, *E. crus-pavonis*, is much like *E. crus-galli* and it is often difficult to distinguish between the two, although a soft, pinkish, or pale purple panicle with crowded spikelets and very long awns is characteristic of *E. crus-pavonis* (Holm *et al*. 1977).

2.5.2.2 Echinochloa colona

There is much variation in this species, which has not yet been properly documented (Michael 2001). However, *E. colona* is mostly recognisable by its small awnless spikelets, the unbranched, rather widely-spaced panicle branches, and the usual absence of bristles on the main axis of the inflorescence (Holm *et al.* 1977; Michael

1994). While it is one of the easier species of *Echinochloa* to recognise, the different forms vary in habit, length of inflorescences and size of spikelets (Michael 2001).

Echinochloa colona has a growth habit ranging from a low spreading form to an erect close-tufted form, with culms to 75 cm tall (Holm *et al.* 1977). The culms are usually kneed towards the base, smooth and hairless, striate, and often branched (Tothill and Hacker 1996) (Figure 2.9).

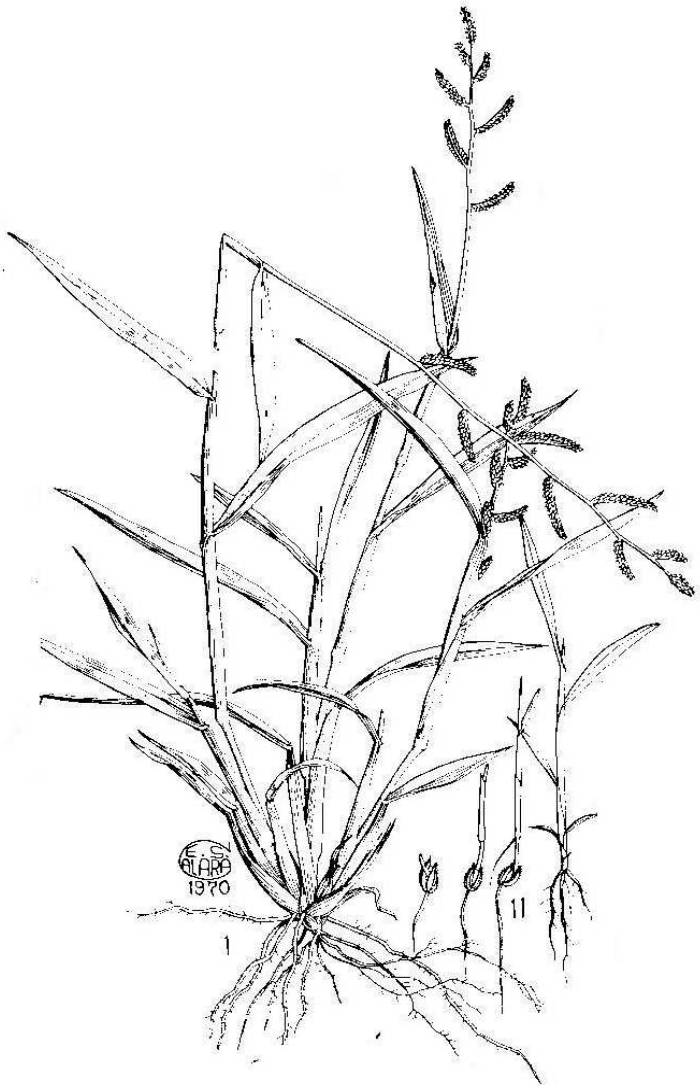


Figure 2.9: Line sketches displaying the seedling and mature habits of *E. colona* (L.) Link (Source: Holm *et al.* 1977).

The stems are slender and hairless, hollow, branched, and may have a purple colouration at the base. The stems often root at the lower nodes with the nodes brown

and thickened. The leaves may have purple, red or black bands running transversely across the surface giving rise to common names such as tiger millet or zebra grass (Hazlewood and Johnson n.d.).

The inflorescence is a panicle of racemes (7 to 40 mm long) that are spread out along the 5 to 15 cm long panicle. At first the racemes are more or less erect on the axis, but later they are slightly spreading (Michael 1983). The spikelets are 2.5 to 3 mm in length, crowded and almost sessile along the raceme, more or less arranged in 3 to 4 rows on one side of the rhachis, ovoid, often tinged with purple, and falling entire and free from the very short pedicels at maturity (Tothill and Hacker 1996).

In contrast to *E. crus-galli*, the seed covering of *E. colona* does not have a stiff bristle-like hair (awn) and the caryopses, which are 1.3 to 2 mm long, are white in colour (Michael 1983).

2.6 Molecular characterisation

There is widespread concern that weed species with high levels of genetic diversity will exhibit considerable potential for adaptation and, therefore, may be able to reduce the effectiveness of control (Jasieniuk and Maxwell 2001). As such, determining the magnitude of variation in weedy species is often considered a high priority (O'Hanlon *et al.* 2000).

Genetic studies are becoming increasingly more common in weed research and can be broadly classified into three areas of investigation: (i) patterns of genetic diversity within invading weeds; (ii) the taxonomic identity of weeds and (iii) determining the origin(s) of introduced weeds (O'Hanlon *et al.* 2000).

Following advances in molecular biology in the last decade, a variety of different methods have been developed for analysis of genetic diversity (Rao 2004). DNA-based markers allow direct comparison of genetic material from individual plants with little or no environmental influences on gene expression (Tasrif *et al.* 2004). Molecular methods used for detecting DNA sequence variation are generally based on

the use of restriction enzymes that recognise and cut specific short sequences of DNA (e.g. restriction fragment length polymorphisms - RFLPs), or polymerase chain reaction (PCR) which involves amplification of target DNA sequences using short oligonucleotide primers (Karp *et al.* 1997). PCR-based techniques, such as random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs or microsatellites), have proven especially important in diversity studies (Rao 2004).

2.6.1 Application of molecular markers in weed taxonomy and research

Molecular markers are well established and currently widely used in weed research to investigate genetic diversity, identify taxonomy and determine the origin of introduced weeds, particularly where multiple origins occur (Nissen *et al.* 1995; O'Hanlon *et al.* 2000). They have been used to resolve taxonomic uncertainties and provide information on the population diversity of many plant species including *Cyperus* biotypes (Tayyar *et al.* 2003), *Aconitum* populations (Cole and Kuchenreuther 2001), *Aegilops cyclindrica* accessions (Pester *et al.* 2003), *Rottboellia cochinchinensis* accessions (Alves *et al.* 2003), *Phalaris minor* populations (McRoberts *et al.* 2005) and *Echinochloa* populations (Danquah *et al.* 2002b; Tasrif *et al.* 2004; Yamaguchi *et al.* 2005; Yasuda *et al.* 2002).

2.6.2 Review of available molecular techniques

In the 1960s biochemical methods based on seed protein and allozyme electrophoresis were introduced and proved useful in the analysis of genetic diversity. However their usefulness was limited due to their inability to detect low levels of variation (Rao 2004). Nissen *et al.* (1995) explored the utility of two genetic marker systems, RAPD and RFLP, for investigating the ecological genetics of weeds. These techniques have become well established and widely used in weed research (O'Hanlon *et al.* 2000).

Four types of DNA markers are now commonly used to assess genetic diversity in plant populations: these are RFLPs, RAPDs, AFLPs and SSRs (Jasieniuk and Maxwell 2001). All are considered selectively neutral and capable of detecting finer

scale genetic variation than allozyme markers (Jasieniuk and Maxwell 2001). These techniques have all been used in studies on genetic diversity in *Echinochloa* species and this review will describe these techniques and contrast their relative advantages and disadvantages.

2.6.2.1 Restriction fragment length polymorphisms (RFLPs)

A non-PCR based method, RFLPs were the first DNA markers to be used by population biologists (Jasieniuk and Maxwell 2001). In RFLP analysis, DNA is digested with restriction enzymes, and the resulting fragments are separated according to molecular weight by gel electrophoresis (Jasieniuk and Maxwell 2001). RFLPs are highly reproducible markers capable of resolving genetic differences in plant species (Jasieniuk and Maxwell 2001), and the same probe enzyme combination on the same samples will give identical results even when carried out in different laboratories (Karp *et al.* 1997). RFLPs are codominant markers in that the different allelic variant bands are visible in heterozygotes, enabling all three genotypic classes to be distinguished (Karp *et al.* 1997). They are useful markers for population studies and diversity classification, provided that sufficient polymorphisms can be detected in the species under study (Karp *et al.* 1997).

Although an advance over earlier allozyme techniques, RFLP analysis also has limitations (Jasieniuk and Maxwell 2001). The procedures are very labour intensive and expensive for the quantity of information obtained and generally involve irradiation (Karp *et al.* 1997). Also, RFLP methods require relatively large amounts of high-quality DNA (10 μg per digestion) and thus are not applicable when very limited amounts of plant material or preserved tissues are available (Jasieniuk and Maxwell 2001). A good supply of probes that can reliably detect variation is required and finding probes for some species can be a problem (Karp *et al.* 1997). If it is not possible to utilise probes from other related species, new probes must be isolated from cDNA or genomic DNA libraries, which requires additional skill and investment of resources (Karp *et al.* 1997).

2.6.2.2 Random amplified polymorphic DNA (RAPD)

Development of PCR in 1985 led to a profusion of new molecular techniques that overcame many of the limitations of probe-hybridisation-based methods such as RFLPs (Jasieniuk and Maxwell 2001; Karp *et al.* 1997). Among these, a subset of closely related techniques was developed, which involved the use of a single 'arbitrary' primer in a PCR reaction, the result of which was the amplification of many discrete DNA products (Karp *et al.* 1997). The most commonly used version is RAPD analysis in which the amplification products are separated on agarose gels in the presence of ethidium bromide and visualised under ultraviolet light (Karp *et al.* 1997).

RAPD analysis has several advantages over allozyme and RFLP techniques: genetic variation at many loci from different regions of the genome can be examined quickly, very small amounts of DNA (10 ng per reaction) are required, no prior template DNA sequence information is required, they are simpler, less costly, sample throughput can be high and the procedure is automatable (Jasieniuk and Maxwell 2001; Karp *et al.* 1997). Because of their ease of use RAPDs have been used in all kinds of diversity studies at all taxonomic levels, including population and phylogenetic studies (Karp *et al.* 1997). RAPD markers are dominant and do not allow the identification of heterozygous individuals, which reduces the accuracy of estimation of population genetic parameters including the estimation of allele frequencies (Jasieniuk and Maxwell 2001). As a result, two to ten times more individuals per locus must be sampled to achieve the statistical power obtained with codominant markers such as RFLPs and allozymes (Jasieniuk and Maxwell 2001).

Further limitations with this procedure result from the fact that it is absolutely critical to maintain strictly consistent reaction conditions in order to achieve reproducible profiles. In practice, band profiles can be difficult to reproduce between and even within laboratories, if personnel, equipment or conditions are changed (Karp *et al.* 1997). It is likely that RAPDs can be used successfully in a single lab when all the operating conditions can be carefully controlled. However, problems in repeatability

suggest that caution should be exercised in including RAPDs data in databases intended for widespread access and use (Karp *et al.* 1997).

2.6.2.3 Amplified fragment length polymorphism (AFLP)

A more recently developed method, which is equally applicable to all species and is highly reproducible is AFLP (Vos *et al.* 1995) that combines restriction digestion and PCR (O'Hanlon *et al.* 2000). Compared with arbitrary-primed PCR, such as RAPDs, AFLPs are performed under high stringency and are therefore theoretically less sensitive to reaction conditions (O'Hanlon *et al.* 2000). Much has been written about the AFLP technique and most of it is overwhelmingly positive (Robinson and Harris 1999).

AFLPs can be generated for any organism without prior DNA sequence knowledge and are thus applicable to a broad range of taxa (Jasieniuk and Maxwell 2001). They provide an effective means of detecting several polymorphisms in a single assay (Karp *et al.* 1997), are highly reproducible and can resolve extremely small genetic differences (Jasieniuk and Maxwell 2001). AFLP markers are as reproducible as RFLP markers, but AFLP analysis is faster, less labour intensive, and generates more information (Jasieniuk and Maxwell 2001). AFLPs are more technically demanding than RAPDs (Karp *et al.* 1997) but are more reproducible and provide a larger genome coverage (Jasieniuk and Maxwell 2001). Only small amounts of DNA (0.3 to 1.0 μg per reaction) are required (Karp *et al.* 1997).

The major limitation of AFLPs is that they are dominant markers and therefore, do not provide as much information in genetic analyses of populations as allozymes, RFLPs or SSRs (O'Hanlon *et al.* 2000). However, Karp *et al.* (1997) stated that heterozygotes can be identified with the use of gel scanners. The cost of this method is relatively high compared with other molecular markers, the number of polymorphisms detected is affected by the restriction enzyme and primers used, and a technical consideration with AFLPs is that high-resolution electrophoresis and radioactive, chemiluminescent or fluorescent labels are required to visualize the fragments (O'Hanlon *et al.* 2000). AFLPs provide less genetic information per locus than co-dominant markers such as

SSRs, but this is somewhat offset by the large number of loci that can be readily screened (O'Hanlon *et al.* 2000).

2.6.2.4 Simple sequence repeats (SSRs) or microsatellites

Microsatellites are an important class of DNA markers because of their abundance and length hypervariability (Gupta *et al.* 1996). An SSR is a form of repetitive DNA consisting of sequences two to ten base pairs long that are repeated a variable number of times in a tandem array at a given locus, such as $(TG)_n$ or $(AAT)_n$ (Karp *et al.* 1997). The variability in the number of such repeats between individuals provides a valuable set of molecular markers for characterising DNA (Karp *et al.* 1997). To conduct SSR analysis, PCR primers for the regions flanking a microsatellite repeat are developed, and the target region is amplified followed by high-resolution electrophoresis of the PCR products including the SSR (Jasieniuk and Maxwell 2001). The DNA of two individuals with SSRs of different lengths at a given locus will yield PCR products of different sizes (Karp *et al.* 1997).

There are several important advantages of microsatellites. They are co-dominant, locus-specific markers that can be detected by PCR, are very robust tools that can be exchanged between laboratories, their data are highly informative and the assay is relatively quick (Hayden and Sharp 2001; Karp *et al.* 1997). Allelic variation attributable to differences in as few as two base pairs can be resolved using this method (Jasieniuk and Maxwell 2001). By virtue of their extreme polymorphism, SSR loci are widely considered to be ideal markers for numerous fields including plant breeding, conservation biology, population genetic studies (O'Hanlon *et al.* 2000), DNA fingerprinting, paternity testing and linkage map construction (Hayden and Sharp 2001).

Microsatellite development necessitates a considerable investment of time and extra skilled expertise and resources (Karp *et al.* 1997). High development cost is a major impediment to the routine application of SSRs in the genetic study of non-commercial species and for identifying markers located in chromosomal regions of interest (Hayden and Sharp 2001). For this reason, the development and application of SSRs

in plants has mostly been restricted to a few of the most agriculturally important crops (O'Hanlon *et al.* 2000). The development of primers requires molecular skills, such as cloning and sequencing, and is labour intensive and time consuming (Jasieniuk and Maxwell 2001). Retrieval of microsatellites has not been easy in plants because of their relatively low abundance compared with animal genomes and a limited cross-transferability to other genera and even to other species within the same genus (Karp *et al.* 1997). However, O'Hanlon *et al.* (2000) stated that there appeared to be moderate to complete cross-species transferability within genera with a success rate ranging from 50 to 100 percent.

Despite these difficulties, microsatellite markers are becoming more widely used because of their high information content. They provide ideal tools for population studies and for assessing diversity among genotypes within species because the markers are codominant. This means that allele frequencies can be determined directly and their rate of change renders them particularly suitable for studies below species levels (Karp *et al.* 1997).

2.6.3 Application of molecular marker techniques to studies of Echinochloa species

Technological advances in molecular biology have greatly increased our knowledge of the genetic diversity existing in plant populations. By measuring genotype, rather than phenotype, genetic markers avoid complicating environmental effects and provide ideal tools for assessing genetic variation, identifying species and other locally adapted forms, and defining genetic relationships (O'Hanlon *et al.* 2000).

In the past, morphological characteristics have been used to identify species and detect biotypes, but this has been difficult with *Echinochloa* species because of the high morphological variation. Despite their world-wide distribution, and the increasing economic losses in food production that result from competition with these weeds, surprisingly little is known about the population genetic structure of members in the genus (Lopez-Martinez *et al.* 1999). Recent developments in molecular techniques provide powerful tools for revealing the differences in DNA sequences

among species and make it possible to establish a practice for identification of *Echinochloa* species.

It is important to appreciate that molecular genetics is a rapidly developing field and technologies are advancing faster than our understanding of their full potential or limitations (Karp *et al.* 1997). It is difficult to compare the different techniques and determine which one is best and for what purpose, because each method has its advantages and disadvantages (O'Hanlon *et al.* 2000) (Table 2.2).

Table 2.2: Comparison of four molecular marker techniques used in genetic diversity studies of *Echinochloa* (Sources: Karp *et al.* 1997; Powell *et al.* 1996).

	RLFPs	**RAPDs**	**AFLPs**	**SSRs**
Development costs	Medium	Low	Low	High
Cost ($ per assay)	High	Low	Medium	Low
Level of polymorphism	Medium	Medium	Medium	Medium
Co-dominant	Yes	No	No	Yes
Repeatability	High	Low	Medium	High
Ease of use	Labour intensive	Easy	Difficult initially	Easy

These techniques vary in the way they resolve genetic differences, in the type of data that they generate, in the taxonomic levels at which they can be most appropriately applied, and in their technical and financial requirements (Karp *et al.* 1997). It is important to recognise that there is no ideal genetic marker and the choice of an appropriate method will be dependent on the aims of the study, technical considerations and the properties of the species.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques were applied to establish a reliable method for identification of *E. oryzicola* and *E. crus-galli* (Yasuda *et al.* 2002). Fukao *et al.* (2004) constructed an RFLP linkage map of an F_2 population (*E. crus-galli* var. *formosensis* x *E. crus-galli* var. *praticola*) to analyse quantitative trait loci (QTLs) affecting flooding tolerance and other physiological and morphological traits. Rutledge *et al*. (2000) and Lopez-Martinez *et al.* (1999) used RAPD markers for the analysis of herbicide resistant

Echinochloa populations. Juraimi *et al.* (2005) and Tasrif *et al.* (2004) found variability among ecotypes of *E. crus-galli* var. *crus-galli* in Malaysia using RAPD markers, and Tsuji *et al.* (2003) used AFLP markers to obtain an insight into the origin and spread of resistant *E. phyllopogon*.

As previously mentioned, microsatellite development necessitates a considerable investment of time, expertise and resources and as such has mostly been restricted to agriculturally important crops. Danquah *et al.* (2002a) developed a microsatellite-enriched library for the isolation of microsatellite loci in *Echinochloa*, with the aim of providing more informative genetic markers. Five primer pairs were developed that amplified microsatellite loci in three agronomically important *Echinochloa* species, *E. colona*, *E. crus-galli*, and *E. crus-pavonis*. All five microsatellites showed inter- and/or intraspecific polymorphism and should be useful markers for studying population genetic structure within *Echinochloa* species and also aid in discriminating among species in the genus.

Since then, limited publications exist that report on the use of these primers to assess genetic diversity in *Echinochloa* species. However SSRs have been used to analyse the genetic diversity and population structure of *Oryza rugipogon* (Zhou *et al.* 2003), to determine relationships among *Lycopersicon esculentum* cultivars (He *et al.* 2003), to investigate the genetic diversity of *Anisantha sterilis* at within- and between-farm scales (Green *et al.* 2001) and to establish genetic relations in *Olea europaea* (Belaj *et al.* 2003). Danquah *et al.* (2002b) used AFLPs and SSRs to assess genetic diversity in *Echinochloa* species from geographic to field levels in Côte d'Ivorie with both methods proving highly informative.

Characterisation for morphological traits cannot, however, be replaced by any of the molecular techniques, and the results of molecular or biochemical studies should be considered as complementary to morphological characterisation (Karp *et al.* 1997).

2.6.4 Taxonomic confusions and difficulties in species identification

The primary objective of plant classification is the grouping of plants and populations into recognizable units with reasonably well-defined boundaries and stable names (Gould 1968). The taxonomic grouping and differentiation of taxa is based on characteristics known or assumed to be genetically controlled, but unfortunately, little genetic information is available concerning the majority of the world's grasses (Gould 1968).

Many varietal differences have caused confusion in the nomenclature of *E. crus-galli*, with no fewer than 60 synonyms being used among the four defined varieties of *E. crus-galli* in North America (Mitich 1990). To add further to the confusion, other genera, in which it has appeared, include *Panicum*, *Setaria*, *Milium*, *Pennisetum*, *Oplismenus* and *Orthopogon* (Mitich 1990).

Taxonomic problems however, are due to several intergrading polymorphic complexes of which *E. crus-galli* is the most widely distributed and difficult to classify (Barrett and Wilson 1981; Maun and Barrett 1986). The apparent diversity of forms is associated with high phenotypic plasticity coupled with a fluent adaptability to the wealth of habitats occurring in seasonally wet and ruderal sites (Maun and Barrett 1986). Variation patterns in North America were further complicated by multiple introductions of alien taxa followed by inbreeding (Barrett and Wilson 1981).

Both *E. colona* and *E. crus-galli* display a high degree of variability in Australia, with different forms or apparent ecotypes existing that vary in characteristics such as growth habit and heading time (Michael 1973). *Echinochloa colona* has been confused with the small-spikelet *E. crus-galli* var. *praticola* and var. *austro-japonensis* (Michael 1983), the latter of which can be identified by their shorter lower glumes and more abundant bristles (Michael 1994).

Echinochloa colona and *E. crus-galli* are similar species that can be difficult to identify especially in the vegetative state (Lambrechtsen 1992). However when

mature, certain morphological characteristics can be used to assist with identification (Table 2.3).

Table 2.3: Principal characteristics distinguishing *E. crus-galli* and *E. colona* (Adapted from Michael 1983; Yabuno 1983).

Characteristic	***E. crus-galli***	***E. colona***
Panicle	Usually nodding	Erect
Branch of rachis	Rather close on a rachis, more or less branched	Rather distant on a rachis, simple
Bristles on rachis	Present	Absent
Spikelet	Often not arranged in rows, length: 3-5 mm	Usually arranged in 4 rows, length: ≤ 3 mm
Awn	Variable in length	Awnless
Caryopsis colour	Brown	White

The presence or absence of long bristles along the main flowering axis and side branches should be noted (Michael 2003), as they are mostly quite prominent in *E. crus-galli* but often absent in *E. colona*. However, weaker bristles may sometimes occur in *E. colona* from subtropical and tropical regions (Michael 2003).

The structure of the individual spikelet is known for most species of *Echinochloa* and the overall shape, size and the relationship between the first glume and the first lemma are very often useful diagnostic features for species identification (Michael 2003). The nature of the caryopsis is also very important, especially the colour, size and shape (Michael 2003). The caryopses of *E. colona*, *E. frumentacea* and some Australian native species are whitish, those of *E. microstachya* are yellowish but most, as in *E. crus-galli*, are brownish (Michael 2003).

According to Yamaguchi *et al.* (2005), taxonomic confusion remains in this genus because of an inadequate understanding of the biosystematic relationship among species and intraspecific taxa, and previous attempts aimed at solving the difficulties have led to a very ambiguous understanding of phylogenetic relationships among taxa.

2.7 Management of *E. crus-galli* and *E. colona* in cropping systems of the northern grain region

2.7.1 Northern grain region (NGR)

The northern grain region of eastern Australia extends from the Liverpool Plains region of northern New South Wales (approx 32°S) to 60 km south of Cooktown in north Queensland (approx 16°S) and extends 100 to 400 km west of the east coast (Webb *et al*. 1997). While some of this belt extends into the tropics, the majority lies in the sub-tropics between Emerald and Tamworth. Cereals are grown within two contrasting physiographic regions of Australia's sub-tropics, the Eastern Highlands and the Murray-Darling Plains (Webb *et al.* 1997). The cropping belt is unique in Australia in that over most of it both summer and winter cereals can be grown, a result of the particular winter-summer rainfall distribution and the nature of the major soil types (Webb *et al.* 1997).

The climate of the NGR is characterised by overlapping influences of summer-dominant rainfall in the tropics and winter-dominant rainfall in the temperate zone (Webb *et al.* 1997). Summer rainfall decreases in a northeast to southwest direction in the summer, while winter rains have a south to north gradient (Freebairn *et al.* 2006).

The major cereal-growing soils in the region are grey, brown and black Vertosols, black, red or brown Sodosols, red and brown Chromosols and Ferrosols (Webb *et al.* 1997). There is a high proportion of Vertosols in the southern section of the region, with Sodosols and Chromosols predominant in central and southern Queensland (Webb *et al.* 1997).

2.7.2 Farming systems

Considerable geographic variation in rain-grown cropping systems exist due largely to variations in annual rainfall distribution with complexities and variability in cropping systems reflecting rainfall reliability (Webb *et al.* 1997). For example, in north

Queensland the systems comprise summer crops almost exclusively, with opportunities for winter cropping increasing with latitude.

A common practice in the northern grain region is the production of summer or winter crops in rotation with a fallow period to store soil water to improve viability and reliability of cropping (Freebairn *et al.* 2006). Fallow refers to a non-crop period of managed freedom from plant growth, the purpose being to store water and accumulate available nitrogen in the soil for later use by crops (Freebairn *et al.* 2006). Growers have traditionally used a bread wheat (*Triticum aestivum*)-sorghum (*Sorghum bicolor*) rotation, producing two crops in three years, with fallow periods varying from 6 to 18 months between crops (Rew *et al*. 2005). Winter crops are often grown after a summer fallow period of around six months and summer crops after a winter fallow (Thomas *et al.* 2007). In addition to wheat and sorghum, other common cereal crops grown are maize and barley, with the proportion of winter crops (wheat, barley) increasing from north to south (Thomas *et al.* 2007). Other crops grown include oilseeds (canola, sunflower), pulses (chickpea, faba bean, mungbean, soybean, peanut, navy bean) and cotton (Thomas *et al.* 2007).

2.7.3 Integrated weed management (IWM) practices

The increased reliance on herbicides to manage weeds in both fallow and in-crop has contributed to the rising concern about herbicide resistant weed populations. A key approach in trying to minimise the occurrence of problematic weeds in crops is the development of an integrated weed management (IWM) system. Management decisions contributing to the development of IWM on a farm are crop rotations, soil and cultivation practices, crop nutrition strategy, other aspects of crop protection, planning, crop hygiene, well-informed and trained staff, and wildlife and landscape management (Naylor and Drummond 2002).

A range of practices is being used across the northern grain region in regard to weed management and while herbicides play a vital role, non-chemical methods such as grazing, tillage, chipping and crop rotation and competition are also being utilised (Osten *et al.* 2007).

2.7.3.1 Non-chemical tactics

Several different types of tillage practices are used in Australia. These include: conventional tillage where the soil surface is totally disturbed and left with little plant residue, conservation tillage systems where at least 30 percent of the soil surface is covered with plant residue after planting, direct drilling in which the crop is sown without previous tillage but where considerable soil disturbance occurs at seeding, and no till where soil disturbance is minimised and the crop sown with narrow soil openers or discs (Chauhan *et al.* 2006). Conservation tillage systems have been progressively replacing conventional tillage systems over the past two decades as the preferred soil preparation method in farming systems (Rew *et al.* 2005). Advantages of conservation tillage systems include increased profits, reduced risk of soil erosion and better salt management if there is a reduction in the through-drainage of water as a result of water usage by the crop (Rew *et al.* 2005). One disadvantage is the increased reliance on herbicides (Rew *et al.* 2005). Weeds can infest fallows and a past practice of controlling these weeds with tillage has been replaced by herbicide applications (Adkins *et al.* 1998).

Crop competition is considered a suitable technique to reduce weed growth. However, very few growers across the northern grain region are adopting this technique as a tool for weed management (Osten *et al.* 2007). In a North American study, Norris *et al.* (2001) observed that increased tomato densities had some effects on the growth of *E. crus-galli* but manipulation of crop density appeared to be useful as a management strategy only when *E. crus-galli* densities were less than about 1.0 plant/m of crop row. In the United States, rotations with rice, soybeans, and/or oats have been effective in reducing the levels of infestation and alternating rice with pasture has been used in Brazil and Australia to reduce *E. crus-galli* populations in succeeding rice crops (Holm *et al.* 1977).

In most crops *E. colona* has the ability to germinate anytime during the growing season, and for this reason, the first flush of weed seeds is often allowed to germinate before the crop is planted and these seedlings are destroyed by cultivation (Holm *et al.*

1977). While chipping is an effective and important control method in cotton, it is ineffective in rice where flooding remains an important control method.

Currently there are no known biological agents to control either *E. colona* or *E. crus-galli* (Holm *et al*. 1977).

2.7.3.2 Chemical tactics

The use of herbicides as substitutes for mechanical tillage has increased dramatically over the last three decades. The introduction of desiccant chemicals such as glyphosate was responsible for more changes in agronomic practice than any other chemical, apart from fertilizers (Freebairn *et al.* 2006). As tillage is reduced, there is decreased soil disturbance, increased crop residue and increased reliance on herbicides for weed control (Chauhan *et al.* 2006). One consequence of this change has been the emergence of herbicide resistance as a factor limiting the use of herbicides and a re-examination of alternative methods of weed control in some cropping systems (Freebairn *et al.* 2006).

Certain species of *Echinochloa* spread rapidly after the introduction of broadleaf-selective phenoxy herbicides in the 1950s (Holm *et al.* 1977). *Echinochloa colona* is now a common weed in summer fallows and, while several post-emergence herbicides offer selective control within the summer crop, few provide economic control during the fallow periods (Adkins *et al.* 1998). Glyphosate is used for broad spectrum fallow weed control and is non-residual and non-toxic to animals (Mueller *et al.* 2005). After more than 25 years of sales, it is the world's largest selling and fastest-growing agrochemical and has become a preferred herbicide to control many types of plants in numerous situations (Mueller *et al.* 2005). However, it does not have activity in the soil, and weeds emerging after treatment are not controlled (Ferrell and Witt 2002).

Wicks *et al.* (2000) indicated that *E. colona* was among several species in northern New South Wales summer fallows that displayed tolerance to recommended field rates of glyphosate and hence adequate weed control was not being achieved. In both

summer and winter fallows in the northern grain region, 83 to 87 percent of growers indicated a very high reliance on glyphosate (Osten *et al.* 2007), and instances of inconsistent control of *Echinochloa* species were reported by Walker *et al*. (2005).

While a suite of management tools are being used to target the entire weed spectrum, some individual species may only be susceptible to one tool and therefore targeted only by that tool (Osten *et al.* 2007). The selection pressure exerted on that weed by that option is therefore very high. As early as 2002 *Echinochloa* was rated as having a moderate to high risk of developing glyphosate resistance due to the high frequency of glyphosate use and limited rotation with differing modes of action herbicides or other weed control options (Walker *et al.* 2004).

2.8 Herbicide susceptibility and the development of herbicide resistance

2.8.1 Herbicide susceptibility and tolerance

The most important herbicides currently used are those capable of controlling a broad spectrum of weeds without affecting the crops. In any given agricultural environment, a weedy species is represented by a heterogeneous population of individuals showing differences in their morphology and other biological features such as development rate, seed production and dormancy (Grignac 1978). Weeds also show a natural variability in sensitivity to herbicides, referred to as herbicide susceptibility, whereas herbicide tolerance indicates a reduced susceptibility that may sometimes result from selection by herbicide (Rao 2000). The term tolerance is frequently used not only to refer to variations in the ability to withstand a herbicide application between different species but also when there is variability within a population of the same species (De Prado and Franco 2004).

The use in a cultivated field of a herbicide with a more or less prolonged residual activity will introduce a powerful selective force that removes a large number of susceptible plants before resistant plants, and allows for a more rapid multiplication of the resistant progeny (Grignac 1978). Repeated treatment of a weed population with the same herbicide can also produce selection favouring the build-up of resistant

biotypes. In many species the development of biotypes resistant to herbicides seems to be a widespread phenomenon when treatments with the same herbicide are repeated for several years and when particular resistance genes exist in the treated population (Grignac 1978).

2.8.2 Herbicide resistance

Herbicide resistance is the inherent ability of a species to survive and reproduce following exposure to a dose of herbicide normally lethal to its wild type (Rao 2000). The development of herbicide resistance in weeds is an evolutionary process and genetic variation for resistance must be present in a susceptible weed population for the evolution of herbicide resistance to occur (Jasieniuk *et al.* 1996). In response to repeated treatment with a particular class or family of herbicides, weed populations change in genetic composition such that the frequency of resistance alleles and resistant individuals increases (Jasieniuk *et al.* 1996). In this way, weed populations become adapted to the intense selection imposed by herbicides. Some individual plants can develop the capability to survive a herbicide application that the same species were susceptible to, this response is known as resistance (De Prado and Franco 2004). In Arkansas, fields that had been sown to rice for more than 20 consecutive years contained *E. crus-galli* populations that were adapted to the intense selection imposed by propanil with plants resistant to 20 times the normal rate of propanil application (Rutledge *et al.* 2000). A reason that resistance has not yet been detected in rice fields in Australia is that no fields have been sown to rice for that period of time (Pratley and Broster 2004).

Herbicide resistance in *Echinochloa* species has been reported for several herbicide groups. The first confirmed case of herbicide resistance in *E. crus-galli* was recorded in the United States in 1978 with plants resistant to photosystem II inhibiting herbicides while the resistance of *E. colona* to ureas and amides was first reported in Costa Rica in 1987 (Heap 2010). Since then, herbicide resistant biotypes of *E. crus-galli* have been reported in 15 countries (Table 2.4) and herbicide resistant biotypes of *E. colona* have been reported in nine countries (Table 2.5).

Table 2.4: Confirmed populations of herbicide resistant *E. crus-galli* indicating mode of action and the country where the resistance occurred (Source: Heap 2010).

Country	Mode of action and the year herbicide resistance was confirmed
USA	Photosystem II inhibitors (1978)
	Urea and amides (1990, 1991, 1994, 1995)
	Synthetic auxins (1998)
	Multiple resistance – synthetic auxins/urea and amides (1999)
	Multiple resistance – ACCase inhibitors/thiocarbamates and others (2000)
Canada	Photosystem II inhibitors (1981)
France	Photosystem II inhibitors (1982)
Greece	Urea and amides (1986, 2000)
Bulgaria	Dinitroanilines and others (1992)
Spain	Photosystem II inhibitors (1992)
China	Chloroacetamides and others (1993)
	Thiocarbamates and others (1993)
Czech Republic	Photosystem II inhibitors (1994)
Poland	Photosystem II inhibitors (1995)
Sri Lanka	Urea and amides (1997)
Thailand	Multiple resistance – chloroacetamides and others/urea and amides (1998)
	ACCase inhibitors (2001)
Brazil	Synthetic auxins (1999)
Italy	Urea and amides (2000)
Yugoslavia	ALS inhibitors (2002)
Philippines	Multiple resistance - Chloroacetamides and others/urea and amides (2005)

Table 2.5: Confirmed populations of herbicide resistant *E. colona* indicating mode of action and the country where the resistance occurred (Source: Heap 2010).

Country	Mode of action and the year herbicide resistance was confirmed
Costa Rica	Ureas and amides (1987)
	ACCase inhibitors (1994)
	Multiple resistance – ACCase inhibitors/ALS inhibitors/ureas and amides (1998)
Colombia	Ureas and amides (1988)
	Synthetic auxins (2000)
El Salvador	Ureas and amides (1999)
Guatemala	Ureas and amides (1999)
Honduras	Ureas and amides (1999)
Panama	Ureas and amides (1999)
Venezuela	Ureas and amides (2000)
Nicaragua	ACCase inhibitors (2000)
Australia	Photosystem II inhibitors (2004)
	Glycines (2007)

In 2007 a population of glyphosate-resistant *E. colona* was confirmed in northern New South Wales (Heap 2010; Preston 2007). No reported resistance to glyphosate occurred in the first 20 years of its use as it had a unique mode of action and an apparently low resistance frequency (Mueller *et al.* 2005). The mode of action of glyphosate is by inhibition of the enzyme 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) which is considered to be predominantly located in plastids such as the chloroplast (Hetherington *et al.* 1998).

Crops resistant to glyphosate have been widely adopted by growers in the United States and repeated use of glyphosate over the years has resulted in the selection of weeds resistant to glyphosate (Koger *et al.* 2005). A common theme among these particular weed species is that they had previously evolved resistance to other herbicides and had an inherent genetic variability that allowed resistance to manifest itself upon the appropriate selection pressure (Mueller *et al.* 2005).

Weed shifts may also result from the widespread use of glyphosate on glyphosate-resistant crops (Hennigh *et al.* 2005). Historically, the repeated use of a single herbicide program has resulted in shifts of the weed spectrum. During the 1950s repeated use of the herbicide 2,4-D shifted the weed communities in several cropping systems from domination by broadleaf weeds to domination by annual grasses (Aldrich 1984). Also in the 1950s, the repeated use of the substituted urea herbicides caused a shift toward broadleaf and perennial weeds, whereas triazine use in the 1960s caused shifts toward late-emerging weeds such as *Panicum dichotomiflorum* (Aldrich 1984).

In recent years, biotypes of several weed species have been confirmed as having evolved resistance to glyphosate applications, including *Lolium rigidum* (Pratley *et al.* 1996), *Conyza canadensis* (Feng *et al.* 2004), *Agrostis* species (Hart *et al.* 2005) and *Ambrosia artemisiifolia, Conyza bonariensis, Eleusine indica, Plantago lanceolata* and *Echinochloa colona* (Heap 2010).

There are limited studies on plants with glyphosate resistance but early studies indicate that two very different mechanisms can endow resistance: target site-based

resistance through changes of the EPSPS gene (an enzyme of the shikimate pathway) conferring an EPSPS weakly resistant to glyphosate, and non-target site resistance where there is reduced translocation of glyphosate to meristematic tissues (Powles and Preston 2006).

The mechanism of resistance identified in glyphosate-resistant populations of goosegrass (*Eleusine indica*) and Italian ryegrass (*Lolium multiflorum*) was target site whereas resistance in Canadian horseweed (*Conyza canadensis*) was identified as reduced translocation. Resistant populations of rigid ryegrass (*Lolium rigidum*) in the United States are due to target site resistant while separate populations in Australia display target site resistance and reduced translocation (Powles and Preston 2006).

Research undertaken by Wakelin *et al.* (2004) and Lorraine-Colwill *et al.* (2003) found that populations of resistant *Lolium rigidum* had altered translocation patterns when compared to susceptible populations and that resistant plants showed a greater tendency for glyphosate to accumulate in the leaf tip and reduced glyphosate accumulation in the critical shoot meristematic zone.

2.9 Conclusion

A review of the literature has provided useful background information on *Echinochloa* species and helped identify gaps in Australian knowledge. Identified as major summer weeds in the northern grain region, *Echinochloa* species have major economic impacts on crop production. However, many reports failed to identify what species were present and which species occurred as the major weed/s. This could be due to similarities in the overall appearance of the grasses and the existence of morphologically intergrading types complicating species identification. In order to develop weed management protocols an understanding of what weed species are present is an essential requirement.

While there were several references in the literature to differing biotypes of *E. crus-galli*, there are limited studies on *E. colona*. The majority of studies have been undertaken overseas but there were two studies on *E. crus-galli* and two on *E. colona*

referring to research conducted in Australia. Pratley and colleagues (2004; 2008) identified the species present in the three major rice-growing regions of New South Wales, and also reported on biotypes of *E. crus-galli* based on awn and panicle size. Webb (1980) compared herbicide susceptibility of different forms of *E. colona* from locations in Western Australia and New South Wales while Michael and van Rijn (1970) also reported on different forms of *E. colona* from New South and Western Australia. There appeared to be no information in the literature pertaining to species or biotypes in farming systems in the NGR. Additionally, there was limited information on the ecology of *E. crus-galli* and *E. colona* in Australia including its germination, seed dormancy, seed production and the environmental processes that influence these processes.

It is therefore necessary to establish if there are morphological characteristics available to assist with identification of the different species in field situations. In this study, I investigate morphological characteristics of *Echinochloa* populations from throughout the NGR to assess the diversity of morphological present in *E. colona* and *E. crus-galli*.

Numerous international studies utilised molecular techniques to identify species, investigate intra- and inter-population variations, and to investigate herbicide resistance in *Echinochloa* species. While the research appeared to be unable to clearly differentiate between resistant and non-resistant strains of *Echinochloa*, they were useful in providing information about the possible origin and movement of resistant biotypes. However there was a lack of information relating to Australian populations and no information readily available on the genetic structure of this genus in this country.

With the recent development of microsatellites (SSRs) for *Echinochloa* species the opportunity exists for more studies to be undertaken into the genetic makeup of this genus in Australia and make comparisons with overseas populations. In this study I use SSRs to assess the genetic differences/similarities between *E. colona* and *E. crus-galli* populations from across the NGR.

This research was largely undertaken because farmers were not achieving good levels of control of *Echinochloa*, and when combined with an increasing concern about the development of herbicide resistant populations in the region, it was necessary to establish if morphology or genetic diversity contributed to the varied levels of control. Since this research commenced, populations of *E. colona* in northern New South Wales have been confirmed as having glyphosate resistance.

This study tests the glyphosate susceptibility of *Echinochloa* populations from throughout the NGR and uses morphological and genetic results to assess whether either factor contributes to varied levels of control.

Chapter 3 Morphological Studies

3.1 Introduction

Many weed species grow over a wide range of environmental conditions and exhibit large variations in size and morphology (Barrett 1982). Morphological traits are used to identify species and detect biotypes across different locations and habitats but this can be difficult with *Echinochloa* due to the morphological variations found within biotypes (Lopez-Martinez *et al.* 1995).

Both *E. colona* and *E. crus-galli* display a high degree of variability in Australia, with the existence of a number of forms or apparent ecotypes that vary in characteristics such as growth habit and heading time (Michael 1973). There are numerous references in the literature to different *E. crus-galli* biotypes but only limited studies on biotypes of *E. colona.* In Australia, Pratley *et al.* (2008) noted differences between forms of *E. crus-galli* collected from rice-growing areas of south-eastern Australia, while Michael and van Rijn (1970) and Webb (1980) reported on different forms of *E. colona* collected from the Namoi River Valley in New South Wales and the Ord River Valley in Western Australia, and various sites in New South Wales and the Kimberley region of Western Australia respectively.

Tsuji *et al.* (2003) compared the morphology of herbicide-resistant and -susceptible biotypes of *E. phyllopogon* using plant height, flag leaf (length, width, margin colour), culm base (colour, diameter), panicle (length, colour) and spikelet characteristics. A clear differentiation between the two biotypes was identified when the combined characteristics were analysed.

Similarly, Tabacchi *et al.* (2006) scored traits including plant height, colour (basal stem, collar region, leaf blade margin, leaf midrib, spikelets), inflorescence habit and spikelet width to classify *Echinochloa* species in Italian rice fields. Analysis separated the plants into two main clusters. When the plants were identified according to the taxonomic key of Pignatti, the first cluster contained only *E. crus-galli*, with the second cluster containing plants identified as *E. erecta* and *E. phyllopogon*. However, when a key developed by Carreter was used, some of the plants previously identified as *E. crus-galli* were identified as *E. hispidula,* and the plants in the second cluster

were identified as either *E. oryzicola* or *E. oryzoides*. Both taxonomic keys are used to classify *Echinochloa* species in Mediterranean countries and this study illustrates that confusion in classification can arise based on a reliance on taxonomic keys.

An early Australian taxonomic key (Stanley and Ross 1989) allowed for the identification of seven *Echinochloa* species in south-eastern Queensland, while Michael (2001) listed 24 species in the Asian-Pacific region including two subspecies and four varieties of *E. crus-galli*, and Sharp and Simon (2002) included 19 species for Australia but only one subspecies of *E. crus-galli*, *E. crus galli* spp. *hispidula*.

This chapter reports on a study comparing the morphology of 30 *E. colona* and 31 *E. crus-galli* populations, collected from three major cropping centres across the northern grain region (NGR) of Australia, when grown under uniform conditions. As the intent was to also compare morphologies and genetic diversity of plants within fields, individual plants were considered to be representative of the population sampled from each collection point in the field. Populations were collected from fallows, crops of sorghum, cotton and mungbean, and non-agricultural areas including roadsides and stock paddocks. Comparisons were made between populations based on morphological characteristics including growth form, plant height, number of tillers, leaves and panicles, and panicle length and width. Selected characteristics were measured at three growth stages: the onset of reproduction, full extension of the flag leaf and the onset of maturity. The onset of reproduction was judged as the emergence of the first panicle, the full extension of the flag leaf referred to a stage when it was possible to measure the flag leaf (first leaf under the first panicle) from the tip of the leaf to the collar region, and the onset of maturity was considered to occur when seeds fell freely from the first panicle that emerged.

In addition, the genetic diversity (Chapter 4) and the response of populations to applications of different rates of glyphosate were examined (Chapter 5). This was to investigate if morphological variations within the two species coincided with genetic differences and responses to herbicide applications (Chapter 6).

3.2 Aim

The objective of the study reported in this chapter was to identify what *Echinochloa* species (in particular *E. crus-galli* and *E. colona*) were present on farms from three regional centres across the NGR, to describe and compare the morphology of the species present, and to establish if biotypes were unique to the centres or similar across the region.

It was designed to answer the following specific questions:

(i) Are there clear differences in morphology between the two species?

(ii) Are differences in the morphology of individual species related to regional centres within the NGR?

(iii) Are there any relationships between species and morphologically different biotypes with location (at farm and field level)?

3.3 Materials and methods

3.3.1 Site selection

Seeds from *Echinochloa* species, thought to be primarily *E. colona* and *E. crus-galli*, were collected from 21 agricultural and non-agricultural fields across the NGR between January and April 2005. Populations were sampled from three major cropping centres of the NGR, at Wowan (central Queensland), Dalby (southern Queensland) and Narrabri (northern New South Wales) (Figure 3.1).

Local agronomists from Wowan and Dalby were asked to provide details of farms within their area suitable for sampling. The farms were to have at least five fields infested with *Echinochloa* and be accessible by conventional vehicle. The Australian Cotton Research Institute (ACRI) was recommended as a suitable location at Narrabri.

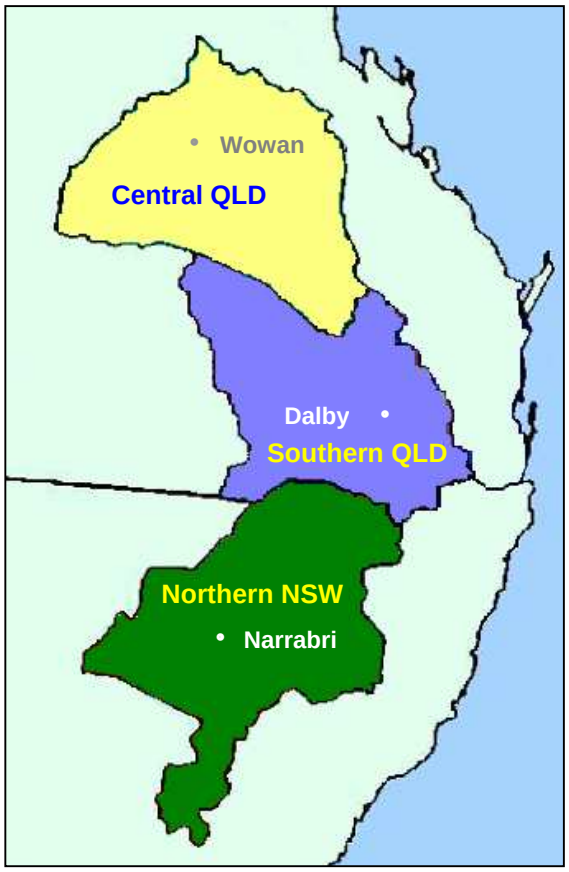


Figure 3.1: The northern grain region of Australia, showing the three major regional centres and the locations where populations were sampled (not to scale).

One farm from each centre was included in the study, and at Dalby and Narrabri the sampling of populations was undertaken in five fields of each farm. Many of the infested fields on the Wowan farm had been ploughed before sampling could be undertaken and no suitable replacement could be found. Therefore three agricultural fields (sorghum, mungbean, fallow) and four non-agricultural areas (horse paddock, cattle yards, roadsides) were sampled on the Wowan farm in order to have a similar

number of samples from each centre. An additional four fields adjoining the Dalby farm were also sampled in order to compare diversity between neighbouring farms.

3.3.1.1 Sampling method and seed collection

In each field, approximately one hectare was pegged out on the edge of the crop or fallow. Depending on the distribution of *Echinochloa* plants within this area, two sampling methods were used. If the infestation was relatively uniform throughout the plot, a series of zigzag transects were made across the sampling area. In fields with patchy distribution, smaller zigzag transects, combined with linear transects were used to sample the patches of *Echinochloa*. The method used for patchy distribution was also used to sample non-agricultural areas at Wowan.

Twenty collection points were established along transects in each agricultural field with the exception of one field at Wowan where only 10 points could be established. In the non-agricultural fields at Wowan fewer collection points (10 or 11) were established. The cattle yards contained four small holding pens and each pen was considered a collection point.

In all cases, except the cattle yards, the distance between collection points was approximately 10 m and at each point a 5 m diameter area was sampled for plants. The seeds of up to five randomly selected plants were collected and packaged separately in paper bags. In field one at Dalby, all the plants within the collection area at one inner and one outer collection point, were sampled to investigate the genetic diversity at a single collection point (Table 3.1).

Table 3.1 Summary of the 21 fields sampled across the northern grain region for *Echinochloa* species, indicating the habitat, the total number of collection points sampled in each field and the number of plants of each species sampled (SQ-southern Queensland, CQ-central Queensland, NNSW-northern New South Wales).

Location	Field number and habitat type	No. of collection points	No. of plants sampled	
			E. colona	*E. crus-galli*
Dalby (SQ) – farm 1	1 - sorghum	20	86	21
	2 - corn	20	97	3
	3 - corn	20	99	1
	4 - mungbean	20	100	0
	5 - sorghum	20	96	1
Dalby (SQ) – farm 2	1 - sorghum	20	75	14
Dalby (SQ) – farm 3	1 - sorghum	20	90	6
Dalby (SQ) – farm 4	1 - sorghum	20	72	0
Dalby (SQ) – farm 5	1 - sorghum	20	72	0
Wowan (CQ) – farm 1	1 - roadside	11	55	0
	2 - horse paddock	10	47	0
	3 - mungbean	20	94	0
	4 - fallow	20	100	0
	5 – sorghum	10	39	0
	6 - cattle yards	4	21	0
	7 - roadside	10	50	0
Narrabri (NNSW) – farm 1	1 - cotton	20	84	16
	2 - fallow	20	96	4
	3 - cotton	20	97	0
	4 - fallow	20	100	0
	5 - tail drain	20	42	58

Due to the shattering nature of *Echinochloa* panicles, many of the mature seeds had fallen off the plants. If shaking the panicle resulted in no seeds being dislodged, seeds were removed by firmly shredding the panicle by hand. As a result both mature and immature seeds were included in the collection.

A population ID comprising a five-digit alphanumeric code was assigned to each plant sampled. The code was based on the sampling method and included the regional centre (D – Dalby, W – Wowan, N - Narrabri), the farm (1 to 5), the field number (1 to 7), the collection point number (1 to 20) and the number of the plant sampled within each collection point (1 to 10). For example, the code D21:13-5 would refer to farm two at Dalby and the seed would have been collected from field one, collection point 13 and from the fifth plant sampled in the area around that point.

Seeds were air-dried in a glasshouse for two weeks before being repackaged in seed packets and stored in a cold room at 10°C. They remained in storage and were removed in both 2005 and 2006, two months before the morphological trials were established.

3.3.2 In-field identification of E. colona and E. crus-galli

In-field identification of the two species was primarily based on two characteristics. The presence of transverse purplish banding on leaves is characteristic of *E. colona* and all plants displaying bands were automatically identified as this species (Photograph 3.1).

Photograph 3.1: *Echinochloa colona* displaying transverse purple banding across the leaves. This plant has a prostrate growth form and stout culms.

However, banding is not present on all *E. colona* plants, so the inflorescences of plants without banding were checked for the presence of long bristles along the racemes. This characteristic is generally absent in *E. colona* (Photograph 3.2a) but present in *E. crus-galli* (Photograph 3.2b). Unbanded plants without bristles were recorded as *E. colona* and those with bristles recorded as *E. crus-galli*.

Photograph 3.2: The inflorescences of *E. colona* (a) and *E. crus-galli* (b) showing the absence/presence of bristles along the racemes.

Phenotypic characteristics including leaf banding, growth form and culm thickness were recorded for each plant from which seeds were collected. These characteristics were incorporated into a coding system (growth category) that was easily identifiable

and allowed comparisons to be made with progeny grown from the collected seeds (Table 3.2).

Table 3.2: Characteristics recorded for *Echinochloa* plants sampled in the field, describing the state of each characteristic and the rating scale used to establish a coding system.

Characteristic	State	Rating Scale
Bristles on raceme	Absence, Presence	nil
Leaf banding	Absence, Presence	0 - 1
Growth Form	Erect, Erect/semi-erect, Semi-erect, Semi-erect/prostrate, Prostrate	1 - 5
Culm build	Stout, Fine	1 - 2

The first characteristic, bristles on the raceme was used to identify a species as *E. crus-galli* and was not part of the coding system. While leaf banding is only found in *E. colona* it was still included in the coding for both species. For example, the plant in photograph 3.1 would have been allocated a code 151; banded, prostrate and with a stout culm. It would already have been identified as *E. colona* based on the presence of transverse banding on the leaves.

3.3.3 Seed characteristics

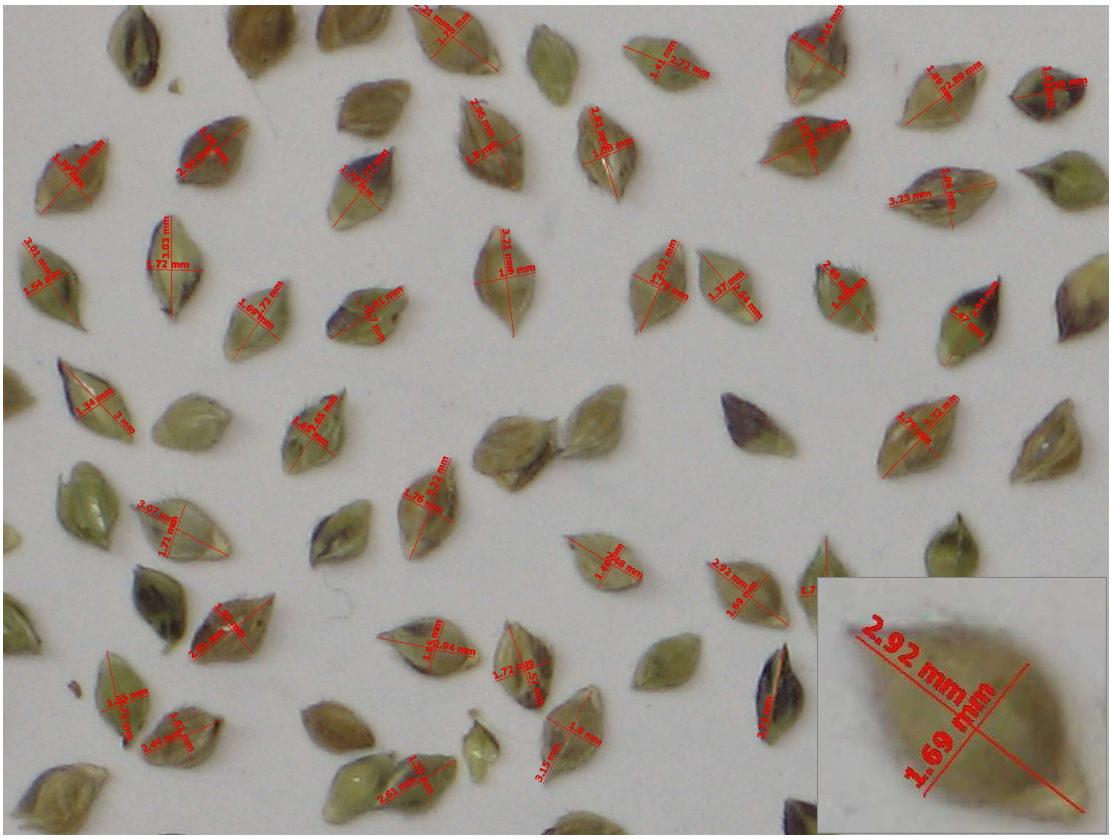
As seed characteristics can be an important tool for differentiation between species of *Echinochloa*, the length and width measurements of the seeds were recorded, as was the caryopsis colour. Thirty seeds from 30 *E. colona* and 26 *E. crus-galli* populations were photographed in 2005 prior to the establishment of the morphological trial.

3.3.3.1 Length and width measurements

A Canon Powershot G6 digital camera was mounted on a tripod at a height of 49 cm from table surface to the front of the camera (excluding lens). Photographs were taken using ‘remote shooting’ and saved onto the computer using Zoombrowser EX 5.0 software (Canon Digital Camera Solution Disc V20.0). The resolution settings that

produced the best images were large superfine with the macro and flash on. These settings photographed an area approximately 3 cm by 2 cm.

Once camera position, settings and image conditions were determined, and before running the acquired images through the AxioVision Release 4.4 software (Carl Zeiss Pty Ltd), a photograph of a clearly marked ruler was taken to establish a scale. Seeds were placed in a seed tray with a thin layer chromatography (TLC) plate inserted, matt side up and photographed. The ruler had also been placed in the tray before being photographed to make it the same height as the seeds. The scale was linked to all the images and allowed for accurate length and width measurements by a 'click and drag' measurement tool incorporated into the program (Photograph 3.3).



Photograph 3.3: Image produced of *E. crus-galli* seeds from one of the plants sampled at Dalby showing the length and width measurements calculated using the AxioVision Release 4.4 software (Inset: close-up).

3.3.3.2 Caryopses colour

Approximately 20 seeds from 10 randomly selected populations of both species were manually scarified with sandpaper to remove the glumes, palea and lemma, and the colour of the caryopses recorded. This was based on a category of colours ranging from white, cream and brown.

3.3.3.3 Data analysis

Seed measurements were analysed using REML variance components analysis (Genstat 2006) on individual seed measurements to compare the effect of all variables (region, population and species) and the co-variate (width) on seed length. Mean comparisons were made on the basis of region, population and species. The effects of the variables (region, population and species) and the covariate (width) were compared using Wald tests for fixed effects (Genstat 2006). One of the *E. colona* populations from Dalby was removed from the analysis, as it was an outlier with a smaller average size than the other populations. Many of the seeds from this population were green and may have been immature thus impacting on the measurements taken.

3.3.4 Morphological trials

A pot trial was established to compare the morphology of progeny grown from seeds collected in the field with that of the parent plants. The trial was conducted under similar environmental conditions to identify if morphology was linked to genetics or environment.

3.3.4.1 Achieving optimal germination

Due to difficulty in getting seeds to germinate, a series of germination trials was conducted using a TRIEL 140-1-SD incubator (Thermoline Scientific) to establish a suitable method for optimum seed germination. This was not a major focus of the thesis but was necessary to ensure adequate replicates could be germinated to set up

the morphology and herbicide susceptibility trials. It was undertaken to establish if there was a single regime that would give good germination rates for both species.

The first experiment compared the germination success of both species at differing temperature regimes (25°C, 28°C, 31°C). The incubator was set at a constant day/night temperature corresponding to one of the above temperatures, and on a 12-hour day/night cycle for 14 days. The two fluorescent lamps (Philips Lifemax TLD 16W 84C) in the incubator operated at a lighting temperature of 4 000 K and produced 1 400 lumens per lamp providing light for the day cycle. The first trial was conducted at the lowest temperature and after each trial, the seeds were removed and the incubator reset at the next temperature level. Each treatment included seeds that had been manually scarified with sandpaper to remove the caryopses and non-scarified seeds. Seed germination was evaluated by placing 50 caryopses/seeds in 9 cm diameter Petri dishes containing a Whatman No. 1 filter paper, moistened with 3 ml of distilled water. Four replicates were established for each treatment and the Petri dishes were placed in clip lock plastic bags to maintain moisture. Additional water was added as required and mouldy seeds were removed.

The second experiment used concentrated (18M) sulphuric acid (H_2SO_4) for 20, 30 or 40 minutes to scarify seeds of both species as per Sung *et al.* (1987). This alternate method was used, as manual scarification was time consuming. The acid-treated seeds were then rinsed under running tap water for three minutes and placed in Petri dishes as described in the previous experiment. This trial was only conducted in the incubator at 25°C and on a 12-hour day/night cycle.

For both experiments, the number of seeds germinated was recorded on a daily basis for a period of 14 days from the initiation of the experiment. The criterion for germination was the emergence of the radicle.

3.3.4.2 Environment

The morphological trials were established in a polyhouse at the Leslie Research Centre in Toowoomba, Queensland in an attempt to mimic natural conditions. The

structure of the polyhouse enabled plants to be exposed to rain and ambient temperature, having a wire dome mesh covering (to exclude birds) and heavy opaque plastic extending approximately 2 m up the sidewalls and covering the doors at each end. Trees growing near the polyhouse provided limited shade early in the morning but otherwise the plants were in full sunlight for most of the day.

Two trials were established to grow *E. colona* and *E. crus-galli* to maturity and assess and compare morphological traits, the first during the summer of 2005/2006 and the second during the spring of 2006/2007.

In the first trial, seedlings of both species were planted out of Petri dishes during the second week of December 2005 but high temperatures resulted in approximately 50 percent of the replicates dying, including all the *E. crus-galli*, during December/January. Additional populations of both species were established during January. The average maximum temperature for December was 40ºC (range 28ºC to 45ºC) and the average minimum was 18ºC (range 12ºC to 22ºC). Over the course of the trial, which ran from December to March, the maximum temperatures in the polyhouse ranged from 27ºC to 47ºC with minima ranging from 11ºC to 22ºC.

Due to the failure to establish sufficient replicates of *E. crus-galli* populations in the first morphological trial, a second trial with only *E. crus-galli* was established in September/October 2006. This trial was established earlier in an attempt to lessen fatalities due to high temperatures. The average maximum temperature for October was 35ºC (range 26ºC to 40ºC) and the average minimum was 12ºC (range 8ºC to 20ºC). The trial ran from September to January and the maximum temperatures in the polyhouse ranged from 26ºC to 45ºC with minima ranging from 5ºC to 22ºC.

A slow release fertiliser (Brunnings Nitrophoska® - 16% N, 3% P, 12.5% K and trace elements) was applied in both trials. In the 2005/2006 trial, a measuring scoop was used to apply approximately 10 g of fertiliser to each pot in February and again in March. In the 2006/2007 trial, the measuring scoop was again used and approximately 10 g of fertiliser were incorporated into the soil at the commencement of the trial and then applied monthly.

In both trials the seedlings were initially watered twice a day, early in the morning and late in the afternoon. This was reduced to a morning watering once the plants became established. Plants were not watered when sufficient rain was received to moisten the soil.

3.3.4.3 Experimental design

The growth categories assigned to the plants in the field were used as the basis to select populations to be included in the morphological trials. Within each growth category, populations were randomly selected from each of the cropping centres. Seeds from plants representing 17 of the 20 growth categories (2 leaf banding x 5 growth forms x 2 culm builds) (Table 3.2) of *E. colona* and four (2005/2006) and five (2006/2007) of the six growth categories of *E. crus-galli* were established in the trials depending on the availability of sufficient seed.

3.3.4.4 Establishment and management

Progeny from 30 *E. colona* populations and 6 *E. crus-galli* populations were successfully established in the first trial in 2005/2006 while 27 *E. crus-galli* populations, including two that had been established in the 2005/2006 trial, were successfully established in the second trial in 2006/2007 (Table 3.3).

Table 3.3: *Echinochloa colona* and *E. crus-galli* populations established in the two morphological trials of 2005/2006 and 2006/2007 indicating the population labels used in Chapter 3 and the original population IDs assigned to the plants sampled in the field. Refer to 3.3.1.1 for description of population ID.

2005/2006 morphological trial		2006/2007 morphological trial	
Population label	Population ID	Population label	Population ID
1	N12: 5-5	1	N15: 20-3
2	N15: 6-1	2	N15: 4-4
3	N11: 1-1	3	N12: 11-5
4	N13: 14-2	4	N11: 15-4
5	D13: 4-2	5	D11: 3-5
6	W16: 4-3	6	N11: 9-1
7	N11: 2-6	7	D11: 6-4
8	N11: 13-3	8	D21: 13-2
9	*N12: 10-3	9	D11: 11-8
10	*N11: 16-3	10	N15: 11-5
11	**N11: 3-5	11	D21: 17-2
12	N15: 10-4	12	N15: 16-2
13	N15: 12-3	13	D11: 20-4
14	D41: 10-4	14	N12: 12-2
15	D21: 4-1	15	N11: 11-2
16	D11: 16-4	16	N15: 2-4
17	W13: 5-2	17	N15: 1-4
18	W13: 17-1	18	D21: 5-5
19	W17: 10-1	19	N15: 7-4
20	W13: 2-1	20	N15: 2-1
21	W13: 11-9	21	**N11: 3-5
22	W14: 1-1	22	**N15: 9-5
23	W14: 6-3	23	N11: 3-3
24	*N15: 5-5	24	N11: 11-4
25	D21: 3-1	25	N15: 14-3
26	D11: 20-1	26	N15: 14-1
27	D51: 6-2	27	N15: 14-4
28	*N15: 13-4		
29	D13: 16-1		
30	N14: 7-4		
31	W12: 9-3		
32	D12: 10-4		
33	W15: 8-1		
34	D14: 12-4		
35	W11: 1-3		
36	**N15: 9-5		

** Echinochloa crus-galli (2005/2006 trial)* *** Echinochloa crus-galli (both trials)*

Seeds used for both trials were germinated in a TRIEL 140-1-SD incubator (Thermoline Scientific) at 28°C and on a 12-hour day/night cycle, as this was found to be the optimal germination method in preliminary trials conducted by the author. In addition optimal germination was achieved when *Echinochloa crus-galli* seeds were manually scarified and *E. colona* seeds were not. Seedlings were planted when the first leaf was approximately 2 cm long and juvenile roots were at least 1 cm long.

In both trials, four replicates of each population were established into 25 cm diameter pots. Each replicate contained four seedlings and this was culled back to one healthy plant per pot. The pots were partially filled with Brunnings potting mix containing composts (including composted pine bark) and other organic gardening materials, onto which a layer of approximately 10 cm deep of black cracking-clay soil from the Department of Primary Industries and Fisheries (DPI&F) farm at Wellcamp (long 151.85074S, lat 27.55360E) was placed. The decision to lay soil on top of the potting mix made the pots lighter and easier to relocate while still providing soil for plant establishment. The growing medium was not sterilized prior to use, but was collected from a field with no history of *Echinochloa* infestation. To identify the *Echinochloa* seedlings that had been planted, a toothpick was placed next to each one. Any additional seedlings emerging during the trial were removed.

During both trials pots became infested with ants, while the *E. colona* plants were infested by aphids. To control the infestations the plants were sprayed as required with Beat-A-Bug™ (active ingredients 7.5 g/L garlic, 2.0 g/L chilli, 0.3 g/L pyrethrens, 1.2 g/L piperonyl butoxide), a naturally based pesticide spray and Mortein ant-sand was sprinkled on the soil at the base of the plants. Neither product appeared to have any effect on the plants in the trial.

In order to overcome any variation in the environment, pots were randomly rearranged in the polyhouse every fortnight over the course of the experiment.

The trials continued for three to four months by which time all plants in both trials had set seed. Seeds were collected from individual plants and one plant from each population was harvested and pressed as a herbarium specimen. Leaf tissue had earlier

been collected from each herbarium specimen to investigate the genetic diversity of the populations in this morphological study (Chapter 4) and to establish if there were relationships between morphology and genetic diversity (Chapter 6).

3.3.4.5 Morphological assessments

Data were collected on a range of morphological characteristics at three stages during plant growth; onset of reproduction (emergence of the first panicle), full extension of the flag leaf (possible to measure the length of the leaf associated with the first panicle to emerge) and the onset of maturity (seeds fell freely from the first panicle that emerged) (Table 3.4).

Table 3.4: Morphological characteristics recorded for *E. colona* and *E. crus-galli* in the morphological trials and the growth stages at which they were measured (R - onset of reproduction, F - flag leaf extended, M - onset of maturity). Ratings devised for characteristics are described below.

Morphological characteristic	Plant growth stage	Rating
Number of leaves	R*	
Plant height (mm)	R* M*	
Number of tillers	R* M*	
Flag leaf length and width (mm)	F M*	
First panicle length and width (mm)	F M*	
Presence of bristles on racemes	M*	yes/no
Number of panicles	M*	
Culm hirsuteness (*E. colona*)	R	0 - 2
Culm pigmentation	R	1 - 4
Culm build	M	1 - 2
Growth form	M	1 - 5
Presence of leaf banding	M	yes/no
Panicle pigmentation (*E. crus-galli*)	M	1 - 3
Presence of awns (*E. crus-galli*)	M	yes/no

*characteristics statistically analysed

Leaves, tillers and panicles

The numbers for each characteristic were counted and recorded.

Plant height

Two measurements were recorded for plant height. The first measurement was a vertical measurement from the soil surface to the highest point of the freestanding plant and referred to as plant height. The second measurement was the extended height and referred to the length of the plant from its base at the soil surface, along the longest part of the plant whether leaf or panicle, to the uppermost point. Both measurements were included in the analyses to reflect the form of the plant.

Flag leaf and first panicle measurements

The length of the flag leaf was measured from the tip of the leaf to the collar region. Panicle length was measured from the tip to below the lowest raceme. Width measurements for both characteristics were taken at the widest point.

Culm characteristics

An obvious difference in the thickness of the culms was observed in both species during the field collection. The bases of the culms were either stout or fine and were given a rating of one or two accordingly (Photograph 3.5). It should be noted that these ratings were only used to provide contrasts between plants of the same species, and a fine culm on an *E. colona* plant was not comparable to a fine culm in *E. crus-galli*. Plants of both species grown in the trials also displayed a variation in the depth of pigmentation at the base of the culm ranging from pale pink to deep red. Plants with no pigmentation were given a rating of one while plants that were highly pigmented and had the pigmentation extending up the stems were given a rating of four (Photograph 3.7) The presence of fine hairs at the base of the culms was also noted. Plants were given a rating of 0 to 2 based on no hairs to hairs highly visible.

Growth form

In the field a gradation of different growth forms was observed and, as a result, plants of both species were categorised into one of five groups. Growth forms were rated on a scale of 1 to 5 ranging from erect (1), erect/semi-erect (2), semi-erect (3), semi-erect/prostrate (4), or prostrate (5) (Photograph 3.4). These codes were then used in the polyhouse trials to allow comparison of the progeny with that of the parent.

Miscellaneous characteristics

The presence of banding on the leaves of *E. colona* plants was recorded. For *E. crus-galli* the presence of bristles along the racemes, the presence of awns and the pigmentation of mature seeds (green, greenish-brown, dark red-brown) were also recorded.

The taxonomic keys developed by Stanley and Ross (1989), Michael (1983) and Sharp and Simon (2002) were used as references in this research.

3.3.4.6 Data analysis

The morphological characteristics included in the data sets for analysis at each of the three stages of plant growth were analysed using the R software (R 2.7.2 © The R Foundation for Statistical Computing, 2004). The morphological characteristics at each stage were averaged and the average values were analysed and compared using cluster analysis based on the Euclidian distance between populations. The resulting dendrograms clustered populations with similar morphologies. As the analyses of the data from the 'full extension of flag leaf' stage contributed no additional information to that obtained from the analyses at the other two growth stages it was omitted from the results.

3.4 Results

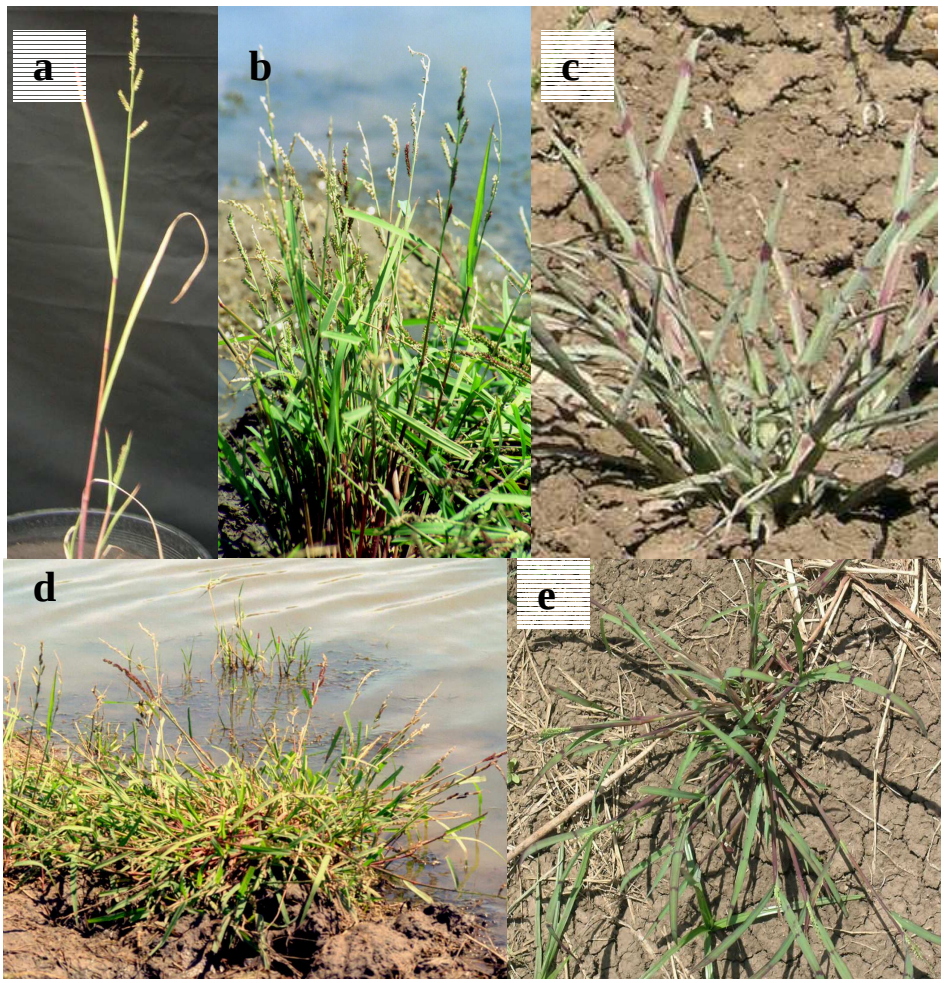
3.4.1 Field collections

3.4.1.1 Distribution of E. colona and E. crus-galli

Echinochloa colona was uniformly distributed across the region and the most commonly occurring species, accounting for 93% of the plants sampled. In central Queensland (Wowan) *E. colona* was the only species to be sampled while in southern Queensland (Dalby) it accounted for 94% of the samples and in northern New South Wales (Narrabri) 84%. *Echinochloa colona* was found in all agricultural and non-agricultural areas sampled throughout the region and, with the exception of the tail drain in Narrabri, was the dominant species (Table 3.1).

3.4.1.2 Morphological characteristics of E. colona and E. crus-galli

There was a large degree of morphological diversity within and between *E. colona* and *E. crus-galli*. In the field a gradation of growth forms was observed for both species. These gradations were categorised into five groups, ranging from erect to prostrate (Photograph 3.4).



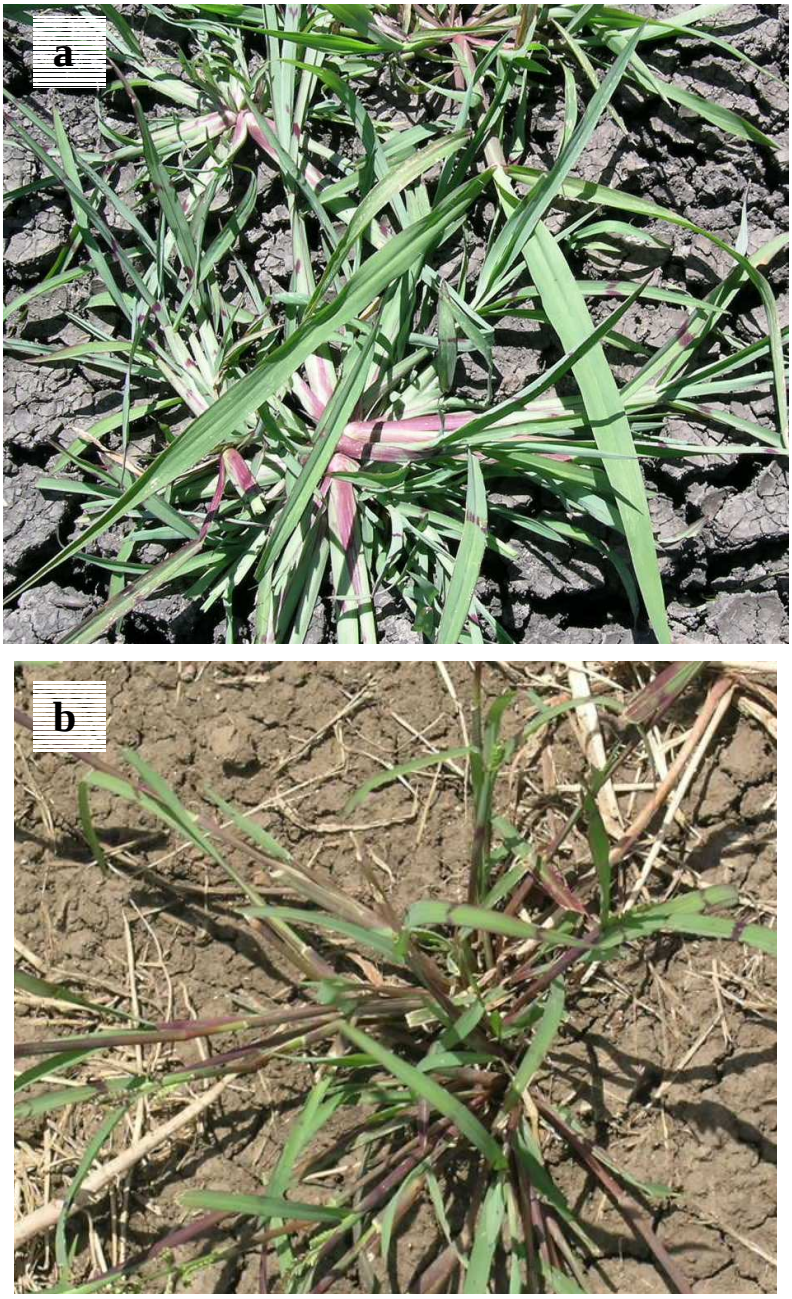
Photograph 3.4: Examples of the five growth forms used to categorise both species (a – erect, b - erect/semi-erect, c – semi-erect, d – semi-erect/prostrate, e – prostrate). All examples shown are *E. colona*.

Populations of *E. colona* varied in growth forms ranging from erect to prostrate, with the majority of plants (50%) being semi-erect/prostrate. The majority of *E. crus-galli* plants were erect (84%) with no plant displaying a prostrate form (Table 3.5).

Table 3.5: Percentage of the total plants sampled categorised according to the growth form observed in the field for *E. colona* and *E. crus-galli*.

Growth categories	Percentage of total collection sampled grouped according to growth category				
	Erect	**Erect/semi-erect**	**Semi-erect**	**Semi-erect /prostrate**	**Prostrate**
E. colona	16	12	13	50	9
E. crus-galli	84	3	3	10	0

An obvious difference was observed in both species in relation to the thickness of the culm base, either stout or fine (Photograph 3.5). The thickness of the culm, in addition to the growth form and the presence of banding on the leaves formed the basis for assigning a growth category. A total of 1 736 plants was sampled and based on the aforementioned characteristics, *E. colona* was classified into 20 growth categories and *E. crus-galli* into six growth categories.



Photograph 3.5: Difference in culm thickness, evident here in *E. colona* (a - stout, b - fine).

<u>*Echinochloa colona* plants</u>

The most common form of *E. colona* sampled in the NGR was semi-erect/prostrate (50%), 41% of these plants had stout culms and of these 24% were unbanded. This meant that the most widespread growth category of *E. colona* in the NGR was a plant that was unbanded, semi-erect/prostrate with a stout culm. The least common form was prostrate accounting for 9% of all the *E. colona* sampled. The least common

growth categories were all banded plants with fine culms and had the growth forms erect (2 plants), erect/semi-erect (1 plant) and prostrate (2 plants) (Figure 3.2).

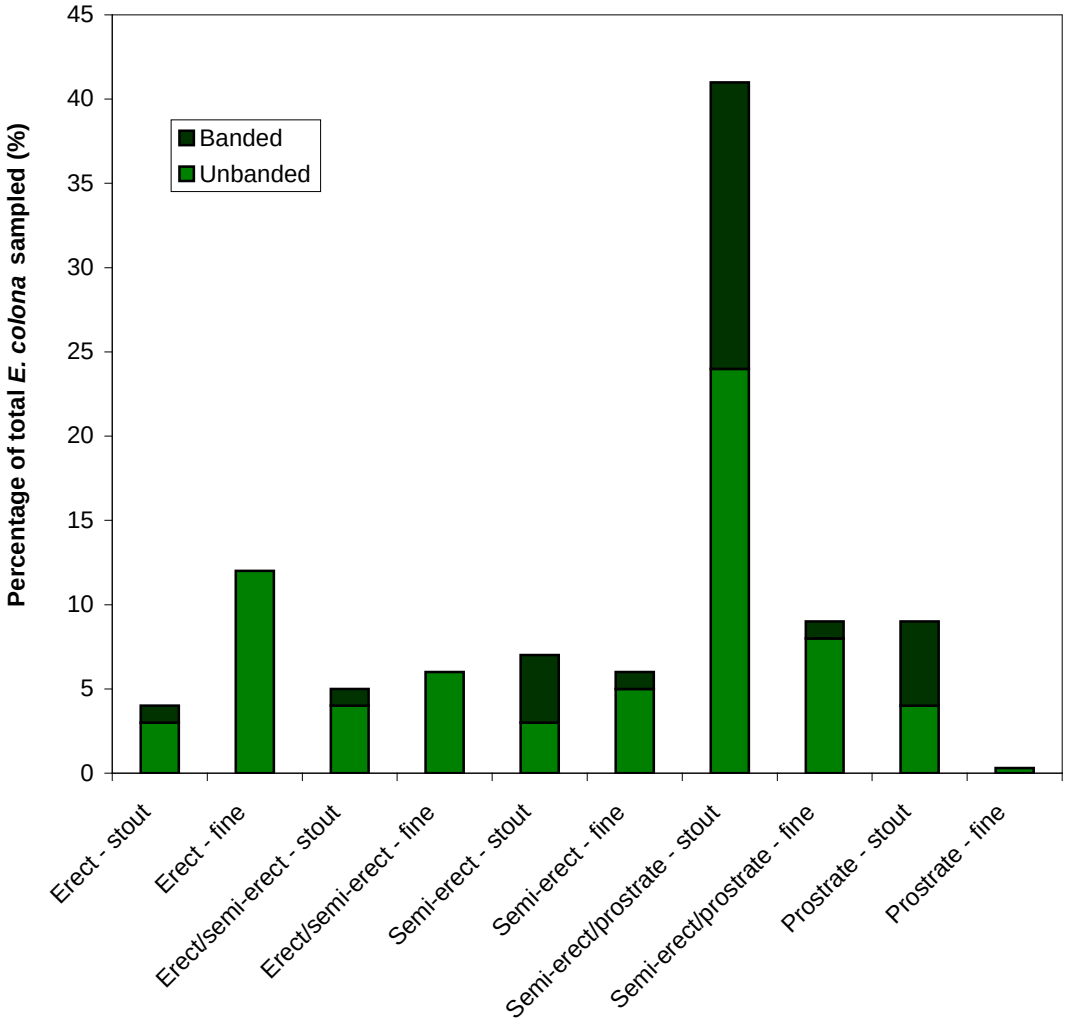


Figure 3.2: *Echinochloa colona* growth categories present in the fields sampled across the three major cropping centres of the northern grain region presented as a percentage of the total number of *E. colona* plants sampled. Three categories are not shown, as their contributions were less than 0.1%.

Across the NGR unbanded plants were more common than banded plants, and accounted for 70% of the *E. colona* sampled. Of these unbanded plants, the percentage of plants with stout culms (38%) was similar to those with fine culms (32%). Of the 30% with leaf banding, the banding was more common in plants with stout culms (28%) compared with those with fine culms (2%).

The populations sampled at Dalby displayed more diversity than from the other two centres, with 19 of the 20 growth categories being present (Figure 3.3). The regional centre from which the *E. colona* was sampled appeared to influence the morphology of the plants. The prostrate form was more common at Dalby (23%) than at Wowan (4%) or Narrabri (<1%), while the erect form was more common in fields at Wowan (31%) and Narrabri (24%) compared with Dalby (2%). While the semi-erect/prostrate form was dominant in all centres, plants with this form sampled at Dalby and Wowan were mostly unbanded whereas the plants from Narrabri were mostly banded (Figure 3.3).

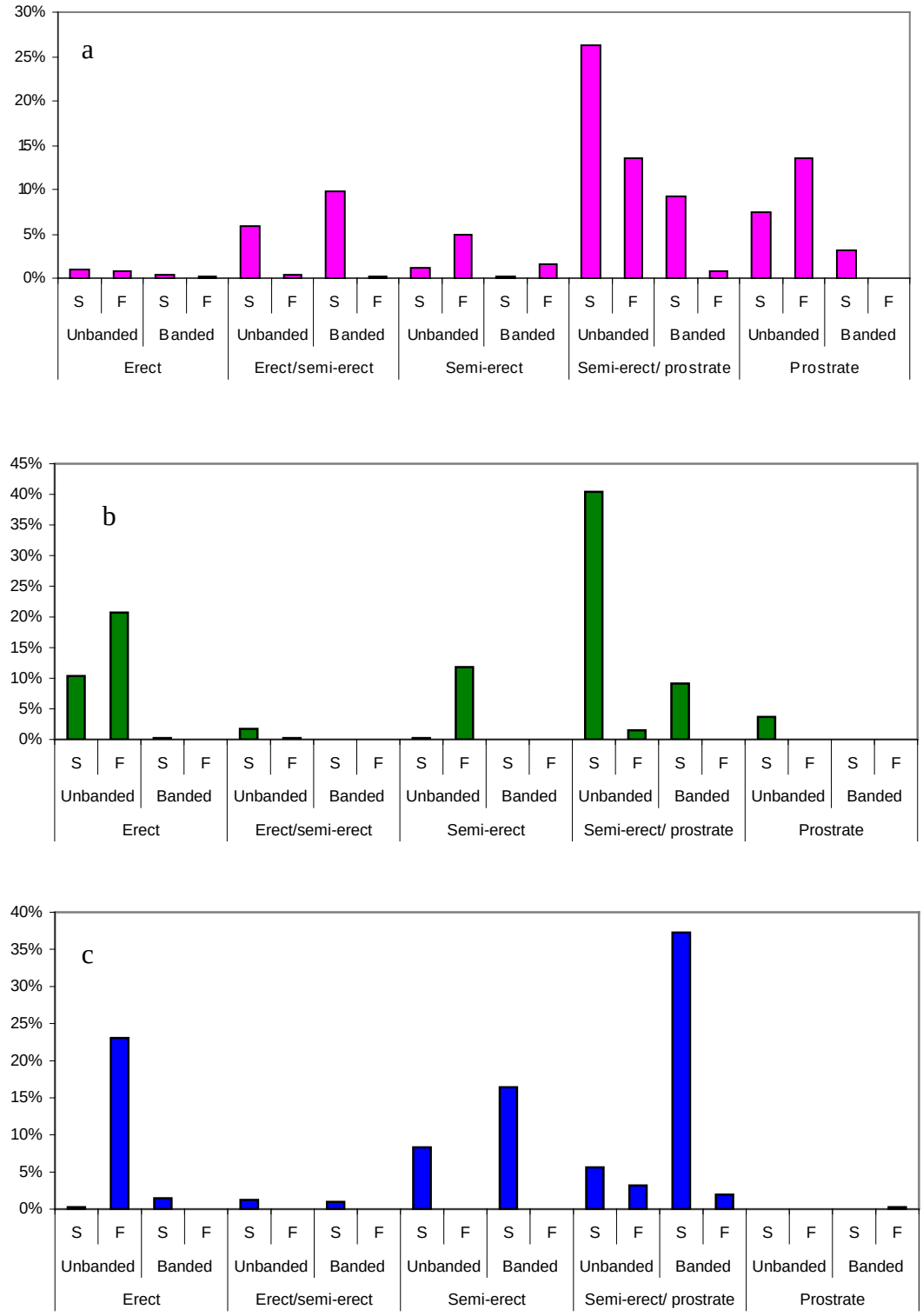


Figure 3.3: Distribution of growth categories of *E. colona* across the three major cropping centres of the northern grain region (a-Dalby, b-Wowan, c-Narrabri) expressed as a percentage of the total number of *E. colona* plants collected (S – stout culm, F – fine culm).

Six agricultural habitats (sorghum, mungbean, corn, cotton, fallow, tail drain) and three non-agricultural habitats (cattle yards, property roads, horse paddock) were sampled across the NGR. The dominant form across the region (semi-erect/prostrate) was present in each habitat with the exception of the horse paddock at Wowan (Figure 3.4).

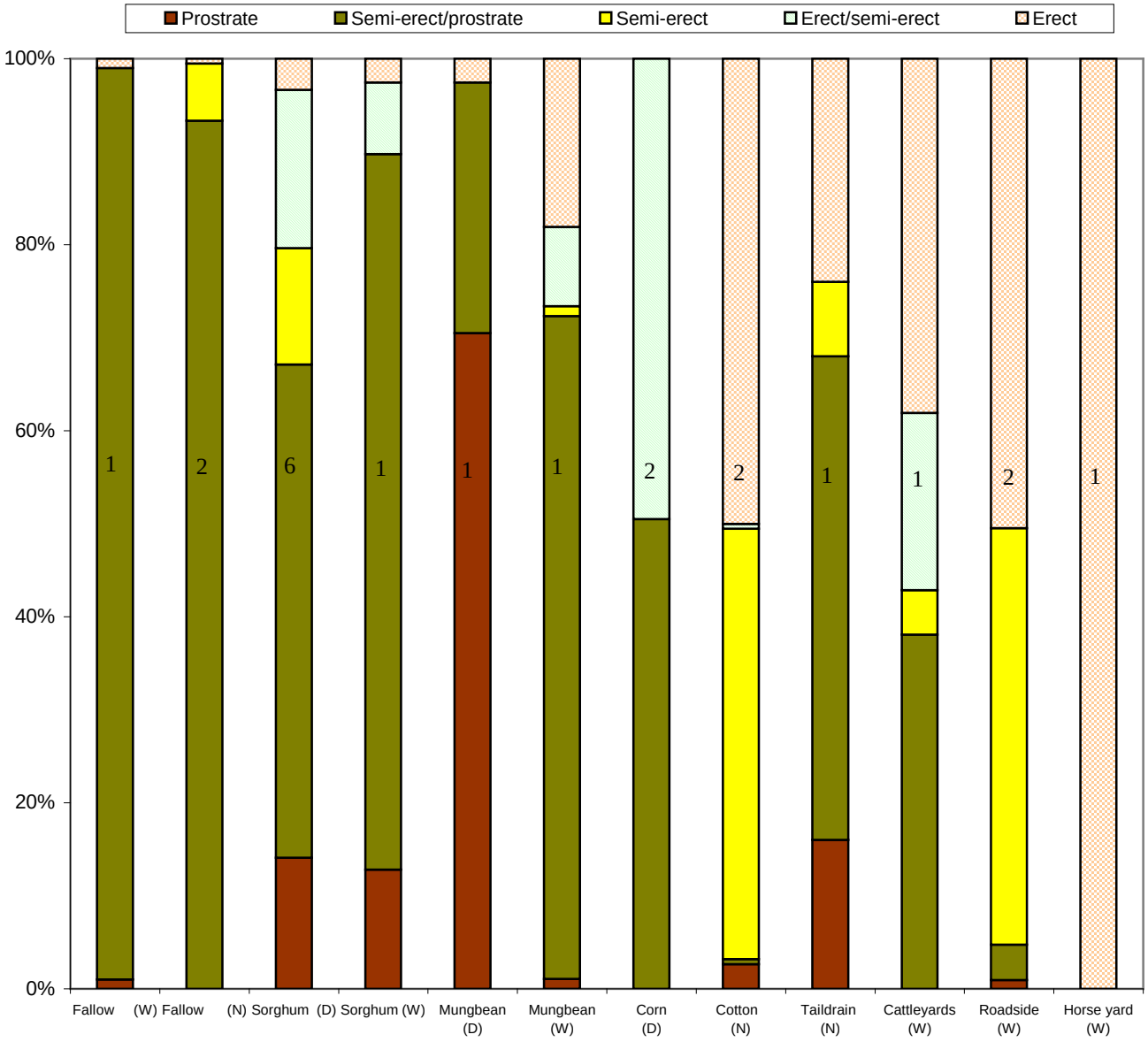


Figure 3.4: The percentage of the five growth forms (erect to prostrate) observed in *E. colona* occurring in the different habitats sampled in each of the regional centres (W – Wowan, D – Dalby, N – Narrabri). Numbers appearing on the bars of the graph indicate the number of fields included in each of the different habitats.

All five growth forms were present in sorghum (Dalby), mungbean (Wowan) and cotton (Narrabri). The horse paddock at Wowan was the only field to display a lack of diversity with 100% of the plants sampled being erect. The erect growth form was also common in other non-agricultural fields at Wowan and also in cotton at Narrabri.

In fallows at Wowan and Narrabri the growth forms present were similar with the semi-erect/prostrate form accounting for over 90% of the plants sampled. There were also similarities between the forms present in sorghum at Dalby and Wowan with the semi-erect/prostrate form being dominant and the prostrate form accounting for nearly 15% of the plants in this habitat from both areas.

The dominant growth forms found in the mungbean crops at Dalby and Wowan were different. While the majority of plants found at Dalby were prostrate (71%) the majority at Wowan (71%) were semi-erect/prostrate. The growth forms found in the remaining agricultural fields, corn (Dalby) and cotton (Narrabri) were dissimilar from the other agricultural fields. However, the forms found in cotton were similar to those sampled from the roadside at Wowan. There were two co-dominant forms, erect (50% in both fields) and semi-erect (46% Narrabri, 45% Wowan).

Echinochloa crus-galli plants

Echinochloa crus-galli was only found in fields at Narrabri and Dalby with 66% of the plants sampled being from Narrabri (Figure 3.5).

Of the plants sampled, 84% had an erect growth form and were evenly divided between those with stout and fine culms. Dalby samples accounted for 26% of the erect plants and all had stout culms. Overall, the majority of plants from Narrabri had fine culms (63%) while stout culms were more common at Dalby (97%). No *E. crus-galli* plants displayed a prostrate growth form.

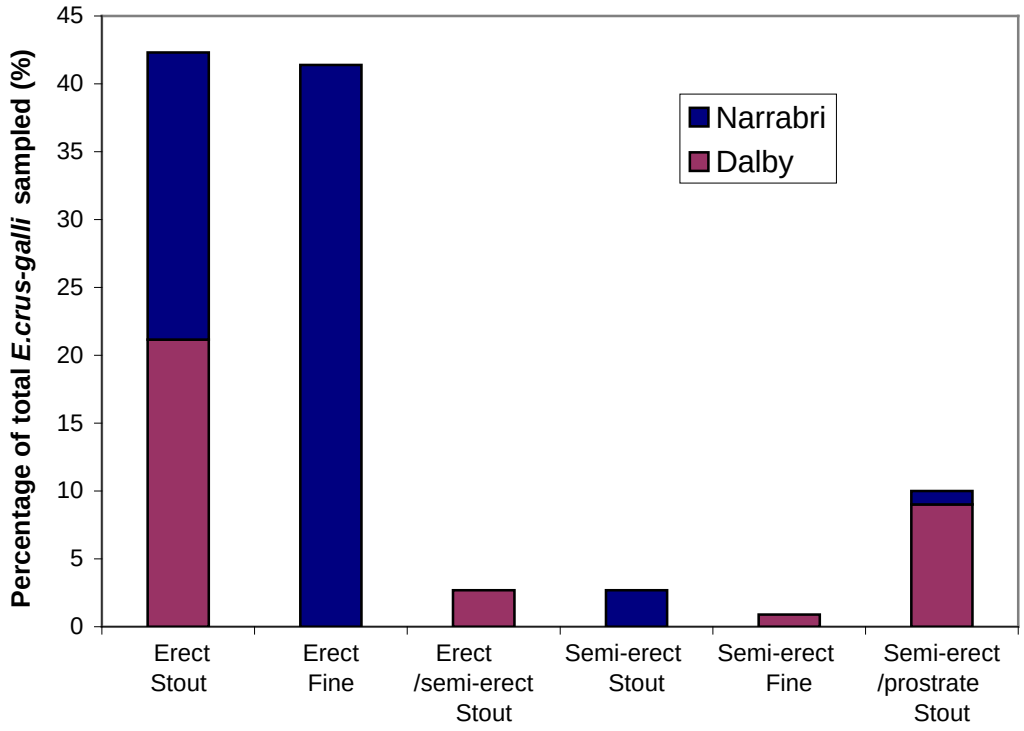


Figure 3.5: *Echinochloa crus-galli* growth categories present in two of the three major cropping centres of the northern grain region, presented as a percentage of the total *E. crus-galli* plants sampled.

There were six growth categories observed in *E. crus-galli* based on the growth form and the thickness of the culm. Both regional centres had a similar diversity with four growth categories occurring in both centres.

Due to insufficient samples the impact of habitat was not investigated.

3.4.1.3 Seed characteristics of E. colona and E. crus-galli

The average length of *E. colona* seeds was 2.30 mm (2.10-2.56 mm) compared with that of *E. crus-galli* at 2.86 mm (2.68-3.09 mm). The average seed widths of the respective species were 1.27 mm (1.18-1.40 mm) and 1.63 mm (1.46-177 mm).

Seeds sizes for both species were not related to the regional centre from which they had been collected. The centre from which the seeds were collected was not significant for either *E. colona* ($p = 0.602$) or *E. crus-galli* ($p = 0.270$), although there

were significant species ($p < 0.001$), width ($p < 0.001$) and species*width ($p = 0.024$) interactions. Because there were no regional centre differences, populations within each species could be considered together to see if there were differences in the seed measurements between populations. Simple linear regression with groups (centre and population) analyses were used to investigate the significance of population differences within each species. There were no significant population*width interactions for either species although there were significant differences between populations ($p < 0.001$) within both species.

Analysis of the relationship between the average length and width measurements of the two species resulted in the formation of two distinct clusters, one containing only *E. colona* and the other *E. crus-galli* (Figure 3.6).

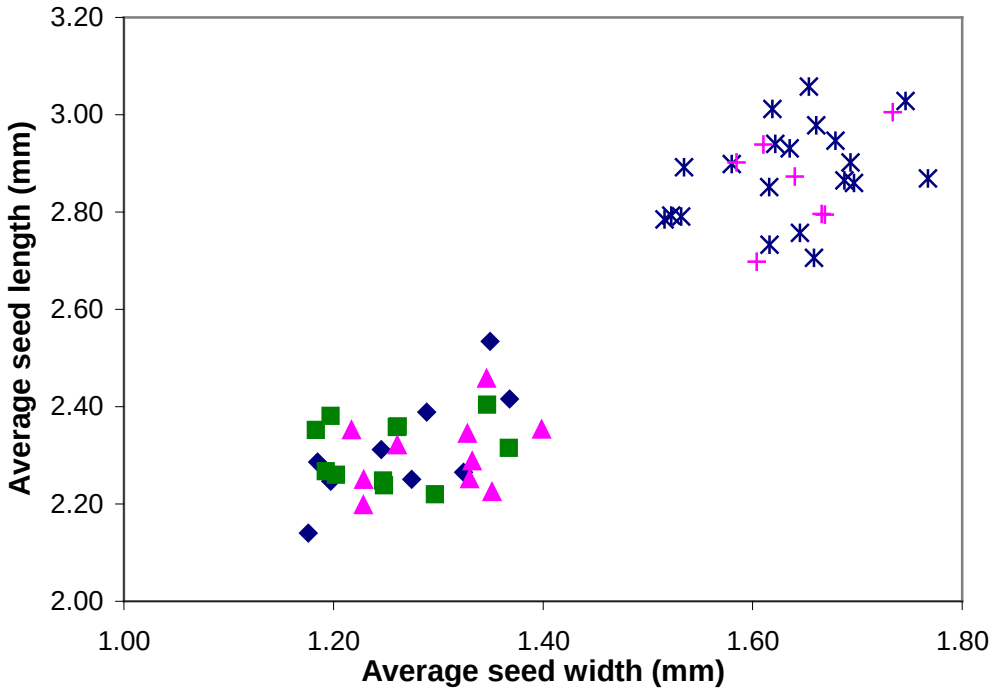


Figure 3.6: Relationship between the average length and width measurements of *E. colona* (▲ — Dalby, ◆ — Narrabri, ■ — Wowan) and *E. crus-galli* (+ — Dalby, ✱— Narrabri) seeds.

To establish which populations were significantly different from each other, REML variance components analyses were used to calculate the least significant difference (LSD) for comparison between the predicted means of the populations (Figure 3.7).

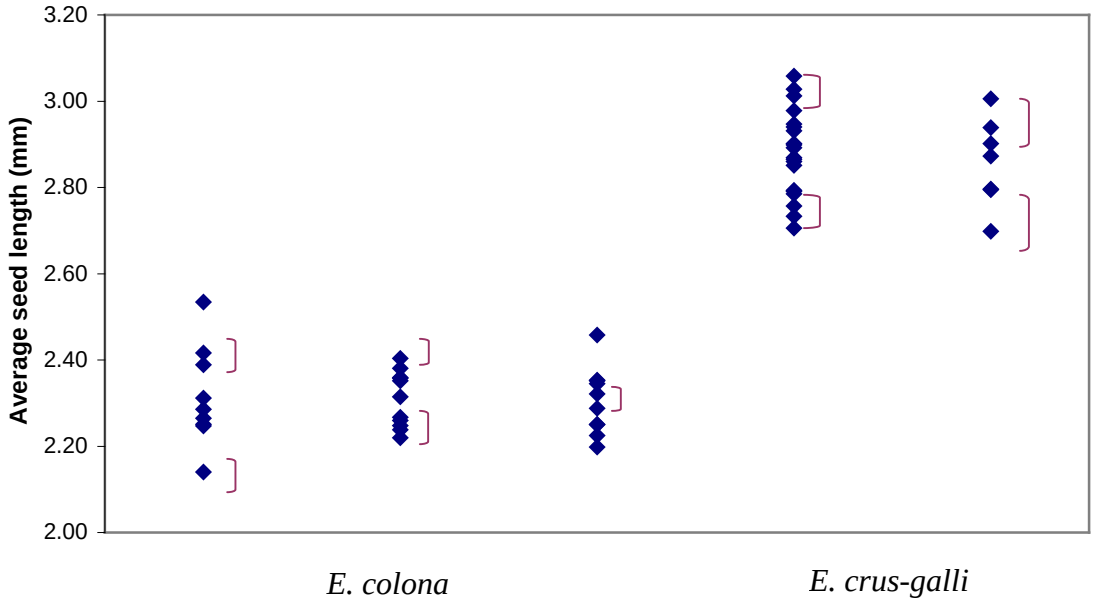


Figure 3.7: Comparison of the average lengths (mm) of 30 seeds from 29 *E. colona* (left to right: Narrabri (9), Wowan (11), Dalby (9)) and 26 *E. crus-galli* populations (left to right: Narrabri (20), Dalby (6)). (LSD for *E. colona* is 0.085 and for *E. crus-galli* is 0.114). Bracketed populations are discussed below.

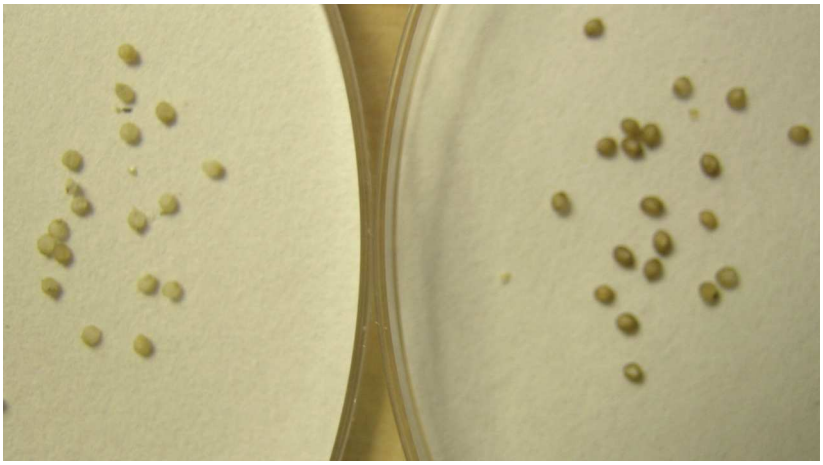
Echinochloa colona

Three populations of *E. colona* from the tail drain site at Narrabri, each from different collection points were included in the analyses. Of these, two populations were not significantly different in average seed length but both were significantly different from the third population. Populations from Wowan separated into two significantly different groups. Each group contained five populations with mungbean and fallow habitats common to both groups. Included in the Dalby populations were three from the same collection point and there were no significant differences in average seed lengths.

Echinochloa crus-galli

Three populations of *E. crus-galli* from the same collection point in a cotton field at Narrabri were included in the analyses. Two populations were not significantly different in average seed length but the third population was significantly different to one but not the other. Populations from Dalby separated into two significantly different groups and each group contained populations from the same collection points in two sorghum fields.

There was a distinct difference noted in the colour of the mature caryopses, *E. colona* were cream and *E. crus-galli* light brown (Photograph 3.6).



Photograph 3.6: Colour comparison of mature *E. colona* and *E. crus-galli* caryopses. Those of *E. colona* are cream in colour (left) whereas in *E. crus-galli* they are light brown (right).

3.4.2 Morphological trials

3.4.2.1 Achieving optimal germination

Germination of *E. colona* seeds was more successful than *E. crus-galli* when the seeds were unscarified, while manual scarification improved germination rates in *E. crus-galli*. Acid scarification failed to improve on the results achieved with manual scarification, and once again the two species reacted differently. The percentage of *E. colona* seeds that germinated increased the longer the seeds had been immersed in acid while the reverse applied to *E. crus-galli* seeds. The optimum results for

germination of *E. colona* were achieved at 28°C using unscarified seeds (60%) while for *E. crus-galli* optimum results were achieved using manually scarified seeds germinated at the same temperature (89%) (Table 3.6).

Table 3.6: Comparison of the percentage of seeds that germinated using unscarified and scarified methods. Two scarification techniques were used – manual (sandpaper) and acid (concentrated sulphuric acid – H_2SO_4). All seeds were incubated using a 12 hr day/night regime. The manual scarification was undertaken using three temperature regimes (25°C, 28°C, 31°C) while the acid scarification was only conducted at 25°C (nd – no data).

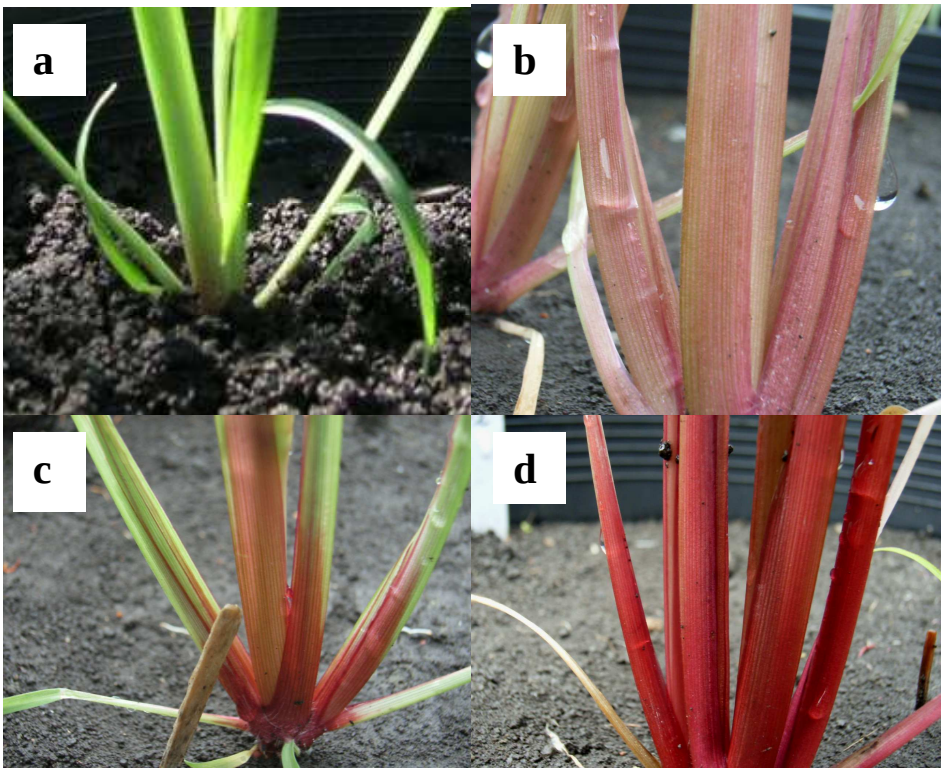
Method of seed preparation and temperature regimes		Percentage of successful germinations	
		E. colona	***E. crus-galli***
Unscarified	25°C	50	0
	28°C	60	15
	31°C	25	22
Scarified - manual	25°C	48	87
	28°C	47	89
	31°C	nd	nd
Scarified – acid	20 min	3	48
25°C	30 min	37	2
	40 min	36	2

3.4.2.2 Echinochloa colona and E. crus-galli - 2005/2006

Echinochloa colona was the only species to survive the December planting and these populations started maturing in late January with the majority maturing in February. Additional populations of both species planted in January matured from mid-February through March, with the majority of *E. colona* and all the *E. crus-galli* maturing in March.

On average, the mature *E. colona* plants were 48 cm high, had produced 7 tillers during their development, and had 14 panicles that were 8 cm long and 2 cm wide. The mature *E. crus-galli* plants had an average height of 36 cm, had produced 7 tillers and had 15 panicles that were 5 cm long and 2 cm wide (Appendix 1).

During the trials it was observed that the pigmentation at the base of the culm varied between and within populations (Photograph 3.7). This characteristic was rated at the reproductive stage while the pigmentation was prominent.



Photograph 3.7: Different pigmentations observed in the culms of *E. colona* and *E. crus-galli* (a – nil, b – pale, c – dark and concentrated at base, d – dark and extending up stem).

The majority of *E. colona* plants (48%) had darkly pigmented culms with the colour concentrated at the base while 31% possessed pale culms. All the *E. crus-galli* plants had pigmented culms with 83% having dark bases with the colour concentrated at the base and the remaining plants being equally divided between the two remaining pigmented classifications.

In both species there was a noticeable difference in the density of hairs visible at the base of the culm at the reproductive stage. The majority of *E. colona* plants (53%) had no obvious hairs, 35% had a few obvious hairs and on 12% of the plants the hairs were highly visible. In *E. crus-galli* the majority of plants (91%) had no obvious hairs.

The *E. colona* plants grown in the morphology trial displayed the five growth forms recorded in the field and 14 of the 20 growth categories (Figure 3.8). The most common form was erect/semi-erect accounting for approximately 38% of the total plants grown, followed by erect (28%). The least common form found in the trial was prostrate (1.7%). The majority of plants had fine culms (83%) and of these 69% had no banding on the leaves.

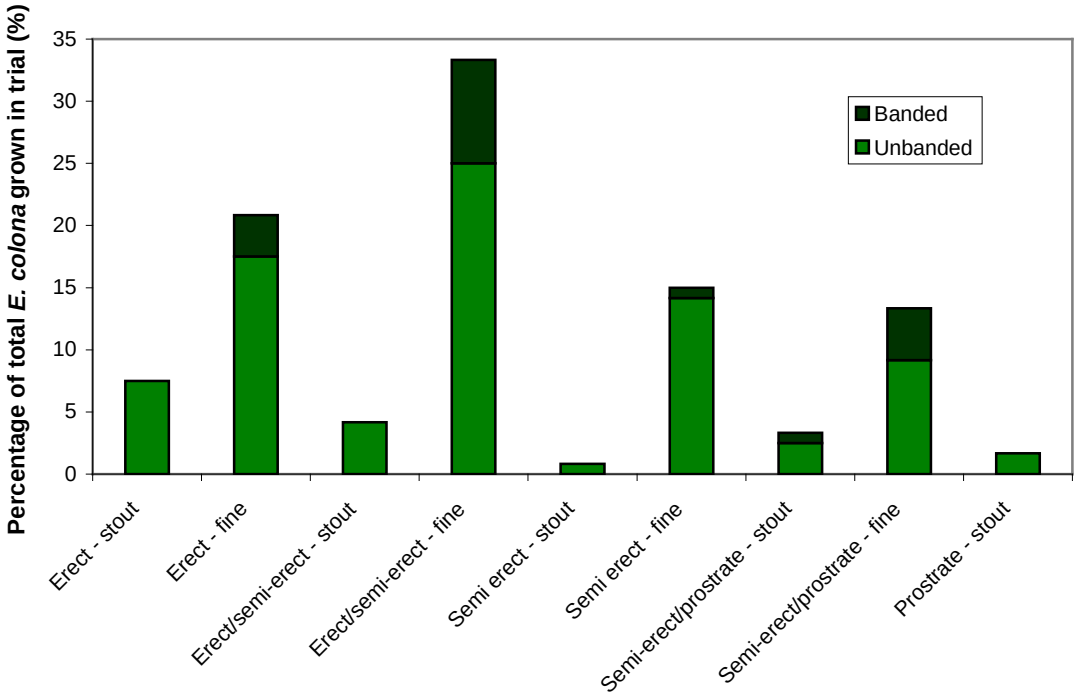


Figure 3.8: *Echinochloa colona* growth categories present in the morphology trial presented as a percentage of the total *E. colona* plants grown from the seeds of 30 populations collected across the northern grain region.

Of the 30 *E. colona* populations established in the morphological trials, 20 came from the seeds of plants with no banding on the leaves and 10 from the seeds of plants with leaf banding. Ninety-eight percent of the replicates grown from the seeds of unbanded plants were unbanded whereas the replicates established from banded plants were closely distributed between banded (52.5%) and unbanded (47.5%). Only three of the populations established from seed from banded plants had leaf banding present in all the replicates. In contrast all the replicates from 18 populations established from the

seed of unbanded plants had no leaf banding. The remaining two populations produced only one plant each with leaf banding.

The majority of *E. crus-galli* plants were erect/semi-erect (29%), with erect and semi-erect/prostrate both comprising 25% of the replicates. Only one of the 24 plants had a stout culm and none of the plants had a prostrate growth form. Due to the limited number of plants successfully established in this trial, data on growth forms and categories are not shown.

Measurements taken at the onset of reproduction and included in the analysis were the number of tillers and leaves, and the two measurements recorded for plant height reflecting the form of the plant. Populations were clustered according to the similarity in the traits included in the analysis and initially split into two main clusters that did not correspond to different species or reflect regional centres.

A threshold of 100 was selected for all the dendrograms produced using the morphological data (Figures 3.9 and 3.10, 3.12 and 3.13) to allow comparisons at the same level of similarity. Further subdivisions at a threshold of 100 resulted in nine clusters that also did not group corresponding to species or regional centres (Figure 3.9).

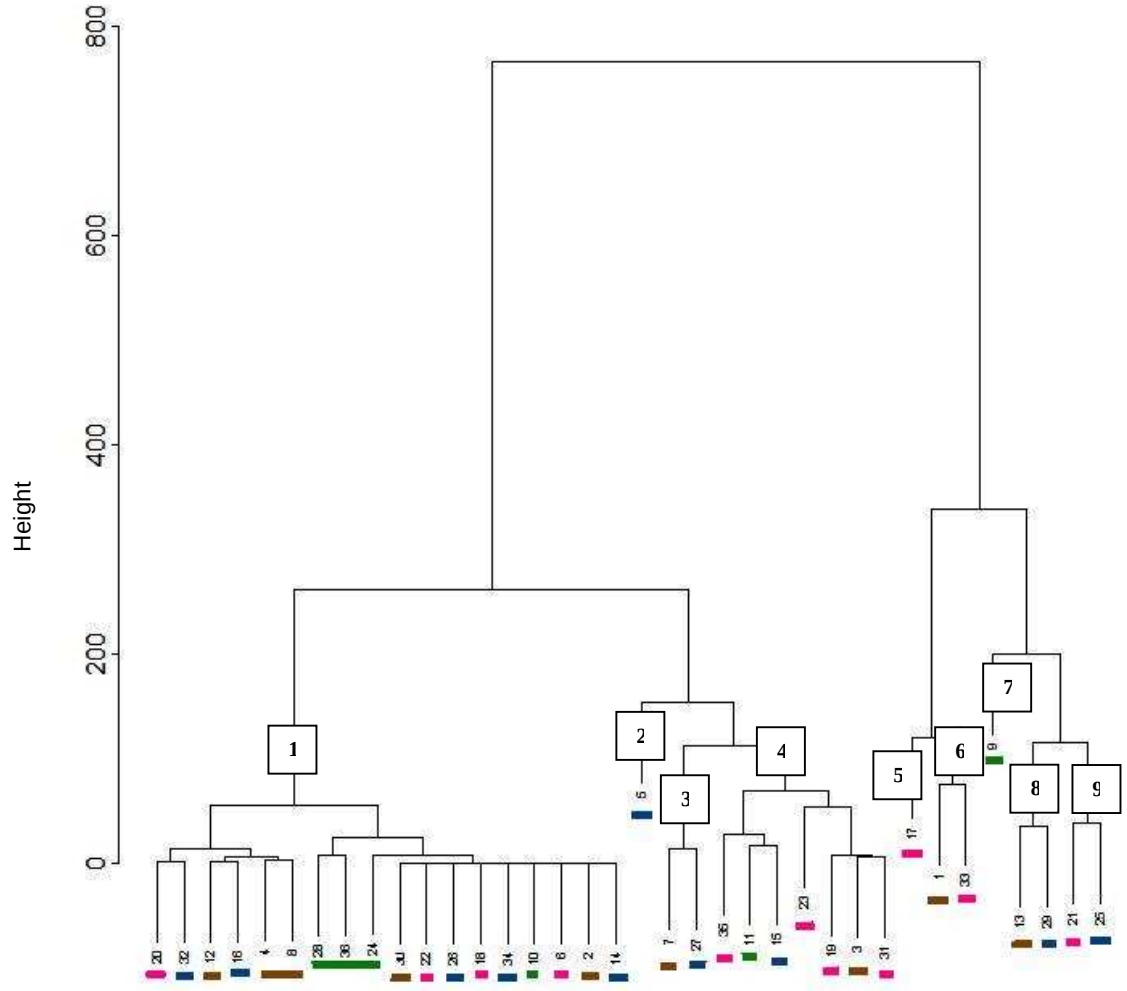


Figure 3.9: Unweighted pair group method (UPGMA) dendrogram using Euclidian distance coefficient of dissimilarity to compare morphological similarities among populations of 30 *E. colona* (Dalby – blue, Wowan – pink, Narrabri – brown) and 6 *E. crus-galli* (green) at the onset of the reproductive phase. A threshold of 100 resulted in nine clusters that are numbered accordingly.

The six *E. crus-galli* populations were all from Narrabri and were spread across three clusters, with four populations appearing in the same cluster. Of these four populations, 28 and 36 were morphologically similar and were both collected from the tail drain. Population 24, also collected from the tail drain had more in common with population 10 (cotton), which in turn had similar morphology to *E. colona* populations from across the region. Population 11 (cotton) and population 9 (fallow) appeared in different clusters with population 9 having more in common

morphologically with *E. colona* from across the region than the other *E. crus-galli* populations.

The *E. colona* populations were spread across eight of the nine clusters and did not group according to regional centre. At this growth stage there appeared to be no species or regional centre trends with regard to the morphological characteristics analysed.

A second analysis, based on measurements taken at the onset of maturity, included the presence/absence of bristles, numbers of panicles and tillers, the length and width of the panicle and flag leaf and the two measurements recorded for plant height reflecting the form of the plant. Populations were clustered according to the similarity in the traits included in the analysis. The populations initially split into two main clusters that did not correspond to different species or reflect regional centre differences. Further subdivisions into six clusters at a threshold of 100 once again did not group according to species or centre (Figure 3.10).

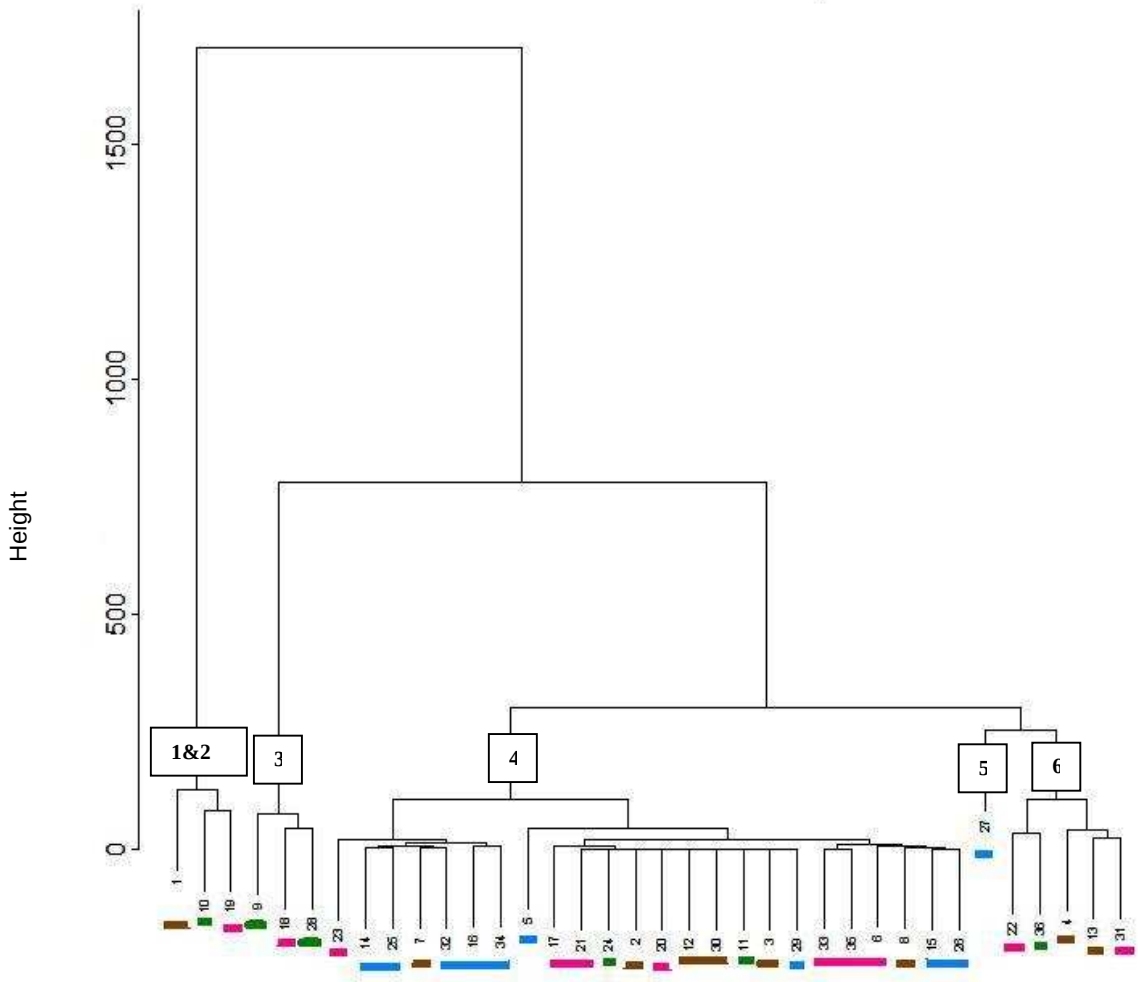


Figure 3.10: Unweighted pair group method (UPGMA) dendrogram using Euclidian distance coefficient of dissimilarity to compare morphological similarities among populations of 30 *E. colona* (Dalby – blue, Wowan – pink, Narrabri – brown) and 6 *E. crus-galli* (green) at the onset of maturity. A threshold of 100 resulted in six clusters that are numbered accordingly.

The six *E. crus-galli* populations were spread across four of the clusters. Population 10 (cotton) was morphologically dissimilar to all the other *E. crus-galli* populations having more in common with two *E. colona* populations, population 19 (Wowan roadside) and population 1 (Narrabri fallow). The third cluster contained *E. crus-galli* populations 9 (fallow) and 28 (tail drain), which were morphologically similar to an *E. colona* population 28 (Wowan mungbean) rather than the three remaining *E. crus-galli* populations from Narrabri. At this growth stage *E. crus-galli* populations 11 and

24 were similar and closer in morphology to *E. colona* populations from across the region.

Echinochloa colona populations were spread across the six clusters. While populations from Wowan appeared in four of the clusters, Narrabri populations appeared in three and Dalby only two. However, the majority of the Dalby populations, with the exception of population 27 (sorghum), were all in the same cluster and therefore relatively similar in morphology. At this growth stage there appeared to be no species or regional centre trends with regard to the morphological characteristics analysed.

3.4.2.3 Echinochloa crus-galli - 2006/2007

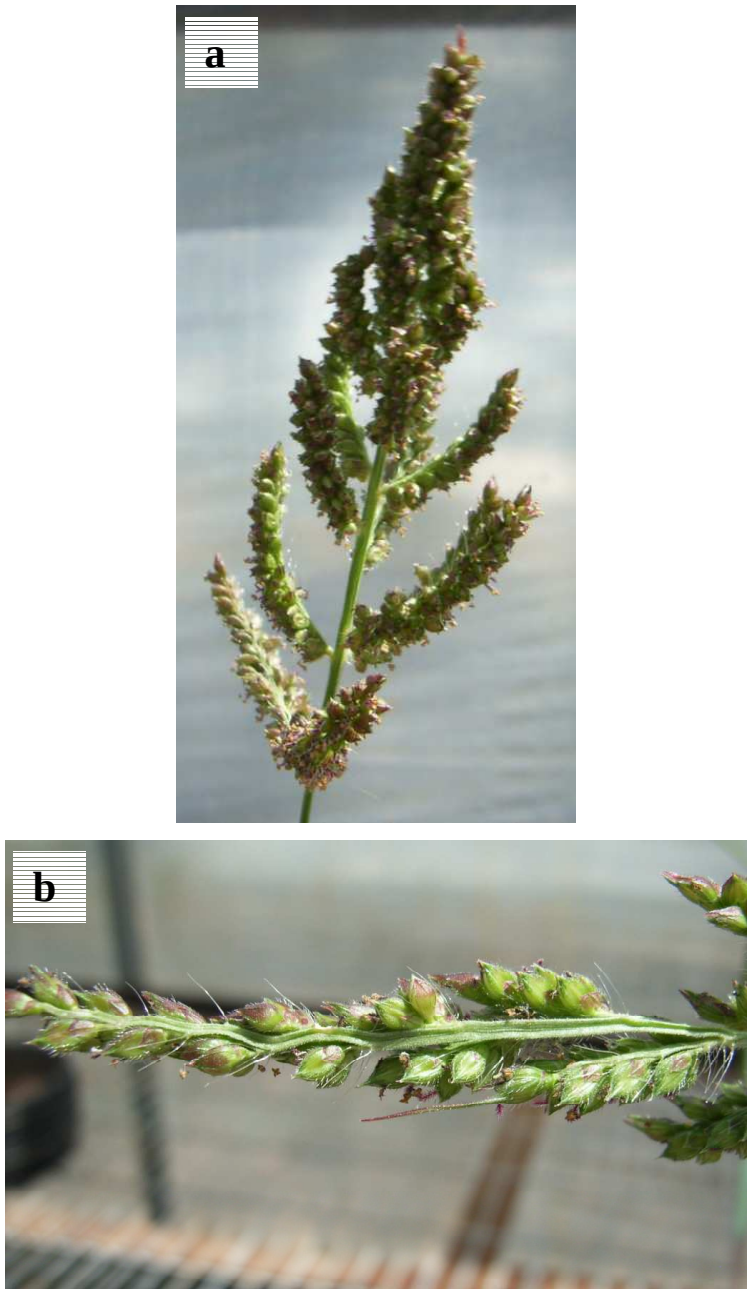
Twenty-seven *E. crus-galli* populations were planted in September/October 2006, with 7 replicates planted in November to replace those that had died. Populations planted in September matured during November with one plant maturing in early December. Those planted in October started maturing in November with the majority maturing in December. Of the seven replicates planted in November, one matured in late December and the others in early January 2007.

On average, the mature *E. crus-galli* plants were 74 cm high, had produced 17 tillers and had 17 panicles that were 12 cm long and 7 cm wide (Appendix 2).

The pigmentation observed at the base of the culms was recorded at the onset of reproduction. It was observed that 3% of the *E. crus-galli* plants had no pigmentation at the base of the culms, 23% were lightly pigmented and 15% had intense pigmentation extending up the culms. The majority of *E. crus-galli* plants (59%) had culms with intense pigmentation concentrated at the base (Photograph 3.7). The density of hairs visible at the base of the culm was also recorded and the majority of *E. crus-galli* plants (88%) had no obvious hairs while 12% had a few.

All the *E. crus-galli* plants grown in the trial, with the exception of two Dalby plants, had some awns present. In all cases the presence of awns was sparse and only present

on spikelets at the top of the panicle. However, all plants had bristles present on the racemes. The panicles displayed two strikingly different forms. The majority of plants had panicles that were an open pyramidal shape (79%) while the remaining 21% had a flatter, thinner columnar shape with the spikelets flattened on one side of the central axis (Photograph 3.8).



Photograph 3.8: Two panicle heads of *E. crus-galli* showing the two forms observed in the morphological trials (a - pyramidal, b - columnar). A single awn, visible in 'b' is indicative of the awning present on the majority of plants grown in the trial.

At maturity, there was a noticeable difference in the pigmentation of the panicles, ranging from a pale green to a dark reddish-brown. While the majority of plants (69%) had panicles containing spikelets that were green with some brown tingeing (Photograph 3.8a), all the replicates in two Narrabri populations had panicles containing spikelets that were a dark reddish-brown. A small proportion of the *E. crus-galli* plants had pale green panicles (19%) (Photograph 3.8b) but only in two populations (one from Dalby and one from Narrabri) were all the replicates this colour.

The *E. crus-galli* plants grown in the morphology trial displayed five of the six growth categories found in the field and an additional two growth categories not sampled in the field; erect/semi-erect and semi-erect/prostrate, both with fine culms (Figure 3.11). The most common form was erect/semi-erect which accounted for approximately 52% of the total plants grown, followed by semi-erect which accounted for approximately 27%. No *E. crus-galli* plant had a prostrate growth form and the least common form grown in the trial was erect (7%). There was more diversity displayed in the Narrabri populations with all seven categories represented compared to Dalby with three.

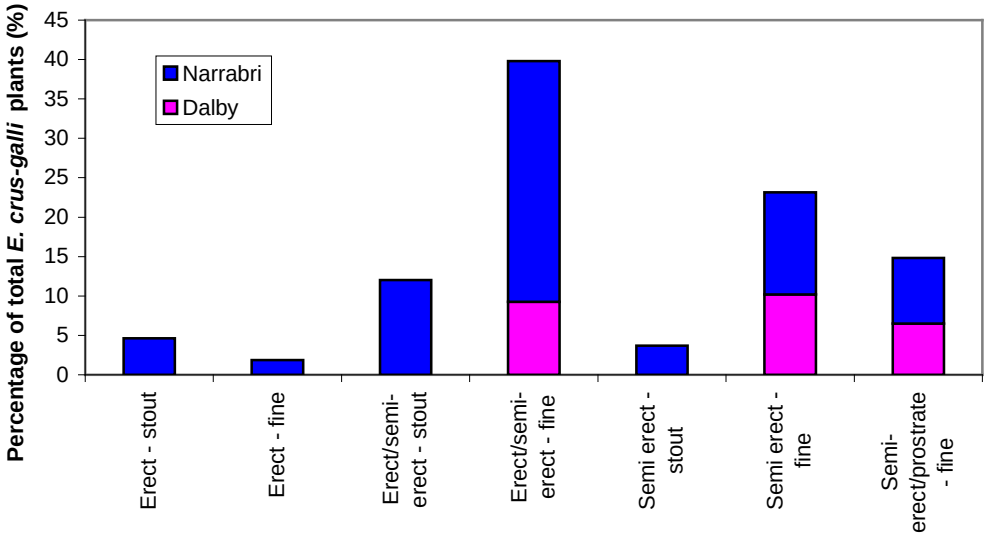


Figure 3.11: Growth categories found in 27 populations of *E. crus-galli* grown in the morphological trial in 2006/2007 shown as a percentage of the total progeny grown.

Measurements taken at the onset of reproduction and included in the analysis were the number of tillers and leaves, and the two measurements recorded for plant height reflecting the form of the plant. Populations of *E. crus-galli* were clustered according to the similarity in the traits included in the analysis. The populations initially split into two main clusters that did not correspond to the two regional centres. Further subdivisions at a threshold of 100 resulted in the creation of four clusters; three contained only Narrabri populations while the remaining cluster contained a mixture of Dalby and Narrabri populations. Even when examined at a lower threshold there was no grouping of the Dalby populations (Figure 3.12).

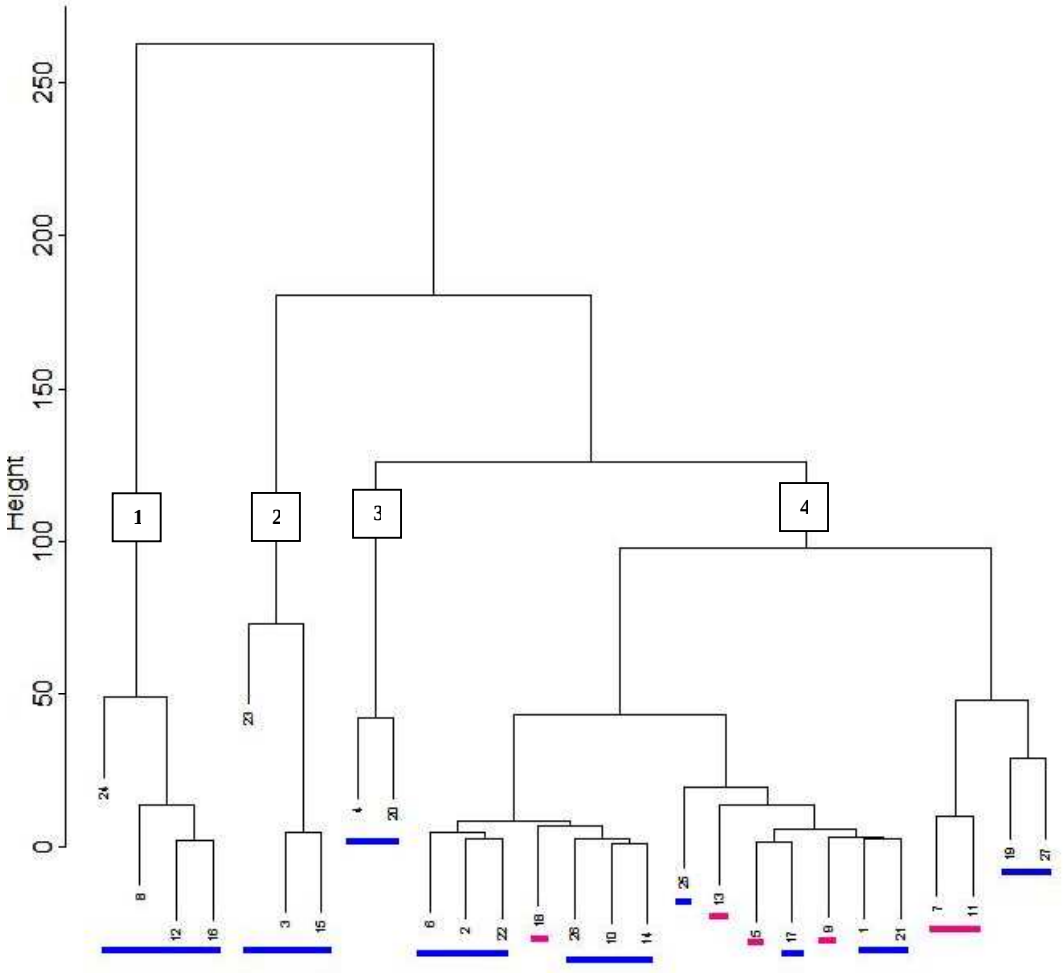


Figure 3.12: Unweighted pair group method (UPGMA) dendrogram using Euclidian distance coefficient of dissimilarity among populations of 27 *E. crus-galli* at the onset of the reproductive phase (Dalby - pink; Narrabri – blue). A threshold of 100 resulted in four clusters that are numbered accordingly.

A second analysis, based on measurements taken at the onset of maturity, included the presence/absence of bristles, numbers of panicles and tillers, the length and width of the panicle and flag leaf and the two measurements recorded for plant height reflecting the form of the plant. Populations were clustered according to the similarity in the traits included in the analysis. The populations initially split into two main clusters that did not correspond to the two regional centres. Further subdivisions at a threshold of 100 resulted in the creation of six clusters and three contained only Narrabri populations. (Figure 3.13).

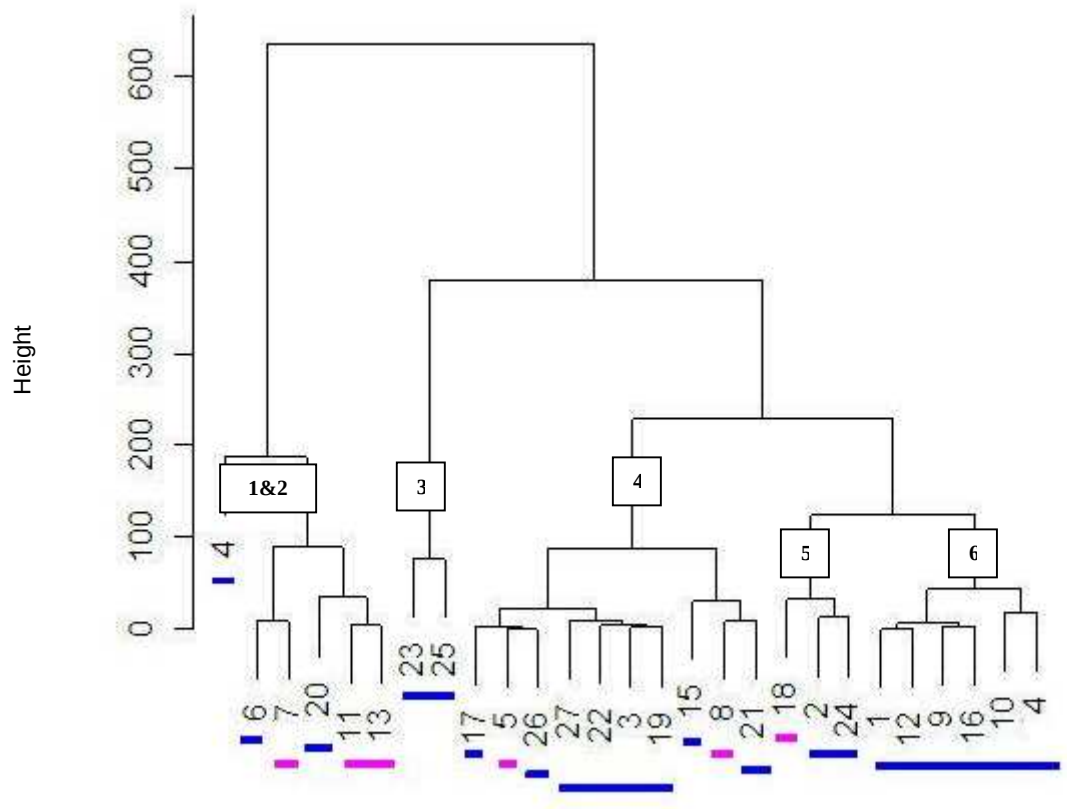


Figure 3.13: Unweighted pair group method (UPGMA) dendrogram using Euclidian distance coefficient of dissimilarity among 27 populations of *E. crus-galli* at the onset of the maturity phase (Dalby - pink; Narrabri – blue) A threshold of 100 resulted in six clusters that are numbered accordingly.

Two populations of *E. crus-galli* from Narrabri were established in both morphological trials (Table 3.7). On average the plants grown in the first trial were smaller in regard to the recorded morphological characteristics and tillered less. In

contrast they produced more panicles. The predominant growth form of the replicates also varied in both trials. In the first trial 50% of the replicates were semi-erect/erect while in the second trial 50% of the replicates had a semi-erect/prostrate growth form. In both years all replicates had fine culms.

Table 3.7: Comparison of the average values for morphological characteristics of two populations of mature Narrabri *E. crus-galli* populations grown in both trials.

	Height (cm)	Tiller number	Panicle number	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length (cm)	Panicle width (cm)
Population 1							
2005/6	55.3	8.5	16.8	7.7	0.7	6.6	1.7
2006/7	96.0	18.5	11.8	15.9	1.2	12.4	8.5
Population 2							
2005/6	28.9	10.5	19.8	5.9	0.5	3.7	1.9
2006/7	84.9	18.5	16.5	17.5	1.3	11.4	7.3

Due to the limited number of populations that were established in both trials these figures were not analysed.

3.5 Discussion

This study established that there were two species of *Echinochloa* occurring as weeds in agricultural and non-agricultural fields in the northern grain region (NGR) of Australia. Characteristics that could provide reliable identification without the need to refer to taxonomic keys included the presence of bristles along the racemes of *E. crus-galli* and the different caryopsis colours and seed sizes of the two species. Reliable identification could not be made based on other aspects including growth habits, plant size, the presence of transverse purple banding across the leaves or the presence of awns.

Echinochloa colona was uniformly distributed across the region and the most commonly occurring species, while *E. crus-galli* was limited to fields in northern

New South Wales (Narrabri) and southern Queensland (Dalby). In southern Queensland the presence of *E. crus-galli* plants was harder to detect as individual plants were commonly interspersed amongst *E. colona* and crop plants. In northern New South Wales however, *E. crus-galli* often occurred in stands and stood out due to the greater height of the plants. The results of this study showed the distribution of these two species across the NGR and are supported by Australian herbaria records. While *E. crus-galli* has been collected in western Queensland and along the Queensland coast, no specimens have been submitted from the central Queensland area (Wowan - long 150.19427E, lat 23.90887S) (Australia's Virtual Herbarium 2010).

Many weed species that grow over a wide range of environmental conditions exhibit large variations in size and morphology (Barrett 1982) and this is evident in *Echinochloa* across the NGR. Field surveys revealed that populations of both species displayed morphological diversity across the region, although there were some general regional centre and habitat trends. *Echinochloa colona* plants had a diversity of growth forms ranging from erect to prostrate, with the majority being semi-erect/prostrate plants. There was a similar diversity found in *E. crus-galli* however the majority of the plants in this species were erect with none having a prostrate growth form.

Populations of both species established during the morphological trials displayed growth forms and characteristics that did not necessarily reflect those of the parent plants. Culm thickness for example, was not always the same in the progeny as the parent plant. In the morphology trial most of the *E. colona* plants had fine culms and only one of the plants with a stout culm had leaf banding. This was in contrast to the field where leaf banding was common in plants with stout culms. Similarly plants grown from seed collected from plants with leaf banding did not always produce banded progeny.

The dissimilarity between the morphology of the parent plants in the field and the progeny grown in pots (including different seasons for two *E. crus-galli* populations) suggests that the environment is a large contributing factor in determining

morphology. Under natural conditions populations can display a range of different characteristics. If representatives from these populations were then grown under the same conditions and displayed uniform characteristics, then the diversity in the field would be the result of phenotypic plasticity responding to environmental factors (Barrett 1982).

Phenotypic plasticity is the ability of individual genotypes to alter their growth and development in response to changes in environmental factors (Barrett 1982). A high phenotypic variation in the field does not necessarily reflect a high level of genetic diversity as a single genotype has the capacity to potentially produce several different phenotypes according to differences in the environment (Holt 1988). However in this study, populations of *E. colona* and *E. crus-galli* did not display uniform characteristics within species when grown in similar environments suggesting that genetics also contributes to the morphology of both species.

It has been acknowledged that there is much variation in *E. colona* (Michael 2001) and in some groups this variability is treated taxonomically by the formal naming of subspecies or varieties (Barrett 1982). More often the variants are simply recognized as ecotypes or ecological races (Barrett 1982). For example Ramakrishnan (1960) reported the existence of two distinct ecotypes of *E. colona* in India, related to the moisture in the substratum, suggesting an environmental reason for different ecotypes/morphology. Similarly, Chun and Moody (1987) investigated morphological and phenological differences of *E. colona* using ecotypes based on the latitude and habitat in the Philippines and found considerable variation in plant height, accumulative tiller length, leaf area, and dry weight among the ecotypes. However the variation in plant height was during the first ten to twenty days of growth with variation decreasing after that time indicating differences in the growth rates of the ecotypes. While the collection centres in this study were located at different latitudes there was no clear distinction between the morphologies of the centres based on the characteristics measured. There have been limited studies on *E. colona* biotypes in Australia (Michael and van Rijn 1970; Ramakrishnan 1960; Webb 1980) and neither attempted to establish whether *E. colona* could comprise subspecies or distinct varieties.

When comparing collection centres across the region, the form common to all *E. colona* populations was a very dense semi-erect/prostrate form. In the two Queensland centres the majority of *E. colona* plants had unbanded leaves whereas in northern New South Wales the majority of plants with this form had banded leaves. The literature infers that banding in the leaves may be the result of stress (Walker and Widderick 2009) although no studies appear to confirm or clarify what stresses are involved. If stress was a contributing factor it could account for the prominence of leaf banding in New South Wales. The majority of plants with this characteristic were located in two fallow fields and the tail drain site. These sites would not have received additional water or fertiliser whereas the fields in Queensland containing unbanded plants were predominantly planted to sorghum but did include one fallow field. The fields planted to sorghum would have received additional fertiliser and irrigation during the development of the crop and *E. colona* could have developed without any of the stresses associated with the development of the Narrabri populations. This does infer that the leaf banding evident in *E. colona* may not be a centre effect but may be related to the habitat in which the weed established. Nevertheless there could be local conditions in northern New South Wales that do contribute to the predominance of leaf banding in this form of *E. colona*.

However, when grown under similar conditions in the morphological trial, eighteen percent of the *E. colona* replicates had leaf banding. While it is difficult to confirm that these plants may have been stressed it cannot be discounted as contributing to the development of the leaf banding. However, as the majority of the plants with leaf banding (91%) were grown from seeds collected from banded plants, this may suggest that leaf banding may also result from genetics and not solely the environment.

As previously stated, the growth forms of *E. colona* observed in the field in this study, may have an association with the habitat from which they were sampled. For example, the semi-erect/prostrate form was dominant in fallows in both Wowan and Narrabri, and the sorghum fields at Dalby and Wowan also contained similar forms. The erect form was dominant in the horse paddock at Wowan and co-dominant in the Wowan roadside sites. In both of these non-agricultural habitats *E. colona* was not the dominant species, having to compete with other grasses and vegetation. These habitats

were also subjected to stock grazing and as noted by Ramakrishnan (1960), a short form of *E. colona* common in dry areas in India, was more frequently subjected to grazing than a tall form that was common in waterlogged areas. The owner of an adjoining property in central Queensland, a horse breeder, could identify *E. colona* and stated that horses did readily graze upon it. However, while the habitat/environment in which *E. colona* was growing appeared to influence the growth forms present in these situations, the dominant growth form found in the mungbean crops at Dalby and Wowan were different, implying that the habitat did not necessarily dictate growth form.

Having made that statement, the crop at Dalby was a much younger crop than the one at Wowan. Therefore, crop competition at different life stages could account for the different forms dominant in the mungbeans crop at Wowan (semi-erect/prostrate) and Dalby (prostrate). Holm *et al*. (1977) noted that one of the characteristics that allowed *E. colona* to effectively compete was that it had a prostrate habit in its early seedling stages that allowed it to root at its nodes, produce sprouts and spread to gain space, but would grow erect when light was limiting. In this study the crop at Wowan was well established compared with a younger crop at Dalby. This may have resulted in an environment where *E. colona* at Wowan developed the semi-erect/prostrate form to compete for light with the larger crop plants, whereas at Dalby smaller crop plants meant that the *E. colona* plants could develop a prostrate form until competition became an issue.

While *E. crus-galli* is the most widely distributed of the two species worldwide, this study established that *E. colona* is the predominant species in the NGR. *Echinochloa crus-galli* exists as several intergrading polymorphic complexes (Barrett and Wilson 1981; Maun and Barrett 1986) and the apparent diversity of forms is associated with high phenotypic plasticity (Maun and Barrett 1986). Two of the most important factors affecting phenological development of *E. crus-galli* are temperature and photoperiod (Swanton *et al.* 2000).

Echinochloa crus-galli populations displayed morphological diversity both in the field and in the morphological trials. In this study, the panicles of *E. crus-galli* plants

displayed a variation in pigmentation from light green, greenish-brown to a very dark reddish-brown. While the majority of the populations from both Dalby and Narrabri had light green of greenish-brown panicles, there were two populations from Narrabri where all the replicates had the intense dark reddish-brown pigmentation. Similarly, Barrett and Wilson (1981) found that *Echinochloa crus-galli* was highly variable with respect to anthocyanin pigmentation. Inflorescences have been described as ranging from green to purplish and culm bases ranging from reddish to dark purple (Halvorson and Guertin 2003) with the intensity of pigmentation in culms being used in Italian rice fields to identify *E. crus-galli* not controlled by quinclorac (Tabacchi *et al.* 2006). There the presence of a reddish basal stem correlated with plants not controlled by this herbicide, whereas those with no red pigmentation were particularly susceptible.

This study found that the panicles of *E. crus-galli* were either an open pyramidal shape or a flatter, thinner and more columnar shape and did not correspond to collection centres. While awns were present on most of the plants grown in the trials reported here, they were sparse and only present on the uppermost spikelets. In addition awning was not as evident on the spikelets of the plants sampled in the field. Pratley *et al.* (2008) also found phenotypic variation within *E. crus-galli* populations in Australia in relation to panicle size and degree of awning. However, in contrast, the presence of awns on the spikelets was roughly related to the areas from which the plants were sampled. Similarly the presence of larger panicles could be related to collection site in their study.

The variation displayed in the morphology of the two Narrabri *E. crus-galli* populations established in both morphological trials implies that environment plays a big role in determining morphology in this species. The plants established in the first trial (summer) were smaller than those established in the second trial (spring). As *Echinochloa crus-galli* is a summer annual (spring-germinating, summer flowering) (Steinmaus *et al.* 2000) and makes optimal growth at high summer temperatures (Rahman and Ungar 1994) the plants in the first trial may have been established too late for optimal development. This is supported by a Canadian study of Barrett and Wilson (1981) where the vegetative shoot biomass of *E. crus-galli* individuals was measured under glasshouse conditions with average temperatures of 25ºC to 30ºC.

Individuals germinated in the spring (April) had three to four times greater biomass that that of individuals germinated in summer (August) and took on average twice as long to flower and also produced more seeds. While biomass was not measured in this current study the plants established during the second trial and planted in late spring were visibly larger and had more leaf matter than those planted the previous year during summer.

In an additional study on temperature treatments undertaken by Swanton *et al.* (2000), it was established that the number of leaves, the number of tillers and the shoot height of *E. crus-galli* were all affected by temperature. For example the number of tillers produced reached a maximum of forty-three at a 29ºC/19ºC day/night treatment but at 35ºC/25ºC had decreased to fewer than twenty, and at 44ºC/35ºC had declined to approximately ten tillers. While the above experiments were undertaken under controlled conditions, it clearly shows how temperature can impact on the growing pattern of *E. crus-galli.*

Seed characteristics for both species were also investigated in this study. While there were significant differences between the two species in relation to the length and width of the seeds, the collection centre was not a significant factor. This is in contrast to studies by Chun and Moody (1987) who found seed size, weight, and number of seeds produced by *E. colona* varied between ecotypes in the Philippines, and Ramakrishnan (1960) who found a marked difference between the seed weight of two forms in India. The *E. colona* seeds in this study ranged from 2.10-2.56 mm in length and 1.18-1.40 mm in width while seeds of *E. crus-galli* ranged from 2.68-3.09 mm in length (excluding awn) and 1.46-1.77 mm in width. These measurements fall within ranges stipulated by various Australian and international authors for these species (Halvorson and Guertin 2003; Michael 2001; Sharp and Simon 2002; Stanley and Ross 1989). Michael (pers. comm.) believes that the majority of *E. crus-galli* in the NGR would be *E. crus-galli* spp. *crus-galli* and reports that seed lengths for this subspecies range from 3-4 mm (Michael 2001).

Variation in weed species also results from genetic differentiation, both within and between populations with the extent of intraspecific variation varying widely among

species (Barrett 1982). In this study the morphology of populations remained dissimilar when grown under the same conditions, indicating that the populations may differ genetically and be responding differently to some selection pressure. However, while the replicates were re-randomised within the polyhouse during the course of the experiments, there may have been microclimates existing that could have contributed to variations during development. Therefore phenotypic plasticity cannot be totally discounted as a contributing factor to the variations observed in plant characteristics in this study.

3.6 Conclusion

Overall in this study, I established that the major species occurring in the NGR, as represented by the sites sampled, is *E. colona* and that currently *E. crus-galli* occurs primarily in northern New South Wales with intrusions into southern Queensland. Morphological differences do exist between the species and include seed characteristics (size and colour) and the presence of bristles along the racemes in *E. crus-galli*. However, there are many common morphological characteristics that could lead to confusion in identification, especially when the plants are small.

There is a high level of diversity in the morphology of populations of both species collected from across the NGR. However, I was unable to establish whether different ecotypes of *E. crus-galli* and *E. colona* existed across the three major centres of the NGR based on the morphological traits measured. A major point to emerge from this study was the fact that while analyses of combined morphological data failed to distinguish between the two species it does confirm that the species share many common features. As the data were collected from plants grown during summer, it can be inferred that if both species were to emerge in the field at this time of year, it would be difficult to identify what species were present prior to maturity and even at maturity a cursory examination of the field would not be sufficient to provide accurate identification of the species present.

Unfortunately when the morphological trial was established the following year (spring) only *E. crus-galli* populations were grown, making a comparison between the

two species at this time of year not available. However, when the morphology of two *E. crus-galli* populations grown in both trials was compared, the summer-grown plants were smaller in height, had smaller leaves and panicles, and produced fewer tillers but more panicles than those grown in spring. If both species had been established during the second trial, at a different time of the year, maybe a different set of data would have provided a clear distinction between the morphologies of *E. colona* and *E. crus-galli*.

However a simple in-field identification tool for mature *E. crus-galli* plants is the presence of bristles on the racemes. The presence of awns may have limited use for identification in the NGR, as when present they were only on a few of the spikelets at the tips of the panicles. The presence of transverse purplish leaf banding is a tool for identifying *E. colona* but it is not present in all plants of the species. If presented with a collection of seeds the length and width measurement as well as the colour of caryopses could be used to distinguish between *E. colona* and *E. crus-galli*.

In addition the results obtained from this study show that the diversity in the morphology of both species is not solely the result of environmental influences and that the genetics of the plants may influence morphology.

Chapter 4 Molecular Studies

4.1 Introduction

The development of molecular markers has provided weed research with the opportunity to differentiate between taxa, investigate genetic diversity and determine the origin of individual introductions. Following advances in molecular biology in the last decade, a variety of different methods have been developed for analysis of genetic diversity (Rao 2004). Methods used for detecting DNA sequence variation are generally based on the use of restriction enzymes for example RFLPs (restriction fragment length polymorphisms) or polymerase chain reaction (PCR). PCR-based techniques include RAPDs (random amplified polymorphic DNA), AFLPs (amplified fragment length polymorphisms) and SSRs or microsatellites (simple sequence repeats). Recently, these techniques have been utilised to conduct taxonomic studies or revisions of genera including *Echinochloa* (Lopez-Martinez *et al.* 1999).

A considerable investment of time, expertise and resources is required to develop microsatellite markers (SSRs) for any particular species, so utilisation of this class of markers has mostly been restricted to agriculturally important crops. Danquah *et al.* (2002a) developed a microsatellite-enriched library for the isolation of microsatellite loci in *Echinochloa,* with the aim of providing population genetic markers more informative than other types of molecular markers, such as allozymes and randomly amplified polymorphic DNA (RAPD) markers. Five primer pairs were developed that amplified microsatellite loci in three agronomically important *Echinochloa* species: *E. colona, E. crus-galli* and *E. crus-pavonis*. All five SSRs showed inter- and/or intra-specific polymorphism and should be useful markers, assisting in the identification of species within the genus and for population genetic studies within *Echinochloa* species.

There is widespread concern that weed species with high levels of genetic diversity have considerable potential for weed adaptation and, therefore, may be able to reduce the effectiveness of weed control (Jasieniuk and Maxwell 2001). Currently little is known about the genetic diversity existing within this genus in Australia.

This chapter reports on a study undertaken utilising the five SSRs developed by Danquah *et al.* (2002a) to identify genetic diversity in *Echinochloa* populations across the northern grain region (NGR) of Australia.

4.2 Aim

The objective of the study reported in this chapter was to determine the genetic variability existing between and within populations of *E. colona* and *E. crus-galli* from three major cropping centres in the NGR. It was designed to answer the following specific questions:

> *(i) Can these SSRs discriminate between Australian accessions of the two species?*
> *(ii) Are the patterns of SSR diversity related to collection site and are there differences in diversity of populations with regard to the positions of populations within paddock and between paddocks (resulting from differences in history of cropping and weed management)?*
> *(iii) Can these SSRs be used to determine the extent of heterozygosity in progeny of individuals as an indication of outcrossing for both species?*
> *(iv) Can this set of SSRs be used to assign individuals to classes based on morphology?*

4.3 Materials and methods

4.3.1 Sample collection, preparation and storage

Three experiments were conducted using the five SSRs developed by Danquah *et al.* (2002a). The method used to collect leaf tissue and prepare the samples for DNA extraction was consistent for all experiments.

A 3 cm length of leaf tissue was collected from each plant at the four to six leaf stage into 1.2 ml microtubes and stored at –80°C until required. The tissue was returned to room temperature then freeze-dried for 24 hours in a Christ Alpha 1-2 LD (Quantum Scientific Pty Ltd). Three 0.3 mm stainless steel ball bearings were placed into each

tube. Racks, containing 96 tubes, were shaken on a Reitch mixer mill for 2 x 60 second cycles, then 2 x 30 second cycles (rotating racks between grinds for even grinding), at a speed of 25 rpm to grind the dried leaf tissue into powder. The ball bearings were carefully tipped out, tubes recapped and gently flicked to loosen the powder. The racks were wrapped in cling wrap to stop moisture being absorbed by the powdered leaf tissue, and held for short-term storage in a –20°C freezer prior to DNA extraction. If the extraction procedure was not being conducted on the same day, the racks were returned to the –80°C freezer.

4.3.2 DNA extraction

Two DNA extraction protocols were used.

4.3.2.1 Wheat and barley extraction protocol

The powdered leaf tissue was removed from the freezer and returned to room temperature. DNA extractions were performed based on the wheat and barley DNA extraction protocol from freeze-dried leaf tissue reported by Fox *et al.* (2003).

After adding 600 µl of extraction buffer (0.1 M Tris-HCl (pH 7.5), 0.05 M EDTA (pH 8), 1.25% SDS) to each of the microtubes containing the powdered leaf tissue, the tubes were resealed and mixed by inverting the racks. Tubes were then incubated at 65°C for one hour in a Hybaid Mini Oven MK II (Integrated Sciences Pty Ltd) before being transferred to the fridge for 30 minutes. When cool, 300 µl of ice-cold 6 M ammonium acetate were added to each tube, the tubes inverted and returned to the fridge for 30 minutes. The tubes were then centrifuged for 15 minutes at 4 000 rpm (Sigma Laboratory Centrifuge 4K15, Quantum Scientific Pty Ltd) to separate the supernatant from the precipitated proteins and plant tissue.

Fresh 1.2 ml microtubes containing 360 µl of iso-propanol were prepared and 600 µl of the supernatant were added. The tubes were sealed and left on the bench for five minutes to allow the DNA to precipitate before being gently inverted and centrifuged for 15 minutes at 4 000 rpm to pellet the DNA. The supernatant was carefully tipped

out and the remaining fluid drained off the DNA pellet by inverting the tubes onto absorbent paper towel.

To wash the pellets, 500 µl of 70% ethanol were added to the tubes and centrifuged for 15 minutes at 4 000 rpm. The resulting supernatant was discarded and the pellet air-dried for 30 minutes before re-suspending in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA (pH 8), 10 mg RNase). The tubes were refrigerated overnight and then the DNA was transferred to a 96-well PCR plate, sealed with an adhesive seal and stored in the fridge until required.

4.3.2.2 CTAB (hexadecyltrimethylammonium bromide) extraction protocol

The powdered leaf tissue was removed from the freezer and returned to room temperature before DNA extractions were performed using the CTAB (hexadecyltrimethylammonium bromide) method as described by Tai and Tanksley (1990) with some modifications.

Fresh extraction buffer was prepared by dissolving 0.6 g sodium metabisulphite and 2.4 g PVP (2% w/v) in 50 ml extraction buffer stock (0.35 M sorbitol, 0.1 M Tris (pH 7.5), 5 mM EDTA), while continually mixing on a heated magnetic stirrer. Once dissolved, 50 ml lysis buffer stock (0.2 M Tris (pH 7.5), 0.05 M EDTA, 2 M NaCl, 2% CTAB) and 20 ml sarcosyl stock (5% w/v) were added and the solution heated to 65°C in a Hybaid Mini Oven MK II (Integrated Sciences Pty Ltd).

The buffer was removed from the oven and 500 µl added to each tube containing the powdered leaf tissue. Tubes were inverted 20 times to mix the leaf powder and buffer and returned to the oven for 40 minutes at 65°C. During this period, the tubes were inverted at 10, 25 and 40 minutes. The tubes were cooled, 500 µl of chloroform/isoamyl (24:1) added to each and then inverted five times. The lids were removed to release pressure build up, replaced and the tubes inverted a further 35 times. Samples were then centrifuged for 30 minutes at 2 500 rpm.

Fresh 1.2 ml tubes were prepared and 450 µl of the resulting supernatant transferred, before adding 360 µl of ice-cold isopropanol (0.8 vol). The tubes were inverted 20 times then centrifuged for 30 minutes at 2 500 rpm. The supernatant was discarded and 500 µl of ice cold 70% ethanol added to each tube. The tubes were gently inverted to dislodge the DNA pellet and then centrifuged for 30 minutes at 2 500 rpm. The ethanol was carefully poured off and the DNA pellet left to dry overnight in a fume hood.

The dry pellets were resuspended in 80 µl of 0.1 TE buffer (10 mM Tris-HCl, 1 mM EDTA (pH 8), 10 mg RNase). The samples were then incubated at 37°C for 45 minutes, and then at 65°C for 30 minutes to inactivate the RNase. The DNA was transferred to 96-well PCR plates, sealed with an adhesive seal and stored in a refrigerator until required.

4.3.3 Polymerase chain reaction (PCR)

Forward and reverse primers, designated EC1 through to EC5 (Danquah *et al.* 2002a) were ordered from Proligo Australia Pty Ltd (Lismore, Australia) (Table 4.1). An additional set of forward primers labelled with appropriate fluoroscenes were also ordered. This was to conduct product sizing using the CEQ 8800 genetic analysis system (Beckman Coulter).

Table 4.1 Sequences of the SSR markers used (Danquah *et al.* 2002a).

Marker		Primer sequences (5′- 3′)
EC1	1-F	5′-ATTACTGGTCAGACGGAAAC-3′
	1-R	5′-GCAGTTATCTCCGTGGGCAC-3′
EC2	2-F	5′-GGCTCCAAACAAGGCAATTC-3′
	2-R	5′-TTCAGGGAATTTAGTACAAG-3′
EC3	3-F	5′-GAAAGGAAATGGGTTGGCTG-3′
	3-R	5′-CTTCGCACCATGATCTTCTC-3′
EC4	4-F	5′-AGTAGAAGGCTGCAAGAAGG-3′
	4-R	5′-TCTCAGCCCACTTTGTATAG-3′
EC5	5-F	5′-CAGAGCCTTCAATCATGGTG-3′
	5-R	5′-TGCTTCAAGTTCTAGGAGAC-3′

The stocks of the unlabelled primers were diluted to final concentrations of 25 ng/µl. The labelled forward primers were diluted to stock concentrations of 50 µM and then further diluted to final concentrations of 5 µM.

The PCR reaction mixture was based on Danquah *et al.* (2002a) with minor modifications. The microsatellite amplification was performed in reaction volumes of 12.5 µl containing 2 µl DNA template (approx 50 ng/µl), 1 µl each of forward and reverse primers (25 ng/µl unlabelled, 5 µM/µl labelled), 1.25 µl PCR buffer including 15 mM $MgCl_2$ (Qiagen), 0.25 µl combined dNTP, 0.1 µl *Taq* polymerase (Qiagen) made up to the total volume of 12.5µl with MilliQ water (Table 4.2).

Table 4.2: PCR reaction mixture including stock and final concentrations of products.

Ingredients		**Stock concentration**	**Final concentration**	**Volume per sample (µl)**
H_2O (MilliQ)		n/a	n/a	6.9
PCR buffer (Qiagen)		10x	1x	1.25
dNTP		10 mM	0.2 mM	0.25
Forward primer (unlabelled)	(OR)	2.5 µg/µl	25 ng/µl	1.0
Forward primer (labelled)		50 µM	5 µM	1.0
Reverse primer		2.5 µg/µl	25 ng/µl	1.0
Taq (Qiagen)		5 U	0.5 U	0.1
DNA				2.0
			Total	12.5 µl

All the ingredients, as well as the PCR plates, were kept on crushed ice during plate preparation. Tubes and plates containing labelled primers were kept covered in alfoil when possible, as the labels were light sensitive.

The reaction was run on a PTC-100™ programmable thermal controller (MJ Research, Watertown, MA, USA) for one cycle 94°C for 5 minutes, then 35 cycles 94°C for 1 minute, 54°C for 1 minute, 72°C for 1 minute and then one cycle 72°C for 10 minutes as per Danquah *et al.* (2002a). PCR products were stored in a –20°C freezer until required.

Unlabelled PCR products were visualised on 8% non-denaturing polyacrylamide gel, while labelled PCR products were sized on a Beckman CEQ 8800 (Beckman Coulter).

4.3.4 Visualisation of DNA and PCR products

4.3.4.1 Visualisation of DNA on 1.5% TAE agarose gel

The DNA extracted from 10 *E. colona* and 12 *E. crus-galli* plants was run on a 1.5% TAE agarose gel to evaluate DNA quality and quantity.

The gel was prepared by adding 1.5 g agarose to 100 ml TAE (0.04 M Tris-acetate, 0.001 M EDTA (pH 8)) and heated in a microwave on medium-high until it started to boil. The mixture was placed on a bench to cool before being poured into an 11 x 14 cm forming tray and inserting the well combs.

Twenty-four 0.2 ml tubes were prepared, each containing 5 µl of dye and 5 µl MilliQ water. To 22 of the tubes, 2 µl of DNA was added and in the remaining two tubes, 2 µl of Lambda DNA marker (Fermentis 0.5 mg DNA/ml) added.

When the gel was set, the combs were removed and 6 µl of the ladder mix added to the first and last well of each row and 15 µl of the DNA mix added to the remaining wells. The gel was run on a Horizon 11-14 horizontal gel electrophoresis system (GibcoBRL Life Technologies) at 90 V for 1 hour with a current of 400 mA. The gel was stained in 0.5 µg/ml ethidium bromide for 15 minutes before being rinsed in water and photographed using a UV light box.

4.3.4.2 Visualisation of PCR products by polyacrylamide gel electrophoresis (PAGE)

The amplified products of five *E. colona* and five *E. crus-galli* were visualised on an 8% non-denaturing polyacrylamide gel, run on a BIO-RAD Sequi-gen sequencing cell at 60 W for 1.5 hours, whereas Danquah *et al.* (2002b) used autoradiography to visualise products run on 6% denaturing polyacrylamide gels for 2 hours at 60 W.

The acrylamide solution contained 12 ml acrylamide (29:1) and 6 ml TBE buffer (0.09 M tris-acetate, 0.08 M Boric Acid, pH 8.3, 0.0026 M EDTA) made up to 60 ml with MilliQ water. The polymerising agents (50 µl TEMED and 500 µl ammonium persulphate (10%)) were added to the solution immediately before the gel was poured. Once set, the gel was pre-run for 15 minutes with 1 x TBE. Loading dye (5 µl) was added to 5 µl of PCR samples and 5 µl individually loaded onto each well. Allele sizes were determined by comparison to a 100 bp ladder (Fermentas GeneRuler).

The gel was silver stained as described by Bassam and Caetano-Anollés (1993). The gel was put in a fixing solution (150 ml 7.5% acetic acid made up to 2 L with MilliQ water) and gently agitated for 20 minutes before being washed in MilliQ water. It was then placed in staining solution (1.5 g silver nitrate, 2.25 ml formaldehyde added to 1.5 L MilliQ water) and gently agitated for 40 minutes, then rinsed with MilliQ water. The gel was developed using a developing solution (1.5 L deionised water, 45 g sodium carbonate anhydrous, 4.5 ml formaldehyde, 750 µl sodium thiosulphate (0.4%)), until bands were clear, but background minimal. The development of the gel was stopped by placing it in acetic acid for 5 minutes. It was then rinsed with MilliQ water and air dried overnight.

4.3.4.3 Visualisation of PCR products by capillary electrophoresis (CEQ)

Capillary electrophoresis is a separation technique that uses narrow-bore fused silica capillaries to separate molecules. The PCR products from nine *E. colona* and seven *E. crus-galli* were sized on a Beckman CEQ 8800, using the manufacturers recommended method. Samples run on the CEQ machine used PCR product containing labelled forward primers and non-labelled reverse primers described in 4.3.3. All additional materials used were provided by Beckman Coulter Inc. and peaks were sized using the manufacturer's software.

The CEQ machine operates on four different dyes: red (Dye 1) for the ladder, and black (Dye 2), green (Dye 3) and blue (Dye 4) allocated to the forward primers. The black dye, the weakest, was allocated to markers EC1 and EC2, green to EC4 and EC5, and blue, the strongest, was allocated to EC3. The decision was made in order to

combine all the PCR products from the same DNA in a single well for electrophoresis on the CEQ, and was based on the banding pattern on the polyacrylamide gel.

The procedure was conducted using 96-well CEQ plates. A master mix was prepared consisting of 30 µl of sample loading solution (CEQ™ SLS) and 0.3 µl of 600 bp ladder (CEQ™ DNA size standard kit – 600 bp) per sample. PCR products containing dye 3 (EC4, EC5) and dye 4 (EC3) were diluted prior to being added to the master mix.

The PCR products EC1 and EC2 were not diluted and 1 µl of each was added straight to the master mix in the wells. The PCR products EC4 and EC5 were diluted 1:1 (1 µl PCR added to 1 µl SLS) and EC3 was diluted 1:3 (1 µl PCR to 3 µl SLS). After completing the dilutions, 1 µl of the diluted PCR product was added to the master mix in the wells.

The PCR products from the 16 *Echinochloa* samples were run through the CEQ using three different mixes to ascertain if the primers could be combined. In the first set, all the markers for each DNA sample were combined together in individual wells. The second mix contained only the markers EC1, EC3 and EC4, and the third mix contained only EC2 and EC5.

4.3.5 Data analysis

The output from the CEQ (peaks) was sized using the manufacturer's software. The peaks, which correspond to fragment size (bp), were tabulated for each sample with values being rounded to the closest whole number.

The data were analysed using R package software (R2.7.2 © The R Foundation for Statistical Computing 2004) using the Jaccard dissimilarity method hierarchical clustering algorithm (HCLUST). Dendrograms were produced based on the unweighted pair group method of arithmetic means (UPGMA) based on the presence/absence of scoreable peaks generated by the five SSRs. Only those plants for which all five primers produced product were included in the analyses.

4.3.6 Experiment one – assessment of SSRs

A series of trials was undertaken to assess the effectiveness of the five SSRs to reliably produce the predicted products of Danquah *et al.* (2002b), using methods modified to suit in-house protocols. The trials included DNA extraction, polymerase chain reaction (PCR), and visualisation of PCR products on gels (1.5% TAE agarose, 8% non-denaturing polyacrylamide) and by capillary electrophoresis (Beckman CEQ 8800) as outlined in 4.3.4.

Leaf tissue used in this experiment was collected from *E. colona* grown from seeds collected from the Department of Primary Industries and Fisheries (DPI&F) farm at Kingsthorpe, Queensland (long 151.81563E, lat 27.47725S), and *E. crus-galli* grown from seeds collected at the Australian Cotton Research Institute (ACRI) in Narrabri, New South Wales (long 149.782904E, lat 30.324876S). The plants were grown to maturity and identified as either *E. colona* or *E. crus-galli* based on morphology. The DNA from 22 plants (10 *E. colona*, 12 *E. crus-galli*) was visualised on 1.5% TAE agarose gel while the DNA from 10 plants (5 *E. colona*, 5 *E. crus-galli*) was visualised by polyacrylamide gel electrophoresis. An additional nine *E. colona* and seven *E. crus-galli* were grown from the same seed collections, and the DNA from these plants visualised using CEQ analysis.

4.3.7 Experiment two – population genetic diversity

In the second experiment, the genetic diversity of *E. colona* and *E. crus-galli* from three major centres across the northern grain region (NGR) was investigated. Initially, the genetic diversity of populations grown in the morphological trials was investigated. Leaf tissue was collected from one replicate of each of the 30 *E. colona* and 31 *E. crus-galli* populations grown during the trials.

In addition, a further 206 *Echinochloa* plants were grown from seeds collected across the three regional centres of the NGR, and leaf tissue sampled to provide a broader study of the genetic diversity in the region.

The procedures for DNA extraction and PCR amplification were performed as outlined in 4.3.2.2 and 4.3.3 respectively. The CEQ analysis was conducted with all markers combined in the same well for each DNA sample using the procedure outlined in 4.3.4.3.

4.3.8 Experiment three – heterozygosity testing

The third experiment was undertaken to investigate the extent of heterozygosity in progeny of individuals as an indication of out-crossing in both species. During the morphological trials, plants grown in 25 cm diameter pots in a polyhouse were allowed to cross freely. The 2005/2006 trial contained both *E. colona* and *E. crus-galli* and the majority of plants flowered in February, while in the 2006/2007 trial only *E. crus-galli* was grown and plants flowered from late November through December.

Five plants from the morphological trial, three *E. colona* and two *E. crus-galli*, were selected representing both species and the regional centres across the NGR. The *E. colona* and *E. crus-galli* from Narrabri were grown from seeds collected from the tail drain site, the seeds of both the Dalby species had been collected from different sorghum fields, and the *E. colona* seeds from Wowan had been collected in a fallow field. As the five plants in the morphological trials matured, seeds were collected and bagged separately. Seeds were air-dried in a glasshouse for two weeks before being repackaged in seed packets and stored in a cold room at 10°C. In September 2007 the seeds collected from the five plants in the morphological trial were mass planted into multiple 25 cm diameter pots and grown to the 4 to 6 leaf stage, harvested and processed as described in 4.3.1. This allowed for the genetic patterns of the progeny to be compared with that of the parent.

Marker 3 (EC3) was run on both species to confirm identity and detect intra-specific variation in *E. colona*, while marker 4 (EC4) was used to detect additional variation in *E. colona* and marker 5 (EC5) was run to detect variation in *E. crus-galli*.

The procedures for DNA extraction and CEQ analysis were undertaken as outlined in 4.3.2.2 and 4.3.4.3 respectively. The PCR amplification as outlined in 4.3.3 was undertaken with minor modification. The reaction was run on a computerised PCR Model CG1-96 (Corbett Research) in lieu of the PTC-100™ programmable thermal controller.

4.4 Results

4.4.1 Experiment one – assessment of SSRs

Aim: To assess DNA extraction methodologies and published SSR markers for applicability and polymorphism in local *Echinochloa* populations.

4.4.1.1 DNA extraction and visualisation of DNA products

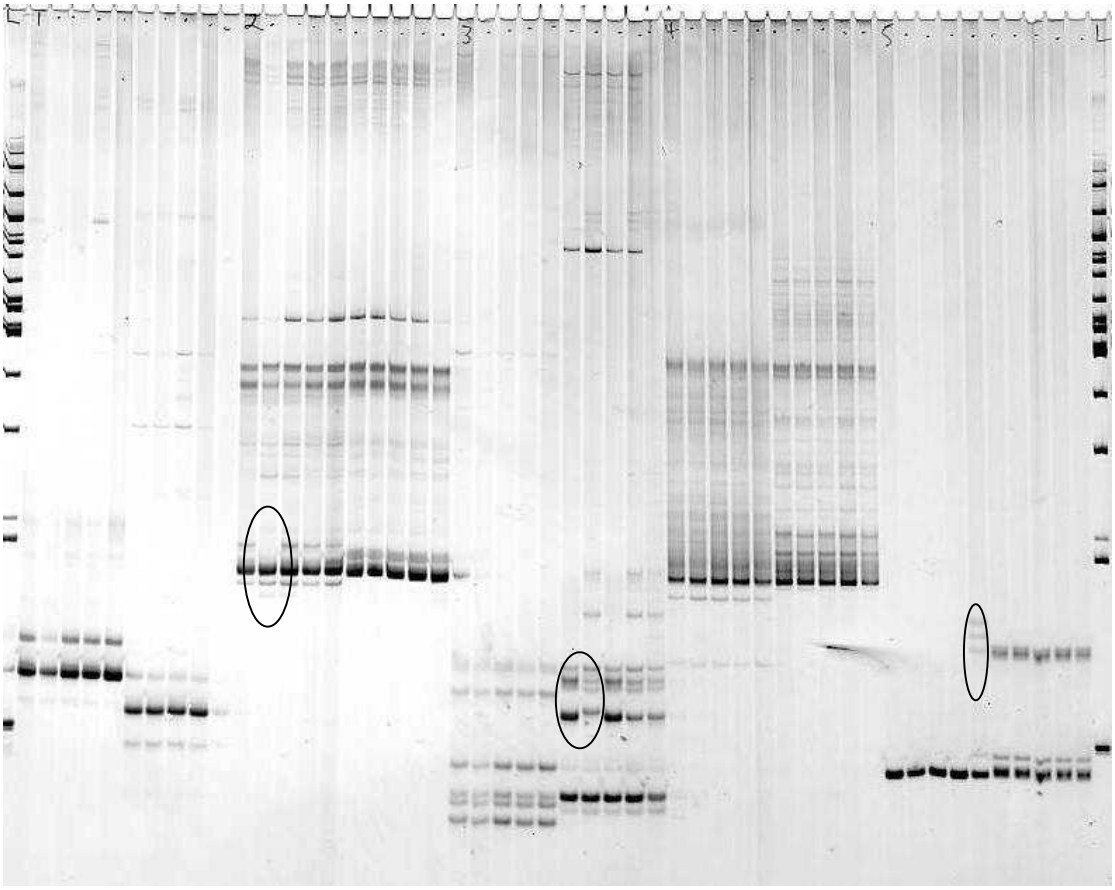
Two DNA extraction methods were compared. The wheat and barley extraction protocol resulted in poor resolution on 1.5% agarose gel but produced satisfactory PCR products (Photograph 4.1). The second method (CTAB) resulted in improved resolution on 1.5% agarose gel and PCR products. The CTAB method was chosen as the preferred DNA extraction method for further studies.

4.4.1.2 PCR and visualisation of DNA products

Marker applicability and polymorphism

Five SSR markers first described by Danquah *et al.* (2002a) were screened on a set of five *E. colona* from Kingsthorpe and five *E. crus-galli* from Narrabri. Non-labelled PCR products were visualised on an 8% non-denaturing polyacrylamide gel (Photograph 4.1).

All five SSRs were able to differentiate between the two species with markers EC2, EC3 and EC5 detecting possible intra-specific variation in this set of accessions.



Photograph 4.1: PCR results of DNA with non-labelled primers using DNA extracted using wheat and barley procedure. The 8% non-denaturing polyacrylamide gel clearly shows differentiation between *E. crus-galli* (left 1-5) and *E. colona* (right 6-10) within each of the five SSRs (EC1-EC5). Lane 10 in EC1 failed to amplify. Possible intra-specific variations indicated in EC2, EC3 and EC5. The 100 bp ladder is shown in the first and last lanes of the gel, each dark bar indicates an increment of 100 bp.

Marker 1 (EC1)

Marker EC1 showed strong banding for *E. crus-galli* at 130 bp in all samples and an additional band at 150 bp in at least four of the samples. All *E. colona* samples had a strong band at 110 bp.

Marker 2 (EC2)

Both species showed a strong band at 180 bp with *E. crus-galli* having an additional weaker band at 170 bp and a possible intra-specific polymorphism at 185 bp.

Marker 3 (EC3)

The banding patterns for all the *E. crus galli* plants were identical with bands at 55, 65, 70 and 80 bp. Further lighter bands were discernable at 125 and 140 bp. The *E. colona* plants had a common dark band at 70 bp with a weaker band at 65 bp. Four of the *E. colona* plants had strong bands at 110 bp, while the remaining plant had a weaker band at 112 bp. All *E. colona* plants shared a similar group of bands between 120 bp and 140 bp.

Marker 4 (EC4)

Both species showed a strong band at 180 bp with *E. crus-galli* having an additional weaker band at 170 bp. *E. colona* had additional weaker banding at 200 bp and 220 bp. No intra-specific variation was evident in this sample set.

Marker 5 (EC5)

Marker 5 showed a strong band at 80 bp for both species and additional bands at 90 bp and 150 bp in *E. colona* only. A possible intra-specific polymorphism is seen in *E. colona*.

Capillary electrophoresis analysis (CEQ)

A similar, but not identical set of samples was amplified using labelled primers and visualised using the CEQ. The patterns were observed within the range 70 bp to 250 bp. A total 26 peaks/complexes, which correspond to base pairs, were scored across all samples. Nine peaks/complexes were scored across the 7 *E. crus-galli* plants (Figure 4.1) and 17 across the 9 *E. colona* plants (Figure 4.2).

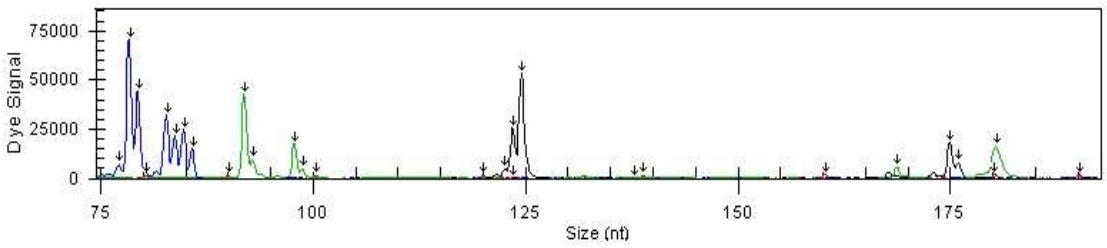


Figure 4.1: CEQ output for one of the Narrabri *E. crus-galli* plants showing 8 peaks/complexes (EC1 & EC2 - black, EC3 - blue, EC4 & EC5 - green, 600 bp ladder - red). The x axis shows the fragment size in base pairs (bp) and the y axis the dye signal corresponding to band intensity.

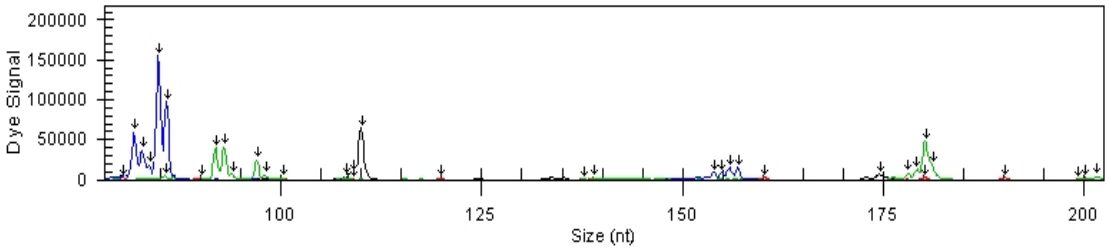


Figure 4.2: CEQ output for one of the Kingsthorpe *E. colona* plants showing 8 peaks/complexes (EC1 & EC2 - black, EC3 - blue, EC4 & EC5 - green, 600 bp ladder - red). The x axis shows the fragment size in base pairs (bp) and the y axis the dye signal corresponding to band intensity.

Using this system, four of the five SSRs were able to clearly differentiate between the two species, with marker 4 (EC4) being the exception. However there were inconsistencies in peak intensity with marker 2 (EC2) that may result in incorrect identification. The output from each marker was analysed separately and the traces from individual plants are shown, being representative of that recorded for each species (Figures 4.3 to 4.7).

Marker 1 (EC1)

Marker 1 showed distinct variation between species with *E. crus-galli* showing a major peak at 124 bp and *E. colona* at 110 bp (Figure 4.3).

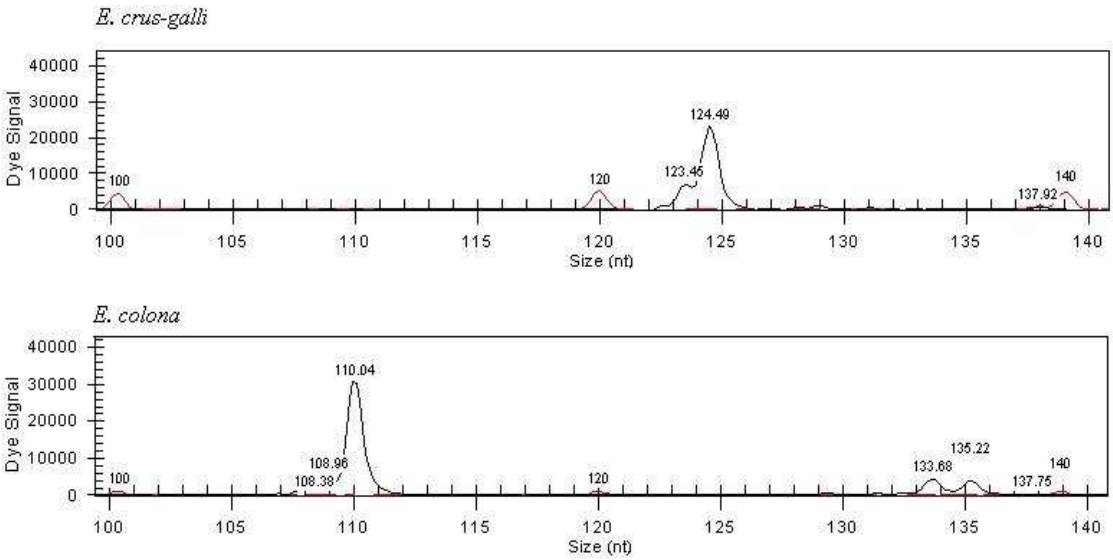


Figure 4.3: CEQ output for EC1 for the two species with a major peak at 124 bp for *E. crus-galli* and at 110 bp for *E. colona*. The x axis shows the fragment size in base pairs (bp) and the y axis the dye signal corresponding to band intensity.

The peak intensity of the complex at 133-135 bp in *E. colona* was inconsistent and often not much higher than the intensity of the ladder. Whilst these banding patterns may have been real product peaks (as opposed to system noise) they were difficult to score. No clear intra-specific variation was evident for either species in these samples using EC1.

Marker 2 (EC2)

Marker 2 showed a major peak at 175 bp for both species (Figure 4.4).

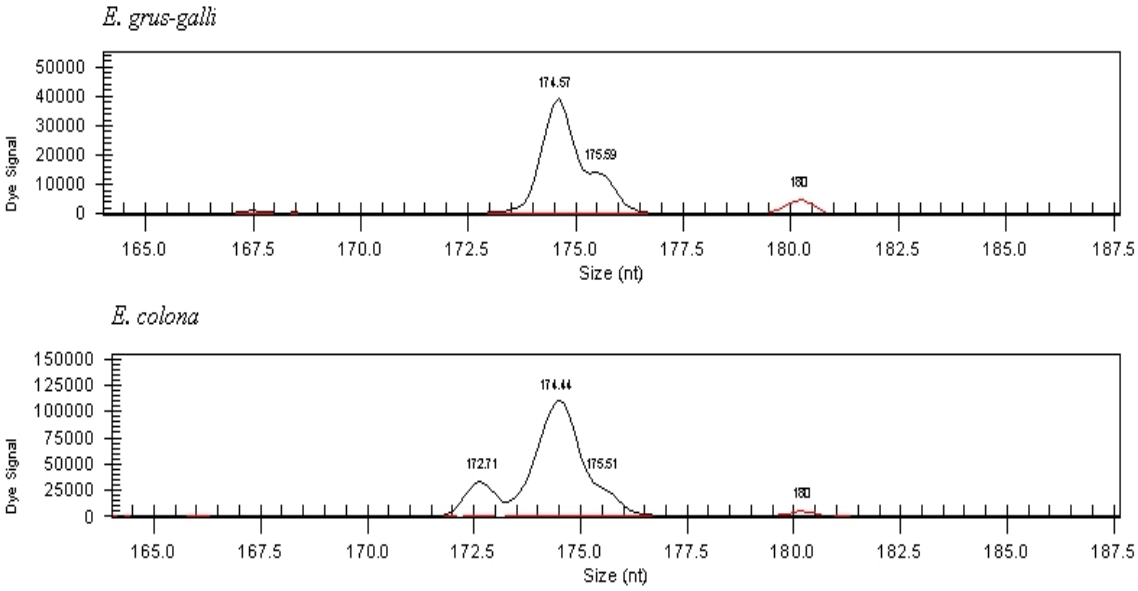


Figure 4.4: CEQ output for EC2 showing a major band at 175 bp for both species. The small peak at 173 bp for *E. colona* was inconsistent in all plants and often close to the height of the ladder. The x axis shows the fragment size in base pairs (bp) and the y axis the dye signal corresponding to band intensity.

The intensity of the peak at 173 bp in *E. colona* was inconsistent and often not much higher than the intensity of the ladder. However, the presence of a peak in *E. colona* at 173 bp is clearly evident in this sample and can distinguish between the species.

Marker 3 (EC3)

Marker 3 showed distinct variation between the two species. *Echinochloa crus-galli* had a major complex comprising of a large doublet at 78/79 bp followed by a large

quad from 83-86 bp. There was also a complex for *E. colona* in this region but it appeared as two doublets at 82/83 bp and 85/86 bp (Figure 4.5).

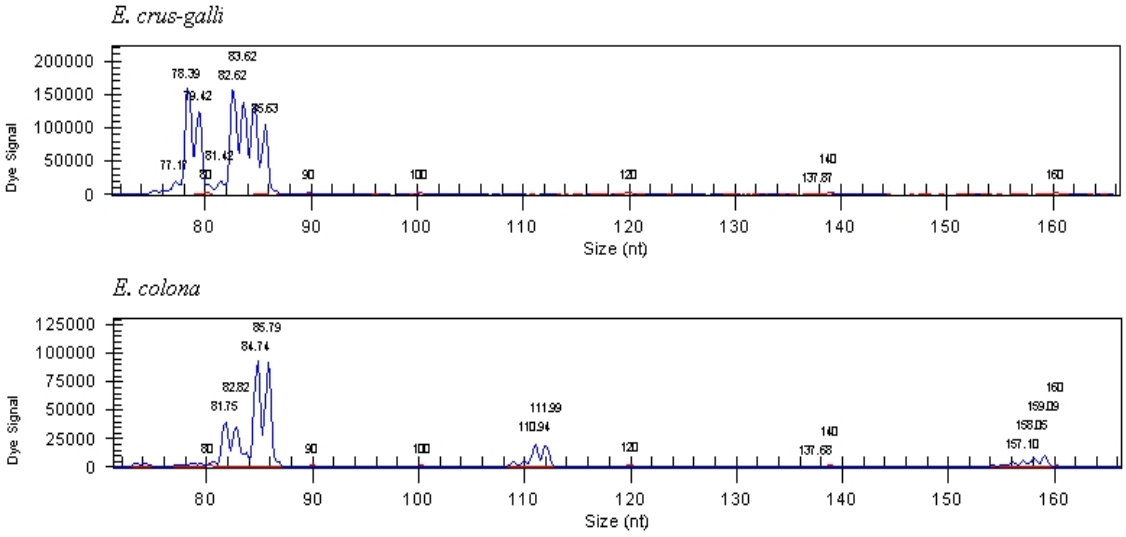


Figure 4.5: CEQ output for EC3 for the two species. *Echinochloa crus-galli* had a large complex comprising a doublet at 78/79 bp followed by a quad at 83-86 bp and *E. colona* a complex with two doublets at 82/83 bp and 85/86 bp. Small peaks are visible in *E. colona* at 109-112 bp and 156-159 bp. The x axis shows the fragment size in base pairs (bp) and the y axis the dye signal corresponding to band intensity.

Additional small peaks were scored for *E. colona* at either the 109-112 bp region or 107-110 bp, but were not present in all plants. Further bands were present at 156-159 bp in some plants and absent in others.

No clear intra-specific variation was evident in *E. crus-galli* using the EC3 marker however intra-specific variation was evident in *E. colona*.

Marker 4 (EC4)

Marker 4 showed peaks at 180 bp for both species (Figure 4.6).

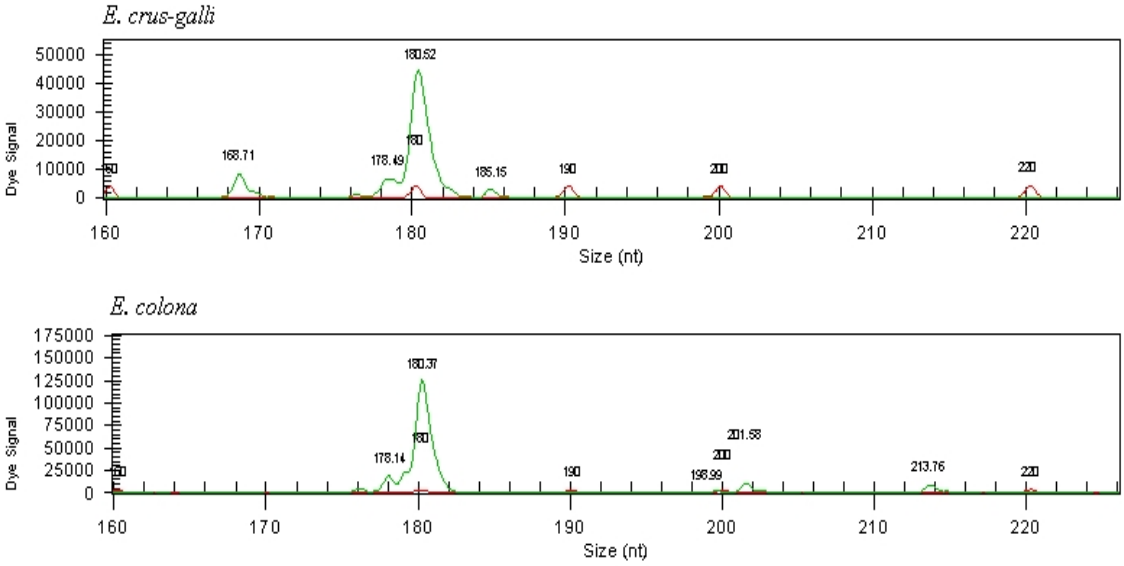


Figure 4.6: CEQ output for EC4 showing a major peak at 180 bp for both species. Small peaks were observed for *E. crus-galli* at 169 bp and 185 bp and for this *E. colona* additional peaks were scored at 202 bp and 214 bp. The x axis shows the fragment size in base pairs (bp) and the y axis the dye signal corresponding to band intensity.

Additional small peaks were observed for *E. crus-galli* at 169 bp and 185 bp but the peak intensity was inconsistent in all plants and often close to the height of the ladder. Whilst these banding patterns may have been real product peaks (as opposed to system noise) they were difficult to score. An additional five small peaks were observed in *E. colona* at 178 bp, 202 bp (variant 204 bp) and 214 bp (variant 216 bp) but were not present in all plants.

No clear differentiation between species was evident in these plants using this marker. However, EC4 showed intra-specific variation in *E. colona* but not in *E. crus-galli*.

Marker 5 (EC5)

This marker revealed a single peak at 92 bp for *E. crus-galli* and a doublet at 92/93 bp for *E. colona* (Figure 4.7).

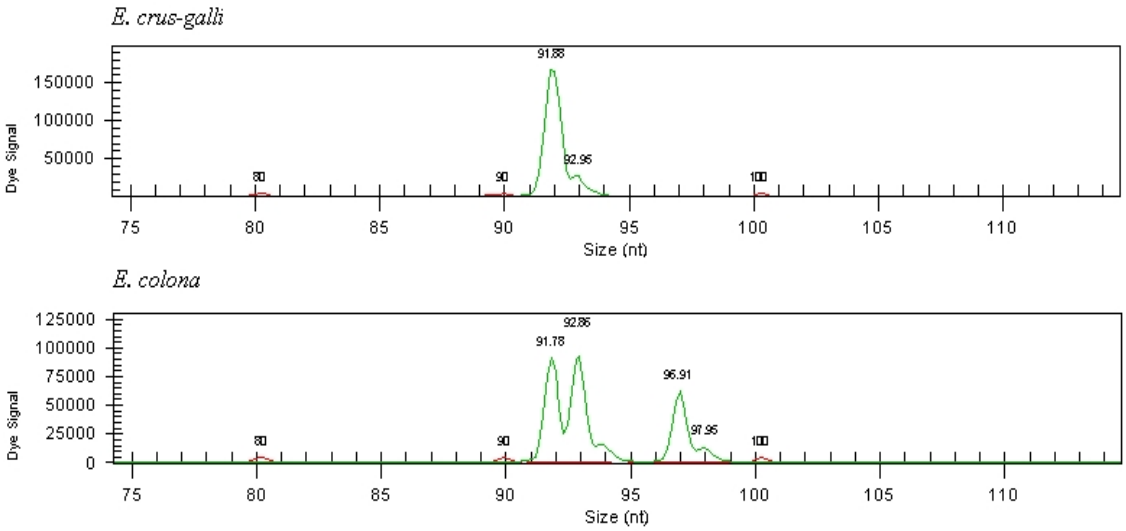


Figure 4.7: CEQ output for EC5 showing a major peak at 92 bp for *E. crus-galli* and a doublet at 92/93 bp for *E. colona*. A smaller peak was also scored for *E. colona* at 97 bp. The x axis shows the fragment size in base pairs (bp) and the y axis the dye signal corresponding to band intensity.

An additional smaller peak was found in all *E. colona* plants at 97 bp but only in some of the *E. crus-galli* plants.

A clear differentiation between the two species was evident in these plants using EC5, however no clear intra-specific differences for either species were detected.

4.4.2 Experiment two – population genetic diversity

Aim: (i) Describe marker polymorphisms of plants in the morphological study.
(ii) Describe marker variability of plants in regional survey and relate to collection location.

4.4.2.1 Morphological study

Markers in the morphological study

Of the 61 leaf samples collected from the morphological trials and run on the CEQ, 16 failed to successfully visualise product for all five SSRs and were excluded from the study. The remaining samples, representing 22 *E. colona* and 23 *E. crus-galli* populations, produced products from which thirty-three peaks/complexes were recorded (Table 4.3).

Table 4.3: *Echinochloa colona* and *E. crus-galli* populations which successfully visualised product for all five SSRs, indicating the population labels used in Chapter 4 and the original population IDs assigned to the plants sampled in the field. Refer to 3.3.1.1 for description of population ID.

E. colona		***E. crus-galli***	
Population label	Population ID	Population label	Population ID
1	D11: 20-1	23	D11: 6-4
2	D13: 4-2	24	D11: 3-5
3	D14: 12-4	25	D11: 11-8
4	D21: 3-1	26	D11: 19-4
5	D21: 4-1	27	D11: 20-4
6	D41: 10-4	28	D21: 5-5
7	D51: 6-2	29	D21: 13-2
8	N11: 2-6	30	D21: 17-2
9	N11: 1-1	31	N11: 3-3
10	N12: 5-5	32	N11: 3-5
11	N15: 10-4	33	N12: 11-5
12	N15: 12-3	34	N12: 12-2
13	W11: 1-3	35	N15: 1-4
14	W12: 9-3	36	N15: 2-1
15	W13: 2-1	37	N15: 2-4
16	W13: 5-2	38	N15: 7-4
17	W13: 11-9	39	N15: 9-5
18	W13: 17-1	40	N15: 11-5
19	W14: 1-1	41	N15: 14-1
20	W14: 6-3	42	N15: 14-3
21	W15: 8-1	43	N15: 14-4
22	W16: 4-3	44	N15: 16-2
		45	N15: 20-3

Twenty-three peaks were recorded for *E. colona* and ten for *E. crus-galli*. All *E. crus-galli* populations had six peaks/complexes in common and all *E. colona* populations had 7 peaks/complexes in common. Only two peaks, 175 bp (EC2) and 180 bp (EC4) were common to both species (Table 4.4).

Table 4.4: Summary of the base pairs (bp) amplified by the 5 SSRs of Danquah *et al.* (2002a) common to all populations of both *E. crus-galli* and *E. colona* run on a Beckman CEQ 8800.

Marker	**EC1**		**EC2**	**EC3**				**EC4**	**EC5**		
Base pairs (bp)	110	124	175	78/79	82/83	85/86	83-86	180	92	92/93	97
E. crus-galli		✓	✓	✓			✓	✓	✓		
E. colona	✓		✓		✓	✓		✓		✓	✓

Marker 1 (EC1)

This marker was monomorphic for both species with peaks scored at 110 bp for *E. colona* and 124 bp for *E. crus-galli*. A small complex of peaks at 133-135 bp observed in *E. colona* was omitted from the analysis, as peak intensity was inconsistent and often not much higher than the intensity of the ladder. Whilst these banding patterns may have been real product peaks (as opposed to system noise) they did not contribute any useful information and were difficult to score.

Marker 2 (EC2)

This marker was monomorphic with a peak for both species shown at 175 bp. Small peaks were observed at 173 bp for *E. colona* but were inconsistent in peak intensity and omitted from analysis.

Marker 3 (EC3)

This marker provided a clear distinction between *E. crus-galli* and *E. colona*. Two of the complexes, a doublet at 78/79 bp and an 83-86 bp quad were unique to *E. crus-galli*, while two doublets at 82/83 bp and 85/86 bp were indicative of *E. colona*.

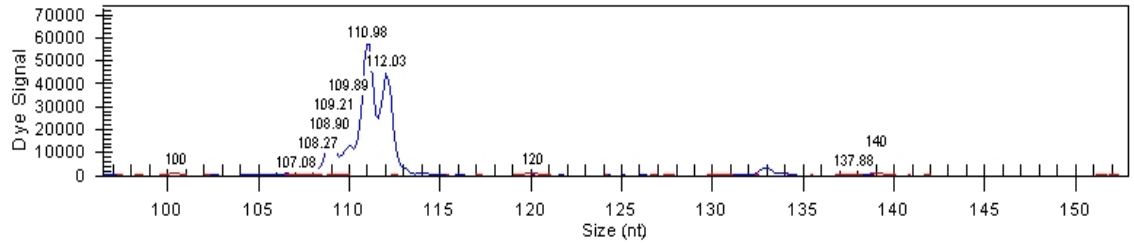
Additional complexes were found in some of the *E. colona* populations in the range 107 bp to 161 bp and appeared to relate to the regional centre from which the seed had been originally collected (Table 4.5).

Table 4.5 The base pair complexes scored using marker EC3 present in the 22 *E. colona* populations included in the morphological study, listed according to regional centre.

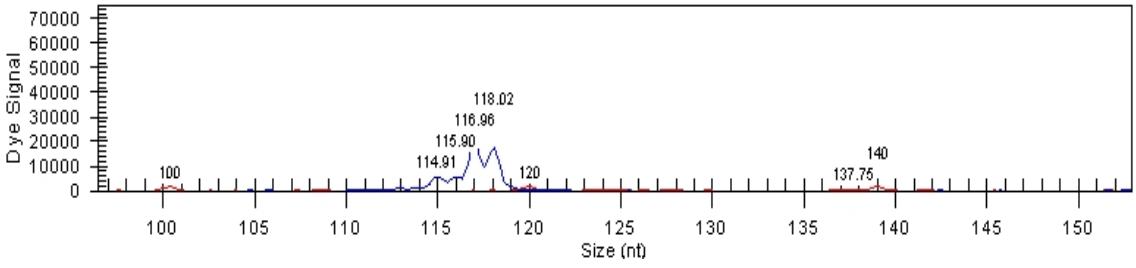
Regional centre	Base pairs (bp)									
	82/83	85/86	107-110	108-112	115-118	143-146	146-150	154-157	156-159	158-161
Dalby	7	7	1	3				2	1	
Wowan	10	10				5	4	2	1	
Narrabri	5	5			3			1		2

The products in the range 107-110 bp and 108-112 bp were only present in Dalby populations. Products in the range 115-118 bp were only present in Narrabri populations and the ranges 143-146 bp and 146-150 bp appeared only in Wowan populations (Table 4.5 and Figure 4.8).

a



b



c

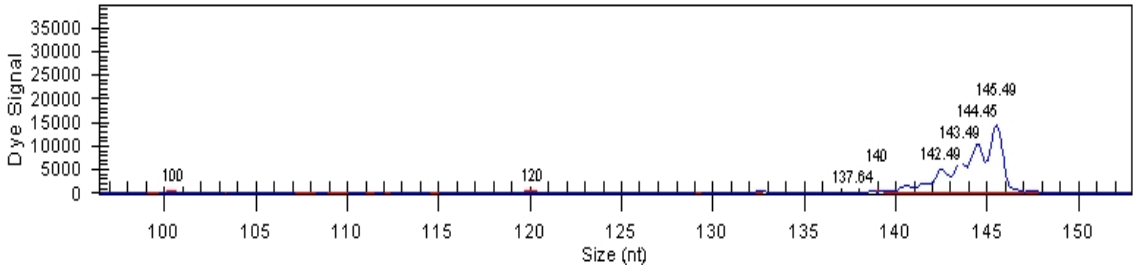


Figure 4.8: The CEQ output of *E. colona* populations scored in the range 100 bp to 150 bp using EC3 (a - Dalby 109-112 bp, b - Narrabri 115-118 bp, c - Wowan 143-146 bp). The x axis shows the fragment size in base pairs (bp) and the y axis the dye signal corresponding to band intensity.

Marker 4 (EC4)

This marker was monomorphic for *E. crus-galli* with a major peak at 180 bp. Smaller peaks were also observed at 169 bp and 185 bp but were inconsistent in intensity, often smaller than the ladder and were omitted from further analyses.

A major peak was present at 180 bp in all the *E. colona* populations, with additional smaller peaks being observed in some populations at 178 bp and between 200 bp and 214 bp. These additional peaks did not appear to be related to regional centres (Table 4.6).

Table 4.6: The intra-specific variations scored using marker EC4 present in the 22 *E. colona* populations included in the morphological study, listed according to regional centre.

Regional centre	Base pairs (bp)					
	178	**200**	**202**	**204**	**212**	**214**
Dalby	7	1	4	1	1	5
Wowan				3	10	
Narrabri	5		4		4	

Marker 5 (EC5)

This marker was polymorphic for *E. crus-galli* with a peak at 92 bp scored in all 23 populations, and an additional peak detected at 97 bp in one Dalby population and at 98 bp in two Narrabri populations. These were the only intra-specific variations found in *E. crus-galli*. The peaks scored for *E. colona* were a doublet at 92/93 bp and a peak at 97 bp.

Of the five SSRs developed by Danquah *et al.* (2002a) only EC2 and EC4 were unable to clearly differentiate between *E. colona* and *E. crus-galli*. While marker 4 (EC4) did provide information that could differentiate between the species it was not consistent or present in all the samples, making this marker unreliable. With regard to intra-species variation this was detected in *E. crus-galli* by EC2 and in *E. colona* by EC3 and EC4 in this set of samples.

Clustering of populations from the morphological study based on molecular characterisation

There was a clear differentiation between *E. crus-galli* and *E. colona* with the populations splitting into two main clusters corresponding to the two species (Figure 4.9).

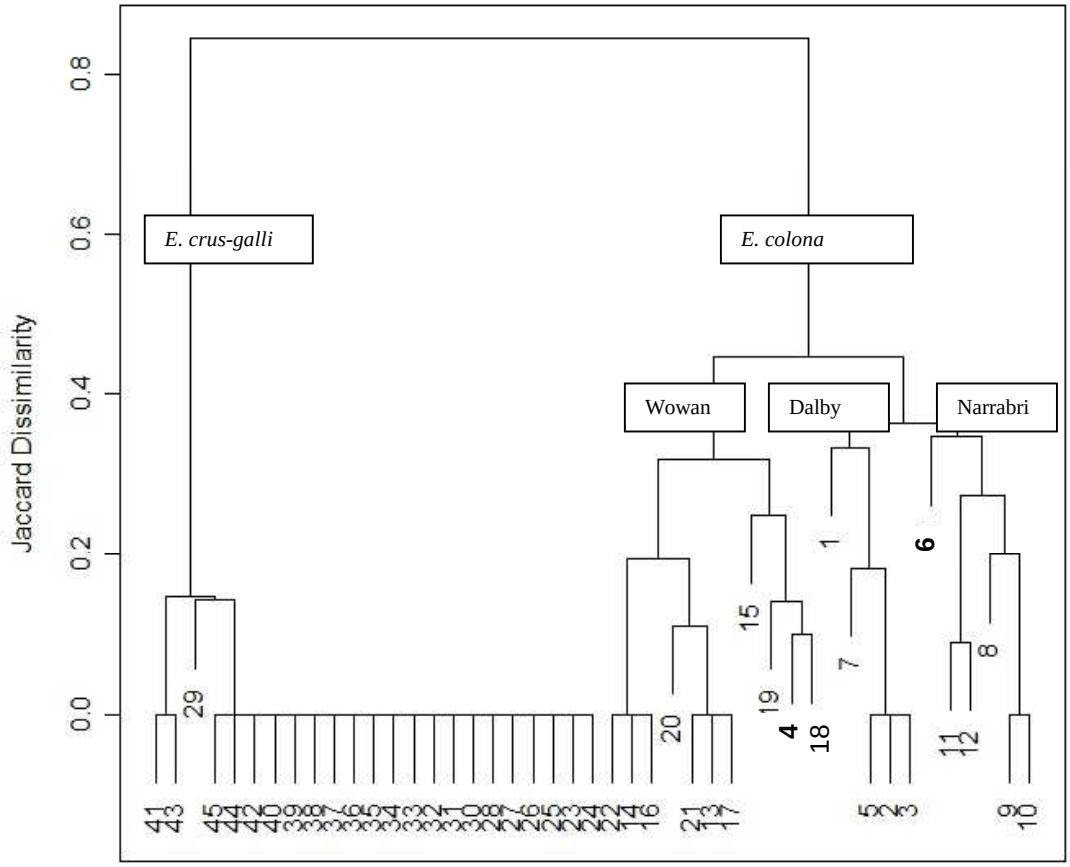


Figure 4.9: Unweighted pair group method (UPGMA) dendrogram using Jaccard dissimilarity among 22 *E. colona* (Dalby 1-7, Narrabri 8-12, Wowan 13-22) and 23 *E. crus-galli* populations (Dalby 23-30, Narrabri 31-45) based on presence/absence of peaks/clusters generated from the five SSRs EC1 to EC5.

Only three of the *E. crus-galli* populations showed any dissimilarity and separated into two distinct smaller clusters. The first contained two Narrabri populations (populations 41 and 43) that expressed the 98 bp band, and the second a Dalby population (population 29) that expressed the 97 bp band from the EC5 marker.

Further subdivisions within *E. colona* related largely to collection sites with more polymorphism evident in *E. colona* than in *E. crus-galli*. The majority of the *E. colona* populations clustered according to the regional centre from which the seeds had been originally collected, with Dalby and Narrabri having more similarities than those from Wowan. The Dalby cluster contained only Dalby populations while the Wowan and Narrabri clusters contained one Dalby population each (populations 4 and 6 respectively).

The Dalby population that appeared in the cluster with the Narrabri samples had no peaks in the 107-112 bp range (present only in Dalby populations) nor in the 200-214 bp range. Additional peaks present at 156-159 bp made it similar to the Narrabri populations which had peaks in the ranges 154-157 bp and 158-161 bp.

The Dalby population included in the Wowan cluster had no peaks in the 107-112 bp range, but did have a peak at 204 bp which was also present in three Wowan populations (populations 15, 18 and 19). These were the only four populations expressing the 204 bp peak from the EC4 marker.

All the populations in the Dalby cluster shared common peaks at 178 bp and 214 bp. Within this cluster, populations 2, 3 and 5 were similar and were the only populations to have peaks from 108-112 bp. These populations were grown from seed sourced from two different farms; all were from different fields and at the time of collection were growing corn, mungbean and sorghum respectively.

4.4.2.2 Regional study of Echinochloa species from across the northern grain region

Markers in the regional survey

In addition to 45 of the populations from the morphological study, a further 206 plants (202 *E. colona*, 4 *E. crus-galli*) were included in a broader experiment to compare the genetic diversity across the northern grain region (NGR). In total, 182 plants produced PCR products for all five SSRs and were included in the analyses.

The 33 peaks/complexes identified in the morphological study were also represented in this study with markers EC1, EC2 and EC5 failing to provide any additional information. Marker 3 (EC3) revealed three additional complexes in some of the Narrabri *E. colona* at 111-114 bp, 113-116 bp and 160-163 bp, while EC4 showed an additional small peak in some Wowan *E. colona* at 170 bp. No additional peaks/complexes were identified in the *E. crus-galli* plants.

Clustering

Dendrograms were produced using the unweighted pair group method of arithmetic means (UPGMA) based on the presence/absence of scoreable peaks. The resulting dendrogram showed a clear differentiation between *E. crus-galli* and *E. colona* with the plants splitting into two main clusters corresponding to the two species. Polymorphism was once again more evident in *E. colona* than in *E. crus-galli* (Figure 4.10).

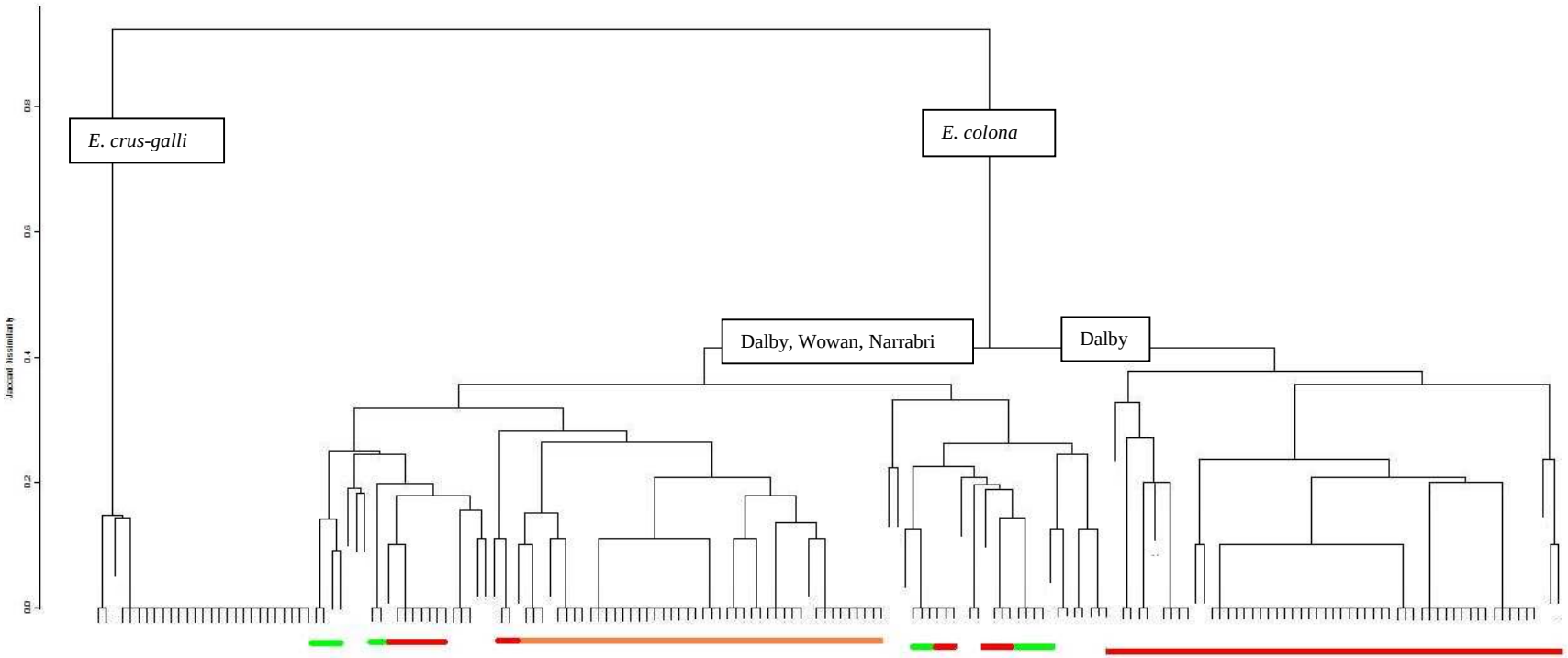


Figure 4.10: Unweighted pair group method (UPGMA) dendrogram using Jaccard dissimilarity among 27 *E. crus-galli* plants (9 Dalby, 18 Narrabri) and 155 *E. colona* plants (25 Narrabri, 50 Wowan, 80 Dalby) showing regional genetic diversity. Some regional centre clusters have been highlighted (Dalby – red, Narrabri – green, Wowan – brown).

The *E. colona* cluster subdivided into two main clusters, one containing only Dalby plants while the second contained plants from the three regional centres. Within this second sub-cluster, there were three smaller clusters. Two contained mainly Dalby and Narrabri plants while the third cluster contained mainly Wowan plants and three from Dalby.

There were only three *E. crus-galli* plants to show any dissimilarity and they appeared as distinct clusters, separating into two Narrabri plants (98 bp) and one Dalby plant (97 bp) the same as in Figure 4.9.

4.4.3 Experiment three – heterozygosity testing

Aim: To determine the extent of heterozygosity in progeny grown from seed collected from individuals of *E. colona* and *E. crus-galli* grown in the morphological trials, as an indication of the degree of outcrossing across the region.

The molecular information for two *E. crus-galli* (Dalby, Narrabri) and three *E. colona* (Dalby, Narrabri, Wowan) plants grown in the morphology trials was compared with that of progeny grown from seeds collected at plant maturity. Marker 3 (EC3) was run on plants of both species to confirm identity and detect intra-specific variation in *E. colona*, while marker 4 (EC4) was used to detect variation in *E. colona* and marker 5 (EC5) was run to detect variation in *E. crus-galli*.

4.4.3.1 Echinochloa colona

Markers EC3 and EC4 both amplified products for 87 *E. colona* plants from Narrabri. While seven peaks were observed in the parent plant, an additional three peaks/complexes were observed in some of the progeny. Forty-four Dalby plants produced products for both markers while 68 plants were successfully amplified for Wowan. An additional doublet at 133/134 bp was present in approximately half of the Dalby progeny, with no additional variation evident in the Wowan progeny (Table 4.7).

Table 4.7: The base pair peaks/complexes scored for three *E. colona* plants included in the morphological study and progeny grown from seed collected from each plant using markers EC3 and EC4, listed according to regional centre (✓ present in parent, × absent in parent).

Regional Centre		EC3 base pairs (bp)								EC4 base pairs (bp)				Peaks complexes scored
		82/83	85/86	109-112	115-118	133/134	142-145	146-150	158-161	178	180	202	212	
Dalby	Parent	✓	✓	✓		×					✓	✓	✓	6
	Progeny	100%	100%	100%		46%					100%	90%	90%	7
Wowan	Parent	✓	✓			✓		✓			✓		✓	6
	Progeny	100%	100%			18%		100%			100%		100%	6
Narrabri	Parent	✓	✓		×	×	×		✓	✓	✓	✓	✓	7
	Progeny	100%	100%		1%	82%	1%		89%	100%	100%	100%	100%	10

The doublet at 133/134 bp was not included in previous studies due to inconsistencies in the intensity of the products but in the three parent plants from this study it was clearly present or absent, so this doublet was scored in the progeny.

4.4.3.2 Echinochloa crus-galli

Markers EC3 and EC5 both amplified products for 83 *E. crus-galli* plants from Narrabri and 23 Dalby plants. While the Narrabri parent plant had a peak at 98 bp, this feature was only present in 41% of the progeny. No additional variation was identified from the progeny of the Dalby plants (Table 4.8).

Table 4.8: The base pair peaks/complexes scored for two *E. crus-galli* plants included in the morphological study and progeny grown from seed collected from each plant using markers EC3 and EC5, listed according to regional centre (✓ present in parent, × absent in parent).

Regional Centre		EC3 base pairs (bp)		EC5 base pairs (bp)			Peaks complexes scored
		78/79	83-86	92	97	98	
Dalby	Parent	✓	✓	✓	×		3
	Progeny	100%	100%	100%	×		3
Narrabri	Parent	✓	✓	✓		✓	4
	Progeny	100%	100%	100%		41%	4

4.5 Discussion

This study established that some of the five SSR markers developed by Danquah *et al.* (2002a) could be used to distinguish between species using both polyacrylamide gel and capillary electrophoresis analysis. Additionally the markers provided an indication of genetic variability existing between and within populations of *E. colona* and *E. crus-galli* from across the northern grain region (NGR) of Australia.

Both product visualisation methods proved useful in differentiating between *E. colona* and *E. crus-galli* and indicated intra-specific variation within the species, although with different levels of precision. This compares with Danquah *et al.* (2002a) where all five microsatellites showed inter- and/or intra-specific polymorphism. Unfortunately the DNA used in the polyacrylamide gel was not the same as that used in the CEQ analysis and therefore direct comparisons cannot be made between the two results. On the polyacrylamide gel all five primers were able to differentiate between the two species with three (EC2, EC3, EC5) detecting possible intra-specific variation. By comparison, when a similar set of plants was visualised using the CEQ, only three of the primers (EC1, EC3, EC5) were able to clearly distinguish between the species, with a fourth (EC2) producing unreliable results in this sample set. Intra-specific variation was observed from three markers (EC3, EC4, EC5) using the CEQ.

The CEQ results for marker 1 (EC1) reflected those of the polyacrylamide gel but did not reproduce the higher band visible at 150 bp in *E. crus-galli* shown in the latter. Using marker 2 (EC2), the polyacrylamide gel showed a minor band at 170 bp in *E. crus-galli* that was not shown by *E. colona*, and a possible intra-specific polymorphism for a band at 185 bp. These minor bands were not consistently found when the CEQ was used to visualise PCR products. This marker did not clearly reflect the gel and showed no clear intra-specific variation. The CEQ output from marker 3 (EC3) was close to reflecting the polyacrylamide gel but missed the higher alleles in both species. The CEQ results reflected the polyacrylamide gel for marker 4 (EC4), while the CEQ results for marker 5 (EC5) matched the polyacrylamide gel with a band missing in *E. colona*.

In Danquah *et al.* (2002a), amplification products were resolved on 6% polyacrylamide denaturing gels and visualised using autoradiography. However, this study used an 8% non-denaturing gel as this was standard laboratory practice. Using denaturing gels for future studies may reduce the number of bands present. This may help identify alternate alleles at particular loci from bands produced from multiple binding sites within the genome for individual primer pairs. Since allelic relationships are not clear in this study, bands/peaks have been scored as single dominant markers.

There were similarities between the results obtained here and those of Danquah *et al*. (2002a). Their study examined the size range of alleles from 76 bp to 181 bp and found that *E. colona* had between one to six alleles per locus compared to *E. crus-galli* with between two to four alleles per locus. This study examined the size range of alleles from 55 bp to 220 bp on the polyacrylamide gel and found between one to seven band sizes per primer pair for *E. colona* and between two to six for *E. crus-galli*. A different range of peaks was examined on the CEQ (78 bp to 216 bp) and two to ten band sizes per primer pair for *E. colona* were observed and one to six for *E. crus-galli*.

Delmotte *et al.* (2001) compared data collected with different automated sequencers and a manual technique (fragment separation in a silver-stained polyacrylamide gel) and found strong discrepancies in allele size of microsatellite loci. The differences between the manual and automated sizing were found to be inversely related to locus size. Therefore it is possible to apply both methods used in this study to successfully reproduce products using the five SSRs (EC1-EC5) and provide insight into the genetic diversity of *E. colona* and *E. crus-galli* populations in Australia. However it is important that any study using microsatellites clearly states the materials and methods used for allele sizing so comparisons of results can be undertaken.

Assessment of the five SSRs established polymorphism was present in *E. colona* plants from the DPI & F farm at Kingsthorpe (south eastern Queensland) and to a lesser extent *E. crus-galli* from Narrabri (northern New South Wales). A larger population study, conducted using populations from the three regional centres of the NGR, revealed a similar set of band sizes in both species. Markers EC1 to EC4 did

not identify any additional peaks in *E. crus-galli*, while an additional peak was observed in one Dalby population using EC5.

Markers EC1, EC2 and EC5 did not identify any additional band sizes in *E. colona*. Additional complexes were found in some of the *E. colona* populations in the range 107 bp to 161 bp (EC3) and appeared to relate to the regional centre from which the seed had been originally collected. Similarly, additional peaks were observed using marker EC4 at 170 bp in some Wowan populations and also in the range 200 bp to 216 bp.

The microsatellites revealed a high level of polymorphism in *E. colona* compared with *E. crus-galli* at all spatial scales studied, including between individuals within a field, between individuals from different fields, and between different farms and regional centres. This is in contrast to Danquah *et al.* (2002b) who assessed the genetic diversity in *E. crus-galli*, *E. colona* and *E. crus-pavonis* from six different geographic origins ranging from Bangladesh to Costa Rica. The *E. colona* samples clustered as a distinct group but the SSR markers were unable to distinguish between the four *E. colona* samples (two each from Costa Rica and Colombia), while polymorphisms were detected using AFLPs. The number of *E. colona* samples in the study was too limited to discount the possibility of detecting polymorphisms using SSRs. Previous studies using other molecular methods have not detected genetic variation in *E. colona* (Danquah *et al.* 2002b) although studies have revealed variation in *E. crus-galli.*

The molecular clustering of *E. colona* populations from the morphological study related largely to the three regional centres, with Dalby and Narrabri populations being genetically similar. This could be due to these regional centres being more climatically similar, thus favouring certain genetic ecotypes. Similarities could be natural, like climate or soil type, or cultural, such as cropping systems. Alternately, it could be due to seed dispersion from one regional centre to another due to practices such as common farm traffic, seed or fodder exchange. However, the sample size is too small to draw any conclusions about genetic ecotypes adapted to cropping or other microenvironments.

When a larger population sample was studied in experiment two the majority of the *E. colona* plants from Wowan still clustered together as did the majority of the Dalby plants. While a combination of the factors discussed above may account for the similarities, it is possible that the marker sets associated with Dalby and Wowan were only genetically linked to genes associated with a selective advantage in that environment. There is no evidence of sub-clustering related to microenvironments, such as if plants were collected from crops or grazing sites. A level of heterozygosity is suggested from the results of experiment three, where banding patterns in progeny were not always identical with parents. Thus a certain degree of random allelic segregation is likely to occur in such populations.

Finally, definitive conclusions could not be drawn regarding the extent of heterozygosity existing in both species due to the small sample size studied. In addition the multiple bands made identification of alternate alleles and hence homozygotes and heterozygotes difficult. However, the differences in banding patterns observed between parents and progeny suggest that a degree of heterozygosity exists in populations of both *Echinochloa* species in the NGR. This is consistent with the report that *E. crus-galli* had a high degree of autogamy but the rate of cross-pollination was sufficient to assure gene exchange amongst populations (Maun and Barrett 1986). This level of outcrossing suggests that populations of weeds could readily adapt to management strategies aimed at dominant ecotypes.

4.6 Conclusion

The research undertaken in this study has confirmed that the SSRs developed by Danquah *et al.* (2002a) are able to discriminate between Australian accessions of *E. crus-galli* and *E. colona* and can determine the genetic variability existing between and within populations across the northern grain region (NGR). If it is determined that weed management practices for *E. colona* and *E. crus-galli* differ, it is important for growers and agronomists to be able to accurately identify the species. If positive identification cannot be made using morphological traits, genetic testing could be used.

A higher level of polymorphism was found to exist in *E. colona* compared with *E. crus-galli* at all spatial scales studied. The molecular clustering of *E. colona* populations could be loosely related to the regional centres from which the seeds were originally collected. However, there is little evidence to support the idea that genetic ecotypes were related to microenvironments, such as if weeds were collected from fallow or cropping situations. The establishment of genetic variation within species suggests that there is the potential for variation in susceptibility to herbicides. The association of genetics with the geographic location of collection site suggests that if genetic diversity is linked to herbicide susceptibility then weed management strategies may need to be region-specific.

Finally, definitive conclusions could not be drawn regarding the extent of heterozygosity existing in both species. A larger sample size may have provided more information although the presence of multiple bands could still make identification of homozygotes and heterozygotes difficult.

Chapter 5 Herbicide Susceptibility

5.1 Introduction

The use of herbicides as substitutes for mechanical tillage has increased dramatically over the last three decades. As tillage is reduced, there is decreased soil disturbance, increased crop residue and increased reliance on herbicides for weed control (Chauhan *et al.* 2006). One consequence of this increased reliance has been the evolution of herbicide resistance as a factor limiting the use of certain herbicides.

In any agricultural environment, a weedy species can be represented by a heterogeneous population of individuals showing differences in their morphology and other biological features such as development rate, seed production and dormancy (Grignac 1978). Some weeds show a natural variability in sensitivity to herbicides, referred to as herbicide susceptibility, whereas a reduced susceptibility which may sometimes result from herbicide selection indicates herbicide tolerance (Rao 2000). The term tolerance is frequently used not only to refer to variations in the ability to withstand a herbicide application between different species, but also when there is variability within a population of the same species (De Prado and Franco 2004).

In response to repeated treatments with a particular class or family of herbicides, weed populations change in genetic composition such that the frequency of resistance alleles and resistant individuals increases (Jasieniuk *et al.* 1996). In this way, weed populations become adapted to the intense selection imposed by herbicides. In many species the evolution of herbicide-resistant biotypes appears to be widespread when treatments with the same herbicide are repeated for several years, and when particular resistance genes exist in the treated population (Grignac 1978). Some individual plants have the capability to survive a herbicide application to which the remainder of the same species were susceptible. This response is known as resistance (De Prado and Franco 2004).

Herbicide resistance in weeds is an evolutionary process (Jasieniuk *et al.* 1996). It evolves as the resistant plants increase as a proportion of the population due to selection pressure by the herbicide. Genetic variation for resistance must be present in a susceptible weed population for the evolution of herbicide resistance to occur

(Jasieniuk *et al.* 1996). The major source of genetic variation in an area where resistance has not been detected previously is likely to be gene mutation (Jasieniuk *et al.* 1996). In general, gene mutations conferring resistance to a specific herbicide class are not induced by application of the herbicide, but rather are believed to occur spontaneously (Jasieniuk *et al.* 1996).

Since the discovery of resistance to the triazine herbicides in common groundsel (*Senecio vulgaris*) in 1968, the incidence of herbicide resistance has increased dramatically. Heap (1997) reported the occurrence of 183 herbicide-resistant weed biotypes (124 different species) in 42 countries, a figure that had increased to 347 resistant biotypes (195 species) by 2010 (Heap 2010). Herbicide resistance has developed rapidly in *Echinochloa* species, particularly *E. crus-galli* and *E. colona* and also is present in *E. crus-pavonis* (Brazil), *E. erecta* (Italy), and *E. oryzicola* and *E. phyllopogon* (California) (Heap 2010). The development of resistance, combined with the confusion in the literature and amongst agronomists concerning the identity and ecological characteristics of taxa within this genus (Barrett and Seaman 1980), has raised concerns over achieving effective weed control (Rutledge *et al.* 2000).

As early as 2002, barnyard grass (*Echinochloa* spp.) was identified as having a high risk of developing resistance to glyphosate in cropping systems of the northern grain region (NGR) (Walker *et al.* 2002). The high frequency of glyphosate use and limited rotation with differing modes of action (MOA) herbicides or other weed control options were identified as contributing factors to the potential development of resistance (Walker *et al.* 2004). In both summer and winter fallows in the NGR, growers indicated a very high reliance on glyphosate, with glyphosate and glyphosate mixes accounting for 92 to 94 percent of the herbicide treatments applied to weeds (Walker *et al.* 2005). In a survey undertaken by Osten *et al.* (2007), barnyard grass (*Echinochloa* spp.) was identified as one of the major weeds in cropping situations across the NGR with instances of inconsistent control being reported by Walker *et al*. (2005). Similarly, Wicks *et al.* (2000) indicated that *E. colona* was among several species in summer fallows of northern New South Wales to display a tolerance to the recommended field rates of glyphosate resulting in inadequate weed control.

To date Australia has 31 weed biotypes that have been confirmed with herbicide resistance including 14 grasses (Heap 2010). Of the grasses, only *Echinochloa colona* (awnless barnyard grass), *Lolium rigidum* (rigid ryegrass) and *Urochloa panicoides* (liverseed grass) have been confirmed as being resistant to glyphosate (Heap 2010). At the commencement of this research in 2005, there were no confirmed glyphosate-resistant populations of *Echinochloa* in Australia. The first documented case of glyphosate-resistant *E. colona* was in 2007, and currently, there are three documented glyphosate-resistant populations of *E. colona* in the NGR (Preston 2009).

Given the inconsistency in control, a detailed knowledge of variability within and between populations and species of *Echinochloa* is necessary in directing appropriate management strategies. Barnyard grass populations are variable in morphology (Chapter 3) and display genetic variability (Chapter 4) that may cause differences in glyphosate efficacy. This chapter reports on a study comparing the response of *E. crus-galli* and *E. colona* populations collected from three major regional centres across the NGR to different rates of glyphosate.

5.2 Aim

The objective of the study reported in this chapter was to determine the responses of populations of *E. colona* and *E. crus-galli* collected from across the NGR to different concentrations of glyphosate. The response of 36 populations to glyphosate was assessed in pot trials and was designed to answer the following specific questions:

(i) Do the two species differ in susceptibility to glyphosate?

(ii) Do populations within a species differ in susceptibility to glyphosate?

(iii) Are any differences in response to glyphosate related to collection location or regional centre?

(iv) Do the differences in herbicide susceptibility have a genetic basis based on the SSRs used?

5.3 Materials and methods

5.3.1 Trial establishment and management

To investigate glyphosate efficacy, 18 populations each of *E. colona* and *E. crus-galli* were subjected to four rates of glyphosate. The populations represented each of the major regional centres across the NGR and were selected to duplicate plants grown in the morphological study (Table 5.1).

Table 5.1: *Echinochloa colona* and *E. crus-galli* populations established in the herbicide susceptibility trial indicating the population labels used in Chapter 5 and the original population IDs assigned to the plants sampled in the field. Refer to 3.3.1.1 for description of population ID.

E. colona		***E. crus-galli***	
Population label	Population ID	Population label	Population ID
DC1	D11: 20-1	DG1	D11: 11-8
DC2	D13: 16-1	DG2	D11: 20-4
DC3	D13: 4-2	DG3	D11: 3-5
DC4	D14: 12-4	NG1	N11: 11-2
DC5	D21: 4-1	NG2	N11: 15-4
DC6	D41: 10-4	NG3	N11: 8-4
DC7	D51: 6-2	NG4	N11: 9-1
NC1	N11: 1-1	NG5	N12: 11-5
NC2	N11: 13-3	NG6	N15: 1-4
NC3	N15: 12-3	NG7	N15: 11-5
WC1	W11: 1-3	NG8	N15: 14-1
WC2	W12: 9-3	NG9	N15: 14-3
WC3	W13: 2-1	NG10	N15: 14-4
WC4	W13: 5-2	NG11	N15: 2-1
WC5	W14: 6-3	NG12	N15: 20-3
WC6	W14: 1-1	NG13	N15: 4-4
WC7	W15: 8-1	NG14	N15: 7-4
WC8	W17: 10-1	NG15	N15: 9-5

The seeds had been stored in paper seed packets in a cold room at 10°C and removed six months prior to the establishment of the herbicide trial. During this period the seed packets were stored at room temperature indoors away from vermin.

The populations were grown in 17 cm pots under polyhouse conditions at the Leslie Research Centre, Toowoomba. The pots were filled with Brunnings potting mix, containing composts (including composted pine bark), potting mixes and other organic gardening materials, onto which a layer of black cracking-clay soil, approximately 8 cm deep was placed. The soil, sourced from the Department of Primary Industries and Fisheries (DPI&F) farm at Wellcamp (long 151.85074S, lat 27.55360E) had not been sterilized and toothpicks were used to mark the seedlings that had been planted. A measuring scoop was used to incorporate approximately 10 g of an all-purpose fertilizer (Brunnings Nitrophoska® - 16% N, 3% P, 12.5% K with trace elements) into the topsoil of each pot. Seeds were germinated in an incubator using the method described in section 3.3.4.4. Four seedlings, each with a leaf approximately 20 mm long and juvenile roots, were planted into each pot between the 18th and 20th January 2007 to establish 16 replicates of each population (Photograph 5.1).

Photograph 5.1: Set-up of pots in replicate groups in polyhouse prior to culling.

A shade cloth, drawn under the roof of the polyhouse during planting, remained *in situ* for three days to provide protection from high temperatures and allow the

seedlings to establish. Seedlings were watered twice daily for the first week and then once a day until the application of herbicide treatments. Seedlings were maintained under polyhouse conditions for approximately three weeks prior to herbicide application. Three days prior to the application of herbicide, the number of plants in each pot was culled to one, and wherever possible remaining plants were of uniform size.

5.3.2 Experimental design

The experiment was a randomised complete block, with four treatments of glyphosate each replicated four times. Herbicide treatments were nil herbicide (control), two sub-lethal doses (0.25 L/ha and 0.50 L/ha) and a lethal dose (1.0 L/ha) of RoundUp® CT (a.i. 450 g/L glyphosate present as the isopropylamine salt) based on the recommended rate of 0.8 to 1.6 L/ha. These were equivalent to glyphosate rates of 0, 112.5, 225 and 450 g a.i./ha, respectively. The herbicide rates were chosen to reflect a treatment within the recommended range and sub-lethal treatments in order to detect subtle differences in response to glyphosate.

5.3.3 Treatment imposition

Treatments were applied mid-morning on 7th February 2007 when the weather was fine, with scattered clouds and an occasional light breeze. The temperature ranged from 28 to 32°C during treatment application. At the time of spraying, the majority of plants had between 6 and 10 emerged leaves. Timing of the application was in keeping with the recommended growth stage of the plant on the label, and representative of the size of plants when farmers would be treating fallows.

Treatments of glyphosate (RoundUp® CT) were applied using a manually pushed spray-bike at a walking rate of 1 m/sec (Photograph 5.2).

Pest Genie (2006), an on-line product, was used to calculate a boom output of 109 L/ha based on a walking speed of 1 m/sec, and to calculate the correct amount of herbicide to add to the tanks for each treatment. The air-pressurised sprayer, set at a

pressure of 200 kPa, was equipped with flat fan nozzles (TT110-01) and delivered a spray volume of 109 L/ha. Replicates were sprayed from lowest to highest dose with the boom set at 50 cm above the height of the plants.

Photograph 5.2: Spray bike used to apply glyphosate treatments to replicates of *Echinochoa* species.

Following the application of herbicide, the pots were returned to the polyhouse and, along with the nil treatment pots, randomised within their replicates and not watered for 24 hours. The experiment was conducted over 14 days and during that period of time the plants were watered daily.

5.3.4 Measurements

5.3.4.1 Plant characteristics

Prior to glyphosate application, seedling height and the number of leaves on each plant were recorded. These measurements were recorded to account for size differences in the plants and used as covariates in subsequent analyses.

5.3.4.2 Visual damage

In order to assess and compare glyphosate efficacy, visual observations were recorded with plants rated as having a degree of damage on a scale of 0 (no injury) to 10 (complete desiccation) (Table 5.2).

Table 5.2: Ratings assigned to individual plants indicating the amount of damage obvious in a visual examination (Adapted from: Australian Weeds Committee 1979).

Rating	Description of damage to plants
0	No damage
1	Negligible
2	Slight
3	Moderate
4	Substantial
5	Majority of plant damaged
6	Substantial necrosis and distortion
7	Severe, much necrosis and distortion
8	Very severe, much necrosis and wilting
9	Mostly discoloured & distorted permanently or desiccated
10	Complete loss of plant

Observations were taken at 3, 6, 9 and 14 days after treatment (DAT). The experiment was terminated after 14 days, as this was sufficient time to record the effects of the herbicide application.

5.3.4.3 Plant biomass

At 14 DAT plants were hand harvested by cutting them off at the base and fresh weight biomass of individual plants measured. The plant matter consisted of both green matter and desiccated plant material. Dry weight biomass per plant was determined after drying the plants for 48 hours in a drying oven at 80°C.

5.3.5 Data analysis

A series of statistics was performed using Genstat (Genstat 2006) to assess the effects of population, species and region on the response to glyphosate at different rates. The dry weight biomass data were log transformed prior to analysis, except for the population cluster analysis, where the percentage biomass of the treatments was expressed as a percentage of the untreated controls. LSDs were calculated at the 5% significance level and used to compare means. Back-transformed data are presented in graphs and, where presented in tables, both the transformed and back-transformed means are included.

5.3.5.1 Effect of population

To investigate the effect of population on the response to glyphosate at different rates, an ANOVA without covariates was initially performed in addition to an ANOVA with covariates of plant height and leaf number at time of spraying. The second analysis was performed to account for difference in plant size and any effect this may have had on the response to glyphosate. To explore further the differences that were found between populations, a third analysis was performed to determine potential groupings (clusters) of populations that responded similarly to glyphosate. For this analysis hierarchical clustering (using average link) based on a similarity matrix (Euclidean distance) was used. A dendrogram was produced with groupings assigned by setting a threshold of 0.97 (Genstat 2006). A one-way ANOVA was also used to compare means across cluster groups, but within treatments.

5.3.5.2 Effect of species

To investigate the effect of species on the response to glyphosate at different rates, an ANOVA without covariates was performed. Secondly, an ANOVA with covariates of plant height and leaf number at time of spraying was performed. It was found that the inclusion of the covariates removed the significant differences between species. Since species differ in their morphology (see Chapter 3), it was considered inappropriate to

include these measurements as covariates for species analysis. Therefore, the ANOVA including covariates was not considered in the results section.

5.3.5.3 Effect of region

To investigate the effect of region on the response to glyphosate at different rates, an ANOVA with covariates (plant height and leaf number) was performed. The inclusion of the covariates removed the significance of the species and allowed the effect of the region to be considered.

5.4 Results

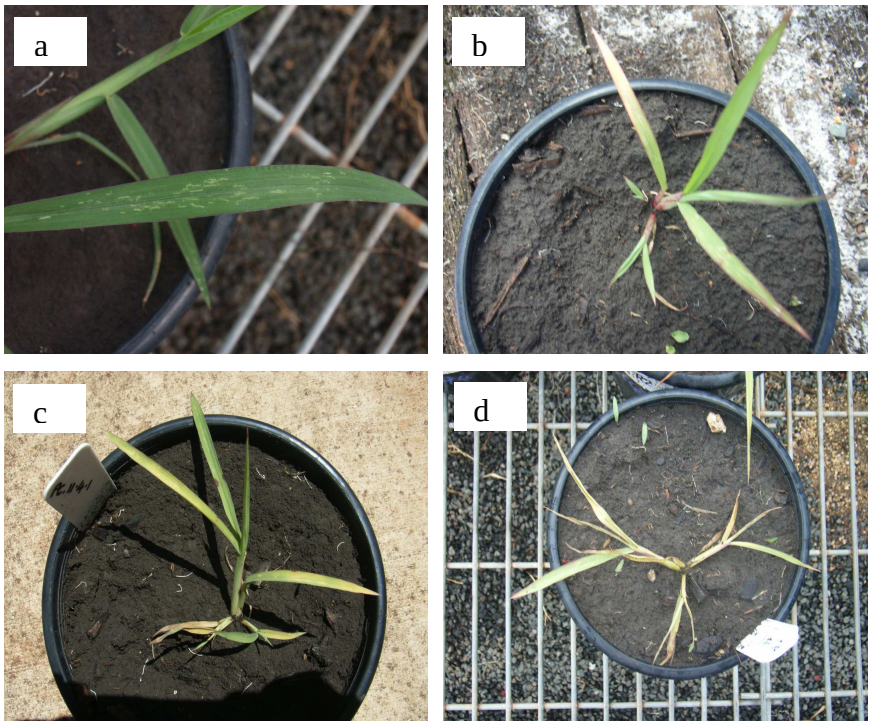
At the time of spraying, the two species were readily distinguishable as *E. crus-galli* plants were generally taller than those of *E. colona*. *Echinochloa crus-galli* had an average height of 19.2 cm and *E. colona* averaged 11.5 cm. The *E. colona* plants had predominantly semi-erect (48%) or erect (36%) growth forms, while *E. crus-galli* were chiefly erect (78%).

Within 48 hours of spraying, some of the plants had begun to show leaf discoloration. On closer examination, the majority of plants showing discoloration were *E. crus-galli* in the 1.0 L/ha. By three days after herbicide application (3 DAT) plants had visual damage ranging from slight discoloration to severe wilting in both species. Visual damage was more obvious in *E. crus-galli* across the three treatments, with all the plants in the 1.0 L/ha treatment displaying some damage (Table 5.3).

Table 5.3: Percentage of the replicates within each treatment displaying visual damage three and fourteen days (DAT) after the herbicide treatments (0.25 L/ha, 0.50 L/ha and 1.0 L/ha).

Herbicide rate	**Percentage of treated plants displaying visual damage**			
	3DAT		**14DAT**	
	E. crus-galli	*E. colona*	*E. crus-galli*	*E. colona*
0.25 L/ha	81	51	90	82
0.5 L/ha	92	75	97	99
1.0 L/ha	100	90	100	100

The damage to *E. crus-galli* ranged from negligible to moderate in the sub-lethal treatments (0.25 L/ha and 0.5 L/ha) while 90% of the plants in the lethal treatment (1.0 L/ha) displayed slight to substantial damage and 5% were severely damaged. By comparison, the visual damage to the majority of *E. colona* plants was negligible to slight across all three treatments (Photograph 5.3).



Photograph 5.3: Examples of *E. colona* showing the visual damage considered to be (a) negligible, (b) slight, (c) moderate, and (d) substantial to severe at 14 DAT.

Echinochloa crus-galli showed signs of damage more quickly, but died off at a slower rate. By 14 DAT the visual damage was similar in both species (Table 5.3).

All plants in the non-treated controls increased in size from the time of herbicide application to the termination of the experiment at 14 DAT, suggesting that management and environmental conditions were conducive for *Echinochloa* growth throughout the experiment. At 14 DAT the non-treated *E. crus-galli* had an average height of 33.7 cm while the *E.* colona plants averaged 21.6 cm. By 14 DAT, seven of the *E. crus-galli* plants and four *E. colona* plants in the non-treated controls had set

seed while two *E. crus-galli* and three *E. colona* plants had set seed in the 0.25 L/ha treatment and one of each species in the 0.5 L/ha treatment.

5.4.1 Effect of population

When average visual ratings across populations were compared graphically, a similar trend of increasing damage with increasing herbicide rates was observed. As the majority of the populations were given the same visual rating, graphing did not readily distinguish between populations.

At 14 DAT the visual damage rating of *E. colona* plants ranged from substantial to severe (4 to 7) at 0.25 L/ha, very severe to plant death (8 to 10) at 0.5 L/ha and complete loss of plant (10) at 1.0 L/ha. For *E. crus-galli*, the visual damage rating was moderate to substantial (3 to 4) at 0.25 L/ha, substantial to complete loss of plant (4 to 10) at 0.5L/ha, with the complete loss of all plants, except one, at 1.0 L/ha.

The average dry weight biomass data have been presented for the two species separately as *E. crus-galli* had bigger plants at the time of spraying and the species differed in their initial responses to treatment.

For the *E. crus-galli* populations there was a general trend of decreasing biomass as the rate of herbicide increased (Figure 5.1).

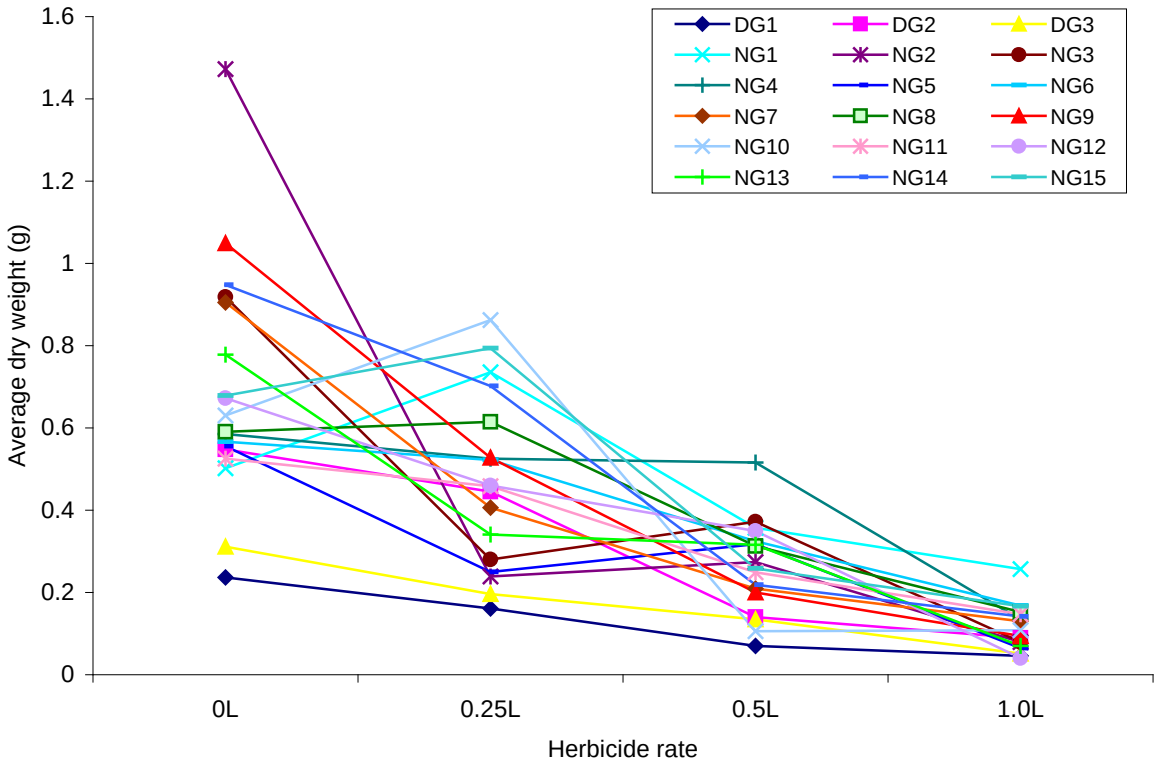


Figure 5.1: Average dry weight biomass of 18 *E. crus-galli* populations at each of the four glyphosate treatments at 14 DAT showing the response of the populations to the different herbicide rates.

There were some exceptions to this where the biomass was greater when compared with the previous lower rate of herbicide application. For example, at 0.25 L (L/ha), there were three populations (NG1, NG10, NG15) that had a greater biomass than the untreated controls. Another noticeable difference in how populations responded to the treatments was the greater biomass of Narrabri population NG1 at the 1.0 L (L/ha) rate. This population had a biomass six times that of the lowest biomass at that rate (Figure 5.1).

The biomass of populations also differed greatly for untreated plants. In particular, population NG2 from Narrabri had a much greater biomass than the other populations. Two Dalby populations (DG1, DG3) were identified as being notably lower in biomass across all treatments, while the third (DG2) also had a biomass that was toward the lower end of populations tested (Figure 5.1).

Similar to *E. crus-galli*, there was also a trend of decreasing biomass as herbicide rates increased for *E. colona* (Figure 5.2).

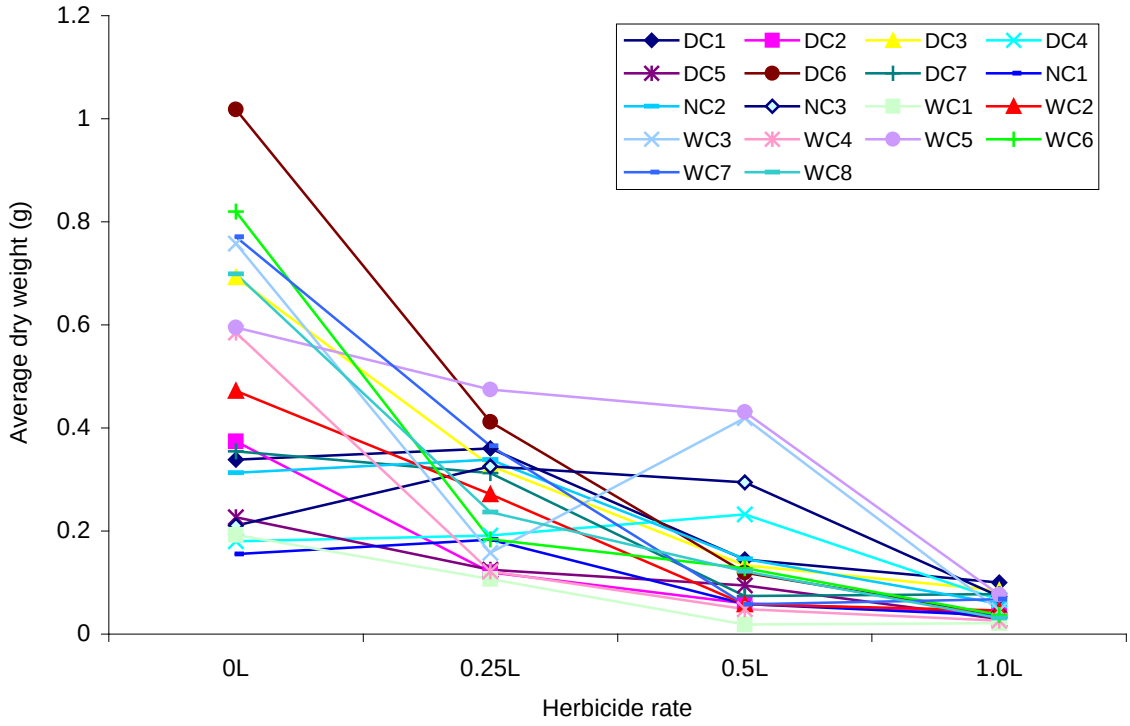


Figure 5.2: Average dry weight biomass of 18 *E. colona* populations at each of the four glyphosate treatments at 14 DAT showing the response of the populations to the different herbicide rates.

Again, exceptions were noted. Notably, two populations from Wowan (WC3, WC5) responded differently. Population WC5 had a higher biomass than other populations at both the 0.25 L and 0.5 L treatments, while population WC3 had a greater biomass at 0.5 L than at 0.25 L. All populations had a similar biomass at the 1.0 L rate showing that there appeared to be no population differences at this rate (Figure 5.2).

A large variability in biomass was also noted in the untreated *E. colona* populations. The three Narrabri populations were all at the lower end of the scale in terms of biomass weight. The majority of the Wowan populations had a greater biomass when untreated and there was no clear trend in the biomass of populations from Dalby.

To account for differences in plant size at spraying, the covariates of plant height and leaf number were included in separate analyses. The inclusion of the covariates removed the previously seen effect that biomass was sometimes greater at higher herbicide treatments, and explains that plant size differences were impacting on biomass results.

The first ANOVA, without covariates, showed there was a significant interaction between population and glyphosate rate at $p = 0.012$. This indicated that the populations were responding differently to the herbicide rates but did not identify which populations.

The second ANOVA, adjusted for the covariates, showed that population and glyphosate rate interaction was still significant at $p = 0.017$. The covariates, leaf number ($p < 0.001$) and plant height ($p < 0.001$), both had highly significant effects on how the populations responded to glyphosate rates.

The back-transformed means of the dry weight biomass have been graphed in order to compare the responses of the two species, and to clearly show the response of individual populations at each herbicide rate (Fig 5.3) that was not clearly evident in figures 5.1 and 5.2.

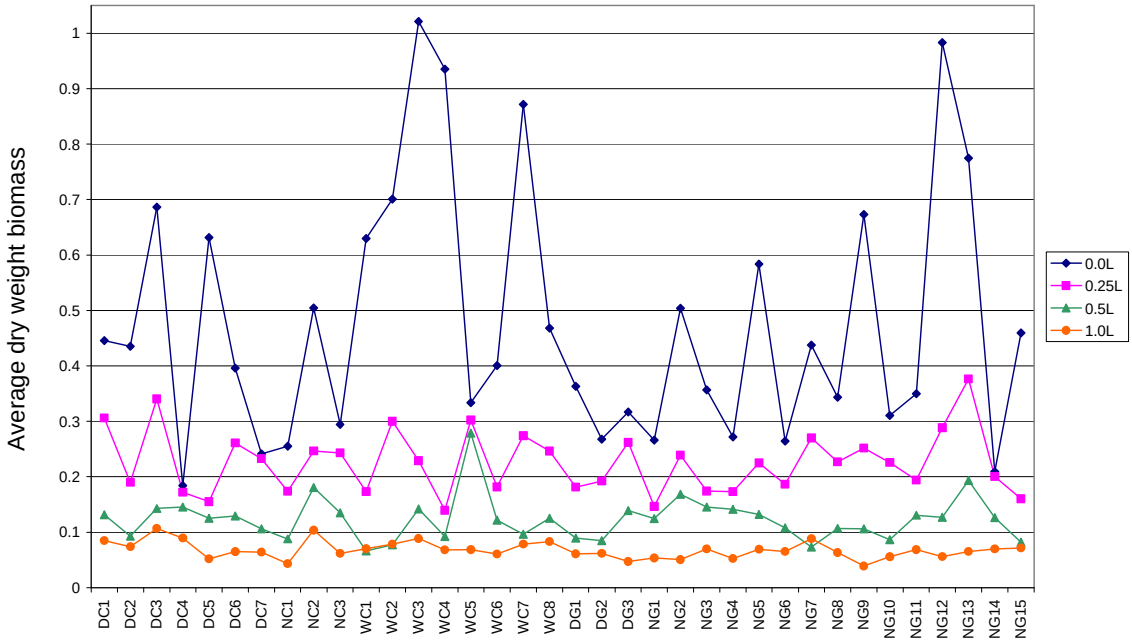


Figure 5.3: The average dry weight biomass of 18 populations each of *E. colona* and *E. crus-galli* at four rates of glyphosate (0 L/ha, 0.25 L/ha, 0.5 L/ha, 1.0 L/ha) using the back-transformed means of ANOVA adjusted for covariates at 14 DAT (LSD on transformed data = 0.7084). (Refer Appendix 4 for the log-transformed data).

The reduction in the dry weight biomass as glyphosate rate increased is consistent across populations of both species, although the magnitudes of the reductions differ (Figure 5.3). For example, in the *E. colona* populations, the reduction in the biomass from the untreated rate to the 0.25 L/ha rate is minimal for DC7 (0.01) but substantial for WC3 (0.79). Similarly in *E. crus-galli* there was a substantial reduction from biomass of the untreated rate to 0.25 L/ha rate for NG12 (0.69) compared to NG14 (0.01).

The susceptibility of the populations to different herbicide rates was analysed to explore potential groupings of populations based on levels of susceptibility. Populations were grouped according to their similarity in herbicide response, based upon dry weight reduction when treated with herbicides in comparison with the dry weight of the untreated control. In total, 10 cluster groups were produced with four clusters (1, 2, 5 and 7) containing 29 of the 36 populations. The remaining populations clustered into six groups with cluster group 4 containing 2 populations and the remaining clusters containing one population each (Figure 5.4).

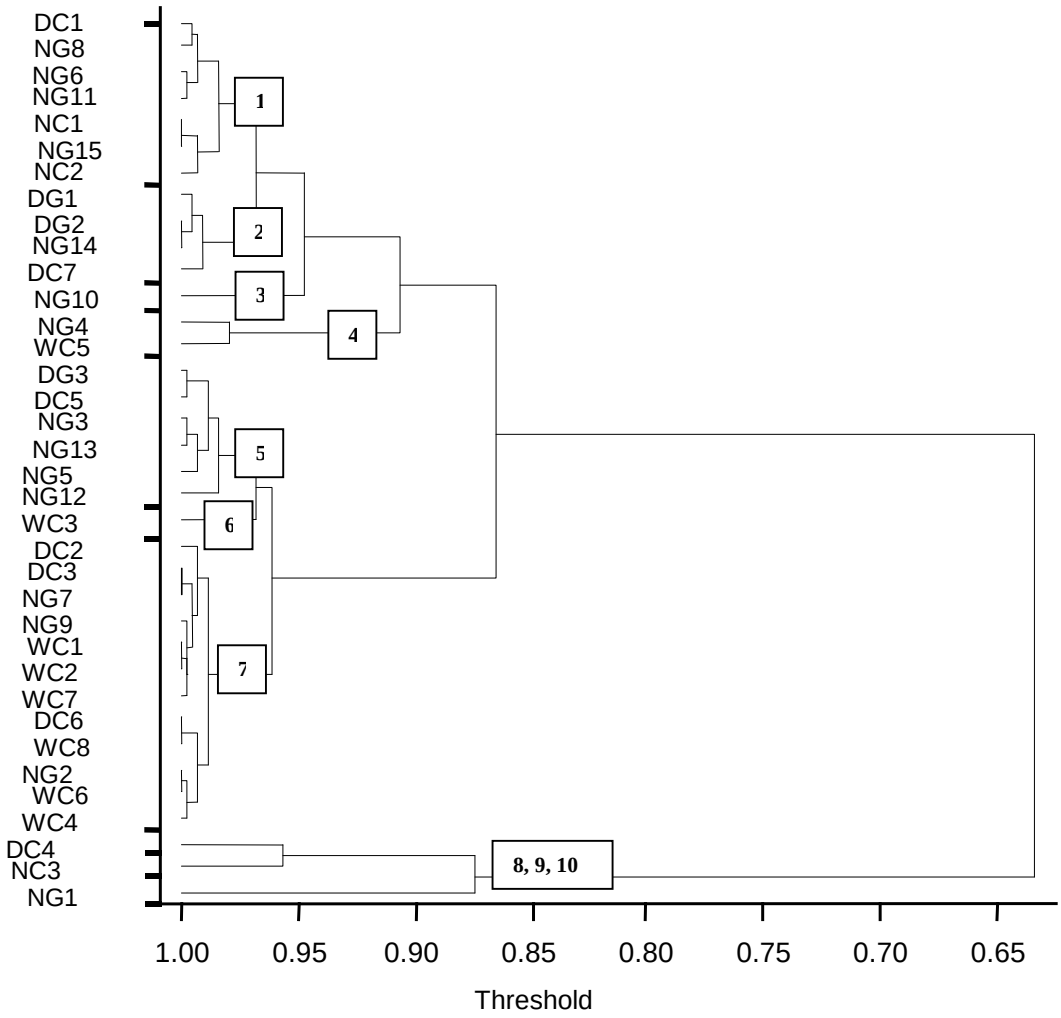


Figure 5.4: Heirarchical clustering of 18 populations each of *E. crus-galli* and *E. colona* from across the NGR. Solid lines on the y axis indicate the populations contained within each of the 10 clusters formed at a threshold of 0.97.

Clusters did not correspond to either species or regions with all clusters, except clusters 3 (*E. crus-galli*) and 6 (*E. colona*), containing both species.

The responses of the individual populations to each of the three herbicide treatments are presented according to the cluster grouping (Figure 5.5).

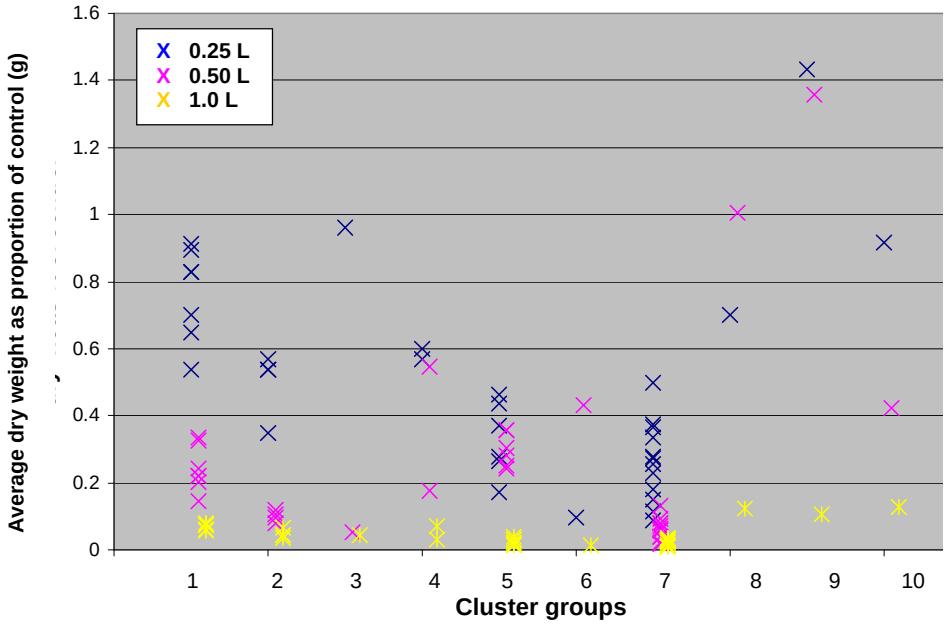


Figure 5.5: The response of the populations contained in each cluster to the three glyphosate treatments (0.25 L, 0.50L, 1.0 L)

Of the major cluster groups, cluster 7 contained twelve populations with high susceptibility to all three glyphosate treatments. Cluster group 1 contained seven populations with moderate susceptibly while cluster groups 2 and 5 showed low to moderate susceptibility, with cluster group 5 having a higher level of tolerance at the 0.5L/ha level than populations in cluster group 2.

Five clusters contained single populations. The population in cluster group 3 (NG10) had a low susceptibility to glyphosate at the 0.25L rate, but higher susceptibility at the 0.5L and 1.0L rates. Conversely the population in cluster group 6 (WC3) had a high susceptibility to glyphosate at the 0.25L and 1.0L rates, but a moderate susceptibility at the 0.5L rate. Similarly the population in cluster group 8 (DC4) also had a higher susceptibility to the 0.25 L rate.

Cluster groups 8, 9 and 10 contained populations that had the lowest susceptibility to the three herbicide treatments.

Figure 5.6 shows how each population within the 10 clusters responded to the lower herbicide treatments of 0.25 L and 0.5 L expressed as the proportion of the average dry weight biomass of the treatments against the dry weight biomass of the control.

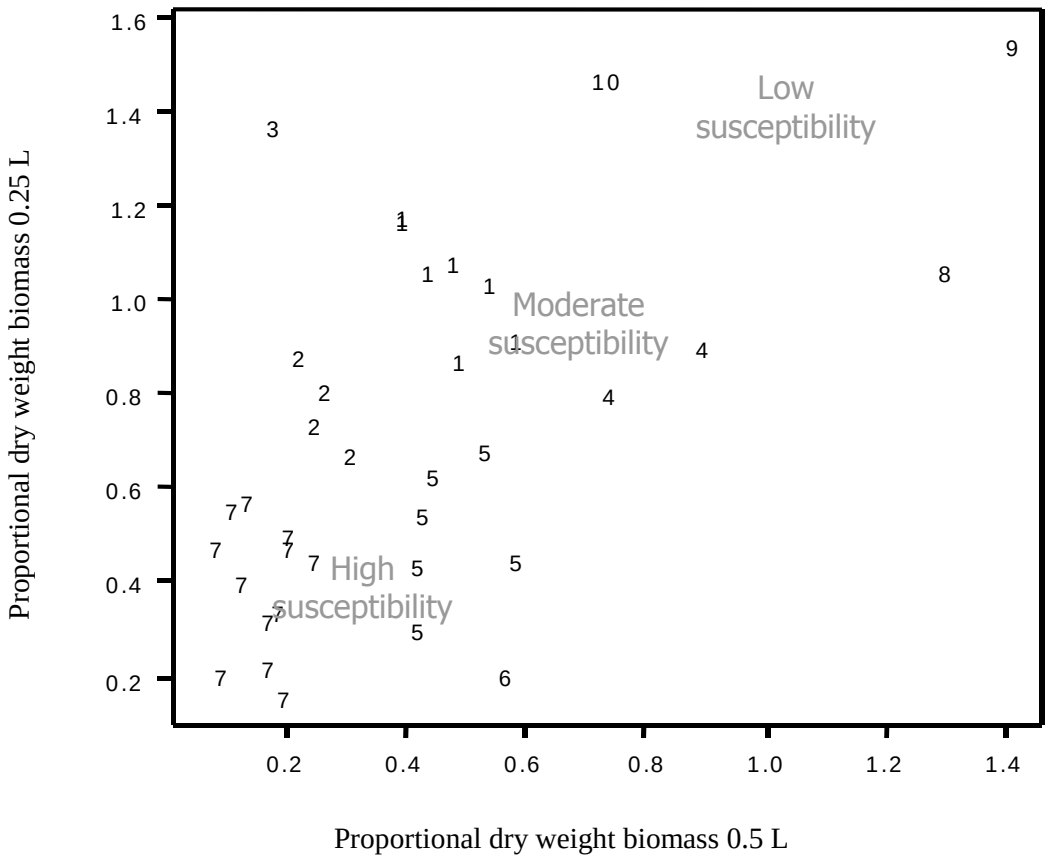


Figure 5.6: Average dry weight biomass for cluster groups for the combination of 0.25L and 0.5L herbicide rates expressed as a proportion of control, indicating levels of susceptibility of each of the populations within the clusters to herbicide treatments at 14 DAT.

By comparing the dry weight biomass of the populations at the two sub-lethal treatments (0.25 L and 0.50 L) it is possible to rate the susceptibility levels of the populations to glyphosate. Populations within clusters 8, 9 and 10 clearly have a lower susceptibility (greater tolerance) to glyphosate treatments at both the lower rates. Clusters 8 and 9 contained *E. colona* populations from Dalby and Narrabri respectively while cluster 10 contained an *E. crus-galli* population from Narrabri. The highly susceptible populations in cluster group 7 comprise nine *E. colona* populations from Dalby and Wowan and 3 *E. crus-galli* populations from Narrabri.

An analysis of variance (ANOVA) across cluster groups compared the biomass of the treatments against the untreated biomass and expressed as a proportion across the cluster groups all showed significant differences ($p < 0.001$). (Table 5.4).

Table 5.4: Comparison of the average biomass (g) of each of the ten cluster groups across each of the three glyphosate treatments (0.25 L, 0.50 L, 1.0 L). Means, within each treatment sharing the same characters are not significantly different ($p < 0.001$).

Cluster group	Average biomass 0.25L		Average biomass 0.5L		Averge biomass 1.0L	
1	1.0478	c	0.4610	d	0.2571	c
2	0.7785	d	0.2478	e	0.1814	d
3	1.3690	ab	0.1679	ef	0.1714	de
4	0.8474	cd	0.8032	b	0.1784	d
5	0.5096	e	0.4586	d	0.1077	ef
6	0.2084	f	0.5531	cd	0.0610	ef
7	0.3904	ef	0.1488	f	0.0823	f
8	1.0596	bcd	1.2885	a	0.3689	b
9	1.5451	a	1.3979	a	0.3492	b
10	1.4659	a	0.7115	bc	0.5122	a

5.4.2 Effect of species

At 14 DAT the average visual damage rating for the two species was similar, with increased damage at the higher rates of herbicide treatment (Figure 5.7).

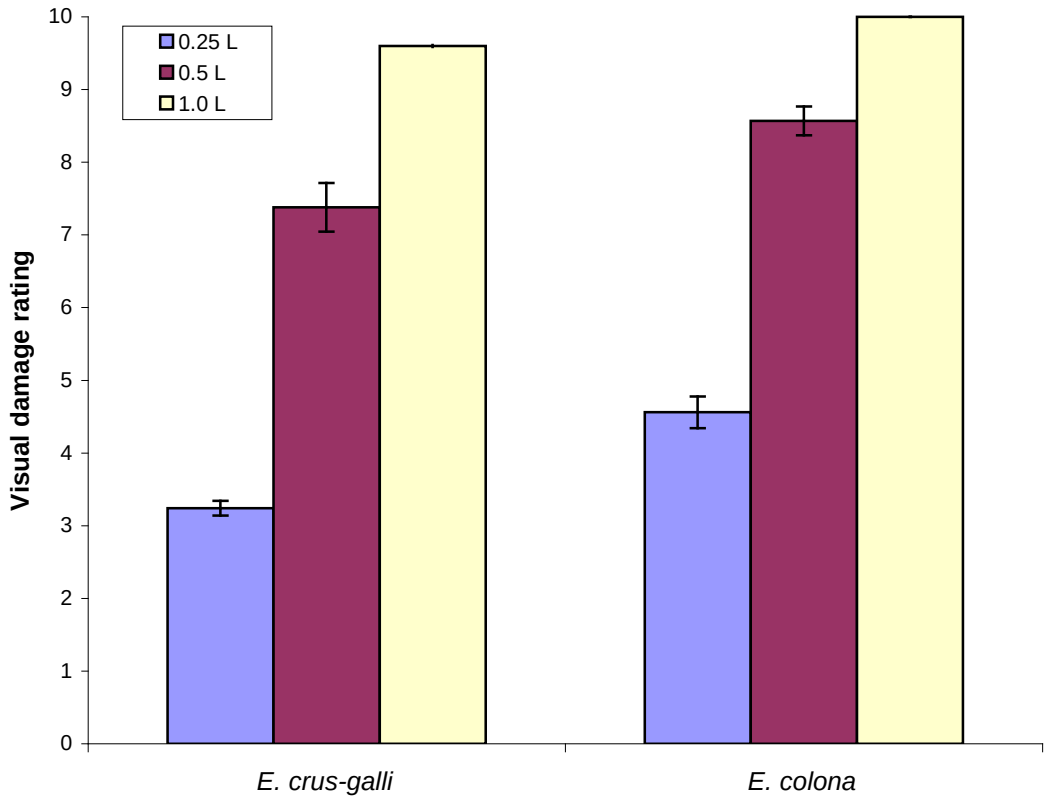


Figure 5.7: Average visual damage ratings to *E. crus-galli* and *E. colona* plants within each of the three herbicide treatments 14 days after treatment (DAT). (Standard errors for 1.0 L treatment were 0.00 and 0.014 respectively).

There was a trend that the visual damage rating was greater for *E. colona* at all three herbicide rates. For both species a rate of 0.5 L was insufficient to provide 100% control, while full control was achieved at the higher rate of 1.0 L only in *E. colona*. The damage to the *E. crus-galli* plants had an average rating of 9.6 with some plants still having some green matter present.

The first ANOVA conducted without covariates showed there was a significant difference in how the two species reacted to the different rates of glyphosate ($p = 0.045$). However this became non-significant ($p = 0.365$) when the covariates were included in the analyses.

5.4.3 Effect of region

At 14 DAT the average visual damage rating of the species was similar with increased visual damage as the herbicide rate increased, irrespective of the region.

There was no noticeable difference in the average visual damage for *E. crus-galli* from Dalby or Narrabri for all the treatments (Figure 5.8).

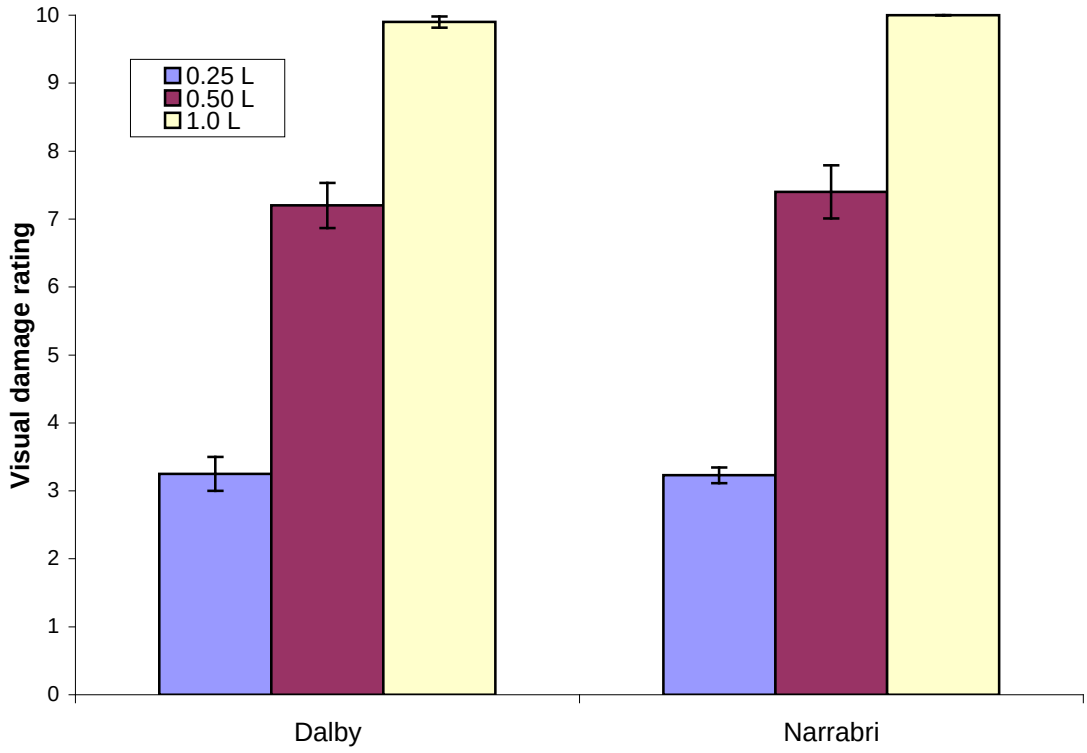


Figure 5.8: Average visual damage rating to *E. crus-galli* plants from the regional centres Dalby and Narrabri at 14 days after treatment (DAT). (Standard error for Narrabri 1.0 L was 0.00).

Echinochloa colona populations from each region also responded similarly to the different herbicide treatments (Figure 5.9). However, populations from Narrabri had a slightly lower visual damage rating at the 0.5L treatment.

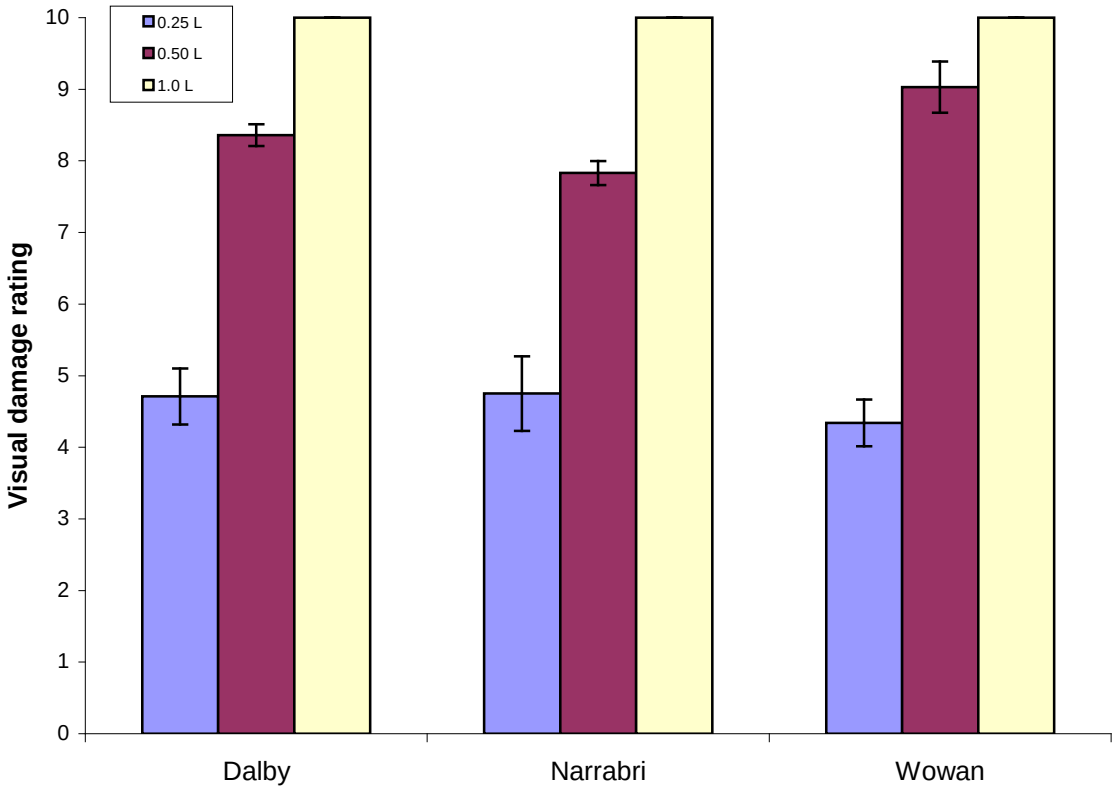


Figure 5.9: Average visual damage rating to *E. colona* plants from the regional centres Dalby, Narrabri and Wowan at 14 days after treatment (DAT). (Standard errors for all 1.0 L treatments were 0.00).

In order to establish if the regional centre was a significant factor in the response of the two species to glyphosate treatments, an ANOVA using log dry weight data analysed 5 areas (2 regional centres (Dalby (SQ) and Narrabri (NNSW)) x 2 species (*E. colona* and *E. crus-galli*), and 1 regional centre x 1 species (Wowan (CQ) and *E. colona*). The covariates, leaf number and plant size were included in the analyses. The result was significant ($p = 0.05$) with the difference between the regions resulting from the dry weight of the untreated controls of the *E. colona* populations from Wowan (Figure 5.10).

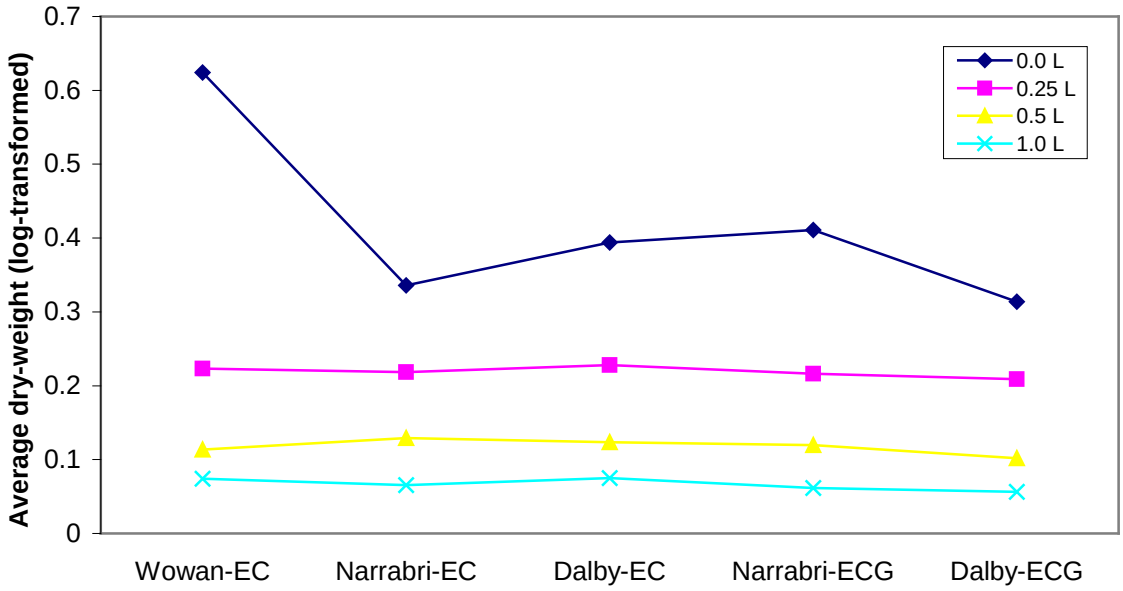


Figure 5.10: The average log-transformed dry weight of all the combined populations of each species (EC – *E.colona*, ECG – *E. crus-galli*) calculated according to the three regional centres and the overall response to the four glyphosate treatments (0.0 L, 0.25 L, 0.50 L, 1.0 L). (LSD = 0.298).

5.5 Discussion

The results from this preliminary study showed that there was a large amount of variability in both visual and biomass measurements for individual populations, as well as between species and across the three major cropping regions of the NGR. This could have been due to the relatively small numbers of individuals in each of the herbicide treatments producing accentuated variability. The percentage mortality was not calculated with the death of the plants being incorporated into the visual damage assessments.

After herbicide application, *E. crus-galli* plants were generally the first to show visual symptoms of herbicide damage, even though *E. crus-galli* plants were larger than *E. colona*. Within two days of spraying, *E. crus-galli* plants in the 1.0 L/ha treatment were beginning to show some discolouration to the leaves and by 3 DAT the degree of visual damage to *E. crus-galli* was more severe across all three treatments. However, by the end of the experiment (14 DAT) the damage was similar for plants across

species at the recommended rate. Generally, herbicides are more effective on younger weeds, but in this case it initially appears to be the opposite. This is likely to reflect a genetic difference between species.

It can be difficult to correctly distinguish between the two species when they are seedlings in the field. The time taken for visual signs of herbicide damage could be used as an indicator to identify species. If farmers have initial poor control with glyphosate this could indicate that the species present is *E. colona*. Also, inconsistent or patchy control could be the result of mixed species and not resistance. To be sure of this, farmers would need to wait approximately two weeks to see the full effect of glyphosate. However, the ability to use response to herbicide to identify species is made difficult by the fact that populations within species differ in their response to glyphosate.

A common trend was as herbicide rates increased, the biomass of populations decreased. This was an expected result and demonstrated that for effective control of *Echinochloa* species, a higher rate of glyphosate is required. Within each of the species, there were differences in biomass of populations after glyphosate application, indicating differences in susceptibility to this herbicide.

There was variability in the dry weight biomass of untreated populations of both species. Generally the *E. colona* populations from Wowan had a higher biomass while those from Narrabri had a lower biomass. For *E. crus-galli* the populations from Dalby generally had a lower biomass than populations from Narrabri. The size of the plants at treatment application may have had an impact on their response to glyphosate. Ideally all plants should be the same size at treatment to remove plant size as a factor impacting on response to herbicide. To take these differences into account, data were analysed with and without plant height and leaf number as covariates and in both analyses, there were significant differences in the population response to glyphosate. This suggests that plant size may not have been the main factor in determining the response to glyphosate and that the genetic make-up of the plant is playing a role in how the populations respond to glyphosate application. The relationship between genetics and herbicide susceptibility is explored in Chapter 6.

There was a large amount of variability in the response of populations to glyphosate at the species, region, and field and collection point levels.

When populations across species were clustered according to their response to herbicides, populations did not cluster according to either species, region of collection, field of collection or even collection point. For example, populations of *E. crus-galli* were represented in seven of the ten clusters, ranging from high to low susceptibility to glyphosate. The three *E. crus-galli* populations from Dalby were all collected from the same field, an area of approximately one hectare, and were spread over two clusters, ranging from moderate to high susceptibility. Three of the *E. crus-galli* plants from Narrabri were collected from the same collection point, an area of approximately 20 m^2, and occur in three separate clusters, ranging from moderate to high susceptibility.

Echinochloa colona occurred in eight of the ten clusters. While there was variability in the response of populations from the same region, the variability seemed less than for *E. crus-galli*. The majority of the *E. colona* populations from Wowan were in the same cluster and all of these populations were highly susceptible to glyphosate at the 0.25 and 0.5 L/ha rates. The two remaining Wowan populations were moderately and highly susceptible. By comparison there seemed to be a large amount of variability in the populations from Dalby, appearing in five of the ten clusters ranging from moderate to high susceptibility.

The *E. colona* populations from Narrabri were in two clusters, with two populations being moderately susceptible and the third having the lowest susceptibility of all populations tested. The two populations occurring in the same cluster were collected from the same field. Similarly, two populations from Dalby collected from the same field were also grouped together in the same cluster. However, populations collected from one field at Wowan occurred in two clusters, both highly susceptible, and a further two populations from another field were in different clusters with moderate and high susceptibility.

The habitat of the collection could be a possible cause of the variability. For example, the least susceptible population in this study was collected from near a tail drain. This population could have been introduced via water coming from neighbouring fields or as seed in water bird faeces. Other factors contributing to the variability in populations could include out-crossing, movement of seed via water, machinery or the contamination of seed and feed. These factors may influence herbicide susceptibility if seeds from areas with different herbicide histories are introduced to the existing populations on the relevant farm.

Because of the variability in control between populations, farmers cannot rely on sub-lethal doses of glyphosate, even though some populations were effectively controlled at 0.25 L/ha. A robust rate of at least 1 L/ha should be used to ensure adequate control of both species. Similar results were achieved in a study by Werth *et al.* (2009) where 99% control of *E. colona* at the 3-5 leaf stage was achieved applying a rate of 1.6 L/ha of glyphosate. The same application, a week later at early tillering, achieved only 52% control, with 48% of the plants surviving the herbicide application. Koger *et al.* (2005) achieved 99% control of *E. crus-galli* at the 2-3 leaf stage and 93% control at the 5-6 leaf stage with glyphosate applications of 840 g/ha. This is nearly twice the rate applied in this study where the 1L/ha application of glyphosate equates to 450g/ha of the active ingredient. Werth (2006) applied glyphosate treatments to *E. crus-galli* at 690 g/ha and achieved full control at both 2-leaf and 6-leaf stages. The worst control was 73% at the 6-leaf stage at 345 g/ha but at this rate 87% control was achieved at the 2-leaf stage.

Applying a robust rate will also ensure that seed production is greatly reduced. In this study no populations survived the 1 L/ha rate or were able to produce seed. However, there were populations that survived and set seed after treatment at both the 0.25 and 0.5 L/ha treatments. For example, an *E. colona* population from Narrabri set seed in both the lower treatments and is the population with the lowest susceptibility to glyphosate at both these rates.

If farmers had applied a sub-lethal dose of glyphosate, resulting in poor control, it would be important for them to apply a follow-up treatment to stop seed-set on

survivors and to prevent possible herbicide resistance through accumulated gene traits (Powles 2007). The follow-up treatment should be herbicide from a different mode of action, or a non-chemical strategy such as cultivation. One approach that is currently being adopted for barnyard grass in the northern region is the double knock tactic. The double-knock is the sequential application of two different tactics such as herbicide and non-chemical or two chemicals with different mode of actions within a short period of time (no more than 21 days). It was developed to stop seed set and also minimise resistance to herbicides (McGilllion and Storrie 2006). While none of the populations tested are resistant to glyphosate, using the double knock tactic will prolong the useful life of glyphosate and delay resistance.

In this study, *E. crus-galli* was generally not as well controlled as *E. colona* with glyphosate, especially at the lower rates. When compared statistically without covariates accounting for morphological differences, there was a significant difference in how the two species responded to this herbicide. Since *E. crus-galli* seems less susceptible to glyphosate, a farmer controlling this species should apply a rate of herbicide well within the recommended rate (0.8-1.6 L/ha) and should not consider applying glyphosate under the recommended rate.

When analysed statistically, there was a significant difference between regions for how both species responded to treatment with glyphosate. This difference can be accounted for by the increased biomass of control plants from Wowan. Taking this into account, it appears that the level of control achieved with glyphosate is relatively consistent across the northern grain region. Therefore, the conclusions drawn from this study apply across the major cropping centres of the NGR.

5.6 Conclusion

This research has identified that there is variability in the response to glyphosate at regional centre, species, paddock and collection point levels. The two species, *E. colona* and *E. crus-galli* responded differently to glyphosate with *E. crus-galli* appearing to be less susceptible. However, the response to this herbicide cannot reliably be used to identify *Echinochloa* species.

While the difference in plant size did not contribute to the difference in response to herbicide, it implies a genetic reason. This may have implications in the future as herbicide resistance develops further across the industry. Irrespective of the species or the region, a robust rate of glyphosate (at least 1 L/ha) should be applied to effectively control both species. In addition, the adoption of a practice such as the double-knock should be implemented to prevent seed set on survivors.

Chapter 6 *General Conclusion*

The aims of this study were to provide reliable methods including morphological and molecular techniques to assist in the correct identification of *Echinochloa* species, to determine the genetic diversity of *Echinochloa* at both geographic and field levels in the NGR, and to investigate the levels of herbicide susceptibility across the region. The purpose of investigating these areas was to provide information that would be useful when devising principles for effective and sustainable management of *Echinochloa* species.

6.1 Morphological and molecular characterisation of *E. crus-galli* and *E. colona*

Results of this study have shown a great deal of variation exists in the morphology of both *E. colona* and *E. crus-galli* across the northern grain region (NGR). For both species, there is morphological variation of plants both between and within centres, farms, fields and individual collection points, making it difficult to characterise species at these levels. *Echinochloa colona* was shown to be genetically diverse based on microsatellite analysis, while *E. crus-galli* is genetically similar across the NGR. The genetic diversity found in some *E. colona* populations could be related to the regional centres with certain ranges of alleles being unique to each centre.

In the morphological study (Chapter 3) it was established that the major *Echinochloa* species occurring in the NGR was *E. colona* and that *E. crus-galli* occurred primarily in northern New South Wales with a few populations established in southern Queensland. In some of the fields sampled both species were present. Plants were identified as either *E. colona* or *E. crus-galli* based on the presence of bristles on the racemes on *E. crus-galli*. This study was the first to investigate which species of *Echinochloa* were present in the NGR. Previous research undertaken in this region does not distinguish between species, and generally group all *Echinochloa* together as 'barnyard grasses' (Osten *et al.* 2007; Walker *et al.* 2002; Walker *et al.* 2005; Walker *et al.* 2004).

Echinochloa crus-galli was found in more temperate regions suggesting that a temperate environment favours the growth and reproduction of this species. In the

first morphological trial established to examine morphological differences between species, the populations were grown in the middle of summer under very high temperatures. In this environment, *E. crus-galli* plants struggled to establish and had a similar growth habit to *E. colona* plants. The following year a second trial investigating the morphology of *E. crus-galli* was established towards the end of spring when temperatures were lower. In this environment *E. crus-galli* plants established readily and were more robust and taller, and visually different from the plants grown in the previous year. Had my study compared the morphology of the two species at this cooler time of the year I may have measured large differences in morphology reflecting the robustness of *E. crus-galli* plants. This may have resulted in differences between species if compared statistically.

The morphology of *Echinochloa* species appeared to be strongly influenced by the environment in which the plant was growing. This study showed that the morphology of *E. colona* plants sampled in the field appeared to be linked to the habitat or environment in which the plants were growing (Figure 3.4). In addition, and as discussed above, when *E. crus-galli* were grown at different times of the year, the growth habits were vastly different.

Based on previous knowledge, when grown in the same environment, a high level of consistency in the morphology of plants from the same population would be expected because these two species are predominantly self-pollinated. It has been reported that *E. crus-galli* plants are self-compatible and highly autogamous resulting in high levels of homozygosity within populations (Maun and Barrett 1986), and similarly *E. colona* is widely believed to be highly selfing. Consistency in the morphology of the two species was not what was observed in this study. This suggests that these species are more outcrossing, and thus more genetically heterozygous, than previously believed (see heterozygousity experiment Chapter 3). Alternately, it could mean that problems existed with the protocols in this study including seed collection (possible contamination between plants) or microclimates within the shade house (eg more/less water and different amounts of fertiliser and potting mix).

When a range of morphological characteristics was measured on both species at the onset of reproduction and at maturity and included in a data set, statistical analyses were unable to distinguish between the species. However, my study shows that individual morphological characteristics can be used to distinguish between the two species. The most reliable characteristic in mature plants is the presence of bristles on the racemes on *E. crus-galli*. I found that an additional identification tool is the difference in the seed size of the two species, and the caryopsis colour. Seeds of *E. crus-galli* are larger and have cream caryopses, whereas *E. colona* are smaller and have light brown caryopses. Taxonomic keys, including that of Michael (1983) show that other *Echinochloa* species have similar seed characteristics to *E. crus-galli* and *E. colona*; therefore care must be taken not to assume that you only have these species present in your populations. Also, in younger plants purple transverse banding on the leaves of *E. colona* can be used as an identification tool. However, banding is not always present on *E. colona* plants, and plants without banding cannot be assumed as being *E. crus-galli*.

In previous studies the absence of awns has been used to distinguish *E. colona* from *E. crus-galli*. This study has shown that this morphological characteristic is not a reliable identification tool in the NGR. Michael (1973) also stated that awning was a variable feature in some forms of *E. crus-galli* and was dependent on environmental conditions. In my study, very few *E. crus-galli* plants sampled in the field had noticeable awns and while awns were present on the majority of plants in the morphological trials, the awns were sparse and limited to the uppermost spikelets on the panicles.

Nevertheless, my study has shown that some morphological characteristics, other than awning, can be used to distinguish between the two species at maturity.

The molecular studies (Chapter 4) have shown that there is a clear differentiation between the two species based on genetics. The microsatellites (SSRs) developed by Danquah *et al.* (2002a) were useful and reliable tools and clearly identified *E. colona* and *E. crus-galli*, and unlike morphological features molecular markers can be used at any stage of the plant's life cycle. The SSRs also provided information on differences

between populations that enabled characterisation of the genetic diversity of the two species to be made at both centre and field levels. While *E. crus-galli* was genetically similar across the NGR, *E. colona* was genetically diverse. This research has indicated that there were distinct genetic groups within the *E. colona* populations in the NGR of Australia.

While *E. crus-galli* was sampled across the NGR, the majority of samples were taken from the same farm in Narrabri. If the sampling of populations had been extended to include more sites in New South Wales where *E. crus-galli* was predominant, more genetic variability may have been observed. Because of the large amount of variability in *E. colona*, more populations need to be sampled and compared to confirm whether the trend established in this study was indicative of the genetic variability within this species across the NGR.

This study has shown that both environment and genetics play a part in the morphological diversity of both species. The morphology of *E. colona* populations sampled in the field appeared to be linked to the habitat or environment in which the plants were growing, while *E. crus-galli* populations grown at different times of the year displayed vastly different growth habits. The populations established in the morphological trials were grown in a common environment and had different growth habits both between and within populations suggesting a genetic difference.

6.2 Relationships between morphology, molecular diversity and herbicide susceptibility

As already indicated, the molecular study showed that there was a clear difference in the genetics of the two *Echinochloa* species. To assess whether the genetic groupings based on Danquah's SSRs of *E. crus-galli* and *E. colona* influenced morphology and herbicide response, a comparison was made of the *E. crus-galli* and *E. colona* molecular cluster (Chapter 4), with the morphological (at onset of reproduction) (Chapter 3) and herbicide response clusters (Chapter 5) (Table 1).

Table 6.1: Comparison of *E. crus-galli* and *E. colona* populations and how the populations clustered, based on similarity of traits, in each of the three studies (morphology, molecular and herbicide response). The cluster number under each heading refers to the cluster in which the population appeared in the relevant chapter. Separate dendrograms were produced for the two morphological trials in 2005/2006 (*E. colona*) and 2006/2007 (*E. crus-galli*). The population ID used in this table is identical to that used in the herbicide study (Table 5.1), identifying the regional centre (W – Wowan, D – Dalby, N –Narrabri), the species (C – *E. colona*, G – *E. crus-galli*) and the population number within each species. Where there are gaps in the table, the population was not tested in the associated experiment due to lack of seed, failure to establish, or failure to generate genetic products. (# Population NG15 was established in both morphological trials).

Species	Population ID	Molecular cluster	Morphological cluster	Herbicide response cluster
E. colona	WC2	4	4	7
	WC4	4	5	7
	WC5	5	4	4
	WC1	5	4	7
	WC7	5	6	7
	WC3	6	1	6
	WC6	7	1	7
	DC1	8	1	1
	DC7	9	3	2
	DC3	10	2	7
	DC4	10	1	8
	DC5	10	4	5
	DC6	11	1	7
	NC3	12	8	9
	NC1	14	4	1
	WC8	-	4	7
	DC2	-	8	7
	NC2	-	1	1
E. crus-galli	NG8	1	4	1
	NG10	1	5	3
	DG1	3	4	2
	DG2	3	4	2
	DG3	3	4	5
	NG11	3	3	1
	NG12	3	4	5
	NG14	3	5	2
	#NG15	3	1($1^{st\ trial}$) 4 ($2^{nd\ trial}$)	1
	NG5	3	2	5
	NG6	3	4	1
	NG7	3	4	7
	NG9	3	4	7
	NG1	-	2	10
	NG2	-	3	7
	NG3	-	-	5
	NG4	-	4	4
	NG13	-	4	5

There were no clear relationships between the observed genetic groupings of *E. colona* or *E. crus-galli* and their observed morphology or response to glyphosate (Table 6.1). The large genetic variability in *E. colona* resulted in only three molecular clusters containing more than one population. Therefore comparison within *E. colona* clusters was limited as was the ability to establish any trends in relationships between the three factors. Because of the large amount of genetic variability in *E. colona*, a larger number of populations across the NGR would have to be compared to properly establish trends. In contrast, the limited molecular diversity in *E. crus-galli* did not correlate with the large amount of variability in morphology and herbicide response.

While this set of SSRs was unable to assign individuals to classes based on morphology, and thus suggest a genetic basis contributed to the different morphologies, it does not preclude a genetic basis. In this study, the morphological traits measured cannot be attributed to these markers. While the genetic markers used were unable to provide any links with morphology and herbicide response, other observations from this study indicate that both morphology and herbicide response are linked to genetics. For example, when plants were grown in an identical environment, they showed morphological differences, both between populations and within populations. Similarly, when plants were grown in the same environment and then treated with the same herbicide, populations responded differently.

The microsatellites were developed by Danquah *et al.* (2002a) in order to differentiate between the genetic make up of three *Echinochloa* species and not used to identify any genetic links with morphology or herbicide response. This study was the first to use these SSRs in an attempt to establish links between genetics and other factors. Five SSRs was a small number of molecular markers upon which to define genetic ecotypes, based on morphology or their response to herbicides. These markers would not be expected to be associated closely with any particular morphological or herbicide response trait, but could provide a general indication of genetic variation and similarities. This study was unable to show a correlation between SSRs and morphological traits and glyphosate response in the classification of *Echinochloa* species present in fields of the NGR. Results from the molecular studies were

therefore only useful to clearly identify species and provide an indication of the genetic variability between populations.

The herbicide study (Chapter 5) showed variability in response to glyphosate at centre, species, paddock and collection point levels, and that response to herbicide could not reliably be used to identify *Echinochloa* species. There were no links made between herbicide response and either morphology or genetics. However, it appeared that *E. crus-galli* was in general less susceptible to glyphosate.

6.3 Future research

With regard to the morphological trials, this study established populations of both species in a polyhouse open to the weather. To remove any variability when examining morphology, future research needs to ensure the environment is identical for all plants. This could be achieved by the use of a glasshouse where the environment can be better controlled and also using scales to more accurately measure out equal amounts of potting mix, fertiliser and water per pot. A large number of the characteristics measured in the morphological trial could not be included in the statistical analyses. Future research may need to look further into how some of these characteristics could be incorporated as they may have influenced the clustering of species and populations.

The genetic diversity of populations of *E. colona* and *E. crus-galli* from locations within the three major cropping centres of the NGR has been presented. As *E. colona* was genetically diverse but did show some centre trends, a broader range of populations need to be tested to confirm or expand on trends. Similarly, the testing of *E. crus-galli* populations over a larger geographical range needs to be conducted to establish if the limited genetic diversity found in this study was reflective of the species in the NGR or limited to the farm sampled in northern New South Wales.

The herbicide susceptibility trial was a single pot experiment and the small number of replicates used per population could have produced an accentuated variability in the results. The experiment would need to be repeated on a larger scale to ascertain if the

trends reported here are repeatable. In addition the replicates were assigned to treatments at the time of planting. A clearer result may have been achieved if plants were allocated to treatments before spraying to achieve a more even distribution of plant sizes between replicates.

6.4 Implications for weed management

The results of this study show that genetics and morphology cannot be reliably used to develop a weed management plan, due to the diversity found in both characteristics and no clear connection with herbicide susceptibility. However, molecular techniques can be used to clearly distinguish between *E. colona* and *E. crus-galli* and morphology can be used to distinguish between the mature plants of both species. Unfortunately this is not ideal for farmers who need to be aware of the composition of species in paddocks and apply herbicide treatments prior to the plants reaching maturity. Delaying herbicide treatment of *Echinochloa* until maturity will result in a lower level of control and increase the likelihood of seeds being produced. This demonstrates the need to consider additional treatments to stop seed set. One such approach is the double knock treatment which targets survivors with the potential to set seed.

Also, in this study there was variability in control of populations ranging from low to high susceptibility, and it was evident that none of the populations tested were resistant to glyphosate. Therefore, in the absence of resistance, most populations of *E. colona* and *E. crus-galli* should be able to be effectively controlled with label rates of this herbicide. It is important for weed managers to remember that these species are at very high risk of developing resistance to glyphosate with three currently confirmed cases in the NGR (Preston 2009). Each of these populations has developed resistance under a system of fifteen years sustained use of glyphosate. Therefore, an integrated weed management (IWM) approach applying different chemical and non-chemical weed control tactics should be used to both improve weed control and prolong the effective life of glyphosate.

In this study I was unable to establish clear relationships between morphology, genetic diversity and glyphosate response. Thus results do not suggest changes to the current recommended integrated weed management plan for *E. colona* and *E. crus-galli*. However, I was able to provide new insights into the composition and morphological diversity and genetic diversity of *Echinochloa* species in the NGR. The results of this study support previous research that robust rates of glyphosate are necessary to control both species in cropping situations.

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Appendices

Appendix 1: Average data for the morphological measurements of *Echinochloa colona* and *E. crus-galli* (2005/2006) taken at the onset on reproduction, emergence of flag leaf, and onset of maturity, where range of values are in parentheses and population labels as used in Chapter 3. Refer 3.3.1.1 for description of original population ID.

Population label	Original population ID	Species	Onset of reproduction					
			Height - form	Height - extend	No. of tillers	No. of leaves	Culm colour	Culm hairiness
1	N12: 5-5	*E. colona*	249.5 (200 – 346)	255.3 (205 – 359)	1.5 (1 – 3)	7.5 (4 – 13)	2.5 (2 – 3)	1.5 (1 – 2)
2	N15: 6-1	*E. colona*	353.8 (312 – 457)	416.8 (357 – 472)	2.5 (1 – 5)	10.5 (6 – 18)	3.0 (3 – 3)	1.5 (1 – 2)
3	N11: 1-1	*E. colona*	387.0 (311 – 436)	443.0 (352 – 499)	2.0 (1 – 3)	11.8 (8 – 19)	3.5 (3 – 4)	1.5 (1 – 3)
4	N13: 14-2	*E. colona*	335.5 (280 – 383)	398.0 (360 – 462)	1.8 (1 – 3)	8.8 (6 – 13)	4.0 (4 – 4)	2.0 (1 – 3)
5	D13: 4-2	*E. colona*	128.5 (103 – 171)	160.3 (106 – 192)	2.0 (1 – 3)	9.3 (4 – 13)	1.8 (1 – 2)	1.0 (1 – 1)
6	W16: 4-3	*E. colona*	360.8 (334 – 378)	409.0 (374 – 438)	8.5 (6 – 12)	38.8 (28 – 58)	2.0 (2 – 2)	1.0 (1 – 1)
7	N11: 2-6	*E. colona*	156.0 (141 – 173)	246.8 (227 – 259)	3.0 (2 – 4)	9.8 (6 – 12)	2.0 (1 – 3)	1.0 (1 – 1)
8	N11: 13-3	*E. colona*	321.0 (257 – 407)	377.3 (265 – 432)	3.8 (2 – 5)	16.0 (9 – 26)	2.5 (2 – 3)	2.5 (1 – 3)
9	N12: 10-3	*E. crus-galli*	399.8 (302 – 516)	443.3 (373 – 531)	1.5 (1 – 3)	10.3 (6 – 15)	2.3 (2 – 3)	1.3 (1 – 2)

Population label	Original population ID	Species	Onset of reproduction					
			Height - form	Height - extend	No. of tillers	No. of leaves	Culm colour	Culm hairiness
10	N11: 16-3	*E. crus-galli*	290.3 (140 – 467)	380.8 (261 – 561)	2.5 (2 – 4)	12.5 (9 – 15)	1.8 (1 – 2)	1.0 (1 – 1)
11	N11: 3-5	*E. crus-galli*	317.0 (109 – 426)	357.3 (147 – 472)	1.3 (1 – 2)	7.5 (6 – 9)	2.3 (2 – 3)	1.0 (1 – 1)
12	N15: 10-4	*E. colona*	378.5 (327 – 452)	426.0 (356 – 524)	4.0 (3 – 6)	19.5 (13 – 25)	3.3 (3 – 4)	1.8 (1 – 2)
13	N15: 12-3	*E. colona*	298.5 (221 – 370)	383.5 (285 – 489)	1.0 (1 – 1)	4.8 (4 – 7)	3.5 (3 – 4)	1.3 (1 – 2)
14	D41: 10-4	*E. colona*	369.3 (303 – 430)	427.8 (330 – 486)	2.3 (1 – 4)	6.5 (4 – 9)	2.8 (2 – 3)	1.8 (1 – 2)
15	D21: 4-1	*E. colona*	321.0 (268 – 360)	382.3 (331 – 444)	1.8 (1 – 2)	6.5 (5 – 8)	2.5 (2 – 3)	1.3 (1 – 2)
16	D11: 16-4	*E. colona*	392.3 (341 – 476)	424.0 (353 – 499)	2.3 (1 – 3)	9.0 (6 – 12)	3.8 (3 – 4)	1.3 (1 – 2)
17	W13: 5-2	*E. colona*	218.8 (176 – 265)	224.0 (179 – 275)	1.0 (1 – 1)	4.5 (4 – 5)	3.3 (3 – 4)	1.3 (1 – 2)
18	W13: 17-1	*E. colona*	455.0 (439 – 482)	486.5 (455 – 528)	2.8 (2 – 3)	11.5 (9 – 15)	2.0 (2 – 2)	1.5 (1 – 3)
19	W17: 10-1	*E. colona*	382.8 (336 – 442)	429.5 (344 – 464)	3.5 (1 – 8)	16.3 (7 – 37)	2.8 (2 – 3)	2.3 (2 – 3)
20	W13: 2-1	*E. colona*	257.8 (154 – 402)	323.8 (236 – 480)	2.8 (1 – 4)	10.8 (6 – 16)	2.0 (2 – 2)	1.5 (1 – 2)

Population label	Original population ID	Species	Onset of reproduction					
			Height - form	Height - extend	No. of tillers	No. of leaves	Culm colour	Culm hairiness
21	W13: 11-9	*E. colona*	285.0 (194 – 400)	339.0 (246 – 426)	1.0 (1 – 1)	6.0 (5 – 9)	3.0 (3 – 3)	1.0 (1 - 1)
22	W14: 1-1	*E. colona*	437.0 (374 – 489)	603.0 (529 – 709)	4.0 (3 – 6)	15.5 (12 – 19)	2.0 (2 – 2)	1.8 (1 – 2)
23	W14: 6-3	*E. colona*	452.0 (221 – 634)	511.0 (276 – 680)	1.5 (1 – 3)	7.5 (4 – 12)	3.0 (3 – 3)	1.8 (1 – 2)
24	N15: 5-5	*E. crus-galli*	53.5 (42 – 68)	65.8 (54 – 84)	1.5 (1 – 2)	5.0 (4 – 7)	2.0 (2 – 2)	1.0 (1 – 1)
25	D21: 3-1	*E. colona*	314.8 (183 – 416)	352.5 (227 – 472)	2.0 (1 – 4)	11.3 (8 – 21)	3.0 (3 – 3)	1.5 (1 – 2)
26	D11: 20-1	*E. colona*	453.5 (314 – 512)	587.8 (317 – 746)	2.3 (1 – 4)	11.0 (4 – 18)	4.0 (4 – 4)	2.5 (2 – 3)
27	D51: 6-2	*E. colona*	243.0 (97 – 454)	266.0 (108 – 489)	1.0 (1 – 1)	7.8 (6 – 10)	3.5 (3 – 4)	1.3 (1 – 2)
28	N15: 13-4	*E. crus-galli*	134. (102 – 156)	146.0 (115 – 164)	1.0 (1 – 1)	6.0 (6 – 6)	2.0 (2 – 2)	1.3 (1 – 2)
29	D13: 16-1	*E. colona*	317.8 (228 – 384)	394.5 (244 – 572)	3.0 (1 – 4)	12.8 (7 – 15)	3.0 (2 – 4)	1.5 (1 – 2)
30	N14: 7-4	*E. colona*	414.8 (372 – 498)	545.5 (421 – 675)	3.8 (3 – 5)	12.0 (10 – 17)	3.5 (3 – 4)	2.5 (2 – 3)
31	W12: 9-3	*E. colona*	382.8 (267 – 481)	434.8 (273 – 651)	1.8 (1 – 4)	8.8 (6 – 15)	3.0 (2 – 4)	1.0 (1 – 1)

Population label	Original population ID	Species	Onset of reproduction					
			Height - form	Height - extend	No. of tillers	No. of leaves	Culm colour	Culm hairiness
32	D12: 10-4	*E. colona*	272.0 (226 – 320)	311.8 (270 – 341)	2.8 (1 – 4)	14.0 (7 – 20)	3.0 (3 – 2)	2.3 (1 – 3)
33	W15: 8-1	*E. colona*	247.75 (192 – 274)	283.73 (266 – 317)	1.0 (1 - 1)	7.0 (6 – 8)	2.5 (2 – 3)	1.25 (1 – 2)
34	D14: 12-4	*E. colona*	394.8 (340 – 428)	477.8 (391 – 547)	3.0 (2 – 4)	10.8 (6 – 13)	3.3 (3 – 4)	2.3 (1 – 3)
35	W11: 1-3	*E. colona*	271.0 (244 – 313)	331.5 (256 – 365)	1.8 (1 – 3)	8.0 (6 – 10)	2.0 (2 – 2)	1.0 (1 – 1)
36	N15: 9-5	*E. crus-galli*	151.3 (86 – 206)	191.3 (127 – 239)	2.0 (2 – 2)	9.3 (8 – 10)	1.8 (1 – 2)	1.0 (1 – 1)

Population label	Original population ID	Species	Emergence of flag leaf			
			Flag leaf length	Flag leaf width	Panicle length	Panicle width
1	N12: 5-5	*E. colona*	80.5 (52 – 126)	7.0 (6 – 8)	56.3 (52 – 65)	22.0 (8 – 38)
2	N15: 6-1	*E. colona*	79.3 (52 – 132)	8.3 (7 – 9)	79.8 (69 – 96)	25.3 (8 – 74)
3	N11: 1-1	*E. colona*	118.0 (81 – 149)	7.8 (5 – 10)	112.5 (83 – 156)	14.8 (8 – 32)
4	N13: 14-2	*E. colona*	94.8 (72 – 129)	8.3 (6 – 10)	84.3 (51 – 103)	18.8 (9 – 26)
5	D13: 4-2	*E. colona*	75.8 (56 – 96)	4.3 (3 – 6)	38.5 (23 – 54)	7.3 (6 – 9)
6	W16: 4-3	*E. colona*	55.5 (48 – 64)	5.0 (4 – 5)	54.5 (51 – 59)	11.3 (9 – 14)
7	N11: 2-6	*E. colona*	89.8 (56 – 115)	5.8 (4 – 8)	44.3 (34 – 54)	8.5 (6 – 11)
8	N11: 13-3	*E. colona*	90.8 (59 – 132)	7.3 (6 – 9)	55.5 (6 – 82)	10.8 (9 – 15)
9	N12: 10-3	*E. crus-galli*	78.0 (65 – 90)	7.3 (5 – 9)	68.0 (44 – 79)	27.8 (9 – 35)
10	N11: 16-3	*E. crus-galli*	95.0 (54 – 141)	9.0 (7 – 11)	70.8 (52 – 93)	46.0 (36 – 66)
11	N11: 3-5	*E. crus-galli*	70.3 (16 – 104)	7.5 (5 – 10)	62.0 (17 – 88)	32.3 (3 – 45)

Population label	Original population ID	Species	Emergence of flag leaf			
			Flag leaf length	Flag leaf width	Panicle length	Panicle width
12	N15: 10-4	*E. colona*	72.3 (49 – 91)	7.0 (5 – 9)	76.3 (56 – 92)	10.8 (8 – 14)
13	N15: 12-3	*E. colona*	120.3 (61 – 173)	8.5 (6 – 11)	90.5 (72 – 115)	18.3 (7 – 31)
14	D41: 10-4	*E. colona*	138.5 (54 – 176)	8.8 (6 – 11)	90.3 (41 – 114)	12.5 (8 – 16)
15	D21: 4-1	*E. colona*	120.0 (91 – 175)	9.3 (8 – 11)	87.8 (65 – 115)	13.8 (7 – 32)
16	D11: 16-4	*E. colona*	92.8 (51 – 142)	7.3 (5 – 9)	68.8 (57 – 86)	9.0 (5 – 12)
17	W13: 5-2	*E. colona*	52.0 (32 – 86)	6.0 (4 – 8)	76.0 (53 – 117)	13.0 (6 – 28)
18	W13: 17-1	*E. colona*	98.3 (81 – 114)	8.5 (8 – 9)	94.5 (87 – 104)	10.3 (8 – 13)
19	W17: 10-1	*E. colona*	74.8 (61 – 94)	7.3 (6 – 9)	65.8 (54 – 86)	9.5 (7 – 15)
20	W13: 2-1	*E. colona*	81.0 (74 – 96)	6.3 (5 – 8)	62.8 (39 – 79)	8.8 (5 – 14)
21	W13: 11-9	*E. colona*	64.5 (49 – 97)	7.8 (6 – 10)	81.0 (49 – 116)	20.0 (11 – 29)
22	W14: 1-1	*E. colona*	111.3 (88 – 146)	9.3 (8 – 10)	82.5 (88 – 94)	10.5 (8 – 15)

Population label	Original population ID	Species	Emergence of flag leaf			
			Flag leaf length	Flag leaf width	Panicle length	Panicle width
23	W14: 6-3	*E. colona*	89.3 (35 – 129)	8.0 (4 – 10)	88.8 (29 – 114)	25.8 (14 – 34)
24	N15: 5-5	*E. crus-galli*	16.5 (9 – 29)	2.8 (2 – 4)	13.5 (6 – 24)	5.3 (3 – 9)
25	D21: 3-1	*E. colona*	70.8 (60 – 82)	7.3 (6 – 9)	63.8 (34 – 110)	11.5 (7 – 19)
26	D11: 20-1	*E. colona*	140.0 (68 – 292)	10.0 (6 – 13)	74.8 (45 – 89)	13.0 (7 – 18)
27	D51: 6-2	*E. colona*	65.3 (42 – 105)	6.3 (5 – 9)	57.3 (32 – 105)	12.0 (9 – 14)
28	N15: 13-4	*E. crus-galli*	35.7 (28 – 48)	4.3 (4 – 5)	32.7 (27 – 36)	9.3 (8 – 11)
29	D13: 16-1	*E. colona*	110.8 (54 – 239)	8.3 (5 – 13)	74.3 (56 – 106)	31.0 13 – 49)
30	N14: 7-4	*E. colona*	148.0 (74 – 259)	12.3 (9 – 15)	85.0 (72 – 107)	44.3 (20 – 59)
31	W12: 9-3	*E. colona*	61.0 (448 – 91)	5.8 (4 – 8)	61.0 (48 – 90)	8.3 (7 – 10)
32	D12: 10-4	*E. colona*	89.8 (64 – 117)	7.8 (7 – 9)	76.8 (61 - 91)	15.3 (9 – 21)
33	W15: 8-1	*E. colona*	33.5 (24 – 47)	4.0 (3 – 5)	28.8 (22 – 38)	10.3 (9 – 11)

Population label	Original population ID	Species	Emergence of flag leaf			
			Flag leaf length	Flag leaf width	Panicle length	Panicle width
34	D14: 12-4	*E. colona*	97.3 (71 – 114)	9.0 (8 – 10)	96.0 (86 – 101)	10.3 (9 – 14)
35	W11: 1-3	*E. colona*	52.0 (43 – 69)	6.8 (6 – 9)	57.3 (46 – 68)	13.3 (10 – 20)
36	N15: 9-5	*E. crus-galli*	51.8 (42 – 74)	5.3 (4 – 6)	50.0 (35 – 88)	23.8 (16 – 32)

Population label	Original population ID	Species	Onset of maturity							
			Height - Form	Height - Extended	No. of tillers	No. of panicles	Panicle length	Panicle width	Flag leaf length	Flag leaf width
1	N12: 5-5	*E. colona*	435.3 (324 – 565)	533.8 (354 – 707)	3.3 (2 – 4)	6.8 (3 – 11)	85.5 (71 – 96)	14.8 (11 – 21)	85.5 (67 – 102)	4.5 (4 – 5)
2	N15: 6-1	*E. colona*	559.8 (411 – 646)	706.5 (499 – 941)	13.5 (9 – 21)	32.5 (22 – 56)	82.0 (74 – 91)	10.8 (8 – 12)	96.8 (44 – 133)	7.0 (5 – 8)
3	N11: 1-1	*E. colona*	585.0 (522 – 650)	671.8 (585 – 822)	5.0 (3 – 10)	8.0 (5 – 13)	105.0 (86 – 121)	14.0 (12 – 16)	125.8 (91 – 149)	6.3 (5 – 8)
4	N13: 14-2	*E. colona*	486.3 (392 – 571)	537.3 (433 – 624)	7.5 (2 – 11)	16.5 (12 – 23)	91.8 (74 – 118)	16.8 (12 – 21)	98.8 (87 – 111)	6.8 (5 – 9)
5	D13: 4-2	*E. colona*	250.3 (151 – 314)	265.3 (151 – 325)	6.3 (2 – 9)	8.5 (2 – 13)	49.5 (31 – 59)	18.0 (9 – 29)	96.0 (45 – 154)	5.0 (4 – 6)
6	W16: 4-3	*E. colona*	551.8 (415 – 608)	591.3 (462 – 665)	9.5 (7 – 12)	10.3 (5 – 17)	90.0 (76 – 98)	26.0 (23 – 33)	149.3 (104 – 176)	8.5 (8 – 10)
7	N11: 2-6	*E. colona*	492.0 (285 – 668)	556.3 (266 – 711)	6.5 (2 – 10)	8.3 (2 – 13)	77.8 (41 – 101)	17.5 (5 – 34)	117.5 (8 – 181)	5.5 (4 – 6)
8	N11: 13-3	*E. colona*	479.0 (408 – 599)	603.8 (467 – 720)	8.8 (6 – 12)	29.3 (10 – 68)	85.0 (63 – 114)	14.8 (12 – 17)	107.0 (71 – 151)	6.3 (5 – 8)
9	N12: 10-3	*E. crus-galli*	646.0 (609 – 681)	737.8 (658 – 832)	7.5 (7 – 8)	15.0 (12 – 20)	82.3 (76 – 90)	20.8 (16 – 29)	90.5 (66 – 117)	6.5 (5 – 8)
10	N11: 16-3	*E. crus-galli*	461.8 (392 – 570)	515.8 (411 – 657)	7.3 (3 – 11)	17.5 (8 – 28)	70.3 (55 – 94)	24.5 (11 – 44)	99.5 (54 – 163)	6.5 (5 – 8)
11	N11: 3-5	*E. crus-galli*	487.0 (169 – 688)	552.8 (271 – 733)	8.5 (4 – 10)	16.8 (10 – 20)	65.5 (43 – 77)	16.5 (8 – 24)	77.3 (74 – 86)	6.5 (6 – 8)

Population label	Original population ID	Species	Onset of maturity							
			Height - Form	Height - Extended	No. of tillers	No. of panicles	Panicle length	Panicle width	Flag leaf length	Flag leaf width
12	N15: 10-4	*E. colona*	643.0 (586 – 737)	704.0 (651 – 745)	6.0 (3 – 9)	12.5 (10 – 16)	78.8 (13 – 106)	17.5 (11 – 26)	128.5 (84 – 174)	6.0 (4 – 7)
13	N15: 12-3	*E. colona*	398.8 (374 – 415)	507.8 (499 – 520)	7.5 (3 – 11)	22.0 (9 – 44)	76.0 (60 – 95)	15.3 (12 – 19)	135.3 (97 – 166)	6.8 (3 – 11)
14	D41: 10-4	*E. colona*	505.0 (475 – 534)	555.8 (525 – 603)	4.5 (2 – 8)	8.8 (6 – 14)	79.3 (58 – 88)	21.3 (13 – 31)	117.3 (83 – 161)	(6 – 8) 6.5
15	D21: 4-1	*E. colona*	434.3 (300 – 542)	515.5 (357 – 625)	5.5 (2 – 11)	14.0 (2 – 29)	85.0 (72 – 97)	19.0 (11 – 35)	130.3 (83 – 166)	6.5 (6 – 7)
16	D11: 16-4	*E. colona*	534.3 (480 – 613)	621.8 (513 – 683)	13.5 (8 – 22)	27.0 (14 – 42)	90.5 (68 – 108)	31.0 (17 – 37)	143.8 (132 – 172)	5.8 (5 – 7)
17	W13: 5-2	*E. colona*	258.3 (216 – 297)	290.5 (256 – 339)	3.8 (3 – 5)	8.5 (6 – 12)	43.3 (36 – 49)	11.8 (11 – 13)	53.8 (46 – 64)	5.5 (4 – 6)
18	W13: 17-1	*E. colona*	654.8 (602 – 702)	695.5 (605 – 767)	4.5 (3 – 6)	9.3 (5 – 15)	122.3 (116 – 130)	18.8 (7 – 34)	141.0 (96 – 196)	7.8 (5 – 10)
19	W17: 10-1	*E. colona*	488.5 (399 – 529)	523.8 (484 – 544)	7.3 (2 – 13)	9.3 (4 – 19)	80.5 (66 – 96)	16.8 (8 – 21)	99.0 (61 – 134)	6.3 (4 – 7)
20	W13: 2-1	*E. colona*	614.8 (585 – 646)	661.3 (637 – 711)	7.0 (4 – 13)	8.8 (4 – 13)	89.0 (62 – 114)	16.5 (9 – 26)	110.0 (74 – 144)	5.0 (4 – 6)
21	W13: 11-9	*E. colona*	402.0 (371 – 446)	459.3 (374 – 558)	5.5 (3 – 8)	16.3 (9 – 28)	64.0 (57 – 71)	19.5 (16 – 26)	71.5 (42 – 136)	6.5 (6 – 8)

Population label	Original population ID	Species	Onset of maturity							
			Height - Form	Height - Extended	No. of tillers	No. of panicles	Panicle length	Panicle width	Flag leaf length	Flag leaf width
22	W14: 1-1	*E. colona*	668.0 (513 – 768)	713.0 (552 – 827)	4.5 (3 – 8)	7.8 (4 – 11)	110.3 (88 – 144)	10.8 (10 – 11)	130.3 (108 – 156)	7.5 (6 – 10)
23	W14: 6-3	*E. colona*	400.3 (151 – 547)	632.0 (464 – 766)	3.8 (2 – 6)	9.0 (3 – 14)	58.3 (29 – 71)	21.3 (12 – 32)	79.0 (40 – 104)	5.5 (4 – 8)
24	N15: 5-5	*E. crus-galli*	72.5 (46 – 116)	97.8 (79 – 126)	6.0 (3 – 8)	10.5 (6 – 14)	16.0 (9 – 20)	5.5 (3 – 8)	39.3 (31 – 46)	3.3 (3 – 4)
25	D21: 3-1	*E. colona*	475.8 (410 – 509)	503.0 (430 – 557)	8.0 (5 – 9)	18.5 (12 – 23)	75.0 (72 – 82)	23.8 (18 – 27)	121.0 (96 – 149)	6.0 (6 – 6)
26	D11: 20-1	*E. colona*	549.5 (408 – 664)	750.8 (452 – 943)	4.0 (4 – 4)	10.5 (3 – 15)	103.8 (49 – 143)	21.0 (15 – 29)	180.5 (92 – 284)	8.8 (6 – 12)
27	D51: 6-2	*E. colona*	406.5 (201 – 518)	442.8 (210 – 613)	8.5 (4 – 20)	15.5 (8 – 36)	73.0 (39 – 93)	27.0 (11 – 39)	109.8 (40 – 174)	6.3 (4 – 8)
28	N15: 13-4	*E. crus-galli*	211.3 (156 – 299)	245.3 (187 – 299)	4.0 (2 – 5)	5.3 (4 – 6)	42.0 (26 – 61)	8.3 (6 – 11)	51.7 (29 – 80)	4.0 (3 – 5)
29	D13: 16-1	*E. colona*	508.8 (413 – 614)	582.3 (422 – 750)	4.8 (1 – 9)	7.5 (1 – 13)	102.0 (72 – 131)	30.3 (20 – 41)	101.8 (63 – 129)	5.5 (3 – 8)
30	N14: 7-4	*E. colona*	616.5 (553 – 716)	736.5 (675 – 798)	7.8 (5 – 14)	21.3 (10 – 30)	126.0 (100 – 150)	25.8 (14 – 41)	125.8 (102 – 151)	6.8 (6 – 8)
31	W12: 9-3	*E. colona*	374.5 (268 – 463)	514.8 (380 – 692)	3.8 (1 – 6)	10.8 (2 – 15)	65.3 (52 – 74)	10.8 (8 – 14)	76.0 (48 – 126)	6.0 (5 – 88)
32	D12: 10-4	*E. colona*	512.8 (434 – 643)	581.8 (465 – 701)	10.5 (3 – 18)	17.5 (6 – 31)	97.3 (66 – 121)	30.5 (10 – 42)	114.5 (82 – 136)	7.0 (6 – 8)

Population label	Original population ID	Species	Onset of maturity							
			Height - Form	Height - Extended	No. of tillers	No. of panicles	Panicle length	Panicle width	Flag leaf length	Flag leaf width
33	W15: 8-1	*E. colona*	324.5 (264 – 389)	361.3 (336 – 401)	4.0 (2 – 7)	8.0 (3 – 17)	60.0 (55 – 66)	17.0 (15 – 19)	97.5 (76 – 114)	6.3 (6 – 7)
34	D14: 12-4	*E. colona*	519.8 (464 – 544)	590.0 (543 – 657)	8.0 (6 – 11)	21.0 (14 – 36)	89.5 (69 – 109)	24.5 (21 – 34)	113.8 (91 – 135)	6.5 (6 – 8)
35	W11: 1-3	*E. colona*	383.0 (271 – 515)	436.5 (294 – 544)	5.0 (3 – 8)	12.3 (6 – 21)	53.5 (45 – 64)	15.8 (12 – 19)	59.3 (42 – 83)	5.8 (5 – 6)
36	N15: 9-5	*E. crus-galli*	255.5 (216 – 320)	289.0 (229 – 337)	10.5 (8 – 12)	19.8 (16 – 25)	37.0 (32 – 43)	18.8 (11 – 27)	59.3 (44 – 76)	5.0 (4 – 8)

Appendix 2: Average data for morphological measurements of *Echinochloa crus-galli* (2006/2007) taken at onset of reproduction, emergence of the flag leaf and onset of maturity, where range of values are in parentheses and population labels as used in Chapter 3. Refer 3.3.1.1 for description of original population ID

Population label	Original population ID	Emergence of 1st panicle					
		Height-form	Height-extend	No. of tillers	No. of leaves	Culm colour	Culm hairiness
1	N15: 20-3	488.5 (124 – 781)	646.25 (314 – 961)	7.25 (5 – 10)	33.25 (19 – 54)	2.75 (2 – 3)	1 (1 – 1)
2	N15: 4-4	558.5 (422 – 724)	730.5 (676 – 840)	7.5 (4 – 12)	37 (27 – 54)	3.5 (3 – 4)	1.75 (1 – 2)
3	N12: 11-5	411 (208 – 535)	540.5 (388 – 685)	5.5 (3 – 9)	21.75 (19 – 26)	2.5 (2 – 3)	1.25 (1 – 2)
4	N11: 15-4	194.25 (131 – 319)	280.5 (175 – 400)	5.75 (4 – 7)	17 (13 – 21)	3 (3 – 3)	1 (1 – 1)
5	D11: 3-5	281.75 (160 – 351)	554 (486 – 694)	9.25 (7 – 13)	42.25 (26 – 69)	3 (3 – 2)	1.25 (1 – 2)
6	N11: 9-1	260 (185 – 320)	467 (296 – 548)	7.75 (5 – 12)	27.25 (18 – 39)	2.75 (2 – 3)	1.5 (1 – 2)
7	D11: 6-4	178.75 (130 – 204)	457.5 (390 – 507)	8.25 (7 – 9)	40.25 (36 – 44)	3 (3 – 3)	1 (1 – 1)
8	D21: 13-2	181 (111 – 234)	623.75 (522 – 709)	9.75 (6 – 13)	53 (38 – 62)	2.75 (2 – 3)	1 (1 – 1)
9	D11: 11-8	231 (211 – 242)	642.25 (540 – 839)	12.25 (12 – 13)	56.75 (54 – 6)	3.75 (3 – 4)	1 (1 – 1)

Population label	Original population ID	Emergence of 1st panicle					
		Height-form	Height-extend	No. of tillers	No. of leaves	Culm colour	Culm hairiness
10	N15: 11-5	436.75 (236 – 592)	665.25 (502 – 842)	10 (7 – 13)	50.25 (37 – 59)	3.5 (2 – 4)	1.25 (1 – 2)
11	D21: 17-2	156 (133 – 193)	409 (312 – 492)	8.25 (5 – 11)	28.5 (13 – 47)	2.75 (2 – 3)	1 (1 – 1)
12	N15: 16-2	333 (250 – 438)	644.75 (470 – 819)	11.5 (8 – 17)	52.75 (30 – 78)	2.75 (2 – 3)	1 (1 – 1)
13	D11: 20-4	255.75 (86 – 385)	413.25 (135 – 587)	6.75 (3 – 16)	22.5 (10 – 49)	2.5 (1 – 3)	1.25 (1 – 2)
14	N12: 12-2	410.5 (270 – 608)	682.5 (549 – 759)	12.5 (10 – 16)	52 (35 – 77)	2.25 (1 – 3)	1 (1 – 1)
15	N11: 11-2	400.5 (312 – 482)	592.75 (542 – 663)	10.25 (9 – 12)	34.5 (26 – 40)	2 (2 – 2)	1.5 (1 – 2)
16	N15: 2-4	335.25 (261 – 429)	639.75 (506 – 742)	12.75 (10 – 16)	52.75 (44 – 67)	2.75 (2 – 4)	1 (1 – 1)
17	N15: 1-4	374.25 (240 – 519)	557.5 (524 – 626)	11.75 (9 – 17)	48.25 (26 – 65)	2.75 (2 – 3)	1 (1 – 1)
18	D21: 5-5	347.25 (195 – 464)	763.25 (498 – 920)	13.25 (9 – 17)	64.75 (40 – 81)	2.75 (2 – 3)	1 (1 – 1)
19	N15: 7-4	312.5 (174 - 444)	542.5 (361 – 644)	11 (6 – 19)	50.75 (21 – 72)	2.25 (2 – 3)	1 (1 – 1)
20	N15: 2-1	217.5 (166 – 255)	378.75 (330 – 455)	5.25 (3 – 8)	22 (11 – 33)	2 (1 – 3)	1 (1 – 1)

Population label	Original population ID	Emergence of 1st panicle					
		Height-form	**Height-extend**	**No. of tillers**	**No. of leaves**	**Culm colour**	**Culm hairiness**
21	N11: 3-5	412.25 (299 – 495)	614.5 (524 – 727)	10 (6 – 14)	46.75 (35 – 63)	3 (3 – 3)	1 (1 – 1)
22	N15: 9-5	279.5 (217 – 390)	545.5 (455 – 639)	9.25 (5 – 11)	37 (19 – 50)	2.75 (2 – 3)	1 (1 – 1)
23	N11: 3-3	576.75 (455 – 671)	915 (884 – 945)	12.5 (10 – 17)	65.25 (59 – 69)	3.5 (3 – 4)	1 (1 – 1)
24	N11: 11-4	517.25 (242 – 657)	743 (589 – 873)	11.5 (3 – 17)	53 (22 – 71)	4 (4 – 4)	1 (1 – 1)
25	N15: 14-3	417.75 (278 – 663)	838.75 (790 – 909)	11.5 (6 – 15)	65 (43 – 80)	3 (3 – 3)	1.5 (1 – 2)
26	N15: 14-1	303.5 (223 – 475)	555.25 (455 – 682)	10.75 (8 – 14)	46.75 (33 – 64)	3.25 (3 – 4)	1 (1 – 1)
27	N15: 14-4	245 (197 – 279)	535.75 (443 – 618)	10.5 (8 – 12)	52.75 (46 – 60)	2.5 (2 – 3)	1 (1 – 1)

Population label	Original population ID	Fully emerged flag leaf			
		Flag Leaf length	Flag leaf width	Panicle length	Panicle width
1	N15: 20-3	127.25 (105 – 168)	12.5 (10 – 17)	118.75 (70 – 163)	58.5 (37 – 72)
2	N15: 4-4	148.25 (87 – 188)	14 (13 – 15)	121.5 (104 – 149)	82.25 (57 – 94)
3	N12: 11-5	151.5 (132 – 181)	10 (8 – 11)	104 (85 – 126)	78 (64 – 98)
4	N11: 15-4	100.75 (66 – 133)	7.75 (6 – 9)	68.25 (45 – 79)	50 (31 – 64)
5	D11: 3-5	135.5 (122 – 150)	10.5 (9 – 11)	110 (94 – 124)	85 (74 – 101)
6	N11: 9-1	161.75 (105 – 195)	9.25 (6 – 13)	92.25 (63 – 119)	66 (35 – 98)
7	D11: 6-4	137 (111 – 160)	10.75 (10 – 11)	104.25 (88 – 115)	64.75 (61 – 69)
8	D21: 13-2	171.5 (116 – 204)	11.75 (11 – 12)	136 (109 – 152)	92.75 (88 – 98)
9	D11: 11-8	196 (138 – 297)	11.5 (10 – 14)	125.25 (106 – 144)	78 (63 – 92)
10	N15: 11-5	154.5 (116 – 206)	14.25 (12 – 17)	121 (101 – 154)	81.75 (68 – 91)
11	D21: 17-2	125 (116 – 137)	10.25 (10 – 11)	78.75 (71 – 86)	47.5 (40 – 53)

Population label	Original population ID	Fully emerged flag leaf			
		Flag Leaf length	Flag leaf width	Panicle length	Panicle width
12	N15: 16-2	185.5 (158 – 225)	13 (11 – 16)	135.25 (109 – 159)	102.25 (75 – 129)
13	D11: 20-4	111.5 (66 – 164)	8.5 (5 – 11)	74.5 (32 – 110)	51.25 (9 – 82)
14	N12: 12-2	173 (151 – 187)	12 (11 – 14)	120.25 (104 – 139)	84 (62 – 107)
15	N11: 11-2	162 (104 – 210)	9.75 (9 – 12)	94.75 (83 – 112)	66.25 (34 – 89)
16	N15: 2-4	196.5 (124 – 267)	15.5 (12 – 18)	126.25 (109 – 143)	100 (76 – 136)
17	N15: 1-4	157.75 (133 – 178)	12.25 (9 – 14)	106.5 (84 – 119)	84.5 (63 – 106)
18	D21: 5-5	170.25 (116 – 209)	12.25 (9 – 14)	131 (94 – 154)	81.5 (72 – 88)
19	N15: 7-4	158 (115 – 199)	13 (11 – 15)	127 (113 – 144)	103.25 (98 – 115)
20	N15: 2-1	147.25 (129 – 170)	11 (9 – 12)	86.5 (76 – 95)	67.75 (64 – 71)
21	N11: 3-5	157.25 (148 – 169)	11.75 (11 – 12)	124.5 (114 – 134)	88.75 (83 – 102)
22	N15: 9-5	173.5 (162 – 189)	12.5 (12 – 14)	102.75 (91 – 119)	75.75 (60 – 88)

Population label	Original population ID	Fully emerged flag leaf			
		Flag Leaf length	Flag leaf width	Panicle length	Panicle width
23	N11: 3-3	155.25 (148 – 162)	11.75 (11 – 12)	127.5 (124 – 133)	90 (85 – 97)
24	N11: 11-4	156 (112 – 223)	14 (13 – 15)	142.75 (131 – 151)	89.75 (62 - 116)
25	N15: 14-3	265 (189 – 311)	17.75 (17 – 18)	166.5 (146 – 182)	123.75 (82 – 153)
26	N15: 14-1	206.25 (150 – 248)	11.5 (10 – 13)	127 (106 – 149)	93.5 (61 – 135)
27	N15: 14-4	187.75 (158 – 210)	11.5 (11 – 12)	120.25 (117 – 127)	85.75 (83 – 92)

Population label	Original population ID	Onset of Maturity								
		Height - Form	**Height - Extended**	**No. of tillers**	**No. of panicles**	**Flag leaf length**	**Flag leaf width**	**Panicle length**	**Panicle width**	**Panicle pigment**
1	N15: 20-3	968.5 (293 – 1603)	1104.5 (5300 – 1664)	11.75 (8 – 16)	10.75 (6 – 19)	127.75 (104 - 175)	12.5 (10 – 17)	117.75 (73 – 162)	55 (41 – 72)	1.75 (1 – 2)
2	N15: 4-4	983 (762 – 1065)	1139 (1040 – 1293)	9.75 (9 – 12)	7.25 (4 – 9)	149.75 (92 – 186)	14 (13 – 15)	121 (102 – 147)	78.25 (51 – 94)	2 (2 – 2)
3	N12: 11-5	739.75 (561 – 1006)	872.75 (691 – 1089)	14.25 (7 – 19)	11.75 (1 – 19)	152.5 (132 – 182)	9.75 (8 – 11)	103.75 (86 – 122)	66 (52 – 74)	1.75 (1 – 2)
4	N11: 15-4	353 (194 – 463)	414.5 (206 – 547)	11.75 (11 – 13)	18.25 (14 – 21)	101.25 (66 – 133)	7.75 (6 – 9)	68.75 (45 – 79)	45.5 (31 – 64)	2 (2 – 2)
5	D11: 3-5	581.25 (487 – 679)	957 (772 – 1121)	15.25 (12 – 19)	14.5 (12 – 22)	136.75 (119 – 151)	10.5 (9 – 12)	110 (94 – 125)	73.75 (52 – 96)	2 (2 – 2)
6	N11: 9-1	487 (284 – 719)	624.25 (336 – 805)	20.25 (16 – 25)	28.5 (19 – 40)	162.5 (106 – 195)	10.5 (7 – 13)	93 (58 – 126)	66 (22 – 98)	1.75 (1 – 2)
7	D11: 6-4	454.75 (381 – 531)	761.5 (565 – 914)	17.5 (10 – 21)	20 (15 – 26)	144.25 (136 – 161)	10.75 (10 – 11)	108.25 (102 – 116)	65 (51 – 74)	2 (2 – 2)
8	D21: 13-2	788.25 (604 – 1007)	1030 (938 – 1129)	15.75 (12 – 19)	11 (6 – 19)	169.5 (1199 – 202)	12 (11 – 13)	132.5 (109 – 149)	83.25 (74 – 92)	2 (2 – 2)
9	D11: 11-8	791.5 (506 – 934)	947.75 (747 – 1053)	20.25 (15 – 24)	18 (8 – 26)	194.75 (134 – 298)	11.5 (11 – 13)	128.75 (105 – 151)	91.75 (77 – 121)	2 (2 – 2)
10	N15: 11-5	823 (542 – 1020)	988 (859 – 1134)	12.5 (7 – 18)	12.25 (4 – 27)	146.25 (116 – 203)	14.25 (12 – 17)	128.5 (101 – 156)	68 (15 – 106)	2 (2 – 2)
11	D21: 17-2	606 (503 – 765)	924 (801 – 1015)	19.5 (15 – 25)	33 (22 – 57)	125 (116 – 137)	10.75 (10 – 11)	78 (71 – 86)	47.5 (40 – 53)	2 (2 – 2)

Population label	Original population ID	Onset of Maturity								
		Height - Form	Height - Extended	No. of tillers	No. of panicles	Flag leaf length	Flag leaf width	Panicle length	Panicle width	Panicle pigment
12	N15: 16-2	728.5 (523 – 1059)	949.75 (795 – 1116)	14.25 (9 – 19)	10.25 (4 – 16)	190.25 (159 – 221)	12 (11 – 13)	140 (106 – 159)	72.5 (39 – 98)	2 (2 – 2)
13	D11: 20-4	421.75 (132 – 644)	573.75 (194 – 825)	10.5 (7 – 15)	15.75 (7 – 32)	111.5 (69 – 162)	8.75 (5 – 11)	74.25 (36 – 109)	41.75 (10 – 61)	2 (2 – 2)
14	N12: 12-2	838.25 (737 – 951)	911.25 (875 – 989)	18.5 (11 – 25)	21.25 (6 – 38)	181.25 (151 – 241)	12.5 (11 – 15)	122.25 (104 – 147)	85 (64 – 112)	1.25 (1 – 2)
15	N11: 11-2	517.25 (417 – 638)	680.75 (560 – 788)	21.25 (16 – 32)	37.75 (20 – 46)	161 (104 – 206)	10 (9 – 13)	94.75 (83 – 112)	67.75 (34 – 89)	3 (3 – 3)
16	N15: 2-4	792.25 (685 – 914)	985.5 (945 – 1077)	16.75 (15 – 19)	21 (14 – 28)	195.5 (123 – 269)	15.75 (13 – 18)	126.25 (106 – 145)	88.75 (56 – 131)	2 (2 – 2)
17	N15: 1-4	697 (510 – 866)	823.25 (665 – 990)	22 (14 – 31)	21.5 (15 – 27)	154.5 (133 – 175)	11.25 (9 – 13)	106 (84 – 120)	77 (62 – 99)	2 (2 – 2)
18	D21: 5-5	611.5 (453 – 799)	1118.5 (870 – 1292)	21.5 (14 – 36)	25.5 (8 – 53)	170.5 (118 – 212)	14 (11 – 19)	132.25 (100 – 154)	69.75 (52 – 84)	1 (1 – 1)
19	N15: 7-4	900.75 (803 – 996)	1028 (876 – 1142)	21 (14 – 28)	25 (15 – 34)	138.25 (117 – 180)	13.25 (12 – 15)	125.75 (111 – 142)	96.75 (89 – 105)	2 (2 – 2)
20	N15: 2-1	586.75 (505 – 680)	676.75 (551 – 796)	13 (5 – 19)	14.2 (6 – 26)	148.25 (129 – 172)	10.75 (9 – 12)	86.25 (75 – 94)	52.25 (33 – 62)	2 (2 – 2)
21	N11: 3-5	787 (559 – 935)	960.25 (929 – 993)	18.5 (16 – 21)	11.75 (6 – 17)	159.25 (148 – 169)	11.75 (11 – 12)	124 (115 – 131)	84.5 (83 – 87)	1.25 (1 – 2)

Population label	Original population ID	Onset of Maturity								
		Height - Form	Height - Extended	No. of tillers	No. of panicles	Flag leaf length	Flag leaf width	Panicle length	Panicle width	Panicle pigment
22	N15: 9-5	754.75 (550 – 1219)	849 (680 – 1228)	18.5 (7 – 30)	16.5 (6 – 24)	175 (159 – 199)	13 (12 – 14)	114.25 (91 – 156)	73 (61 – 92)	2 (2 – 2)
23	N11: 3-3	827 (634 – 912)	1043.5 (991 – 1127)	18.5 (16 – 21)	10 (6 – 15)	159.25 (149 – 168)	11.5 (11 – 12)	121.5 (115 – 129)	84.5 (83 – 87)	3 (3 – 3)
24	N11: 11-4	1271.75 (1222 – 1336)	1329 (1286 – 1447)	12.5 (6 – 15)	9.75 (3 – 13)	130 (111 – 171)	14 (12 – 16)	147.75 (135 – 168)	84.5 (64 – 116)	1 (1 – 1)
25	N15: 14-3	1018 (940 – 1105)	1323.75 (1159 – 1743)	12.75 (10 – 15)	6.75 (5 – 8)	259.25 (172 – 309)	17.5 (16 – 18)	164 (146 – 183)	120.75 (78 – 159)	1.75 (1 – 2)
26	N15: 14-1	814 (678 – 921)	877.25 (749 – 1031)	18 (14 – 21)	12.75 (6 – 21)	206.5 (149 – 249)	12 (10 – 14)	126.25 (103 – 152)	91 (55 – 148)	1.25 (1 – 2)
27	N15: 14-4	692 (561 – 796)	852 (745 – 912)	14.5 (12 – 20)	8 (4 – 10)	186.25 (159 – 208)	11.25 (11 – 12)	119.5 (109 – 136)	80.75 (79 – 82)	3 (3 – 3)

Appendix 3: Average dry weight biomass of *Echinochloa colona* and *E. crus-galli* 14 days after treatment with different rates of glyphosate (450 g/L) showing range of dry weights in parentheses and population labels as used in Chapter 5. Refer 3.3.1.1 for description of original population ID.

Population label	Species	Original population ID	Rate of glyphosate (L/ha)			
			0 (control)	**0.25**	**0.50**	**1.00**
DG1	*E. crus-galli*	D11: 11-8	0.237 (0.082 - 0.473)	0.161 (0.07 – 0.28)	0.070 (0.037 – 0.152)	0.046 (0.018 – 0.074)
DG2	*E. crus-galli*	D11: 20-4	0.548 (0.106 - 1.493)	0.445 (0.148 – 1.262)	0.140 (0.08 – 0.255)	0.090 (0.034 – 0.1998)
DG3	*E. crus-galli*	D11: 3-5	0.311 (0.09 - 0.651)	0.196 (0.103 – 0.3)	0.135 (0.065 – 0.25)	0.051 (0.037 – 0.082)
NG1	*E. crus-galli*	N11: 11-2	0.502 (0.171 - 1.116)	0.736 (0.414 – 1.34)	0.357 (0.1118 – 0.651)	0.257 (0.103 – 0.351)
NG2	*E. crus-galli*	N11: 15-4	1.472 (0.68 - 1.776)	0.239 (0.117 – 0.406)	0.274 (0.085 – 0.798)	0.080 (0.027 – 0.139)
NG3	*E. crus-galli*	N11: 8-4	0.918 (0.117 – 2.052)	0.280 (0.169 – 0.37)	0.372 (0.14 – 0.808)	0.080 (0.056 – 0.106)
NG4	*E. crus-galli*	N11: 9-1	0.585 (0.27 – 1.118)	0.525 (0.315 – 0.782)	0.516 (0.125 – 0.831)	0.134 (0.065 – 0.23)
NG5	*E. crus-galli*	N12: 11-5	0.556 (0.226 – 0.897)	0.250 (0.094 – 0.408)	0.317 (0.03 – 1.022)	0.065 (0.026 – 0.1)
NG6	*E. crus-galli*	N15: 1-4	0.567 (0.318 – 1.112)	0.522 (0.227 – 1.155)	0.325 (0.173 – 0.728)	0.168 (0.104 – 0.196)

Population label	Species	Original population ID	Rate of glyphosate (L/ha)			
			0 (control)	**0.25**	**0.50**	**1.00**
NG7	*E. crus-galli*	N15: 11-5	0.905 (0.571 – 1.23)	0.406 (0.201 – 0.8)	0.210 (0.123 – 0.267)	0.130 (0.082 – 0.191)
NG8	*E. crus-galli*	N15: 14-1	0.591 (0.18 – 0.986)	0.615 (0.232 – 1.222)	0.313 (0.12 – 0.702)	0.153 (0.096 – 0.193)
NG9	*E. crus-galli*	N15: 14-3	1.049 (0.844 – 1.257)	0.528 (0.264 – 1.103)	0.200 (0.103 – 0.351)	0.094 (0.022 – 0.1540
NG10	*E. crus-galli*	N15: 14-4	0.630 (0.063 – 1.024)	0.863 (0.375 – 1.858)	0.106 (0.026 – 0.157)	0.108 (0.073 – 0.164)
NG11	*E. crus-galli*	N15: 2-1	0.525 (0.27 – 0.873)	0.459 (0.255 – 0.615)	0.249 (0.109 -0.501)	0.147 (0.108 – 0.171)
NG12	*E. crus-galli*	N15: 20-3	0.673 (0.498 – 0.957)	0.460 (0.23 – 1.041)	0.350 (0.036 – 1.208)	0.041 (0.016 – 0.053)
NG13	*E. crus-galli*	N15: 4-4	0.778 (0.475 – 1.020)	0.341 (0.102 – 0.602)	0.316 (0.042 – 1.044)	0.070 (0.02 – 0.116)
NG14	*E. crus-galli*	N15: 7-4	0.948 (0.596 – 1.192)	0.702 (0.264 – 1.539)	0.219 (0.058 – 0.396))	0.141 (0.119 – 0.174)
NG15	*E. crus-galli*	N15: 9-5	0.679 (0.117 – 1.125)	0.794 (0.657 – 1.14)	0.258 (0.134 – 0.508)	0.167 (0.159 – 0.175)
DC1	*E. colona*	D11: 20-1	0.339 (0.167 – 0.485)	0.360 (0.158 – 0.831)	0.145 (0.029 – 0.371)	0.100 (0.083 – 0.121)
DC2	*E. colona*	D13: 16-1	0.374 (0.223 – 0.653)	0.120 (0.047 – 0.216)	0.060 (0.014 – 0.177)	0.041 (0.026 – 0.056)

Population label	Species	Original population ID	Rate of glyphosate (L/ha)			
			0 (control)	**0.25**	**0.50**	**1.00**
DC3	*E. colona*	D13: 4-2	0.692 (0.543 – 0.864)	0.327 (0.072 – 0.851)	0.133 (0.069 – 0.198)	0.085 (0.059 – 0.126)
DC4	*E. colona*	D14: 12-4	0.180 (0.08 – 0.458)	0.191 (0.084 – 0.391)	0.232 (0.042 – 0.585)	0.067 (0.037 – 0.092)
DC5	*E. colona*	D21: 4-1	0.227 (0.095 – 0.42)	0.125 (0.012 – 0.42)	0.095 (0.009 – 0.303)	0.029 (0.01 – 0.074)
DC6	*E. colona*	D41: 10-4	1.018 (0.622 – 1.77)	0.412 (0.172 – 0.722)	0.119 (0.045 – 0.281)	0.037 (0.1 – 0.068)
DC7	*E. colona*	D51: 6-2	0.355 (0.147 – 0.56)	0.312 (0.195 – 0.472)	0.074 (0.055 – 0.085)	0.078 (0.02 – 0.136)
NC1	*E. colona*	N11: 1-1	0.155 (0.051 – 0.296)	0.184 (0.062 – 0.236)	0.059 (0.01 – 0.139)	0.036 (0.014 – 0.069)
NC2	*E. colona*	N11: 13-3	0.313 (0.066 – 0.449)	0.339 (0.043 – 1.09)	0.146 (0.072 – 0.253)	0.059 (0.039 – 0.078)
NC3	*E. colona*	N15: 12-3	0.211 (0.148 – 0.273)	0.325 (0.044 – 1)	0.294 (0.025 – 1.024)	0.074 (0.033 – 0.157)
WC1	*E. colona*	W11: 1-3	0.192 (0.074 – 0.255)	0.107 (0.01 – 0..339)	0.019 (0.011 – 0.031)	0.021 (0.014 – 0.028)
WC2	*E. colona*	W12: 9-3	0.472 (0.332 – 0.729)	0.271 (0.088 – 0.433)	0.058 (0.017 – 0.117)	0.046 (0.029 – 0.064)
WC3	*E. colona*	W13: 2-1	0.758 (0.288 – 1.089)	0.158 (0.085 – 0.261)	0.419 (0.04 – 1.468)	0.046 (0.019 – 0.082)

Population label	Species	Original population ID	Rate of glyphosate (L/ha)			
			0 (control)	**0.25**	**0.50**	**1.00**
WC4	*E. colona*	W13: 5-2	0.585 (0.436 – 0.717)	0.122 (0.012 – 0.41)	0.049 (0.046 – 0.051)	0.026 (0.02 – 0.0338)
WC5	*E. colona*	W14: 6-3	0.595 (0.073 – 2.042)	0.474 (0.074 – 1.096)	0.431 (0.054 – 1.045)	0.076 (0.029 – 0.124)
WC6	*E. colona*	W14: 1-1	0.820 (0.36 – 1.654)	0.184 (0.094 – 0.301)	0.128 (0.027 – 0.201)	0.038 (0.014 – 0.056)
WC7	*E. colona*	W15: 8-1	0.771 (0.461 – 1.062)	0.365 (0.059 – 0.698)	0.058 (0.019 – 0.077)	0.068 (0.04 – 0.105)
WC8	*E. colona*	W17: 10-1	0.699 (0.537 – 0.944)	0.237 (0.111 – 0.321)	0.122 (0.057 – 0.236)	0.031 (0.029 – 0.034)

Appendix 4: Table of the log transformed dry weight biomass means of 18 populations each of *Echinochloa crus-galli* and *E. colona* at four rates of glyphosate adjusted for covariates (number of leaves, plant height). (LSD = 0.7084).

Population label	Species	Original population ID	Rate of glyphosate (L/ha)			
			0 (control)	0.25	0.50	1.00
DG1	*E. crus-galli*	D11: 11-8	-1.013	-1.707	-2.415	-2.795
DG2	*E. crus-galli*	D11: 20-4	-1.317	-1.648	-2.466	-2.779
DG3	*E. crus-galli*	D11: 3-5	-1.149	-1.339	-1.974	-3.054
NG1	*E. crus-galli*	N11: 11-2	-1.324	-1.921	-2.082	-2.926
NG2	*E. crus-galli*	N11: 15-4	-0.686	-1.431	-1.781	-2.987
NG3	*E. crus-galli*	N11: 8-4	-1.030	-1.748	-1.928	-2.661
NG4	*E. crus-galli*	N11: 9-1	-1.303	-1.752	-1.957	-2.947
NG5	*E. crus-galli*	N12: 11-5	-0.538	-1.491	-2.020	-2.666
NG6	*E. crus-galli*	N15: 1-4	-1.330	-1.678	-2.226	-2.729
NG7	*E. crus-galli*	N15: 11-5	-0.826	-1.309	-2.610	-2.425
NG8	*E. crus-galli*	N15: 14-1	-1.068	-1.483	-2.233	-2.758
NG9	*E. crus-galli*	N15: 14-3	-0.396	-1.380	-2.242	-3.241
NG10	*E. crus-galli*	N15: 14-4	-1.169	-1.488	-2.447	-2.886
NG11	*E. crus-galli*	N15: 2-1	-1.050	-1.638	-2.033	-2.676

Population label	Species	Original population ID	Rate of glyphosate (L/ha)			
			0 (control)	0.25	0.50	1.00
NG12	*E. crus-galli*	N15: 20-3	-0.017	-1.243	-2.065	-2.879
NG13	*E. crus-galli*	N15: 4-4	-0.255	-0.977	-1.642	-2.728
NG14	*E. crus-galli*	N15: 7-4	-1.564	-1.607	-2.068	-2.660
NG15	*E. crus-galli*	N15: 9-5	-0.778	-1.830	-2.505	-2.635
DC1	*E. colona*	D11: 20-1	-0.809	-1.184	-2.026	-2.465
DC2	*E. colona*	D13: 16-1	-0.832	-1.661	-2.377	-2.606
DC3	*E. colona*	D13: 4-2	-0.377	-1.077	-1.944	-2.234
DC4	*E. colona*	D14: 12-4	-1.693	-1.759	-1.927	-2.413
DC5	*E. colona*	D21: 4-1	-0.459	-1.864	-2.076	-2.955
DC6	*E. colona*	D41: 10-4	-0.926	-1.342	-2.048	-2.733
DC7	*E. colona*	D51: 6-2	-1.421	-1.458	-2.242	-2.745
NC1	*E. colona*	N11: 1-1	-1.365	-1.750	-2.430	-3.136
NC2	*E. colona*	N11: 13-3	-0.684	-1.399	-1.710	-2.266
NC3	*E. colona*	N15: 12-3	-1.223	-1.413	-2.001	-2.779
WC1	*E. colona*	W11: 1-3	-0.462	-1.751	-2.713	-2.656

Population label	Species	Original population ID	Rate of glyphosate (L/ha)			
			0 (control)	**0.25**	**0.50**	**1.00**
WC2	*E. colona*	W12: 9-3	-0.356	-1.204	-2.561	-2.544
WC3	*E. colona*	W13: 2-1	0.021	-1.474	-1.949	-2.423
WC4	*E. colona*	W13: 5-2	-0.067	-1.968	-2.383	-2.685
WC5	*E. colona*	W14: 6-3	-1.097	-1.197	-1.278	-2.679
WC6	*E. colona*	W14: 1-1	-0.914	-1.705	-2.104	-2.798
WC7	*E. colona*	W15: 8-1	-0.137	-1.294	-2.338	-2.542
WC8	*E. colona*	W17: 10/1	-0.759	-1.400	-2.078	-2.489